Lab to Pilot Scale Assessment on the Pretreatment of Empty Fruit Bunch Using Anhydrous Ammonia

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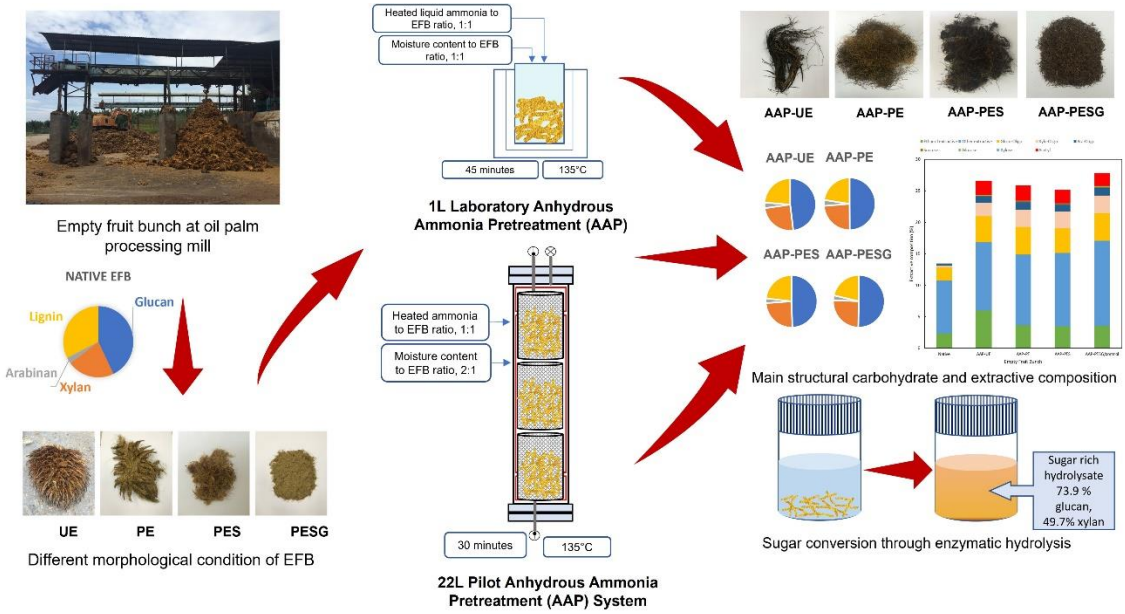
GRAPHICAL ABSTRACT



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Laboratory and pilot scale pretreatment processes were assessed for empty fruit bunch (EFB) at different morphologies using anhydrous ammonia pretreatment (AAP). The AAP was used to deconstruct the complex structure of EFB through physical and chemical reaction to promote efficient conversion of the carbohydrates to monomeric sugars. Different morphologies of EFB samples used were unpressed EFB (UE), pressed EFB (PE), pressed and shredded EFB (PES), and lastly pressed, shredded and ground EFB (PESG). The APP process was optimized using a 1.0 L laboratory scale reactor and further scaled up to a 22 L pressure vessel (AAPB). AAP-PESG contained 76.2%, and AAP-PES contained 75.5% of structural carbohydrates, showing no significant difference. AAP-UE showed the lowest glucan conversion of 28%. The optimal laboratory conditions adopted were 135 °C, 30 min, moist to dry EFB loading of 2:1, and ammonia to dry EFB loading of 1:1. Glucan conversion of AAP-PES were 87%, 80%, and 62% at 1%, 3%, and 6% glucan loadings, respectively. The AAP-PES detected acetamide concentration at 7.3 mg/g, while AAPB-PES was only at 4.4 mg/g. Chemical composition, Fourier transform infrared spectroscopy, Brunauer Emmett Teller surface area, and scanning electron microscopy supported the assessment of AAP and AAPB processes.

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Keywords: Anhydrous ammonia pretreatment; Empty fruit bunch; Pilot-scale; Chemical composition; Enzymatic hydrolysis

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INTRODUCTION

As one of the world's biggest palm oil exporters, Malaysia produces abundant empty fruit bunch (EFB) from an oil palm tree (*Elaeis guineensis*) in the palm oil mill. The EFB accounts for 25% to 30% of solid waste generated for every tonne of fresh fruit bunch (FFB) processed. The volume increases annually, with only a small fraction of EFB being employed for value-added products, while most are underutilized (Barlianti *et al.* 2015). The abundance of EFB produced has created many environmental issues, such as fouling and attraction of pests, which can pose challenges for processing mills to handle. Managing and disposing of large quantities of EFB can be time-consuming and costly (Suhartini *et al.* 2022). Additionally, if not managed properly, the high amounts of EFB can lead to operational issues or interfering with production processes. To address these environmental issues, it is important for the palm oil industry to implement effective EFB management practices. On top of current efforts, which utilize soil mulching using EFB and composting

treatment as enriched biofertilizer, pretreatment of EFB can lead to more efficient and environmentally friendly ways to process and utilize EFB in the palm oil mill plant.

In general, for successful operation of an oil palm mill with a composting plant facility, the EFB must go through a pressing process to recover the oil palm and subsequently increase the crude palm oil (CPO) recovery. Additionally, the EFB yields valuable byproducts, including nutrient rich content for biofertilizer and as feed for animals (Dolah *et al.* 2021). The bunch-size unpressed EFB (UE) contains approximately 2% of the oil compared to pressed EFB (PE), with only 0.2% oil content (Chang 2014; Law *et al.* 2017). Additionally, PE is further subjected to the shredding process prior to the composting process that yields shredded PE (PES) as loose and strand fibre with a length between 50 and 250 mm. Consequently, different morphology and size of EFB in the form of UE, PE, and PES from the oil mill causes limitation in utilizing them all. Although these different types of UE, PE, and PES are abundantly available, thus far these different EFB samples have not been efficiently utilized, and most are used for low-value products that result in quick gain profit. The different morphology and type of EFB will give different effectiveness of utilization due to the characteristics such as moisture content, bulk density, and chemical composition. Moreover, additional processing of the EFB will require higher energy and cost, while the larger size of EFB is difficult to handle for storage and mobilization. Many studies have focused on utilizing the small sizes of EFB (Abdul *et al.* 2016; Tan *et al.* 2016), which requires an energy-intensive grinding process that is not economical and tedious to oil palm millers for process of commercialisation. Thus, the most prevailing practice for EFB pretreatment is hydrothermal treatment due to its technology availability and its potential to enhance oil recovery from EFB (Diyanilla *et al.* 2020).

In the effort for an efficient and sustainable utilization of the biomass as the new green resource, the anhydrous ammonia based pretreatment (AAP) has been developed, in the laboratory as well as in the pilot scale, to mainly alter and open up the EFB cell wall structure. The deconstruction of the lignin-carbohydrate matrix improved the hemicellulose (arabinoxylan) hydrolysability, increased the cellulose crystallinity, and finally made the cellulose more accessible to enzymes in the hydrolysis process to produce fermentable sugars, such as hexose (C6) and pentose (C5), in the biorefinery scheme process (Harun *et al.* 2013). Previous studies have shown that the AAP has significant effect on the glucose (C6 sugar) yield from different lignocellulosic biomass. Furthermore, because of the high volatility of anhydrous ammonia, it provides a dry-to-dry pretreatment process, and the used ammonia can easily be recovered and reused for the subsequent batches of pretreatment (Lau *et al.* 2010). However, the referenced study primarily investigated the performance of ammonia pretreatment on standard laboratory size with milled-size EFB, while this current research evaluates EFB with different morphologies at the lab scale and assessing their performance during scale-up.

There are several common issues when utilizing EFB as a source of energy or other products in palm oil mill. The EFB contains high moisture, and drying of the fibre can be energy-intensive and add to the overall cost of operation. Additionally, EFB has relatively low energy density; thus, it requires a large volume of biomass to produce certain amount of energy, which can be a challenge in terms of handling, transportation, and storage. Thus, to achieve the economics of scale and high bioconversion efficiencies can be difficult, especially for smaller scale operations (Chiew and Shimada 2013).

Most of the utilization studies of the lignocellulosic biomass have been evaluated and reported using alkaline, acid, and organic solvent as the pretreatment agent in breaking the plant cell wall structure (Lau *et al.* 2010; Harun *et al.* 2013; Abdul *et al.* 2016; Tan *et al.* 2016; Law *et al.* 2017). However, with respect to different morphology and type of EFB for examples, such as UE, PE, and PES, there are limited reports exploring on the utilization of EFB, particularly with this different type of morphology and size using AAP and comparing the results and opportunity for the large-scale utilization. The focus of this work was to verify that the finding observed at small scale can be successfully replicated and maintained consistently at larger scales. A further goal was to observe the potential of continuous process by reusing ammonia, which holds promise for viable commercial applications. Therefore, a comprehensive pretreatment study up to pilot scale system is required so that it can show as a proof-of-concept technology and be resourceful for the industry (Akhlisah *et al.* 2021; Morais *et al.* 2022).

This study aimed to assess different EFB morphology using anhydrous ammonia pretreatment from laboratory to pilot-scale. The pretreatment process was optimized at lab-scale and series of experiments were conducted to determine the optimum process parameter in the AAPB system. The effectiveness of pretreatment was evaluated *via* structural and non-structural carbohydrates content, morphology changes, sugar conversion, and inhibitor formation from the enzymatic hydrolysis. Although some EFB in the processing mill already has been utilised for value-added products, proper and efficient biomass utilisation is important for sustainable and economic impacts in the biomass industry.

EXPERIMENTAL

Raw Material

The EFB was collected from Flemington oil palm mill of Sime Darby Plantation Berhad (Bhd) in Bagan Datuk, Perak, Malaysia. There were three categories of the samples taken, as shown in Fig. 1(a), which were UE, PE, and PES. All samples were freshly produced in the oil palm mill on the same day of collection. Samples were properly sealed in gunny bags and transported to the laboratory within 4 to 5 hours and were immediately keep in a cool room at 4 °C overnight. Moisture content and mass of EFB upon collection were also recorded.

