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Research paper

Influence of Deep Eutectic Solvent (DES) Pretreatment on Various Chemical Composition of Empty Fruit Bunch (EFB)

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Abstract

Chemical composition of empty fruit bunch (EFB) is an important chemical property that determines its utilization performance in bioconversion process. Various chemical compositions of EFB have been reported, and these differences in composition affects EFB utilization in terms of conversion and product yield. Complex structure and recalcitrant characteristic of lignocellulosic biomass (LCB) also affects its utilization that leads to pretreatment system requirement. Pretreatment using deep eutectic solvent (DES), a group of ionic liquid (IL), has attracted scientific interest due to its exceptional ability in hemicellulose and lignin removal. This research determined the chemical composition of six native EFB sample collected in Malaysia, and identified composition difference among samples using *t-test*. The work further determined the influence of DES01 pretreatment on selected pretreated samples using enzymatic hydrolysis process. Chemical compositions of six native EFB samples, collected in Perak, Selangor and Negeri Sembilan in dry and wet seasons, were determined using National Renewable Laboratory Analysis (NREL) protocol. Enzymatic hydrolysis of pretreated EFB samples were conducted following NREL protocol using Cellic CTec2 and Cellic HTec2 enzymes for 72 hours. The *t-test* analysis on structural carbohydrate (SC) content of native EFB showed Sg. Siput sample (SSD) in dry season and Bahau sample (BW) in wet season had statistically significant difference where native SSD contained the highest SC while native BW had the lowest. Enzymatic hydrolysis results of DES01 SSD and DES01 BW samples indicated the influence of DES01 pretreatment. DES01 SSD substrate produced higher glucan and xylan conversion after 72 hours of hydrolysis with 92.40% and 68.71% respectively compared to the DES01 BW sample with 75.82% and 18.78% only. This could be correlated with higher glucan and lower lignin contents in However, the different composition of native EFB also affected the hydrolysis of pretreated EFB. The variable pressure scanning electron microscopy (VPSEM) analysis showed that EFB structures were destroyed by the hemicellulose and lignin removal after the pretreatment and enzymatic hydrolysis.

Keywords: *Compositional analysis; DES; EFB; environmental factors; enzymatic hydrolysis*

1. Introduction

Malaysia is the second largest palm oil producer after Indonesia. Thus the country has vast plantation of oil palm tree (*Elaeis guineensis* jacq.) and produces huge quantity of oil palm biomass waste approximately 4 million tonnes per year [1]. Empty fruit bunch (EFB), a lignocellulosic biomass (LCB), is produced in abundance as a residue product after the palm oil extraction process in palm oil mill. EFB is generated around 1.07 tonne with every tonnage of crude palm oil produced [2], and has become a potential raw material for utilization in bioconversion technology to produce bio-based products.

Chemical composition of EFB is an important chemical property that determines its utilization performance in the bioconversion process. Based on the literature review, various chemical composition of EFB has been reported, and these differences in composition can affect the EFB utilization in terms of material conversion and product yield [3-8]. Variation in chemical composition of EFB is contributed by several factors such as plantation season, geographical location, plant species, soil characteristics, fertilization system and several others. Some studies have reported that differences in harvest time and geographical location can affect the chemical composition of corn stover [9-10]. Although many literature have reported different EFB chemical compositions, but there is limited information on how these differences can affect the utilization of EFB in the process as this is important to establish a sustainable bioconversion design and operation.

EFB is composed of three main polymers which includes 35-45% of glucan, 25-35% of hemicellulose, and 15-25% of lignin [11]. It is considered a good source of lignocellulosic carbohydrate that can be converted into high-value intermediates and products such as fermentable sugars, succinic acid and few others. Glucan, primarily cellulose, and hemicellulose can be hydrolyzed into monomeric

sugars through acid or enzymatic hydrolysis process which is then fermented to produce bio-ethanol, bio-hydrogen, or any bulk chemicals such as succinic acid [12]. In addition, the complex structure and recalcitrant characteristic owing to the lignin-carbohydrate complex (LCC) in the cell wall structure also affect the LCB utilization that leads to requirement of pretreatment system when processing LCB. It is hypothesized that the LCCs are key elements in explaining the impact of lignin on cell-wall degradation. The recalcitrant lignin is a major barrier in utilizing LCB because it is not easily separated from cellulose. The pretreatment process is a crucial step to enhance the accessibility of cellulolytic enzymes to cellulose and hemicelluloses in biomass during hydrolysis process [13].

