Hydrolysis Empty Fruit Bunch (EFB) Using Green Solvent

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Abstract. Empty fruit bunch (EFB) contained high amount of cellulose and can be further utilized for nanocrystalline cellulose (NCC) production. Increasing awareness on environment has attracted attention on green solvent compared to the usage of conventional strong acid in nanocellulose isolation. In this study, NCC has been isolated from raw EFB using environmental friendly solvent, natural deep eutectic solvent (NADES). The purpose of this study is to investigate the effect of different types of NADES on NCC isolation. Three different types of NADES based on three different organic acids (citric acid, malic acid and lactic acid) have been used in this study. The solubility of NCC in these NADES was measured while their physical characterization was determined using Fourier transform infrared (FTIR), scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM). From the results, EFB dissolution in NADES made up of citric acid shows higher solubility with a value of 2.74%. FTIR spectra shows similar spectra between treated EFB and NCC isolated using NADES. From FESEM micrograph, citric acid based NADES is able to produce NCC in the range of 25 to 37 nm. Based on the results, NCC has been successfully isolated using NADES and further improvement can be applied to enhance its production.

1. Introduction

Over the last 25 years, Malaysia has been one of the global oil palm producer contributing about 40-60% of the world oil palm [1]. Consequently, this industry generates lots of lignocellulosic waste such as empty fruit bunch (EFB). EFB from oil palm consist of 44.4% of cellulose, 30.9% of hemicellulose and 14.2% of lignin [2]. Inexpensive, readily available and high cellulose content are the factors that have raised the interest in utilizing them in energy production, medical and pharmaceutical products, and industrial biodegradable products [3]. Besides, the high cellulose content in EFB can be used to produce nanocrystalline cellulose (NCC).

NCC is also known as cellulose nanowhiskers and cellulose microcrystals [4]. NCC are rod like particles and highly crystalline with a diameter of 1-100nm while its length varies between tens to hundreds of nanometers[5]. NCC are generated when polysaccharides bound at the fibril surface are removed which result in the cleavage and destruction of the vulnerable amorphous region [6]. NCC possesses remarkable properties such as large surface area, superior mechanical strength, high specific surface area, non-toxic and biodegradability which make it as one of the most promising new class of nanomaterial [7]. NCC is usually produced by conventional method using strong acid hydrolysis to remove amorphous parts so that highly crystalline NCC can be obtained [7]. However, concerns are raised due to the toxicity of the NCC produced through conventional method as the utilization of
concentrated acid and alkali are hazardous and detrimental to environment [8]. Thus, a new solvent named natural deep eutectic solvent (NADES) is seen as an alternative due to its biodegradability, low toxicity and low cost [9].

NADES is a sub-class of deep eutectic solvent (DES) and it is a mixtures between hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) components, which comprised of two or three natural compounds [10]. NADES is synthesized from low-cost and naturally available chemicals which is sustainable, biodegradable and has a good physicochemical properties as solvent [9]. Besides, it has low toxicity as it is usually made of compounds that are naturally occurred in the environment[11]. Therefore, to fulfill the increasing interest on green technology, NADES is the most promising solvent to be studied for its potential in the hydrolysis of EFB to obtain NCC. However, the potential of NADES in isolating NCC has yet to be investigated. Thus, the main aim of this study is to discover the potential of NADES in NCC production.

2. Materials and method
EFB was collected from Taclico Company Sdn. Bhd. Chemicals used in this study including sodium hydroxide (NaOH), sodium chlorite (NaClO₂), acetic acid (>95%), choline chloride (99%), citric acid 1-hydrate (99.5-100%), DL-malic acid (99+%), DL-lactic acid (85%) and ethanol (95%) were acquired from A.R. Alatan Sains (K) Sdn. Bhd., Malaysia and Modern-Lab Chemicals Sdn. Bhd., Malaysia.

2.1. EFB Pre-treatment
Prior usage, a miniature grinder was used to grind EFB into smaller size and then sieved to get 250µm particle size. Lignin was removed using alkaline treatment by heating 10 g of EFB in 200 mL of 3% (w/v) NaOH solution at 85°C for 1 to 2 hours [12,13]. The mixture was filtered and washed with distilled water until the pH of the wash reached pH 7. Alkaline-treated EFB was mixed with bleaching solution at a solid to liquid ratio of 1:30 and heated at 70˚C for 1 hour. 1% (w/v) of NaClO₂ was used as bleaching solution and was prepared by putting few drops of glacial acetic acid in the solution to obtain pH 4 [14]. The mixture was filtered and washed with distilled water repeatedly to achieve pH 7.

