Transformation of oil palm fronds into pentose sugars using copper (II) sulfate pentahydrate with the assistance of chemical additive

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Keywords: inorganic salt, cellulose, hemicellulose, lignin.

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Abstract

Of late, the current trend of biomass valorization focuses on the transformation of lignocellulosic biomass into reducing sugars, so that the latter can be further used as building blocks in the production of value-added products such as biofuels and commodity chemicals. As such, researchers have established many pretreatment technologies in order to overcome the recalcitrance of the biomass, which is essential to ensure more efficient components utilization and subsequently sugars recovery. Among the chemical pretreatment methods, inorganic salt is considered to be a relatively novel but simple reagent that has been proven to offer comparable pentose (C5) sugar recoveries, mainly xylose and arabinose, in the hydrolysate as the conventional dilute acid hydrolysis pretreatment. Hence, this study investigated the effects of different concentrations (1.5 – 6.0% (v/v)) of hydrogen peroxide or sodium persulfate in facilitating copper (II) sulfate pentahydrate pretreatment for improving pentose sugar hydrolysis and recovery from oil palm fronds. The best result was observed when 0.2 mol/L of copper (II) sulfate pentahydrate was integrated with 4.5% (v/v) of sodium persulfate to recover 8.2 and 0.9 g/L of monomeric xylose and arabinose, respectively in the liquid fraction. On the other hand, an addition of 1.5% (v/v) of hydrogen peroxide yielded poorer result than sodium persulfate, in which a reduction of approximately 74% of total pentose sugars were observed as compared to using 4.5% (v/v) sodium persulfate. By using copper (II) sulfate pentahydrate alone (control), only 0.8 and 1.0 g/L xylose and arabinose, respectively could be achieved. The results were mirroring the importance of using chemical additives together with inorganic salt pretreatment of oil palm fronds. In conclusion, at 120°C and 30 min, an addition of 4.5% (v/v) of sodium persulfate during copper (II) sulfate pentahydrate pretreatment of oil palm fronds was able to attain a total pentose sugar yield up to ~40%.

1 Introduction

Lignocellulosic biomass mainly exist in the forms of agricultural wastes, energy crops, as well as forestry residues [1]. Biomass is currently exploited as a renewable source of fermentable sugars, which are considered as one of the main building blocks for bio-fuels and specialty chemicals. In Malaysia, up to 26.2 million tons of oil palm fronds (OPF) are produced annually, for each million ton of fresh fruit bunch (FFB) processed [2]. In practice, OPF are usually left to degrade as mulch in the oil palm plantations after pruning process, in order to improve soil properties and water retention [3]. However, this typical practice offers a limited value to the industry since OPF shows potential for sugar recovery with its chemical composition of 40-50%, 34-38%, and 20-21% of cellulose, hemicellulose, and lignin, respectively [4]. Furthermore, sugar recovery from OPF would provide an environmentally friendly alternative in biomass handling, instead of degrading OPF on the open land.

Nevertheless, cellulose, hemicellulose, and lignin form a complex structure which causes the biomass to be highly resistant to hydrolysis and microbial digestion [5]. Lignin, in particular, is a phenolic macromolecule which acts as the main physical barrier in the recalcitrance of biomass [6]. As such, Mosier et al. [7] necessitates the hydrolysis of the lignin structure during pretreatment process, in order to render the biomass more suitable for downstream fermentation. Recently, the use of inorganic salts such as KCl, NaCl, $CaCl_2$, $MgCl_2$, $CuCl_2$, $FeCl_3$, and $AlCl_3$ has been explored as one of the more novel ways of pretreating biomass [8-12], as compared to the conventional systems such as dilute acid and alkali pretreatment [6]. Trivalent salts appeared to have superior sugar recoveries, as $FeCl_3$ was able to selectively hydrolyze the hemicellulose from the corn stover [13]. Inorganic salt provides several advantages over the traditional dilute acid hydrolysis, namely lesser corrosiveness for certain salts [14] as well as the possibility of recycle and reuse of salt during pretreatment [15]. Among all the inorganic salts studied, $CuSO_4$ remains as one the least explored. Hence, in this study, the performance of copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) would be tested for sugar conversion from OPF. Leipner et al. [16] reported that inorganic hydrate salts such as $ZnCl_2.4H_2O$ and $FeCl_3.6H_2O$ caused cellulose dissolution by transforming the cellulose I to II. Furthermore, the relatively low cost of many hydrated metal salts coupled with their air/moisture insensitivity makes their use in large scale industrial processes viable [17].

One of the bottlenecks for most inorganic salt pretreatments, like dilute acid hydrolysis, is the need to operate at high temperatures (150 to 200°C) in order to achieve significant hydrolysis of hemicellulose. Previously, Diaz et al. [18] reported an improvement in sugar recovery up to 75% when hydrogen peroxide (H_2O_2) was added to alkaline pretreatment of rice husk. The rate of enzymatic hydrolysis was also accelerated by optimizing the concentration ratio of H_2O_2 and transition metal ions Fe^{2+} under room temperature [19]. Another oxidizing agent worth investigating is sodium persulfate ($Na_2S_2O_8$), which is commonly used for surfactant removal or groundwater remediation [20, 21]. Sulfate radicals ($SO_4^{-}\bullet$) which are generated by $Na_2S_2O_8$ have been reported to be stronger and more selective oxidants than the hydroxyl radicals ($OH\bullet$) which are produced from H_2O_2. Interestingly, transition metal ions were useful in aiding the efficient generation of radicals from $H_2O_2/Na_2S_2O_8$ [22, 23]. To the best of our knowledge, there have been little to no studies regarding the use of inorganic salt coupled with oxidizing agent for biomass pretreatment purpose. It was postulated that H_2O_2 and $Na_2S_2O_8$ were able to aid $CuSO_4.5H_2O$ hydrolysis of OPF by acting as delignification agents during inorganic salt pretreatment. Thus, the main objective of this study was to recover pentose sugars (xylose and arabinose) from OPF using $CuSO_4.5H_2O$ pretreatment under moderate conditions (120°C and 30 min), and also to evaluate the effects of H_2O_2 and $Na_2S_2O_8$ additives in enhancing sugar recovery during the pretreatment process.