Pretreatment of LCB using deep eutectic solvent (DES), a group of ionic liquid (IL), has attracted a widespread of scientific interest due to its exceptional ability in hemicellulose and lignin removal [14-17]. While IL has outperformed other chemical pretreatments in terms of its chemical properties, thermal stability as well as being an environment-friendly solvent [18-20], the usage of IL is subjected to several constraints due to its high cost, high energy requirement for IL recycling, production of toxic and high viscosity [14]. Although the physical and chemical properties of DES are similar with IL, the former is favorable for it is more environment-friendly and cheaper cost [21]. The estimated cost of DES is approximately 20% of a comparable worth of IL. This has been the driving factor for replacing IL with DES as green alternative for LCB pretreatment and cellulose dissolution [22, 23]. DES is a mixture of two or three ionic compounds which interact with each other by self-association to form a eutectic mixture that has a melting point below than that of each compound [24-26]. Moreover, it has been reported that DES is effective for enzymatic activity [27].

At present, there is little information available on how the pretreatment can influence differences in EFB chemical composition. Hence, this present study focuses on two main objectives where the first objective is to determine the chemical composition of EFB collected from different geographical location and season in Malaysia, and identify any difference in these compositions using statistical method. The second objective is then to determine the influence of DES pretreatment on these composition in terms of enzymatic hydrolysis performance of the pretreated EFB.

2. Materials and methods

2.1. Collection and preparation of EFB sample

There were six native untreated EFB samples collected from northern to southern of part of Malaysia in two different seasons. Two samples were collected in each (1) Sg Siput, Perak, (2) Bestari Jaya, Selangor and Bahau, Negeri Sembilan (Fig. 1) in dry (January-February) and wet season (October-November). The season classification is based on Oldeman method using monthly rainfall as the basic element where dry season has rainfall below 100 mm and wet season has rainfall above 200 mm [28]. The ecotype of all locations was in lowland due to their altitudes which are lower than 200 m above sea level [29]. Malaysia has a large lowland areas and usually planted with oil palm trees [30]. All EFB samples were collected from oil palm trees, aged between 9-18 years old, during peak-yield period for these trees (Ferdous et al. 2015). Samples collected were pressed (after oil recovery process) and non-shredded type. However, the specific palm species for all samples could not be identified. Table 1 shows the collection information of EFB samples.

Table 1: Collection of EFB samples

Sites Information	Descriptions	Sg Siput, Perak	Bestari Jaya, Selangor	Bahau, Negeri Sembilan
Ecotype	Altitude (m above sea level)	82 Lowland	12 Lowland	65 Lowland
EFB Type	After oil recovery process	Pressed, non-shredded	Pressed, non-shredded	Pressed non-shredded
Oil Palm Tree	Age (Year)	9-18	9-18	9-18
Season	Dry	February, 2016	February, 2016	February, 2016
	Rainfall (mm)	57	109	84
	Wet	October, 2015	October, 2015	October, 2015
	Rainfall (mm)	358	292	111

Collected EFB samples were dried naturally under the sun until its moisture content reduced to less than 10% (dry weight basis, dwb). Moisture content of each EFB sample was analysed using IR-35 Moisture Analyser (Denver Instrument). Dried EFB sample was ground using cutting mill (FRITSCH Universal Cutting Mill PULVERISETTE 19), sieved with a 2 mm mesh size. Ground EFB samples were stored in an airtight plastic bag and kept in a cold room for further use.

2.2. Soil analysis

Approximately 0.1 g of the sample was weighed and placed in containers. Then, 10 mL of nitric acid (HNO_3) and 5 mL of hydrochloride acid (HCL) were added to the sample. The sample was then heated in a microwave for 1 hour. Sample then was removed from the microwave and further dissolved in 45 mL of 1% HNO_3. Samples were then analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The percentage of elements is calculated based on Eq. 1 below:

$$\text{Element (\%)} = (\text{ICP-OES reading} \times \text{Dilution} \times \text{Volume (mL)} \times 100) / (\text{Weight of sample (gram} \times 10^6) \quad (1)$$

2.3. Chemical compositional analysis

The chemical composition analysis of six native untreated EFB samples with average size of 2 mm were based on Laboratory Analysis Protocol (LAP) developed by the National Renewable Energy Laboratory (NREL) (Golden, Colorado USA). Several LAPs were used to determine the composition of structural constituents in the samples in terms of glucan, xylan, arabinan, and lignin content. Composition of structural constituents from all samples were compared and inferential statistic method of t-test was utilized to determine the

difference in the composition of EFB samples based on the significance level at 0.05. Differences with p-values of less than 0.05 were considered to be significantly different. The same NREL compositional analysis was conducted on the DES pretreated EFB.

