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DELIGNIFICATION OF OIL PALM EMPTY FRUIT BUNCHES USING DEEP EUTECTIC SOLVENT IN PRESSURIZED REACTOR

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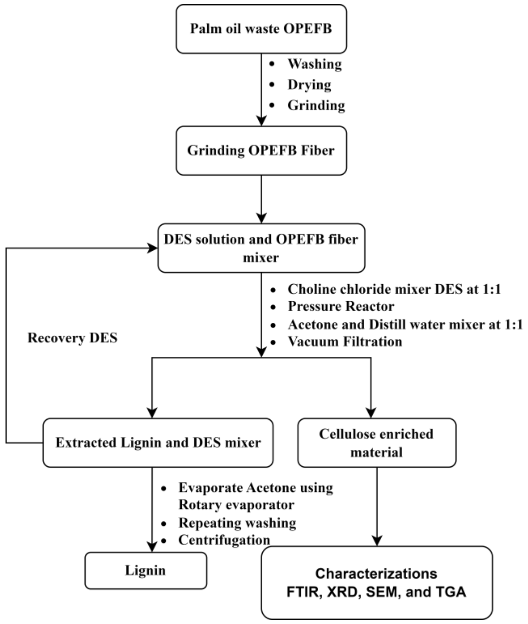
Graphical abstract



Abstract

Although lignin from oil palm empty fruit bunches (OPEFB) is a sustainable bioresource with several value-added uses, it is still difficult to extract lignin from lignocellulosic biomass economically. Lignin fractions are required for high-value applications and subsequently increase the feasibility of lignocellulosic biorefineries. Besides, the remaining cellulose could be further valorized into various chemical products. In this study, the delignification efficiency of OPEFB under inert and pressurized conditions has been studied as an effective pretreatment method for OPEFB. The optimal conditions of the high-pressure reactor to remove lignin from OPEFB were determined to be 80 °C at 30 bars with the presence of deep eutectic solvents (DES). It was identified that pressure affects the removal of lignin with the least amount of structural breakdown and the digestibility of cellulose. The effective removal of lignin from the OPEFB was established under mild conditions employing an inert nitrogen gas pressurized system. This work selectively removes lignin from the OPEFB of palm oil biomass plants using DES under mild extraction conditions. Solid residues were characterized by using FTIR, SEM, TGA, and XRD to investigate fractions under various reaction conditions, such as removal temperature, time, and pressure. The thermal stability of lignin was determined by TGA. In this study, 36.68% of lignin was removed under optimum pressure conditions at the lowest process temperature of 80 °C to determine how these factors influence the efficiency of lignin extraction in a high-pressure reactor.

Keywords: Lignocellulose, biorefineries, lignin, deep eutectic solvent, high-pressure reactor

Abstrak

Walaupun lignin daripada tandan kosong kelapa sawit (OPEFB) merupakan sumber biojisim yang mampan dengan pelbagai kegunaan nilai tambah, ianya masih sukar untuk mengeluarkan lignin daripada biojisim lignoselulosa secara ekonomi. Lignin digunakan dalam aplikasi bernilai tinggi dan seterusnya meningkatkan kebolehlaksanaan biorefineri melibatkan lignoselulosa. Selain itu, penggunaan selulosa boleh diperluaskan lagi ke dalam pelbagai produk kimia. Dalam kajian ini, kecekapan delignifikasi OPEFB dalam keadaan lengai dan bertekanan telah dikaji sebagai kaedah prarawatan yang berkesan untuk OPEFB. Keadaan optimum reaktor tekanan tinggi untuk mengeluarkan lignin

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daripada OPEFB dikenal pasti pada suhu 80 °C dan tekanan 30 bar dengan kehadiran pelarut eutektik dalam (DES). Tekanan mempengaruhi penyingkiran lignin dengan jumlah pecahan struktur yang paling sedikit dan kebolehcernaan selulosa. Penyingkiran lignin yang berkesan daripada OPEFB telah diwujudkan dalam keadaan sederhana menggunakan sistem bertekanan gas nitrogen lengai. Kajian ini secara selektif berjaya mengeluarkan lignin daripada OPEFB menggunakan DES di bawah keadaan pengekstrakan ringan. Sisa pepejal dicirikan dengan menggunakan FTIR, SEM, TGA, dan XRD untuk pencirian pecahan OPEFB di bawah pelbagai keadaan tindak balas, seperti suhu, masa dan tekanan semasa proses delignifikasi. Kestabilan haba lignin ditentukan oleh TGA. Dalam kajian ini, 36.68% lignin telah diekstrak dalam keadaan tekanan optimum pada suhu 80 °C untuk menentukan bagaimana faktor-faktor ini mempengaruhi kecekapan pengekstrakan lignin dalam reaktor tekanan tinggi.