2 Materials and Methods

2.1 Biomass Feedstock and Chemicals Preparation

Fresh OPF were obtained from an oil palm plantation, owned by Universiti Kebangsaan Malaysia and their leaflets were removed. A sugarcane press machine was used to extract the solid petioles residue, after that the petioles were sun-dried for 2 days. Next, these residues were ground in a pulverizer set at 6000-7000 rpm, in which the particles of size ≤0.5 mm were collected after passing through a mechanical siever. After that, the OPF were dried at 55°C for 48 h in an oven to remove any remaining moisture, and stored in a container with tight-fitting lid filled with desiccants at room temperature until further use. High grade monomeric sugars (99%) (D (+) glucose, D (-) xylose, and L (+) arabinose) were used for calibration of standard curves, while the $CuSO_4.5H_2O$, oxidizing agents H_2O_2 and $Na_2S_2O_8$, as well as other chemicals used in this study were analytical grades.

2.2 Chemical composition of OPF

The chemical composition of OPF was determined using the standard laboratory analytical procedure (LAP) from the National Renewable Energy Laboratory (NREL) [24]. Soxhlet extraction was performed using 200 mL of distilled water to determine the amount of water extractives, in which the water extracted samples further underwent 200 mL of ethanol extraction to determine the amount of ethanol extractives. On the other hand, OPF samples in porcelain crucibles were burned at 575°C for 24 h, whereby the ash content was determined by the weight of the remaining solids after undergoing calcination. The cellulose, hemicellulose, and lignin contents were also determined using the acid hydrolysis method given in the NREL LAP protocol.

2.3 $CuSO_4.5H_2O$ pretreatment with/without H_2O_2 or $Na_2S_2O_8$ additives

Firstly, 2.5 g of OPF samples were transferred into 50 mL Schott bottles. The $CuSO_4.5H_2O$ aqueous solutions were prepared at a range of 0.2-0.8 mol/L concentrations. The inorganic salt solutions were then transferred into the Schott bottles containing OPF samples at a fixed solid-to-liquid ratio of 1:10 (w/v). Next, the mixtures were sent for reaction at 120°C and 30 min. After the completion of reaction, the mixtures were then removed at a cooling temperature of approximately 75°C and air cooled to quench the reaction. A small portion of the pretreatment hydrolysate was extracted and centrifuged at a speed of 13,500 rpm for 10 min using a Mini 1312M Micro Centrifuge, before passing through a 0.22 μm syringe filter for sugar content analysis. In order to study the effects of oxidizing agents, the same procedure was repeated but with an addition of H_2O_2 or $Na_2S_2O_8$ at varying concentrations (1.5-6.0% (v/v)) to the $CuSO_4.5H_2O$ solution before the reaction at 120°C and 30 min.

2.4 Analytical methods

2.4.1 Analysis of Sugar Content in Hydrolysate using High Performance Liquid Chromatography (HPLC)

The yield of monomeric sugars (glucose, xylose, arabinose) was analyzed using an Agilent series 1200 Infinity HPLC system equipped with Refractive Index (RI) detector and a Bio-Rad Aminex HPX-87H column. The column was operated at a flow rate of 0.6 mL/min with 0.005 mol/L H_2SO_4 as the mobile phase. The temperature of the column was set at 65°C, while the RI detector was set at 40°C to minimize the effects of temperature gradient. The concentration of the sugars was analyzed in g/L by comparing the chromatogram with the concentration of known sugar standards. Lastly, the % recovery of monomeric sugars was calculated using the formula adapted from Kamireddy et al. [15] and Pappas et al. [25]:

$$\%\ \text{recovery of sugar} = \frac{\text{sugar recovered (g/L) x volume of solvent used (L)}}{\text{OPF carbohydrate composition (\%) x mass of OPF used in pretreatment (g)}} \text{ x } 100\%$$

2.4.2 Analysis of Biomass Solids using Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscope (FE-SEM), and Brunauer-Emmett Teller (BET)

The raw and pretreated OPF samples from the best pretreatment conditions (control, H_2O_2-assisted, $Na_2S_2O_8$-assisted) were dried and subjected to FTIR analysis using a Thermoscientific Nicolet iS10 spectrometer. The OPF were pressed into a disc with attenuated total reflection (ATR), and the sample spectra were obtained using an average of 64 scans with a spectral resolution of 4 cm^{-1}, over the wavelength range of 525 and 4000 cm^{-1}. The raw and pretreated OPF samples from the best pretreatment conditions (control, H_2O_2-assisted, $Na_2S_2O_8$-assisted) were firstly adhibited to a specimen stub using double-coated tape and sputter coated with Platinum. The samples were then sent for FE-SEM imaging using a Hitachi SU8010 machine, in order to observe any microstructural changes in the biomass surface morphology after pretreatment process. The raw and pretreated OPF samples from the best pretreatment conditions (control, H_2O_2-assisted, $Na_2S_2O_8$-assisted) were subsequently sent for BET analysis in order to analyze the changes in the specific surface area. The analysis was performed using a Micromeritics ASAP 2020 machine, with N_2 as the adsorbate at 77.3K. The samples were degassed at 90°C for 2 h, and subsequently at 110°C for 22 h prior to the adsorption analysis [26].