2.4. Selection of EFB sample for DES pretreatment

The efficiency of bioconversion productivity of EFB strongly depends on the biomass composition particularly the structural carbohydrates. Therefore, it is crucial to choose the biomass which can be converted efficiently. In this research, EFB samples identified with the highest and lowest carbohydrate content were selected for further study on the influence of DES pretreatment on different compositions of EFB samples.

2.5. DES pretreatment

DES solvent was prepared using choline choride and imidazole with molar ratio of 3:7 [14] referred as DES01. Choline chloride and imidazole (≥ 98% mass fraction purity) were purchased from Scienfield Expertise PLT (Selangor, Malaysia). EFB sample weighed 10 g of mass was added into DES01 at ratio 1:5 solvent loading (SL), soaked and heated at 160 ℃ (unpublished data) for 2 hours. The process was conducted at atmospheric pressure under constant stirring condition. Cotton wool and aluminium foil were used to cover the bottle cap to ensure minimal vaporisation of the samples. Upon completion of the pretreatment process, the pretreated EFB was separated from the black liquor using a cheese cloth in gravimetric filtration followed by oven drying of DES pretreated EFB at 50 ℃ until constant weight was achieved.

2.6. Enzymatic hydrolysis

The enzymatic hydrolysis method at 1% glucan loading (GL) was performed based on the Laboratory Analysis Protocol (LAP 009) developed by the National Renewable Energy Laboratory (NREL). Two enzymes, Cellic CTec2 and Cellic HTec2, were purchased from Novozymes A/S (Bagsvaerd, Denmark). Cellic CTec2 was combined with Cellic HTec2 at a volume ratio of 1:1 (v/v). The activity of Cellic CTec2 was at 15 FPU/g of glucan. Hydrolysis was conducted in scintillation vial at 15 mL working volume, 50 ℃, 150 rpm, and pH 4.8 using 1 M citrate buffer for 72 hours. The hydrolysate samples were taken upon completion of the hydrolysis, and heated at 100℃ for 15 minutes to denature the enzymes. Samples were then centrifuged at 7000 rpm for 10 minutes, and filtered using 0.22 μm Whatman membrane syringe filter prior to HPLC analysis.

2.7. High performance liquid Chromatography (HPLC) for carbohydrate quantification

All monomeric sugars were analysed using High Performance Liquid Chromatography (HPLC) (UltiMate 3000 LC system, Dionex, Sunnyvale, CA) equipped with a refractive index (RI) detector (RefractoMax 520, ERC, Germany) at 40 ℃ oven temperature. The Rezex ROA-Organic acid column (300 mm × 7.8 mm, Phenomenex, USA), with guard column (50 mm × 7.8 mm) was used for quantifying the concentration of sugar in the acid hydrolysed and enzymatic hydrolysate samples. The mobile phase used was DI water at a flow rate of 0.6 mL/min and the sample injection volume was fixed at 20 μL. Calibration curves were established using an analytical grade of individual standard for glucose, xylose, and arabinose (Sigma-Aldrich, Germany). The calibration curves and sample concentrations were performed using the Chromeleon v. 7.2.2.6686 software (Dionex, Sunnyvale, CA).

2.8. Glucan conversion from sugar concentration

The efficiency of the carbohydrate EFB converted into monomeric sugar was based on glucan conversion calculated using the sugar concentrations in the hydrolysate samples using the following Eq. 2:

$$\text{Glucan conversion (\%)} = (C_S / C_{MaxTheo,S}) \times 100\% \quad (2)$$

where C_S is the actual sugar concentration in unit g/L determined using HPLC, and $C_{MaxTheo,S}$ is the maximum theoretical sugar concentration in the hydrolysis sample at 1% of glucan loading.

2.9. Variable pressure scanning electron microscopy (VPSEM)

Morphological analysis of native untreated EFB, DES pretreated EFB and unhydrolyzed sample obtain after enzymatic hydrolysis was conducted by scanning the samples using Variable Pressure Scanning Electron Microscopy, VPSEM (EVO MA10 CARL ZEISS, UK). The native untreated EFB samples according to Section 2.4. All samples were freeze-dried before VPSEM analysis. Each sample was put on aluminium stubs and covered with gold using the sputter coater system Model Q150 RS (Quorum Technologies, UK). Samples were observed in the magnification range of 50-1000x.

