# Improvement of Delignification, Desilication and Cellulosic Content Availability in Paddy Straw via Physico-chemical Pretreatments

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*Authors' contributions*

*This work was carried with collaboration between all authors. Author MGMFS conducted all experiments protocol. Author AGNS assisted in the pre-treatment experiment. Author MZNAI consulted on mycology works. Author AANA managed on the statistical analysis. Author MM designed the study and consulted on the study approach. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** Paddy straw consists of cellulose and hemicellulose as their plant materials leading to their potential to produce bioethanol through several processes such as pretreatment, enzymatic hydrolysis and ethanol fermentation. Among these processes, pretreatment of paddy straw is particularly important for enzymatic hydrolysis process as they are being limited by the presence of ash and silica content. This study was set to observe the effect of different pretreatments on cellulose, hemicellulose, lignin and ash content of paddy straw.
**Place and Duration of Study:** This study was conducted in Department of Biology, Faculty of Science, Universiti Putra Malaysia, between October 2015 and June 2016.
**Methodology:** Pretreatments comprises the combination of physical (mechanical) and chemical

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treatments to modify the lignocellulosic structure while reduce lignin and separate silica content in paddy straw fibre. Paddy straw was prepared into three different sizes (2mm, 5mm and 8 mm) for physical treatment. Autoclave, boiled and four different concentrations (0.5%, 1%, 2% and 5% (v/v) and (w/v) respectively) of nitric acid and sodium hydroxide, respectively for chemical treatment were used on paddy straw.

**Results:** Size five millimeter paddy straw showed the highest cellulose content (35.61%) compared to the other sizes and when the paddy pretreated with 2% (w/v) sodium hydroxide (NaOH), the percentage of cellulose content escalated to 72.47%. Pretreatment of 2% (w/v) NaOH have performed the most efficient delignification and desilication process (1.02% lignin; 5.44 ash content); and the performance was supported with SEM images on surface area of the paddy straw with large distortion caused by the treatment.

**Conclusion:** Therefore, a physico-chemical pretreatment of size 5 mm and 2% (w/v) NaOH was found to be the most suitable condition to break the cellulose-lignin complex and make the paddy straw becomes feasible for biofuel production.

## 1. INTRODUCTION

Agro-industrial wastes are deposited in landfills and burning of the residues has increased the production of greenhouse gases [1]. Over 90% worldwide production of this biomass is found in the Asia region [2]. The waste produced by industries including municipal waste, pulp and paper, agriculture, food, forestry and animal wastes is in huge amount and can potentially be treated to produce more valuable products like animal feed, chemicals and biofuels [3]. The production of biofuels from agro wastes stand a good prospective as the raw materials do not compete with other food-source materials and they are rich in sugar and starch [4]. Rice is the most preferred food and energy source among Malaysian than any other agricultural crops [5]. Prior to consumers' demand, recent technologies in rice production have been developed to increase its productivity. However, the increase of paddy rice production has also raised the bulks of its by-products including paddy straw.

As a lignocellulosic material, paddy straw comprises cellulose, hemicelluloses and lignin as its major component with a potential to produce bioethanol through bioconversion process of lignocellulosic biomass to fermentable sugar [6]. This has leads to the need in developing a biotechnical process using a low cost cellulolytic enzyme production that is economically feasible [7]. While it has been known that paddy straw is useful in bioethanol production, Ibrahim [8] concluded in his study that this process is limited by the existence of high ash and silica content in paddy straw, which makes it difficult for the paddy straw to be converted into ethanol. Generally, paddy straws differ from other cereal straws as they have a relatively high amount of silicon dioxide (SiO2) in their ash content but with low amount of lignin content [9]. According to Liu et al. [10], the ash is made up of 75% $SiO_2$, 3% $P_2O_5$, 1.5% CaO, 2% $Fe_2O_3$, 10% $K_2O$ and small portions of Na, Mg, and S. Although silicon plays significant roles in rice production, such as grain yield and phenolic synthesis, in addition to serving as a shield for the cell wall of the plant [9], this compound has become a limiting factor for pretreatment and enzymatic digestion of straw.

