

# Insight into the Deep Eutectic Solvent Pretreatment of Oil Palm Empty Fruit Bunches: Effects of Temperature, Empty Fruit Bunch to Solvent Ratio, and Time

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This work aimed to comprehensively examine the pretreatment efficiency of oil palm empty fruit bunches (EFB) using two different types of deep eutectic solvent (DES) mixtures, *i.e.*, choline chloride/imidazole (DES-I) and choline chloride/glycerol (DES-G) in terms of pretreated EFB structural composition and enzymatic hydrolysis. The influence of the pretreatment temperature (55 °C, 90 °C, 125 °C, 160 °C, and 195 °C), EFB to solvent ratio (1:5, 1:10, 1:15, and 1:20), and pretreatment time (2 h, 4 h, and 6 h) on the performance of pretreated EFB and the generated black liquor was examined. The optimal conditions for EFB pretreatment were 160 °C, 1:5 ratio, and 2 h using DES-I solvent, and 160 °C, 1:10 ratio and 4 h using DES-G solvent. The structural carbohydrates of empty fruit bunch pretreated with DES-I, DES-I EFB1 and DES-G, DES-G EFB2 increased to 66.1%, and 64.6%, respectively. The enzymatic hydrolysis of DES-I EFB1 resulted in higher glucan conversion (92.4%) compared to DES-G EFB2, indicating that DES-I solvent was more efficient than DES-G for EFB pretreatment. X-ray diffraction, Fourier transform infrared spectroscopy, and variable-pressure scanning electron microscopy confirmed the removal of lignin and hemicelluloses from EFB during pretreatment and enzymatic hydrolysis.

*Keywords:* Empty fruit bunch; Deep eutectic solvents; Dissolution; Pretreated EFB; Enzymatic hydrolysis; Glucan conversion

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## INTRODUCTION

Malaysia is the second largest crude palm oil producer in the world, after Indonesia, in which palm oil has become the single-largest commodity crop earner to Malaysia. The large-scale production of crude palm oil has consequently generated a large amount of palm-based by-products. It is reported that the palm oil processing only utilizes 10% of a palm oil tree for conversion into palm oil products, while the remaining 90% of the tree is converted into biomass residue, which is still underutilized (Hafyan *et al.* 2020). The solid waste produced from palm oil processing include empty fruit bunches (EFB), palm oil trunk (OPT), palm oil frond (OPF), and mesocarp fiber. The Malaysia Biomass Industries Confederation (MBIC) reported that there was approximately 19.5 million tons of EFB generated in 2015 (MBIC 2016). The abundant amount of EFB, if not managed properly, can eventually cause environmental problems, despite being a resource with high value

applications. Hence, there is a need to convert EFB to value-added chemical feedstock that can benefit the palm oil industry.

The EFB consist of three primary components: cellulose (37% to 59.7%), hemicellulose (16% to 28%), and lignin (10% to 31%) (Safri *et al.* 2017; Aini *et al.* 2018). Although EFB is regarded as a high-potential feedstock, it is still utilized for low value biomass-based products, *e.g.*, fibers, compost, fuel, and fuel pellets. The complex and recalcitrant structure of the EFB posed by the intertwined carbohydrate-lignin structure has become the major hurdle for high value applications, signifying the need of a pretreatment as a key upstream process prior to high value applications such as biomass-derived fuels and chemicals (Lee *et al.* 2014; Hassan and Mutelet 2017; Tan *et al.* 2018). The pretreatment alters and opens up the EFB cell wall structure *via* hydrolyzing the hemicelluloses, degrading the lignin matrix, reducing the cellulose crystallinity, and finally making the cellulose more accessible to enzymes or acid during the hydrolysis process for producing fermentable sugars, which improves the carbohydrate conversion (Abdul *et al.* 2016).

Several types of pretreatments have been employed to pretreat EFB including chemical pretreatment, mechanical pretreatment, and biological pretreatment. Although chemical pretreatment using acid or alkali is the most common technique used to pretreat EFB, it also has some drawbacks. The acid pretreatment is known to be less effective in removing lignin and results in lower sugar yield due to carbohydrate degradation into the enzyme-inhibiting byproducts, *e.g.*, furfural, 5-hydroxymethylfurfural (HMF), formic acid, and levulinic acid (Cara *et al.* 2007; Jeong *et al.* 2012). Alkaline pretreatment causes the loss of hemicelluloses, and the formation of inhibitors under severe conditions. It also causes the formation of salts upon neutralization of the pretreated biomass, which may present challenges with its disposal during product purification (Prado *et al.* 2012)

Due to the environmental awareness, there is an increasing interest in utilizing ionic liquid (IL) as the pretreatment solvent. Ionic liquid fits the criteria of a green solvent, *e.g.*, non-volatility and negligible vapor pressures (Wilkes 2002). However, it has the following disadvantages: a high cost, complex preparation of the solvent, low moisture tolerance, and toxic character (Degam 2017). The discovery of deep eutectic solvent (DES) may substitute for the role of IL due to its similar physico-chemical properties, and also has additional advantages, *e.g.*, lower cost, easy synthesis, renewability, recyclability, high purity, low toxicity, non-flammability properties, and the fact that it is a biodegradable solvent (Zhang *et al.* 2012; Thi and Lee 2019). A DES is an eutectic liquid mixture of two or three components, which has the ability to establish a hydrogen bond interaction between the hydrogen bond donor (HBD) and the hydrogen bond acceptor (HBA) (Kalhor and Ghandi 2019). Majová *et al.* (2017) discussed the recovery of DES after pretreatment of biomass for further use, by subsequent evaporation of anti-solvent or simple freeze-drying of the washed solutions. Recently, DESs have been proposed as worthy alternatives for lignocellulose biomass dissolution (Zhao *et al.* 2013; Xia *et al.* 2014). Moreover, Lehmann *et al.* (2014) reported that DESs are good solvents for enzyme activity.

Recent work on EFB pretreatment using DES has been demonstrated by several researchers, who mostly reported on the efficient pretreatment using Type III DESs, *i.e.*, choline chloride-lactic acid (Tan *et al.* 2018; Thi and Lee 2019; Sai and Lee 2019; Quek *et al.* 2020). Tan *et al.* (2018) used six different DESs, varying from acidic to basic in nature, and reported the combination of choline chloride-lactic acid produced the best result by achieving 100% hemicellulose extraction and 88% delignification compared to raw EFB.

Thi and Lee (2019) compared three combination of Type III DES using choline

chloride, choline chloride-lactic acid (ChCl-LA), choline chloride-urea (ChCl-U), and choline chloride-glycerol (ChCl-G) to pretreat EFB at a temperature of 120 °C for 3 h, with a solid to liquid ratio of 1:10 (w/v). Thi and Lee (2019) concluded that the pretreatment using acidic ChCl-LA was the best, which produced the highest reducing sugars yield (20.7%). Sai and Lee (2019) further optimized the conditions of EFB pretreatment using ChCl-LA at a temperature of 100 °C for 1 h with a solid to solvent ratio of 1:10 (w/v) and managed to achieve a maximum reducing sugars yield of 51.1% higher than dilute acid, alkaline, and organosolv pretreatments, which indicated the efficiency of DES pretreatment. Quek *et al.* (2020) demonstrated the delignification of EFB using the ultrasound-assisted ChCl-LA pretreatment for 30 min at a temperature of 50 °C in a sonication bath that resulted in the reduction of total lignin content in the EFB from 31.6% to 18.8%.

Imidazole, another green solvent in the amine group, can be used as a HBD in Type III DES to create a novel and sustainable approach in LCB pretreatment. It is an amphoteric molecule with base-dominating characteristics and previously has been used to pretreat corncob and wheat straw (Procentese *et al.* 2015; Morais *et al.* 2015). Procentese *et al.* (2015) used three different DES systems with choline chloride as the HBA and varied the HBD. They reported the pretreatment using choline chloride-imidazole conducted at a temperature of 115 °C, 15 h, and a solid to liquid ratio of 1:16 (w/v) produced a glucose and xylose yields of 94% and 84%, respectively, after enzymatic hydrolysis. The DES systems used were able to remove lignin, increase the biomass crystallinity, and reduce the cellulose crystallinity depending on the chosen DES. Morais *et al.* (2015) also reported the use of imidazole as a novel solvent for wheat straw fractionation and delignification to produce cellulose- and hemicellulose-rich fractions and depolymerized lignin. The highest recovery of cellulose-rich material was obtained at a temperature of 170 °C for 2 h, achieving a 62.4% cellulose composition in the pretreated wheat straw compared to the 38.8% cellulose composition in native wheat straw. The enzymatic hydrolysis of imidazole-pretreated wheat straw resulted in a 99.3% glucan to glucose yield and extensive delignification (up to 92%) for the cellulose-rich material.

Thus far, the DES mixture combination of choline chloride-imidazole has not been fully investigated in terms of EFB pretreatment. Although Tajudin *et al.* (2019) reported on the use of a choline chloride-imidazole mixture for pretreating different native EFB, there has been no comprehensive study on the effect of different pretreatment conditions, *i.e.*, temperature, biomass to solvent ratio, and time, on the performance of pretreated EFB and the DES liquor residues after the pretreatment. Thus, the aim of this study was to further explore the efficiency of the EFB pretreatment using two different Type III DES mixtures, *i.e.*, choline chloride-imidazole (DES-I) and choline chloride-glycerol (DES-G) based on the pretreated EFB structural composition and enzymatic hydrolysis. Hence, the work was intended to initially evaluate the effect of the pretreatment conditions using DES-I and DES-G on the performance of pretreated EFB in the following ways: (1) EFB dissolution and chemical composition; (2) glucan conversion during enzymatic hydrolysis; and (3) the inhibitors in the DES liquor, which would be used to determine the optimal pretreatment conditions for EFB using DES-I and DES-G mixtures. The efficiency of the EFB pretreatment at optimal DES-I and DES-G conditions were determined based on the enzymatic hydrolysis and structural characterization of the pretreated EFB. To the best of the authors' knowledge, this is a pioneer comprehensive work that reports the usage of a choline chloride-imidazole eutectic mixture as a pretreatment agent during the pretreatment of EFB.

## EXPERIMENTAL

### Empty Fruit Bunch (EFB) Collection and Sample Preparation

The native empty fruit bunch (EFB) sample used in this study was collected from the Sungai Siput Palm Oil Mill in Sungai Siput, Perak, Malaysia. The native EFB was dried under sunlight until the moisture content was less than 10% (dry weight basis of the EFB). A moisture analyzer (IR-35, Denver Instrument, Arvada, CO) was used to measure the moisture content of the EFB samples. Then, the dried native EFB samples were ground using a universal cutting mill (PULVERISETTE 19, FRITSCH Universal, Idar-Oberstein, Germany), and sieved with a 2 mm sieve. The ground milled EFB samples were kept in a sealed plastic bag and stored in a 4 °C cold room until further use. The chemical composition of the native EFB samples was determined according to the National Renewable Energy Laboratory standard protocols as described in later section (Sluiter *et al.* 2012).

