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Research Article

Effects of Dilute Acid and Alkaline Pretreatments on Enzymatic Saccharification of Palm Tree Trunk Waste for Bioethanol Production

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Abstract

The sugar palm tree (*Arenga pinnata)* was abundant in Indonesia and has high cellulose contents for bioethanol production. However, the lignin content was the major drawback which could inhibit saccharification enzymes and therefore removing the lignin from the biomass is important. This paper evaluated the effects of pretreatments using nitric acid (HNO_3) and ammonium hydroxide (NH_4OH) at 2 to 10% (v/v) on reducing sugar and ethanol contents and compared with the effects of steam pre-treatment. The pretreated samples were hydrolyzed using cellulase enzymes at pH 5.0 with a substrate concentration of 10% (w/v) for 24 to 72 h at 50 °C. Subsequent assessments of enzymatic saccharification following pre-treatment with 10% (v/v) HNO_3 showed maximum reducing and total sugar contents in palm tree trunk waste of 5.320% and 5.834%, respectively, after 72 h of saccharification. Following pretreatment with 10% (v/v) of NH_4OH, the maximum reducing and total sugar contents of palm tree trunk waste were 2.892% and 3.556%, respectively, after 72 h of saccharification. In comparison, steam pretreatments gave maximum reducing sugar and total sugar contents of 1.140% and 1.315% under the same conditions. Simultaneous saccharification and fermentation (SSF) was conducted at 37 °C (pH 4.8) and 100 rpm for 120 h using 10% (v/v) *Saccharomyces cerevisiae* and cellulase enzyme with a substrate concentration of 10% (w/v). The result showed the highest ethanol content of 2.648% was achieved by using 10% (v/v) HNO_3. The use of 10% (v/v) NH_4OH gained a yield of 0.869% ethanol while the steam pretreatment could obtained 0.102% ethanol.

Keywords: Bioethanol; lignocellulose; substrate concentration; dilute acid pretreatment; alkaline pretreatment; SSF

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1. Introduction

Population growth has led to increased consumption of fossil fuels globally. According to energy information, global petroleum consumption was 88.216 thousand barrels per day in 2010 and was increased to 91.253 thousand barrels per day by the 2013 [1]. In 2014, energy from fossil fuels accounted for 86.3% of global energy consumption, and renewable energy contributed only 9.2% [2]. Increasing fossil fuel consumption depletes reserves and causes global warming and climate change. Therefore, alternative environmentally sustainable energy sources are urgently needed to meet the increas-

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ing demand for fuel oil [3].

The importance of alternative energy sources has increased due to the continuous depletion of limited fossil fuel stocks and due to the need for a safer environment. Among alternative energy sources, bioethanol can be produced from biomass [4]. Bioethanol is a promising renewable energy source that can be exploited to reduce environmental impacts [5]. However, the use of crops for energy production will be a burden on the agricultural sector, especially in developing countries such as Indonesia [6].

Conventionally, bioethanol was produced from starch and sucrose containing materials, such as corn and sugar cane, using first-generation technologies [7]. But the associated impacted on food supply [8] warrants the use of other raw materials. Lignocellulose is commonly found in byproducts of agricultural residues, such as wheat straw, grains, corn stover, and forestry residues, such as sawdust, and can be used to produce bioethanol with second-generation technologies [9]. Because lignocellulosic materials are abundant, these approaches could reduce environmental impacts, because their use is associated with very low greenhouse gas emissions [10].

Lignocellulose comprises the three major molecule class's lignin, cellulose, and hemicellulose. Lignin contributes strength and rigidity to plants and prevents swelling of lignocellulosic materials [11]. Palm trees are among the most abundant sources of lignocellulosic biomasses, and according to Sahari *et al*. sugar palm trunks comprise 46.4% lignin and 53.6% cellulose, of which 61.1 % is holocellulose [12]. In Klaten Indonesia, many home industries extract starch from palm tree trunks for use as a raw materials for *bihun* noodle production. In this process, solid extracts from palm tree trunks are soaked in water and then filtered to separate soluble and insoluble components. Starch is obtained from the water insoluble solids, and the water-soluble solids can be further processed into bioethanol. Palm tree trunk wastes are not fully utilized and are found in many remote areas. These resources could be used to produce bioethanol in second-generation biomass conversion processes.