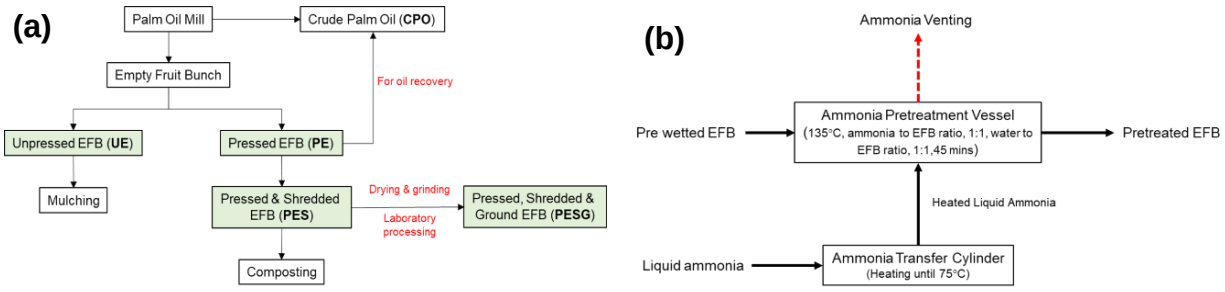

Fig. 1. (a) Flow of different categories of EFB samples from oil palm mill, (b) Schematic diagram of anhydrous ammonia pretreatment process

Preparation of EFB Samples

There were four categories of EFB, as shown in Fig. 1(a), to be analyzed in this study. These were UE, PE, PES, and PESG. Each morphology the EFB was dried for three to four consecutive days until the moisture content reduced to 10 ± 3% (dry weight basis, DWB). The EFB was sun-dried in an open environment, with daytime temperatures ranging from 30 to 35 °C (9 am to 4 pm) and nighttime temperatures between 25 and 28 °C. During the night, the drying process occurred under a roof but in an open area. To facilitate the drying process, all EFB samples were exposed to direct sunlight. Given the substantial quantity collected (approximately 100 kg wet weight) and the large size of the whole fruit, drying them inside a laboratory oven was not feasible. The PESG was ground into approximately 2-mm particle size using a universal cutting mill (Pulverisette 15 Fritsch, Germany) with a 2-mm screen mesh to achieve uniform particle size. Then, PESG was packed in a sealed bag and stored at 4 °C to maintain the moisture content until further use.

Moisture Content of EFB Samples

The moisture content of each EFB category was recorded at a different stage of preparation which are as follows: (1) collection at the mill, (2) followed by receiving the EFB at the laboratory, (3) after the drying process, and (4) after the pretreatment process. The purpose of moisture sampling was to observe the loss and gain of moisture content from the EFB throughout the experiment. Samples were periodically flipped up and down every 2 h to quicken the drying process. Any spoilt samples were removed immediately to ensure no biological contamination in the samples. The activity for the moisture content profiling of UE, PE, and PES was taken every 12 h to observe moisture loss during the drying process. The moisture content was determined *via* a Moisture Analyser (Denver Instrument, Germany) and recorded based on DWB.

Anhydrous Ammonia Pretreatment

Laboratory-scale experiment

As in ammonia fiber explosion (AFEX) pretreatment (Lau *et al.* 2010), a similar set up was also applied in the AAP process to pretreat these different morphological EFB samples. The pretreatment process was performed in a 1-L closed stainless steel vessel reactor (Ammar Reactor, India). Each pre-wetted EFB sample was loaded into this reaction vessel and clamped shut for the preheating process. The ammonia was transferred into this vessel using 1-L transfer cylinder prior to preheating process. Both the transfer cylinder and pressure vessel were preheated simultaneously until stable heat and pressure was achieved. The pretreatment conditions were based on the previous AFEX study for EFB pretreatment (Abdul *et al.* 2016) with (1) pretreatment temperature of 135 °C, (2) ammonia to EFB loading ratio of 1 to 1, (3) water to EFB loading ratio of 1 to 1, and (4) pretreatment time of 45 min. The 45 min residence time started once the pressure vessel reached the pretreatment temperature of 135 °C. Once the time was completed, the pressure in the vessel was slowly released by gradual opening of the vessel valve, causing the ammonia vapour to be evaporated out of the vessel. The vessel cooling process started once the pressure inside reached the atmospheric pressure, and it was cooled to the room temperature or until it was safe to unclamp and open the vessel. Subsequently, the pretreated EFB was unloaded from the pressure vessel and allowed to dry overnight under the fume hood for removal of excess residual ammonia. Pretreated EFB was then stored at 4 °C until further use. Figure 1(b) illustrates the schematic diagram of the AAP process.

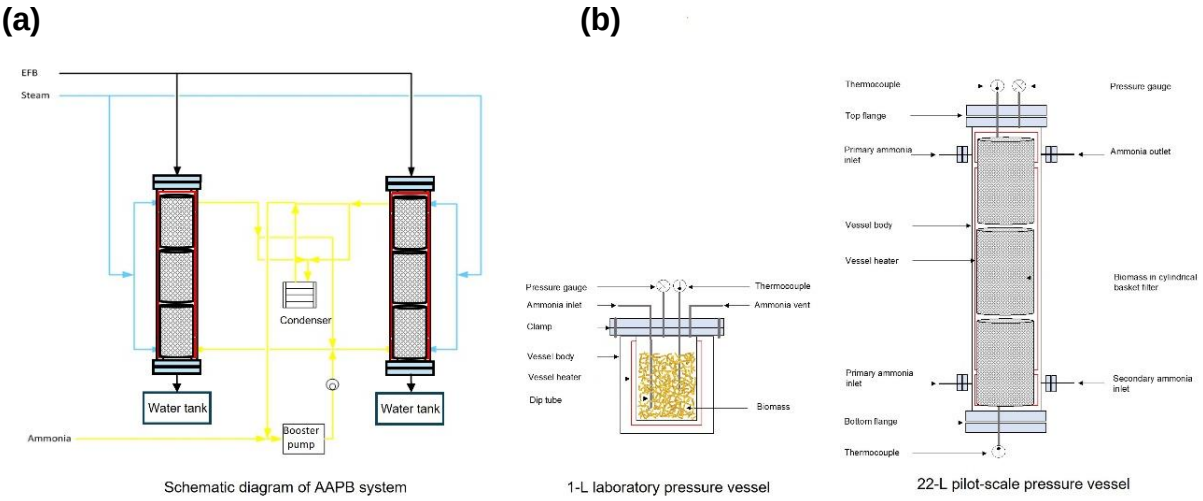

Fig. 2. (a) The schematics of pilot scale diagram of batch AAP (AAPB) system, (b) Configuration of laboratory and pilot scale pretreatment vessels

Determination of different pretreatment parameter conditions at laboratory scale.

The optimum pretreatment parameters in this study were determined through a one factor at a time method, where different components within the pretreatment condition were optimised while the other conditions remained constant. Due to high resistance from contamination and storage easiness PES morphology was chosen as the biomass for the determination of optimal conditions study. The parameters involved were pretreatment time (15, 30, 45, 60, and 75 min), the ratio of moisture content to dry EFB (0.5:1, 1:1, 2:1, 3:1, and 4:1), pretreatment temperature (95, 115, 135, 155, 175, 195, and 215 °C), and the ratio of ammonia loading to dry EFB (0.5:1, 1:1, 1.5:2, 2:1, and 2.5:1). Each parameter pretreatment efficacy was evaluated based on the sugar concentration and conversion from 1% glucan loading enzymatic hydrolysis.

Pilot-scale set-up

The AAP process was scaled up in a pilot set up at Demo Plant Zero-Waste technology at KKS Tennamaram, Selangor. The pilot scale of batch AAP (AAPB) system consists of 22-L pretreatment vessel, equipped with a booster pump, a steam generator, and a condenser, as shown in Fig. 2(a). Two identical pretreatment vessels were used in the process design, aiming for continuous operation with ammonia reuse. Notably, the operating pressure at the pilot scale was significantly lower (8 to 10 bar) compared to the laboratory scale, which operated at 35 bar. The vessel was equipped with top and bottom flange covers, three cylindrical perforated baskets for biomass loading, and electrical heating jacket. Sample of PES were loaded into each basket in the vessel and densified by hand pressure. The top flange cover was closed once loaded with EFB for the pre-steaming process using saturated steam (pressure of steam). The outlet at the bottom flange cover was opened to allow displaced air to escape from the pressure vessel. Once the inside vessel temperature reached 70 °C, the steam supply was stopped and the outlet at the bottom flange cover was closed. Immediately, the anhydrous vapour ammonia was directly loaded into the pressure vessel through the booster pump. This mixture of EFB and ammonia in the vessel was heated using the electrical jacket once the ammonia to EFB loading ratio was attained. The pretreatment conditions for the critical parameters were according to the

optimised conditions obtained using the laboratory scale set up and was further tuned for the best operating conditions. Pretreatment process stops by releasing ammonia into the water container underneath the pretreatment vessel, preventing ammonia from escaping to the environment. The vessel was allowed to cool for a while until safe to open, and EFB samples were unloaded for drying purposes, letting the ammonia residue evaporate overnight. The pretreated samples were labelled according to the position of cylinder basket filter as B1-AAPB-PES, B2-AAP-PES, and B3-AAP-PES, respectively. The schematic diagrams for the configuration of both laboratory and pilot scale pretreatment vessels are shown in Fig. 2(b).

Chemical Composition Analysis of EFB

The chemical composition analysis was performed on all samples of untreated and pretreated EFB according to the Laboratory Analysis Protocol (LAP) developed by the National Renewable Energy Laboratory (Golden, CO, USA). All samples were subjected to a two-stage extraction process in Accelerated Solvent Extraction (ASE, Dionex 350, Sunnyvale, CA, USA) with water and 95% methanol as solvents for the respective extractions in the protocol. The extractives from water and ethanol extractions, including non-structural sugar, inorganic compound, nitrogenous compound, and waxes, must be removed to avoid disturbance in the structural carbohydrates and lignin determinations during acid hydrolysis. The acid hydrolysis was a two-stage process begins with a higher concentration of 75% HCl, followed by a subsequent with 4% HCl. Then, the hydrolysate was drawn and filtered for monosaccharides content analysis using high-performance liquid chromatography (HPLC) (Dionex Ultimate 3000, Thermo Scientific, Waltham, MA, USA). The remaining hydrolysate was collected *via* vacuum pump as acid-soluble lignin, and the residue was dried, weighed, and recorded as acid-insoluble lignin.

High-Performance Liquid Chromatography Analysis

The HPLC method was used to analyze the concentrations of the monosaccharides, such as glucose, xylose, and arabinose, in the hydrolysate samples obtained from the chemical compositional analysis. The HPLC device was equipped with an autosampler and refractive index (RI) detector (RefractoMax 520, ERC, Germany). Rezex ROA-Organic acid column (300 mm × 7.8 mm, Phenomenex, USA) with guard column (50 mm × 78 mm) was used for the separation of monosaccharides. The column temperature was maintained at 60 °C, and 0.005 M sulphuric acid (filtered and degassed) was used as the mobile phase with a flow rate of 0.6 mL/min and 10 µL injection volume. The standard curve was generated using different concentration of analytical grade sugar mixture (glucose, xylose, and arabinose).

