INTRODUCTION

Napier grass (*Pennisetum purpureum* Schum) is also known as elephant grass. This perennial plant that grows in tropical, subtropical, and temperate regions is native to East Africa and is an important forage crop. The grass often grows in plains (sea level), mountains (elevation of 1500 m), riverbeds, arable land, roadsides, and wasteland. It grows in upright clusters, up to 2-5 m in height with a stem diameter up to 3-4 cm. Similar to other C4 grasses such as *Miscanthus*, Napier grass grows fast, regenerates easily, and is tolerant to drought, pests, and diseases. It is generally propagated by the cut-stem implantation method or ramets. This grass has a high biomass productivity with an annual dry matter yield often exceeding 40 t/ha. In Florida, USA, a report showed that the annual production of this grass is about 45 t/ha, which is much higher
than the yield of sugarcane 21 t/ha per year or corn 13 t/ha per year. In some strain, the administration with 3-month inter-cutting interval, a mean of 50.2 t dry matter (DM)/ha/year are achieved. Napier grass has cellulose content up to 40%-45% dry base weight, which makes it a great potential source for renewable energy production, as well as a substrate for the production of bio-based chemicals, for example, monosaccharides, oligosaccharides, xylitol, alcohols, organic acids, etc.

Generally, the refining of Napier grass or others of lignocellulosic biomass has to hydrolyze cellulose into fermentable sugars by enzyme, and then these sugars are converted into products by microorganism. However, it is shows a low utilization of enzymatic hydrolysis when the lignocellulosic biomass is used directly, because the cellulose fiber is covered with the lignin. Thus, a pretreatment step is needed to render it more accessible for hydrolytic enzyme. Numerous physical, physico-chemical, chemical, and biological methods have been tested for the pretreatment of Napier grass. These pretreatments afford the recovery of cellulosic content from grass biomass, the removal of structural components such as lignin, pectin, or hemicellulose, and the breakdown of the crystal region in the cellulose bunch to render it digestible for cellulolytic enzymes. Chemical methods for Napier grass biomass pretreatment involve the use of sodium hydroxide, liquid ammonia, dilute sulfuric acid, dilute acetic acid, and hydrogen peroxide. Napier grass biomass has also been subjected to hot water pretreatment at 100°C for a period of 25 minutes in a batch autoclave reactor. The use of acid or alkali, combined with heat or microwave, has also been proposed for the pretreatment of Napier grass. Biological pretreatment is another method that uses lignin-degrading fungi to decompose lignin and breakdown the recalcitrant linkage between lignin and hemicellulose. After microbial pretreatment, cellulose is thus more exposed to hydrolysis and the saccharide yield from Napier grass can be enhanced. In addition, two-stage pretreatment processes have often been used. Biomass of Napier grass was presoaked with 1% H₂SO₄, or 1% NaOH, and then exposed to 75 and 150 kGy of electron beam irradiation. Another example is a steam explosion on grass biomass, followed by 5% NaOH pretreatment.

The pretreatment of lignocellulosic biomass generally causes the removal of lignin and hemicellulose, leaving the cellulose (glucan) in the solid. For example, the diluted acid pretreatment can effectively hydrolyze hemicellulose, while the pretreatment with alkaline solution can dissolve lignin from the Napier grass. An additional alkaline pretreatment applied to the remaining solid fraction from the diluted acid pretreatment can improve the lignin removal rate.

This study evaluated the chemical pretreatments efficiency including diluted sulfuric acid, sodium hydroxide, and two-stage by diluted sulfuric acid and sodium hydroxide successively on non-cellulosic components removal. The yield cellulosic materials were used to produce ethanol by simultaneous saccharification and fermentation (SSF) that is an effective process for bioethanol production from lignocellulosic feedstock after pretreatment. Afterward, the ethanol production using SSF was influenced by the removal efficiency of non-cellulosic fractions via different pretreatment methods was comparatively studied in the present work.

