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ORIGINAL ARTICLE

Utilization of Potassium Carbonate-Ethylene Glycol as Deep Eutectic Solvent to Delignification Oil Palm Empty Fruit Bunch for Furfural and Ethanol Production

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ABSTRACT – Pretreatment using deep eutectic solvent (DES) was done to reduce the lignin content in oil palm empty fruit bunch (OPEFB). DES solution was prepared by combining potassium carbonate (K_2CO_3) as hydrogen bond acceptors (HBA) and ethylene glycol (EG) as hydrogen bond donors (HBD). This study aimed to obtain optimum conditions in the pretreatment process and determine the levels of lignin, cellulose, hemicellulose, and glucose in OPEFB. The pretreatment stage was performed at different temperature parameters, 100°C, 120°C, and 150°C for 60 minutes to produce optimum conditions. Biomass pretreated with DES was hydrolyzed by cellulase complex to obtain glycose and xylose. The results showed that the pretreatment optimum condition value achieved with DES K_2CO_3:EG at 150°C for 60 minutes could degrade lignin by 46.06%, hemicellulose by 4.08%, and increase cellulose by 60.21%. The glucose and xylose content reached 58.48 g/l and 26.60 g/l, respectively. This sugar has the potential for ethanol production from glucose and furfural production from xylose.

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INTRODUCTION

Lignocellulosic biomass is abundant renewable energy and a promising bioresource that can be transformed into various products such as chemicals and energy materials. One of the large lignocellulosic biomasses is oil palm empty fruit bunches (EFB). Oil palm empty fruit bunches are formed from 1 ton of fresh fruit bunches at a rate of 23% or 230 kg [1]. EFB biomass contains cellulose, hemicellulose, and lignin that can be utilized. Cellulose stands as one of the most abundant organic compounds on Earth. It comprises linear homopolysaccharides consisting of β-1,4-linked D-glucopyranoside residues with 3,000 or more glucose units [2]. Through hydrolysis, cellulose can be converted into glucose, which then can be fermented to create bioethanol [3]. Hemicellulose is a mixture of various sugars, including five-carbon sugars like xylose and arabinose and six-carbon sugars such as glucose, mannose, and galactose. It also includes the six-carbon deoxy sugar, rhamnose [4]. Hemicellulose has a lower degree of polymerization compared to cellulose. It can be hydrolyzed to produce xylose and dehydrated to form furfural [5]. However, the high lignin content in EFB (30–35%) can hinder the utilization process of cellulose and hemicellulose, necessitating a pretreatment process aimed at reducing lignin content. Pretreatment can be done using chemical methods such as acids, bases, oxidation, organosolv, ozonolysis, and ionic liquids. However, these methods have several drawbacks, including substantial water for washing the pretreatment, longer treatment times, non-selective oxidants, expensive solvent costs, and higher waste recovery expenses. Additionally, the non-biodegradable nature of the byproducts makes these methods economically and environmentally less viable [6].

Deep eutectic solvent (DES) can be utilized as a chemical solution for pretreatment, boasting numerous advantages, including lower costs, easy synthesis, renewability, recyclability, high purity, low toxicity, non-flammability, and the fact that DES is a biodegradable solvent [7]. DES is a solvent with hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) properties, thereby supporting the breaking down of target analyte molecules. Standard HBA components are quaternary ammonium salts, while HBD includes amines, carboxylic acids, alcohols, polyols, or carbohydrates [8].

Previous research conducted by Atikah et al. [9] on the pretreatment of EFB using a DES solution choline chloride (ChCl)-Urea demonstrated that at a temperature of 110°C with a processing time of 4 hours, it yielded glucose at 60.47 mg/mL. In a study by Majová et al. [10], pretreatment using pulp and a DES solution consisting of a ChCl-Oxalic Acid mixture resulted in the degradation of 38.7% of lignin in the pulp. Another study by Okur & Koyuncu [11] focused on rice husk delignification using a DES solution ethylene glycol-citric acid, achieving optimal results at a temperature of 120°C and a processing time of 4 hours, which showed 57.33% of lignin degradation. Pretreatment using DES potassium carbonate (K_2CO_3) and glycerol was carried out by Suopajärvi et al. [12] on wheat straw and corn stover biomass. At a temperature of 100°C for 16 hours, the wheat straw sample exhibited a reduction in lignin content from 22% to 12.1%, while the corn stover lignin content decreased from 24% to 16.3%. While few publications are related to DES K_2CO_3-EG, the application of this DES for EFB pretreatment appears to be limited based on our current knowledge. This study focuses on the pretreatment process of EFB using DES K_2CO_3-EG on EFB, investigating its impact on lignin. Additionally, enzymatic hydrolysis of the pretreated pulp was conducted to produce glucose and xylose as raw materials for ethanol and furfural production.

