Delignification of oil palm empty fruit bunch via ultrasound-assisted deep eutectic solvent pretreatment

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Abstract. Lignin is the complex organic polymers contain in lignocellulosic biomass, acting as structural support of the biomass. Its presence making the biomass recalcitrance to bioprocessing. Therefore, pretreatment is essential in removing the lignin content to enhance the sugar extraction and bioethanol production from lignocellulosic biomass. In this work, pretreatment of oil palm empty fruit bunch (OPEFB) using ultrasound-assisted deep eutectic solvents (DESs) was performed. Three types of DESs, namely choline chloride-lactic acid (ChCl-LA), choline chloride-glycerol (ChCl-G) and choline chloride-urea (ChCl-U) in delignification were studied. The pretreatment was conducted with the presence of ultrasonication. The DESs recyclability were also performed in this work. ChCl-LA exhibited the best delignification properties, in which ChCl-LA pretreated OPEFB had the lowest total lignin content of 18.8%, followed by ChCl-U (19.4%) and ChCl-G (21.2%). All the three DESs were successfully recycled with slight decrement in delignification performance after two recycles. The recyclability of the DESs would improve the economic feasibility of the pretreatment process.

1. Introduction

Malaysia being the second largest palm oil producer in the world, generating large amount of lignocellulosic biomass wastes from oil palm plantation and milling activities, including oil palm empty fruit bunch (OPEFB), palm kernel shells and many more. These wastes accounting up to an amount of 46.6 MT [1,2]. OPEFB contains approximately 75% of carbohydrates with remaining 23% of lignin [3], could be an alternative resource to traditional petroleum refinery in producing a wide range of bio-based chemicals and fuels.

In view of the recalcitrant nature of the biomass, pretreatment is vital to improve bioprocessing efficiency [4,5]. The purpose of pretreatment is to alter the biomass structure through partial removal of lignin component in order to expose the cellulosic fraction within biomass [6]. There are various types of pretreatment techniques available, including physical pretreatment, chemical pretreatment, biological pretreatment and physico-chemical pretreatment. Among them, chemical pretreatment is the most widely explored and applied technique in the industries in view of its efficacy in biomass pretreatment. Despite that, chemical pretreatment encountered obstacles such as high cost of chemicals, low recyclability and reusability, possesses negative environmental impact and generation of inhibitors during the pretreatment. Therefore, researches start focusing on developing potential green solvent for biomass processing and pretreatment. Two widely explored green solvents are ionic liquids (ILs) and deep eutectic solvents (DESs) [3,7,8]. The latter is preferred in view of its low price, environment friendly, easily accessible and low generation of inhibitors [9].

DES is formed from a combination of two or more anionic and/or cationic species through hydrogen-bonding interaction. As a result, a eutectic mixture, *i.e.* liquid with lower melting point compared to their individual compounds, will be formed. DESs are categorized into four categories based on the combination of compounds. Among them, DES type III undergone extensive research in view of their inexpensive raw materials and environmental-friendly properties. These led to its popular interests across different industries, in particular biomass processing [3,7,10].

To further enhance the chemical pretreatment, incorporation of non-conventional energy such as ultrasound was explored [11]. In ultrasound pretreatment, combination of physical and chemical effects take place. In the presence of ultrasound, cavities or bubbles are formed through the rarefaction cycle, which stretches the liquid molecules apart [12]. These bubbles expand, rise to the liquid surface and coalescence due to the wave compression. When these bubbles collapse, powerful hydromechanical shear force is produced. During this process, high local temperature and pressure are generated. These actions assisted in biomass surface and morphology disruption, which could promote better reaction rate [13].

The aim of work is to study the effectiveness of biomass pretreatment by incorporating ultrasound in DES pretreatment. Choline chloride-lactic acid (ChCl-LA), choline chloride-glycerol (ChCl-G) and choline chloride-urea (ChCl-U) were selected as the pretreatment solvent. Their nature and properties in affecting the biomass pretreatment was evaluated based on their delignification capability. As far as to authors' knowledge, none of the work has been conducted to evaluate pretreatment performance for recycled DES. Therefore, the recyclability and reusability of DESs was also included in this work to further enhance the economic feasibility of the process.

2. Methodology

2.1. Preparation of oil palm empty fruit bunch

OPEFB was donated by Kwantas Oil Sdn. Bhd., Malaysia. It was washed with tap water for dirt and foreign particles removal, followed by drying in an oven overnight at 70 ℃. The dried OPEFB was grinded and screened through a mesh size of 0.5 mm. The OPEFB sample was stored in a sealed container placed at room condition prior to pretreatment.