### Chemicals

The analytical grade chemicals used for the DES-I and DES-G pretreatment were choline chloride, (+98%), glycerol (+99%), and imidazole (99%), sourced from Alfa Aesar (Haverhill, MA). The analytical grade chemicals used for the analysis including the chemical composition, sugar, and inhibitor determination analyses were D(+)-glucose, D(+)-xylose, L(+)-arabinose, sulphuric acid (95% to 99%), 5-Hydroxymethyl-2-furaldehyde (99%), acetic acid, furfural, *p*-coumaric acid, and ferulic acid sourced from Sigma-Aldrich (St. Louis, MO). The tri-sodium citrate dihydrate and citric acid monohydrate used for buffer preparation were sourced from Thermo Fisher Scientific (Waltham, MA). All these chemicals were purchased from Scienfield Expertise PLT (Selangor, Malaysia).

### Commercial Enzymes

Two commercial enzyme cocktail mixtures were used in this study, *i.e.*, Cellic<sup>®</sup> CTec2 (cellulase) and Cellic<sup>®</sup> HTec2 (hemicellulase), which were purchased from Novozymes A/S (Bagsvaerd, Denmark). The Cellic<sup>®</sup> CTec2 contained a cocktail mixture of exo-1,4- $\beta$ -glucanases (cellobiohydrolases), endo-1,4- $\beta$ -glucanases,  $\beta$ -glucosidases and xylanase, and the Cellic<sup>®</sup> HTec2 mainly consisted of endo-1,4- $\beta$ -xylanases. The respective enzyme activity for Cellic<sup>®</sup> CTec2 was 142 FPU/mL  $\pm$  2.97 FPU/mL (with a protein concentration of 279.32 mg/mL  $\pm$  1.10 mg/mL).

### Synthesis of the Deep Eutectic Solvent (DES) Mixture

Two different DESs were prepared using a mixture of choline chloride and imidazole at a molar ratio of 3:7, which was identified as the DES-I mixture, and choline chloride and glycerol at a molar ratio of 1:2, which was identified as the DES-G mixture. The respective DES synthesis, conducted in a 2 L glass beaker and covered with aluminum foil, was heated to a temperature of 100 °C and stirred at 500 rpm for 2 h to 4 h at atmospheric pressure until it formed a homogeneous liquid. The DES mixtures were left to cool down to room temperature and then stored in sealed laboratory glass bottles.

## Effect of Different Pretreatment Conditions and the Determination of Optimal Conditions

The two eutectic mixtures synthesized in the previous section (DES-I and DES-G) were used as the solvents in the EFB pretreatment. First, 10 g of dried empty fruit bunches (EFB) was added into individual 2 L flasks containing the respective DES-I and DES-G solvents at different EFB to solvent ratios (1:5, 1:10, 1:15, and 1:20) and soaked at different temperatures (55 °C, 90 °C, 125 °C, 160 °C, and 195 °C) for various amounts of time (2 h, 4 h, and 6 h). The pretreatment process was conducted on a hot plate at atmospheric pressure and stirred constantly at 500 rpm. Each bottle was sealed with cotton wool and aluminum foil to prevent vaporization of the sample during the pretreatment process. The pretreated EFB and DES liquor residues were separated *via* vacuum filtration and centrifugation after pretreatment. Finally, the pretreated EFB samples were dried overnight in an oven at a temperature of 40 °C, then were stored in a cold room at a temperature of 4 °C until needed for further use and analysis. The performance of the pretreated EFB at different temperatures, ratios, and times were analyzed in terms of the EFB dissolution (%), EFB chemical composition, and the glucan conversion during the 1% enzymatic hydrolysis. Additionally, the DES liquor residues were characterized to determine the presence of common inhibitors in the pretreatment. The optimal EFB pretreatment conditions for the respective DES-I and DES-G mixtures were determined using the one-factor-at-a-time method based on the EFB dissolution (%), EFB chemical composition, and the glucan conversion during the 1% enzymatic hydrolysis.

### Empty Fruit Bunch (EFB) Dissolution during Deep Eutectic Solvent (DES) Pretreatment

The EFB dissolution indicated the amount of solids loss to the solvent during the DES pretreatment and was determined based on the reduction in the dried mass recovered after the respective DES pretreatment, as shown in Eq. 1,

$$EFB \text{ Dissolution (\%)} = \left(1 - \frac{m_{EFB,after}}{m_{EFB,before}}\right) \times 100\% \quad (1)$$

where  $m_{EFB,before}$  and  $m_{EFB,after}$  are the dried weight basis of the EFB mass (dwb) before and after the DES-I and DES-G pretreatment, respectively.

### Compositional Analysis of Native Empty Fruit Bunches (EFB) and the EFB Pretreated With Choline Chloride-Imidazole (DES-I) and Choline Chloride-Glycerol (DES-G)

The chemical composition analysis was performed on the native EFB and the DES pretreated EFB using the laboratory analysis protocol (LAP) developed by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2012). All samples ( $n = 3$  for each type of sample) were extracted with distilled water and 95% ethanol using an accelerated solvent extractor (Dionex ASE 350, Thermo Scientific, Sunnyvale, CA) for the extractive removal before quantifying the structural carbohydrates and lignin during the acid hydrolysis step. The compositions of the EFB samples were characterized based on the structural carbohydrates, *i.e.*, glucan, xylan, and arabinan, and lignin.

## Enzymatic Hydrolysis of Choline Chloride-Imidazole (DES-I) and Choline Chloride-Glycerol (DES-G) Pretreated Empty Fruit Bunches (EFB)

The enzymatic hydrolysis of native EFB, DES-I, and DES-G pretreated EFB, as well as EFB pretreated under optimal conditions for DES-I and DES-G (DES-I EFB1 and DES-G EFB2), were performed at a 1% glucan loading (w/v) in a 15 mL reaction volume (using 20 mL scintillation vials) in an incubated shaker (Infors HT Ecotron Incubator, Surrey, UK). The hydrolysis samples were buffered using a 1 M citrate buffer (with a pH of 4.8) and loaded with Cellic CTec2 cellulase at 15 FPU/g of glucan and combined with Cellic HTec2 hemicellulase at a ratio of 1:1 (v/v). All samples were incubated at a temperature of 50 °C, 150 rpm, and a pH of 4.8 for 72 h. Upon completion, the hydrolysis samples were placed in capped tubes and heat-treated for 15 min at a temperature of 100 °C to denature the enzymes and terminate the hydrolysis reaction. All samples were centrifuged at 7000 rpm for 10 min and then filtered through 0.22 µm Whatman membrane syringe filters prior to high performance liquid chromatography (HPLC) carbohydrate analysis.

## High Performance Liquid Chromatography (HPLC) for Carbohydrate Quantification

All carbohydrate monomers, *i.e.*, glucose, xylose, and arabinose, were analyzed using high performance liquid chromatography. The HPLC system consists of a Dionex Ultimate 3000 HPLC (Thermo Fisher Scientific, Waltham, MA) equipped with a refractive index detector (ERC RefractoMax 520, Riemerling, Germany). A Rezek-RPM monosaccharide column (Phenomenex, Torrance, CA) with a de-ashing guard cartridge (Phenomenex, Torrance, CA) was used for monomeric sugar concentration analysis in water extractive samples for compositional analysis. Degassed HPLC grade water was used as the mobile phase at a flow rate of 0.6 mL/min at a column temperature of 40 °C. An additional Rezek-ROA monosaccharide column (Phenomenex, Torrance, CA) was used to quantify the sugar concentration of the acid hydrolysis samples. Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (5 mM) was used as the mobile phase at 0.6 mL/min at a column temperature of 60 °C. The HPLC sample injection volume was 20 µL. The standard calibration curves at different concentrations of individual and mixed sugars, *i.e.*, glucose, xylose, arabinose, sucrose, fructose, and mannose, and sample quantifications were generated using Chromeleon chromatography data system (CDS) software (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA).

## Carbohydrate Conversion

The carbohydrate conversion of the native and pretreated EFB during enzymatic hydrolysis were reported in terms of glucan and xylan conversions and calculated using the actual monomeric and oligomeric sugars produced during the hydrolysis over the available theoretical sugar in the EFB samples, as shown in Eq. 2,

$$\text{Carbohydrate Conversion (\%)} = \frac{C_{\text{Mono}} + C_{\text{Oligo}}}{TSC} \times V \quad (2)$$

where  $C_{\text{Mono}}$  and  $C_{\text{Oligo}}$  are the monomeric and oligomeric sugar concentrations (g/L), respectively,  $V$  is the volume of enzymatic hydrolysis reaction (L), and  $TSC$  is the theoretical sugar content in the EFB at the specified glucan loading (g). The glucan and xylan conversions were determined using the respective monomeric and oligomeric C6 and C5 sugars.

## Characterization of Native Empty Fruit Bunches (EFB) and Choline Chloride-Imidazole (DES-I) and Choline Chloride-Glycerol (DES-G) Pretreated (EFB) at Optimal Pretreatment Conditions

### *X-ray diffraction (XRD) analysis for the crystallinity index measurement*

The oven-dried 2 mm native EFB and DES pretreated EFB samples at optimal conditions for the DES-I and DES-G pretreatments (identified as DES-I EFB1 and DES-G EFB2), and the unhydrolyzed samples of DES-I EFB1 and DES-G EFB2 after hydrolysis were prepared for X-ray diffraction (XRD) scanning. Commercial high purity microcrystalline cellulose (Avicel® PH-101, CAS: 9004-34-6, Product Number: 11363) and lignin (Lignin, alkali, CAS: 8068-05-1, Product Number: 471003) were used as the control for comparison purposes.

The respective sample was spread evenly on the specimen disc, followed by crystallinity measurement using an Advance XRD device (Bruker AXS Inc., Fitchburg, WI) with Cu  $\alpha_1$  radiation source at a wavelength of 1.5406 Å from a generator operating at 40 kV and 40 mA. All samples were analyzed at a speed of 0.25 °/s at the range of  $2\theta$  from 20° to 80°. The XRD data were used to determine the crystallinity index (CrI) by the ratio of the scattering maximum intensity of the crystalline peak ( $I_{002}$ ) and the scattering minimum intensity of the amorphous peak ( $I_{am}$ ), according to Eq. 3,

$$\text{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (3)$$

where  $I_{002}$  is the intensity of the peak at the 002 lattice diffraction (crystalline region,  $2\theta = 22.6^\circ$ ) and  $I_{am}$  is the intensity of the peak at the 001 amorphous phase (amorphous region,  $2\theta = 18^\circ$ ) (Oh *et al.* 2005).