2 | MATERIALS AND METHODS

2.1 | Feedstock preparation

The feedstock preparation is following Yeh et al. Briefly, stems of Napier grass harvested from Chiayi, Taiwan, were pressed by a roll mill to remove juice, and then cut into sticks ca. 2 cm in length. These Napier grass sticks were dried at a ventilated place until constant weight as the feedstock for pretreatments and ethanol fermentation.

2.2 | Alkaline pretreatment

Napier grass sticks were incubated with 10% NaOH at solid to liquid ratio of 1:20 (w/v) at 90°C for 1 hour, and then the biomass (insoluble fraction) were collected and washed with tap water until the pH became neutral. After the removal of free water using a dehydrator (Gen Fong BM900, 8 kg capacity), the pretreated biomass was stored in sealed plastic bags at 4°C. To calculate its dried weight, the pretreated biomass was dried in an oven at 65°C for 24 hours.

2.3 | Dilute acid pretreatment

Napier grass sticks were incubated with H₂SO₄ (1, 5, 10% w/v) at a variable ratio of solid to liquid (w/v) between 1:5 and 1:20 with different temperature (120, 150, and 180°C) for 90 minutes in a reactor that is a tailor-made high-pressure stainless steel cooker equipped with a heating coil that can generate a temperature up to 400°C in the vessel (ca. 500 mL). The pretreated biomass (insoluble fraction) were collected and washed with tap water. After the removal of free water using a dehydrator, the pretreated biomass was stored in sealed plastic bags at 4°C. To calculate its dried weight, the pretreated biomass was dried in an oven at 65°C for 24 hours.

2.4 | Two-stage pretreatment

Napier grass sticks were incubated in the above-mentioned reactor with 1% H₂SO₄ at a solid to liquid ratio of 1:10 (w/v) at 120°C for 1 hour. Treated biomass (insoluble fraction) were collected and then washed with tap water. The resultant solids were then treated again with 2% NaOH with a 1:10 solid to liquid ratio at 80°C for 6 hours. After the removal of free water using a dehydrator, the pretreated biomass was stored in
sealed plastic bags at 4°C. To calculate its dried weight, the pretreated biomass was dried in an oven at 65°C for 24 hours.

2.5 | Enzymatic saccharification

Enzymatic saccharification of pretreated Napier grass biomass was done by using commercial cellulase Celly® CTec2 and HTec2 Enzymes (Novozymes, Denmark). Pretreated biomass were incubated in 0.05 mol/L citrate buffer at pH 4.8, to produce a final water-insoluble solids (WIS) concentration of 10% (w/w) and then autoclaved at 121°C 30 minutes. Cellulase of 0.08 mL/g WIS (corresponding to 12 FPU/g-WIS) was loaded into each substrate mixture and then incubated in water bath at 50°C for 96 hours. After enzyme hydrolysis, the solid and hydrolysate were separated by centrifugation at 4170 × g for 30 minutes. The hydrolysate was stored at 4°C for fermentation. The enzyme saccharification protocol follows the application sheet of Celly® CTec2 and HTec2 Enzymes for hydrolysis of lignocellulosic materials (Novozymes, Denmark).

2.6 | Simultaneous saccharification and fermentation (SSF) for bioethanol production

Simultaneous saccharification and fermentation of pretreated Napier grass biomass was done by using the commercial cellulase Cte2 and Ethanol Red™ (a commercial active dry yeast, mainly composed of Saccharomyces cerevisiae), respectively. Pretreated biomass was transferred to a 250-mL flask containing 0.05 mol/L citrate buffer at pH 4.8, to produce a final WIS concentration of 10% (w/w), and then autoclaved at 121°C for 30 minutes. For SSF, cellulase (0.08 mL/g-WIS) and dry yeast (1 g/L) were loaded into each substrate mixture, and then incubated in water bath at 40°C, 150 rpm for 72 hours.