Kata kunci: Lignoselulosa, biorefineries, lignin, pelarut eutektik dalam, reaktor tekanan tinggi

1.0 INTRODUCTION

The most prevalent natural biodegradable polymer on Earth is lignocellulosic biomass. Malaysia is the world's second biggest crude palm oil producer. Each year, about 17.7 million tons of palm oil are produced across 4.5 million hectares, resulting in a significant amount of waste [1]. Oil palm empty fruit bunch (OPEFB) is a source of waste generated by the oil palm industry. As a lignocellulosic biomass, OPEFB is mostly made up of between 24 and 65% cellulose, 21 to 34% hemicellulose, and 21 to 31% lignin [2]. OPEFB is a possible raw material for the production of second generation bioethanol due to its high availability and chemical makeup [3]. Along with its significant opportunity for use as a polymer, it is predicted to play a significant role in the environmentally friendly manufacture of other basic components, or aromatic compounds, including ferulic acid, vanillin, and phenol [4]. The improper management of OPEFB waste has negative effects on the environment, including the need for a large amount of space to dispose it and its combustion releases greenhouse gases like carbon dioxide (CO_2) and methane (CH_4) gases, which are significant contributors to climate change[5].

Lignin as one of the OPEFB components has many applications including dispersants, food additives, carbon fibers, copolymers in bio-composite materials, reinforcement for polymers, adhesives, emulsifiers, bio-based thermosets used in printing technology, natural antioxidants and antibacterials, prebiotics, fuels and platform chemicals, nanocomposites, battery electrodes, and adsorbents for heavy metals and toxic organic compounds in the environment [6]. Lignin is also typically burned to generate heat and power in lignocellulosic biorefineries because it is a low-value stream in these facilities [7]. Consequently, more focus has been placed on the complete utilization of lignocellulose-derived value-added products. In fact, resin and nanofibers with antioxidant activity can all be made from lignin, provided it is of high enough purity [8].

Therefore, pretreatment technologies that permit both effective extraction from lignocellulosic biomass and facile extraction into a large lignin component could make a significant contribution to the viability of lignin bioproducts [9]. Hence, lignin valorization is critical to biorefinery profitability for optimizing biomass usage and reducing waste [10]. A variety of pretreatment techniques have been explored, including mechanical, chemical, and biological methods [11]. The primary issues with these techniques are their high setup and operating costs, safety concerns, and the disposal of waste. The biological process takes time, and the laccase enzyme is expensive because it needs more facilities and tools.

Lignin valorization depends on the efficient fractionation of lignin from lignocellulosic biomass. Deep eutectic solvents (DESs) as one of the promising solvent have received a lot of interest recently as environmentally friendly solvents for biomass processing [12]. Recent year, microwave energy heating with DES as solvent has drawn interest for extracting lignin from lignocellulosic biomass due to its efficiency and minimal risk [13, 14]. DES are prepared by mixing hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) at different ratios. DES can be modified based on the hydrogen bond donor (HBD) to meet the needs of the application [15]. Several forms of DESs at different ratios (HBA: HBD) have been utilized in biomass pretreatment [16]. The most were being studied at HBA: polyol and HBA: carboxylic acid ratios [17, 18]. The performance of lignin removal varied depending on whether the DESs were acidic or basic. It was reported that acidic DES could delignify more easily than basic DES [19]. Indeed, the OPEFB was more successfully delignified by DES consisting of choline chloride: oxalic acid [13].

DES pretreatment has been extensively covered in the literature [20, 21, 22]. However, more efforts should be made to minimize the cost of these pretreatment methods so they can be used commercially. DES is considered a more cost-effective solvent for lignocellulosic biomass pretreatments than ionic liquids. Indeed, it can be recovered as a crystal, which reduces toxicity and simplifies possible recycling. Thus, DES's promising properties make it the most convenient and environmentally friendly solution for biomass processing.

In this study, the pressured heating system's ability to extract lignin from OPEFB is introduced through the utilization of DES. Pressurized heating systems in inert environments allow selective bond breaking during lignin depolymerization and have a more compact molecular weight distribution. This technology can turn biomass or agro-residue waste into profitable products.

2.0 METHODOLOGY

2.1 Materials and Chemicals

The raw OPEFB waste was collected from the local palm oil industry. The OPEFB fiber was washed with distilled water and dried in sunlight. The dried raw OPEFB samples were ground in an electric grinder machine. The OPEFB composition was determined using the standard TAPP1 T203, T204, T211, T421, and T222 methods, and it approximately contains 40% cellulose, 22% hemicellulose, 25% lignin, and 13% other components (extractives/fat, ash, and moisture) [13]. All other chemicals that were used in this study are distilled water, nitrogen gas, acetone (ACS reagent 99.5%), choline chloride (Reagent grade 98%), and oxalic acid (Reagent grade 98%).