Production of bioethanol from lignocelluloses is performed in pretreatment, saccharification, and fermentation steps. During the pretreatment process, lignin is broken down and cellulose is made accessible to enzymes that convert polymers into fermentable sugars [13]. Saccharification is performed enzymes that convert cellulose into glucose, which is required for fermentation. The use of enzymes for saccharification is preferred to the use of chemicals, because enzymes are highly specific and operate under mild process conditions [14]. Most cellulase enzymes are relatively unstable at high temperatures, but maximum activities of cellulases have been shown at 40-50 °C and pH 4.5-5.0 [15]. In this study, enzyme activity was decreased at temperatures above 65 °C and was almost completely abolished at 80 °C [16]. Glucose is commonly fermented using microorganisms, and the most commonly used yeast for bioethanol production is *Saccharomyces cerevisiae*, which has low optimum pH and high tolerance to ethanol and inhibitors, and does not require oxygenation [17].

Various processes have been developed for ethanol production. Among these, SSF requires little energy [18] compared to separated hydrolysis and fermentation (SHF) procedures. SSF reportedly gives greater ethanol yields than SHF, reflecting less inhibition during saccharification [19]. Yet SSF is hampered by differing optimal temperatures for saccharification (45-50 °C) and fermentation (20-30 °C), and is typically performed at 35-38 °C so that saccharification and fermentation can proceed simultaneously [20]. Many studies have used SSF to convert various lignocellulose biomasses to bioethanol. Herein, we investigated the use of SSF to convert pretreated palm tree trunk waste as the lignocellulose biomass for bioethanol production. Palm trees are very common in Indonesia and palm tree trunk wastes contain starch, cellulose, hemicellulose, and lignin. The use of solid palm tree trunk waste is limited, especially as feedstock for the production of bioethanol [21]. Palm tree trunk waste is a solid byproduct and is produced following starch extraction, which is performed in many home industries in Klaten, Central Java Indonesia. Biomasses from residues of extracted palm tree trunks have an environmental impact because they remain in the fields around palm starch industries.

Mixtures of cellulase and *S. cerevisiae* were previously employed in SSF processes, in which cellulose and hemicellulose fractions of lignocelluloses were hydrolyzed into fermentable sugars and fermentation into ethanol was performed simultaneously [22]. Our previous study of starch free sugar palm trunks showed the temperature effects of pretreatments with dilute nitric acid (HNO_3) or ammonium hydroxide (NH_4OH) on hydrolysis at a substrate concentration of 1% v/v, and subsequent fermentation. SSF and SHF methods were also compared in a previous investigation [21]. But for

novelty, we investigated the effects of 24, 48, and 72 h pretreatments with different concentrations of HNO_3 or NH_4OH (2%, 4%, 6%, 8%, and 10% v/v) on reducing sugar and total sugar contents during saccharification and subsequent ethanol yields from fermentation. We also investigated the lignocellulosic contents and morphological structures of palm tree trunk waste before and after pretreatments.

2. Materials and Methods

2.1 Preparation of Raw Material

2.1.1 Palm tree trunk waste

Palm tree trunk waste was obtained as a wet coarse powder from a home industry in Klaten, Central Java. Raw materials were oven-dried at 80 °C until a constant weight was achieved, and were then milled into flour with a size of ± 40 mesh. Flours were then stored at room temperature for further use.

2.1.2 Enzyme and yeast

Commercially available cellulase enzyme (SQzyme CS) was purchased from Suntaq International Limited (Nanshan District, China) and was used for saccharification of palm tree trunk wastes. This acid cellulase enzyme is produced by a strain of *Trichoderma reesei* and is supplied as a concentrated liquid used in saccharification and fermentation processes. *S. cerevisiae* was used for ethanol production and was obtained from Gadjah Mada University, Yogyakarta Indonesia.