3 Results and Discussion

3.1 Chemical Composition of Raw OPF

Using the standard NREL procedure, the compositional analysis of raw OPF is shown in Table 1. Although the composition of OPF used in this study was found to be different from the other findings [27-30], the variation could be explained by the different maturity and geographical location where the OPF was obtained. It was crucial to identify the components in the OPF, since the theoretical amount of recoverable pentose sugars (xylose and arabinose) was directly dependent on the hemicellulose available in the biomass itself. In addition, high compositions of crystalline cellulose and lignin could hinder the access of chemical solvent into the OPF structure, causing the hydrolysis to be inefficient during pretreatment process.

Table 1 Chemical composition of OPF used in this study

Biomass component	Percentage (%)
Glucan	45.2 ± 0.5
Xylan	20.0 ± 0.4
Arabinan	2.7 ± 0.4
Lignin	21.5 ± 1.9
Ash	2.8 ± 0.3
Water extractives	12.7 ± 0.8
Ethanol extractives	3.8 ± 0.5

3.2 Effect of $CuSO_4.5H_2O$ concentration

The preliminary study of this work firstly involved the investigation of inorganic salt pretreatment using $CuSO_4.5H_2O$ ranging between 0.2 and 0.8 mol/L. The yields of monomeric sugars after undergoing inorganic salt pretreatment are shown in Fig. 1. It was important to highlight that higher concentration of salt was not studied due to economic reasons, as excessive amount of salt would incur additional costs and/or require further purification steps before fermentation. According to Fig. 1, the xylose and arabinose recovered after 0.2 mol/L of $CuSO_4.5H_2O$ pretreatment were 0.8 (4.1%) and 1.0 (35.2%) g/L, respectively. Moreover, there were insignificant changes in the yield of xylose and arabinose when the concentration of $CuSO_4.5H_2O$ increased from 0.2 to 0.8 mol/L, as proven by one-way analysis of variance. These results were unexpected, since it was initially postulated that an increase of inorganic salt would result in the enhancement of pentose sugar hydrolysis. Furthermore, the yield of pentose sugars presented here were considerably lower than the previous studies using $FeCl_3$ [8, 31]. Interestingly, high sugar recoveries were often achieved only when trivalent salts were employed, due to their stronger catalytic activity as compared to mono- and divalent salts [32]. Besides, Fe salts usually had higher hydrolysis efficiency due to the high density charge of Fe ions, enabling them to coordinate with the oxygen donor parts of the carbohydrates in biomass without losing their charge from the hydroxyl groups of the ligand [33].

Nevertheless, the low recovery of pentose sugars was in agreement with Awosusi et al. [34], in which a strict relationship between the hydration levels and solvating ability was observed. It was reported that salts with lower hydration levels were more effective in cellulose dissolution. Specifically, the monomeric glucose yields in the hydrolysate of zinc hydrate salt pretreatments were 10.8, 3.4, and 0% for $ZnCl_2.3H_2O$, $ZnCl_2.4H_2O$, and $ZnCl_2.5H_2O$, respectively [34]. Moreover, Leipner et al. [16] observed only minor swelling of cellulose when the pretreatment was carried out using $LiCl/5H_2O$ system, but the cellulose I structure was retained. On the other hand, $LiCl/2H_2O$ pretreatment caused major swelling of cellulose and the modification of structure into cellulose II. Therefore, one possible reason for the low pentose sugar yields after undergoing $CuSO_4.5H_2O$ pretreatment was the high saturation of water molecules, causing the competition between carbohydrate polymers and water in the coordination sphere of the metal ion. According to Leipner et al. [16], a less saturated cation would provide “free” coordination sites for the hydroxyl group of the cellulose to coordinate to the cation, subsequently functioning better as a dissolution solvent.

Hence, it was suspected that the divalent hydrate metal salt $CuSO_4.5H_2O$, coupled with its saturated cation coordination sphere, was not severe enough to break the hemicellulosic structure of the OPF even at higher concentrations. As a result, 0.2 mol/L of $CuSO_4.5H_2O$ was chosen as the recommended concentration for further investigation, with xylose and arabinose recoveries of 0.8 (4.1%) and 1.0 (35.2%) g/L, respectively. On the contrary, the monomeric glucose recovery experienced a slight decrease from 2.7 (6.0%) to 2.1 (4.6%) g/L with an increase of $CuSO_4.5H_2O$ concentration. The reduction of glucose were probably due to non-structural carbohydrates that were more prone to degradation.

