Original Research Article

Improvement of Delignification, Desilication and Cellulosis Content Availability in Paddy Straw via Physico-Chemical Pre-treatments

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 pre-treatment, 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 pre-treatments 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: Pre-treatments comprises the combination of physical (mechanical) and chemical 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 pre-treated with 2 % (w/v) sodium hydroxide (NaOH), the percentage of cellulose content escalated to 72.47%. Pre-treatment 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, 2 % (w/v) NaOH was found to be the most suitable condition to break the cellulose-lignin complex.

Keywords: Acid pre-treatment; alkali pre-treatment; ash content; bioethanol; SEM images.

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, [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 [10], the ash is made up of 75% SiO2, 3% P2O5, 1.5% CaO, 2% Fe2O3, 10% K2O and small portions of Na, Mg, and S. Silicon has played many significant roles for paddy such as grain yield, phenolic synthesis, as well as serving as the shield for plant cell wall [9]. Nevertheless, silicon has become a limiting factor in paddy straw pre-treatment for enzymatic digestion.

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]. 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. Lignin acts as a matrix for the cellulose microfibrials made up by polymer chains, which contain tightly packed and crystalline regions [14].

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]. Pre-treatment process is consequently crucial to remove lignin, silica, reduce cellulose crystallinity, and to maximize the accessible surface area of materials [17].

Pre-treatment, 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 [18], many pre-treatment techniques have been developed to improve this conversion process. The effectiveness of a pre-treatment 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, pre-treatment 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]. Hence, this study attempts to determine the effectiveness of various physical and chemical pre-treatments 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. MATERIAL 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 [21].

2.2 Pre-treatment of Paddy Straw

Combination of physical and chemical pre-treatments 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 pre-treated 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.

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 [21]. 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 ± 25 ºC after being treated with 72% (v/v) sulfuric acid (H_2SO_4) for 3 hours [22].

2.5 Determination of ash content

The percentage of ash content was determined and calculated from the method by [21]. 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 ± 25 ºC 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 [23]. The images were then viewed and observed using SEM at 1.0 K magnification.

Table 1. Pre-treatments on paddy straw.

Pre-treatments	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; autoclaved (121°C ± 0.5; 15 minutes)
0.5% (v/v) HNO_3	Soaked in 0.5% (v/v) HNO_3 (4 hours); autoclaved (121°C ± 0.5;15 minutes)

1.0% (v/v) HNO_3	Soaked in 1.0% (v/v) HNO_3 (4 hours); autoclaved (121°C ± 0.5;15 minutes)
2.0% (v/v) HNO_3	Soaked in 2.0% (v/v) HNO_3 (4 hours); autoclaved (121°C ± 0.5;15 minutes)
5.0% (v/v) HNO_3	Soaked in 5.0% (v/v) HNO_3 (4 hours); autoclaved (121°C ± 0.5;15 minutes)
0.5% (w/v) NaOH	Soaked in 0.5% (w/v) NaOH (4 hours); autoclaved (121°C ± 0.5;15 minutes)
1.0% (w/v) NaOH	Soaked in 1.0% (w/v) NaOH (4 hours); autoclaved (121°C ± 0.5;15 minutes)
2.0% (w/v) NaOH	Soaked in 2.0% (w/v) NaOH (4 hours); autoclaved (121°C ± 0.5;15 minutes)
5.0% (w/v) NaOH	Soaked in 5.0% (w/v) NaOH (4 hours); autoclaved (121°C ± 0.5;15 minutes)

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 [24]. In this study, the percentages of cellulose, hemicellulose, lignin and ash content were used as parameters to measure the effectiveness of physical and chemical pre-treatment of paddy straw. Theoretically, pre-treatment could further enhance the delignification and desilication processes on lignocellulosic material [25]. Therefore, both cellulose and hemicellulose will be exposed for further saccharification by selected conversion agent.

3.1 Physical pre-treatments

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 pre-treatment 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 pre-treatment by producing an amount of 34.64% of cellulose. However, the percentage of lignin in 8 mm slightly went up to 10.92%. The 2 mm paddy straw has failed to indicate better result in which they were only able to produce 33.55% cellulose compared to the percentage of cellulose produced by 5 mm and 8 mm (Table 2). Paddy straw with size 2 mm also displayed the highest 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 [26, 27]. 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 channeling effect [28]. Channeling is a process that limits the path of a charged particle in a crystalline solid [29]. 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 [25, 30]; 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 [31]. 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.

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 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*

3.2 Pre-treatments with different concentration of acid and alkali

Physico-chemical pre-treatment were referred as a combination of physical and chemical pre-treatments. In this study, the combination of both pre-treatment was carried out to enhance the lignocellulosic material availability for bioconversion process in later stages (Table 1). Autoclaved at 121 ± 5 ºC 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 pre-treatment were based on the lignocellulosic composition of the paddy straw and pre-treatment of 2% (w/v) NaOH has shown tremendous cellulose content improvement from 35.61% to 72.47%; which doubles the amount of cellulose compared to untreated paddy straw. The amount of cellulose increased has also resulting in removal of ash and lignin content as well as reducing the hemicellulose content. The pre-treatment of 2% (w/v) NaOH for 4 hours has reduced the amount of lignin to 1.02% from 10.79% of lignin as in untreated paddy straw. The delignification process was improved up to 90.54% and in comparison to a study by [32], their best pre-treatment for delignification was 12% (w/v) NaOH (1 hour); in which yields the highest delignification of only 79.6%. The value is rather lower than the yield produced by pre-treatment of 2% (w/v) NaOH. Aside from their high efficiency, pre-treatment with dilute concentration of alkali was preferred due to their economic feasibility and the residual of alkali on the substrate can be easily removed compared to a more concentrated alkali. Not only the amount of lignin was reduced, the ash content which made more than 70% of silica has also been reduced to 5.44% compared to the percentage of ash content in untreated paddy straw of 12.17%. The percentage of hemicellulose has been reduced from 26.96% to 19.42% when pre-treated with 2% (w/v) NaOH compared to untreated paddy straw.