EXPERIMENTAL METHOD

Materials and Instruments

The empty fruit bunch (EFB) utilized in this study was sourced from oil palm plantations in South Sumatra, Indonesia. To prepare it for the deep eutectic solvent (DES) pretreatment process, physical pretreatment was done by grinding the EFB until its size was reduced to less than 5 mm. To prevent an increase in moisture content caused by water absorption, the processed EFB was securely stored in a plastic bag. The DES precursor, consisting of potassium carbonate and ethylene glycol, is obtained from Sigma Aldrich. All other chemicals used for analytical purposes were of analytical grade and were sourced from Merck and Sigma Aldrich.

Method and Procedure

Synthesis of Deep Eutectic Solvent (DES)

DES used in this study consisted of potassium carbonate (K_2CO_3) and ethylene glycol with a molar ratio of 1:10 (DES K_2CO_3-EG). The steps were mixing potassium carbonate with ethylene glycol at 80ºC for 60 minutes in a beaker glass. The mixing of DES used a magnetic stirrer with a speed of 200 rpm to form a homogeneous, colorless, or transparent solution [13]. Then, DES is cooled at room temperature until ready to use as a reaction medium for the pretreatment of EFB. The physical characteristics of the DES were determined through several measurements. Viscosity was measured using a Tamson Instrument BV, density was assessed with a DMA 4100 M, and the pH of the DES was determined using a pH meter from Mettler Toledo.

Delignification EFB by DES

Oil palm empty fruit bunches (EFB) with 0.5 grams were placed into screw-capped reaction tubes, and specific DES solvents were added in a 1:10 ratio. The mixture was heated at different temperatures (100°C, 120°C, and 150°C) for 60 minutes. Each reaction tube was sealed to prevent sample evaporation during the pretreatment process. The pretreatment of the samples in the reaction tubes in an ECO8 thermoreactor with occasional stirring every 15 minutes. After pretreatment, the EFB residue and DES were separated through vacuum filtration. The residue from the pretreatment process was subsequently dried in an oven at 50°C. Before conducting composition analysis and enzymatic hydrolysis, the pretreated EFB was stored in plastic containers.

Enzymatic Hydrolysis of DES-Pretreated EFB

One gram of pretreated EFB was added to a 10 ml solution of 0.05 M citric acid buffer (pH 4.8). The substrate was sterilized using an autoclave at a temperature of 121°C for 30 minutes and then cooled to 50°C. After that, two enzymes, H-Tech and C-Tech, were added to the sample at a concentration of 30 FPU (filter paper units) per gram of dry substrate weight. The saccharification process was carried out in a shaking incubator at 150 rpm and a temperature of 50°C for 72 hours. The samples obtained from this process were subsequently analyzed for glucose and xylose content using high-performance liquid chromatography (HPLC).

Analysis

The composition of EFB was analyzed before and after DES pretreatment following the National Renewable Energy Laboratory/NREL method[14]. In this process, 300 mg of either EFB or pretreated EFB was combined with sulfuric acid to assess the levels of lignin, cellulose, and hemicellulose. The lignin content was further categorized into acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). Total lignin was determined as the sum of AIL and ASL. AIL was measured through gravimetry using a Sartorius BS224S balance, while ASL was measured using a UV/Vis Spectrophotometer (Optizen 2120 UV) at 205 nm. After hydrolysis, the filtrate was used to measure the glucose and xylose content through HPLC with a Waters e2695 instrument equipped with an HPX-87P column (Bio-RAD, CA, USA) and an RI detector. The mobile phase flowed at a 0.6 mL/min rate using a five mM H_2SO_4 solution. The glucose and xylose measurements were converted to cellulose and hemicellulose content through anhydrous correction. The samples obtained from enzymatic hydrolysis were analyzed using the same method as the component analysis.