2.7 | Analytical methods

The composition of the Napier grass samples before and after pretreatment was determined according to a previously published method. The concentrations of ethanol, acetate, furfural, and monosaccharides in the hydrolysate and SSF mixture were determined using a Jasco PU-1580 HPLC equipped with an RI detector (Shodex RI-71). The sample mixture was separated by a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm i.d.) that was operated at 65°C using a mobile phase of 5 mmol/L H₂SO₄ at the flow rate of 0.6 mL/min. The theoretical ethanol yield was estimated by following formulae.

\[
\text{Ethanol yield (\%)} = \frac{\text{Ethanol produced (g) in SSF}}{\text{Glucose content in pretreated biomass (g) × 0.511 × 100.}}
\]

3 | RESULTS AND DISCUSSION

3.1 | Pretreatment of Napier grass

Table 1 shows the change in Napier grass biomass composition after different pretreatments. Prior to pretreatment, the stems of Napier grass were composed of 41.8% cellulose (glucan), 23.2% hemicellulose (including xylan and arabinoxylan), and 25.0% lignin on a dried mass basis (Table 1). As expected, the cellulose content in the biomass increased significantly after alkaline pretreatment due to the removal of hemicelluloses (67.1% from dried raw material) and lignin (63% from dried raw material). After the alkaline pretreatment at 90°C for 1 hour, the cellulose content increased to 62.1% w/w, meaning that about 70.3% glucan were remained. Similar results were reported by Yeh et al., where Miscanthus floridulus stems were used as the feedstock. After the alkaline treatment, there were displayed 81.6% and 61.6% removal of the hemicellulose and lignin with 77% glucan remaining. A 10% NaOH solution was used as we previously used in the alkaline pretreatment of cogongrass, sugarcane bagasse, and Miscanthus floridulus to produce ethanol. At this higher NaOH concentration, the alkaline solution can be

| Composition | Pretreatment method | Cellulose | Hemicellulose | Lignin | Ash | Extractive | Solid recovery |
|-------------|---------------------|-----------|---------------|--------|-----|------------|---------------|
| Untreated   | 41.8 ± 2.2          | 23.2 ± 1.3 | 25.0 ± 0.3    | 1.0 ± 0.2 | 8.1 ± 1.2 | –            |
| Alkaline    | 62.1 ± 2.7          | 16.1 ± 0.8 | 19.5 ± 1.7    | 0      | 1.0 ± 0.5 | 47.4 ± 0.6   |
| Dilute acid | 47.8                | 8.5        | 26.8          | 0.4    | 11.2      | 50.0 ± 0.5   |
| Two-stage   | 67.6                | 4.0        | 17.0          | 0.2    | 11.5      | 38.1 ± 1.1   |

Data were shown with mean ± SD (n = 3). Recoveries of cellulose and hemicellulose in spent liquor after dilute acid pretreatment were 5.68 and 16.74 g/g dry biomass, respectively. The total recovery yields of cellulose and hemicellulose after dilute acid pretreatment are then 70.8% and 90.5%, w/w, respectively.
repeatedly used for five times\textsuperscript{19} which is conducive to saving alkali and water.

Pretreatment with dilute acid mainly led to a decrease in the hemicellulose content (81.1\% from dried raw material, as shown in Table 2) due to the degradation caused by acid-catalyzed hydrolysis. The delignification was relatively less severe, thus resulting in a slight increase in cellulose content after dilute acid pretreatment (57.2\% glucan remaining from dried raw material). A similar finding that lignin content 19.4\% decreased to 13.4\% by dilute acid pretreatment on elephant grass has been reported in the literature.\textsuperscript{20} The calculated lignin removal rate (46.2\% from dried raw material) was higher because of the low solid recovery rate (50.0\%, w/w only). As shown in previous work,\textsuperscript{17} some solid factions were lost after pretreatment because of inefficient recovery in the steps of solid-liquid separation and washing. However, the combined use of alkaline and dilute acid on Napier grass resulted in the removal of hemicellulose and lignin about 93.4\% and 74.1\%, respectively, and 61.4\% glucan being preserved from dried raw material. Similar results have also been reported, where the removal of hemicellulose and lignin were 83\% and 58\% from initial feedstock with glucan preservation about 88\%.\textsuperscript{15}

| Pretreatment | Alkaline | Dilute acid | Two-stage |
|--------------|----------|-------------|-----------|
| Solid recovery (\%, w/w) | 47.4 ± 0.6 | 50.0 ± 0.5 | 38.1 ± 1.1 |
| Yield of cellulose recovery in the solid after pretreatment (\%, w/w) | 62.1 ± 2.7 | 47.8 | 67.6 |
| Removal ratio of hemicellulose (\%, w/w) | 67.1 ± 0.2 | 81.1 | 93.4 |
| Removal ratio of lignin (\%, w/w) | 63.0 ± 3.2 | 46.2 | 74.1 |