Table 3. Percentage of lignocellulosic composition of 5mm 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 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.*

The second best pre-treatment 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 pre-treatment 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 [33]. Despite the increased percentage of cellulose in dilute acid pre-treatment, 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 pre-treatment 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 pre-treatment 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 [34, 35]. The autoclave pre-treatment 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) pre-treatment 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 [24].

In chemical pre-treatments, dilute nitric acid (HNO_3) and sodium hydroxide (NaOH) were used at various concentrations to observe the effectiveness of the treatments in removing ash and lignin content in order to enhance the accessibility towards cellulose and hemicellulose of the paddy straw. In 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 [36]. According to [37], the most common alkali pre-treatments are using hydroxyl derivatives of sodium, potassium, calcium, and ammonium salts; where NaOH were selected based on the report by [38], in which the effects of SO_2, Na_2CO_3, and NaOH pre-treatments were tested on the enzymatic digestibility of grape marc and found out the greatest degrading effects were obtained by pre-treatment with 1% (w/v) NaOH solution.

In this study, the best pre-treatment of paddy straw with size 5 mm was by 2% (w/v) NaOH. The mechanism of dilute NaOH pre-treatment 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 [39]. The effectiveness of 2% (w/v) NaOH has been reported by [40], where higher enzymatic hydrolysis and glucose were detected with the use of substrate pre-treated 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 pre-treated. This explains why pre-treatment of 5% (w/v) NaOH becomes less effective in exposing cellulosic materials as the percentage of cellulose dropped to 48.32%.

Acid pre-treatment 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 [41]. However, the concentration of the acid influenced their efficiency as the percentage of hemicellulose increases along with the amount of acid concentration. Acid pre-treatments also produces low amount of cellulose aside from concentration of 0.5% (v/v). 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 [42, 43]. 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 resulting the sample weigh more than 50% of control samples [44]. This statement helps us to understand the factor contributes the percentage of lignocellullosic composition of acid treatment to be more than 100% in total. The pre-treatment of HNO_3 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 [45]. According to [46], another drawback of acid pre-treatment is the production of fermentation inhibitors like furfural and hydroxymethyl furfural (HMF) that reduces the effectiveness of the pre-treatment method and further processes.

3.3 SEM images of cell wall structure of paddy straw

Physico-chemical pre-treatments with various concentrations of HNO_3 and NaOH has significantly increased the cellulose and hemicellulose content and reduced the lignin. Figure 1 depicts the impact of acid and alkali in pre-treatment 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 [47, 28]. Dilute alkali treatments reduce the recalcitrant nature of lignocellulosic biomass by digesting cellulose fibers and maximize the cellulose accessibility [48]. In comparison to acid pre-treatment, alkali pre-treatment has caused a larger surface area of distortion, which further increased the accessibility towards cellulose and hemicellulose.

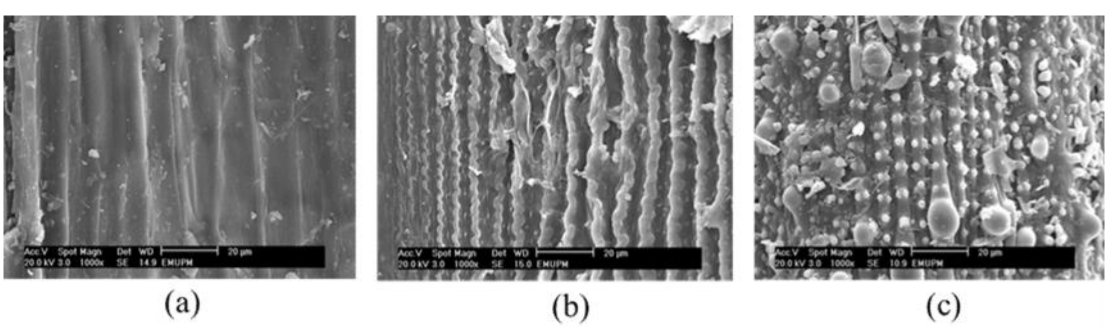

Fig. 1: Scanning electron micrographs of 5mm paddy straw fibres. (a) Untreated (b) Treated with 2% (w/v) sodium hydroxide (NaOH) (c) Treated with 0.5% (v/v) nitric acid (HNO_3)

4. CONCLUSION

Based on the percentage of lignocellulosic composition and ash content analysis, a physico-chemical pre-treatment 2% (w/v) NaOH was chosen as the best pre-treatment for paddy straw since it exhibits the highest cellulose percentage which is 72.47%; 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 that were torn as a result of pre-treatment with dilute alkali. Cellulose has become the important key for the selection as it is the main component to be converted into glucose in bioethanol production.

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