2.1.3 Preparation of innoculums

Innoculums of *S. cerevisiae* were maintained in medium containing glucose, peptone, yeast extract, and agar, at 20, 20, 10, and 15 g/L, respectively. Culture media were sterilized using an autoclave at 121 °C for 20 min and were then incubated at 30 °C for 72 h. *S. cerevisiae* was characterized by the appearance of white patches on medium. *S. cerevisiae* pre-culture medium contained $(NH_4)_2HPO_4$, urea, KH_2PO_4, and $MgSO_4.7H_2O$ at 2, 6.4, 2, and 1 g/L, respectively. The pre-culture growth medium was sterilized for 20 min at 121 °C and was incubated at 150 rpm and 30 °C for 48 h prior to experiments. The main-culture growth medium was prepared on a rotary shaker at 150 rpm and 30 °C for 72 h, and contained treatment substrate, yeast extract, peptone, $(NH_4)_2HPO_4$, urea, KH_2PO_4, and $MgSO_4.7H_2O$ at 20, 10, 20, 2, 6, 2, and 1 g/L, respectively. Pre-culture and main-culture media were used for fermentation.

2.2 Experimental Methods

2.2.1 Pretreatment of palm tree trunk waste

Steam pretreatment was performed by soaking 25 g samples of dry palm tree trunk waste in distilled water. Dilute HNO_3 and NH_4OH pretreatments were prepared by adding 25 g of dry palm tree trunk waste to solutions containing 2%, 4%, 8%, and 10% (v/v) HNO_3 or NH_4OH with a solid to liquid ratio of 1:20 (w/v) in 500 mL glass beakers. Mixtures were stirred to produce a palm tree trunk slurry, which was then autoclaved at 121 °C for 60 min. Residues were removed from solutions using filter paper on a vacuum filtration unit and were washed with distilled water to obtain neutral pH. Pretreated substrates were washed with water to reduce inhibitory product concentrations [23]. Residues were then dried until a constant weight was achieved and were used for the subsequent processing steps.

2.2.2 Enzymatic saccharification

Dried pretreated palm tree trunk waste samples of 10 g were placed in 250 mL erlenmeyer flasks and were mixed well with 100 mL of citrate buffer at pH 5.0. The slurry was then sterilized at 121 °C for 20 min and 2 g aliquots of cellulase enzyme (200 FPU/g) were added to substrate at 10% (w/v) and the mixtures were incubated at 50 °C and 150 rpm for 24, 48, and 72 h. Reactions were stopped by boiling the samples for 5 min and total sugar and reduced sugar contents were then determined.

2.2.3 Simultaneous saccharification and fermentation

Glucose from the saccharification process was converted to ethanol by fermentation using the yeast *S. cerevisiae*. Fermentation reactions were performed in 250 mL erlenmeyer flask containing 10 g of dry pretreated palm tree trunk waste in 1 M citrate buffer (pH 4.8). After sterilizing for 20 min at 121 °C, 10 mL of pre-culture, 10 mL of main-culture, and 20 FPU/g of cellulase enzyme were added to flasks and the mixtures were incubated at 37 °C for 120 h. Fermented medium was then distilled to determine ethanol contents.

2.3 Analysis

2.3.1 Determination of lignin contents

Lignin contents of 0.3 g dried samples were determined in 3 mL aliquots of 72% H_2SO_4 in glass tubes at room temperature. Mixtures were stirred every 30 min for 2 h to provide

complete hydrolysis. Subsequently, 84 mL of distilled water was added and samples were autoclaved for 1 h. After cooling to room temperature, samples were filtered to separate solids and filtrates. Residues were then dried at 105 °C and, accounting for ashing, acid insoluble lignin contents were determined by burning the hydrolyzed sample at 575 °C in a furnace. Acid soluble lignin contents were determined according to absorbance of samples at 320 nm. Total lignin contents were determined as the sum of acid insoluble and soluble lignin contents as described previously [24].

2.3.2 Determination of hemicellulose contents

To 250 mL erlenmeyer flasks, 150 mL of 500 mol/m^3 NaOH and 1 g dried samples were added. Mixtures were then boiled for 3.5 h and were then cooled to room temperature. After washing and neutralization of pH, mixtures were filtered and residues were dried to a constant weight in an oven at 105 °C. Hemicellulose contents (%w/w) were determined by calculating weight differences between samples before and after treatment [24].

