INTRODUCTION

Mitigating global climate change and CO₂ emissions has pushed for the continuous search of right types of biomass for the production of cellulosic fuels and chemicals (Albers et al., 2016; Lynd, 2017). This biomass selection has used crop wastes (e.g., corn stover and rice straw) and wood residues to avoid competition with foodstuffs. For an effective CO₂ removal with minimum land use change, energy-dedicated crops such as switchgrass and miscanthus are considered (Schmer et al., 2008; Searchinger et al., 2018). Meanwhile, the chemical composition of hydrolysates (i.e., partially degraded biomass) greatly diverges depending on the type of biomass (Mahdieh & Sudip, 2020; Nguyen Duc et al., 2020; Park et al., 2020).

Abstract

Kenaf (Hibiscus cannabinus) is an annual fiber crop grown mainly in India and China. This crop is becoming a new bio-based energy source because of its fast growth rate, excellent CO₂ absorption ability, and large productivity per unit area. In this study, we evaluated 10 different cultivars of kenaf for their potential as biomass for cellulosic ethanol production. First, kenaf samples were hydrolyzed using dilute sulfuric acid, which is the most simple and cost-effective pretreatment method. Next, simultaneous saccharification and fermentation (SSF) of the hydrolysates were performed by wild-type and engineered xylose-fermenting yeast strains. The results of compositional analysis of the biomass, the hydrolysates, and the fermented products suggested that ethanol yield and productivity were significantly affected by a type of kenaf cultivars, which was not predictable based on the biomass compositions. Also, the ethanol production was maximized when the xylose fraction was utilized by engineered yeast under the control of pH to avoid acetate inhibition. Considering the sugar compositions and their fermentability, kenaf can be a promising energy-dedicated crop for cellulosic ethanol production.

KEYWORDS

lignocellulosic biomass, Saccharomyces cerevisiae, xylose fermentation

Abbreviations: PIs, plant instructions; SSF, simultaneous saccharification and fermentation.
Therefore, optimizing cellulosic treatment process is necessary for a specific type of biomass considering the seasonal and regional variations.

Kenaf (Hibiscus cannabinus) is a textile crop similar to cotton and flax, but kenaf fibers have rough and inflexible properties only suitable for rope, fishing nets, and paper products. There are several other properties that make kenaf attractive alternative biomass for the production of biofuels and chemicals (Ayadi et al., 2017). First, kenaf can grow up to 4–5 m tall and be harvested up to three times a year (Islam, 2019). Second, the plantation cost of kenaf is low since the plant can grow in a variety of climate conditions (Ramesh et al., 2018). The annual production yield of kenaf is 10.9–27.2 tons/ha (Kamaruddin & Othman, 2012), which is larger than any other lignocellulosic biomass including agricultural wastes and forestry residues (see Table 1). Although the annual productivity of agricultural wastes is high, the production per unit area is lower than other lignocellulosic biomass because most parts of crop is consumed as food and feed resources. In contrast, production yield of forestry residue is similar to kenaf, but has lower annual productivity due to slow growth (De Bhowmick et al., 2018). Third, kenaf represents a more efficient and sustainable energy source since the entire plant is used as an energy crop. Moreover, CO₂ absorption is excellent due to the fast growth rate of kenaf (Baghban & Mahjoub, 2020).

To date, kenaf has surprisingly received limited attention for lignocellulosic biomass. A few studies have demonstrated bioethanol production from the cellulose fraction of kenaf hydrolysates (Guo et al., 2014; Ruan et al., 2011), and this process might be improved by physical detoxification (Shah et al., 2019) and/or chemical delignification (Wan Azelee et al., 2014). Here, we systematically investigated the quality of kenaf biomass hydrolysates based on the fermentation capacity and ethanol yields from 10 different cultivars of kenaf.

