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Oil Palm Empty Fruit Bunch Waste Pretreatment with Benzotriazolium-Based Ionic Liquids for Cellulose Conversion to Glucose: Experiments with Computational Bibliometric Analysis

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ABSTRACT

This study aims to utilize benzotriazolium salt-ionic liquids (ILs) as solvents in the oil palm empty fruit bunch (EFB) waste pretreatment along with bibliometric analysis using VOSviewer. Three ILs have been synthesized and tested as EFB solvents by a microwave-heating method. Those are organic salts of 1,3-methyl-octyl-1,2,3-benzotriazolium ([MOBzt]$^+$) cation with three kinds of anions such as bromide ([Br]$^-$), acetate ([CH_3COO]$^-$), and thiocyanate ([SCN]$^-$). The bibliometric analysis showed that new research needs to be conducted to improve the development of research relating to biomass pretreatment. The highest solubility of EFB is in [MOBzt]CH_3COO is about 7,5% w/w. The effect of anions on the ability to dissolve EFB is CH_3COO^->SCN^->Br^-. When subject to ILs pretreatment, EFB exhibited increased cellulose crystallinity, changed in the structure of cellulose I to cellulose II, reduced particle size, and decreased lignin content compared to untreated one, improving the glucose yield from enzymatic hydrolysis. The highest glucose yield (1,237 mg/mL) was obtained when the EFB was pretreated by [MOBzt]CH_3COO with enzymatic hydrolysis for 24 hours. This research is expected to contribute to the development of new biomass pretreatment methods.

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1. INTRODUCTION

The diminishing fossil fuel resources and increasing global warming due to greenhouse gas emissions make biomass efficiency increasingly important (Antar *et al.*, 2021). There is a lot of available biomass that can be converted into renewable-based biofuels. Thus, most of the fossil fuels currently consumed in the transportation sector can be replaced (Kaltschmitt, 2019; Oke *et al.*, 2022). Biomass is a cheap, renewable, abundantly available resource. Biomass and agro-waste are prospective major global renewable energy sources.

In Indonesia, oil palm is the main part to support the food industry to produce edible oil (Khatiwada *et al.*, 2021; Qaim *et al.*, 2020). Besides that, oil palm empty fruit bunch (EFB) is a byproduct of the palm oil mill (Yoo *et al.*, 2019). As with other biomass, EFB consists of three main components, namely cellulose, hemicellulose, and lignin (Shamsudin *et al.*, 2022). EFB is composed of 40-50% cellulose and about 20-30% of hemicellulose and 15-20% of lignin (Teow *et al.*, 2020). EFB is the largest organic waste generated by oil palm plantations and has the potential to be used as a substitute biofuel for fossil fuels (Dolah *et al.*, 2021).

In general, to be able to convert lignocellulosic materials into biofuels, three steps usually need to be carried out, namely (1) pre-treatment of lignocellulose so that the digestibility of enzymes or microbial components of polysaccharides increases; (2) cellulose and hemicellulose hydrolysis so fermentable reducing sugars can be obtained; and (3) fermenting sugar into liquid fuel or other fermentation products (Baig *et al.*, 2019; Hoang *et al.*, 2021; Machineni, 2020; Mahmood *et al.*, 2019; Zhou *et al.*, 2021). The current pretreatment method used for decrystallization of cellulose is considered incapable and ineffective due to the loss of sugar so that sugar from biomass becomes expensive. Several factors that influence the effectiveness of the biomass pretreatment method include the crystallinity of cellulose, the presence of lignin, and covalent cross-links between lignin and hemicellulose in plant cell walls. (Albornoz-Palma *et al.*, 2020; Beig *et al.*, 2021; Mankar *et al.*, 2021). These factors affect the enzymatic hydrolysis of polysaccharides into sugars. Several strategies can be done to make the pretreatment efficient, namely (1) the lignin and hemicellulose cross-linked matrix that attaches to the cellulose fiber needs to be removed or disrupted, (2) hydrogen bonds in crystalline cellulose also need to be disturbed, and (3) surface area and porosity of cellulose need to be increased for further extraction and hydrolysis (Xia *et al.*, 2020). Therefore, the development of pretreatment methods, selection of operating conditions, and design of pretreatment equipment is usually an empirically trial-and-error process (Yiin *et al.*, 2021).

Dilute acids such as HCl are commonly used in pretreatment methods of the paper and pulp industry (Chen *et al.*, 2019), and the others are H_2SO_4 (Yuan *et al.*, 2022), hot water (Chen *et al.*, 2022; Wells *et al.*, 2020), lime (Yuan *et al.*, 2022), ammonia fiber expansion (Zhao *et al.*, 2020), and organic solvents (Halder *et al.*, 2019). Nowadays, the conversion of cellulose to water-soluble sugars using dilute acids is also attracting attention, but this can lead to waste originating from polysaccharide degradation products so that downstream fermentation organisms are often hampered as well as decreased overall sugar yield (Liu *et al.*, 2020). Furthermore, after dilute acid pretreatment, condensed lignin remains on the surface of crystalline cellulose, potentially preventing enzyme access to the substrate for sugar production (Huang *et al.*, 2022).

Ionic liquids (ILs), a new type of non-volatile solvent, have recently been used as non-derivatizing media in lignin dissolution (Dias *et al.*, 2020). At low temperatures (100 °C), ILs are liquids and ILs are organic salts. ILs

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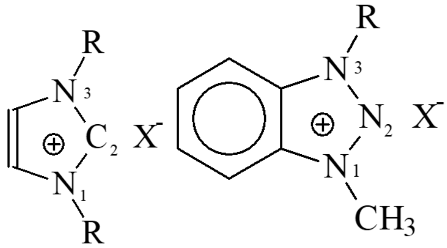
are very effective at crystalline cellulose solubilization (Salama & Hesemann, 2020; Sayyed *et al.*, 2019) and increasing subsequent saccharification rates (Chuetor *et al.*, 2022). The cellulose regenerated from ILs was discovered to be essentially amorphous and porous, making it much more susceptible to degradation by cellulases (Ben Hmad & Gargouri, 2020; Krugly *et al.*, 2022; Tu *et al.*, 2020). Overall, this process is simpler to operate, requires less energy, and is more environmentally friendly than existing commercial dissolution processes such as the viscose method (Adu *et al.*, 2021). ILs are almost entirely recovered after use in the process and can be reused for biomass dissolution. Research related to the use of ILs in the process of dissolution of biomass is still focused on the imidazolium salts as shown in **Figure 1**.