2.3.3 Determination of cellulose contents

Assuming that extracted hemicellulose, lignin, ash, and cellulose are the only components, cellulose contents (%w/w) were calculated as the difference [23].

2.3.4 Determinations of reducing sugar contents

Reducing sugar contents were measured using dinitrosalicylic acid (DNS) as described previously [25]. Briefly, 3 mL aliquots of DNS reagent were added to 3 mL samples in a test tube and the mixtures were incubated at 90 °C for 10 min. Subsequently, 1 mL aliquots of Rochelle salt solution were and the mixtures were cooled to room temperature. Absorbance was then measured at 575 nm. Blanks were prepared by substituting sugar solution with distilled water in the same procedure. Reducing sugar contents were then determined with reference to a standard curve.

2.3.5 Determination of total sugar contents

Total sugar contents were measured using phenol sulfuric acid by adding 0.05 mL of 80% phenol and 5 mL of concentrated H_2SO_4 to 2 mL sample solutions in colorimetric tubes [26]. After 10 min, mixtures were shaken in a water bath at 25-30 °C for 15 min before measuring absorbance at 490 nm for hexoses and 480 nm for pentoses and uronic acids. Blanks were prepared by substituting sugar solution with distilled water and following the same procedure. Total sugar contents were determined with reference to a standard curve.

2.3.6 Determination of ethanol contents

Ethanol contents were determined by direct injection into a Shimadzu GC-8A (Shimadzu Corporation, Japan) gas chromatography instrument. Flow rates of H_2, air, and N_2 (carrier gas) were set at 30, 300, and 2 mL/min, respectively. The temperature of the FID detector was 285 °C and the injection volume was limited to 0.1 µL. Into 1 mL sample vials, 50 mg aliquots of internal standard solution were added to 0.5 mL samples. After mixing, 0.1 µL aliquots of sample solutions were injected directly into the GC instrument using a syringe. Ethanol contents were then calculated as reported previously [27].

2.3.7 Scanning Electron Microscopy (SEM) observations

A JEOL JSM-6510 SEM (JEOL Ltd. Tokyo, Japan) with 1000 × magnification was used to observed morphological changes in biomass samples (raw materials and treated biomass samples of palm tree trunk waste). Dried samples were placed on aluminum specimen mounts using conductive carbon tape. Sputter gold coating was then performed to prevent charging. All specimens were examined using a SEM under vacuum conditions at an accelerating voltage of 5 kV [23].

3. Results and Discussion

3.1 Effects of Pretreatments on Lignocellulose Contents

Pretreatment of lignocellulosic biomasses is an important process because it removes lignins and hemicellulose and improves access of enzymes to cellulose fibers, and hence, the reactivity of cellulose [28]. Pretreatments with dilute acid and alkaline are the most widely used methods for lignocellulosic materials. Dilute acid pretreatments can be performed with inexpensive chemicals, mild operating conditions, and simple procedures. In contrast, alkaline pretreatments increase the accessibility of enzymes to cellulose by removing lignin and some hemicellulose during saccharification [29].

In our hands, lignin and hemicellulose contents were decreased after pretreatment, whereas cellulose contents were slightly in-

creased (Figure 1). Lignin contents were decreased from 24.25% before pretreatment to 17.85% and 16.50% after pretreatment with 10% (v/v) NH_4OH and 10% (v/v) HNO_3, respectively. Under the same conditions, hemicellulose contents were decreased from 26.98% before pretreatment to 22.61% and 22.23%, respectively. Cellulose contents were also slightly increased from 20.57% before pretreatment to 36.70% and 63.53% after pretreatment with NH_4OH and HNO_3, respectively. Hence, the dilute acid pretreatment enhanced the digestibility of cellulose by solubilizing lignin and hemicellulose [30]. Alkaline pretreatments result in saponification of the intermolecular ester bonds crosslinking xylan hemicelluloses and other components (lignin and other hemicellulose) [30], leading to removal of lignin and hemicellulose contents and increased access of the enzyme to cellulose [31].