Furfural, hydroxymethyl furfural (HMF), formic acid, acetic acid, and acetamide content were analyzed from the pretreatment hydrolysate. Rezex ROA-Organic acid column (300 mm × 7.8 mm, Phenomenex, USA) and C18 column (Phenomenex, USA) were used to separate organic acids and furfural, respectively. The column temperature was maintained at 40 °C using 0.005 M sulphuric acid (filtered and degassed) as the mobile phase with a flow rate of 0.6 mL/min and 20 µL injection volume.

Structural Carbohydrate Content

The structural carbohydrate content in the EFB samples was determined based on the concentration of monosaccharides in the hydrolysate samples using the following equations:

$$Glucan\ content\ (\%) = \frac{0.9\ \times\ g\ of\ total\ release\ glucose}{Sugar\ recovery\ standard\ of\ glucose\ (g)}\ \times\ 100\%$$

$$Xylan\ content\ (\%) = \frac{0.88\ \times\ g\ of\ total\ release\ xylose}{Sugar\ recovery\ standard\ of xylose\ (g)}\ \times\ 100\%$$

$$Arabinan\ content\ (\%) = \frac{0.88\ \times\ g\ of\ total\ release\ arabinose}{Sugar\ recovery\ standard\ arabinose\ (g)}\ \times\ 100\%$$

where 0.9, 0.88, and 0.88 are the conversion factor of glucose, xylose, and arabinose to the equivalent molecular weight of glucan, xylan, and arabinan, respectively.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) analysis on untreated EFB, pretreated EFB, and unhydrolyzed pretreated EFB samples were performed using a Nicolet 6700 FTIR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with the Attenuated Total Reflectance (ATR) method. All samples were scanned from 4000 to 400 cm^{-1} at a wavelength resolution of 2 cm^{-1} and recorded in transmission mode. The aim was to determine the lateral order index (LOI), total crystalline index (TCI), and hydrogen bond index (HBI). The LOI, TCI, and HBI were obtained using height absorbance ratios at 1437/899, 1378/2900, and 2208/1330, respectively.

Brunauer Emmett Teller Analysis

Brunauer Emmett Teller (BET) surface area, total pore volume, and average pore diameter of PES, OAAP-PES, and AAPB-PES were analyzed using the benchtop ChemBET PULSAR TPR/TPD analyser (Quantachrome Instruments, USA) with adsorption-desorption of nitrogen. The sample analysis was conducted using liquid nitrogen at temperature of 77.35 °K. The specific surface area was calculated from the adsorption isotherms changes in pressure using pressure transducers by the BET method, while the volume of the pore was estimated from the volume of nitrogen held at the highest relative pressure (P/P_o).

Scanning Electron Microscopy

Morphological changes analysis of PES, OAAP-PES, and AAPB-PES were performed using scanning electron microscopy (SEM) (EVO MA10, Carl Zeiss, UK). All samples were freeze-dried prior to scanning analysis. The samples were positioned on aluminium stubs and sputter-coated with gold using sputter coater system (Model Q150 RS, Quorum Technologies, UK). The result was observed at a magnification range of 250 to 5000X. Elemental analysis was performed using an energy-dispersive X-ray (EDX). The X-ray map was recorded based on the penetration of electron beam into sample.

Enzymatic Hydrolysis

Sugar conversion for both untreated and pretreated EFB were assessed through enzymatic hydrolysis process using the NREL standard protocol (LAP-009). The conditions of enzymatic hydrolysis used were at 50 °C in an incubated shaker (Infros HT Ecotron, Switzerland) at 150 rpm for 48 h. The enzymatic hydrolysis condition was optimized by the method of Farahin Abdul Rahman *et al.* (2018). All samples were loaded

with a low glucan mass equivalent to 1% (w/v) glucan loading suspended in 20 mL scintillation vials with 50 mM citrate buffer (pH 4.8), loaded with enzymes (Novozymes, Denmark) of Cellic CTec2 cellulase in 15 FPU/g of glucan and combined with Cellic HTec2 hemicellulase at a ratio of 1:1 (v/v). The enzyme activity for Cellic CTec2 was 142 ± 2.97 FPU/mL (279.32 ± 1.10 mg/mL protein concentration). Enzymatic hydrolysis process was ended by immersing the hydrolysis vial in cold ice for 60 min. The process causes enzyme activity to cease quickly, making it possible to continue separating the hydrolysate and unhydrolyzed EFB by centrifuging at 7000 rpm for 10 min. The hydrolysate samples were filtered through 0.22 μm Whatman membrane syringe filter, and the concentration of the sugar were determined by HPLC using the same conditions as describe above. Sugar conversion of glucan and xylan were represented in percentages using the following equations:

$$Glucan\ conversion\ (\%) = \frac{0.9 \times V \times glucose\ concentration}{\%\ of\ glucan\ composition \times dry\ weight\ sample} \times 100$$

$$Xylan\ conversion\ (\%) = \frac{0.88 \times V \times xylose\ concentration}{\%\ of\ xylan\ composition \times dry\ weight\ sample} \times 100$$

V: $volume\ of\ enzymatic\ hydrolysis\ reaction$

RESULTS AND DISCUSSION

Moisture Content of EFB Samples

The EFB produced in the mill was wet and warm (40 to 45 °C), with bunches and shredded strands. The warm touch of EFB is attributable to the sample being taken after sterilization and stripping of fresh fruit bunches in the oil palm mill. The initial moisture content of the UE, PE, and PES showed a range of moisture content from 59% to 65%. The UE had the highest moisture content, which was due to the influence of oil content in the fibre compared to the PE and PES. Once recorded, the EFB was securely sealed and transferred to the Universiti Kebangsaan Malaysia (UKM) laboratory. No significant amount of water was lost from EFB during the transfer process. Although the moisture attempted to evaporate, the EFB was kept inside the sealed bag, thereby trapping the moisture, such that the water would be re-absorbed by the EFB. The initial moisture content recorded were 64.6%, 62.6%, and 59.4% for UE, PE, and PES. Approximately 12 h after samples collection, the UE, PE, and PES batches showed 62.8%, 61.6%, and 58.3% moisture content, respectively.

The moisture content decreased with water removal from the EFB *via* evaporation as the time exposure increased. After 24 h, the moisture content for both UE and PE was approximately 35% to 40%. However, after 48 h, it decreased to 26.4% and 20.3% respectively (Fig. 3).

Meanwhile, PES showed a significant drop in moisture content to 12.1% at 48 h. The significant difference was merely due to the loose structure of shredded EFB, making it have more surface area to dry off. For the next 60 and 72 h, the PES showed stagnant moisture content at 9.4% and very minimal reduction until the 96th h. In contrast, the UE

and PE took longer than 84 h to reach below 10% and maintain its moisture content. The moisture content inside EFB had reached equilibrium, where the EFB neither gained nor lost any more moisture due to the surrounding environment factors: humidity and temperature. The remaining moisture inside the EFB can be held either as a vapor incorporated in the cell wall cavities or as a compound bound within the cell wall structure. Figure 3 shows the decreasing trend of moisture content for EFB over 96 h.

Moisture content is the most cardinal parameter before particle size reduction. The EFB with high moisture content makes the structure soft and resistant to grinding. In addition, this study observed that EFB with less exposure during drying was contaminated easily with rot fungi within 24 h of the process was worse for EFB in whole bunch size because the stranded fiber held closely together and trapped the moisture favoring fungal growth (Kamcharoen *et al.* 2014; Hau *et al.* 2020). Therefore, it is impossible to handle EFB in large amounts and have it prepared in the laboratory unless using industrial equipment for drying.

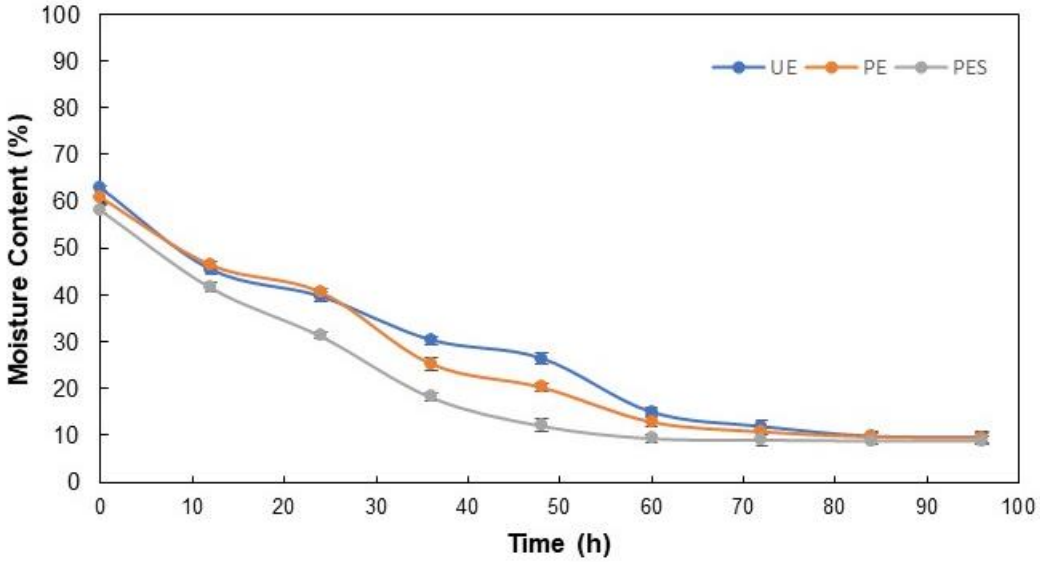

Fig. 3. The profile of moisture contents of different EFB at UKM processing laboratory before pretreatment

Morphology Appearance of Untreated and Pretreated EFB

Pretreated EFB showed a very distinct physical appearance compared to untreated EFB. The shape remained the same, but the color changed slightly to dark brown, and a powerful ammonia residue odor was detected. Figure 3 shows the physical structure of untreated and pretreated EFB under different conditions. The dark brown color was caused by the lignin condensation and re-localization process that increased the residual lignin to the surface of the solid particle (Abdul *et al.* 2016; Latif *et al.* 2018). The significant presence of residual lignin contributes to the browning effect. The study reported that lignin modification (demethylation) occurs at high temperature and pressure by melting, coagulation, and reprecipitation of cellulose fibers (Syed Abdullah *et al.* 2009). Upon completion of pretreatment, the pretreated EFB was put to rest and dried under a fume hood to release all the ammonia residue. The moisture content of pretreated EFB was found in the range of 45% to 65%, indicating the loosening of the cell wall structure and showing that the pretreatment works on EFB. The AAP-PES and AAP-PESG (also the control group) showed an even distribution of darker tone, while AAP-UE and AAP-PE showed

uneven darker tone distribution. The ammonia may not penetrate and react with the entire fiber strand due to the bunch structure. Figures 4(b) of AAP-PE shows some lighter color of the internal structure, which indicates the pretreatment may not completely cover the entire bunch. If the EFB was found dry in the pressure vessel, the ammonia failed to penetrate the cellulose structure, thus giving off the ammonia effect. The moisture inside the EFB acts as a competing catalyst for breaking the acetyl bond and solubilizing the ammonia (Bals *et al.* 2010; Zhao *et al.* 2020). As a result, the AAP had made the physical characteristics of EFB to be more pliable, which increased the digestibility during enzyme hydrolysis.