1,3-alkylmethyl benzotriazolium salt (**Figure 1** (2)) has a structure similar to the class of N, N-dialkyl-imidazolium (**Figure 1** (1)). In addition to the type of atom at position 2, the difference lies in the presence of two clusters of benzene on benzotriazolium structure that would expand the positive charge delocalization cation so that it will lead to the weakening of the cation-anion Coulomb interaction. Cation-anion Coulomb interaction in compound (**Figure 1** (2)) is weak. It is expected to lead to a more powerful and efficient the compound (**Figure 1** (2)) to dissolve the biomass. The object of this study is to investigate the performance of pretreatment methods in degrading EFB lignocellulose structure with ILs based on benzotriazolium salt as solvents in the pretreatment step, effect of anions such as bromide ($[Br]^-$), acetate ($[CH_3COO]^-$), and thiocyanate ($[SCN]^-$on the ability to dissolve EFB, to understand the underlying mechanism(s) of dissolution. The recovered product was characterized to determine the glucose levels of EFB obtained from the ILS pretreatment technique using a spectrophotometer. Physical properties including cellulose crystallinity were measured using X-Ray Diffraction (XRD); lignin structure, lignin content and morphology of EFB were also characterized and compared using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

To ensure this research is important, we also added bibliometric analysis. The analysis is important because it is to see the extent to which research developments are related to the topic being studied so that the topic in this research is worthy of research. Many reports regarding bibliometric have been reported, including bibliometric analysis in nanocrystalline cellulose production research as drug delivery system (Fauziah and Nandiyanto, 2022), nanocrystalline cellulose synthesis for packaging application research (Maulidah and Nandiyanto, 2021), techno-economic education (Ragadhita and Nandiyanto, 2022), and magnetite nanoparticle production research (Nugraha and Nandiyanto, 2022). This bibliometric is important for understanding the position of the research being carried out in the midst of the development of the relevant research topic.



1 2

Figure 1. (1) Imidazolium salt structure and (2) benzotriazolium salt structure.

2. METHODS

2.1. Materials

Oil palm empty fruit bunch (EFB) with particle size about 0.1–2 mm were thoroughly washed with water, then heated in a oven for 24 h at 50 °C prior to use, 1H-Benzotriazole p.a, dimethyl sulfate p.a, octyl bromide p.a, sodium hydroxide p.a, ethyl acetate, hexane, sodium acetate were purchased from Aldrich, Fluka, Merck and Bratachem. Cellulase enzymes were purchased from Sigma–Aldrich.

2.2. Synthesis of Ionic Liquids

[MOBzt]Br, [MOBzt]SCN, and [MOBzt]CH3COO was synthesized according to the method conducted by Forsyth *et al* (2003) with some modifications (Forsyth & MacFarlane, 2003).

2.3. Dissolution of Wood in Ionic Liquid

The ILs were put into a porcelain crucible. EFB sample with a particle size of about 0.1–2 mm was added to the ILs rapidly. The mass of the EFB sample is 1% of the mass of the ILs. After that, the mixture is immediately transferred to a microwave reactor with a certain power until the EFB dissolved.

2.4. EFB Regeneration

The previously prepared EFB solution was gradually added to the methanol, then stirred rapidly. The precipitate that appears was then filtered using a Buchner funnel and washed thoroughly with methanol. Next, sun drying was carried out on the sample to determine its solid content.

2.5. Enzymatic Hydrolysis

The residue was then treated according to the method developed by Lee *et al*. (2008) (Lee *et al*., 2009). The enzymatic hydrolysis reaction catalyzed by cellulase was carried out in a 20 mL bottle in a water bath shaker at a speed of 200 rpm and a temperature of 37°C. This reaction was carried out in 3.5 mL and the cellulase concentration was 34 U/mL in 50 mM citrate buffer which had a pH of 4.7. To quell the enzymatic reaction, a 50 mL sample was removed periodically and boiled for 3 minutes.

2.6. Glucose Determination

The glucose analysis of hydrolysis samples was determined using a Somogyi-Nelson method (Pramanik *et al.*, 2021). Measurement of glucose concentration was conducted by spectrophotometer. All reactions were carried out in duplicate.

2.7. Biomass Spectroscopy

EFB needs to be analyzed before pretreatment using ILs and after regenerating cellulose using methanol which acts as an anti-solvent to carry out pretreatment biomass studies using ILs. Cellulose or regenerated cellulose samples were made into clear KBr pellets before being tested using the FTIR spectrum (Shimadzu). The JEOL JSM-6360LA microscope was used to obtain SEM images of untreated and pre-treated EFB solids (dry powder). EFB samples were also tested using XRD (Panalytical) in which the cellulose crystallinity index (CrI) was determined via Eq. (1) below.

$$CrI\ (\%) = \left[\frac{I_{002} - I_{18.0^\circ}}{I_{002}}\right] x\ 100 \qquad (1)$$

CrI is the crystalline index, $I_{18.0°}$ is the intensity diffraction at 18.0° 2ϑ degrees, and I_{002} is the maximum intensity of the (002) lattice diffraction. Detailed information on how to interpret XRD, SEM, and FTIR is explained elsewhere (Fatimah *et al.*, 2022; Yolanda and Nandiyanto, 2022; Nandiyanto *et al*., 2019).

2.8. Bibliometric Analysis

The bibliometric analysis was carried out using VOSviewer with the help of Publish and Perish reference manager application for article data searching in Google Scholar-indexed journals. The keywords that were used for article data searching were biomass pretreatment, cellulose, ILs, and oil palm

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empty fruit bunch waste. The search for articles is only limited to 2012-2022 (the last 10 years), then the data for these articles is mapped using VOSviewer to visualize and develop a trend map between the items. The frequency of occurrence of the keyword is set to a minimum of 10 times so that 36 terms were obtained from 314 articles.