The effects of HNO_3 pretreatment on lignin and hemicellulose removal and cellulose enhancement were similar to those reported previously [32]. In their study, rice straw pretreatments at different HNO_3 concentrations (0.2%-1.0%), increasing temperatures (120-160 °C), and reaction times (1-20 min) were performed for subsequent fermentation to ethanol. Hemicellulose fractions were increasingly removed by HNO_3 pretreatments that were performed at a high temperatures and for longer times [32]. However, when acid concentrations or reaction times were too high, decomposition products of xylose, such as furfural, become inhibitory. Another study reported the effects of pretreatments of oat hulls with a 4% (w/w) nitric acid solution at atmospheric pressure [33]. These studies showed that pretreatments increased cellulose contents by 2 fold, diminished hemicellulose contents by 4.2 times and lignin contents by 1.5 times, and increased ash contents by 1.3 fold. Previous researcher reported cellulose, hemicellulose and lignin contents after dilute sulfuric acid pretreatments of bagasse pith [34]. They found that sulfuric acid pretreated solid residues had higher cellulose contents than control solid residues than that were pretreated without acid [34], indicating removal of hemicellulose fractions. Lignin and hemicellulose removal by NH_4OH treatments was also considered in a study of H_2SO_4 acid, NaOH, and NH_4OH pretreatments of Pteris (fern) biomasses [35] . In this study, NH_4OH treatments gave the highest reducing sugar concentrations, suggesting greater removal of lignin and unfettered hydrolysis using ammonia. Our fermentation reactions were performed with *S. cerevisiae* (Baker's yeast) and resulted in the production of ethanol to 0.333 mg/L. Production of bioethanol from sugarcane bagasse (SCB) using NH_4OH-H_2O_2 pretreatment and simultaneous saccharification and co-fermentation was reported previously [36]. NH_4OH is a weak alkali that has been used to extract lignin from lignocellulosic materials, and is known to retain high amounts of both glucan and xylan in solid fractions. Moreover, NH_4OH pretreatment has advantages of mild reaction conditions that prevent the formation of various toxic compounds, such as furfural and hydroxymethyl furfural. These compounds are generated from the decomposition of sugars during most other pretreatments [36].

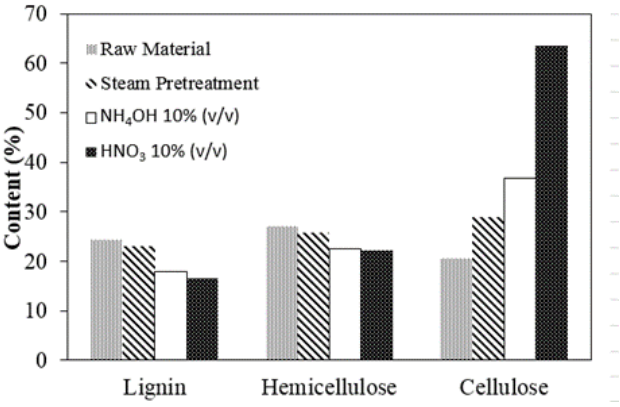

Figure 1. Lignocellulosic contents of palm tree trunk waste before and after pretreatment

3.2 Effects of Dilute Acid and Alkaline Concentrations on Saccharification

Figures 2 and 3 show that acid or alkaline concentrations of pretreated samples influence on sugar contents (reducing sugar and total sugar) during enzymatic saccharification. Under these conditions, sugar contents are increased with increasing acid or alkaline concentrations of pretreated samples, reflecting increased frequencies of random collisions between substrate and active sites of enzymes [37]. Comparisons of these graphs indicate that the highest sugar contents were achieved after 72 h of enzymatic saccharification with 10% (v/v) HNO_3, with reducing sugar and total sugar contents of 5.320% and 5.834%, respectively.

The effects of pretreatments with 0.65% HNO_3 at 158.8 °C for 5.86 min on enzymatic hydrolysis were previously shown in a study of rice straw [32]. These study found that HNO_3-treated rice straw had much higher enzymatic digestibility than untreated controls, indicating that HNO_3 improves enzyme accessibility to cellulose. In their study, the highest glucose

yield was achieved after 72 h hydrolysis (47.7 g/L) and was higher than that with H_2SO_4 (43.8 g/L) [32].