3. Result and discussion

3.1. Chemical composition of native EFB sample and soil analysis

The compositional analysis result of six native EFB sample is summarized in Table 2. The total structural carbohydrate content in dry season samples were observed to be higher than in wet season samples. The increase of carbohydrate content in various plant tissues response to the water stress in dry season. Previous study reports that this could be due to the accumulation of the soluble sugars in the plant tissues, and suggests these sugars might contribute to osmoregulation, where the plants regulate the osmotic pressure during water stress [31]. Other studies indicates that higher sugar in plant tissues during dry season is due to the plant drought tolerance under water stress [32]. Higher level of UDP-Glc in the expression of sucrose *synthase* (*SuSy*) and UDP-glucose *pyrophosphorylase* (*UGPase*)

encoding genes in cotton under drought stress, shows a potentially higher cellulose biosynthesis [32]. The increase in cellulose synthesis could be a means to indicate cell wall integrity and cell turgor pressure are maintained, thus allowing continuous cell growth under low water potential.

Wet season results in a lower oxygen level in the root zone due to this molecule's low diffusion rate in water [33], and alters cell wall polysaccharides. FTIR analysis performed on maize seedlings exposed to wet season showed a degradation of cell wall polysaccharides and a decrease in pectin content [34]. A similar trend was observed in azuki bean seedling (*Vigna angularis*), where a decrease in cell wall sugar content was observed, correlated with cell wall thinning in rainy season [35].

In contrast, the acid insoluble lignin (AIL) in dry season samples was lower than wet season samples. Dry season alters lignification as shown by the decrease in lignin deposition. The lignin was reduced because drought stress caused disruption of lignin deposition in EFB. In wet season, AIL was higher because the cell wall becomes more compact, tight and less permeable to water. This is due to avoid cell wall damage when plants are exposed to long wet season. The compositional variability in EFB samples seemed to be more depending on rainfall than geographical location**.**

Table 2: Compositional analysis of native EFB samples

	Dry Season			Wet Season		
	Sg. Siput	Bestari Jaya	Bahau	Sg Siput	Bestari Jaya	Bahau
1. SC	61.15 ± 1.95	60.98 ± 0.1	59.79 ± 1.5	58.79 ± 2.39	57.33 ± 0.04	54.57 ± 2.26
Glucan	37.57 ± 0.41	38.30 ± 0.79	38.94 ± 1.68	37.15 ± 2.9	33 ± 1.06	31.85 ± 1.07
Xylan	22.63 ± 0.2	22.36 ± 0.26	19.16 ± 0.28	20.80 ± 0.82	23.35 ± 0.72	22.72 ± 1.19
Arabinan	0.93 ± 1.32	0.31 ± 0.43	1.69 ± 0.1	0.83 ± 0.31	0.98 ± 0.37	0
2. AIL	22.3 ± 0.83	23.21 ± 0.3	23.58 ± 0.33	29.74 ± 1.65	28.72 ± 0.73	30.21 ± 1.16

*SC= Structural carbohydrate, AIL= Acid insoluble lignin

Table 3: Statistical significance on structural carbohydrate of EFB samples using *t-test* analysis at significance level of 0.05

SC.	SS	Dry	-	0.2	0.31	0.22	0.26	**0.01**
	SS	Wet	0.2	-	0.25	0.24	0.67	0.11
	BJ	Dry	0.31	0.25	-	0.04	0.14	0.07
	BJ	Wet	0.22	0.24	**0.04**	-	0.26	0.11
	B	Dry	0.26	0.67	0.14	0.26	-	0.06
	B	Wet	**0.01**	0.11	0.07	0.11	0.06	-
			Dry	Wet	Dry	Wet	Dry	Wet
			SS	SS	BJ	BJ	B	B

*SC= Structural Carbohydrate, SS= Sg Siput, BJ= Bestari Jaya, B=Bahau

The bold values are the p-values that are less than 0.05. The components with <0.05 p-values were considered to be significantly different.

The soil samples were also analysed to determine its effect on the composition of the EFB sample. Table 4 shows the characteristics of all soils samples. The soil samples were characterised in terms of pH as well as major elements such as phosphorous (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), and sodium (Na).