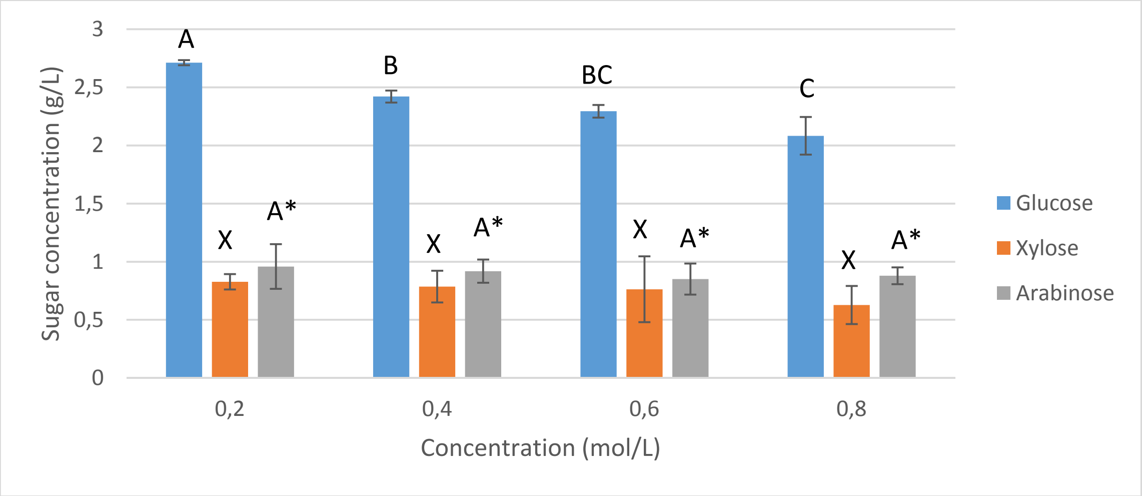


Fig. 1 Monomeric sugar yields of $CuSO_4.5H_2O$ pretreatment. Values annotated with different letters represents different significance levels (one-way ANOVA, Tukey's test: $P<0.05$).

3.3 Effect of H_2O_2 or $Na_2S_2O_8$ concentration

3.3.1 H_2O_2-assisted pretreatment

A recent study conducted by Peng et al. [35] revealed that the hydrogen from the hydroxyl groups of hemicellulose backbones could be abstracted by free radicals, resulting in the disintegration of the hemicellulose structure in the biomass. Thus, H_2O_2 represented a suitable source of hydroxyl radicals (OH•) and superoxide anion radicals (O_2^-), especially in the presence of iron and copper transition metals [36]. As such, the effects of H_2O_2 on $CuSO_4.5H_2O$ pretreatment (120ºC, 30 min) was an important factor to be investigated in this study. Fig. 2 shows the effect of H_2O_2 in the range of 1.5-6.0% (v/v), using 0.2 mol/L of $CuSO_4.5H_2O$ (Fig. 1).

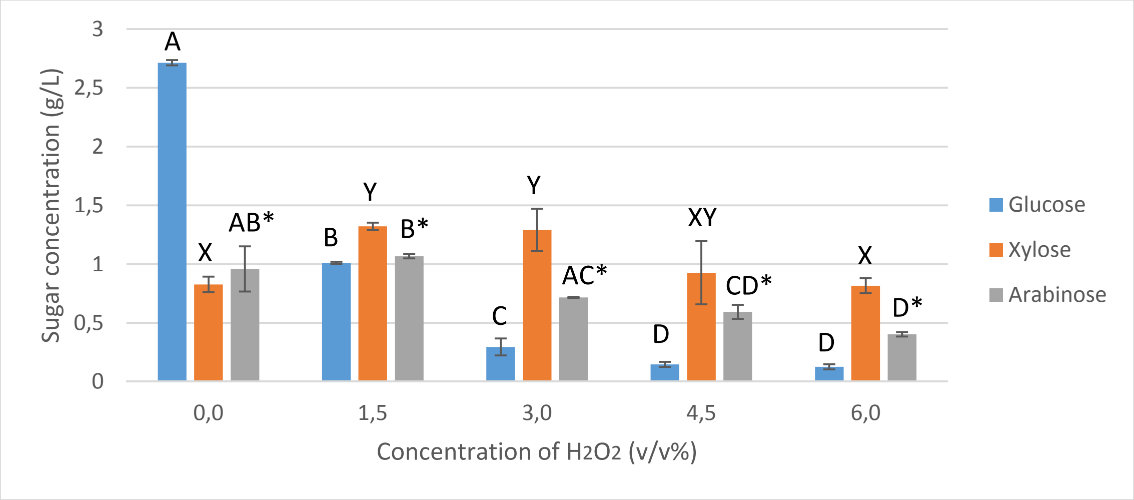


Fig. 2 Monomeric sugar yields of H_2O_2-assisted $CuSO_4.5H_2O$ pretreatment. Values annotated with different letters represents different significance levels (one-way ANOVA, Tukey's test: $P<0.05$).

According to Fig. 2, additions of H_2O_2 from 1.5 to 6.0% (v/v) obviously decreased the monomeric glucose yield. Specifically, the glucose yield decreased from 2.7 (6.0%) to 0.1 (0.3%) g/L, highlighting the negative impact of H_2O_2 to the glucose recovery during $CuSO_4.5H_2O$ pretreatment. As mentioned earlier, the decrease in glucose yield could be due to the dehydration of existing non-structural glucose. Therefore, an addition of H_2O_2 caused greater reduction of glucose as compared to when the $CuSO_4.5H_2O$ concentration was increased. However, the aforementioned addition of H_2O_2 at 120°C and 30 min was still not harsh enough to hydrolyze cellulose into glucose, justified by the observations in which the monomeric glucose yield did not improve. On the other hand, an addition of 1.5% (v/v) H_2O_2 seemed to slightly enhance the yield of xylose and arabinose from 0.8 (4.1%) to 1.3 (6.6%) g/L and 1.0 (35.2%) to 1.1 (39.1%) g/L, respectively. Howeveran increase of H_2O_2 higher than 1.5% (v/v) not only did not improve the yields of both xylose and arabinose, but reductions of these sugars could be observed (Fig. 2). According to one-way analysis of variance, there was an insignificant difference between the xylose recoveries obtained for 1.5 and 3.0% (v/v) H_2O_2. As such, the best condition for H_2O_2-assisted pretreatment was 0.2 mol/L of $CuSO_4.5H_2O$ + 1.5% (v/v) H_2O_2.