2.2 DES Preparation

The DES was prepared by mixing oxalic acid as a hydrogen bond donor (HBD) and chlorine chloride as a hydrogen bond acceptor (HBA) in a 1:1 molar ratio [13]. The mixture was stirred at 90 °C until a transparent solvent was formed. Then the DES was cooled to prevent moisture absorption.

2.3 OPEFB Delignification Under Pressurized system

Figure 1 depicts the flow diagram for the investigation of lignin extracted from OPEFB using DES under pressurized conditions. A high-pressure batch reactor was used to extract the lignin from the OPEFB fiber. In a typical process, 10 g of OPEFB fiber was mixed with 100 g of DES and placed in a pressure reactor. The delignification was carried out at the respective extraction nitrogen atmosphere pressures of 1, 20, 30, 40, and 50 bars and at 80 °C. After 1 hour of the extraction process, 50 mL of acetone: water (1:1 v/v) were mixed with the dark-brown liquid and filtered by vacuum filtration to separate the soluble and insoluble DES fractions. After lignin precipitation and separation of the solid fraction, DES was recovered by removing acetone and water using a rotary evaporator. The lignin sample and cellulose enriched material were dried at 50 °C in an oven for 24 hours and stored at room temperature.

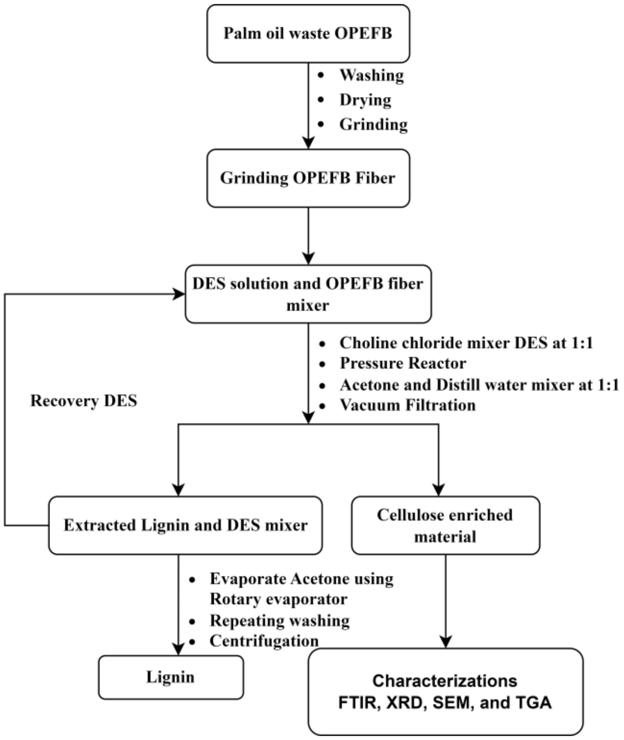

Figure 1 Flow chart of lignin Extraction

2.4 Calculation of Extracted Lignin

The amount of extracted lignin from OPEFB biomass via a high- pressure system was calculated based on TAPPI T222 (Equation 1).

$$L\% = \frac{m_{isolated}}{m_{TAPPI}} \times 10g\ EFB \qquad (1)$$

Where L is the percentage of OPEFB lignin removed, $m_{isolated}$ (gm) is the weight of lignin obtained by high-pressure reactor, and m_{TAPPI} (gm) is the weight of OPEFB lignin estimated using the TAPPI method.

2.5 Washing the Solid Fractions

The solid fractions obtained after the removal of lignin were repeatedly washed using distilled water in a beaker, followed by drying in the oven at 50 °C. The dried fractions were mashed with a mortar and pestle until they became powder.

2.6 Characterization of Treated solid OPEFB and Extracted Lignin

The crystallinity of ground OPEFB fibres (both untreated and DES-treated solid fractions) was evaluated by X-ray diffraction in the range between 10° and 80° angle. The crystallinity index is a quantitative indicator of crystallinity calculated using equation 2.

$$\text{Crystallinity Index}(CrI) = \frac{\text{Area of the all crystalline peaks}}{\text{Area of all the crystalline and amorphous peaks}} \qquad (2)$$

Similar samples also were subjected to FTIR analysis (Perkin Elmer Spectrum 100 FT-IR spectrometer) to examine the functional group alterations carried on by the high-pressure DES treatment. The spectral range was between 400 - 4000 cm^{-1} with 4 cm^{-1} resolution. The surface morphology of OPEFB fibers (both untreated and DES-treated solid fractions) was investigated using a high-resolution electron microscope (HITACHI TABLETOP, Model-TM3030Plus SEM) operating in high vacuum at an accelerated voltage of 15 kV to identify their surface properties. TGA was used to analyse thermal behaviour of lignin and OPEFB fibre. The thermal stability of raw OPEFB and lignin was determined by thermogram analysis in a nitrogen atmosphere and the heating rate was 10 °C/min to 600 °C.