### *Fourier transform infrared (FTIR) analysis for functional group determination*

The Fourier transform infrared (FTIR) analysis on all the oven-dried native EFB, DES-I EFB1, DES-G EFB2, UH DES-I EFB1 and UH DES-G EFB2 samples were performed using a Nocilet 67000 FTIR spectrophotometer (Thermo Fisher Scientific, Waltham, MA) based on the method of attenuated total reflectance (ATR). All samples were scanned with the range from 4000 to 400  $\text{cm}^{-1}$  at a wavelength resolution of 2  $\text{cm}^{-1}$  and recorded in transmission mode. The FTIR analysis was also used to determine the ratio of the lateral order index (LOI), total crystallinity index (TCI), and hydrogen-bond intensity (HBI). The LOI was determined by the wavelength ratio of 1437 to 899  $\text{cm}^{-1}$ , the TCI was determined by the wavelength ratio of 1378 to 2900  $\text{cm}^{-1}$ , and the HBI was ascertained by the wavelength ratio of 308 to 1330  $\text{cm}^{-1}$  (Oh *et al.* 2005; Shah *et al.* 2020).

### *Variable pressure scanning electron microscopy (VPSEM) analysis for empty fruit bunch (EFB) morphological changes*

Morphological analysis of the native EFB, DES-I EFB1, DES-G EFB2, UH DES-I EFB1, and UH DES-G EFB2 samples were conducted using variable pressure scanning electron microscopy (VPSEM) (EVO MA10 CARL ZEISS, Cambridge, UK). All samples were freeze-dried before the scanning analysis. The samples were placed on aluminum stubs and coated with gold using a sputter coater system (Q150R S, Quorum Technologies, Lewes, UK). The magnification range was between 50× and 1000× for each sample.

## RESULTS AND DISCUSSION

### Chemical Composition of Native Untreated Empty Fruit Bunches (EFB)

The primary structural components of EFB are made up of cellulose, hemicellulose, and lignin, where the cellulose and hemicellulose formed the structural carbohydrate (SC) in the biomass. The chemical composition of native untreated EFB was found to be consisting of  $61.2\% \pm 1.95\%$  SCs and  $22.3\% \pm 0.8\%$  lignin, where the SC was formed of  $37.6\% \pm 0.41\%$  glucan,  $22.6\% \pm 0.2\%$  xylan, and  $0.9\% \pm 1.32\%$  arabinan. This composition result obtained was consistent and fell within the range of reported compositions for native EFB (Abdul *et al.* 2016).

### Effects of the Pretreatment Temperature

#### *Empty fruit bunch (EFB) dissolution and chemical composition of deep eutectic solvent (DES) pretreated EFB*

Figure 1a and Fig. 1b show the effects of the pretreatment temperature on the EFB dissolution and the chemical composition of the pretreated EFB after DES-I and DES-G pretreatment conducted at a EFB to solvent ratio of 1:10 for 4 h at a temperature range of 55 to 195 °C. Generally, the temperature influenced the DES-I and DES-G solvents during pretreating and dissolving the EFB into the liquid. The EFB mass dissolution in the solvent gradually increased for the EFB pretreated with DES-I (as shown in Fig. 1a) from 16.4% to 45.7%, and from 10.2% to 44.5% for the EFB pretreated with DES-G (as shown in Fig. 1b) when the temperature increased from 55 to 195 °C.

The increasing temperature has been reported to alter the ionic characteristics and the molecular polarity of the DES solvent, promoting the breakage of the intramolecular hydrogen bond network, and enhancing the solubility of the carbohydrates and lignin in the DES solvents (Chen *et al.* 2019). The dissolution of the solids into the liquid could also be affected by the decreasing viscosity of the DES solvent as the temperature increased (Degam 2017). Additionally, it can be observed that the EFB dissolution was slightly higher in DES-I compared to DES-G, which was likely due to the hydrogen bond basicity, which promoted better dissolution. During the pretreatment, it was observed that the pH of DES-I ranged from 8.59 to 8.19, while the pH of DES-G ranged from 6.41 to 6.28.

The increase in temperature also modified the chemical composition of the DES-I and DES-G pretreated EFB, since the amount of hemicelluloses (xylan and arabinan) and lignin retained in the pretreated EFB were reduced compared to native EFB. As the temperature was increased from 55 to 195 °C, the hemicellulose fraction in the DES-I and DES-G pretreated EFB was reduced from 23.5% in the native EFB to  $15.4\% \pm 0.5\%$  and  $14.3\% \pm 0.0\%$ , respectively, at a temperature of 195 °C (as shown in Fig. 1a and Fig. 1b). A similar trend was also observed for lignin, where the fraction in DES-I and DES-G pretreated EFB was reduced from 22.3% in the native EFB  $12.8\% \pm 0.6\%$  and  $13.6\% \pm 0.5\%$  respectively, at a temperature of 195 °C (as shown in Fig. 1a and Fig. 1b). A greater reduction in the lignin fraction was observed in the DES-I pretreated EFB, which was most likely due to the hydrogen bond basicity of the DES-I solvent compared to DES-G (Degam 2017; Hassan and Mutelet 2017; Horváth *et al.* 2017).

While the temperature had an inverse effect on the hemicellulose and lignin compositions, a different trend was observed in the structural glucan composition in both the DES-I and DES-G pretreated EFB. The glucan fractions in both pretreated EFB gradually increased as the temperature increased from 55 to 160 °C (as shown Fig. 1a and Fig. 1b) and reached the highest glucan composition ( $45.1\% \pm 0.4\%$  and  $48.9\% \pm 0.8\%$ ,



with respect to DES-I and DES-G pretreated EFB) at a temperature of 160 °C. This increasing trend in the cellulose fraction of the pretreated EFB was primarily due to the dissolution of the hemicellulose and lignin in the DES solvent. However, further increase of the temperature from 160 to 195 °C resulted in a sudden reduction of the glucan fraction from  $45.1\% \pm 0.4\%$  to  $37.8\% \pm 0.2\%$  in DES-I pretreated EFB (as shown in Fig. 1a), with a 16.2% reduction, and from  $48.9\% \pm 0.8$  to  $38.3\% \pm 0.5\%$  in DES-G pretreated EFB (as shown in Fig. 1b), with a 21.7% reduction. This reduction could be due to the further dissolution and degradation of the cellulose chain at high temperatures (Arumugam *et al.* 2020). Other studies have reported that at higher temperature ranges, certain IL are capable of dissolving cellulose molecules by breaking down the inter- and intra-molecular hydrogen bond networks between cellulose molecules and making a new IL-cellulose hydrogen bond network, which promotes cellulose dissolution. This also could be contributed by the high interaction energy, hydrogen bond formation, bond acidity, or basicity and polarity between the cellulose and the solvent (Brandt *et al.* 2013; Hassan and Mutelet 2017).

#### *Glucan conversion of deep eutectic solvent (DES) pretreated empty fruit bunches (EFB)*

Figure 1a and Fig. 1b show the effect of the pretreatment temperature on the glucan conversion obtained during the 1% enzymatic hydrolysis of both DES-I and DES-G pretreated EFB. The result showed that the highest glucan conversion was attained when the temperature was at 160 °C (67.8% and 71.4% for DES-I and DES-G pretreated EFB, respectively). The pretreatment conditions (a temperature of 160 °C, a 1:10 ratio, and 4 h) that yielded the highest glucan fraction for both DES-I and DES-G pretreated EFB were the result of the solubilization of a majority of the hemicelluloses (27% to 32%) and lignin (34% to 40%) into the DES solvents. Glucan conversion was declined at further increase in temperature (at 195 °C), which resulted in the formation of inhibitors such as HMF and furfural in both DES-I and DES-G pretreated EFB samples, as shown in Fig. 1c and Fig. 1d.

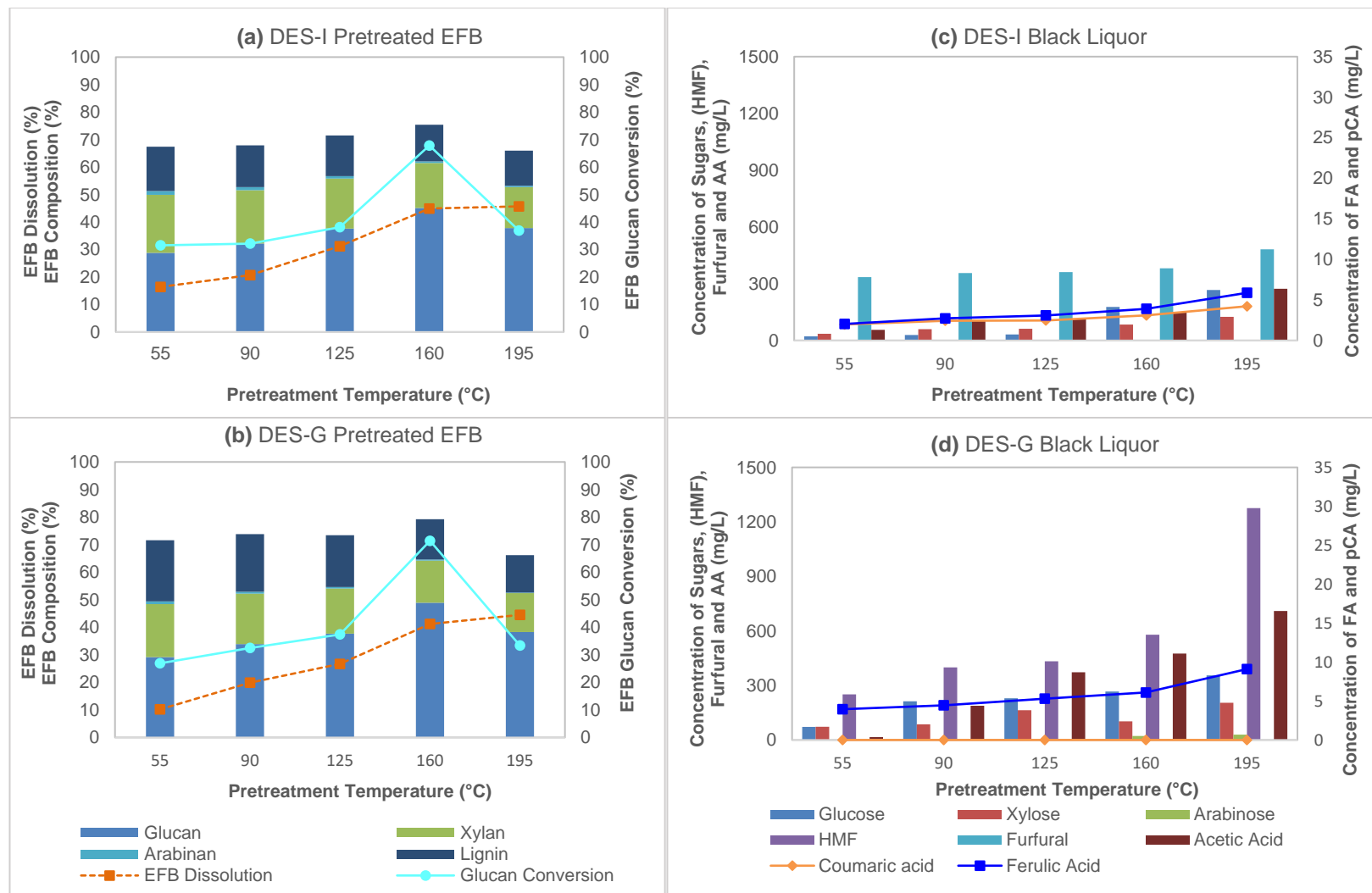
#### *Inhibitors in the deep eutectic solvent (DES) black liquor*