The slight improvement in the pentose sugars recovery may be caused by the presence of Cu^{2+} ions, which underwent continuous redox reaction to generate OH• radicals, as shown in Equation (1) and (2) [23]. It was worth noting that the Cu^{2+} with organic degradation intermediates decomposed easily when the Cu^{2+} were exposed to OH• radicals. The OH• would then participate in the oxidative delignification of the OPF structure, as proven by the past studies [37, 38]. Subsequently, the breakage of the lignin structure improved the access of $CuSO_4.5H_2O$ solvent into the hemicellulosic structure of OPF for better sugar hydrolysis. However, higher concentrations of H_2O_2 were not studied since excessive amounts of H_2O_2 have been proven to promote undesired secondary reactions [39], which could explain the decrease of pentose sugars recovery at higher H_2O_2 dosages in this study.

$$Cu^{2+} + H_2O_2 \rightarrow Cu^{+} + HO_2\bullet + H^{+} \qquad \text{Equation (1)}$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH\bullet + OH^{-} \qquad \text{Equation (2)}$$

3.3.2 $Na_2S_2O_8$-assisted pretreatment

Similarly, $Na_2S_2O_8$-assisted pretreatment was carried out using the same concentration range of 1.5-6.0% (v/v), using 0.2 mol/L of $CuSO_4.5H_2O$. The pretreatment was performed under the same conditions (120°C, 30 min) and the results are shown in Fig. 3.

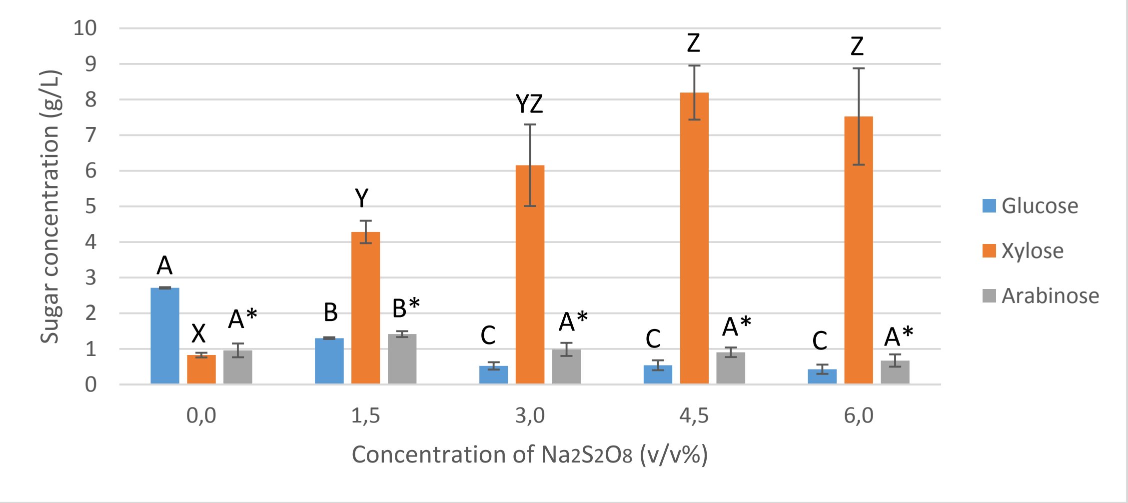


Fig. 3 Monomeric sugar yields of $Na_2S_2O_8$-assisted $CuSO_4.5H_2O$ pretreatment. Values annotated with different letters represents different significance levels (one-way ANOVA, Tukey's test: $P<0.05$).

When $CuSO_4.5H_2O$ was assisted with 4.5% (v/v) $Na_2S_2O_8$, a peak xylose recovery of approximately 8.2 (41.0%) g/L was achieved. One-way analysis of variance proved that the synergistic effect of 0.2 mol/L of $CuSO_4.5H_2O$ plus 4.5% (v/v) $Na_2S_2O_8$ resulted in significantly higher xylose recovery than using $CuSO_4.5H_2O$ alone. However, there was a negligible decrease in the arabinose recovery, from 1.0 (35.2%) to 0.9 (33.1%) g/L. This phenomenon could be explained by the production of sulfate radicals ($SO_4^{-}\bullet$) and sulfate ions (SO_4^{2-}) [40] when the $S_2O_8^{2-}$ oxidizing agent was activated by Cu^{2+} ions. The formation of these radicals subsequently led to higher rates of OPF delignification, causing better access of $CuSO_4.5H_2O$ solvent access into the OPF for improving sugar hydrolysis. Thus, the superior effects of $Na_2S_2O_8$ over H_2O_2 could be attributed to the reaction mechanisms shown in Equation (3), in which Cu^{2+} ions were oxidized into Cu^{3+} by the existing $S_2O_8^{2-}$ ions [22], as well as Equation (4), whereby $OH\bullet$ were formed via a secondary reaction [40]. Hence, it was proposed that the oxidative delignification during $Na_2S_2O_8$-pretreatment was not only contributed by $SO_4^{-}\bullet$, but also $OH\bullet$ simultaneously.