Fig. 4. Physical structures of: (a) untreated EFB, and (b) pretreated EFB
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Laboratory Scale Experiment

Structural constituent analyzes of morphologically different EFB samples

The analysis of carbohydrate contents in EFB is the most important value for the sugar conversion. In general, untreated EFB and all pretreated EFB samples did not show significant changes in the structural carbohydrate contents of glucan, xylan, and arabinan (Elgharbawy *et al.* 2018). This finding of unchanged sugar composition was supported by previous studies, which validated the promising use of AAP (Teymouri *et al.* 2005; Lau *et al.* 2010; Harun *et al.* 2015; Abdul *et al.* 2016). The main objective of performing this pretreatment was to preserve all the structural components and utilize the EFB as much as possible. Figure 5 shows that the native EFB is composed of 36.8% glucan, 18.7% of xylan, and 2.4% of arabinan; an almost similar composition was found in AAP-UE, AAP-PE, AAP-PES, and AAP-PESG for their respective components. This finding shows that the mechanism of ammonia pretreatment would result in swelling and delignification effect towards cellulose, thus preventing the lignin from absorbing cellulases and beneficial

during enzymatic hydrolysis. Typically, the lignin fragments are linked to the carbohydrate fragment in the lignin-carbohydrate complex *via* the ether and ester linkages. Through this AAP, the glucan composition remained constant due to the dry nature condition of the pretreatment that prevented the loss of holocellulose during pretreatment (Chundawat *et al.* 2010; Yoo *et al.* 2011).

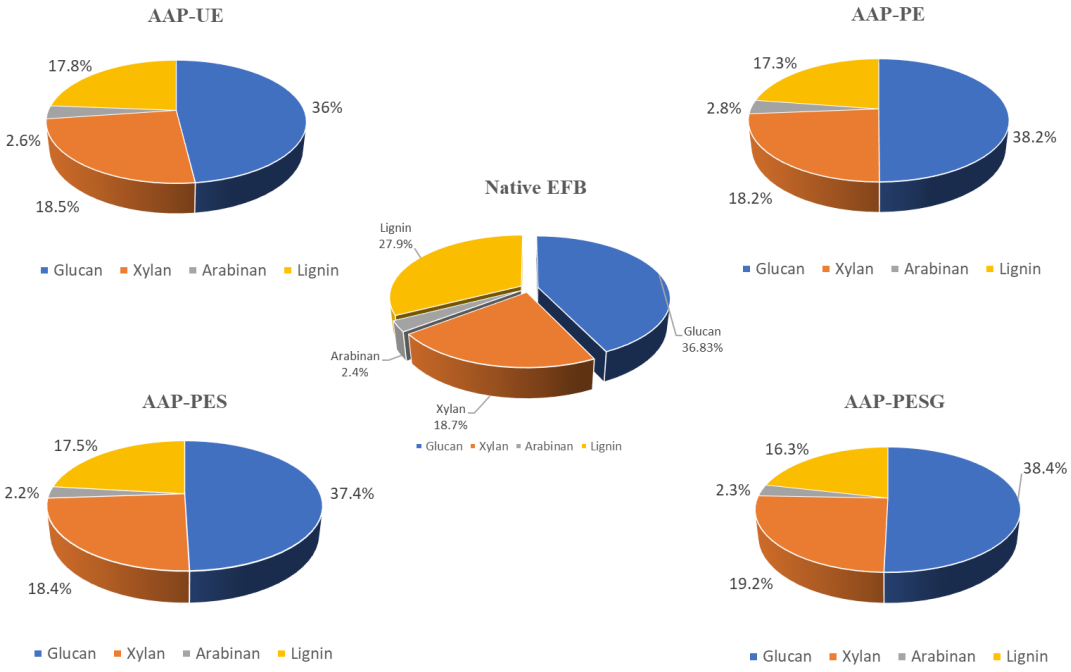

Fig. 5. Main structural compositions of native and morphologically different pretreated EFBs
U UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Ammonia had cleaved the lignocellulosic chemical linkages, particularly the lignin-carbohydrate complex (LCC). These can be seen through the disappearance of carboxylic linkage and the carbonyl linkage in Fourier transform infrared spectroscopy (FTIR) after AAP (Sun and Cheng 2002). This study found that the content of lignin was significantly reduced after pretreatment. Native EFB recorded 27.9% of lignin. Lignin contents of 17.8%, 17.3%, 17.5%, and 16.3% were obtained for AAP-UE, AAP-PE, AAP-PES, and AAP-PESG, respectively, which is approximately 40% of lignin loss. Pretreatment had caused the detachment of lignin and re-deposited on the surface, which dissolved into the extractive component of pretreated EFB (Harun *et al.* 2015; Latif *et al.* 2018). The re-deposited lignin can be found in acid-soluble lignin that is significantly shown in the increment of total extractives. AAP-PESG showed the highest lignin loss compared with other pretreated EFBs, mainly due to more surface area being exposed. However, the lignin loss from long fibres, such as AAP-PES, only showed 1% less than AAP-PESG. Thus, this insignificant value does not reflect the need for grinding the EFB into a smaller size.

Liquid extractive analysis of morphologically different EFBs

The liquid extractives contain extraneous components from the two-stage extraction process apart from the insoluble cell wall based on the soluble components namely, non-structural sugars, debris, and protein. Meanwhile, the ethanol extraction

would remove waxes, fat acids, phenolic substances, and chlorophyll (Tan *et al.* 2016). Extractives must be removed to avoid cross reaction, with acid hydrolysis that could be a part of the components in acid insoluble. In Fig. 6, all conditions of pretreated EFB showed an increase in total extractive component compared with native EFB, which only showed 13.4% of total extractives.

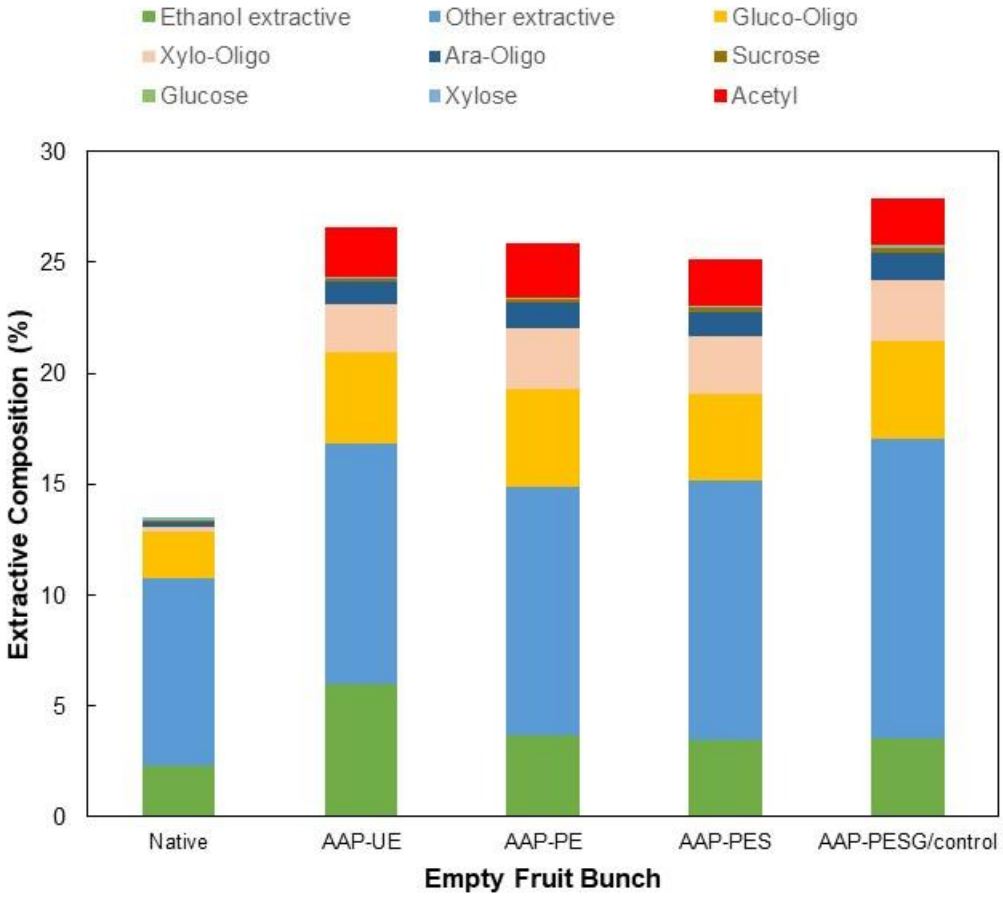

Fig 6. Extractive compositions of native and morphologically different pretreated EFBs
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Generally, more than 90% of extractive components were extracted after being pretreated. The increase of extractive components demonstrates that the AAP process had successfully broken down the external structure of the cell wall by dignifying and exposing the cellulose and hemicellulose structures. This finding was similar in any AAP using various types of biomasses because of the dry-to-dry process nature (Harun *et al.* 2013; Shao *et al.* 2013: Abdul *et al.* 2016; Mokomele *et al.* 2018). Furthermore, AAP-UE showed a distinct increase in ethanol extractive because of the oil content present in the EFB extracted. AAP-PESG/control was the only EFB that was pretreated at a standard laboratory size of 2 mm, yielding the highest total extractives, similar to the findings reported in other studies (Lau *et al.* 2010; Abdul *et al.* 2016). Moreover, AAP-PE and AAP-PES were almost identical in their yield of the respective extractive component due to a similar group of pressed EFB, except that AAP-PES had the loose free structure. It is expected to have a different composition, but due to chemical composition protocols, the results may represent a small fraction of the pretreated EFB. Nevertheless, the real

difference of AAP-PESG was only the other extractive, which was due to higher lignin removal that can be extracted on smaller particle size fibers.