Severe pretreatment conditions can possibly cause degradation of the glucans and hemicelluloses in the biomass, causing the formation of inhibitors, *e.g.*, hydroxyl-methyl furfural (HMF), furfural, acetic acid (AA), *etc.*, and the depolymerization of lignin releasing phenolic compounds, *e.g.*, gallic acid, ferulic acid (FA), and *p*-coumaric acid (pCA), in the solvent turning it to black liquor (Jönsson *et al.* 2013; Mahmud *et al.* 2017; Bukhari *et al.* 2019). Figure 1c and Fig. 1d show the effects of the pretreatment temperature on the concentration of the inhibitors released from the EBF, with respects to the DES-I and DES-G solvents at a 1:10 solvent ratio and a 4 h pretreatment time.

The concentration of inhibitors increased as the temperature increased, which corresponded to greater EFB dissolution in the solvent at higher temperatures. The concentration of glucose, xylose, and AA gradually increased as the temperature increased; however, their concentrations were consistently lower in the DES-I black liquor compared to the DES-G black liquor throughout the temperature interval. The highest concentration of glucose, xylose, and AA in the DES-I black liquor were 266.5, 125.3, and 273.6 mg/L at a temperature of 195 °C (as shown in Fig. 1c) compared to 355.7, 205, and 709.5 mg/L in the DES-G black liquor at the same temperature (as shown in Fig. 1d). Furfural, HMF, FA, and pCA were observed to be selectively present in different liquors. While furfural steadily increased and dominated the DES-I black liquor with a highest concentration of

481.8 mg/L at a temperature of 195 °C, HMF was observed to dominate the DES-G black liquor with its abrupt increase in concentration from 579 mg/L at a temperature of 160 °C to 1276 mg/L at a temperature of 195 °C. Menegazzo *et al.* (2018) reported that under some pretreatment conditions using IL, the biomass could directly transform to HMF in a one step process. Ferulic acid was found to appear in both the DES-I and DES-G black liquors (as shown in Fig. 1c and Fig. 1d), while pCA was detected only in the DES-G black liquor (as shown in Fig. 1d). Previous work indicated that FA acts a “bridge” between the lignin *via* etherified bonding, and between the carbohydrates *via* esterified bonding in the lignin carbohydrate complex (LCC). Hence, FA can therefore be released either under acid or alkaline conditions through its acid labile etherified bonding or alkaline labile esterified bonding (Buranov and Mazza 2008; Koshijima and Watanabe 2013).

The pCA binds with lignin or carbohydrates primarily through esterified bonds, which are only labile under alkaline conditions (Linh *et al.* 2017; Li *et al.* 2019). The pH of the respective DES-I and DES-G solvents was recorded at 8.4 and 6.4, which explained why only FA was released in the acidic EFB pretreatment using the DES-G solvent. Linh *et al.* (2017) observed that FA was released considerably faster than pCA. Although pCA had a broad distribution throughout the lignin matrix, it tended to be densely located in the lignin fraction that is not easily solubilized *via* the alkaline pretreatment. Figure 1c indicates that the highest concentration of FA and pCA in the DES-I black liquor was 5.9 and 4.2 mg/L, respectively, while the FA concentration in the DES-G black liquor was 9.1 mg/L at a temperature of 195 °C.



**Fig. 1.** Effect of the pretreatment temperature on (a) DES-I pretreated EFB, (b) DES-G pretreated EFB, (c) DES-I black liquor, and (d) DES-G black liquor at a EFB to solvent ratio of 1:10 and a treatment time of 4 h

### *Optimal temperature for subsequent choline chloride-imidazole (DES-I) and choline chloride-glycerol (DES-G) pretreatment*

The performance of the pretreated EFB in terms of the chemical composition and the glucan conversion showed that a temperature of 160 °C was the optimal pretreatment temperature for DES-I and DES-G to achieve the highest glucan composition and conversion of the DES-I and DES-G pretreated EFB. The temperature was applied to the subsequent DES-I and DES-G pretreatments to further determine the effect of the EFB to solvent ratio and pretreatment time.

### **Effects of the Empty Fruit Bunch (EFB) to Solvent Ratio**

#### *Empty fruit bunch (EFB) dissolution and chemical composition of deep eutectic solvent (DES) pretreated EFB*

Figure 2a and Fig. 2b show the effect of the EFB to solvent ratio on the EFB dissolution and chemical composition during the DES-I and DES-G pretreatment of EFB at a ratio of 1:5 to 1:20 at a temperature of 160 °C for 4 h. The rate of EFB mass dissolution in the DES-I solvent gradually increased from 31.7% to 49.9% when the ratio increased from 1:5 to 1:15; the dissolution rate was maintained when the ratio was further increased to 1:20 (as shown in Fig. 2a). However, for the DES-G solvent, the EFB mass dissolution continuously increased from 25.9% to 48.8% when the ratio increased from 1:5 to 1:20 (as shown in Fig. 2b).

Technically, a ratio of solid to solvent should be kept high to facilitate greater throughput of material through a pretreatment process, thus yielding higher production rates. However, a higher solid to solvent ratio could also result in higher production cost. Hence, sufficient amount of solvent should be used to dissolve both lignin and hemicelluloses as well as to retain most of the crystalline cellulose in the remaining EFB solid. Figure 2a and Fig. 2b show the effect of the EFB to solvent ratio on the composition of the DES-I and DES-G pretreated EFB as it increased from 1:5 to 1:20. The hemicellulose (xylan and arabinan) fraction was drastically reduced from 23.5% in the native EFB to 16.4% and 15.7% in the respective DES-I and DES-G pretreated EFB at a ratio of 1 to 10 (as shown in Fig. 2a and Fig. 2b). However, for both DES pretreatments, a further increasing of the ratio resulted in an increase in the hemicellulose fraction.

When the ratio was increased from 1:5 to 1:20, the lignin composition fraction was observed to steadily reduce over the ratio interval, reaching its lowest composition of 11%  $\pm$  0.8% and 12.8%  $\pm$  0.4% in the DES-I and DES-G pretreated EFB at a ratio of 1:20 (as shown in Fig. 2a and Fig. 2b). This resulted in a lignin reduction of 50.6% and 42.6% from the native EFB in comparison to DES-I and DES-G pretreated EFB, respectively. A higher lignin reduction was observed in the DES-I pretreated EFB, owing to the alkaline conditions of the solvent, which allowed the cleavage of the alkaline labile ester bonding in the lignin fraction.

The trend of the structural glucan composition in both the DES-I and DES-G pretreated EFB is interesting enough to be highlighted. The glucan composition in the DES-I pretreated EFB attained the highest composition (48.4%  $\pm$  0.4%) at the lowest ratio of 1:5. It is clearly shown that the lowest ratio, when coupled with the high pretreatment temperature at 160 °C, caused the DES-I pretreated EFB to experience considerable removal of the hemicellulose (28%) and lignin (40%) fractions, which resulted in a higher composition of the glucan fraction (as shown in Fig. 2a). Further increasing of the ratio gradually reduced the glucan composition, which is most likely caused by the dissolution of some amorphous structure in the glucan fraction. Roy *et al.* (2020) reported that

imidazole-based IL pretreatment significantly helps the removal of the cellulose portion of the biomass as well as cleaving the  $\beta$ -O-4 linkages.

However, the glucan fraction in the DES-G pretreated EFB increased when the ratio was increased to 1:10, with a glucan fraction of  $48.9\% \pm 0.8\%$ , but this fraction was subsequently reduced as the ratio was increased to 1 to 20, which was likely caused by the amorphous region dissolution in the solvent.

Rahim *et al.* (2021) reported that the low viscosity of 1-(2-methoxyethyl)-3-methylimidazolium chloride (MOE-MImCl) is a primary factor in improving the dissolution of bamboo in ILs, since it boosts the mass transfer of the solute, thus leading to a higher percentage of biomass dissolution. Thus, the DES viscosity can influence the biomass to solvent ratio during the pretreatment process. A low viscosity DES-I solvent required a low EFB to solvent ratio since it could easily diffuse into the biomass structure and boost the solute mass transfer. The experimental data indicated that at a temperature of 160 °C, the DES-I solvent had a lower viscosity compared to DES-G, which is likely the reason for the low ratio required for the DES-I solvent.