3.0 RESULTS AND DISCUSSION

Figure 2 shows the lignin removal from OPEFB using DES under inert and high-pressure conditions. The temperature and time were kept constant at 80 °C and 60 min, respectively, whereas the pressure varied from 1 to 40 bars. Based on the data presented in Figure 2, it appears that the high-pressure environment was able to facilitate lignin removal in comparison to the analogous test conducted at atmospheric pressure. At a temperature of 80 °C, the higher lignin removal is 36.68% of the lignin as compared to an analogue microwave heating system reported in our previous work [23]. It was also found that pressure has a crucial function in lignin removal, with a rising trend from 1 to 30 bars. But more than 30 bar pressures have no impact on removing the highest lignin from OPEFB. During the process of lignin depolymerization, a pressure reactor was utilized to control the reaction and exert influence over the targeted breakdown of a chemical bond. This was accomplished by applying different pressures to the reactor. Inert nitrogen gas pressure permits the transfer of the heating energy and internal (e.g., inter-atomic bond stretching) energies of the molecules, influencing the dynamical and structural properties, such as bond length distributions, of the system. The pressure results in appreciable lignin delignification due to influence of pressure in synergizing the interaction between OPEFB and DES. These findings confirm that DES is a reliable solvent for lignin removal from biomass in a high-pressure and inert-gas atmosphere. Therefore, it can be said that extracting lignin from OPEFB in a pressure reactor is a simple and relatively affordable technique.

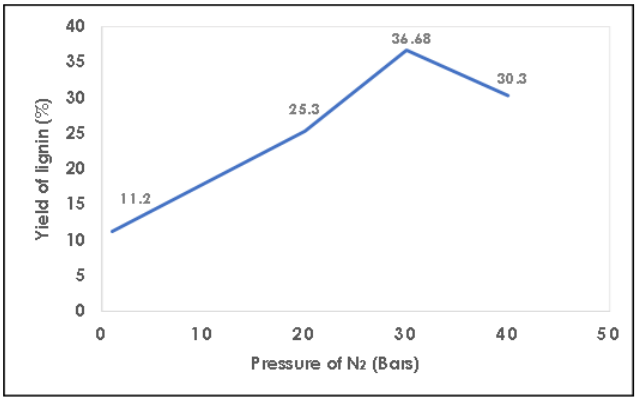

Figure 2 Delignification of OPEFB at different pressures

3.1 Crystallinity Index Analysis by XRD

The crystallinity index (CrI) of untreated and treated OPEFB fibers is depicted in Figure 3. A broad peak in the raw OPEFB indicated evidence of both lignin and hemicellulose, the peaks at 15.61°, 22.61°, and 34.84° were attributed to the cellulose-I characteristic peak [14]. The crystalline components were amplified and intensified following DES treatment at a ratio of 1:10 fiber to DES.

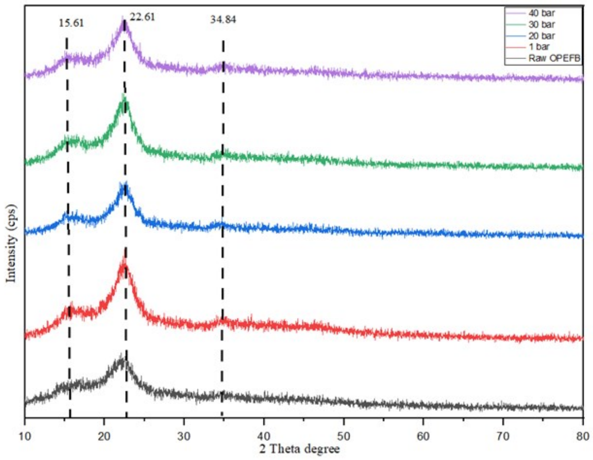

Figure 3 XRD of Pretreated OPEFB

Additionally, the percentage of cellulose crystallinity was calculated using the peak area equation (2), resulting in Table 1. Low crystallinity (37.94%) of raw OPEFB may be attributable to the presence of hemicellulose and lignin, whereas it is 49.49% for treated fibers. It was an obvious sign that non-cellulosic (non-crystalline) components had been removed from the OPEFB fiber structure. The breakdown of the amorphous portion and chemical modifications to the cellulose, hemicellulose, and lignin structures are reflected in the rise in cellulose crystallinity. According to a prior study, the removal of non-cellulosic elements enhances the stress relaxation of the cellulose chains [24]. As a result, cellulose fibers were modified. After DES treatment, polymer chain structure and surface characteristics could be altered due to the disruption of intermolecular hydrogen bonds in cellulose, resulting in cellulose dissolution due to the binding of DES to the cellulose. In addition, the 2θ signal at position 15.61° corresponds to the amorphous component of cellulose, and the highly strong signal at position 22.61° corresponds to the crystalline area. The CrI value has significantly changed when comparing the XRD diffraction patterns of the raw sample with the sample treated with high-pressure nitrogen gas. XRD diffraction patterns were used to compare cellulose crystallinity before and after treatment. At high pressure, acidic DES could break down the hemicellulose network, which increases the crystallinity of the cellulose. Under conditions of 30 bars, 1 hour, and 80 °C, the CrI of the sample was 49.49%, which showed that DES could remove the amorphous parts of the OPEFB. From the above discussion, it has been demonstrated that nitrogen pressure affects the removal of lignin and hemicellulose, whereas excessive pressure has the opposite effect. The treatment with high-pressure nitrogen increases the amount of cellulose that is brought into contact with the solvent, which accelerates the hydrolysis process.