The structure of cellulose and hemicellulose is heavily packed with layers of lignin to protect them against enzymatic hydrolysis [11]. Lignin, on the other hand, is an aromatic biopolymer with an integral cell wall component in all vascular plants built up by the oxidative coupling of three-major C6-C3 (phenypropanoid), forming a randomized assembly in a tri-dimensional system inside the cell wall [12,13]. Lignin acts as a matrix for the cellulose microfibrials made up by polymer chains, which contain tightly packed and crystalline regions [14]. The hydrogen bond between polysaccharides and adhesion of lignin to the polysaccharides has caused them to be insoluble in water and partially soluble in organic solvent.

The complex structure of a cell wall makes it hard for paddy straw to be digested using anaerobic microorganisms, resulting in low digestion rate for biogas production [15]. The main problem in the consumption of lignocellulosic biomass is the disruption of complex matrix of polymers; liberating the monosaccharides and improvement of pretreatment methods to maximize the material digestibility for the following enzymatic hydrolysis [16]. Pretreatment process is consequently

crucial to remove lignin, silica, reduce cellulose crystallinity, and to maximize the accessible surface area of materials [17].

Pretreatment, or other known as the delignification, is a process that usually involves physical, physiochemical, chemical and thermal methods, as well as the combination of these method to break the lignin layers of protection that limit the accessibility of enzymes to celluloses and hemicelluloses. According to Zhu et al. [18], many pretreatment techniques have been developed to improve this conversion process. The effectiveness of a pretreatment technique has been defined as the ability to rupture the matrix of cell-wall that includes the connectivity between lignin and carbohydrates, along with depolymerizing and solubilizing hemicellulose polymers [19]. Among these techniques, pretreatment is believed to be predominantly important, considering the difficulties of lignocellulosic structure for enzymatic hydrolysis as well as one of the most expensive processing steps over the whole conversion process [20]. Several pretreatments methods have been widely studied over the years such as steam explosion, acid pretreatment, thermal pretreatment, microwave and alkali pretreatment. Steam explosion is the most common pretreatment method used in commercial biorefineries. However, it works much less effective for softwood, which is not suitable for a feedstock in bioconversion

processes due to its high lignin content and recalcitrance [21]. Due to low cost and rapid reaction of chemical pretreatment, most of the studies have been focusing on the dilute acid and alkali pretreatment [22]. Hence, this study attempts to determine the effectiveness of various physical and chemical pretreatments used to increase the accessibility to the cellulose and hemicellulose content in paddy straw by reducing the lignin shield and separating the silica that encloses the monosaccharide.

## 2. MATERIALS AND METHODS

### 2.1 Substrate Preparation

Paddy straw was washed to remove the undesired particles, sundried and kept in a dry container. Extractives were removed by Soxhlet extraction for 2 hours using toluene-ethanol (2:1) mixed solvent [23].

### 2.2 Pretreatment of Paddy Straw

Combination of physical and chemical pretreatments was carried out on the paddy straw. The physical treatment includes grinding the paddy straw in hammer mill to several mesh sizes (8 mm, 5 mm and 2 mm). All grinded paddy straws were treated in treatment listed in Table 1, respectively. All pretreated paddy straws were washed with distilled water until their pH values reach at almost neutral before being dried in an oven at 60°C until reaching a constant weight.

**Table 1. Pretreatments on paddy straw**

| Pretreatments | Conditions |
|---|---|
| Control (Untreated) | No treatment given |
| Autoclaved | Autoclaved (121°C ± 0.5; 15 minutes) |
| Boil | Soaked in boiled water (100°C ± 0.5) for 4 hours at room temperature and autoclaved (121°C ± 0.5; 15 minutes) |
| 0.5% (v/v) $HNO_3$ | Soaked in 0.5% (v/v) $HNO_3$ for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 1.0% (v/v) $HNO_3$ | Soaked in 1.0% (v/v) $HNO_3$ for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 2.0% (v/v) $HNO_3$ | Soaked in 2.0% (v/v) $HNO_3$ for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 5.0% (v/v) $HNO_3$ | Soaked in 5.0% (v/v) $HNO_3$ for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 0.5% (w/v) NaOH | Soaked in 0.5% (w/v) NaOH for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 1.0% (w/v) NaOH | Soaked in 1.0% (w/v) NaOH for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 2.0% (w/v) NaOH | Soaked in 2.0% (w/v) NaOH for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |
| 5.0% (w/v) NaOH | Soaked in 5.0% (w/v) NaOH for 4 hours at room temperature and autoclaved (121°C ± 0.5;15 minutes) |