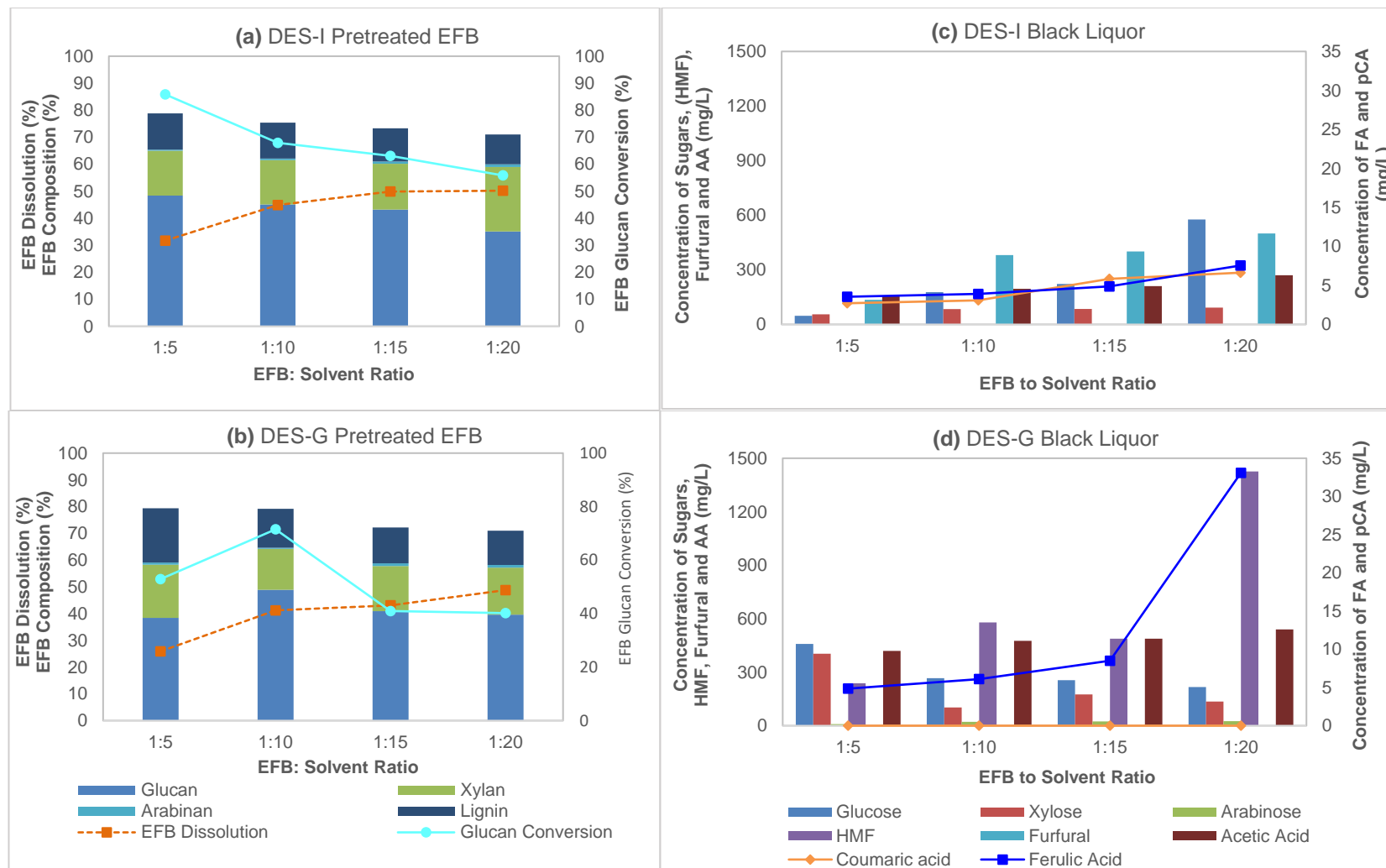
#### *Glucan conversion of deep eutectic solvent (DES) pretreated empty fruit bunches (EFB)*

Figure 2a and Fig. 2b show the effects of the EFB to solvent ratio on the glucan conversion during the 1% enzymatic hydrolysis of both the DES-I and DES-G pretreated EFB at a temperature of 160 °C and a treatment time of 4 h. The hydrolysis of the DES-I pretreated EFB attained the highest glucan conversion (85.9%) at a ratio of 1:5, while the hydrolysis of the DES-G pretreated EFB required a higher ratio (1:10) in order to attain its highest glucan conversion rate of 71.5%. The composition of the DES-I and DES-G pretreated EFB at the respective ratios indicated the solubilized amount of hemicellulose and lignin were 27% and 40% in the DES-I liquor, respectively, and 32% and 34% in the DES-G liquor, respectively.

#### *Inhibitors in the black liquor of the deep eutectic solvent (DES) pretreatments*

Figure 2c and Fig. 2d show the effects of the EFB to solvent ratio on the concentration of the inhibitors released into the liquors during the DES-I and DES-G pretreatment of EFB at a temperature of 160 °C and a treatment time of 4 h. The concentration of the inhibitors, particularly glucose, xylose, and AA, increased in the DES-I liquor when the ratio increased, which was likely due to additional EFB dissolution into the solvent at higher ratios (as shown in Fig. 2c). However, the opposite trend was observed in the DES-G liquor (as shown in Fig. 2d). As the ratio increased, the concentration of glucose and xylose gradually decreased, but AA was observed to maintain a slow increase in concentration. Furfural dominated the DES-I black liquor with the highest concentration of 500 mg/L at a 1:20 ratio, while HMF dominated the DES-G black liquor with its concentration suddenly increasing from 487.9 mg/L at a ratio of 1:15 to 1426.1 mg/L at a ratio of 1:20.

The FA was found in both the DES-I and DES-G liquors (as shown in Fig. 2c and Fig. 2d) while pCA was only found in the DES-G liquor (as shown in Fig. 2d), as described in a previous section. The formation of these inhibitory products during pretreatments were below the inhibition ranges tested on the cell growth of microorganisms, *e.g.*, *Saccharomyces cerevisiae*, at a range of 500 mg/L to 4000 mg/L of furfural, and *Zymomonas mobilis*, at a range of 960 mg/L to 2000 mg/L (Banerjee *et al.* 1981; Delgenes *et al.* 1996).



**Fig. 2.** Effect of the EFB to solvent ratio on (a) DES-I pretreated EFB, (b) DES-G pretreated EFB, (c) DES-I black liquor, and (d) DES-G black liquor at a temperature of 160 °C and a treatment time of 4 h

### *Optimal empty fruit bunch (EFB) to solvent ratio for subsequent choline chloride-imidazole (DES-I) and choline chloride-glycerol (DES-G) pretreatment*

The EFB to solvent ratio also had a considerable effect on the chemical composition as well as the glucan conversion of the DES-I and DES-G pretreated EFB. Values of 1:5 and 1:10 were the optimal ratios for the DES-I and DES-G pretreatment to attain the highest glucan composition and conversion during the enzymatic hydrolysis. The optimal temperature of 160 °C and a ratio of 1:5 and 1:10 were applied in the subsequent DES-I and DES-G pretreatments to further determine the effect of the treatment time on the pretreated EFB.

### **Effect of the Pretreatment Time**

#### *Empty fruit bunch (EFB) dissolution and chemical composition of deep eutectic solvent (DES) pretreated EFB*

Figures 3a, b show the effects of pretreatment time on the EFB dissolution and the chemical composition of the DES-I and DES-G pretreated EFB, which ranged from 2 to 6 h, at 160 °C. The EFB to solvent ratios were 1:5 for the DES-I pretreatment and 1:10 for the DES-G pretreatment. When the pretreatment time was increased from 2 to 6 h, the EFB dissolution in the solvent gradually increased from 29.9% to 46.2% for DES-I pretreated EFB (Fig. 3a), and from 23.6% to 44.4% for the DES-G pretreated EFB (Fig. 3b).

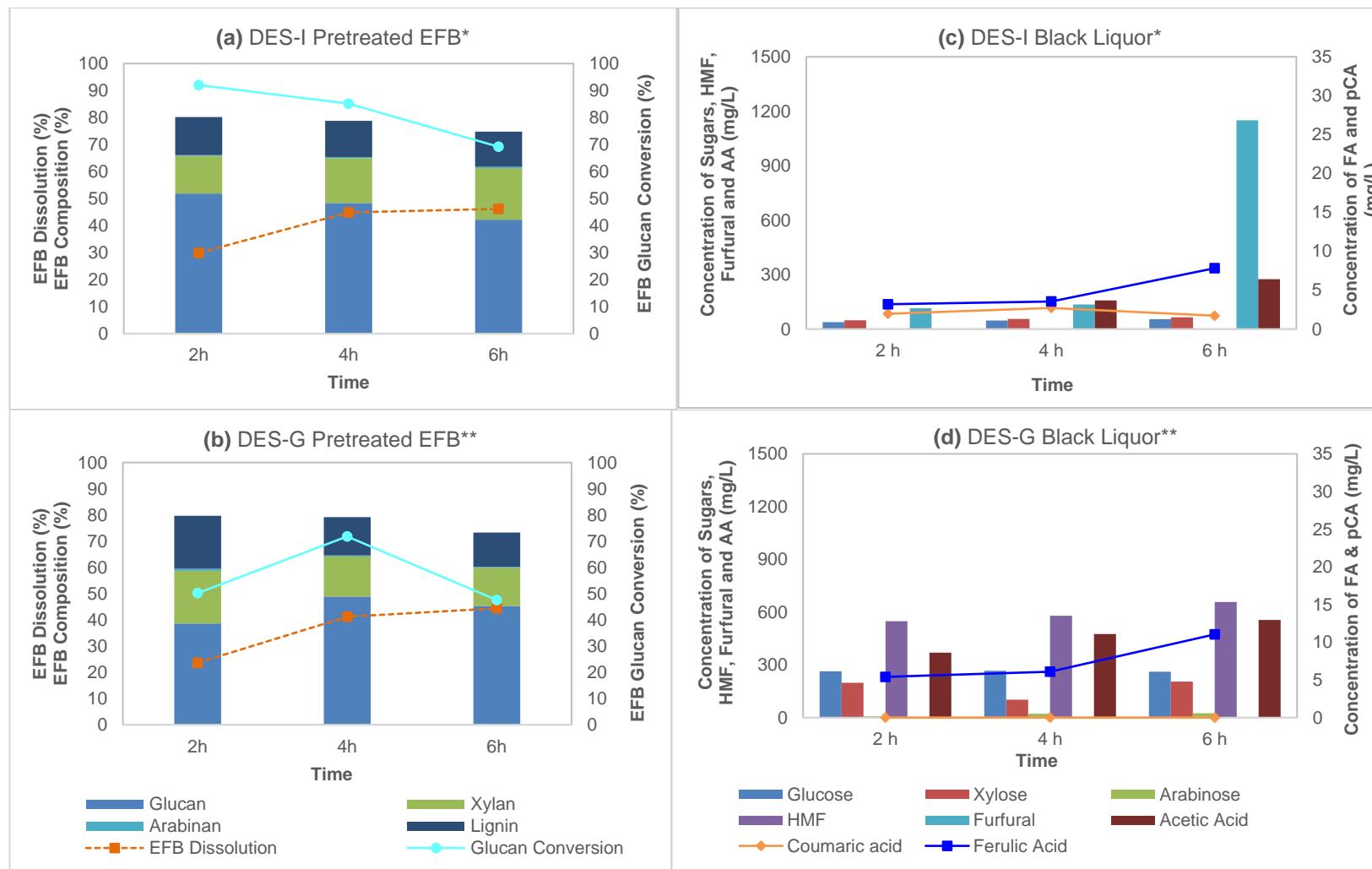
Although increasing the pretreatment time caused an increase in the solid-liquid interaction that resulted in a higher removal of lignin (approximately 41% in the DES-I pretreated EFB), it also reduced the glucan composition from 51.9%  $\pm$  0.2% at 2 h to 42.2%  $\pm$  0.5% at 6 h, which caused the hemicellulose fraction to increase. The treatment time of 2 h was already sufficient to remove 37% of the lignin in the DES-I pretreated EFB. However, for the DES-G pretreated EFB, a longer time was required to remove a greater amount of lignin from the EFB, due to the higher viscosity of the DES-G solvent. When the EFB was pretreated with DES-G for 4 h, 34.5% of the lignin were removed from the biomass, which resulted in the highest glucan composition of 48.9%  $\pm$  0.8%, and a further increased in pretreatment time would only cause glucan solubilization.