4-day enzyme hydrolysis

| Glucose productivity\textsuperscript{a} (g/L/h) | 4.08 ± 0.12 | 2.21 ± 0.38 | 3.77 ± 0.10 |
| Glucose concentration at 96 h (g/L) | 51.6 ± 2.0 | 29.2 ± 3.4 | 56.9 ± 3.7 |
| Glucose yield (%)\textsuperscript{b} | 75.4 ± 2.9 | 55.4 ± 6.5 | 76.4 ± 5.0 |
| Glucose (g)/raw material (g)\textsuperscript{c} | 0.245 ± 0.009 | 0.146 ± 0.017 | 0.217 ± 0.014 |
| (n = 2) | (n = 3) | (n = 2) |

3-day SSF\textsuperscript{d}

| Ethanol productivity\textsuperscript{a} (g/L/h) | 0.743 ± 0.159 | 0.473 ± 0.149 | 0.75 ± 0.05 |
| Residual solids fraction after SSF (\%, w/w) | 27.6 ± 1.3 | 64.3 ± 2.6 | 21.5 ± 1.4 |
| Ethanol concentration at 72 h (g/L) | 30.2 ± 1.2 | 15.0 ± 0.5 | 30.6 ± 1.6 |
| Ethanol yield (%)\textsuperscript{e} | 86.6 ± 3.4 | 56.8 ± 1.8 | 80.5 ± 4.1 |
| Ethanol (g)/raw material (g)\textsuperscript{f} | 0.143 ± 0.006 | 0.075 ± 0.003 | 0.116 ± 0.006 |
| (n = 3) | (n = 3) | (n = 2) |

\textsuperscript{a}Glucose productivities and ethanol productivities were slopes that measured from 0 to 8 h.

\textsuperscript{b}Data measured on the basis of theoretical glucose produced from cellulose in pretreated biomass.

\textsuperscript{c}Percentage of yield was based on dried raw material.

\textsuperscript{d}SSF was conducted by loading 100 g dry pretreated biomass to 1-L of enzyme mixture.

\textsuperscript{e}Data measured on the basis of theoretical ethanol produced from cellulose in pretreated biomass.

\textsuperscript{f}Data measured on the basis of theoretical ethanol produced from cellulose in pretreated biomass.

Figure 1 illustrates the influence of operating conditions on releasing soluble fraction in lysate after dilute acid pretreatment. The released glucose, xylose, and their acetic acid and furfural derivatives in lysate were investigated. As expected, dilute acid caused the hydrolysis of cellulose and hemicellulose to yield glucose and xylose into soluble fraction, respectively. Differently, hemicellulose (xylan) was more susceptible to hydrolysis to form monosaccharide than cellulose (glucan). As shown in Figure 1A, the released glucose concentration increased gradually from 6.3\% to 9.3\% (g/g dried biomass), when the sulfuric acid concentration increased from 1\% to 10\%. However, the xylose concentration in lysate decreased depending on dilute acid concentration. Higher sulfuric acid concentration resulted in the formation of acetic acid, furfural, and hydroxymethylfurfural (HMF). Therefore, 1\% sulfuric acid was considered to be the most suitable concentration to prevent the hydrolysis of cellulose and the formation of fermentative inhibitors (acetic acid and furfural). When 1\% sulfuric acid was employed, Napier grass were treated at 120°C with solid-to-liquid ratio of 1:10 for 60 minutes yielded 16.9 ± 0.3 g/L xylose, representing 74.3 ± 0.01\% hemicellulose hydrolyzing. At the
same time, 6.3 ± 0.8 g/L glucose, 3.9 ± 0.1 g/L acetic acid, 0.31 ± 0.03 g/L furfural, and 0.63 ± 0.06 g/L HMF were produced in the lysate.