### Table 1: Annual production yield per unit area of different types of lignocellulosic biomass

| Type              | Biomass        | Annual yield metric tons per hectare | Reference                  |
|-------------------|----------------|--------------------------------------|----------------------------|
| Energy-dedicated  | Kenaf          | 10.9–27.2a                           | Ayadi et al. (2017)        |
|                   | Switch grass   | 14                                   | Yang et al. (2018)         |
| crops             |                |                                      |                            |
| Agriculture       | Corn stover    | 8.4                                  | Yang et al. (2018)         |
| wastes            | Corn cob       | 6.5                                  | Harsanti et al. (2019)     |
|                   | Wheat straw    | 6                                    | Yang et al. (2018)         |
|                   | Rice straw     | 4.1–6.2                              | Abraham et al. (2016)      |
| Forestry          | Hybrid poplar  | 14                                   | Yang et al. (2018)         |
| residues          | Hardwood stems | 4.5b                                 | De Bhowmick et al. (2018)  |
|                   | Softwood stems | >4.5b                                | De Bhowmick et al. (2018)  |

aProduction yield for 6–8 months.

bBased on dried weight (metric ton).

The sugar content of the dilute acid hydrolysates and fermentation profiles were compared, focusing on how efficiently the xylose fraction of the hydrolysates can be fermented into ethanol. With a comparison to other types of lignocellulosic biomass, the potential of kenaf for lignocellulosic ethanol production is discussed.

## 2 | MATERIALS AND METHODS

### 2.1 | Yeast strain and culture conditions

The xylose-fermenting yeast strain, *Saccharomyces cerevisiae* SR8 and the *S. cerevisiae* D452-2 (wild-type) was used for expressing a heterologous xylose pathway and as control strain, respectively. The former one originated from *Pichia stipitis* developed previously (Kim, Serker, et al., 2013). Yeast were precultivated in YP medium (10 g/L yeast extract and 20 g/L Bacto™ Peptone) containing 20 g/L glucose for 24 hr at 30°C under oxygenated conditions (250 rpm). The yeast pellets were harvested by centrifugation at 3,134 × g for 5 min and then washed with distilled water. The initial cell concentration was adjusted to an optical density at 600 nm (OD₆₀₀) of either 1.0 or 20.0, which corresponds to an initial cell density of 0.5 or 10 g dry cell weight/L, respectively. The collected cell pellets were inoculated into the kenaf hydrolysate-like medium or lignocellulosic hydrolysates.