2.2. Synthesis of deep eutectic solvents

ChCl-LA, Ch-G and ChCl-U were prepared by mixing choline chloride and lactic acid (for ChCl-LA) or glycerol (for ChCl-G) or urea (for ChCl-U) at respective molar ratio of 1:5, 1:2 and 1:2. The mixtures were heated under continuous stirring (150 rpm) at 80 ℃ to form a clear homogenous solution. The synthesized DESs were kept in separate chemical storage bottles and placed in a desiccator.

2.3. Ultrasound-assisted deep eutectic solvent pretreatment

OPEFB of 10% (w/v) solid loading was added into a test tube containing DES. The mixture was placed in a sonication bath (SK2510 HP, KUDOS®, China). Reaction was carried out for 30 min at 50 ℃ in the sonication bath at constant power of 240 W. The pretreated OPEFB was vacuum filtered. The filtrate was subjected to DES recovery, which will be discussed in subsequent section, and the solid residue was washed to remove DES residual prior to drying at the oven.

2.4. Recovery of deep eutectic solvents

DESs were recovered by subjecting the filtrates to rotary evaporator followed by centrifugation for 15 min at rotational speed of 3500 rpm. Supernatants were collected as recovered DESs.

2.5. Lignin determination

Lignin content in the OPEFB was determined following the standard procedure in the National Renewable Energy Laboratory (NREL) report [14]. The samples were hydrolysed with 72% sulfuric acid at 30 ℃ for 1 h, followed by dilute acid hydrolysis with 4% sulfuric acid at 121 ℃ for 1 h using an autoclave. The total lignin content can be measured by determining the acid soluble lignin (ASL) and acid insoluble lignin (AIL) content in the liquid and solid samples respectively. ASL was determined through absorbance measurement at 240 nm while AIL was gravimetrically determined. The equations involved in computing %ASL and %AIL are expressed in Equation (1) and (2) respectively:

$$\%ASL = (UV_{abs} \times V_{filtrate} \times \text{Dilution factor} / \varepsilon \times W_{sample} \times \text{Pathlength}) \times 100 \quad (1)$$

$$\%\ AIL = [(W_{crucible + dried\ solid} - W_{crucible}) - (W_{crucible + ash} - W_{crucible})] / W_{sample} \times 100 \quad (2)$$

where UV_{abs} represents the average UV-Vis absorbance for the sample, $V_{filtrate}$ represents the volume of filtrate (86.73 mL), ε is the absorptivity of biomass, W_{sample} represents the weight of sample in mg, Pathlength represents the pathlength of UV-Vis cell in cm (1 cm), $W_{crucible + dried\ solid}$, $W_{crucible}$ and $W_{crucible + ash}$ represent the weight of crucible with dried biomass sample, weight of empty crucible, and weight of crucible with ash, respectively.

3. Results and discussion

3.1. Performance of deep eutectic solvents in delignification

The total lignin content (%) for different DES-pretreated OPEFBs was tabulated in Table 1. Untreated OPEFB was included in the table to illustrate the delignification performance of different DESs. From the table, untreated OPEFB had total lignin content of 31.6% comprising 31.4% of AIL and 0.24% of ASL. ASL is the fraction of lignin that soluble in 72% sulfuric acid and it is typically present in relatively small amounts in most biomass sample. Meanwhile, AIL, or commonly known as Klason lignin contributes to majority of the lignin content in the biomass. In this study, the ASL difference was insignificant to delignification performance study while the AIL showed significant reading for direct comparison. Therefore, the delignification ability of pretreatment can be directly reflected through the %AIL of the pretreated biomass.

Table 1. Total lignin content in untreated and DES pretreated OPEFBs.

Sample	%AIL	%ASL	Total lignin content (%)
Untreated OPEFB	31.4	0.24	31.6
ChCl-LA pretreated OPEFB	18.7	0.11	18.8
ChCl-U pretreated OPEFB	19.3	0.16	19.4
ChCl-G pretreated OPEFB	21.1	0.14	21.2

By comparing the total lignin content of the pretreated OPEFBs to the untreated OPEFB, reduction in total lignin content was observed for all DESs suggesting that delignification had taken place during ultrasound-assisted DES pretreatment. Among the DESs investigated, ChCl-LA pretreated OPEFB had the lowest total lignin content, *i.e.* 18.8%, followed by ChCl-U at 19.4% and ChCl-G at 21.2%. This suggested that ChCl-LA and ChCl-U has better performance in the terms of lignin removal ability compared to ChCl-G. This could be due to the properties of ChCl-LA and ChCl-U being acidic and alkaline respectively. As reported by Tang *et al*. [15], ChCl-U has weak alkali properties of pH 9 which allows it to increase the cellulose digestibility by solubilizing lignin content of biomass. Similarly, ChCl-LA with mild acidic properties also promotes lignin solubility. In addition, ChCl-LA with the lowest viscosity, provided better mass transfer of solvent to the biomass and aided in biomass delignification [16]. The viscosity was part of the reason behind the inefficiency of ChCl-G in lignin removal as it is the most viscous mixture among all three DESs.