#### *Glucan conversion of deep eutectic solvent (DES) pretreated empty fruit bunches (EFB)*

Figures 3a and 3b also showed the effect of pretreatment time on glucan conversion during 1% enzymatic hydrolysis of the DES-I and DES-G pretreated EFB from 2 to 6 h. The glucan conversion of the DES-I pretreated EFB reduced from 92.4% after 2 h to 69.2% after 6 h (Fig. 3a). For the DES-G pretreated EFB, the glucan conversion reached its highest conversion rate (71.8%) when the EFB was pretreated for 4 h, due to the highest glucan composition in the biomass (Fig. 3b). However, this conversion was much lower compared to the glucan conversion of the DES-I pretreated EFB with a 2 h pretreatment time.

#### *Inhibitors in the black liquor of the deep eutectic solvent (DES) pretreatments*

Figures 3c and 3d show the effect of pretreatment time on inhibitor concentration in the DES-I and DES-G black liquors, with a pretreatment time ranging from 2 to 6 h. The DES-I liquor yielded a low concentration of glucose, xylose, furfural, AA, FA, and pCA at 2 and 4 h pretreatment times, with the concentration of each inhibitor less than 150 mg/L (Fig. 3c). However, the concentration of all inhibitors was consistently higher in the DES-G liquor, ranging from a pretreatment time of 2 h up to 6 h, particularly glucose, xylose, AA, HMF, and FA (Fig. 3d). The higher concentration of inhibitors also could cause the lower glucan conversion during the hydrolysis of the DES-G pretreated EFB.



**Fig. 3** Effect of the pretreatment time (a) DES-I pretreated EFB, (b) DES-G pretreated EFB, (c) DES-I black liquor, and (d) DES-G black liquor at a temperature of 160 °C and a EFB to solvent ratio of 1 to 5 for DES-I and 1 to 10 for DES-G



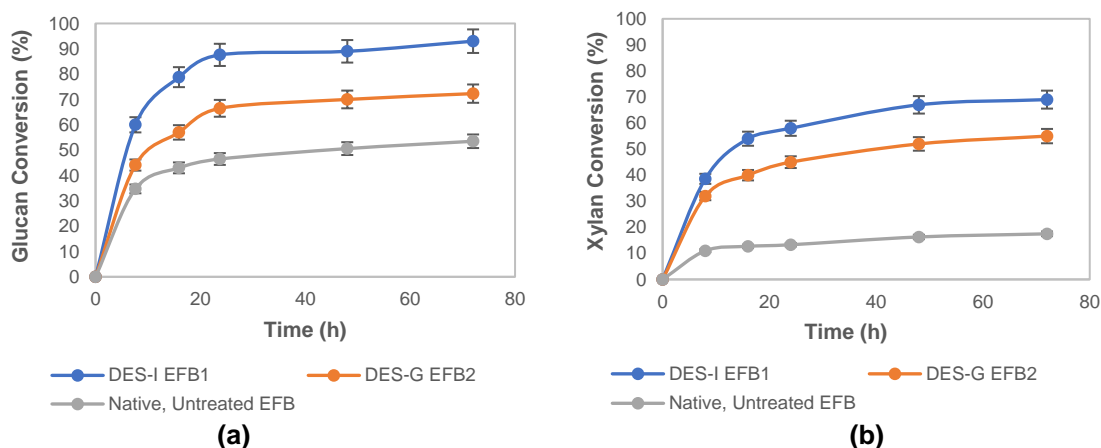
*Optimal choline chloride-imidazole (DES-I) and choline chloride-glycerol (DES-G) pretreatment conditions for empty fruit bunches (EFB)*

The results indicated that the optimal conditions for DES-I pretreatment were a temperature of 160 °C, a EFB to solvent ratio of 1:5, and a pretreatment time of 2 h. The optimal DES-G pretreatment conditions were a temperature of 160 °C, a EFB to solvent ratio of 1:10, and a pretreatment time of 4 h. Further analyses were conducted on the DES-I EFB1 and DES-G EFB2 samples, which were pretreated at the optimal pretreatment conditions of DES-I and DES-G to compare and determine the efficiency of these pretreatments.

**Performance of Choline Chloride-Imidazole (DES-I) and Choline Chloride-Glycerol (DES-G) Pretreated Empty Fruit Bunches (EFB) at Optimal Pretreatment Conditions**

*Enzymatic hydrolysis of the DES-I EFB1 and DES-G EFB2 samples*

Figure 4 illustrates the performance of the 1% enzymatic hydrolysis of the DES-I EFB1 and DES-G EFB2 samples using Cellic CTec2 and Cellic HTec2 enzymes. The DES-I EFB1, pretreated using DES-I with a EFB to solvent ratio of 1:5, a temperature of 160 °C, and a treatment time of 2 h, was successfully hydrolyzed by the enzymes, and it yielded 10.26 g/L  $\pm$  0.01 g/L of glucose and 4.50 g/L  $\pm$  0.04 g/L of xylose after a total of 72 h of hydrolysis (as shown in Fig. 4a). These were equivalent to the glucan and xylan conversions of 93% and 68.7%, respectively. The hydrolysis profile also showed that after 48 h of hydrolysis, the conversion of glucan in the DES-I EFB1 sample already reached 89% and 67% for the glucan and xylan conversions. In contrast, the enzymatic hydrolysis of the DES-G EFB2 sample, pretreated using DES-G with a EFB to solvent ratio of 1:10, a temperature of 160 °C and a treatment time of 4 h, produced lower sugar concentrations (7.99 g/L  $\pm$  0.03 g/L glucose and 3.59 g/L  $\pm$  0.04 g/L xylose). These corresponded to 72.3% and 55.6% of the glucan and xylan conversions, respectively (as shown in Fig. 4b).



**Fig. 4** Enzymatic hydrolysis of the DES-I EFB1 and DES-G EFB2 pretreated at a 1% glucan loading: (a) glucan conversion and (b) xylan conversion

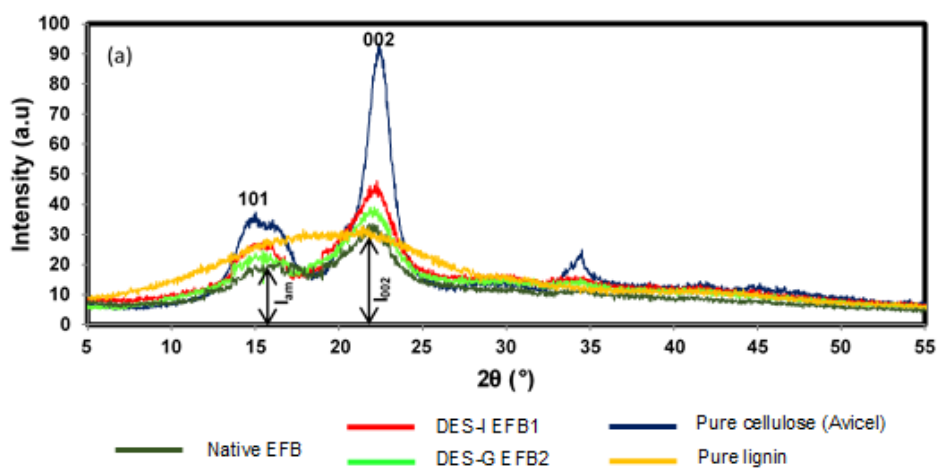
Both the DES-I EFB1 and DES-G EFB2 samples showed an improvement in terms of carbohydrate hydrolysis when compared to the carbohydrate hydrolysis in the native EFB. The glucan hydrolysis increased by 73% and 35.1%, respectively, for the DES-I

EFB1 and DES-G EFB2 samples, exhibiting the potential of DES-I as a pretreatment solvent over DES-G. The improvement in the enzymatic hydrolysis was affected by the reduction in the lignin content and the low inhibitor content that resulted in an increase in pores sizes and surface area, which facilitated the carbohydrate digestion process.

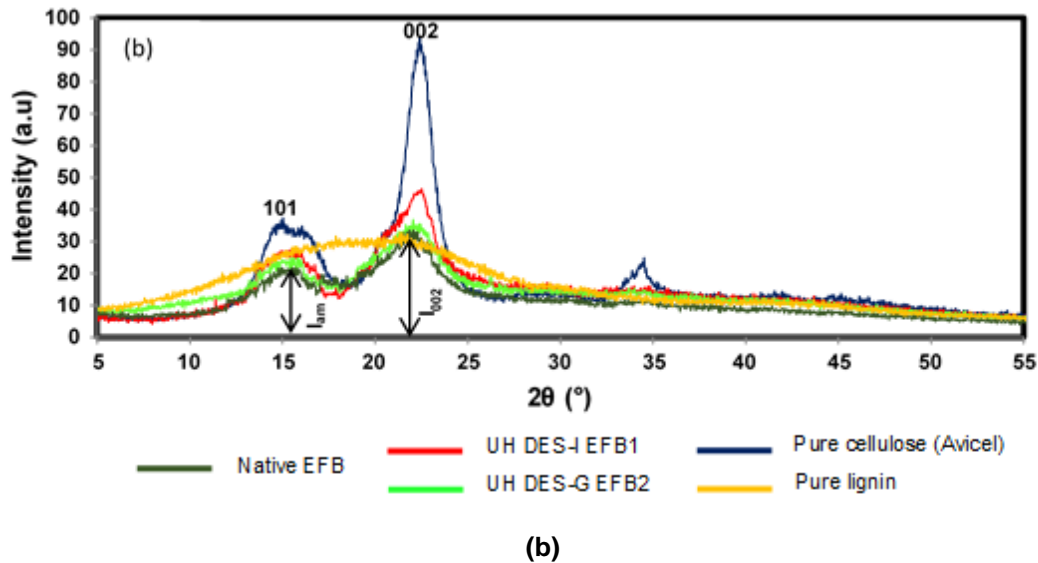
#### *X-ray diffraction (XRD) analysis*

The degree of crystallinity (CrI) and structural changes in cellulose are the major factors that influence the effectiveness of the enzymatic saccharification of lignocellulosic biomass (Çetinkol *et al.* 2010). Figure 5 compares the x-ray diffractogram of the native EFB, DES-I EFB1, and DES-G EFB2, as well as the remaining unhydrolyzed solid residues, UH-DES-I EFB1 and UH-DES-G EFB2. In addition, commercial samples of pure cellulose (Avicel) and pure lignin were used as the control for comparison purposes.