3. RESULTS AND DISCUSSION

3.1. Bibliometric Analysis

3.1.1. Biomass pretreatment publication data

314 articles that met the criteria were obtained from the search results using the Publish or Perish reference manager application. The articles were obtained in the form of metadata consisting of the number of citations, rank, authors, title, year, journal name, publisher, and links. The 20 most relevant articles with the highest number of citations were taken as sample data as shown in **Table 1**. The total number of citations owned by these articles is 13,177 with the number of citations per year being 1317.70, while the number of citations per article is 41.70. The number of authors per article is 3.71. The total h-index of all articles is 53 and the g-index is 107.

Table 1. Biomass pretreatment publication data.

No.	Authors	Title	Year	Cites	Ref.
1.	Z Anwar *et al.*	Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review	2014	847	(Anwar *et al.*, 2014)
2.	HV Lee *et al.*	Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process	2014	601	(Lee *et al.*, 2014)
3.	EC Bensah and M Mensah	Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations	2013	322	(Bensah and Mensah, 2013)
4.	AA Elgharbawy *et al.*	Ionic liquid pretreatment as emerging approaches for enhanced enzymatic hydrolysis of lignocellulosic biomass	2016	275	(Elgharbawy *et al.*, 2016)
5.	RK Mishra *et al.*	Materials chemistry and the futurist eco-friendly applications of nanocellulose: Status and prospect	2018	224	(RK Mishra *et al.*, 2018)
6.	Y Lu *et al.*	Structural characterization of lignin and its degradation products with spectroscopic methods	2017	203	(Lu *et al.*, 2017)
7.	G Salehi Jouzani and MJ Taherzadeh	Advances in consolidated bioprocessing systems for bioethanol and butanol production from biomass: a comprehensive review	2015	202	(Jouzani and Taherzadeh, 2015)
8.	H Xie *et al.*	Recent strategies in preparation of cellulose nanocrystals and cellulose nanofibrils derived from raw cellulose materials	2018	199	(Xie *et al.*, 2018)
9.	SS Chen *et al.*	Valorization of biomass to hydroxymethylfurfural, levulinic acid, and fatty acid methyl ester by heterogeneous catalysts	2017	178	(Chen *et al.*, 2017)

Table 1. **(Continue).** Biomass pretreatment publication data.

No.	Authors	Title	Year	Cites	Ref.
10.	YL Loow *et al.*	Recent advances in the application of inorganic salt pretreatment for transforming lignocellulosic biomass into reducing sugars	2015	145	(Loow *et al.*, 2015)
11.	RA Sheldon	Biocatalysis and biomass conversion in alternative reaction media	2016	134	(Sheldon, 2016)
12.	WY Cheah *et al.*	Pretreatment methods for lignocellulosic biofuels production: current advances, challenges and future prospects	2020	133	(Cheah *et al.*, 2020)
13.	JJ Liao *et al.*,	Current advancement on the isolation, characterization and application of lignin	2020	126	(Liao *et al.*, 2020)
14.	HT Tan and KT Lee	Understanding the impact of ionic liquid pretreatment on biomass and enzymatic hydrolysis	2012	121	(Tan and Lee, 2012)
15.	Yunpu *et al.*	Review of microwave-assisted lignin conversion for renewable fuels and chemicals	2016	115	(Yunpu *et al.*, 2016)
16.	LAZ Torres *et al.*	Lignin as a potential source of high-added value compounds: A review	2020	112	(Torres *et al.*, 2020)
17.	M Jablonský *et al.*	Extraction of value-added components from food industry based and agro-forest biowastes by deep eutectic solvents	2018	101	(Jablonský *et al.*, 2018)
18.	YH Chan *et al.*	Bio-oil production from oil palm biomass via subcritical and supercritical hydrothermal liquefaction	2014	100	(Chan *et al.*, 2014)
19.	D Tian *et al.*	Acidic deep eutectic solvents pretreatment for selective lignocellulosic biomass fractionation with enhanced cellulose reactivity	2020	86	(Tian *et al.*, 2020)
20.	AK Rana *et al.*	Cellulose nanocrystals: Pretreatments, preparation strategies, and surface functionalization	2021	86	(Rana *et al.*, 2021)

3.1.2. Biomass pretreatment research development

The number of research related to biomass pretreatment published in journals indexed by Google Scholar was 314 articles in 2012-2022 with an average of about 28 articles per year, as shown in **Table 2**. From year to year, the number of articles experienced fluctuating conditions with the following details: 4 articles (2012); 6 articles (2013); 7 articles (2014); 12 articles (2015); 27 articles (2016); 19 articles (2017); 33 articles (2018); 28 articles (2019); 46 articles (2020); 87 articles (2021); and 45 articles (2022).

Based on the level of research development depicted in **Figure 2**, research related to biomass pretreatment experienced a drastic decline in 2022 compared to the previous year, so interest in this research is declining.

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Table 2. Biomass pretreatment research development.

Year	Number of Publications
2012	4
2013	6
2014	7
2015	12
2016	27
2017	19
2018	33
2019	28
2020	46
2021	87
2022	45
Total	**314**
Average	**28,54545**

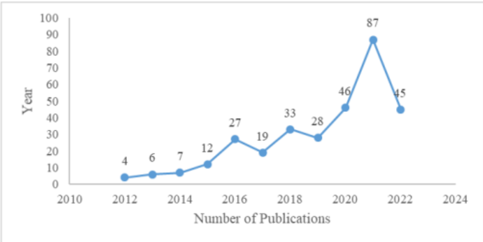

Figure 2. Level of biomass pretreatment research development.

3.1.2. Biomass Pretreatment Visualization using VOSviewer

Computational mapping of article data is done using VOSviewer. Based on the results of the computational mapping, 36 items were found and divided into 5 clusters as follows.

(i) Cluster 1 has 9 items marked by red circles and consists of acid, deep eutectic solvent, extraction, ionic liquid, oil palm empty fruit bunch, OPEFB, pre-treatment, use, and waste biomass.
(ii) Cluster 2 has 9 items marked by green circles and consists of an aqueous solution, biomass, cellulose, hemicellulose, lignin, overview, removal, study, and waste.
(iii) Cluster 3 has 7 items marked by blue circles and consists of effect, lignocellulosic biomass, lignocellulosic waste, pretreatment, production, recent advance, and review.
(iv) Cluster 4 has 6 items marked by yellow circles and consists of biomass waste, EFB, empty fruit bunch, oil palm, oil palm biomass, and oil palm frond.
(v) Cluster 5 has 5 items marked by purple circles and consists of agricultural waste, application, characterization, nanocellulose, and preparation.