### 2.2 | Lignocellulosic hydrolysate preparation

Ten plant introductions (PIs) of kenaf samples were planted in early April 2019, and harvested the following November (Jeju Island, South Korea) and the information about each PI can be searched by IT number (see Table 2) in the Plant
| No. | Entry | IT number | Component of dried kenaf<sup>a</sup> | After acid pretreatment<sup>b</sup> | After enzyme hydrolysis (24 hr)<sup>b</sup> | Ethanol production after SSF (24 hr)<sup>b</sup> |
|-----|-------|-----------|-------------------------------------|---------------------------------|---------------------------------|---------------------------------|
|     |       |           | Cellulose  | Hemicellulose  | Glucose | Xylose | Acetate | Glucose | Xylose | Acetate | Glucose | Xylose | Acetate | Wild-type | Engineered (pH 5) | Engineered (pH 6) |
| 1   | WIR276 | 207883    | 45.4 ± 0.5 | 13.5 ± 0.8   | 5.7 ± 0.5 | 4.6 ± 0.3 | 3.5 ± 0.2 | 19.4 ± 0.9 | 8.5 ± 0.3 | 3.5 ± 0.1 | 10.0  | 12.0  | ND     |
| 2   | WIR360 | 207885    | 47.3 ± 0.1 | 13.4 ± 1.4   | 5.0 ± 0.3 | 5.0 ± 0.3 | 3.1 ± 0.1 | 19.1 ± 0.4 | 9.9 ± 0.1 | 3.5 ± 0.0 | 8.0   | 9.3   | ND     |
| 3   | WIR453 | 207888    | 51.6 ± 1.2 | 14.3 ± 0.2   | 2.3 ± 0.0 | 4.3 ± 0.2 | 3.6 ± 0.0 | 18.1 ± 0.2 | 9.4 ± 0.5 | 3.7 ± 0.2 | 7.0   | 8.1   | ND     |
| 4   | WIR275 | 207882    | 51.2 ± 0.7 | 14.0 ± 0.6   | 3.3 ± 0.7 | 7.4 ± 5.1 | 4.4 ± 1.2 | 20.2 ± 3.4 | 12.7 ± 5.9 | 4.6 ± 1.4 | 7.4   | 7.6   | 10.9   |
| 5   | WIR333 | 207884    | 49.5 ± 0.4 | 13.5 ± 0.1   | 3.9 ± 0.1 | 4.7 ± 0.0 | 3.6 ± 0.0 | 18.1 ± 2.2 | 8.9 ± 0.7 | 3.7 ± 0.1 | 7.4   | 7.4   | ND     |
| 6   | ET1   | Cultivar  | 41.4 ± 0.6 | 13.0 ± 0.2   | 11.6 ± 0.5 | 5.8 ± 0.2 | 2.4 ± 0.0 | 22.3 ± 1.2 | 8.2 ± 0.4 | 2.3 ± 0.2 | 9.7   | 11.3  | ND     |
| 7   | Kenaf | Cultivar  | 47.2 ± 0.2 | 12.8 ± 0.0   | 5.0 ± 0.2 | 5.5 ± 0.2 | 2.3 ± 0.0 | 18.0 ± 0.0 | 7.6 ± 0.0 | 2.7 ± 0.8 | 7.3   | 8.9   | ND     |
| 8   | Local | Cultivar  | 50.3 ± 0.4 | 13.5 ± 0.4   | 8.3 ± 3.7 | 10.2 ± 1.6 | 4.8 ± 0.3 | 22.6 ± 2.0 | 12.6 ± 0.7 | 4.1 ± 0.0 | 9.3   | 10.8  | 15.1   |
| 9   | PI468075 | 181219  | 50.0 ± 0.1 | 13.7 ± 0.3   | 3.1 ± 0.0 | 4.3 ± 0.1 | 3.3 ± 0.2 | 17.1 ± 0.4 | 8.0 ± 0.3 | 3.0 ± 0.2 | 8.9   | 10.8  | ND     |
| 10  | WIR19 | 207877    | 42.6 ± 0.7 | 12.1 ± 0.5   | 7.1 ± 0.2 | 6.1 ± 0.2 | 2.6 ± 0.1 | 20.5 ± 0.3 | 8.8 ± 0.2 | 2.4 ± 0.1 | 10.9  | 11.4  | ND     |
|     | Average |           | 47.7 ± 3.6 | 13.4 ± 0.6   | 47.7 ± 3.6 | 5.8 ± 1.7 | 3.4 ± 0.8 | 19.5 ± 1.8 | 9.4 ± 1.7 | 3.3 ± 0.7 | 8.6   | 9.8   | 13.0   |

Abbreviation: ND, not determined.

<sup>a</sup>Content (% w/w) of cellulose and hemicellulose; g/100 g dried weight.
<sup>b</sup>Content (w/v) of glucose, xylose, acetate, and ethanol; g/L.
DB Search tap provided by the National Agrodiversity center (http://genebank.rda.go.kr/eng/pla/pds/SimpleSearch.do, Republic of Korea). The kenaf samples were dried at 60°C for 24 hr and milled, and then stored at −80°C until used. Two grams of kenaf powders were pretreated in 1% (w/v) H₂SO₄ at 121°C for 30 min. For simultaneous saccharification and fermentation (SSF), the whole pretreatment slurry was neutralized to pH 5.0 or 6.0 using 7.5 M of NaOH, and then sterilized using an autoclave (SANYO MLS-3781L; Panasonic).

2.3 Yeast-mediated SSF (simultaneous SSF)

Saccharification and fermentation was performed with engineered xylose-utilizing strain S. cerevisiae SR8 and wild-type (S. cerevisiae D452-2) in the 10 different PIs of kenaf hydrolysates, including a no yeast control group for the enzymatic hydrolysis. Next, yeast cells at OD₆₀₀ of 20.0 and 80 filter paper cellulase units (FPU/g) biomass of Cellic® CTec2 (Novozymes) were added to 20 ml of kenaf hydrolysates (based on 10% [w/v] solids). Each fermentation was performed in 100 ml Erlenmeyer flasks for 24 hr at 30°C under oxygen-limited conditions (80 rpm) and in biological duplicates.