2.2. NADES Preparation
Three types of choline chloride based NADES was prepared by mixing all components and heated at 80°C for 1 to 2 hours until homogenous liquid NADES was formed [15] 10 wt% of water was added to ease the homogenization of the mixture [16]. Table 1 shows the ratio of NADES prepared in this study.

| HBA       | HBD     | Ratio | NADES |
|-----------|---------|-------|-------|
| Choline  | Citric  | 2:1   | 1     |
| chloride | acid    |       |       |
|          | Malic   | 2:1   | 2     |
|          | acid    |       |       |
|          | Lactic  | 2:1   | 3     |
|          | acid    |       |       |

2.3. EFB dissolution
EFB was dissolved in NADES to obtain NCC by referring to the method used by previous study [5,17]. Treated EFB was dissolved in NADES at a ratio of 1:40 and heated at 85°C. The mixture was heated and stirred vigorously for 6 hours. Three different NADES as shown in Table 1 before was used to dissolve the treated EFB. Cold distilled water was added into the mixture after 6 hours of heating to stop the reaction. The mixture was washed with ethanol first and then distilled water using centrifuge at 8000 rpm for 10 minutes. During centrifugation step, NCC suspension was collected until
there was no more cloudy suspension obtained. NCC suspension was then dried to obtain NCC powder for characterization and solubility measurement. Solubility of NCC in NADES was calculated as the equation (1) shown below.

\[
\text{Solubility} (\%) = \frac{\text{Weight of dried NCC}}{\text{Initial weight of treated EFB}} \times 100
\]  

(1)

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

NCC obtained was analyzed to observe changes in their functional group using Shimadzu IR spectrophotometer model Prestige-21. NCC was mixed with potassium bromide (KBr) to form a pellet and then viewed in the spectrometer using the range of 500 cm\(^{-1}\) to 4000 cm\(^{-1}\)[18].

2.5. Field Emission Scanning Electron Microscope (FESEM)

The morphology of NCC obtained was determined using field emission scanning electron microscope (Carl Zeiss Leo Supra 50 VP). Scanning electron microscope (Zeis Supra 35VP) was used to evaluate the morphology of raw EFB, alkaline treated EFB and bleached EFB. Platinum coating was used to coat all samples prior analysis.

3. Results and discussion

3.1. Solubility

The ability of NADES to dissolve EFB to produce NCC has been measured in this study. Figure 1 shows the solubility of EFB obtained using different types of NADES. From the results obtained, it is found that NADES 1 that is made up of cellulose and consequently produce more nanocellulose compared to the other NADES. In previous study, fructose has been successfully converted to HMF when NADES made up of choline chloride and citric acid is used.[20]. This shows that NADES is able to hydrolyze carbohydrates such as cellulose into NCC. NADES is formed by hydrogen bonding between HBA and HBD.

![Figure 1. Nanocellulose solubility based on different types of NADES.](image)

It is known that NADES is able to donate or accept electron or proton, making it as an excellent dissolution agent [19]. Besides, NADES also able to promote the hydrolysis of inulin to fructose and followed by the dehydration of fructose to hydroxymethylfurfural (HMF) [20]. Based on Figure 1, NADES 1 is able to dissolve 2.74% of cellulose and consequently produce more nanocellulose compared to the other NADES. In previous study, fructose has been successfully converted to HMF when NADES made up of choline chloride and citric acid is used.[20]. This shows that NADES is able to hydrolyze carbohydrates such as cellulose into NCC. NADES is formed by hydrogen bonding between HBA and HBD. This creates a competition between NADES and cellulose in terms of hydrogen bonding. From the point of view of molecular structure of organic acid used, citric acid contains higher numbers of carbonyl groups and hydroxyl group as compared to malic acid and lactic acid. The amount of carbonyl group and hydroxyl groups in NADES is imperative for the formation of hydrogen bonding with cellulose and consequently increased cellulose solubility [21].
3.2. FTIR Analysis