The equation used in this research is as follows:

$$\text{Pretreated biomass solid recovery (\%)} = \frac{weight\ pretreated\ EFB}{weight\ untreated\ EFB} \times 100\% \quad (1)$$

$$\text{Delignification (\%)} = \frac{(lignin\ untreated - lignin\ pretreated)}{lignin\ untreated} \times\ 100\% \quad (2)$$

RESULT AND DISCUSSION

Deep Eutectic Solvent (DES) Characterization

The production of alkaline DES using potassium carbonate (K_2CO_3) as the hydrogen bond acceptors (HBA) and ethylene glycol (EG) as the hydrogen bond donors (HBD) requires an appropriate molar ratio composition to create a homogeneous DES solution. The molar ratio of K_2CO_3:EG for forming a homogeneous solution is 1:10. At ratios below 1:10, a white precipitate still forms after the cooling process, indicating incomplete DES formation. Figure 1 illustrates the mechanism of DES formation due to the interaction between the electronegative O^- atom in K_2CO_3 and the H atom in EG, forming a hydrogen bond [15]. The characteristics of the DES were tested based on pH value, density, and viscosity. The results of the DES characteristics measurements can be seen in Table 1.

Table 1. Characteristics of DES

HBA	HBD	Molar ratio	pH	Density (g/ml)	Viscosity (mm^2/s)
K_2CO_3	EG	1:10	13.74	1.31	43.76

The viscosity of DES K_2CO_3-EG has a higher value than DES in another study conducted by Ratnasari and Lustiyani [16], where DES ChCl: EG (1:1) had a viscosity of 37 mm^2/s. In the study by Lim et al. [15], the viscosity of K_2CO_3:glycerol DES (1:5) was 17.61 mm^2/s. Meanwhile, DES K_2CO_3-EG (1:10) density is 1.31 g/mL. According to research conducted by V. Fischer and W. Kunz [17], the density of DES ranges between 1.1 g/mL and 2.4 g/mL. In another study by Naser et al. [18], the synthesis of DES from K_2CO_3:glycerol (1:5) resulted in a 1.40 g/ml density. The high viscosity of the DES may hinder mass movement during biomass pretreatment when compared to DES with lower viscosity, which could consequently impact fractionation efficiency.

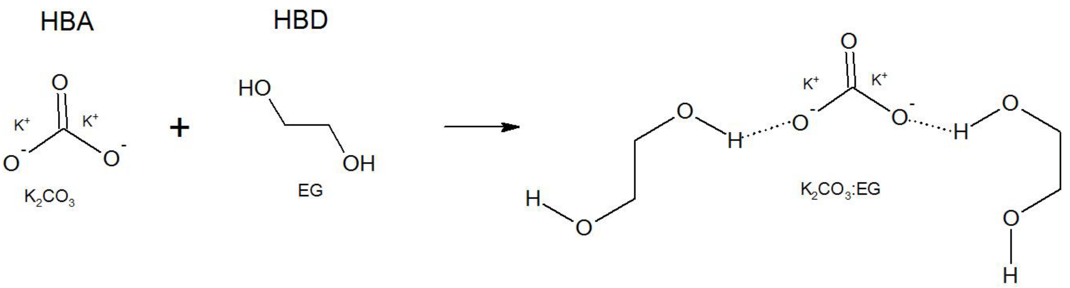

Figure 1. Interaction for the formation of K_2CO_3:EG DES

Delignification of Oil Palm Empty Fruit Bunches (EFB)

Before pretreatment, EFB undergoes a chemical characterization to identify the lignocellulosic components and other compounds. The EFB content in this study is predominantly composed of 39% lignin, followed by 32.21% cellulose. The levels of hemicellulose and ash are 8.87% and 4.99%, respectively. Various previous studies have demonstrated differing lignocellulosic content in EFB. For instance, Isroi [19] reported lignin content at 35.82%, hemicellulose at 20.05%, and cellulose at 40.37%. Septevani et al. found lignin at 38.5%, hemicellulose at 23.9%, and cellulose at 37.6%. Khairiah et al. [20] discovered that lignin was 14.7%, hemicellulose was 21.23%, and cellulose was 46.6%. The variations in the lignocellulosic content of EFB are attributed to factors such as the type or variety of oil palm plants, environmental conditions of oil palm growth, and the maturity level of oil palm plantations.