## 2.3 Determination of Cellulose and Hemicellulose Content Availability Percentages

The percentage of cellulose and hemicellulose content was determined and calculated adopting the method proposed by Han and Rowell [23]. The calculations involved expressed data on loss of dry matter and dry matter basis that occurs in processing steps.

## 2.4 Determination of Lignin Content

The lignin content of paddy straw was determined by using Acid-Detergent Lignin (ADL) procedure. The process requires preparation of Acid-Detergent Fiber (ADF) before carrying out the ADL process and the calculations were carried out based on the amount of loss upon ignition at 500 ± 25ºC after being treated with 72% (v/v) sulfuric acid ($H_2SO_4$) for 3 hours [24].

## 2.5 Determination of Ash Content

The percentage of ash content was determined and calculated from the method by Han and Rowell [23]. The fibers were weighed and burned over a low flame of Bunsen burner until well and fully carbonized. The fibers were ignited in the furnace at 575 ± 25ºC for 3 hours. The calculations were based on moisture-free weight of the fibre.

## 2.6 Physical Observation Using Scanning Electron Microscope (SEM)

The SEM viewing of untreated (control) and pretreated (2% (w/v) NaOH and 0.5% (v/v) $HNO_3$) paddy straws was done in Microscopy Unit at the Institute of Bioscience, Universiti Putra Malaysia using Phillips XL30 SEM. For SEM viewing, the paddy straw was oven dried at 60°C for 24 hours [25]. The images were then viewed and observed using SEM at 1.0 K magnification.

## 3. RESULTS AND DISCUSSION

The main approach used to improve enzymatic hydrolysis is by removing lignin and silica while increasing the cellulosic accessible surface area [26]. In this study, the percentages of cellulose, hemicellulose, lignin and ash content were used as parameters to measure the effectiveness of physical and chemical pretreatment of paddy straw. Theoretically, pretreatment could further enhance the delignification and desilication processes on lignocellulosic material [27]. Therefore, both cellulose and hemicellulose will be exposed for further saccharification by selected conversion agent.

## 3.1 Physical Pretreatments

Lignocellulosic materials contain two types of surface area, which are internal and external. In order to increase the external surface area, the size particle of paddy straw was reduced. Thus, three different fiber sizes were chosen (2 mm, 5 mm and 8 mm) to demonstrate the effect of physical pretreatment on paddy straw by comparing the delignification of fiber in each treatment.

The best size of paddy straw with highest cellulosic composition (35.61% cellulose; 26.96% hemicellulose) and accessibility was displayed by 5 mm with lowest amount of lignin, 10.79%. Paddy straw size 8 mm showed second best physical pretreatment by producing an amount of 34.64% of cellulose. In comparison of size 5 mm to 8 mm, there is no significant difference between the percentage of cellulose, hemicellulose and lignin. The selection has been done according to the percentage of cellulose produced as cellulose plays major roles in the production of bioethanol. However, the percentage of ash content produced by size 8 mm is significantly lower than 5 mm. The 2 mm paddy straw has failed to indicate better result in which they were only able to produce 33.55% cellulose and significantly high lignin percentage (12.14%) compared to the percentage of cellulose and lignin produced by 5 mm and 8 mm (Table 2). Paddy straw with size 2 mm also displayed significantly high amount of ash content and lignin; 12.14% and 13.68% respectively. This further suggests that there is a limit in substrate's size in achieving optimum delignification.