Enzymatic hydrolysis of different morphological EFB

The enzymatic hydrolysis process in this study was run at 1% glucan loading because mixing and mass transfer at lower glucan loading had not significantly affected the hydrolysis performance. Mass transfer efficiency is due to the water content that acts as a medium for enzyme reactions. In a 1% hydrolysis run, the water content is much higher than the substrate content; thus, it gave an added advantage in the process of diluted hydrolysis. Figure 7 shows a sugar conversion profile from zero to 72 h of glucan and xylan for all different EFB morphology where the pretreated EFB was significantly different from the untreated EFB. On average, the conversion has increased more than double after being pretreated with anhydrous ammonia except for AAP-UE, and this finding was found similar with other work (Teymouri *et al.* 2005; Abdul *et al.* 2016), both using AFEX pretreatment on EFB. The samples PE, PES, and PESG showed 33.0%, 33.0%, and 33.8% of glucan conversion, respectively, while only 23.5% from UE after 72 h. Findings also showed 17.8%, 20.8%, 22.0%, and 22.5% xylan conversion for UE, PE, PES, and PESG. Both glucan and xylan conversions from UE showed much lower conversion than others due to the oil content inside the fiber that prevents water absorption. The increase in sugar conversion shows that the pretreatment method did have an effect in facilitating the digestibility of carbohydrate content. As predicted, AAP-PESG converted the highest amount of glucan and xylan at 66.5% and 44.7%, followed by AAP-PES (64.4% of glucan, 41.9% of xylan), AAP-PE (63.5% of glucan, 39.2% of xylan), and lowest conversion by AAP-UE (29.5% of glucan, 18.2% of xylan). However, the enzymatic hydrolysis finding in this study was not comparable to the previous studies (Abdul *et al.* 2016; Farahin Abdul Rahman *et al.* 2018), which reported a higher glucan conversion at 90% after 72 h of hydrolysis time. The pretreatment in this study may require some process improvement and adjustment (optimization) according to the current resources (EFB) to increase the extents of glucan and xylan conversion. All ammonia-based pretreatment showed a promising result on retaining the sugar inside the pretreated biomass, which eventually increases the total sugar yield during enzymatic hydrolysis (Bals *et al.* 2010; Farahin Abdul Rahman *et al.* 2018).

Theoretically, the size of the EFB will affect the performance of enzymatic hydrolysis. The smaller the size of the EFB, the larger the surface area of the EFB will be exposed to the enzyme digestibility, which will increase the extent of sugar conversion (Wang *et al.* 2022).

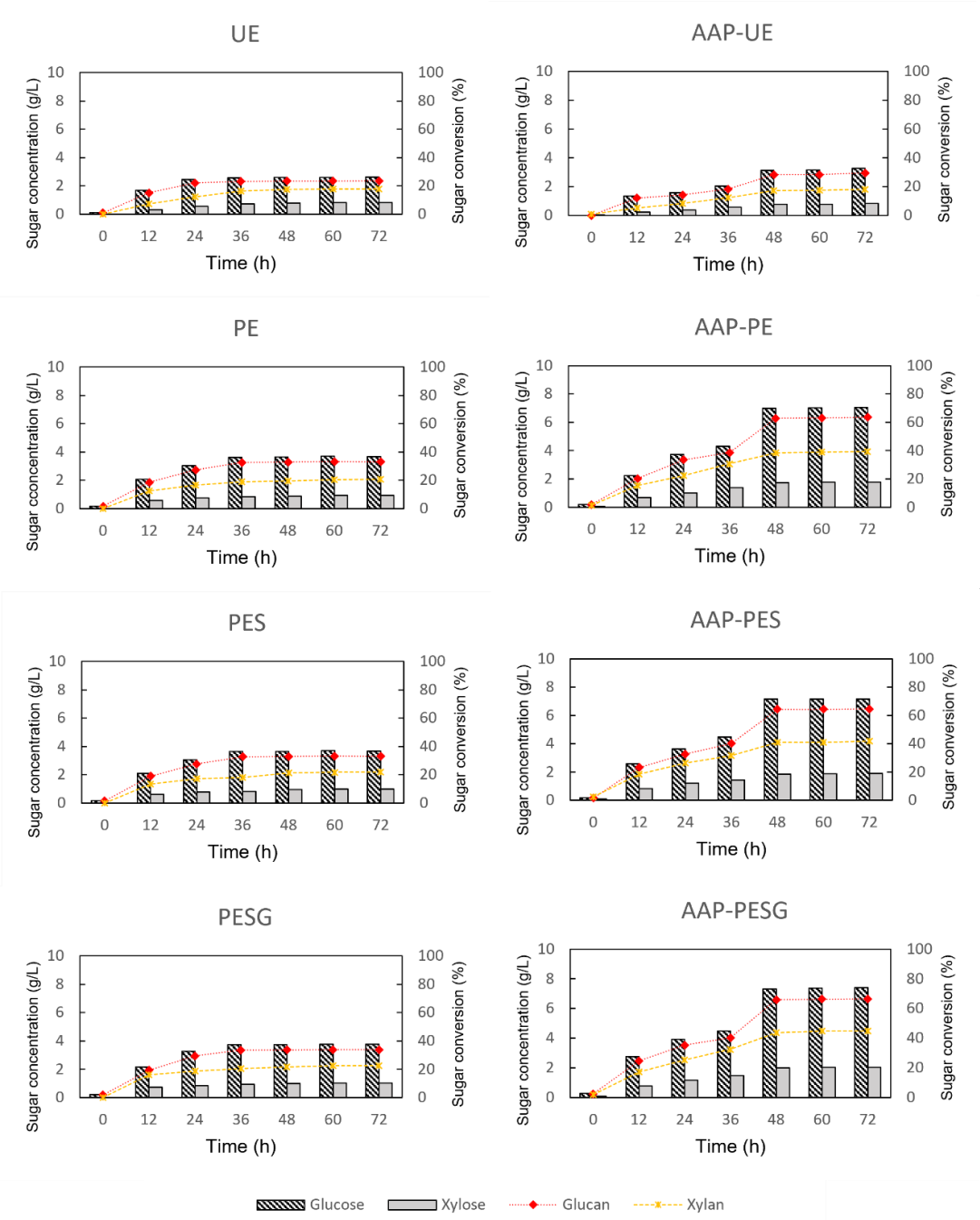

Fig. 7. Enzymatic hydrolysis profile on morphologically different EFBs of native and pretreated UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

This study showed a similar trend as the theory; however, AAP-PE and AAP-PES showed insignificant differences compared to AAP-PESG, which relatively was ground to a significantly smaller size (2 mm). Thus, the size reduction showed no increased pretreatment effectiveness when measuring sugar conversion. The sugar conversion from AAP-PE and AAP-PES showed a comparable value to AAP-PESG. These findings could be a strong justification of larger biomass size producing high sugar conversion but lower energy consumption during biomass size reduction process, which may increase the better

biorefinery process and economic value of EFB. Some researchers reported that EFB should be soaked in detergent overnight to prevent fungal contamination and then washed the next day to remove oil and dirt before pretreatment (Shamsudin *et al.* 2012). The concept is ideal for a small scale, but the industry would not favor practicing additional steps. This process led to lower sugar recovery yield at the end of enzymatic hydrolysis due to some sugar solubilization during the soaking and washing process.

Fourier transform infrared spectroscopy of morphologically different EFBs

The purpose of FTIR analysis in this context was to show LOI and TCI indices that represent the crystallinity ratio, and the HBI was carried out to show the hydrogen bonding between certain hydroxyl groups in cellulose. The LOI was obtained from the peak-height 1437/899 absorbance ratio, where 1437 cm^{-1} is associated with crystalline cellulose I and 899 cm^{-1} is associated with amorphous cellulose. Likewise, the TCI ratio given by 1378 cm^{-1} is attributed to CH deformation and 2900 cm^{-1} associated with CH and CH_2 stretching. Thus, TCI ratio was proportional to the crystallinity degree of the fiber cellulose. Finally, the HBI ratio was determined using wavelengths 3308 and 1330 cm^{-1} (Rafidison *et al.* 2020). Table 1 shows LOI, TCI, and HBI of untreated, pretreated, and unhydrolyzed (UH) pretreated EFB.

Table 1. LOI, TCI, and HBI Indices Ratio from FTIR Spectra

Samples	LOI	TCI	HBI
Crystallinity Indices	**(1437/899 cm^{-1})**	**(1378/2900 cm^{-1})**	**(2208/1330 cm^{-1})**
UE	0.92	1.09	0.79
AAP-UE	0.95	1.12	0.86
UH-AAP-UE	0.95	1.14	0.69
PE	0.94	1.07	0.75
AAP-PE	1.07	1.17	0.82
UH-AAP-PE	1.14	1.22	0.76
PES	0.86	1.1	0.55
AAP-PES	0.98	1.26	0.84
UH-AAP-PES	1.15	1.31	0.68
PESG	0.95	1.09	0.66
AAP-PESG	1.11	1.22	0.92
UH-AAP-PESG	1.17	1.33	0.76

UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

The values for all samples increased after AAP, except that the UE samples showed no changes. The increased value of LOI, TCI, and HBI indicated that pretreatment increased the structure of the crystal in the pretreated EFB (Lee *et al.* 2014); however, AAP-PESG showed a slightly higher ratio value compared to UE, PE, and PES. UH-AAP-

PE, UH-AAP-PES, and UH-AAP-PESG also showed an increase in LOI and TCI due to more crystalline in the structure that failed to be hydrolyzed by the enzyme. The UH samples contained the highest crystallinity degree and highly ordered cellulose structure because the AAP action removed the amorphous portion of the lignin and hemicellulose (Oh *et al.* 2005; Rafidison *et al.* 2018). The conditions of PESG may have influenced the pretreatment effect, but it still did not significantly affect the pretreatment process.

Determination of optimal AAP condition – laboratory scale

The biomass used in this experiment was PES, as it is the best and the most economical morphological condition to practice. The aim was to determine the best optimal condition of AAP on PES. Figure 8 shows the effects of residence time, the ratio of moisture content, temperature, and ammonia loading on the sugar concentration and sugar conversion during 1% enzymatic hydrolysis. Experimental data in Fig. 8a show different residence times from 15 to 75 min of pretreatment time. The 30-min residence time achieved 70% glucan conversion and showed no significant increase at 45, 60, and 75 min, while 15-min residence time only gave 53.8% glucan conversion. Thus, 30 min was selected for the subsequent experiment as the shortest pretreatment time given at the best conversion.