Enzymatic hydrolysis of untreated and nitric acid pretreated oat hull feedstock was investigated previously [33]. The lowest concentration of reducing sugars was observed for untreated oat hulls, and after pretreating oat hulls with nitric acid solution and washing until neutral pH was achieved, the concentration of reducing sugars rose by a factor of 4.3. An- other study conducted previously [34] shows the hydrolysis of pretreated bagasse pith solids. Compared with control pretreatments, acid pretreatments led to higher glucose yields in enzymatic hydrolysates [34]. The glucose yield improved from 14% (control) to 49% and glucose yields of enzymatic hydrolysis after acid pretreatment were higher, reflecting: (1) hemicellulose removal through the breakdown of lignocellulosic structures, and 2) increased porosity and surface area of cellulose, providing greater accessibility for the enzyme. Previous study [38] reported a nitric-acid hydrolysis of *Miscanthus giganteus* to sugars and subsequent fermented to bioethanol. These study reported removal of hemicellulose and lignin after the first pretreatment step. In their first step, *M. giganteus* was treated with 0.5 wt.% nitric acid at temperatures between 120 °C and 160 °C for 5-40 min. In the second pretreatment step, 0.5 or 0.75 wt.% nitric acid was used at temperatures between 180 °C and 210 °C for less than 6 min. After second-step pretreatments, maximum glucose was obtained at 195 °C after 3 min using 0.5 wt.% nitric acid, indicating hydrolysis of hemicellulose and cellulose to fermentable sugars [38].

In our experiments, saccharification rates were significantly higher after 72 h than after 24 and 48 h. Similarly with previous study [39] pretreated rice straw with ionic liquid showed that saccharification times of 24 and 72 h produced glucose yields of 75.4% and 90.9%, re-

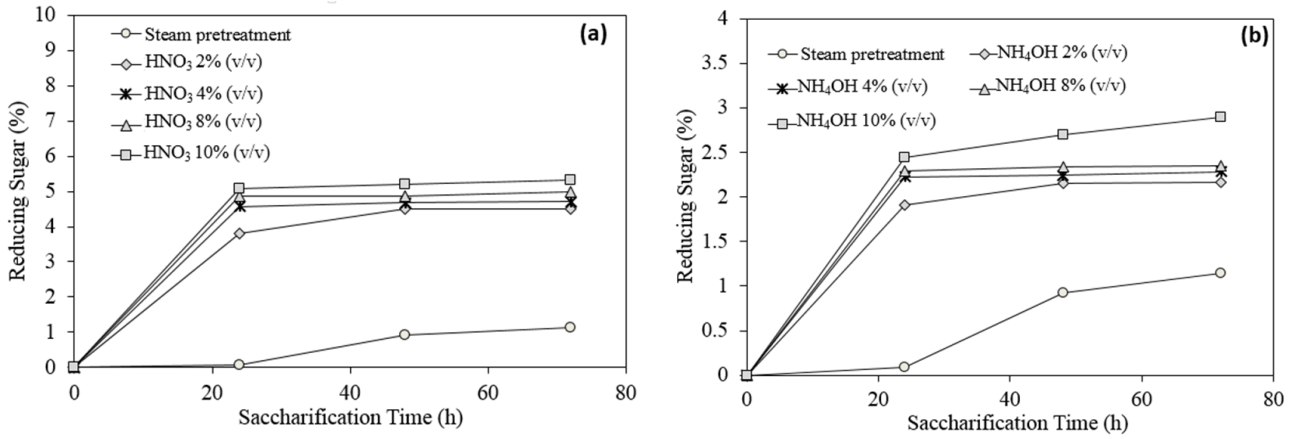

Figure 2. Reducing sugar levels after (a) HNO_3 pretreatment and (b) NH_4OH pretreatment; saccharification was performed by 20 FPU/g cellulase at pH 5.0 and 50 °C