Reducing the particle size of paddy straw by grinding is expected to assist the delignification process by facilitating heat and mass transfer in enzymatic hydrolysis [20]. Hypothetically, reduction in paddy straw particle size would give a higher surface volume ration, delignification and enhanced enzyme accessibility for hydrolyzation, thus increases the availability of lignocellulosic compound [28,29]. However, the size of 2 mm may impose negative effects since very fine particles prefer to form clumps during the following steps involving liquid, causing a

**Table 2. Percentage of lignocellulosic composition of untreated paddy straw fibres for all sizes**

| Size | Ash Content (%) | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|---|
| 2 mm | 13.68±0.97[a] | 33.55±0.71[b] | 20.09±0.25[b] | 12.14±0.16[a] |
| 5 mm | 12.17±0.44[b] | 35.61±0.80[a] | 26.96±3.31[a] | 10.79±0.27[b] |
| 8 mm | 11.52±0.34[c] | 34.64±0.39[ab] | 21.85±1.50[ab] | 10.92±0.28[b] |

*Values are means of three replicates of pretreatment with ±SD.*
*\*Means in each column with same superscript letter are not significantly different amongst themselves when Tukey test were used at 5% significance level*

channeling effect [30]. Channeling is a process that limits the path of a charged particle in a crystalline solid [31]. On the other hand, 8 mm paddy straw may have a low surface area and poor in eliminating mass and has a difficulty in transferring heat during hydrolysis reactions, thus leading to a less effectiveness in delignification and lignocellulosic availability.

Size 5 mm may have the traits to maximize the availability of specific surface area and reduce the degree of polymerization (DP) by accompanying the formation of more cellulose ends available to the exoglucanase [27,32]; as they were able to exposed highest of cellulose availability (35.61%). The particle size also plays important role in determining the power requirement for mechanical comminution (a reduction process of raw materials from one particle size to a smaller). A study proposes if particle size held to the range of 3-6 mm, the energy input for comminution can be kept below 30 kWh per ton of biomass [33]. Too small, less than 3 mm, requires more energy inputs in substrate preparation. Not only size 5 mm was able to produce the highest amount of cellulose among other sizes, the study also strongly suggests that medium size requires lower energy inputs; thus, making the whole conversion process becomes more cost-effective.

## 3.2 Pretreatments with Different Concentration of Acid and Alkali

Physico-chemical pretreatment were referred as a combination of physical and chemical pre-treatments. In this study, the combination of both pretreatment was carried out to enhance the lignocellulosic material availability for bioconversion process in later stages (Table 1). Autoclaved at 121 ± 5ºC for 20 minutes has showed improved amount of cellulose content from untreated paddy straw of size 5 mm; increased to 46.52% from initial percentage of untreated paddy which is 35.61%. This treatment also has encouraged the delignification process

by dropping to 9.66% from 10.79% of untreated paddy straw. In order to further investigate the effectiveness of autoclave treatment, combination of boiled water were tested and result shows boiled water has promotes larger delignification and desilication process (8.46% lignin; 9.44% ash content) but no further improvement of cellulose content from only autoclave treatment.

A combination of physical and chemical pre-treatments of paddy straw with size 5 mm treated with various concentrations of acid and alkali pretreatments were carried out (Table 3). The best pretreatment were based on the lignocellulosic composition of the paddy straw. Pretreatment of 2% (w/v) NaOH (4 hour) has shown 2 folds of cellulose content and almost 90.54% reduction of lignin from 35.61% to 72.47% and 1.02% from 10.79% compared to untreated paddy straw, respectively. The percentage of hemicellulose has also been reduced from 26.96% to 19.42% when pretreated with 2% (w/v) NaOH compared to untreated paddy straw. The pretreatment with NaOH with other agro waste such as wheat straw results in slight degradation of hemicellulose, whereas the contents of lignin after alkali pretreatment were particularly low (121°C, 4% NaOH, 2.2%), indicating that alkaline solutions efficiently remove lignin by breaking ester bonds, thereby increasing the porosity of biomass [34]. A study by Harun and Geok [35] was only managed to achieve the highest delignification of 79.6% using more concentrated alkali (12% (w/v) NaOH for 1 hour). Aside from their high efficiency, pretreatment with dilute concentration of alkali was preferred due to their economic feasibility and the residual of alkali on the substrate that can be easily removed compared to a more concentrated alkali. The determination of ash content is vital as the quality of ash determines the total amount of recoverable silica due to it is the largest constituent in the ash. This would be particularly useful in the case of paddy, which the material is initially considered unsuitable for a

biofuel feedstock as they contain high percentage of silica [36]. However, with the pretreatment of 2% (w/v) NaOH, the content of ash has been reduced to 5.44% compared to the percentage of ash content in untreated paddy straw of 12.17%.