$$Cu^{2+} + S_2O_8^{2-} \rightarrow Cu^{3+} + SO_4^{-}\bullet + SO_4^{2-} \qquad \text{Equation (3)}$$

$$SO_4^{-}\bullet + H_2O \rightarrow SO_4^{2-} + OH\bullet + H^{+} \qquad \text{Equation (4)}$$

The formation of highly unstable Cu^{3+} ions enhanced the hydrolysis of hemicellulose in the OPF into monomeric xylose via the breakage of glycosidic linkages between the cellulose and hemicellulose, yielding more xylose in the process. The findings were in agreement with Sun et al. [32], in which trivalent salts were proven to have stronger catalytic ability in hydrolyzing the compounds of lignocellulosic biomass. Moreover, $SO_4^{-}\bullet$ had the capability to further attack the hemicellulose structure to a certain extent by removing the hydrogen atom from the hemicellulose hydroxyl groups [35]. In addition, the use of H_2O_2 as an additive was not as effective as $Na_2S_2O_8$ because the ability of $OH\bullet$ to hydrolyze biomass has been demonstrated to function the best under alkaline environments [18, 41], but the inorganic salt $CuSO_4.5H_2O$ produced an acidic environment. In fact, Liu et al. [22] proclaimed that the metal activation of $Na_2S_2O_8$ for $SO_4^{-}\bullet$ generation reached its peak under acidic conditions, implying that the $SO_4^{-}\bullet$ were able to function better than $OH\bullet$ for OPF delignification when combined with the inorganic salts. Nevertheless, a higher $Na_2S_2O_8$ concentration of 6.0% (v/v) caused a reduction in xylose recovery to 7.5 (37.6%) g/L. Thus, too high concentration of $Na_2S_2O_8$ was undesirable, due to unwanted reactions which competed to consume the $SO_4^{-}\bullet$ when oxidants were present in excess [21]. These results concurred with the work of Nfodzo and Choi [42], who stated that the decomposition of triclosan improved with higher concentration of persulfate, as long as the oxidant/transition metal ratio was adjusted to 1:1. On the contrary, glucose and arabinose yields were relatively low at 0.54 (1.2%) and 0.9 (33.1%) g/L, respectively when 4.5% (v/v) $Na_2S_2O_8$ was added. The addition of $Na_2S_2O_8$ could have produced an environment which was too harsh for the preservation of monomeric sugars, excessively dehydrating the glucose and arabinose. Nonetheless, the ability of $SO_4^{-}\bullet$ to be activated via transition metals and temperature pathways was a favourable trait during OPF delignification as compared to its $OH\bullet$ counterpart. However, extreme reaction temperatures should be avoided to achieve the ideal synergistic effects between Cu^{2+} and temperature during formation of free radicals [40].

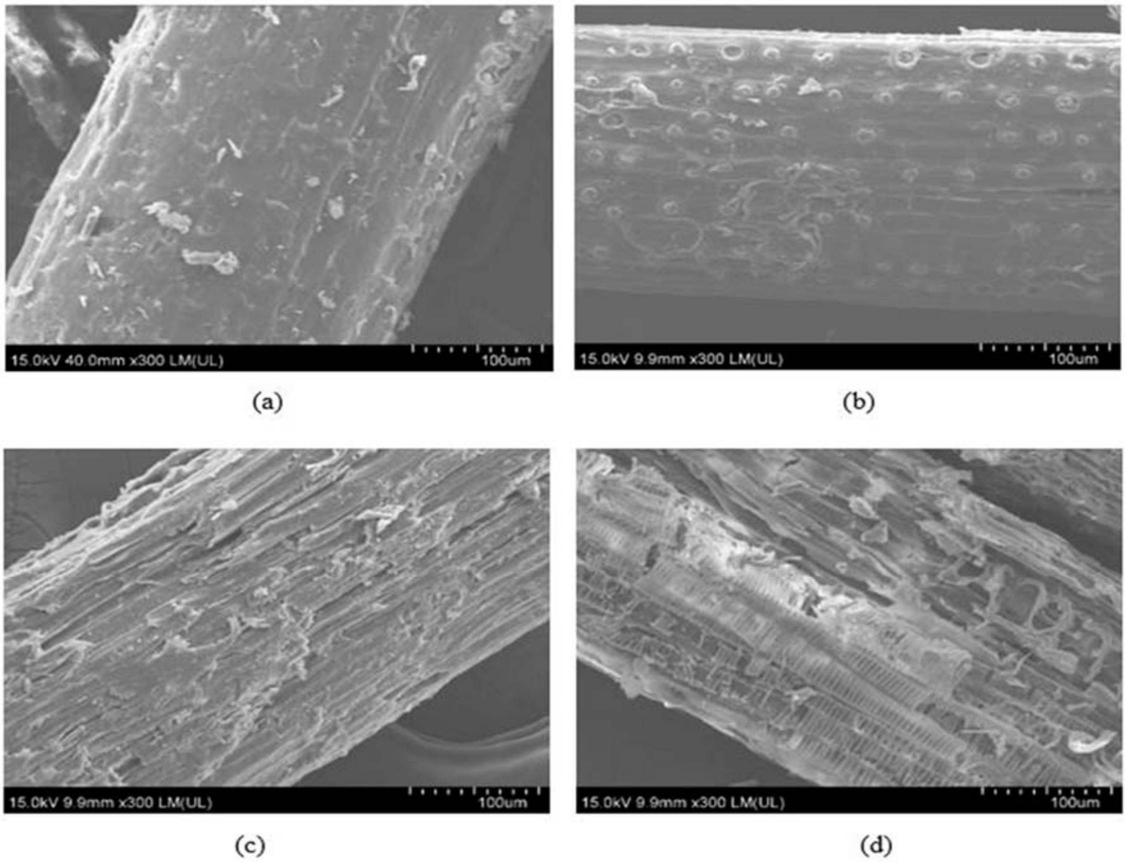
3.4 Characterization studies of Raw and Pretreated OPF

3.4.1 FE-SEM and BET

Surface morphology of the OPF before and after pretreatment is shown in Fig. 4. In Fig. 4(a), the raw OPF was comprised of smooth fibrils with low porosity, which limited the penetration of $CuSO_4.5H_2O$ solvent into the OPF. The well-shaped and rigid fibrils of the untreated OPF were also observed by Kristiani et al. [43] as well as Lai and Idris [44].