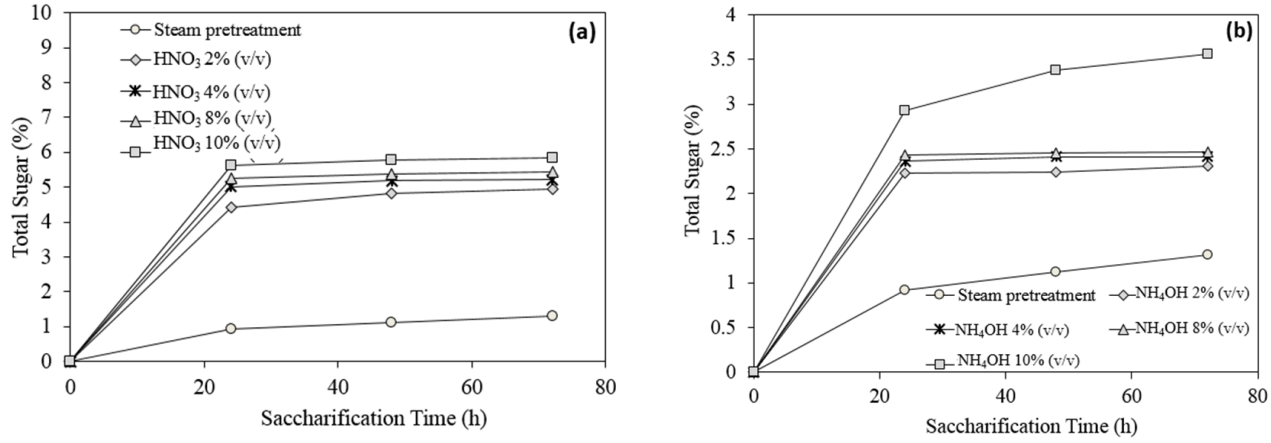

Figure 3. Total sugar contents after (a) HNO_3 pretreatments and (b) NH_4OH pretreatments; saccharification was performed with 20 FPU/g cellulase at pH 5.0 and 50 °C

spectively, at 45 °C using 20 FPU cellulose and 30 IU ß-glucosidase per g of substrate.

According to previous researcher [23] total reducing sugar (TRS) yields from hydrolysis of sugarcane bagasse and spent citronella biomasses increased with acid concentrations and reaction times. The highest TRS yield from citronella and bagasse was achieved after 48 h at 50 °C with 10 FPU of cellulase. Under these conditions, yields from citronella and bagasse biomasses were 226.99 and 282.85 mg/g, respectively. In comparison, hydrolysis at 24 h for 50 °C in the presence of cellulase at 10 FPU produced TRS yields of 204.56 mg/g from citronella and 246.96 mg/g from bagasse [23].

3.3 Effects of Dilute Acid and Alkaline Concentrations on Fermentation

Figure 4 shows slight increases in ethanol contents with increasing acid or alkaline concentrations of pretreated samples. In these experiments, the highest ethanol concentration of 2.6476% was achieved following pretreatment

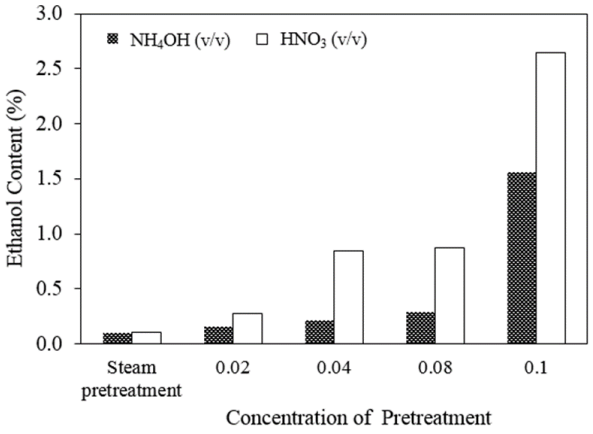

Figure 4. Ethanol contents after simultaneous saccharification and fermentation (SSF) with *S. cerevisiae* and 20 FPU/g cellulase at 37 °C and pH 4.8 for 120 h