The second best pretreatment in this study was 0.5% (v/v) $HNO_3$. The composition of cellulose obtained was 60.72%; which is behind 2% (w/v) NaOH by 11.75%. The percentage of hemicellulose composition has been reduced significantly to only 10.79%; with a total of loss of 59.97%, making this pretreatment as the lowest producers of hemicellulose. For bioethanol production, removal of hemicellulose in initial stage is important to reduce structural constrains for further enzymatic cellulose hydrolysis [37]. Despite the increased percentage of cellulose in dilute acid pretreatment, the treatments failed to reduce the amount of lignin and ash content in the paddy straw. The values rose higher than the percentage of untreated paddy straw.

Out of all various concentration of acid pretreatment were used, the least effective concentration of $HNO_3$ was 5% (v/v); which only produced 44.83% cellulose. The percentage of cellulose decreases as the concentration of acid becomes higher. This pattern can also be seen in the percentage of lignin and ash content of the treatment; both of the parameter increases as the concentration of the treatment exposed to the paddy straw become more concentrated.

In this study, a combination of physical, autoclaved (by means of steaming without explosion) and chemical pretreatment were used to heighten up to their possible maximum capacity in degrading the cellulosic materials of paddy straw. Steaming with or without explosion (autohydrolysis) is known to their function in removing hemicellulose and helps to elevate the mean pore size of the substrate for the probability of the cellulose to become hydrolyzed [38,39]. This process is totally different from steam explosion. Steam explosion seems to be suitable for hardwoods, but become limited for softwoods that contain a comparatively large amount of condensed-type lignin [40]. There are some limitations of steam explosion which includes the incomplete disruption of the lignin–carbohydrate matrix [41]. The autoclave pretreatment has successfully displayed their efficiency in increasing the accessibility of cellulose up to 46.52%; with an increment of 10.91% from untreated paddy straw. Meanwhile, boiled or liquid hot water (LHW) pretreatment does improved delignification and disilication process but failed to improve the cellulose availability. This event occurred as water under high pressure penetrate into the biomass to hydrate the cellulose but somehow causing the removal of hemicellulose and part of the lignin [26].

Dilute nitric acid ($HNO_3$) and sodium hydroxide (NaOH) were used at various concentrations to observe the effectiveness of the treatments in

**Table 3. Percentage of lignocellulosic composition of 5 mm paddy straw fibres after treated with different pretreatment**

| Treatment | Ash content (%) | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|---|
| Untreated | 12.17±0.44[d] | 35.61±0.80[d] | 26.96±3.31[bcd] | 10.79±0.27[de] |
| Autoclaved without chemical treatment | 12.99±0.74[cd] | 46.52±1.91[c] | 20.47±2.94[cde] | 9.66±0.30[ef] |
| Boil with autoclaving | 9.44±2.23[e] | 43.97±0.45[c] | 28.90±2.37[bc] | 8.46±1.23[f] |
| **Acidic treatment with autoclaving** | | | | |
| 0.5% (v/v) $HNO_3$ | 13.33±1.11[cd] | 60.72±1.08[b] | 10.79±5.25[f] | 12.68±0.08[c] |
| 1.0% (v/v) $HNO_3$ | 15.11±0.79[bc] | 58.09±0.43[b] | 16.50±3.85[ef] | 12.90±0.43[c] |
| 2.0%(v/v) $HNO_3$ | 16.03±0.11[b] | 46.10±4.09[c] | 28.73±3.12[bc] | 15.43±1.04[b] |
| 5.0% (v/v) $HNO_3$ | 18.99±1.57[a] | 44.83±2.69[c] | 39.48±3.33[a] | 18.66±0.40[a] |
| **Alkaline treatment with autoclaving** | | | | |
| 0.5% (w/v) NaOH | 13.74±0.69[bcd] | 58.57±0.38[b] | 17.47±2.27[ef] | 11.55±0.50[cd] |
| 1.0% (w/v) NaOH | 7.22±0.25[ef] | 46.42±0.16[b] | 34.16±0.28[ab] | 2.38±0.27[g] |
| 2.0% (w/v) NaOH | 5.44±2.87[f] | 72.47±2.06[a] | 19.42±2.14[de] | 1.02±0.09[g] |
| 5.0% (w/v) NaOH | 7.09±6.88[ef] | 48.32±2.05[c] | 23.20±4.05[cde] | 1.45±0.13[g] |