The increase in CrI can be interpreted either as an increase in the crystalline phase or a decrease in the amorphous phase of the EFB. Figure 6 compares the CrI for all the samples. The native EFB sample had the lowest CrI (0.42), and the value increased to 0.6 and 0.53 for the DES-I EFB1 and DES-G EFB2 samples after DES pretreatment, respectively. Both pretreated EFB samples demonstrated that the DES pretreatment successfully removed the amorphous components, lignin, and hemicelluloses, which resulted in a higher crystallinity in the sample. The reason behind that could be that the strong hydrogen bond interactions in cellulose were weakened because of competing hydrogen bond formation between the chloride ions (Cl<sup>-</sup>) in DES and the (-OH) groups in carbohydrates, consequently breaking the intramolecular hydrogen bond network. Furthermore, lignin removal by DES treatment would have exposed cellulose on the surface of the DES-treated EFB (Kwon *et al.*, 2020). Kim *et al.* (2003) reported that enzyme saccharification responds positively with an increase in the CrI value.

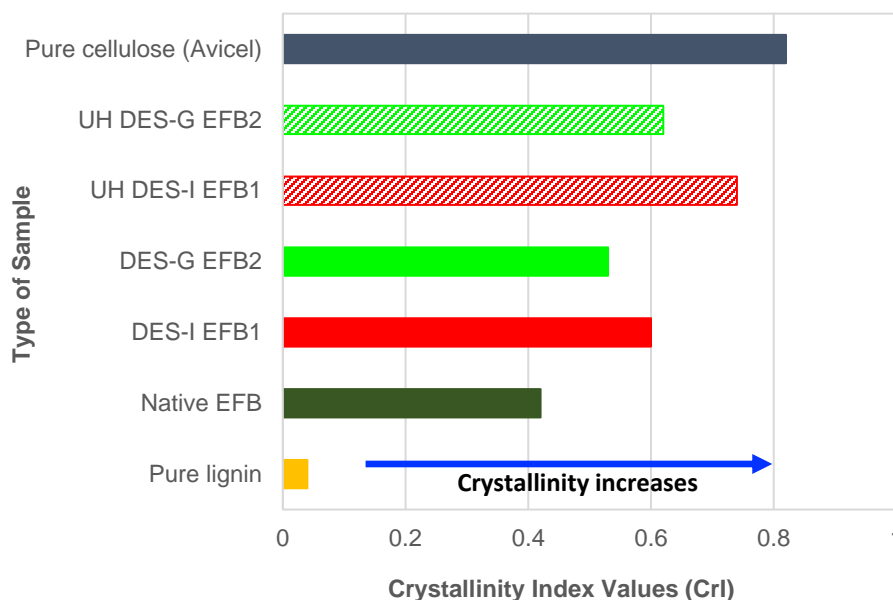


(a)



**Fig. 5.** X-ray diffractogram of the (a) native EFB, DES-I EFB1, DES-G EFB2, pure cellulose, and pure lignin; and (b) native EFB, UH-DES-I EFB1, UH-DES-G EFB2, pure cellulose, and pure lignin.

During enzymatic hydrolysis, more chemical linkages in the pretreated EFB were hydrolyzed, which degraded the pretreated EFB samples. This led to highly crystalline samples in the unhydrolyzed solid residues of UH DES-I EFB1 and UH DES-G EFB2 with CrI values of 0.74 and 0.62, respectively. Commercial cellulose (Avicel) and lignin were used as the controls to show comparisons of the highest and lowest CrI in all the samples (as shown in Fig. 6).



**Fig. 6.** Crystallinity index (CrI) value for native EFB, DES-I EFB1, DES-G EFB2, UH-DES-I EFB1, UH-DES-G EFB2, pure cellulose, and pure lignin

*Fourier transformation infra-red (FTIR) analysis*

Figure 7 illustrates the FTIR spectra for the native EFB (1), DES-I EFB1 (2), DES-G EFB2 (3), pure cellulose (4), pure lignin (5), UH DES-I EFB1 (6), and UH DES-G EFB2 (7) samples. There were seven peaks identified on the spectra ranging from 1020 to 3500  $\text{cm}^{-1}$ , labeled as Peak A to Peak G, that can be used to describe the intensity of the functional groups in all samples. Table 1 shows the characterization of the signal assignment for these seven peaks from the respective samples, as either a strong (+), decreased (-), or diminished (NI) intensity of the related functional group. Notably, the varying intensity of the peak from DES-I EFB1 (2), DES-G EFB2 (3), UH DES-I EFB1 (6), and UH DES-G EFB2 (7) were primarily decreased or diminished in comparison to the peak intensity for native EFB (1) and pure lignin (5), signifying the effect of the DES-I and DES-G pretreatments. This was particularly obvious for the DES-I EFB1 (2) and UH DES-I EFB1 (6) samples when the peak intensity resembled the peak intensity of pure cellulose (4), which indicated the efficiency of the DES-I pretreatment in removing hemicelluloses and lignin (as shown in Table 1).

Table 2 compares the lateral order index (LOI), total crystallinity index (TCI), and hydrogen-bond intensity (HBI) from the FTIR spectra for the native EFB (1), DES-I EFB1 (2), DES-G EFB2 (3), pure cellulose (4), pure lignin (5), UH DES-I EFB1 (6), and UH DES-G EFB2 (7) samples. The TCI is proportional to the degree of crystallinity in cellulose, and the LOI is correlated to the overall degree of order in cellulose. In addition, the HBI is related to the crystallinity system and degree of intermolecular regularity.

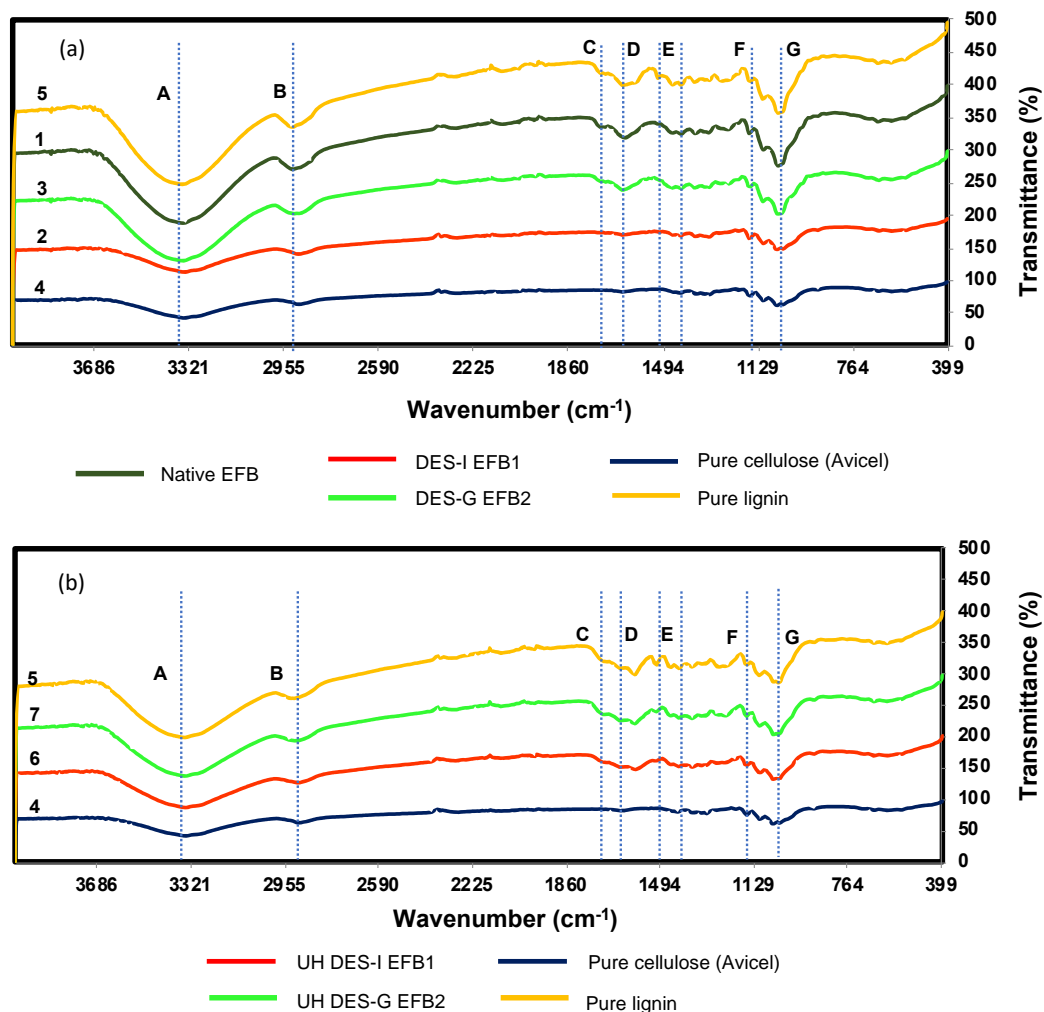
**Table 1.** Characterization of the Signal Assignment for Peaks from Empty Fruit Bunch Samples and Controls