The pH value acted as an indicator to determine the status of the soil whether acidic, neutral, or alkali, and identified changes in either biological or chemical activity in the soil **[**36**]**. The pH of the soil samples ranged between 4 to 5 indicates that the soil is acidic. Previous studies reports that the suitable pH of the soil for oil palm plantation are in the range of 3 to 5 [37-38]. Another study conducted in Southeast Asia shows that almost 95% of oil palm trees are estimated to grow on acidic soil which has a pH value of less than 5 **[**39**]**.

During dry season, the contents of P, K and Ca were higher than in dry season for EFB sample in Sg Siput and Bestari Jaya. P, K and Ca contents in wet season was lower due to the removal of top soils or lost due to the soil erosion during heavy rain **[**40**]**. On the other hand, element such S, Mg and Na contents of all soil samples did not shows a lot of differences. Major elements in the soil have significant role on the quality of EFB samples, where high carbohydrate content of EFB samples in dry season (Table 2) could have been the result of high content of major element in the soil samples.

Table 4: Topsoil (0 to 30 cm depth) characterisation for soil samples

Season	Dry			Wet		
Location	Sg. Siput	Bestari Jaya	Bahau	Sg. Siput	Bestari Jaya	Bahau
pH	4.58	4.32	4.02	4.88	4.27	3.99
Major elements						
Phosphorous (P)	0.23	0.24	0.03	0.02	0.03	0.03
Potassium (K)	0.22	0.23	0.05	0.04	0.16	0.04
Sulphur (S)	0.04	0.04	0.05	0.01	0.04	0.05
Calcium (Ca)	0.37	0.40	0.04	0.03	0.05	0.04
Magnesium (Mg)	0.04	0.04	0.03	0.05	0.02	0.03
Sodium (Na)	0.01	0.01	0.01	nd	0.01	0.01

The inferential statistic using *t-test* analysis on structural carbohydrate of EFB samples (Table 3) showed the EFB samples in dry season in Sg Siput (SSD) and wet season in Bahau (BW) had lowest p-values with value of 0.01. This indicated that EFB sample of SSD contained the highest structural carbohydrate (SC) content which corresponded to the lowest lignin content. EFB sample of BW had the lowest SC content with the highest lignin content (Table 2). Therefore, these two EFB samples of SSD and BW were selected for further study to investigate the influence of DES pretreatment on EFB with different compositions.

3.2. Chemical compositional changes in pretreated EFB

Table 5 shows the compositions of native EFB of SSD and BW samples and pretreated DES01 SSD and DES01 BW EFB samples. The composition of the structural carbohydrates (SC) and glucan content of the native untreated SSD sample was 61.2% and 37.6%, while

native untreated BW sample was 54.6 % and 31.9%, respectively. The SC increased by 8% and 12% in both DES01 SSD and DES01 BW respectively owing to the increased in glucan content. The glucan content increased by 38% and 35.1% in the respective DES01 SSD and DES01 BW. The increase of glucan content in both sample could be accounted for the removal of hemicellulose and lignin in the pretreated samples.

Hemicellulose and lignin were decreased after DES01 pretreatment for both EFB samples. This is due to the pretreatment role to break the chemical bonding and remove the hemicellulose and lignin fractions which could possibly improve the cellulose accessibility in the enzymatic hydrolysis. DES01 pretreatment had the ability to reduce lignin as reported in corn cob pretreatment using imidazole-based DES **[**14**]**. This is due to the interaction between imidazole ring and phenyl ring of lignin. Based on other study, DESs has ability and polarity of H-bond acceptor that contributes in dissolving the lignin [41]. DESs would promote the cleavage of ether bonds cause degradation of lignin [42]. Similar effects are also observed using imidazolium-based IL pretreatment. The IL of imidazolium cation had played a role in delignification [43]. Comparing to native EFB of SSD and BW, lignin contents were reduced by 36.7% and 46.7% in DES01 SSD and BW respectively (Table 5). Generally, DES01 pretreament was an effective method to pretreat EFB it was able to remove both hemicellulose and lignin, and therefore resulted in an increase in the glucan fraction in both DES01 SSD and BW samples.