*Values are means of three replicates of pretreatment with ±SD.*
*\*Means in each column with same superscript letter are not significantly different amongst themselves when Tukey test were used at 5% significance level*

removing ash and lignin content in order to enhance the accessibility towards cellulose and hemicellulose of the paddy straw. Dilute acid pre-treatments, $HNO_3$ were chosen over other acid treatments such as sulfuric acid ($H_2SO_4$) and hydrochloric acid (HCl) due to their ability to produce highest glucose content and good in cellulose-to-sugar conversion process [42]. According to Kumar and Sharma [43], the most common alkali pretreatments are using hydroxyl derivatives of sodium, potassium, calcium, and ammonium salts. The selection of NaOH were based on the report by Vaccarino et al. [44], in which the effects of $SO_2$, $Na_2CO_3$, and NaOH pretreatments were tested on the enzymatic digestibility of grape marc and found out the greatest degrading effects were obtained by pretreatment with 1% (w/v) NaOH solution.

In this study, the best pretreatment of paddy straw with size 5 mm was by 2% (w/v) NaOH. The mechanism of dilute NaOH pretreatment works by rupturing the structural linkages between lignin and carbohydrates of hemicelluloses, leading to a decrease in the lignin polymerization degree, a decrease in cellulose crystallinity, and a separation of the hemicellulose sugars [45]. The effectiveness of 2% (w/v) NaOH has been reported by Rahnama et al. [46], where higher enzymatic hydrolysis and glucose were detected with the use of substrate pretreated with 2% (w/v) NaOH compared to higher NaOH concentration. Concentration more than 2% (w/v) could cause loss of carbohydrate through solubilization while the substrate was being pretreated. This explains why pretreatment of 5% (w/v) NaOH becomes less effective in exposing cellulosic materials as the percentage of cellulose dropped to 48.32%.

Acid pretreatment is known for their ability to remove hemicellulose efficiently from straws to provide high yields of fermentable sugars and have been used as parts of overall processes in fractionating the components of lignocellulosic biomass [47]. However, the concentration of the acid influenced their efficiency as the percentage of hemicellulose increases along with the amount of acid concentration. The acid concentration of more than 0.5% (v/v) showed increased percentage of lignin and ash content compared to the percentage of the untreated paddy straw; as the structure of lignin changes due undesirable reaction of the simultaneous depolymerization and repolymerization of lignin

during acid-catalyzed treatment of biomass [48, 49]. However in the context of ash, more than 100% was detected where the apparent increased of silicon content may due to the mechanism of $HNO_3$ in removing organic and inorganic (non-silicon) components in paddy straw during soaking process, thus resulting the sample weigh more than 50% of control samples [50]. In some cases, the pretreatment of $HNO_3$ also involved in the leaching process instead of dissolution of $SiO_2$ in which they are significantly better at removing impurities, carbon and obtained high purity of silica with 99% and more [51]. According to Brodeur et al. [52], another drawback of acid pretreatment is the production of fermentation inhibitors like furfural and hydroxymethyl furfural (HMF) that reduces the effectiveness of the pretreatment method and further processes.

## 3.3 SEM Images of Cell Wall Structure of Paddy Straw

Physico-chemical pretreatments with various concentrations of $HNO_3$ and NaOH has significantly increased the cellulose and hemicellulose content and reduced the lignin. Fig. 1 depicts the impact of acid and alkali in pretreatment where the surface structure of untreated paddy straw was densely packed (Fig. 1a) compared to rough surface of pre-treated paddy straw (Fig. 1b & 1c).