Peak #	Peak (cm <sup>-1</sup> )	Signal Assignment	Intensity Strength Strong = (+), Decreased = (-), Diminished = (NI)							Ref.
			Native EFB (1)	DES-I EFB1 (2)	DES-G EFB2 (3)	UH DES-I EFB1 (6)	UH DES-G EFB2 (7)	Pure cellulose (4)	Pure lignin (5)	
A	3200 to 3500	O-H stretching in aromatic and aliphatic groups of cellulose, hemicellulose, and aromatic lignin	+	-	-	-	-	-	+	Agrawal <i>et al.</i> 2017
B	2800 to 2940	C-H vibrations in CH <sub>3</sub> and CH <sub>2</sub> groups	+	-	-	-	-	-	+	Agrawal <i>et al.</i> 2017
C	1718 to 1734	C=O stretching in hemicelluloses (ester group)	+	NI	-	-	-	NI	+	Sim <i>et al.</i> 2012; Kurian <i>et al.</i> 2015
D	1622 to 1648	C=O stretching in lignin (ester group)	+	-	+	-	-	-	+	Sim <i>et al.</i> 2012; Kurian <i>et al.</i> 2015
E	1450 to 1550	Aliphatic and aromatic ring skeleton vibration in lignin	+	NI	-	NI	-	NI	+	Sim <i>et al.</i> 2012; Li <i>et al.</i> 2018
F	1140 to 1160	1. C-O-C asymmetry ring vibrational stretching in (b-1,4)-glycosidic bond in cellulose and hemicellulose; 2. C-H stretching vibration of syringyl units/coumaric acid ester in lignin	+ <sup>1,2</sup>	+ <sup>1</sup>	+ <sup>1,2</sup>	+ <sup>1</sup>	+ <sup>1,2</sup>	+ <sup>1</sup>	+ <sup>2</sup>	Musule <i>et al.</i> 2016; Sim <i>et al.</i> 2012; Li <i>et al.</i> 2018
G	1020 to 1040	1. C-H bending vibration of guaiacyl units; 2. C-O, C-C, C=C, C-C-O and C-OH stretching vibrations of cellulose, hemicellulose and lignin	+	-	+	-	-	-	+	Li <i>et al.</i> 2018; Sim <i>et al.</i> 2012

**Table 2.** Crystallinity Indices Ratio from the FTIR Spectra

Crystallinity Indices	Lignin (Control – Low)	Native EFB	DES-I EFB 1	DES-G EFB2	UH DES-I EFB1	UH DES-G EFB2	Cellulose-Avicel (Control – High)
LOI	0.55	0.72	0.96	0.85	1.01	0.89	1.13
TCI	0.61	0.85	1.17	0.99	1.25	1.18	1.29
HBI	0.35	0.69	1.47	1.34	1.58	1.46	1.69

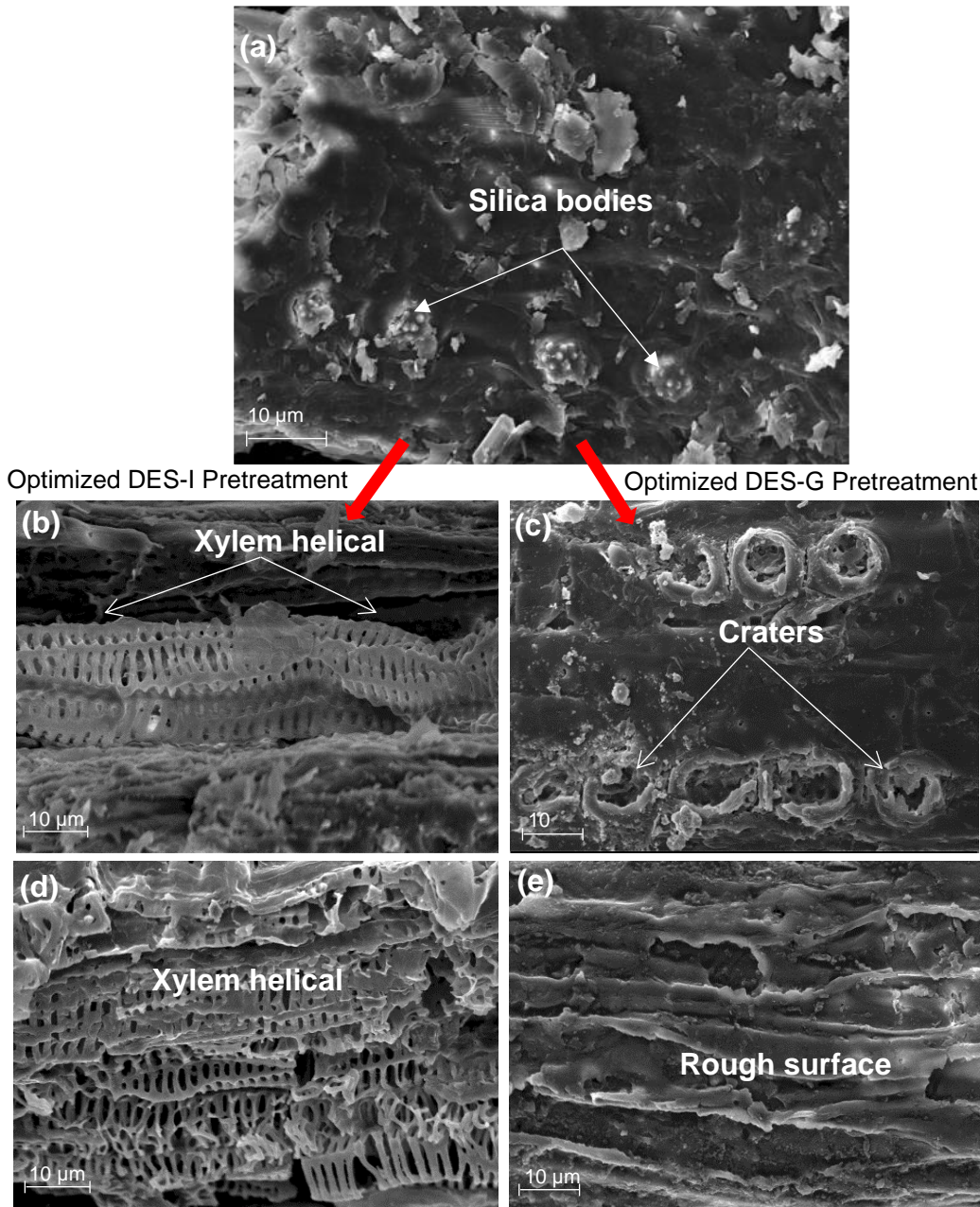
The LOI, TCI, and HBI values in the samples increased after the DES-I and DES-G pretreatments, and they continued to increase after the enzymatic hydrolysis, which implied that the samples were more crystalline in structure. This was mostly contributed by the cellulose remaining in the DES-I EFB1 (2), DES-G EFB2 (3), UH DES-I EFB1 (6), and UH DES-G EFB2 (7) samples after the removal of the fragmented amorphous structure, composed of hemicelluloses and lignin, during the DES pretreatment process (Kim *et al.* 2003; Lee *et al.* 2014). Both the DES-I EFB1 and unhydrolyzed UH DES-I EFB1 samples showed greater LOI, TCI, and HBI values compared to the DES-G EFB2 and UH DES-G EFB2 samples. These results might indicate that the removal of a considerable amount of the amorphous portion of the lignin and hemicelluloses during the DES-I pretreatment caused both the DES-I EFB1 and UH DES-I EFB1 samples to have the highest crystallinity degree as well as highly ordered cellulose structure, which leads to a higher hydrogen bond intensity between the neighboring hydrogens in the cellulose chain (Polleto *et al.* 2012).



**Fig. 7.** FTIR spectra for the samples of the (a) native EFB, DES-I EFB1, DES-G EFB2, pure cellulose, and pure lignin, and (b) native EFB, UH-DES-I EFB1, UH-DES-G EFB2, pure cellulose, and pure lignin.

#### *Morphological properties of the samples using variable pressure scanning electron microscopy (VPSEM)*

Figure 8 shows the exterior surface morphology of native EFB, DES-I EFB1, DES-G EFB2, UH DES-I EFB1, and UH DES-G EFB2 using VPSEM. The surface of the native untreated EFB (as shown in Fig. 8a) shows that most of the cuticle and silica layers were broken during the milling process, and the surface was contaminated by the broken cuticle debris due to the milling effect. A clear structure of silica bodies (SB) was observed on the native EFB fiber surface.



**Fig. 8.** Surface morphology via VPSEM of the (a) native EFB, (b) DES-I EFB1, (d) UH DES-I EFB1, (c) DES-G EFB2, and (e) UH DES-G EFB2 samples

The DES-I EFB1 sample showed the removal of a considerable amount of broken cuticle debris and silica bodies, which clearly exposed the helical coiled cellulosic microfibrils that reinforce the tracheary elements in plant xylem (as shown in Fig. 8b) (Gray 2015). The presence of cellulosic xylem helical structure became more obvious after the UH DES-I EFB1 sample underwent enzymatic hydrolysis (as shown in Fig. 8(d)). The cellulose fiber structure was clearly exposed to the surface, which indicated the complete destruction and removal of the cuticle and silica layers (Bonnett 1972).

Figure 8c shows the visual observation of the DES-G EFB2 sample with some cuticle debris and craters. However, none of the cellulosic xylem helical structures were



present in the DES-G EFB2 sample, showing the inefficiency of the DES-G pretreatment in deconstructing the microstructure of the EFB. The SEM observation of the surface of the UH DES-G EFB2 sample (as shown in Fig. 8e) only showed the rough surface structure, even after undergoing enzymatic hydrolysis, and it was not the same as the surface of the UH DES-I EFB1 sample. The optimal DES-I pretreatment removed greater amounts of lignin compared to the optimal DES-G pretreatment. The epidermal layer of the DES-I EFB1 was torn, peeled, and considerably ruptured. Most of the craters of the silica body initially found on the surface of the surface of the native EFB were destroyed. The delignification not only removed the relatively hydrophobic lignin but also the cuticle debris and silica on the EFB surface, causing the defibrillated inner microfibrils to become considerably exposed.

## CONCLUSIONS

1. Both the DES-I and DES-G deep eutectic solvent pretreatments were shown to be effective in reducing the lignin content and cellulose crystallinity, which enhanced the enzymatic digestibility of empty fruit bunch.
2. Even so, DES-I demonstrated a 92.4% and 68.7% glucan and xylan conversion rate, respectively, which was higher than the DES-G pretreatment glucan and xylan conversion rate (72.3% and 55.6%, respectively).
3. In addition, the DES-I pretreatment could be applied at a low loading ratio of EFB to DES (1:5) at a temperature of 160 °C for only 2 h. However, the use of the DES-G pretreatment required a higher ratio of biomass to DES (1:10) loading at a temperature of 160 °C for 4 h to achieve similar results.
4. The optimum pretreatment conditions applied in this study for both DES-I and DES-G resulted in low inhibitors concentration in the resulting black liquor from the DES pretreatment.
5. The Fourier transform infrared (FTIR) and X-ray diffraction (XRD) analyses also showed verification of hemicellulose degradation after the DES pretreatment.
6. The morphological analyses of the DES-I pretreated EFB indicated that the removal of a considerable amount of cuticle and silica bodies made it highly exposed to enzymatic hydrolysis.
7. Thus, the DES pretreatment seems to be a promising method as a bioconversion processes for empty fruit bunches.

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