with 10% (v/v) HNO_3, as shown in a previous study of HNO_3 pretreated biomass samples. In a study of rice straw fermentation, the previous study [32] showed that higher ethanol concentrations were achieved after pretreatment with 0.65% HNO_3 and suggested that nitrate in the medium enhanced fermentation [32]. Ethanol production from fermentation was also investigated previously [33] by using SHF and dSSF methods with nitric acid pretreated oat hull pulp. In this study, SHF processing led to an ethanol concentration of 22.1 g/L and an ethanol yield of 0.128 g/g oat hull. In comparison, the dSSF process led to an ethanol concentration of 27.6 g/L and an ethanol yield of 0.159 g/g oat hull. This comparison of SHF and dSSF methods for bioethanol synthesis demonstrates that dSSF enhances yields by a factor of 1.2 compared with SHF. In another study of second-step hydrolysate samples that were collected using 0.5% nitric acid at 195 °C for 3 min, fermentation was performed with the yeast strain *S. cerevisiae* SA-1, and resulted in 0.46 g ethanol/g glucose [33]. These data show that the byproducts of nitric acid hydrolysis do not affect fermentation, because ethanol yields were close to theoretical levels [38]. Another study [40] shows an increasing biomass concentrations between 1% and 20% led to increasing ethanol concentrations. Yet, lower yields were achieved at bio-mass concentrations of > 20%, perhaps due to the absorption of liquids by the biomass [40].

3.4 Scanning Electron Microscope Analysis

Figure 5a shows the morphology of palm tree trunk waste before pretreatment. These specimens were compact and had smooth surfaces. The most apparent effect of HNO_3 (Figure 5b) and NH_4OH pretreatments (Figure 5c) was the formation of pores on these surfaces. Moreover, with substantial loss of lignins, these surfaces became very rough. The ob-

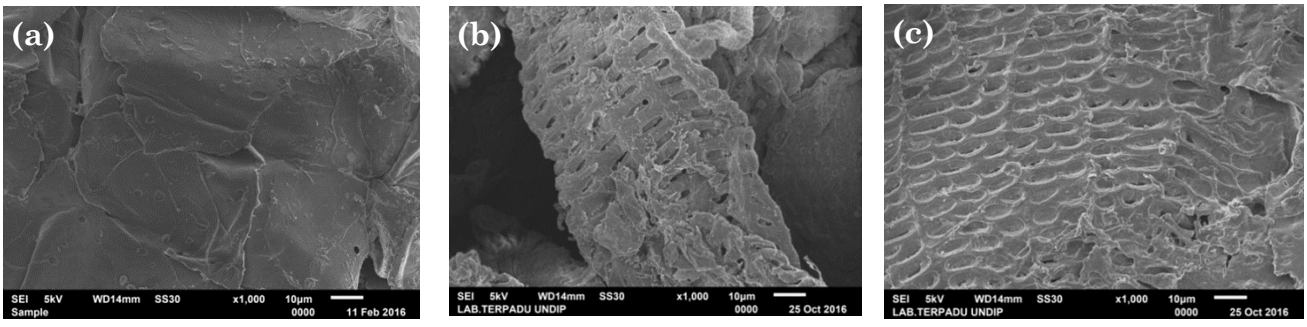

Figure 5. Morphological structure of palm tree trunk waste at 1000 × magnification; (a) raw material, (b) pretreated with 10% (v/v) HNO_3, (c) pretreated with 10% (v/v) NH_4OH; scale bars represent 10 µm.

served degradation of cell walls indicates that pretreatment processes successfully break down lignin and hemisellulose contents of lignocellulose biomasses [41]. Morphologies of untreated and NH_4OH-H_2O_2 pretreated sugarcane bagasse (SCB) samples were previously analyzed using SEM [36]. This study showed that untreated SCB samples had smoother surfaces than pretreated SCB samples, and a difference in porosity was noted between treated and untreated samples. Moreover, the effects of the pretreatment were clearly evident in SEM images of vascular bundles.

4. Conclusion

Palm tree trunk wastes contain substantial amounts of cellulose and offer potential raw materials for bioethanol production, which could be produced from high concentrations of substrate. Sugar and ethanol contents were also increased with acid or alkaline concentrations of pretreated samples concentrations. The present pretreatment, saccharification, and fermentation procedure achieved a maximal ethanol content of 2.6476% following pretreatment with 10% (v/v) HNO_3. This study represents a step toward the development of pretreatments that optimize ethanol production from palm tree trunk wastes and other lignocellulosic materials.

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