Table 1 The crystallinity index of Raw EFB and solid Fractions

Sample	Ac	Aa+Ac	CrI (%)
Raw OPEFB	841.02	2217.03	37.94
1 bar	1133.65	2767.96	40.96
20 bars	776.76	1732.93	44.82
30 bars	1101.04	2235.66	49.49
40 bars	908.77	2025.25	44.87

3.2 FT-IR Analysis Pre-treated Solid Fiber

FTIR was used to characterize the functional groups and structural features of the pretreated solid fiber and raw OPEFB fiber. The Perkin Elmer spectrometer was used to obtain FTIR spectra by applying the wave number range of 400 cm^{-1} to 4000 cm^{-1} with KBr pellets containing finely ground pre-treated solid fiber and raw OPEFB fiber. FT-IR spectroscopy reveals that the structure of cellulose in all treated fibers was slightly changed (Figure 4). Table 2 displays the functional group designation of FT-IR spectrum wavenumbers for raw and treated fibers. The FT-IR spectrum at 897 cm^{-1} corresponds to the (1-4) glycosidic linkages of cellulose in polysaccharides [25]. All pre-treated samples exhibited connections in the 897 cm^{-1} wavelength range, as shown in Figure 4. It means that the cellulose composition of all the pre-treated OPEFB fibers changed significantly. The functional group identification is based on the wavenumbers of the FT-IR spectra of both raw and pre-treated fibers. The peaks at 1718 cm^{-1} and 1244 cm^{-1} refer to the ester and carbonyl (C-O) groups of hemicellulose and lignin, respectively. The pre-treated OPEFB structure changed between wavelengths 1100-1650 cm^{-1} (assigned C=C, C-H, C-H, CH_2 wagging, C-O-C asymmetric stretching or bending vibrations of various groups present in lignin, and 2750–3750 cm^{-1}).

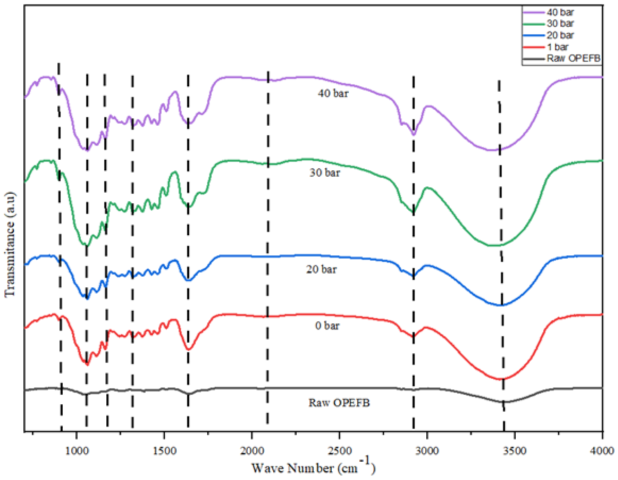

Figure 4 FT-IR Analysis of DESs treated solid OPEFB Fractions

Table Error! No text of specified style in document. Functional groups identification based on the wavenumbers of FT-IR spectra of both raw and pre-treated fibers

Functional group	Wave number (cm^{-1})	Identification
OH stretching	3413	Polysaccharides
C-H symmetrical stretching	2917	Polysaccharides
C–O stretching	1718	Hemicellulose
C=C stretching	1630	Lignin
C-H bending	1459	Lignin
C-H bending	1374	Cellulose
CH_2 Wagging	1319	Cellulose
C-O stretching	1244	Lignin
C-O-C asymmetric stretching	1162	Cellulose
C-C, C-OH, C-H vibrations of side groups and ring	1059	Hemicellulose
Glycosidic linkages symmetric ring stretching	897	Polysaccharides

On the other hand, the typical peak of hemicellulose occurred at 1059 cm^{-1} (C-C, C-OH, C-H vibrations of the side groups and ring). The domain between 3200 cm^{-1} and 3700 cm^{-1} exhibited broad peaks induced by the stretching vibrations of hydroxyl (-OH) functional groups of various organic compounds, including phenols and alcohols [14].