Moisture plays an essential role in AAP, as it binds the biomass and ammonium ion and forms hydrogen bonds with cellulose to cause swelling in the crystalline cellulose structure. Figure 8b shows different moisture ratios to the dry weight of PES. The previous ratio by Lau *et al.* (2010) was a 1:1 ratio, and this study found that the 2:2 ratio resulted in a better glucan conversion of 88.3% compared to 71.4% at a 1:1 ratio. The higher conversion may be due to more moisture inside PES to hold and react with ammonia. However, as the moisture ratio increased, it showed no significant effect on conversion as the biomass may have reached maximum ability to absorb more moisture.

The different temperature effects of AAP are shown in Fig. 8c. Glucan and xylan conversions increased with increasing temperature from 95 to 155 °C, at which they maximized at about 89% and 67%. The increase in temperature, causing an increase in chemical reactions, cleaving the internal bonds within the PES. The sugar conversion showed a decreasing pattern when heating more than 175 °C, and the appearance of AAP-PES at 215 °C showed a slight burn on some of the fiber. A higher temperature than 175 °C can cause depolymerization and hemicellulose solubilization, which could account for the decline in conversion (Zhao *et al.* 2014, 2020). At extreme temperatures during alkaline pretreatment, higher temperature may convert the xylose to furfural or other degradation products. Pretreatment at 135 °C in this study has shown optimal conversion; further temperature increases did not benefit the process significantly and will cause unnecessarily higher energy consumption.

Figure 8d shows the effect of different ammonia loadings on sugar conversion of AAP-PES. The glucan and xylan conversion increased significantly with ammonia loading from 0.5 to 1.0 and remained about 89% of glucan conversion when ammonia loading was at 1.5 to 2.5 ratio to dry biomass. The AAP-PES showed a great improvement in sugar concentration and conversion compared to PES (24% glucan and 18% xylan conversion). Even though ammonia can cleave the alkaline chemical bonds between lignin and hemicellulose, the higher ammonia loading can disrupt the lignin, causing large pores and deposition hemicellulose and lignin to cell corners and surface of the cell wall (Chundawat *et al.* 2010). In addition to this, anhydrous ammonia alone can also interact directly with cellulose for phase changes in the crystal structure from cellulose I to cellulose III (Mosier

et al. 2005). Moreover, the higher the ammonia loading, the higher the operating pressure involved with the hazardous chemical usage. Therefore, 1.0 ammonia loading was chosen for the most optimal condition on the operation AAP system.

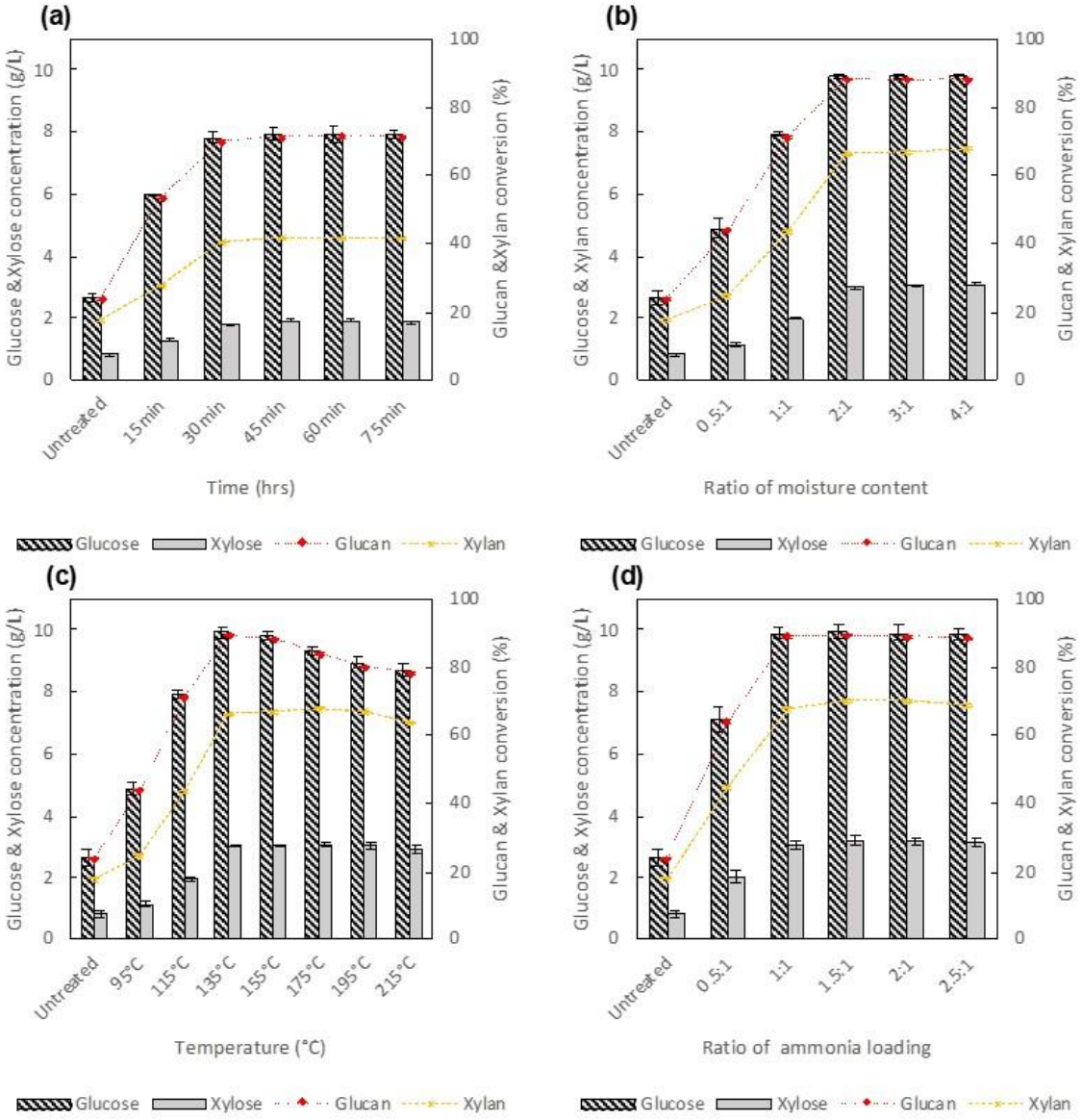

Fig 8. Effect of different pretreatment time (a), ratio of moisture content (b), temperature (c), and ratio of ammonia loading on glucose/xylose concentration and glucan/xylan conversion
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Performances of 1%, 3%, and 6% glucan loadings of optimized AAP

Figure 9 shows enzymatic hydrolysis for 72 h from low to high glucan loadings (1%, 3%, and 6%) per dry weight of EFB for AAP-PES. Although the optimum enzymatic hydrolysis time was 48 h, the goal of this study was to determine if the hydrolysis profile at various glucan loading will produce differently until 72 h of hydrolysis time. After 72 h of hydrolysis time, the glucose and xylose concentrations produced at 1% glucan loading was 9.79 g/L and 3.99 g/L, respectively. The concentration increased to 26.8 g/L and 12.0 g/L at 3% for glucose and xylose. At 6%, the hydrolysate concentration yielded 47.9 g/L

for glucose and 19.1 g/L for xylose. The glucose and xylose concentrations increased because the glucan and xylan contents were more hydrolyzed than low glucan loading hydrolysis.

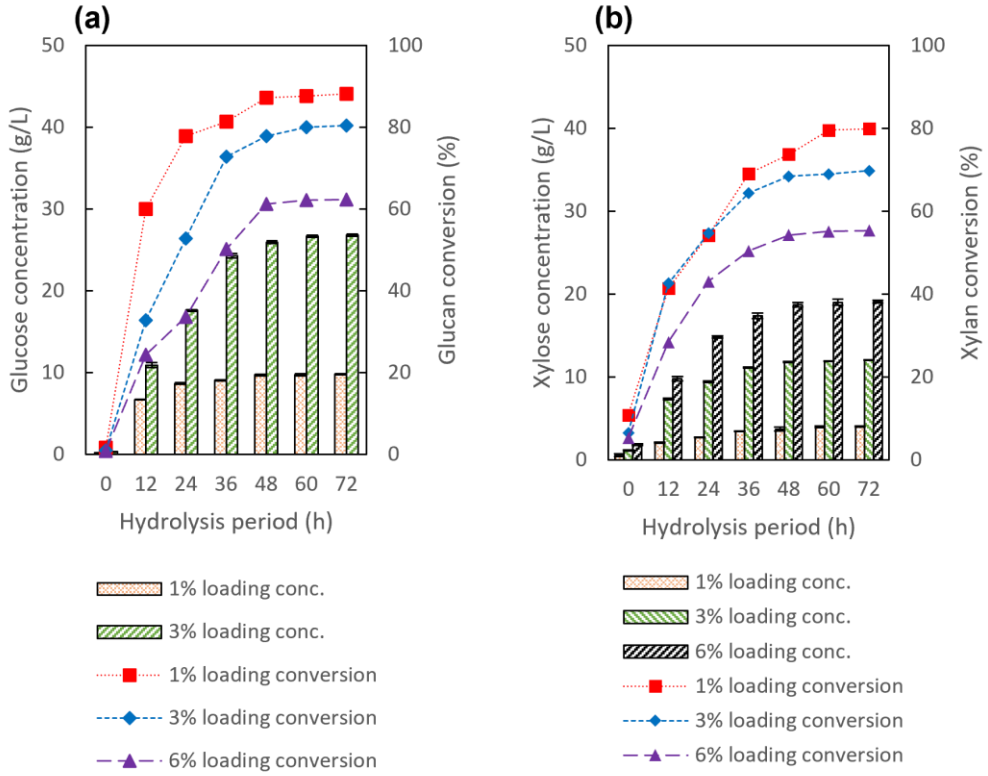

Fig. 9. Enzymatic hydrolysis profile of (a) glucose concentration and glucan conversion, (b) xylose concentration and xylan conversion at 1%, 3%, and 6% glucan loadings for 72 h
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

The sugar conversion at 1% glucan loading was at 88%; then it dropped to 80% and 62% for 3% and 6% glucan loadings, respectively. Higher glucan loading required less water ratio and made the hydrolysis process slightly dryer than low glucan loading. Water acts as a medium for solubilization and mass transfer; the rate of enzyme movement decreased with less water during hydrolysis, slowing the absorption of the enzyme process to the cellulose fiber. The decline in conversion shows that the rate of cellulose digestibility dropped at higher glucan loading. The purpose of experimenting with a high glucan loading (3% and 6%) was to mimic the industrial activities and understand the behavior of the concentrated enzymatic process. The results show that the glucan and xylan conversion reached the maximum yield as early as 48 h (as expected) of hydrolysis and did not increase significantly even for higher glucan loading at 72 h. Nevertheless, at 3% glucan loading, the yield was found at 80% glucan and 69% xylan, which was still at a favorable level and comparable with other previous reported work (Farahin Abdul Rahman *et al.* 2018).