Table 5: Compositional analysis of native and pretreated EFB of each season and location

Location	Sg. Siput		Bahau	
Season	Dry		Wet	
Chemical Components	Native EFB	Treated SSD	Native EFB	Treated BW
1. Struc. Carb.	61.2 ± 2.0	66.1 ± 0.0	54.6 ± 2.3	61.6 ± 0.5
Glucan	*37.6 ± 0.4*	*51.9 ± 0.2*	31.9 ± 1.1	*43.1 ± 0.1*
Xylan	*22.6 ± 0.2*	*13.9 ± 0.2*	22.7 ± 1.2	*18.5 ± 0.4*
Arabinan	*0.9 ± 1.3*	*0.3 ± 0.0*	0	*0*
2. Lignin	22.3± 0.8	14.1 ± 0.1	30.2 ± 1.2	16.1 ± 0.2

3.3. Enzymatic hydrolysis of native and DES pretreated EFB samples

The physical structure of pretreated EFB changed significantly after DES pretreatment due to the removal of undesirable components such as hemicellulose and lignin that increased the porosity of biomass structure. Enzymatic hydrolysis of cellulolytic biomass hydrolyses the cellulose into fermentable sugar, which can be further converted into biobased products using microbial fermentation process. Fig. 2 shows the time profiling of (A) glucan conversion and (B) xylan conversion of 1% glucan loading for native and DES01 pretreated SSD and BW EFB samples.

Fig. 1: Study area in a) Ladang Elphil, Perak, b) Ladang Tennamaram, Selangor and c) Ladang Kok Foh, Negeri Sembilan

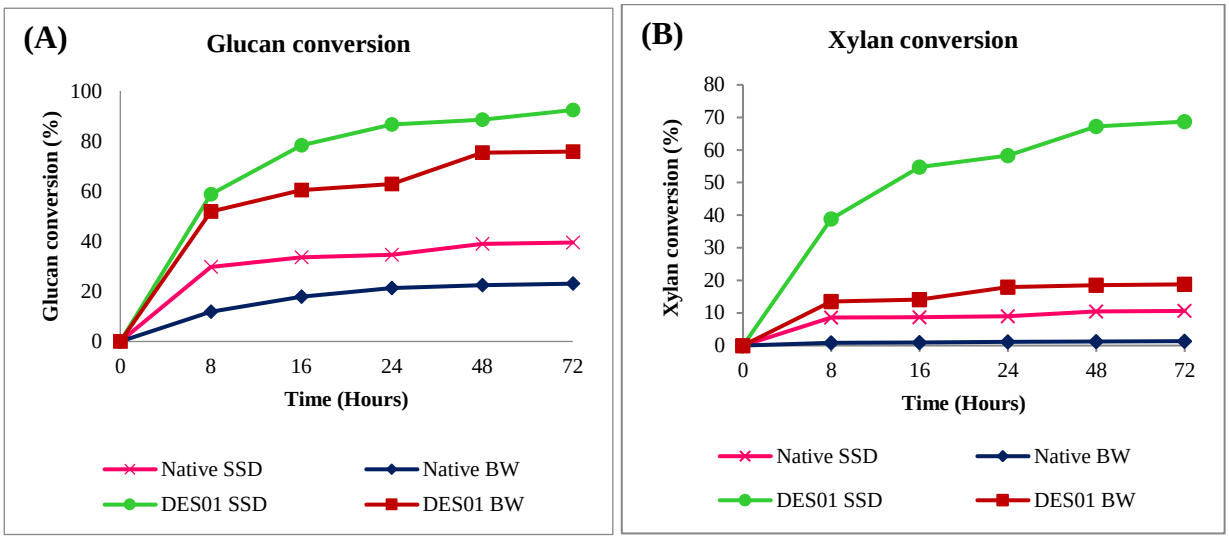

Fig. 2: Time profiling of (A) glucan conversion and (B) xylan conversion for native and DES01pretreated EFB for SSD and BW sample.

Glucan and xylan conversion increased dramatically during the first 8 hours of the hydrolysis process. The sugar production rate slowly decreased as the hydrolysis process continued [44]. The enzymatic hydrolysis model is divided into two levels where at the first level that is the initial hydrolysis rate is almost linear. On the other hand, at the second level, the hydrolysis rate is flat and can slowly decreases [45].

The hydrolysis of DES01 SSD sample proceeded at high initial hydrolysis rate during the first 8 hours compared to DES01 BW sample. The glucan and xylan conversion from 0 to 8 hours for DES01 SSD sample was 0% to 58.73% and 0% to 38.89% respectively, whereas the glucan and xylan rate for DES01 BW sample was 0% to 51.89 and 0% to 13.48% respectively. Hence, the high hydrolysis rate of DES01 SSD substrate produced higher glucan and xylan conversion after 72 hours of hydrolysis with 92.40% and 68.71% respectively compared to the DES01 BW sample with 75.82% and 18.78% only (Fig. 2).