Based on the strength of the absorption vibration at the treated fibers, it may be assumed that some lignin and hemicellulose were removed from the raw OPEFB. At 30 bars, the peak intensity was higher than in other treated OPEFB. This could be because of the high pressure, which could speed up the breakdown of lignin and hemicellulose. The characteristics of the extracted lignin were confirmed via H-NMR, FTIR, and DSC analysis and reported in our previous work. [23].

3.3 Morphology Analysis by Scanning Electron Microscope (SEM)

The SEM image (Figure 5(a, b)) of raw OPEFB illustrates the presence of non-cellulosic substances such as lignin and hemicellulose on the microfibrils of the initial ground OPEFB fiber. Figures 5 (c, d) and (e, f) illustrate the rough and porous surface layer of DES-pretreated OPEFB fiber. The non-cellulosic components, such as lignin and hemicellulose, were removed to a certain extent by DES treatment. The red box represents the zoomed in area of each sample. It is clear that the surface of the untreated OPEFB was smooth and had no visible cracks. On the contrary, it can be seen that the treated sample had cracks and damage to the surface. The results show that high-pressure nitrogen gas increases DES's capacity to penetrate biomass and dissolve lignin and hemicellulose.

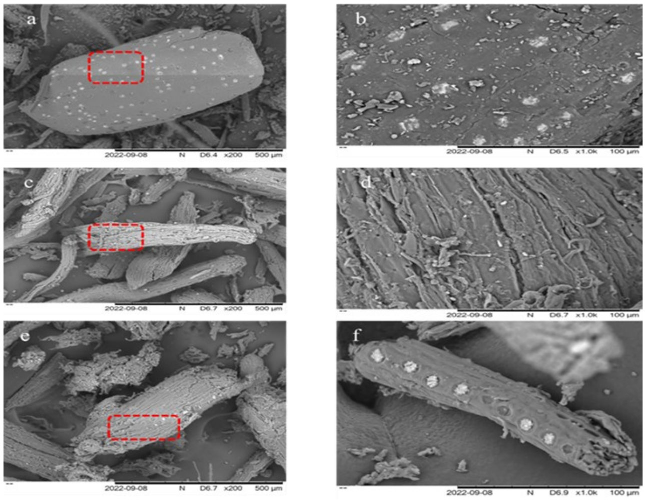

Figure 5 SEM image of raw OPEFB fiber (a, b) DES treated, OPEFB fiber (c, d) and (e, f)

3.4 TGA Analysis of Lignin and OPEFB

Figures 6a and b present the TG curves of raw OPEFB and lignin, respectively. The thermal breakdown process of natural fibers is affected by their varied chemical constitutions. The TGA can split the thermal stability of raw OPEFB fiber and lignin into three thermal phases. In the first phase, the evaporation of water occurred between 50-150 °C for each sample. Due to the thermal decomposition of a-or β-aryl-alkyl ether bonds, the weight loss of raw OPEFB fibers was detected at approximately 250-350 °C, whereas the thermal stability of lignin showed an earlier weight loss, which began at approximately 180 °C and reached a maximum decomposition at 300 °C [26]. In the second phase, lignin loses around 98% of its weight, which differs significantly from raw OPEFB (86.16%). At the third phase, the weight loss of lignin is about 36.21% at 434.14 °C, in contrast to the weight loss of raw OPEFB is 41.53% at 352.29 °C. DES has affected the thermal stability of lignin to a maximum of 150 °C, illustrating that DES in a pressure reactor is appropriate for the extraction of OPEFB [27]. Additionally, the thermogravimetric study showed that the lignin is highly stable at 300 °C which is dominated by acetone and water-extracted lignin [13]. A high temperature is required to break down the lignin compound than the raw OPEFB. The degradation continues until each of the samples is reduced to ash.