Each term is labeled by a colored circle and the relationship between the terms is indicated by its cluster. The frequency of occurrence of each term in the title and abstract of the article will affect the size of the label circle where the more often the term appears, the larger the size of the label circle. **Figures 3, 4,** and **5** shows a visualization mapping analysis. **Figure 3** shows a network visualization that illustrates the relationship between terms in an interconnected network. This visualization shows the clusters of each term that are often studied when it comes to the topic of biomass pretreatment. From this visualization also, the biomass pretreatment research topics related to this study can be divided into 4 terms. First, there is the biomass term (**Figure 6**) which is included in cluster 2. The frequency of occurrence of this biomass term is 127 with a total of 35 links and a total link strength of 561. Next, there is the cellulose term (**Figure 7**) which also includes cluster 2 with the frequency of occurrence of as many as 142 and the total links are also 35, but the total link strength is 620. The ILs term (**Figure 8**) which belongs to cluster 1 has a frequency of occurrence of 124 with a total of 33 links and a total link strength of 529, while the oil palm empty fruit bunch term (**Figure 9**) which is also included in cluster 1 has a frequency of occurrence of 72 with a total of 35 links and a total link strength of 319.

Figure 4 shows the visualization of the overlay between the terms which indicates the novelty of the research. From the visualization of the overlay, this pretreatment biomass research is most often carried out from mid-2019 to mid-2020. Thus, the level of popularity of this topic has passed and has recently decreased, so new research on the topic of biomass research is urgently needed. The density visualization in **Figure 5** shows the frequency level of occurrence of a term where the lighter the yellow color and the size of the term label, the more often that term appears. From **Figure 5**, terms such as biomass, cellulose, waste, ILs, pretreatment, and oil palm empty fruit bunch are some terms that often appear, which means that many studies related to these terms have been carried out.

The relationship between the biomass term and other terms is shown in **Figure 6**. Based on the network visualization, the biomass term is related to cellulose, waste, empty fruit bunch, pretreatment, EFB, study, oil palm frond, review, overview, aqueous solution, removal, agricultural waste, biomass waste, hemicellulose, nanocellulose, preparation, application, lignin, oil palm, characterization, deep eutectic solvent, oil palm empty fruit bunch, extraction, ILs, acid, lignocellulosic biomass, production, recent advance, lignocellulosic waste, effect, use, waste biomass, oil palm biomass, and OPEFB.

Cellulose term (**Figure 7**) relates to the biomass, waste, empty fruit bunch, pretreatment, EFB, study, oil palm frond, review, overview, aqueous solution, removal, agricultural waste, biomass waste, hemicellulose, nanocellulose, preparation, application, lignin, oil palm, characterization, deep eutectic solvent, oil palm empty fruit bunch, extraction, ILs, acid, lignocellulosic biomass, production, recent advance, lignocellulosic waste, effect, use, waste biomass, oil palm biomass, and OPEFB terms.

ILs term (**Figure 8**) relates to the biomass, waste, empty fruit bunch, pretreatment, EFB, study, oil palm frond, review, overview, agricultural waste, biomass waste, hemicellulose, nanocellulose, preparation, application, lignin, oil palm, characterization, deep eutectic solvent, oil palm empty fruit bunch, extraction, acid, lignocellulosic biomass, production, recent advance, lignocellulosic waste, effect, use, waste biomass, oil palm biomass, and OPEFB terms.

Last is oil palm empty fruit bunch term (**Figure 9**) relates to the biomass, waste, cellulose, empty fruit bunch, pretreatment, EFB, study, oil palm frond, review, overview, agricultural waste, biomass waste, hemicellulose, nanocellulose, preparation, application, aqueous solution, removal, lignin, oil palm, characterization, deep eutectic solvent, oil palm empty fruit bunch, extraction, acid, lignocellulosic biomass, production, recent advance, lignocellulosic waste, effect, use, waste biomass, oil palm, and OPEFB terms. Based on those findings, the four terms determined tend to be related to each other and have high relevance to various terms so this will have a major impact on the novelty of the research to be carried out.

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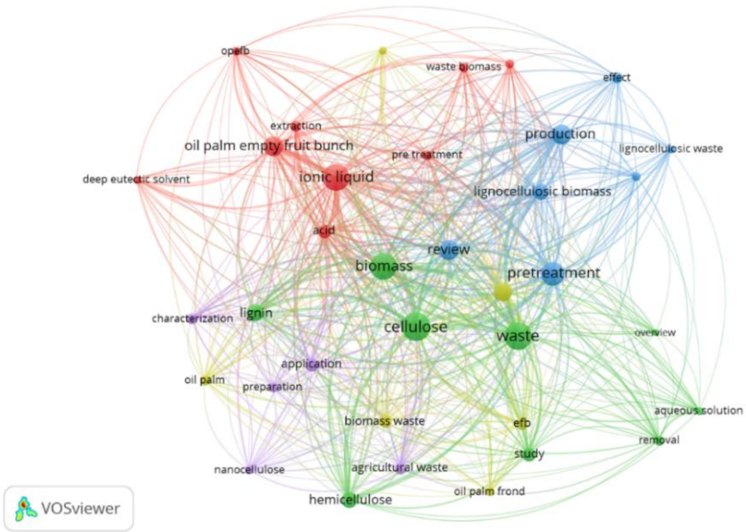

Figure 3. Network visualization of biomass pretreatment research.

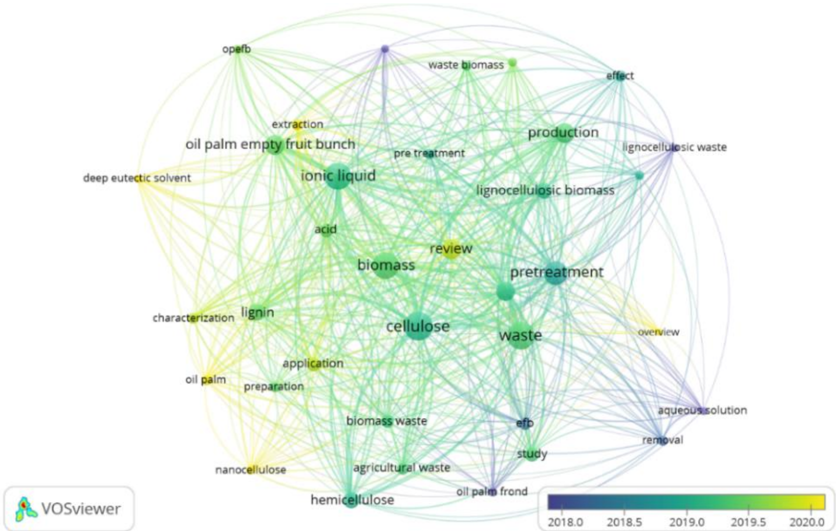

Figure 4. Overlay visualization of biomass pretreatment research.