Increased duration of dilute acid pretreatment resulted in a significant increase in the concentration of released sugars. However, acetic acid and furfural were gradually produced over time, as shown in Figure 1B. This suggests that the release of acetyl group from hemicellulose and conversion of sugar to furfural increased with treatment time. The influence of solid (biomass) to liquid (dilute acid) ratio was found relatively minor (Figure 1C). At different solid to liquid ratios, the yields from hydrolysis of cellulose and formation of acetic acid and furfural were almost the same. The hydrolysis of xylan was slightly lower with the solid to liquid ratio of 1:5, in comparison with hydrolysis at solid to liquid ratios of 1:10, 1:15, and 1:20, respectively. Unexpectedly, the incubation temperature could significantly alter the lysate patterns. In comparison with 120°C, higher temperatures could not result in the accumulation of more sugars, but production of more acetic acid and furfural from xylose in the lysate (Figure 1D). A higher temperature (180°C) promoted hydrolysis of cellulose and increased glucose accumulation in lysate (16.3% g/g dried biomass). However, all xylose were converted into acetic acid and furfural. Therefore, the best condition of dilute acid pretreatment in this work was determined that using 1% sulfuric acid and a solid to liquid ratio of 1:10 at 120°C for 60 minutes would yield the highest xylose concentrations and the lowest concentrations of acetic acid and furfural with the highest cellulose retained in solid fraction. These results are similar to other reports using the dilute acid to pretreat other different feedstock like *Sorghum bicolor* straw and *Parthenium hysterophorus*. The conditions of pretreatment of *S. bicolor* for maximum hemicellulose solubilization were 2% H$_2$SO$_4$ for 60 minutes at 121°C. For *P. hysterophorus*, the most efficient treatment conditions were autoclaving of biomass under 121°C at 15 psi pressure for 30 minutes in 1% v/v H$_2$SO$_4$ environment.

The cellulose content in biomass increased from 41.8% to 47.7% (w/w) after dilute acid pretreatment, but the treated biomass still contained some lignin. A further treatment by alkaline solution on the dilute acid pretreated biomass could remove more lignin, resulting in higher cellulose content.

**FIGURE 1** Results from dilute acid pretreatment at different conditions by changing (A) acid concentration, (B) incubation time, (C) solid-to-liquid ratio, and (D) temperature. The basic conditions for the pretreatment were as follows: 1% sulfuric acid, solid-to-liquid ratio of 1:10, temperature of 120°C, and incubation time of 60 minutes.
(67.7%, w/w) in the residual solid. As shown in Table 2, solid recovery rates after sole dilute acid and alkaline pretreatment are 50.0% and 47.4% respectively, due to the removal of hemicellulose and lignin in dilute acid and alkaline pretreatments. Two-stage pretreatment resulted in the removal of both hemicellulose and lignin, but resulted in the lowest solid recovery (38.1%). Considering the glucan recovery, the alkaline and two-stage pretreatments could retain, respectively 70.3% and 61.4%, of glucan from raw material.

### 3.2 Enzymatic hydrolysis of Napier grass biomass after different pretreatments

Figure 2 illustrates the enzymatic hydrolysis of pretreated biomass by dilute acid, alkaline, and two-stage pretreatments. Enzyme saccharification was carried out at 50°C for 96 hours. Based on the same loadings of pretreated biomass and enzyme, the two-stage pretreated biomass yielded the highest concentration of glucose (56.9 g/L) along with the lowest concentration of xylose (4.2 g/L) among these three pretreatment methods. The dilute acid pretreated biomass yielded relatively lower concentrations of glucose (29.2 g/L) and xylose (5.1 g/L) because a relative large portion of lignin remained in the biomass. However, after the enzymatic hydrolysis, alkaline-pretreated biomass yielded higher concentrations of both glucose (51.6 g/L) and xylose (13.5 g/L). The glucose yields from dilute acid and alkaline pretreated biomass after enzyme saccharification were 55.4% and 75.4% (w/w), respectively, while the glucose yield from biomass after two-stage pretreatment was 76.4% w/w (Table 2). These data suggest that the enzyme, cellulase CTec2, worked after two-stage pretreatment was 76.4% w/w (Table 2).