Scale-up Process

The scale-up study of AAP was demonstrated at oil palm processing mill in KKS Tennamaram. The pressure vessel size has increased from 1 L to 22 L and can load up to 2.5 kg of dry weight biomass (PES). The process was attempted under the same parameter as the lab scale and PES as the biomass. In test 1 of AAPB, the temperature and pressure

were carefully monitored to ensure the stability of the pretreatment process. Pretreatment ended by releasing the ammonia vapor into the storage reactor. The cooldown process continued until the pressure vessel was safe to open. Then, PES was unloaded to the drying rack and was found dry in all cylindrical basket filters with moisture content recorded in a range of 15% to 25% instead of 45% to 65% based on the laboratory experiment. Dry EFB indicates that the pretreatment was inefficient because less ammonia can penetrate the fiber for the binding and reaction. The PES could be dried during the heating process inside the pressure vessel and during the pretreatment time. Quick moisture loss may be due to a low moisture content ratio; thus, test 2 on AAPB was conducted by increasing the moisture loading to 3:1 of dry weight biomass. The new strategy for more efficient pretreatment continued as other parameters remained the same. The top and bottom baskets were found sufficiently wet as an indicator of a successful pretreatment process. Unfortunately, the middle basket was still dry (25% to 40% moisture content) with slight improvement. The investigation was performed, and it was found that the heating element at the middle part of the pressure vessel was heated at higher voltage, causing an increase in temperature at the center.

To have an optimum process, the pretreatment temperature setting was reduced to 115 °C for a uniform heating distribution. Finally, the indicator of wet biomass was found in all baskets after pretreatment in test 3 of AAPB. However, evaluation from 1% glucan loading of enzymatic hydrolysis indicated that AAPB-T3 did not yield glucan and xylan conversions at optimum level and evenly. Figure 10 (a) shows that the second and third baskets yielded much lower conversion (less than 60% for glucan and 30% for xylan), suggesting insufficient ammonia during the reaction. Therefore, test 4 was conducted by doubling the ammonia loading for better distribution and reaction of biomass and ammonia. As a result, Fig. 10 (b) shows that the glucan/xylan conversion was increased to more than 70% and 50% for all baskets. Table 2 summarizes the parameter changes during the fine-tune activities of AAPB.

Table 2. Operating Conditions of Pilot Scale AAP System

Parameter	AAP	AAPB-T1	AAPB-T2	AAPB-T3	AAPB-T4
Time (min)	30	30	30	30	30
Temperature (°C)	135	135	135	115	115
Ratio: Moisture Content to Biomass	2:1	2:1	3:1	3:1	3:1
Ratio of Ammonia to Biomass	1:1	1:1	1:1	1:1	2:1
Pressure (Bar)	35	10	10	10	12

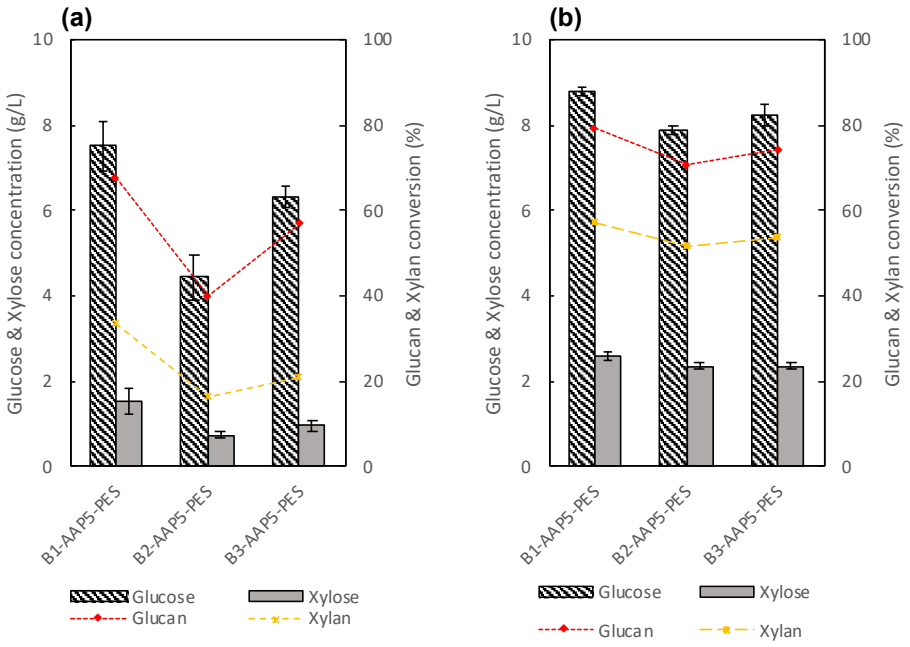

Fig. 10. Enzymatic hydrolysis of (a) AAPB-T3 and (b) AAPB-T4 at 1% glucan loading for each cylindrical basket filters
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Performances of AAP and AAPB on PES

Chemical composition

Table 3 describes the chemical compositions of PES, AAP-PES, UH-AAP-PES, and B1, B2, B3-AAPB-PES. In general, the compositions of the structural components of PES were made up of structural carbohydrates (58.3%) and Klason lignin (27.9%). The carbohydrates were composed of glucan (36.8%) and xylan (18.7%), respectively, as the main components, while arabinan content was only 2.7%. The ash accounted for 1.83%, and there were 13.8% of total extractives for the non-structural components of PES. The AAP-PES and all AAPB-PES showed no significant changes in structural carbohydrates than PES. For each sample, the glucan, xylan, and arabinan components remained approximately at 37%, 18%, and 2%, respectively. The AAP and AAPB prevented the loss of holocellulosic components during pretreatment due to the dry-to-dry process. Similar findings reported that using AFEX for various types of biomasses (rice straw, corn stover, bamboo, EFB, and sweet sorghum) yield 100% of structural carbohydrates after pretreatment (Teymouri *et al.* 2005; Li *et al.* 2010; Harun *et al.* 2013).

The AAP and AAPB processes showed consistency in chemical composition even though the process parameter was slightly different after fine-tune activities. However, the lignin composition between PES and AAP-PES showed significant change from 27.9% to 18.08%. AAPB-PES offered the same lignin reduction and showed that ammonia pretreatment successfully detaches the lignin. Even though AAPB was performed at lower pressure and temperature than AAP, the ammonia loading ratio was double, leading to more ammonia penetrating the cellulose, causing lattice transformation and crystal plane widening, known as a swelling effect (Bals *et al.* 2011) . This process led to breaking down the ester linkages and resulting in solubilization of lignin residue and re-deposition on the

biomass surface. In contrast, the composition of UH-AAP-PES from the enzymatic hydrolysis process showed 22.4% of structural carbohydrates with the chemical components were from the crystalline phase of carbohydrates that was not digestible by the enzyme. The 13.0% of lignin that is still available inside the cellulose fiber after pretreatment caused inefficiency during the enzymatic hydrolysis process, as lignin acts like a glue in the cell wall of EFB. Hence, this observation showed that it is challenging to hydrolyze 100% of pretreated fiber without removing the lignin.

Table 3. Chemical Composition of PES, UH-AAP-PES, and AAPB-PES

Type of Component in EFB	EFB Composition Analysis (% of EFB on DWB)					
	PES	AAP- PES	UH-AAP-PES	B1-AAPB-PES	B2-AAPB-PES	B3-AAPB-PES
1. Structural Carbohydrates						
Glucan	36.83 ± 0.01	38.86 ± 0.03	18.76 ± 0.21	37.41 ± 0.01	36.24 ± 0.03	37.01 ± 0.02
Xylan	18.71 ± 0.12	18.54 ± 0.09	3.64 ± 0.34	18.12 ± 0.17	14.9 ± 0.09	18.3 ± 0.04
Arabinan	2.7 ± 0.21	2.34 ± 0.04	0.38 ± 0.19	2.45 ± 0.19	0.56 ± 0.09	2.32 ± 0.07
2. Lignin	27.91 ± 0.11	18.08 ± 0.02	12.95 ± 0.23	18.30 ± 0.21	18.92 ± 0.11	18.81 ± 0.01
3. Non-structural Components						
Ash	1.83 ± 0.05	0.82 ± 0.09	1.03 ± 0.13	1.13 ± 0.09	1.07 ± 0.07	1.17 ± 0.39
Total extractive	13.87 ± 0.01	25.54 ± 0.13	21.34 ± 0.41	22.5 ± 0.11	20.01 ± 0.08	22.4 ± 0.03
Water Extractive						
Gluco- Oligo	2.08 ± 0.01	4.28 ± 0.11	2.36 ± 0.43	3.44 ± 0.03	2.37 ± 0.17	2.43 ± 0.13
Xylo- Oligo	0.24 ± 0.02	2.23 ± 0.01	0.89 ± 0.52	2.42 ± 0.01	2.31 ± 0.11	2.85 ± 0.07
Ara- Oligo	0.25 ± 0.09	1.14 ± 0.02	n.a	1.1 ± 0.06	0.89 ± 0.21	1.25 ± 0.08
Acetyl	n.a	3.16 ± 0.03	n.a	1.27 ± 0.01	1.64 ± 0.11	1.64 ± 0.11
Sucrose	n.a	n.a	n.a	n.a	n.a	n.a
Glucose	n.a	n.a	n.a	n.a	n.a	n.a
Fructose	n.a	n.a	n.a	n.a	n.a	n.a
Ethanol extractive	2.27 ± 0.08	2.26 ± 0.21	2.78 ± 0.13	2.54 ± 0.09	1.84 ± 0.19	2.76 ± 0.32
Other extractive	8.43 ± 0.13	12.47 ± 0.13	15.32 ± 0.32	11.73 ± 0.13	10.96 ± 0.13	11.47 ± 0.13

*n.a: not available
UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

BET surface area measurements

The surface area of PES, AAP-PES, and AAPB-PES was analyzed to determine whether the pretreatment process affects the structural morphology of the biomass. The BET surface area measurements revealed that AAP-PES and AAPB-PES resulted in a more porous material with a higher surface area than PES. The average surface area of AAP-PES was 12 times larger than PES, which were 386 and 31.5 m^2/g, respectively. AAPB-PES also showed a similar BET surface area trend a minimum of ten times larger than PES. From the results of this study, the increase of BET surface area and total pore volume were significantly influenced by the severity condition (temperature, pressure, and chemical addition) of pretreatment effect between the fibers (Md Yunos *et al.* 2017). The pore diameter in this study was significantly reduced from 113 Å of PES to 64.1 Å of AAP-PES. Typically, the initial pore structure has low porosity. However, after ammonia pretreatment, the swelling effect enhanced and widened the existing pores while creating many new ones. Additionally, the removal of lignin from the pretreated EFB can also contribute to the swelling effect. As more pores are created, the pore diameter decreases due to the narrowing effect caused by the sudden depressurization of ammonia. This leads to the development of micropore volume (Kumar *et al.* 2009; Chandra *et al.* 2012). The BET surface area, total pore volume, and average pore diameter are shown in Table 4.