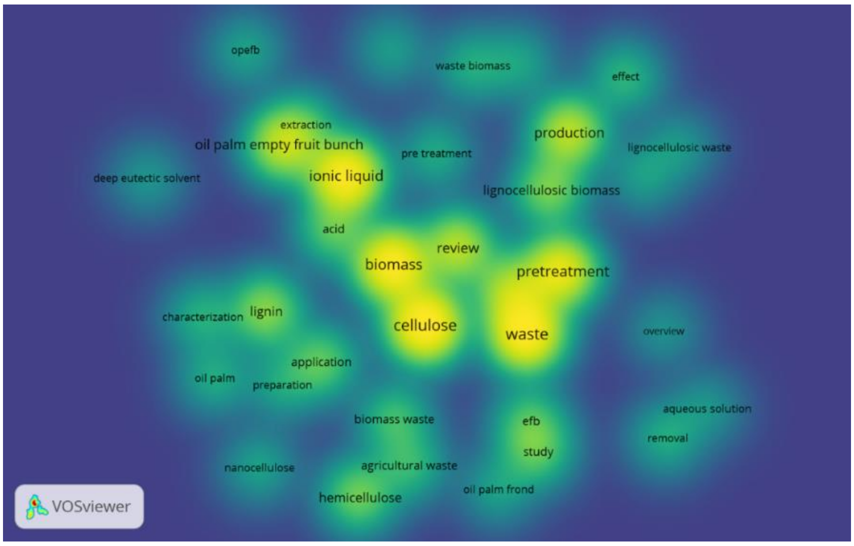

Figure 5. Density visualization of biomass pretreatment research.

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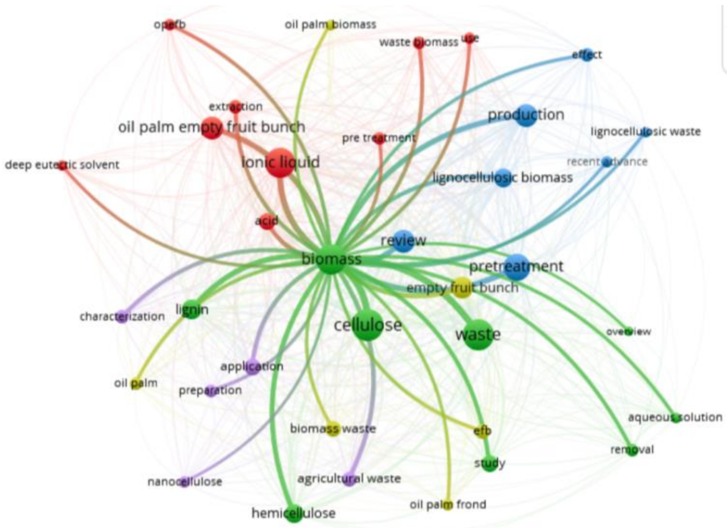

Figure 6. Network visualization of biomass term.

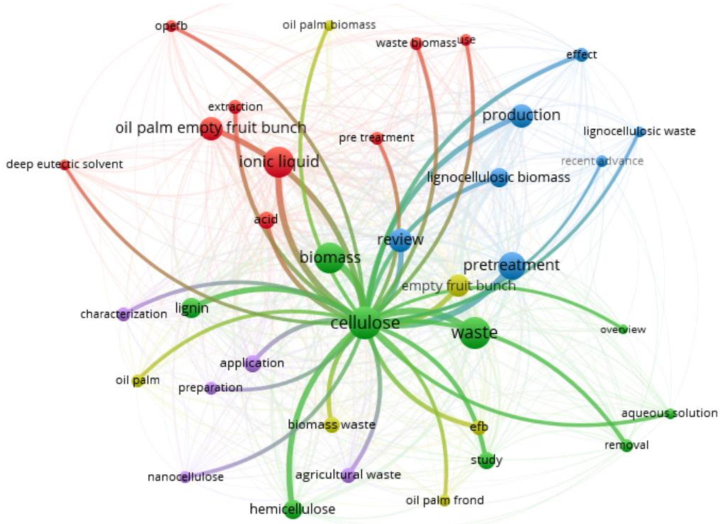

Figure 7. Network visualization of cellulose term.

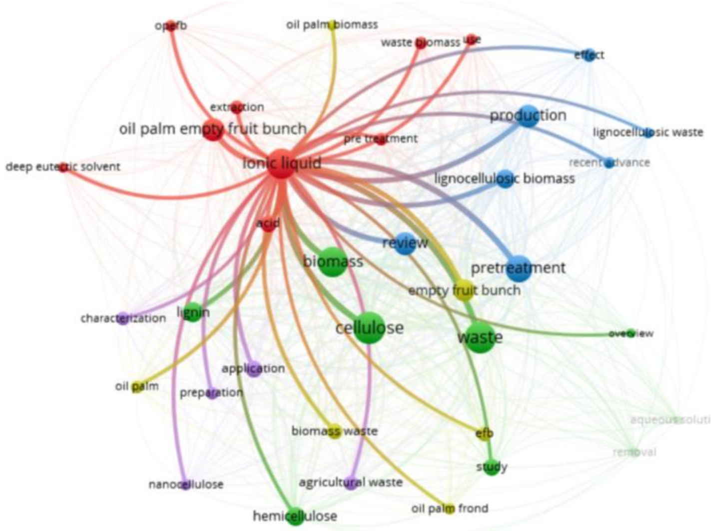

Figure 8. Network visualization of ILs term.