The pretreatment process uses DES at temperatures of 100°C, 120°C, and 150°C with a constant heating time of 60 minutes. After the process, the solid material from the pretreated EFB is filtered and dried to obtain the remaining biomass. The recovered EFB biomass processed with DES K_2CO_3-EG experiences a reduction of 22% to 65%, as shown in Figure 2 (a). The chemical pretreatment process with DES aims to reduce the lignin content and acetyl group compounds and partially dissolve hemicellulose [3]. The reduction in lignin, hemicellulose, and other chemical compounds results in a decrease in the produced EFB mass. Generally, EFB undergoing pretreatment will lose nearly half of its initial mass. The temperature of the pretreatment process affects the EFB recovery; the higher the process temperature, the lower the EFB mass recovery. This is due to the increased solubility of lignin in the black liquor.

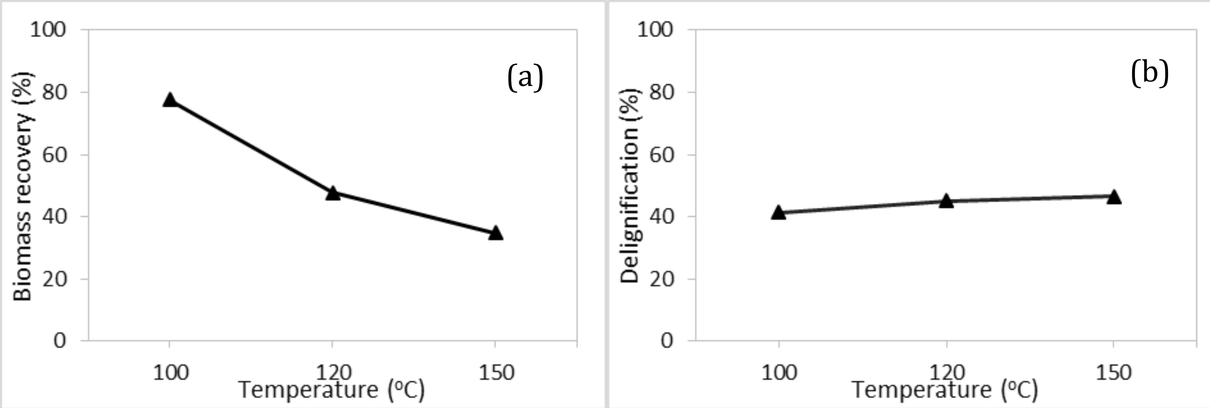

Figure 2. Biomass (a) recovery and (b) delignification after 60 minute pretreatment

Figure 2 (b) illustrates the delignification graph of EFB, indicating that the higher the process temperature, the greater the delignification of EFB. The highest delignification is achieved with DES K_2CO_3-EG at 150°C, reaching 46%. The increased delignification of EFB by DES is due to the higher temperature leading to more degraded lignin and other components, such as hemicellulose or cellulose, which also dissolve. The complex bonds within lignocellulose were broken due to the hydrogen bond interactions between DES and hydroxyl groups [21]. Another factor influencing lignin degradation is the pH of the DES used. This aligns with the study by Suopajärvi et al. (2020) [12] on delignification using K_2CO_3:glycerol-based DES, which is alkaline, on wheat straw and corn stover samples at 100°C for 16 hours. The results obtained from the wheat straw sample showed 45% lignin degradation, while the corn stover sample had 32% lignin degradation. The findings from our prior study employing oxalic acid and ethylene glycol (OAEG) as DES resulted in a delignification rate ranging from 30% to 44% [22]. The utilization of K_2CO_3-EG yielded slightly greater outcomes compared to OAEG. This phenomenon can be attributed to the fact that potassium carbonate dissolves lignin [23]. This demonstrates that the delignification process is more effective in an alkaline-based DES than in an acidic one. However, the delignification results achieved are still lower when compared to using a NaOH base, which can reach up to 76.74% [3]. This difference is due to the high solubility of lignin in NaOH. Nevertheless, the use of NaOH not only reduces lignin content but also impacts the reduction of hemicellulose content.