These data suggest that the enzyme, cellulase CTec2, worked equally well on alkaline- and two-stage pretreated biomass, but less efficiently on the dilute acid-pretreated biomass. The removal of lignin helped cellulase to access cellulose, contributing significantly to the hydrolysis of glucan and xylan into monosaccharides. Based on the per unit gram of dried raw material, 0.245 g glucose could be produced from Napier grass after alkaline pretreatment and enzymatic hydrolysis. This value was higher than the use of dilute acid pretreatment (0.146 g/g) or two-stage pretreatment (0.217 g/g). These results suggest that alkaline pretreatment was superior to other two methods, because both lignin and hemicellulose could be removed to a higher extent and glucan could be retained. In other two methods, the use of dilute sulfuric acid at high temperature caused cellulose hydrolysis to glucose and also glucose degradation to HMF, resulting in lower glucose recovery yields from raw material. The glucose yield is also higher than that from elephant grass biomass after hydrothermal pretreatment (57.8%) and much higher than that from milled biomass without pretreatment (24.8%).

The removal of hemicellulose could also play a dominate role for enzyme accessibility and efficiency of enzymatic saccharification as suggested by Leu and Zhu. As shown in the present work, even the single use of alkaline pretreatment could result in the removal of 67.1% hemicellulose, leading to efficient lignocellulose saccharification.

Since the biomass of Napier grass after alkaline pretreatment resulted in the highest glucose yield, ethanol production via this pretreatment thus showed more potential than other methods. Higher concentrations of fermentable sugars in the hydrolysate are favored to achieve higher ethanol concentration by fermentation. Higher sugars concentration could be obtained by repeating the enzymatic hydrolysis of the pretreated biomass in one pot. Figure 3 shows the accumulation of reducing sugars during the repeated enzymatic hydrolysis of alkaline pretreated biomass. After three repeats, the final concentrations of glucose and xylose reached 87.1 ± 1.6 g/L and 25.2 ± 5.0 g/L respectively. However, the yields of total sugar (glucose and xylose) were decreased with the number of repeats because of the activities of beta-glucosidase and cellulase were inhibited by glucose and celllobiose, respectively.

### 3.3 Production of ethanol from pretreated Napier grass by SSF

SSF combines the enzyme saccharification and ethanol fermentation in a single stage. Pretreated Napier grass by dilute acid, alkaline, and two-stage methods were used as the substrates, and the ethanol concentrations after 72 hours SSF were 15.0 ± 0.5 (n = 3), 30.2 ± 1.2 (n = 3), and 30.6 ± 1.6 g/L (n = 2), respectively (Table 2). These values corresponded to ethanol yields of 56.8%, 86.6%, and 80.5%, based on the theoretical ethanol produced from glucan in pretreated biomass. The ethanol yield based on the glucan in pretreated biomass using sole alkaline pretreatment was higher than that from dilute acid and two-stage pretreatments. These SSF results

![Figure 2](image-url)
were consistent with the data of enzymatic hydrolysis on pretreated biomass. The alkaline pretreatment with NaOH solution led to the highest glucose yield after enzymatic hydrolysis basis on per unit mass of dried raw material (0.245 g/g raw material). The ethanol yield of SSF on alkaline-pretreated Napier grass obtained in this work (75.4%) was also higher than that from Napier grass pretreated by low-moisture anhydrous ammonia (LMAA)\(^5\) and 1% NaOH.\(^{26}\) The dilute acid pretreatment yielded biomass that was relatively difficult to be hydrolyzed in the SSF process, in comparison with those treated by alkaline and two-stage methods. Results suggested that the removal of lignin from the lignocellulose feedstock favored the hydrolysis by cellulases and also the SSF process on the pretreated biomass.