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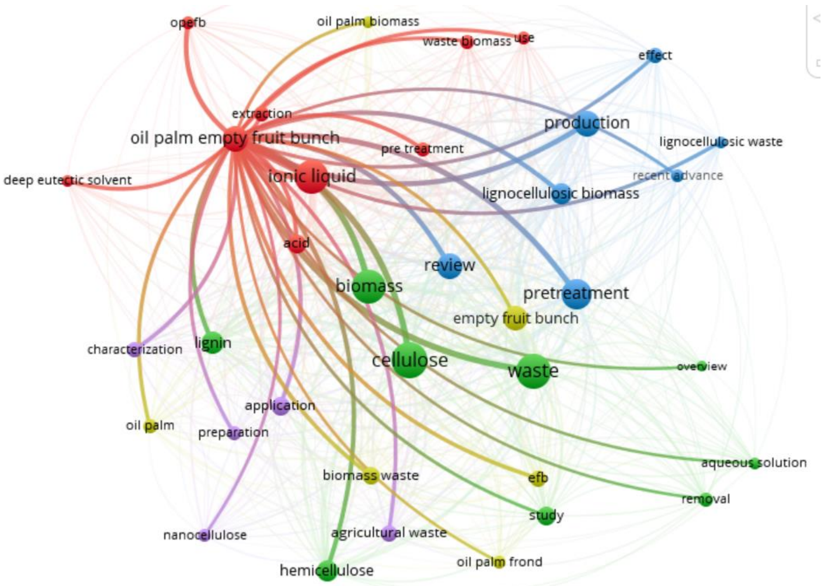

Figure 9. Network visualization of oil palm empty fruit bunch term.

3.2. Dissolution of EFB into Ionic Liquids

Separation of the ILs solvent mixture from EFB can be done by centrifugation because the EFB has been dissolving in ILs and precipitated with anti-solvent. The ILs and anti-solvents are also recoverable and easily separated so that they can be recycled. The solubilities of EFB in ILs are shown in **Table 3**. The solubilization efficiency of the EFB in ILs was found to be of the order [MOBzt] CH_3COO > [MOBzt]SCN >[MOBzt]Br. The highest EFB solubility was obtained using [MOBzt] CH_3COO.

The dissolution mechanism of EFB in benzotriazolium salt-based ILs can be caused by the large nature of the benzotriazolium cation, in addition, the electronegativity and basicity of the anion are also relatively strong. The dissolving capacity of EFB in benzotriazolium-based cations also depends on the associated anion. The effect of anions on the ability to dissolve EFB is CH3COO->SCN->Br-. EFB soluble in ILs may occur due to the strong hydrogen bond basicity of the acetate anion. The anion acts as a hydrogen bond acceptor that will interact with the cellulose hydroxyl groups. The dissolution rate of EFB in ILs is influenced by the particle size of the EFB sample. This is due to the complex and compact structure of the EFB cell wall. In addition, lignin, cellulose, and hemicellulose will inhibit the diffusion of ILs into the molecule.

3.2. XRD of Raw EFB and EFB Treated with [MOBzt]Br

XRD was used to determine the structural changes of EFB after treatment using ILs. Based on the XRD pattern, there was an increase in crystallinity for samples treated with ILs when compared to samples not treated with ILs. The intensity of the diffraction peak decreases at $2\theta=21°$. In **Figure 10**, the crystallinity of cellulose in EFB was reduced due to the ILs dissolving regeneration treatment.

Table 3. Dissolution behavior EFB in different benzotriazolium-based ILs.

Entry	ILs	Solubility (% wt)
1	[MOBzt] Br	6,92 %
2	[MOBzt] SCN	6,98 %
3	[MOBzt] CH_3COO	7,50 %

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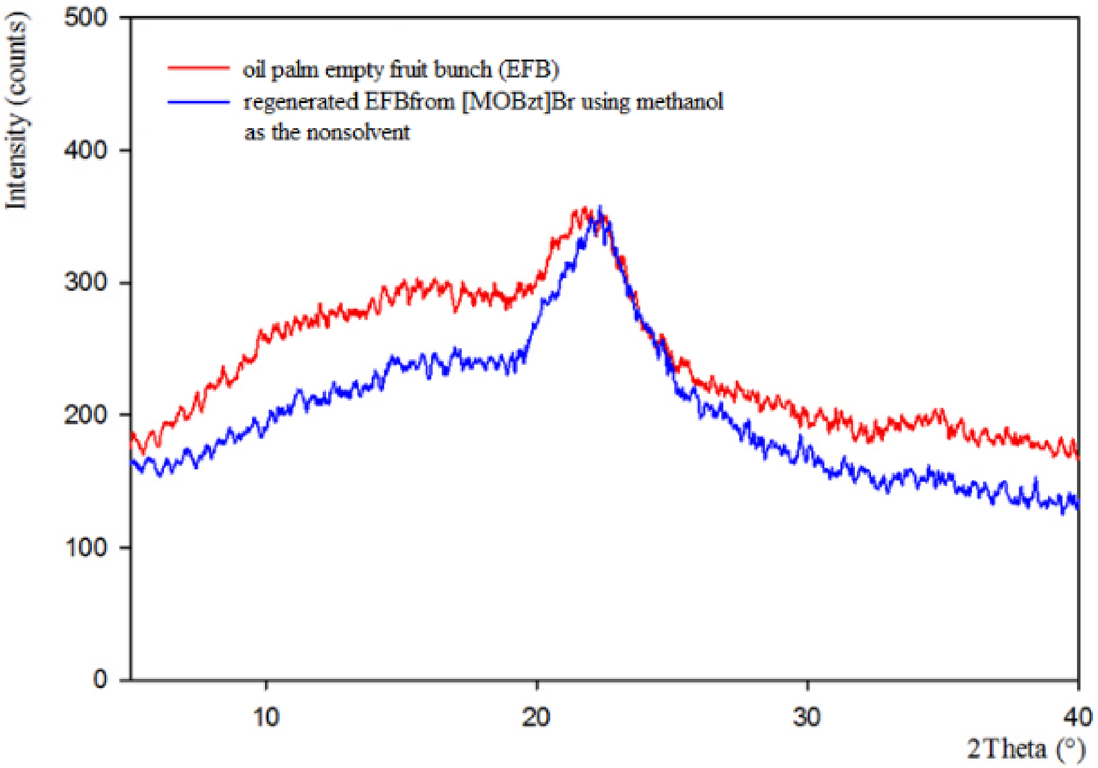

Figure 10. X-ray spectra of (a) oil palm empty fruit bunch (EFB) and (b) regenerated EFB from [MOBzt]Br using methanol as the nonsolvent.