The degradation reaction of lignin with DES solution involves several stages depending on the type of DES used and reaction conditions. However, the lignin degradation process with DES generally comprises three main stages. The first stage is the dissolution of lignin in the DES solution. The DES solution contains compounds capable of forming hydrogen bonds with lignin and dissolving it in the solution. This occurs because DES possesses polar and non-polar properties that enable it to dissolve organic compounds like lignin. The second stage is the cleavage of lignin bonds. This involves chemical reactions between the DES solution and lignin that help break down the chemical bonds within lignin. This reaction depends on reaction conditions such as temperature, pH, and reaction time. One common reaction that occurs is deacetylation, resulting in phenolate compounds. The final stage is the separation of degradation products from the DES solution. The degradation products are separated from the DES solution using filtration techniques [24].

Table 2 shows the chemical composition of EFB before and after the pretreatment process. EFB processed with K_2CO_3:EG experienced an increase in cellulose content by 29%, from 31% to 60%. The rise in cellulose corresponds with a decrease in lignin content after the pretreatment process, as shown in Figure 2 (b). The reduction in lignin content leads to an increase in cellulose content. This indicates that DES K_2CO_3:EG is selective in dissolving lignin and does not dissolve cellulose. Lim et al. (2019) [15] researched the pretreatment process of wheat straw using K_2CO_3:glycerol, which could enhance cellulose content by 39%. The ash content in all EFB pretreatments decreased due to the leaching of minerals from the EFB during the pretreatment and washing processes. Interestingly, the decrease in ash content did not exhibit a clear trend with increasing pretreatment temperature. Surprisingly, there is a lack of studies discussing the effect of pretreatment temperature on reducing ash content in EFB. Nevertheless, it has been demonstrated that increasing the volume of washing fluid and extending the washing time lead to a greater reduction in ash content in EFB [25].

Table 2. Chemical Composition of EFB after Pre-Treatment

DES	t (min)	T (°C)	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Ash (%)
Raw EFB			39.13	31.21	8.87	5.07
K_2CO_3-EG (1:10)	60	100	22.95	58.44	7.06	1.66
	60	120	21.48	59.70	5.22	3.03
	60	150	21.11	60.21	4.08	2.48

Figure 3 shows the surface morphology of EFB before and after pretreatment. In untreated EFB, the surface tends to be rugged, solid, and surrounded by wax and silica. Based on research by Santi et al. (2019) [26], the silica (SiO_2)

content in fresh EFB reaches 11.03%, is spread over every surface, and binds between fibers. However, after pretreatment using DES K_2CO_3:EG, the EFB surface becomes porous, indicating reduced silica and wax content. Additionally, the structure of the EFB appears more fibrous and brittle. This suggests that DES K_2CO_3:EG can reduce the lignin content and change the morphology of EFB.