2.4 Kenaf hydrolysate-like medium

The 20 ml of kenaf hydrolysate-like medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 10 g/L xylose and 0–5 g/L acetic acid) was adjusted to pH 5 or 6 using 7.5 M NaOH, with the medium containing no acetic acid having no pH adjustment. The fermentation was performed in 100 ml Erlenmeyer flasks for 24 hr at 30°C under oxygen-limited condition (80 rpm) with a starting OD₆₀₀ of 1.0 for S. cerevisiae SR8 strain.

2.5 Analytical methods

The contents of cellulose and hemicellulose were calculated based on neutral detergent fiber (NDF; cellulose, hemicellulose and lignin) and acidic detergent fiber (ADF; cellulose and lignin). The NDF, ADF and lignin were analyzed using Van Soest method (Van Soest et al., 1991). For a neutral detergent solution, dissolve 30 g of sodium lauryl sulfate, 18.61 g of disodium ethylenediaminetetraacetic acid, 6.81 g of sodium borate decahydrate, and 4.56 g of anhydrous sodium phosphate dibasic in 1 L of distilled water, add 10 ml of 2-ethoxyethanol, and adjust the pH to 6.9–7.1 with H₃PO₄. One gram of sample is added in crucible with 100 ml of neutral detergent solution and 2 ml of decahydronaphthalene. The crucible is installed on a fiber analyzer (Fibertec 2010 FOSS Analytical), and boiled for 60 min. The precipitate was washed three times with hot distilled water (90–100°C) using a suction filtration method, followed by filtration two to three times with acetone until the solution no longer had a color. After drying overnight in a dry oven at 105°C, the residue was measured, and the NDF content was measured as follows:

\[
\text{NDF (\%)} = \frac{W_1 \times 100}{S},
\]

where \(S\) is the weight of the sample (g) and \(W_1\) is the weight of the residue after drying (g).

Acidic detergent fiber was dissolved in an acidic detergent solution in the same method as NDF analysis, and the mass of the remaining material was measured. Lignin was treated with 72% sulfuric acid for 3 hr, filtered, dried, and analyzed through an ashing process, and cellulose was calculated as the value of ADF minus lignin content.

The determination of glucose, xylose, acetate, and ethanol concentration in the kenaf hydrolysate and the kenaf hydrolysate-like medium was performed using high performance liquid chromatography (1260 series; Agilent Technologies) equipped with a Rezex-ROA Organic Acid H+ column (8%, 150 mm × 4.6 mm; Phenomenex Inc.). Columns were eluted with 0.005 N H₂SO₄ at 0.6 ml/min at 50°C, as described previously (Kim et al., 2019). The pH meter (F-51; HORIBA) was used for the determination of hydrogen ion concentration. The statistical significance of mean differences at \(p < .05\) was calculated using the Student’s \(t\) test with Microsoft Excel software.

3 RESULTS

3.1 Dilute acid pretreatment of kenaf

Physicochemical pretreatments are necessary to make lignocellulose more susceptible to enzymatic hydrolysis of the polysaccharides into sugars (Park et al., 2020). Among various types of the pretreatment methods, hydrothermal pretreatment using dilute acid is known as the most simple and effective way (Park et al., 2020). For each cultivar of kenaf, the stem parts were collected and prepared (Figure 1). As a result of analyzing the cellulose, hemicellulose contents of 10 dried kenaf samples, the cellulose and hemicellulose contents were 41%–51.5% (w/w) and 12.1%–14.3% (w/w), respectively, which was 3.6-fold higher in cellulose than hemicellulose on average (Table 2). Unlike most other lignocellulosic biomass, kenaf plants are known to be richer in noncellulose (hemicellulose, lignin, pectin, etc.) content (Akil et al., 2011). Therefore, in this biomass, xylose, degraded
from hemicellulose, is a major sugar component as much as glucose, so application of the xylose-utilizing yeast strain is more appropriate to improve ethanol production. The content of cellulose and hemicellulose between the 10 samples was not significantly different ($\alpha = 0.05$, $t$ test), but, after acid treatment and enzymatic hydrolysis, the range of degraded glucose and xylose contents varied widely. Therefore, it is important to optimize the saccharification process in order to compare sugar content and other components that affect fermentation between different cultivars of kenaf.