As shown in Figure 4, during the process of SSF, glucose was produced by enzymatic hydrolysis of glucan and accumulated in the mixture because the fermentation rate was initially low. However, after 48 hours, almost no glucose was found in the SSF mixture, but there was some ethanol produced, suggesting that the glucose derived from enzyme hydrolysis in the late phase was readily converted into ethanol by the yeast. Since xylose produced in the SSF mixture by the enzymatic hydrolysis could not be utilized by the yeast, xylose concentration remained constant during the late phase of SSF. Among these pretreatment methods, an ethanol yield of 0.143 g/g-dried raw material was observed after 72 hours SSF on dried raw material of Napier grass under alkaline pretreatment. This ethanol yield is the highest one among those reported in the literature (Table 3). A recently published paper also reported a high ethanol yield (0.1415 g/g raw material) from SSF on NaOH pretreated elephant grass.\(^{20}\) However, they used a higher dose of enzyme (30 PFU/g-substrate of Accellerase 1500) and the alkaline pretreatment was done at higher temperature (120°C). Contrarily, the dilute acid and two-stage pretreatments resulted in relatively low ethanol yields of 0.075 and 0.116 g/g-dried raw material, respectively. The ethanol yield form Napier grass obtained in the present work (0.143 g/g dried raw material) is very close to that from others grasses. SSF of pretreated \textit{Miscanthus × giganteus} and \textit{Miscanthus lutarioriparius} results in experimental ethanol yields of 0.13 and 0.15 g/g-raw material, respectively.\(^{27}\)
| Pretreatment                  | Enzymatic hydrolysis                          | Sugar yield sugar (g)/sugar of treated biomass (g) | Ethanol yield | Ethanol concentration by SSF (g/L) | Reference |
|------------------------------|----------------------------------------------|---------------------------------------------------|---------------|-----------------------------------|-----------|
| 7% NaOH for 4 h              | Cellulase from Trichoderma reesei ATCC 26921 | 81.8% glucose (24 h)                              | 38%, SHF by E. coli strain K011                     | —         | [6]       |
| 1% NaOH presoaked biomass for 48 h and expose to the electron beam irradiation (EBI) with 150 kGy | 15 FPU/g DM of cellulase from T. reesei ATCC 26921 (Sigma) | 59% glucose (120 h)                              | —            | —                                   | [13]     |
| 2% NaOH at 120°C for 1 h with and with 1:20 solid to liquid ratio | 30 FPU/g DM of commercial enzyme Accellerase 1500 (Genecor) | 82% sugar (45 h)                                | 0.1415 g/g raw material when SSF by Saccharomyces cerevisiae | 26.1      | [25]     |
| 3% NaOH at 121°C for 15 min with a 1:20 solid to liquid ratio | 15 FPU/g DM of Penicillium echinulatum strain | 0.285 sugars g/g raw material (72 h)              | —            | —                                   | [7]       |
| 1% NaOH at 95°C for 1 h with MeOH presoaked biomass | Acremozyme cellulase | 87.5% hexose, 34% pentose (120 h)                | 0.121 g/g, SSF by S. cerevisiae                    | —         | [24]     |
| 7.5% H2O2 was adjusted the pH to 11.5 with NaOH, treatment at 35°C for 24 h with continuous stirring 250 rpm | T. reesei TISTR 3081 Cellulase (60 U/g substrate), xylanase (1200 U/g substrate) | 69.2% sugar (72 h)                              | 0.120 g/g raw material when SSF by S. cerevisiae and Pichia stipitis | —         | [9]       |
| Bio-pretreatment: 10^5 spore/mL Phanerochaete chrysosporium added into grass material at room temperature for 3-4 wks | Cellulase from T. reesei ATCC 26921 | 67.1% glucose (24 h)                              | 24%, SHF by E. coli strain K011                     | —         | [7]       |
| Bio-pretreatment: 5% (v/v) microbial consortia MCI (Clostridium straminisolvens CSK1) added into a grass material at 50°C for 3 wks | 70 IU/g DM of Cellulase (NS50013), 7 IU/g DM of β-glucosidase (NS50010), 7 IU/g DM of xylanase (NS2002) | 83.2% sugar (48 h)                              | —            | —                                   | [12]     |