Having had the lignin removed from the OPEFB, the biomass was concentrated with cellulose and hemicellulose compounds. These compounds are made of sugars monomers, and hence can be saccharified to sugars and subsequently fermented to high value product of biofuel. There are also numerous studies reported that lignin content in the biomass could inhibit the enzymatic saccharification of biomass [17,18]. Therefore, with the removal of lignin from OPEFB, a higher conversion to sugars is expected during enzymatic saccharification.

3.2. Recovery and recyclability of deep eutectic solvents

Although DES is categorized as a reasonably cheap, green solvent, its recyclability is still an important issue to be addressed to further improve its economic value in low cost-high volume industrial applications. According to Xu *et al*. [19], DESs are more readily to be recycled than other solvents due to its regeneration process does not involve any further chemical reactions. In this research work, the DESs were recovered through simple separation using rotary evaporator. The recovered DESs were then used to pretreat the OPEFB and its degree of delignification was re-evaluated. Figure 1 shows the delignification performance of recycled DESs. As observed from the figure, there is a slight decrement in degree of delignification for recycled DESs. The performance of delignification dropped approximately 8% to 11% for all DESs after first recycle. The drop in the degree of delignification of pretreated OPEFB continued to take place when the DESs were recycled for the second time. These findings concluded that the increase in number of recycle leads to lower delignification efficiency. This indicates that the recycled DESs have lower ability in disrupting and solubilizing the lignin of OPEFB, thus lower cellulose fraction accessibility. Earlier studies also suggested that the recycled DESs exhibited reduction in lignin removal efficiency [20]. The drop of degree of delignification in all recycled DESs was due to the presence of water and other impurities such as lignin in the recycled DESs. The presence of lignin in recycled DES reduced the number of active sites available for reaction and hence decreased its delignification efficiency. Nevertheless, removal of lignin still took place as the total lignin content obtained in recycled DESs were lower than untreated OPEFB. The sequence of the delignification performance remained the same as the fresh DESs where ChCl-LA exhibited the best delignification capability, followed by ChCl-U and ChCl-G.

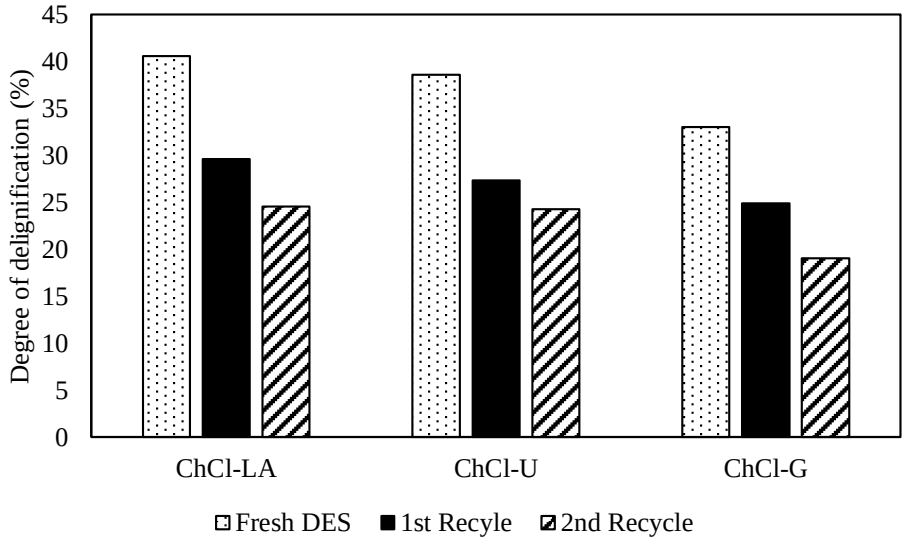

Figure 1. Delignification performance of recycled DESs.

4. Conclusion

In this study, oil palm empty fruit bunch (OPEFB) pretreated with acidic choline chloride-lactic acid (ChCl-LA) had the lowest total lignin content of 18.8%, followed by choline chloride-urea (ChCl-U, 19.4%) and choline chloride-glycerol (ChCl-G, 21.2%). This identified that ChCl-LA exhibited the best delignification properties among all due to its nature of acidic properties. Recycled deep eutectic solvents (DESs) shown decrement in delignification efficiency with its efficacy dropped with number of recycles. The drop of delignification efficiency was likely due to the presence of water as well as other impurities in the recycled DESs. Further investigation on the isolation and characterization of lignin is recommended in converting the lignin into valuable chemicals.

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