The crystallinity index (CrI) was also found to decrease substantially for all samples treated with ILs. This crystallinity can be interpreted as the ratio of the amount of crystalline cellulose to the total amount of sample material. These include crystalline and amorphous cellulose (Chen *et al.*, 2020). The crystallinity of cellulose was also found to increase from 15.9% (without ILs treatment) to 28.6% (with ILs treatment).

Highly crystalline cellulose has also long been known to be more difficult to access by cellulases than amorphous cellulose. This increased crystallinity of cellulose indicates that the recovered product is highly crystalline. Unfortunately, the increase in cellulose crystallinity in this study may occur due to hydrolysis of the amorphous area which is larger than the crystal area so that the peeling reaction in the amorphous area occurs. This causes the relative crystallinity (with ILs treatment) to increase. Thus, ILs are known to destroy the crystalline area of cellulose so that the ratio of pore and the inner surface area becomes larger.

3.3. FTIR of Raw EFB and EFB Treated with [MOBzt]Br

As shown by the FTIR spectrum in **Figure 11**, pretreatment using [MOBzt]Br affected the EFB structure. The presence of absorption at 3411 cm^{-1} indicates a reduced stretching of the -OH group after treatment using ILs. This proves that the cellulose partial hydrogen bonds have been destroyed so that the accessibility of cellulose to the reagents also increases (Li *et al.*, 2021).

The peak characteristics of cellulose are also at a peak of around 1000—1200 cm^{-1} (Vârban *et al.*, 2021). The absorption at about 1160 cm^{-1} represents the antisymmetric bridge strain of the C-O-C group in cellulose and hemicellulose, whereas the absorption near 1247 cm^{-1} may be due to the weak C-O strain in hemicellulose.

The change of intermolecular was also detected in the 898 cm^{-1} band, which signifies *β*(1-4)-glycosidic linkages and intramolecular hydrogen bond breakage.

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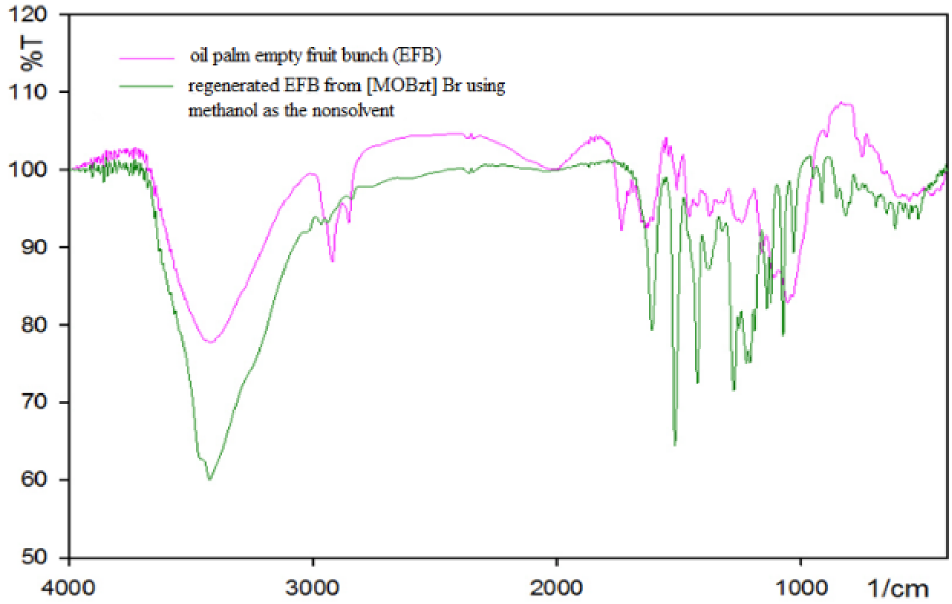

Figure 11. FTIR spectra of (a) oil palm empty fruit bunch (EFB), (b) regenerated EFB from [MOBzt]Br using methanol as the nonsolvent.

Intramolecular and intermolecular hydrogen bonding loss in amorphous cellulose results in increased surface area, which leads to improved enzyme accessibility and increased binding sites in regenerated cellulose fibers. FTIR spectroscopy analyses revealed that the functional groups of lignin changed significantly during IL pretreatment.

Carbonyl (C=O) stretching unconjugated ketones were found at the 1735 cm^{-1} band. Carbonyls were found primarily in the side chains of lignin structural units and serve as an important functional group in the side chains, which contain either aldehyde or keto groups. The absence of such bands showed that the lignin side chain was broken down throughout ILs treatment (Liu *et al.*, 2019). Methoxyl stretching is represented by the prominent band at 1247 cm^{-1}. The content of methoxyl in the lignin of the ILs-treated EFB was found to be lower than that of the untreated EFB. After ILs pretreatment, some bands, such as the band at 1051cm^{-1}, appeared. The aromatic ring deformation in the C-H plane is represented by this band. Its appearance suggested that the aromatic compound content had been increased (Md Salim *et al.*, 2021). When the ILs-pretreated EFB spectrum is compared to the untreated EFB spectrum, the bands at 1510 cm^{-1} (aromatic skeletal from lignin) decrease significantly. FTIR spectroscopies revealed that ILs not only broke down interunit linkages but also changed the lignin functional groups. Changes in lignin structure would affect EFB biodegradability. The FTIR results revealed that the peaks of cellulose, lignin, and hemicellulose were lower than those of the controls, demonstrating that some of the cellulose, hemicellulose and lignin had been degraded. When EFB was treated with [MOBzt]Br regenerated from methanol, its structure changed from I to II in the FTIR.

3.4. SEM of Raw EFB and EFB Treated with [MOBzt]Br

SEM images of raw EFB (untreated) and regenerated EFB from ILs (pretreated) were taken at 50x magnification as shown in **Figure 12**. According to the findings, untreated EFB has a highly fibrillar, intact morphology. When compared to raw EFB, regenerated EFB has a smaller particle size. However, ILs pretreatment does not affect cell wall structure.