This could be correlated with higher glucan and lower lignin contents in DES01 SSD pretreated sample. Lignin removal also enhanced of the enzymatic hydrolysis in this study. The enzymatic hydrolysis results of DES01 SSD and DES01 BW samples indicated the influence of DES01 pretreatment on both samples. This can be observed on the improvement of the hydrolytic performance of both DES01 SSD and DES01 BW samples over the native untreated samples.

However, the glucan and xylan conversion obtained during hydrolysis for both pretreated samples also depended on the initial composition of the native EFB. Comparing the hydrolysis of the native SSD with native BW sample, the former sample had higher glucan and lower lignin content initially with 37.6% and 22.3% respectively thus providing higher glucan and xylan conversions with 39.5% and 10.62% respectively. Native BW had lower glucan and higher lignin content (Table 5), hence resulting with lower glucan and xylan conversions with 23% and 1.3%, more than 10% lower than in native SSD.

Therefore, while it is clear that DES01 pretreatment able to influence the SC and glucan composition in the respective pretreated EFB samples to give better hydrolysis performance, the initial composition of the native EFB also affected the degree of improvement made by DES01 pretreatment.

3.4. VPSEM analysis of native, DES pretreated EFB and unhydrolysed DES of EFB

Fig. 3 shows the VPSEM analysis of native untreated EFB samples of SSD (Native EFB SSD) and BW (Native EFB BW), DES01 pretreated EFB samples of SSD (DES01 SSD) and BW (DES01 BW), and unhydrolysed DES01 EFB samples of SSD (UH DES01 SSD) and BW (UH DES01 BW). The surface of native untreated EFB had silica bodies on the fibre surface. DES01 SSD, the EFB sample after pretreatment, showed the presence of xylem helical (Fig. 3A2). The UH DES01 SSD showed the fibre surfaces changed and xylem helical appeared to more exposed after enzymatic hydrolysis (Fig. 3A3). Fig. 3B2 demonstrates that DES01 pretreatment able to remove silica from the native EFB as observed in DES01 BW sample, pretreated EFB sample of BW, thereby leaving fibres with holes known as craters. VPSEM observation of Fig. 3B3 on unhydrolysed sample of DES01 BW (UH DES01 BW) shows the porous materials of cellulose.

Higher amount of lignin removal was observed in DES01 SSD sample than in DES01 BW sample. The epidermal layer of the DES01 SSD sample was torn peeled and ruptured significantly. Generally, DES01 pretreatments roughened the secondary cell wall surface of the fibre and was able to disrupt the structure of the lignin [46]. Other research using ionic liquid pretreatment, which has similar properties with DES, has shown similar results where the surface of the biomass became more porous and the enzymes managed to gain easy access during enzymatic hydrolysis [47].

Most of the silica body's craters initially found on the surface of the native untreated EFB's surface were destroyed. Delignification removed the waxy lignin on the EFB surface causing the defibrated inner microfibrils to become significantly exposed.