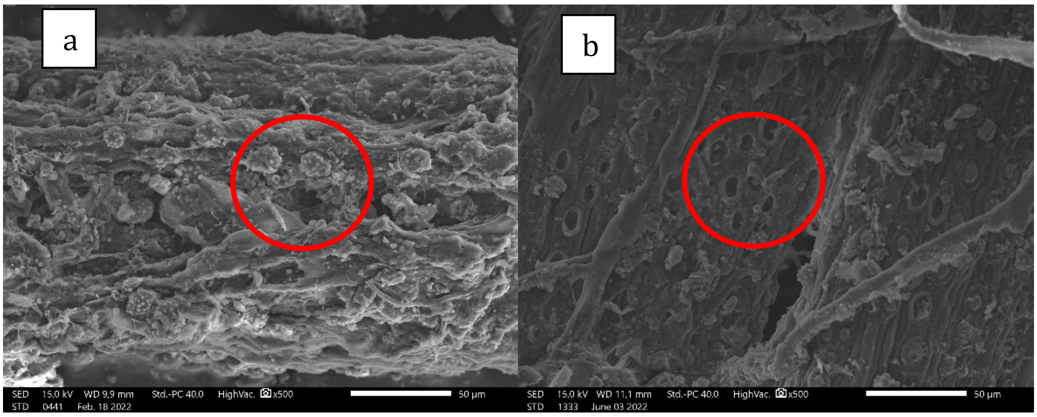

Figure 3. SEM image of OPEFB (a) before and (b) after pretreatment

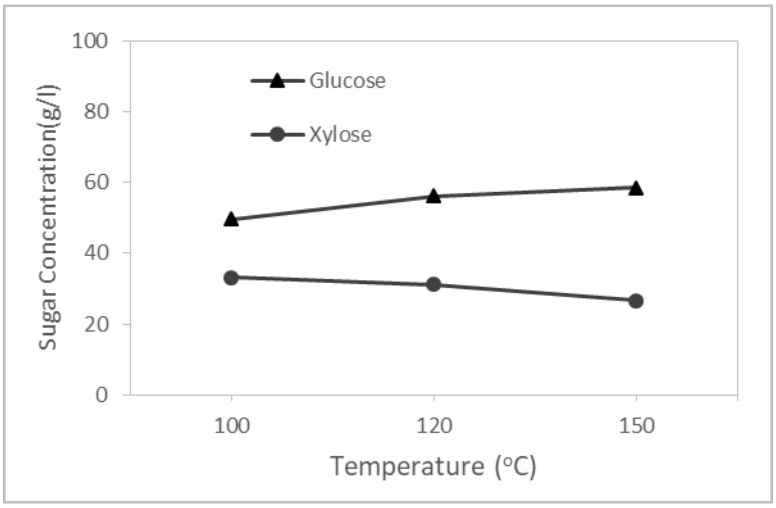

Figure 4. Sugar content from saccharification

Enzymatic Hydrolysis of Pretreated EFB

Figure 4 illustrates the enzymatic hydrolysis of pretreated EFB at different temperatures. The increase in cellulose content following pretreatment using different DES compositions and pretreatment temperatures is visible. This phenomenon is attributed to the rise in temperature, which aids in removing lignin and hemicellulose, subsequently improving enzyme accessibility and expediting cellulose hydrolysis [27]. Figure 4 illustrates the glucose content from saccharification using C-Tech and H-Tech enzymes over 72 hours on pretreated EFB. Increasing the temperature during the pretreatment process leads to a higher glucose yield in the saccharification process. Consequently, all samples pretreated at 150°C attained the highest glucose content. Pretreated EFB samples using DES K_2CO_3:EG yielded a glucose content of 58.48 g/l and a xylose content of 26.60 g/l. In a study by Atikah et al. [9], pretreatment of EFB using DES ChCl-Urea resulted in optimal glucose content at 110°C for 1 hour, reaching 66.33 mg/mL. Enzymatic activity is influenced by the presence of hemicellulose and lignin, where the pretreatment using DES aims to reduce hemicellulose and lignin to optimize enzyme performance. This glucose and xylose content holds potential for bioethanol and furfural production. Glucose can be converted into bioethanol through fermentation using *Saccharomyces cerevisiae.* Stoichiometrically, each 1 mol of glucose yields 0.51 mol of ethanol. Meanwhile, acidic solvents can transform xylose into furfural via dehydration reactions. Aside from mineral acids like H_2SO_4 and HCl, DES can serve as a solvent and catalyst in furfural formations [28].

CONCLUSION

The synthesis of DES using a K_2CO_3:EG mixture was successfully achieved at a molar ratio of 1:10. The DES K_2CO_3-EG has a pH value of 13.74, density of 1.31 g/mL, and viscosity of 43.76 mm^2/s. Pretreatment of EFB using

K_2CO_3:EG DES resulted in lignin delignification of 46.06% with a biomass yield of 34.88% at a temperature of 150°C for 60 minutes. The cellulose content increased to 60.21%. Enzymatic hydrolysis using cellulase enzyme produced a glucose content of 58.40 g/l and a xylose content of g/l. DES K_2CO_3-EG holds potential as a solvent in the EFB pretreatment process to produce pulp for ethanol and furfural production.

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