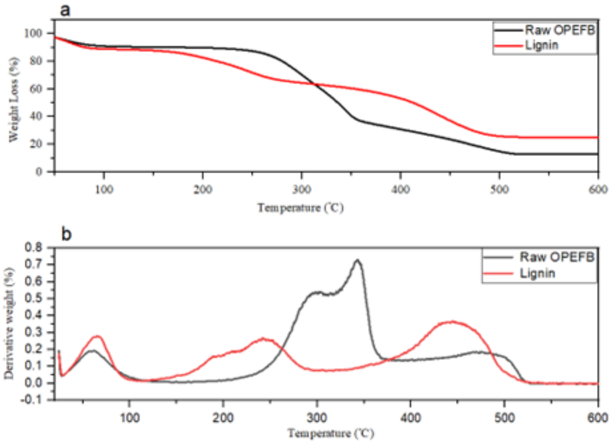

Figure 6 TGA Thermogram of raw OPEFB and lignin

4.0 CONCLUSION

DES was successfully utilized to pre-treat OPEFB under high pressure to remove lignin and develop cellulose-enriched fibers for potential strategic purposes. The findings demonstrated that high pressure could successfully eliminate lignin and hemicellulose, increasing the cellulose material. The resistant lignocellulosic structure was altered, enhancing the permeability of the material, due to the high-pressure nitrogen gas's dual effects of intense penetration and blasting during the pressure exerted. The effective processing conditions for OPEFB at 80 °C and 1 hour resulted in a lignin yield of 36.68%. The thermogram indicated the thermal stability of lignin. The XRD spectra of the treated solid EFB are more significant in terms of crystallinity and lignin removal. 80 °C and 30 bar are the optimum conditions at which the crystallinity of treated OPEFB is higher than that of raw OPEFB. Future research can be extended by focusing on the recycling of DES, subsequently further supporting the circular economy initiative.

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Conflicts of 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.

References

[1] S. H. Chang. 2014. An Overview of Empty Fruit Bunch from Oil Palm as Feedstock for Bio-oil Production. *Biomass and Bioenergy*. 62: 174-181. Doi: 10.1016/j.biombioe.2014.01.002.

[2] S. H. Anita *et al.* 2020. Optimization of Microwave-Assisted Oxalic Acid Pretreatment of Oil Palm Empty Fruit Bunch for Production of Fermentable Sugars. *Waste and Biomass Valorization*. 11(6): 2673-2687. Doi: 10.1007/s12649-018-00566-w.

[3] J. J. Liao, N. H. A. Latif, D. Trache, N. Brosse, and M. H. Hussin. (2020). Current Advancement on the Isolation, Characterization and Application of Lignin. *International Journal of Biological Macromolecules*. 162: 985-1024. Doi: 10.1016/j.ijbiomac.2020.06.168.

[4] P. Kahar *et al.* 2022. An Integrated Biorefinery Strategy for the Utilization of Palm-oil Wastes. *Bioresource Technology*. 344: 126266. Doi: 10.1016/j.biortech.2021.126266.

[5] S. Sethupathy *et al.* 2022. Lignin Valorization: Status, Challenges and Opportunities. *Bioresource Technology*. 347: 126696. Doi: 10.1016/j.biortech.2022.126696.

[6] E. Miliotti, S. Dell'Orco, G. Lotti, A. M. Rizzo, L. Rosi, and D. Chiaramonti. 2019. Lignocellulosic Ethanol Biorefinery: Valorization of Lignin-rich Stream through Hydrothermal Liquefaction. *Energies*. 12(4): Doi: 10.3390/en12040723.

[7] A. W. Bassett *et al.* 2019. Synthesis and Characterization of Molecularly Hybrid Bisphenols Derived from Lignin and CNSL: Application in Thermosetting Resins. *European Polymer Journal*. 111: 95-103. Doi: 10.1016/j.eurpolymj.2018.12.015.

[8] D. Kai *et al.* 2016. Engineering Poly(lactide)-Lignin Nanofibers with Antioxidant Activity for Biomedical Application. *ACS Sustainable Chemistry and Engineering*. 4(10): 5268-5276. Doi: 10.1021/acssuschemeng.6b00478.

[9] K. Mondylaksita, J. A. Ferreira, R. Millati, W. Budhijanto, C. Niklasson, and M. J. Taherzadeh. 2020. Recovery of High Purity Lignin And Digestible Cellulose from Oil Palm Empty Fruit Bunch using Low Acid-catalyzed Organosolv Pretreatment. *Agronomy*. 10(5). Doi: 10.3390/agronomy10050674.

[10] C. E. de A. Padilha *et al.* 2021. Production and Application of Lignin-based Chemicals and Materials in the Cellulosic Ethanol Production: An Overview on Lignin Closed-Loop Biorefinery Approaches. *Waste and Biomass Valorization*. 12(12): 6309-6337. Doi: 10.1007/s12649-021-01455-5.

[11] G. P. Naik, A. K. Poonia, and P. K. 2021. Chaudhari, Pretreatment of Lignocellulosic Agricultural Waste for Delignification, Rapid Hydrolysis, And Enhanced Biogas Production: A Review. *Journal of the Indian Chemical Society*. 98(10): 100147. Doi: 10.1016/j.jics.2021.100147.

[12] V. Z. Ong, T. Y. Wu, C. B. T. L. Lee, N. W. R. Cheong, and K. P. Y. Shak. 2019. Sequential Ultrasonication and Deep Eutectic Solvent Pretreatment to Remove Lignin and Recover Xylose from Oil Palm Fronds. *Ultrasonics Sonochemistry*. 58(May): 104598. Doi: 10.1016/j.ultsonch.2019.05.015.

[13] I. S. Muryadi, M. N. A. Yaakob and R. Roslan. 2021. Extraction of Lignin from Empty Fruit Bunch Fiber via Microwave-Assisted Deep-Eutectic Solvent Heating. *Current Science and Technology*. 1(2): 18-25. Doi: 10.15282/cst.v1i2.6708.