This rough surface is an evidence of cellulose crystallization resulted from the breakdown of hemicellulose, lignin and ash by hydrolyzing uronic acid and acetic esters and also by the swollen cellulose layer [53,30]. The initial structure of untreated paddy straw (size 5 mm) is coarse and brown in color, but after been treated with 2% (w/v) NaOH, the structure become softer and green in color.Dilute alkali treatments reduce the recalcitrant nature of lignocellulosic biomass by digesting cellulose fibers and maximize the cellulose accessibility [54]. According to Zhang and Cai [55], pretreatment with 2% (w/v) NaOH on paddy straw has caused the microfibril separated from the initial connected structure (Fig. 1a) and fully exposed, causing the increment in the external surface area and the porosity of the rice straw. In comparison to acid pre-treatment, alkali pretreatment has caused a larger surface area of distortion, which further increased the accessibility towards cellulose and hemicellulose.
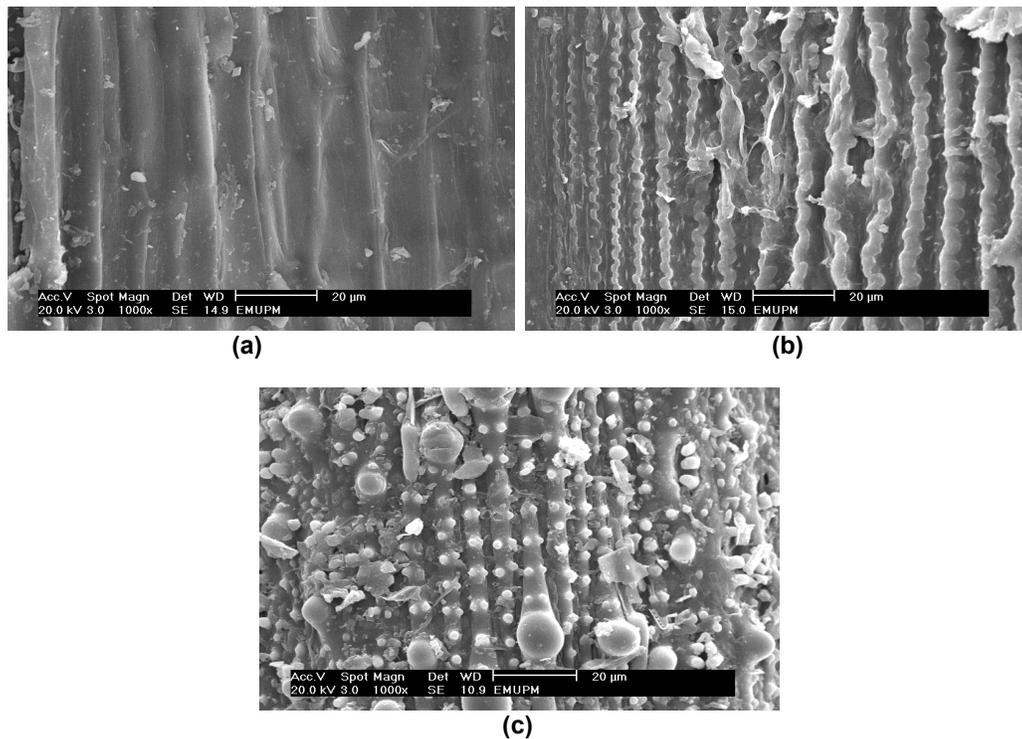
**Fig. 1. Scanning electron micrographs of 5 mm paddy straw fibres. (a) Untreated (b) Treated with 2% (w/v) sodium hydroxide (NaOH) (c) Treated with 0.5% (v/v) nitric acid (HNO₃)**

## 4. CONCLUSION

Based on the percentage of lignocellulosic composition and ash content analysis, a physico-chemical pretreatment of size 5 mm and 2% (w/v) NaOH was chosen as the best pretreatment for paddy straw since it exhibits the highest cellulose percentage which is 35.61% and 72.47%, respectively; due to their ability to performed desilication and delignification efficiently (5.44% ash content; 1.02% lignin). The performance of 2% (w/v) NaOH was supported by SEM images on surface structure of paddy straw.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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