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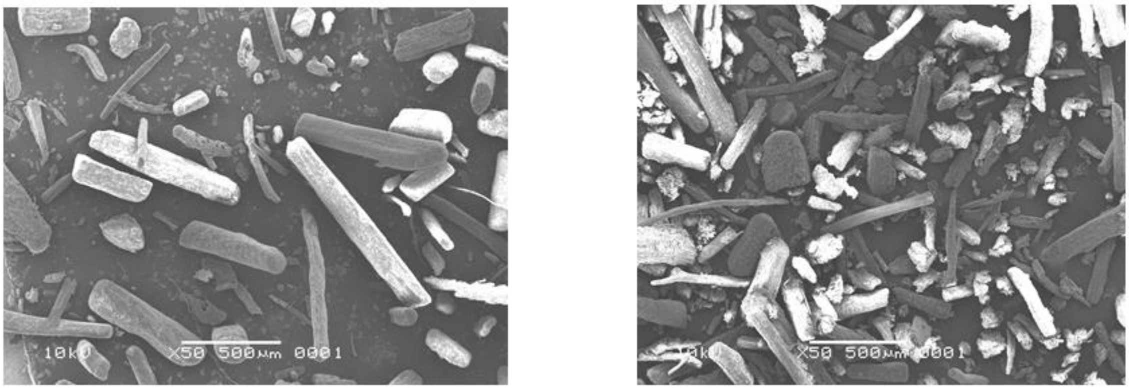

(a) (b)

Figure 12. SEM micrographs of (a) raw EFB and (b) regenerated EFB from [MOBzt]Br using methanol as the nonsolvent.

3.5. Effect of [MOBzt]Br, [MOBzt]SCN, and [MOBzt]CH_3COO Pretreatment on Enzymatic Hydrolysis of EFB

After hydrolyzing the regenerated EFB samples in a water bath shaker, the conversion of cellulose to glucose was calculated using glucose concentration measurements taken at various time intervals. **Figure 13** shows incubation time appears to have little effect on the formation rate of glucose. The rates of glucose formation differ significantly between untreated and regenerated EFB. Under the specific experimental condition, the enzymatic hydrolysis rate of EFB was significantly improved after treatment with ILs based on benzotriazolium salt (**Figure 13**). The improvement in the hydrolysis rate of raw EFB was less than that of EFB with ILs. Before 24 hours, the hydrolysis rate of EFB treated with ILs was greatly improved. The removal of lignin by ILs treatment thus increases the cellulose accessible fraction. The hydrolysis results demonstrated that soluble sugars were released more quickly and to a greater extent in the ILs-pretreated samples.

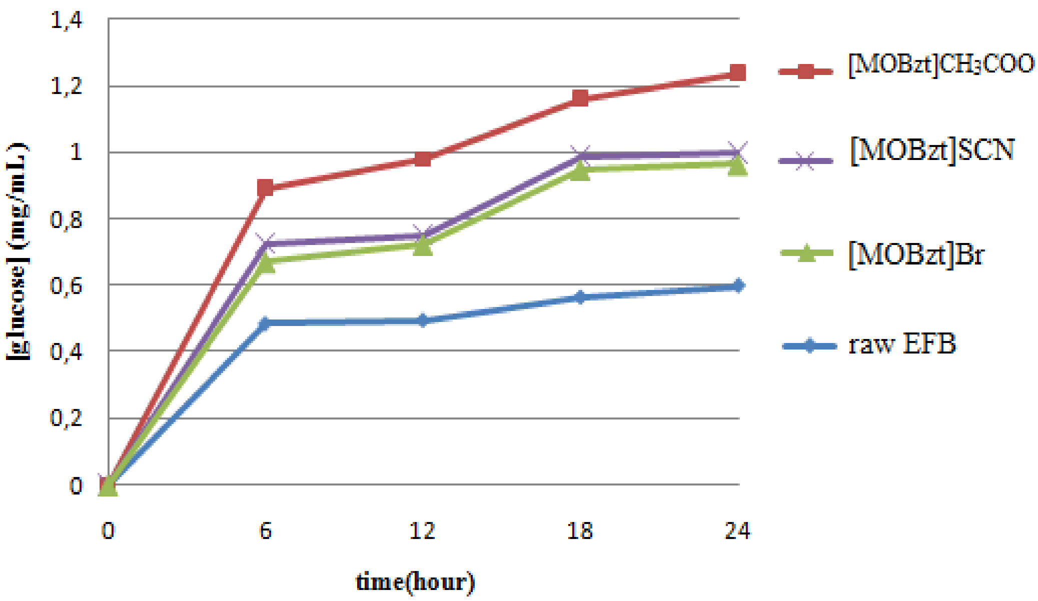

Figure 13. Effect of ILs on the enzymatic hydrolysis of EFB.

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However, the results of hydrolysis EFB are not as expected, where the cellulase enzyme can only hydrolyze a little cellulose. This might be due to the excess components of lignin that can inhibit the access of enzyme cellulase. By acting as a physical barrier between the cellulase enzyme and its substrate, lignin inhibits cellulose degradation. As a result, the lignin content has an inverse relationship with the rate and extent of enzymatic cellulose degradation in lignocellulosic materials (Barron *et al.*, 2021; Candido & Gonçalves, 2019).

4. CONCLUSION

The results of the bibliometric analysis show that research related to biomass pretreatment has increased in 2012-2016, then experienced fluctuations in 2017-2020, increased dramatically in 2021, but also decreased significantly in 2022, so the interest in this research experienced a decline in today's so that new research on the topic still has a high chance. Therefore, research related to biomass pretreatment is very much needed. To reduce the lignin content and cellulose crystallinity of EFB, ILs based on benzotriazolium salt were used. [MOBzt]Br did not completely dissolve the EFB, but it did allow for easy extraction of the lignin. ILs pretreatment was a promising alternative process for obtaining high sugar yields from the recovered product. The highest glucose yield (1,237 mg/mL) was obtained when the EFB was pretreated by [MOBzt]CH_3COO with enzymatic hydrolysis for 24 hours. EFB which was given pretreatment using ILs based on benzotriazolium salt more easily hydrolyzed by the cellulase enzyme and glucose yield is higher than without pretreatment.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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