On the other hand, after undergoing $CuSO_4.5H_2O$ pretreatment, the structure of the OPF was still visibly enclosed even though the formation of holes was apparent on the surface as shown in Fig. 4(b), indicating that the $CuSO_4.5H_2O$ alone was not severe enough to breakdown the OPF. Awosusi et al. [34] stated that the swelling-triggering interaction between the hydrate metal salts and sugar polymers did not necessitate the latter's dissolution, coinciding with the low pentose sugars recovery using only $CuSO_4.5H_2O$ in the pretreatment process. Fig. 4(c) shows the OPF structure became more disordered and rough with an addition of H_2O_2, due to the effects of oxidative delignification by OH• radicals. Nevertheless, the addition of $Na_2S_2O_8$ caused the most drastic rupture of OPF, attributed to the greater extent of lignin and hemicellulose removal (Fig. 4(d)) as compared to the other pretreatments. As a result, the breakage of lignocellulosic structure revealed the inner cavity of cellulose and hemicellulose, thereby increasing the surface area for improved solvent access and sugar recovery [45].

This claim was further proven via BET analysis, in which the specific surface area of raw OPF increased from 0.3752 to 0.4587 m^2/g after undergoing 0.2 mol/L of $CuSO_4.5H_2O$ control pretreatment. Thus, although $CuSO_4.5H_2O$ was ineffective in hydrolyzing the structural carbohydrates, it was generally effective as a swelling media for the OPF, as proven by Fischer et al. [46] using $LiCl.xH_2O$ inorganic hydrate salts. Using both $CuSO_4.5H_2O$ and H_2O_2, the oxidative delignification effects further increased the surface area to 0.4872 m^2/g. However, the increase in the specific surface area of the OPF (0.6952 m^2/g) was the most remarkable $Na_2S_2O_8$ was used. The greater increase in the surface area after the addition of oxidizing agents could be attributed to the more severe breakage of lignocellulosic structure, as mentioned earlier.



(a) (b)

(c) (d)

Fig. 4 FE-SEM images of (a) raw, (b) control (0.2 mol/L of $CuSO_4.5H_2O$), (c) H_2O_2-assisted (0.2 mol/L of $CuSO_4.5H_2O$ + 1.5% (v/v) H_2O_2), and (d) $Na_2S_2O_8$-assisted (0.2 mol/L of $CuSO_4.5H_2O$ + 4.5% (v/v) $Na_2S_2O_8$) pretreated OPF at 300x magnification.

3.4.2 FTIR

The FTIR spectra of raw and pretreated OPF are shown in Fig. 5. According to the spectral trend of raw OPF, a small sharp band was observed at 900 cm^{-1}, representing the β-glycosidic bonds between sugar polymers in cellulose and hemicellulose [47]. Moreover, there were small but noticeable peaks at 1420 cm^{-1} due to the bending of the symmetrical CH_2 groups found in the cellulose [28], while the –OH group stretching vibration in cellulose was characterized by the broad 2900 cm^{-1} peak [48]. Large bands observed at 1031 cm^{-1} was attributed to the C-O-C stretching vibration of the main components in OPF, particularly cellulose and hemicellulose. Insignificant changes were observed for the aforementioned peaks as the conditions were not severe enough to hydrolyze cellulose, which usually decomposes at high temperatures in the range of 320-360°C [49]. However, the oxidizing agents-assisted pretreatment were effective in removing amorphous lignin for better solvent access into the lignocellulosic structure. This claim was justified by the disappearance of the 1600 cm^{-1} peak, often caused by the aromatic skeletal vibrations of lignin, in the cases of H_2O_2- and $Na_2S_2O_8$-assisted pretreatments [50]. Furthermore, the band peak at 1235 cm^{-1} became narrower and less obvious, especially after an addition of $Na_2S_2O_8$. This phenomenon could be due to the disruption of the C-O-C aryl-alkyl ether bond in lignin [51]. A significant change was also evident at the 1735 cm^{-1} peak [52], which represents the C=O acetyl group in hemicellulose carbonyl ester, whereby the peak diminished remarkably after the addition of $Na_2S_2O_8$. This phenomenon was probably due to the greater removal of lignin, leading to a better access of the $CuSO_4.5H_2O$ solvent into the OPF for improving hemicellulose hydrolysis.