| Hot liquid water for 20 min | 65 FPU/g DM of Cellulast 1.5 L and 17 IU/g DM of Novozyme 188 | 89.7% glucose (72 h)                              | 94.4%, SHF by S. cerevisiae                       | —         | [10]     |
| Two-stage: 210°C steam explosion for 20 min followed by 5% NaOH at 80°C for 30 min at a 4% (w/w) solid content | 20 FPU/g-glucan of cellulast 1.8 L, 50 FPU/g-glucan of novozyme 188 | Enzymatic digestibility of 96.1% (72 h) | —            | —                                   | [14]     |
| Two-stage: 1.5% H2SO4 at 121°C with 1:10 solid to liquid ratio for 60 min followed by 2% NaOH with 1:6 solid to liquid ratio at 80°C for 6 h | 25 FPU/g DM of cellulase complex NS50013 and 10 CBU/g DM of β-glucosidase NS50010 | 81% sugar (130 h)                                | 0.128 g/g raw material when SSF by S. cerevisiae | 24         | [15]     |
| 10% NaOH with 1:20 solid to liquid ratio at 90°C for 1 h | 12 FPU/g substrate of CTec2 with 1:10 solid to liquid ratio at 50°C | 75.4% glucose 73% xylose (96 h)                      | 0.143 g/g raw material when SSF by S. cerevisiae | 30.2       | This work |
| 1% H2SO4 with 1:10 solid to liquid ratio at 120°C for 60 min | 12 FPU/g substrate of CTec2 with 1:10 solid to liquid ratio at 50°C | 55.4% glucose 53% xylose (96 h)                      | 0.075 g/g raw material when SSF by S. cerevisiae | 15.0       | This work |
| 2% NaOH with 1:10 solid to liquid ratio at 120°C for 60 min and followed by 2% NaOH with 1:10 solid to liquid ratio at 80°C for 6 h | 12 FPU/g substrate of CTec2 with 1:10 solid to liquid ratio at 50°C | 76.4% glucose 93% xylose (96 h)                      | 0.116 g/g raw material when SSF by S. cerevisiae | 30.6       | This work |
Two-stage pretreatment failed to yield a higher ethanol concentration than that obtained from one-stage alkaline solution pretreatment of Napier grass. Similar to previously reported data, the ethanol yield from the SSF on Napier grass after two-stage pretreatment was 0.128 g/g raw material. Based on the results summarized in Table 3, SSF on alkaline-pretreated Napier grass could yield ethanol in the range from 0.121 to 0.143 g/g-dried raw material. Our results suggested that alkaline pretreatment was superior to dilute acid and two-stage pretreatment methods under same SSF conditions and raw materials. Furthermore, the pretreated solid after the alkaline-based method also contained xylan, which can be hydrolyzed to xylose. If a xylose-fermenting strain like Escherichia coli KO11 or recombinant yeast were used instead of conventional yeast Saccharomyces cerevisiae, the ethanol yield from SSF could be much higher, as the xylose would also be converted to ethanol. Finally, a study on the nonspecific binding of cellulase to lignin disclosed that enzymatic saccharification of lignocelluloses can be enhanced at an elevated pH of 5.5-6.0. The increase of pH to 5.5 for SSF could be a reasonable option.

4 | CONCLUSION

Compared with dilute acid and two-stage pretreatments, alkaline pretreatment was found to be superior based on the ethanol yield from dried biomass of Napier grass. Alkaline-pretreated Napier grass biomass could be converted to glucose at 0.245 g/g-raw material in 96 hours through enzyme hydrolysis. Applying SSF to the alkaline-pretreated biomass resulted in an ethanol yield of 0.143 g/g-raw material, resulting in the highest yield among those that have been published in the literature. Both high lignin and hemicellulose removal rates by alkaline pretreatment alone could make biomass more accessible to enzyme hydrolysis and lead to higher ethanol production. The ethanol yield reached 86.6% of the theoretical yield based on the glucan in pretreated biomass using sole alkaline pretreatment, which was higher than that from the two-stage pretreatment (80.5%). The results also indicated that Napier grass is a potential biomass that can be converted into bioethanol.

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