[14] B. Ahmed, Q. Wu, H. Lin, J. Gwon, I. Negulescu, and B. Cameron. 2022. Degumming of Hemp Fibers using Combined Microwave Energy and Deep Eutectic Solvent Treatment. *Industrial Crops and Products*. 184(February): 115046. Doi: 10.1016/j.indcrop.2022.115046.

[15] Y. T. Tan, G. C. Ngoh, and A. S. M. Chua. 2019. Effect of Functional Groups in Acid Constituent of Deep Eutectic Solvent for Extraction of Reactive Lignin. *Bioresource Technology*. 281: 359-366. Doi: 10.1016/j.biortech.2019.02.010.

[16] E. L. Smith, A. P. Abbott, and K. S. Ryder. 2014. Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews*. 114(21): 11060-11082. Doi: 10.1021/cr300162p.

[17] Y. Liu *et al.* 2017. Efficient Cleavage of Lignin–Carbohydrate Complexes and Ultrafast Extraction of Lignin Oligomers from Wood Biomass by Microwave-Assisted Treatment with Deep Eutectic Solvent," *ChemSusChem*. 10(8): 1692-1700. Doi: 10.1002/cssc.201601795.

[18] S. Zulkefli, E. Abdulmalek, and M. B. Abdul Rahman. 2017. Pretreatment of Oil Palm Trunk in Deep Eutectic Solvent and Optimization of Enzymatic Hydrolysis of Pretreated Oil Palm Trunk. *Renewable Energy*. 107: 36-41. Doi: 10.1016/j.renene.2017.01.037.

[19] Y. T. Tan, G. C. Ngoh, and A. S. M. Chua. 2018. Evaluation of Fractionation and Delignification Efficiencies of Deep Eutectic Solvents on Oil Palm Empty Fruit Bunch. *Industrial Crops and Products*. 123: 271-277. Doi: 10.1016/j.indcrop.2018.06.091.

[20] A. K. Kumar, B. S. Parikh, and M. Pravakar. 2016. Natural Deep Eutectic Solvent Mediated Pretreatment of Rice Straw: Bioanalytical Characterization of Lignin Extract and Enzymatic Hydrolysis of Pretreated Biomass Residue. *Environmental Science and Pollution Research*. 23(10): 9265-9275. Doi: 10.1007/s11356-015-4780-4.

[21] C. Y. Ma *et al.* 2021. Microwave-assisted Deep Eutectic Solvents (DES) Pretreatment of Control and Transgenic Poplars for Boosting the Lignin Valorization and Cellulose Bioconversion. *Industrial Crops and Products*. 164: 113415. Doi: 10.1016/j.indcrop.2021.113415.

[22] A. Satlewal, R. Agrawal, S. Bhagia, J. Sangoro, and A. J. Ragauskas. 2018. Natural Deep Eutectic Solvents for Lignocellulosic Biomass Pretreatment: Recent Developments, Challenges and Novel Opportunities. *Biotechnology Advances*. 36(8): 2032-2050. Doi: 10.1016/j.biotechadv.2018.08.009.

[23] Md. Arfat, A. H. Mustafa, V. R. Madduluri, R. Roslan, M. H. Ab Rahim. 2024. Lignin Extractions from Oil Palm Empty Fruit Bunch under Pressurized and Inert Conditions. *International Journal of Chemical and Biochemical Sciences*. 25(13): 158-164.

[24] G. Viscusi, G. Barra, and G. Gorrasi. 2020. Modification of Hemp Fibers through Alkaline Attack Assisted by Mechanical Milling: Effect of Processing Time on the Morphology of the System. *Cellulose*. 27(15): 8653-8665. Doi: 10.1007/s10570-020-03406-0.

[25] G. Kalisz, B. Gieroba, O. Chrobak, M. Suchora, A. L. Starosta, and A. Sroka-Bartnicka. 2021. Vibrational Spectroscopic Analyses and Imaging of the Early Middle Ages Hemp Bast Fibres Recovered from Lake Sediments. *Molecules*. 26(5): 1-16. Doi: 10.3390/molecules26051314.

[26] Y. Song, H. Ji, X. Shi, X. Yang, and X. Zhang. 2020. Successive Organic Solvent Fractionation and Structural

Characterization of Lignin Extracted from Hybrid Poplar by Deep Eutectic Solvent for Improving the Homogeneity and Isolating Narrow Fractions. *Renewable Energy*. 157: 1025-1034. Doi: 10.1016/j.renene.2020.04.159.

[27] K. Kohli, S. Katuwal, A. Biswas, and B. K. Sharma. 2019. Effective Delignification of Lignocellulosic Biomass by Microwave Assisted Deep Eutectic Solvents. *Bioresource Technology*. 303. Doi: 10.1016/j.biortech.2020.122897.