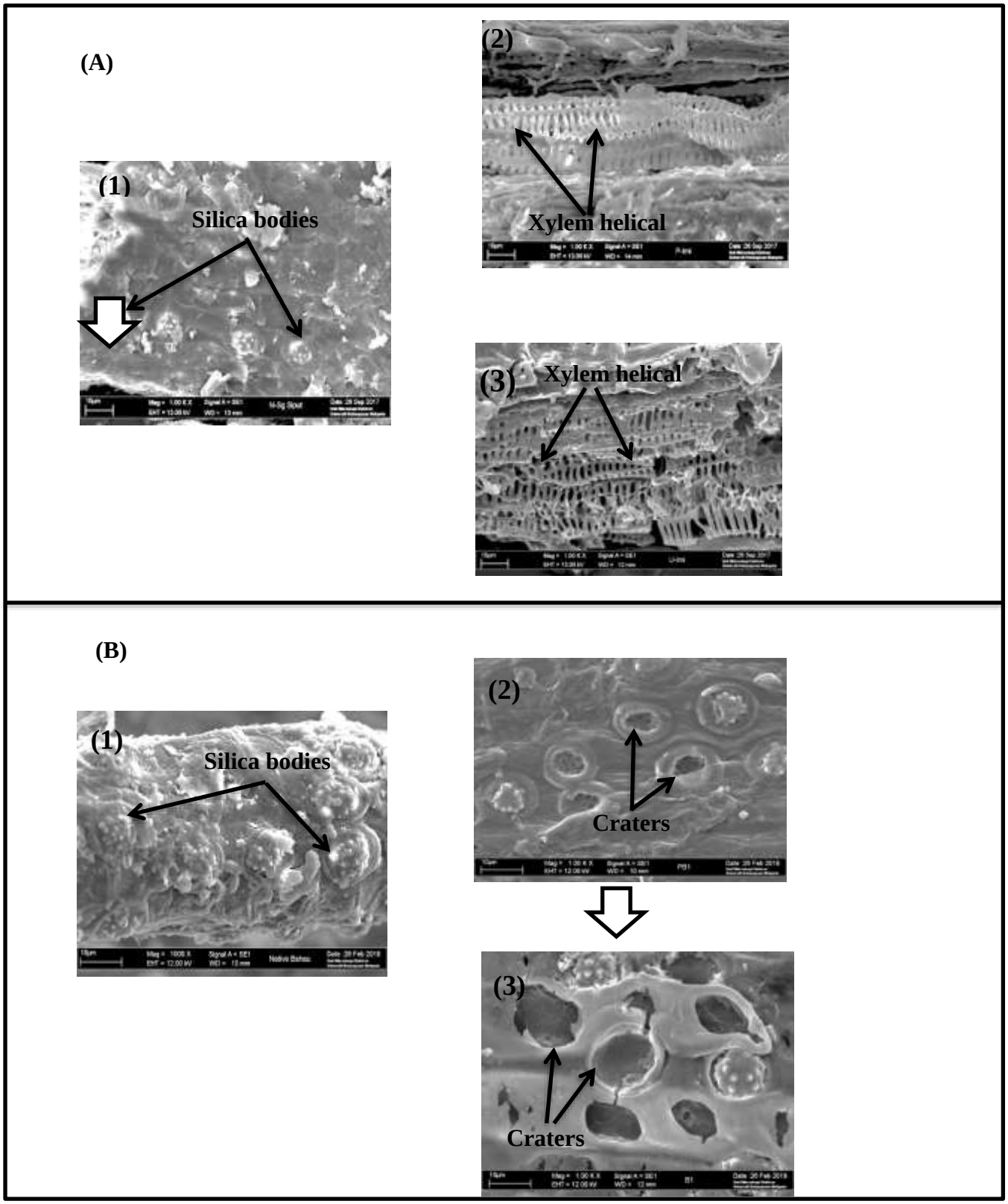

Fig. 3: Surface morphology at 1000x magnification of (A) SSD for (1) Native EFB SSD (2) DES01 SSD, (3) UH DES01 SSD and (B) BW for (1) Native EFB BW, (2) DES01 BW (3) UH DES01 BW.

4. Conclusion

The research work determined the composition of six native EFB samples collected from Sg Siput, Perak, Bestari Jaya, Selangor and Bahau, Negeri Sembilan in dry and wet season using National Renewable Energy Laboratory (NREL) protocols. The inferential analysis using *t-test* conducted on structural carbohydrate (SC) content of native EFB showed Sg. Siput sample (SSD) in dry season and Bahau sample (BW) in wet season had statistically significant difference where native SSD contained the highest SC while native BW had the lowest SC. Compared to native EFB of SSD and BW samples, after DES01 pretreatment the SC increased by 8% and 12% in both DES01 SSD and DES01 BW respectively owing to the increased in glucan content. The glucan content increased by 38% and 35.1% in the respective DES01 SSD and DES01 BW samples and this increase of glucan could be accounted for the removal of hemicellulose and

lignin after pretreatment. High hydrolysis rate of DES01 SSD substrate produced higher glucan and xylan conversion after 72 hours of hydrolysis with 92.40% and 68.71% respectively compared to the DES01 BW sample with 75.82% and 18.78% only. This could be correlated with higher glucan and lower lignin contents in DES01 SSD pretreated sample. The enzymatic hydrolysis results of DES01 SSD and DES01 BW samples indicated the influence of DES01 pretreatment on both samples. While it is clear that DES01 pretreatment able to influence the SC and glucan composition in the respective pretreated EFB samples to give better hydrolysis performance, the different composition of the native EFB also affected the hydrolysis of pretreated EFB. Variable pressure scanning electron microscopy (VPSEM) analysis showed that EFB structures were destroyed by the hemicellulose and lignin removal after the pretreatment and enzymatic hydrolysis.

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