Table 4. BET Surface Area of PES, AAP-PES, and AAPB-PES

	PES	AAP- PES	B1-AAPB-PES	B2-AAPB-PES	B3-AAPB-PES
BET Surface Area (m^2/g)	31.52	386.15	347.04	297.47	324.32
Total Pore Volume (cm^3/g)	0.089	0.6184	0.4482	0.2971	0.4317
Average Pore Diameter (Å)	112.9	64.1	51.7	40	53.2

UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

Scanning electron microscopy

Figure 11 shows the SEM images of the exterior surface morphology of PES, AAP-PES, and AAPB-PES. The SEM images reveal a significant physical change on the epidermal surface structures of PES (Figs. 11: 1A, 1B, and 1C), which appeared to be a rough surface, rigid structure, and numerous craters filled with spiky silica bodies. The finding was similar with all untreated EFB that has been studied before (Suraya Rosli *et al.* 2017). The silica bodies are essential in the growth of the plant cell, as they act as a defense mechanism for bacteria and fungus. These silica bodies were made up through sedimentation of soil minerals and silica polymerization during water uptake inside the plant cell wall, thus silica bodies were well distributed on the PES epidermal surface. However, the silica bodies together with lignin prevent the enzyme from digesting the lignocellulosic material.

AAP is the process improvement for PES to have higher enzymatic accessibility. Figure 11 in 2A (lab scale) and 3A (pilot-scale) showed significant changes on the surface morphology through the disappearance of silica bodies due to the re-deposition of lignin extractive. The removal of silica bodies exposed the craters making more silica pores available as empty cavities and increasing the surface area for the enzymatic reaction

(Zulkiple *et al.* 2016). According to Abdul *et al.* (2016), the exposed silica pores were caused by high pressure, high temperature, and ammonia that diffused into the epidermal layer and led to the swelling effect. The expansion of ammonia exerts a force on the internal structure and exposes the internal microfibril of PES. Figure 11 (2C) shows complete destruction and removal of the cuticle and silica bodies under 5000 magnifications. Furthermore, a noticeable globule on the surface of AAP-PES was observed, and it is known as the re-deposition of lignin after pretreatment. The AAP and AAPB removed a significant amount of hydrophobic lignin, silica bodies, and cuticle debris, causing the epidermal layer of PES to look ripped, peeled, and exposed to the inner microfibrils.

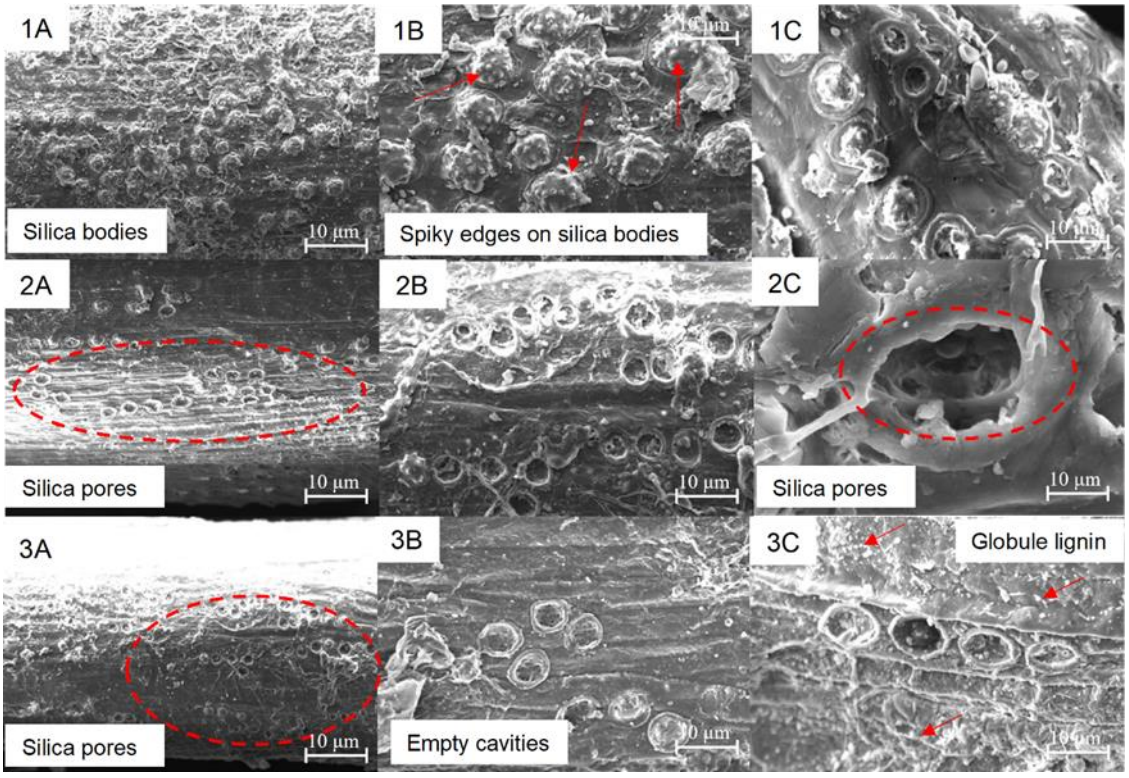

Fig. 9. Surface morphologies *via* SEM of PES (1A, 1B, and 1C), AAP-PES (2A, 2B, and 2C), and AAPB-PES (3A, 3B, and 3C) at 250X, 700X, 1000X, and 5000X magnifications

Inhibitor formation from the enzymatic hydrolysis

Inhibitors are commonly found during hydrolysis, resulting from the degradation of pentoses and hexoses. It is common to detect inhibitors from various pretreatment methods such as steam explosion, dilute acid pretreatment or another thermo-chemical method. In this study, the enzymatic hydrolysis of AAP-PES and AAPB-PES produced a few amounts of inhibitors and may restrict the growth of microorganisms during fermentation. There was very little concentration of (Table 5) furfural and 5-hydroxymethylfurfural (HMF) found in the hydrolysate. However, HPLC analysis confirmed that the concentrations found were too low and negligible. In contrast, acetic acid showed a slight increase in concentration from PES to AAP-PES and AAPB-PES. Acetic acid formation resulted from the saponification of the acetyl group in hemicellulose (Sari *et al.* 2021).

The most visible finding in this inhibitor study was the detection of acetamide. Acetamide was produced from ammonization during pretreatment that caused the reaction

of acetyl linkage within the cell wall (Jönsson and Martín 2016). The concentration of acetamide was not detected in PES but significantly produced 7.3 mg/g in AAP-PES. While acetamide in B1, B2, and B3-AAPB-PES were detected but observed to be lower. AFEX studies that used ammonia-based pretreatments on various biomass show similar detection of acetamide (Bals *et al.* 2019). The finding may result from two factors, excess ammonia added to the available acetate content in the PES, and the severity of pretreatment condition. The AAP was conducted by using liquid ammonia at 135 °C that reflected higher pressure operation, while AAPB used ammonia vapor at 115 °C, making a slightly lower pressure and safer operation. Thus, the difference between lab-scale and pilot-scale limited the amount of acetamide produced during pretreatment. Apart from this, AAP and AAPB made almost inhibitor-free materials, which are highly beneficial when converted to fermentable sugar.

Table 5. Inhibitors Present in the Hydrolysate of PES, AAP-PES, and AAPB-PES

	PES	AAP- PES	B1-AAPB-PES	B2-AAPB-PES	B3-AAPB-PES
Furfural (mg/g)	0.001 ± 0.05	0.003 ± 0.09	0.002 ± 0.21	0.003 ± 0.14	0.002 ± 0.17
Hydroxymethyl-furfural (HMF) (mg/g)	0.029 ± 0.01	0.142 ± 0.07	0.063 ± 0.01	0.048 ± 0.06	0.032 ± 0.01
Formic acid (mg/g)	0.034 ± 0.05	0.092 ± 0.01	0.061 ± 0.01	0.054 ± 0.09	0.058 ± 0.24
Acetic acid (mg/g)	0.83 ± 0.04	1.23 ± 0.21	1.06 ± 0.03	1.11 ± 0.03	1.04 ± 0.01
Acetamide (mg/g)	ND	7.3 ± 0.11	4.43 ± 0.18	3.31 ± 0.09	3.46 ± 0.07

UE: Unpressed EFB; PE: Pressed EFB; PES: Pressed and shredded EFB; PESG: Pressed shredded and ground EFB; AAP: Anhydrous Ammonia Pretreatment

CONCLUSIONS

1. This study showed promising improvements for the palm oil industry regarding the process of oil palm empty fruit bunch (EFB) utilization using anhydrous ammonia pretreatment.
2. The lab-scale anhydrous ammonia pretreatment (AAP) and pilot-scale (AAPB) processes successfully showed that EFB could be pretreated and well preserved. The main carbohydrate constituents, such as glucan (36.8%), xylan (18.7%), and arabinan (2.7%), remained intact and recovered from the pretreated cell wall structure while 35% to 40% lignin was rendered soluble.
3. Different morphologies of EFB (PE and PES) also demonstrated potential for utilization using the AAP method, which can simplify the industry process on a larger scale.
4. Even though AAPB was performed using ammonia vapor compared to liquid ammonia in AAP, the AAPB pretreated EFB was able to hydrolyze and give similar sugar conversion as to laboratory set-up.

5. Moreover, AAPB with minor severity pretreatment conditions minimized the production of inhibitors, creating more opportunity in biorefinery platforms.
6. The dual design of the AAPB pretreatment vessel offers the possibilities for continuous process and enables the recycling of anhydrous ammonia.
7. Thus, assessment of the lab to pilot scale performance of anhydrous ammonia pretreatment has provided good process understanding for future full scale operation design.

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