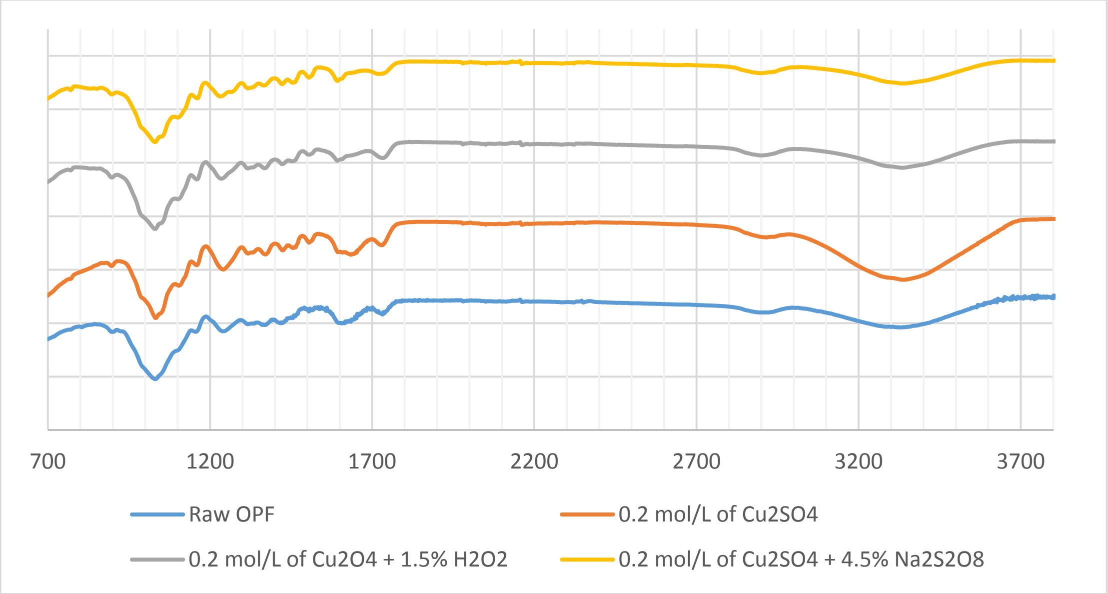


Fig. 5 FTIR spectra of raw and pretreated OPF.

3.4 Comparison with past studies

The results obtained in this study are tabulated in Table 2. In comparison with the past studies using OPF of similar particle sizes [29, 53], the pentose sugar yields after undergoing both $CuSO_4.5H_2O$ and $Na_2S_2O_8$ pretreatment was noticeably higher. Besides, the percentage sugar recoveries in this study were greater than previous literature which required harsher conditions, such as longer durations [30] or higher temperatures [54]. Nonetheless, the results in this study were very preliminary and required further investigations.

Table 2 Pretreatment of OPF using various pretreatment techniques

Feedstock	Pretreatment conditions	Sugar recovery	Ref.
841 µm OPF particles	1) Soaked in 2.0 mol/L of NaOH at room temperature for 24h 2) Acid hydrolysis with 10.0% (v/v) H_2SO_4 for 121°C and 30 min	1) Maximum reducing sugar concentration of 0.0811 g/L	29
<1 mm OPF particles	1) Auto-hydrolysis for 121°C and 1h 2) Enzymatic hydrolysis using 16 U xylanase for 48h	1) Maximum xylose concentration of 0.795 g/L	30
0.5 mm OPF particles	1) Auto-hydrolysis for 121°C and 60 min 2) Enzymatic hydrolysis using 4 U Trichoderma viride endo-(1, 4)-β-xylanase/100mg hydrolysate, at 40°C and 48h	1) Arabinose and xylose yields of 19.24% (w/w) and 25.64% (w/w), respectively	53
<1 mm OPF particles	1) Hot compressed water for 175°C and 12.5 min	1) Highest concentration of 0.4434 g/L xylose and 0.0633 g/L glucose	54
125-706 µm OPF particles	1) Soaked in 7% (w/w) aqueous ammonia for 80°C and 20h 2) Simultaneous saccharification and fermentation using 60 FPU Accellerase 1000/g glucan and 30 CBU β-glucosidase/g glucan, at 38°C and 48h	1) Xylose concentration of 7.6 g/L (62.4% recovery)	55
≤0.5mm OPF particles	1) 0.2 mol/L of $CuSO_4.5H_2O$ + 4.5% (v/v) $Na_2S_2O_8$ reaction at 120°C and 30mins	1) Xylose concentration of 8.2 g/L (41.0% recovery) and arabinose concentration of 0.9 g/L (33.1% recovery)	This study

4 Conclusion

In this study, an integrated biomass pretreatment system consisting of inorganic salt and oxidizing agents under reduced severity was investigated. The combination of 4.5% (v/v) $Na_2S_2O_8$ with 0.2 mol/L $CuSO_4.5H_2O$ exhibited the best synergistic effects to obtain a total pentose sugar yield of approximately 40%. The result showed a significant increase as compared to using $CuSO_4.5H_2O$ alone (control), due to the in-situ production of Cu^{3+} ions when $Na_2S_2O_8$ was added. The results from the characterization studies such as FTIR, FE-SEM, and BET further validated the importance of incorporating $Na_2S_2O_8$ into inorganic salt pretreatment. Specifically, the FE-SEM and BET results showed the physical effects of oxidizing agents on the lignocellulosic structure of the OPF, as a consequence of the oxidation and breakage of lignin structure. Moreover, the FTIR spectra provided a representation of the chemical effects of the oxidizing agents in aiding inorganic salt pretreatment, in which the changes in chemical structure of the OPF coincided with the proposed pretreatment mechanism in this study.

Acknowledgements

The funding of this research is provided by Universiti Kebangsaan Malaysia and supported by Department of Higher Education, Malaysia under Long Term Research Grant Scheme (LRGS/2013/UKM-UKM/PT/01). In addition, the authors would like to thank Monash University Malaysia for providing Y.-L. Loow with a postgraduate scholarship.

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