FTIR spectra of raw EFB, alkaline treated EFB and bleached EFB are portrayed in Figure 2. From the results obtained, EFB undergoes some changes on its chemical structures and functional groups. A broad band between 3000-3500 cm\(^{-1}\) appears in raw, alkaline treated and bleached EFB indicating the stretching vibration on free \(-\text{OH}\) group resulting from the hydrogen bonding in cellulose molecules [5]. The peak that appeared at 1635-1646 cm\(^{-1}\) shows the \(-\text{OH}\) bending vibration of the absorbed water molecules. Meanwhile, the stretching vibration of saturated C-H in cellulose and hemicellulose can be identified by the peak that occurred in the range of 2903 cm\(^{-1}\) to 2918 cm\(^{-1}\) [22]. Besides, the peak at 1508 cm\(^{-1}\) in raw EFB indicates the presence of C=C stretching vibration in the aromatic lignin. This particular peak is not observed in the bleached sample proving that lignin has been removed successfully [23]. The removal of lignin also can be confirmed by the disappearance of peak 1246 cm\(^{-1}\) which signify the C-O-C stretching of aromatic ether linkages in raw EFB [24]. The deformation of the vibration of C-H and C-O-C pyranose can be found prominently in bleached EFB at 1105 cm\(^{-1}\) suggesting the removal of hemicellulose and lignin [25].

![Figure 2. FTIR spectra of raw EFB, alkaline treated EFB and bleached EFB.](image)

FTIR spectra of NCC shows similar cellulose fingerprint region as FTIR spectra of treated EFB indicating that NADES is able to maintain cellulose structure (Figure 3). The peak found at 897 cm\(^{-1}\) indicates the deformation of C-H glycosidic bond with a ring vibration contribution and the O-H bending. These shows the presence of \(\beta\)-glycosidic linkage between the anhydroglucose units of cellulose which can also be known as the amorphous region [26]. Based on the samples, NCC obtained from NADES 1 shows the highest transmittance value suggesting that more \(\beta\)-glycosidic bonds have been destroyed. Cellulose structure is further confirmed by the appearance of peak near 1044 cm\(^{-1}\) which represents the C-O and C-H stretching vibration [27]. From the spectra obtained, NADES 1 shows higher intensity of this peak which signifies higher crystallinity of the as-produced NCC. The spectra obtained from all treatments suggest that nanocellulose obtained is in cellulose I form due to the presence of peak at 1424 cm\(^{-1}\) and 1163 cm\(^{-1}\) [18].
3.3. SEM and FESEM Analysis
The SEM and FESEM micrographs of raw EFB, treated EFB and NCC obtained from NADES are shown in Figure 4. In Figure 4(a), SEM micrograph of raw EFB shows a smooth, compact structure and non-fibrous component due to the presence of lignin and hemicellulose [28]. These materials hold together bundles of individual fibris tightly like a cement [29]. Silica can also be found on the surface of raw EFB and has been removed during alkaline treatment as shown in Figure 4(b). This is indicated by the formation of empty holes which is previously occupied by silica. It is important to remove silica during pre-treatment process to ease the penetration of hemicellulose [30]. Further treatment with bleaching agent reduces the fiber diameter by defibrillation as shown in Figure 4(c). Defibrillation process initiates by oxidizing the lignin and promote the formation of hydroxyl carbonyl and carboxylic group which help to enhance lignin solubilisation [23].
Figure 4(d) shows the FESEM micrograph of NCC obtained using NADES 1. The size of NCC obtained ranged of 25 to 37 nm resulting from the breakdown of cellulose fibril into nano-sized cellulose by disruption of hydrogen bonding. The inter-cellulose hydrogen bonding competes with hydrogen bonding formed between carbonyl group of NADES 1 and hydroxyl group of cellulose [21]. Thus, the ability of NADES 1 to form more hydrogen bonding with cellulose has shown its capability to hydrolyse cellulose into NCC.

4. Conclusion
A green solvent synthesized from natural components is able to hydrolyze raw EFB into NCC based on the results obtained in this study. NADES 1 synthesized from the mixture of citric acid and choline chloride has shown better results as compared to other NADES types. NADES 1 able to dissolve more treated EFB into NCC with a solubility value of 2.74%. Moreover, the usage of NADES in producing NCC from EFB does not change the cellulose structure, as proved by similar FTIR spectra of NCC to those of treated EFB. Besides, FESEM micrograph of NCC obtained using NADES 1 shows that NADES 1 able to hydrolyze cellulose into NCC with a size ranging from 25 to 37 nm. The increasing awareness on environment and attention on biodegradable as well as biocompatible NCC has seen NADES as a promising green solvent. The potential of NADES in isolating NCC from lignocellulosic materials should be further investigated to improve its efficiency.

Acknowledgement
The authors would like to acknowledge the financial support given by the Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme (FRGS) with a grant number of FRGS/1/2015/TK10/UNIMAP/03/05.

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