All samples were treated with 1% (w/v) sulfuric acid solution at 121°C for 30 min, which is equivalent to a 2.0 combined severity factor, calculated as described previously (Zhang, Vancov, et al., 2016). The released sugar profiles of the pretreated kenaf samples varied greatly (Table 2); ranging from 2.3 to 11.6 g/L glucose and 4.3 to 10.2 g/L xylose, suggesting differences in the structural compositions of each cultivar and possible variations in the recalcitrance of the biomass (Figure 2a). Furthermore, the ratio between glucose and xylose averaged 1.0 among the samples, with the ET1 cultivar having 2.0 ratio at the highest glucose concentration (11.6 g/L), and the Local sample had a 0.8 ratio with the highest xylose concentration (10.2 g/L). Although there was no significant correlation between xylose and acetate

![Figure 1](image1.png)

**FIGURE 1** Cellulosic ethanol production using kenaf. Representative workflow of one kenaf cultivar for its sugar and fermentation profiles of the hydrolysates. Fermentation was performed by wild-type and engineered *Saccharomyces cerevisiae* yeast at pH 5 or 6, with only the engineered yeast strain capable of fermenting xylose.

![Figure 2](image2.png)

**FIGURE 2** Variations in sugar and fermentation profiles of 10 kenaf cultivars. (a) Sugar content of xylose and glucose after acid pretreatment and enzyme saccharification. (b) Ethanol production after simultaneous saccharification and fermentation by wild-type and engineered yeast *Saccharomyces cerevisiae* at pH 5 (*p < .05*)
concentrations ($\alpha = 0.05$, $t$ test), only the two samples with the highest xylose concentration, Local and WIR275, had acetate levels higher than 4 g/L. These results suggest that the degree of acetylation might be different among PIs (Johnson et al., 2017).

### 3.2 | Saccharification of pretreated kenaf samples

The pretreated kenaf hydrolysates were then subjected to enzyme hydrolysis by a mixture of cellulase and hemicellulase (see Section 2). Most saccharification (95%) was completed in 24 hr, yielding 25.0–35.1 g of total fermentable sugars (glucose and xylose) from 100 g of dried kenaf stem samples (Figure 2a). The variations in the sugar profiles of the enzyme-treated hydrolysates still varied greatly, and the sugar concentrations before and after the hydrolysis showed statistically significant but weak correlations ($\alpha = 0.05$, $t$ test), suggesting that multiple parameters might be affecting the pretreatment and hydrolysis yields differently.

Average glucose and xylose concentration increased by 3.6-fold and 1.7-fold after enzyme treatment, respectively, which led an increase in the ratio between glucose and xylose from 1.0 to 2.1. There was no significant change in average acetate concentration (3.3 g/L; Table 2). These results suggest that most of the hemicellulose was solubilized already during the dilute acid pretreatment, and further hydrolysis of xylose oligomers occurred during enzyme treatment. Indeed, there was significant correlation between xylose and acetate concentrations after enzyme treatment ($\alpha = 0.05$, $t$ test).

### 3.3 | SSF of kenaf hydrolysates

To evaluate the fermentability of the kenaf hydrolysates and their xylose fractions, simultaneous SSF was performed by two yeast strains: wild-type D452-2 and the xylose-fermenting engineered strain SR8. By using SSF method, since glucose and xylose could be consumed simultaneously as they are released, the effect of feedback inhibition could be reduced to effectively increase enzymatic hydrolysis activity (Zhang, Kumar, et al., 2016). The wild-type strain and the engineered strain produced average 8.59 and 9.75 g/L ethanol, respectively, after 24 hr of fermentation at an initial cell concentration of 10 g/L. Thus, the xylose fraction of the hydrolysates provides an additional 12% increase in ethanol production (Figure 2b). Beyond 24 hr of fermentation there was no significant increase in ethanol despite an average 49% of xylose remaining, suggesting there was some inhibition factor to the xylose fermentation of the kenaf hydrolysates. Meanwhile, the wild-type strain consumed average 3.7 g/L xylose during fermentation and converted some into xylitol, which is catalyzed by endogenous xylose reductase activity when cell density is high (Kim, Kwee, et al., 2013).

Residual xylose concentrations after the SSF of the kenaf hydrolysates did vary among the PIs, but also strongly correlated with the initial acetate concentration ($R^2 = .893$). Meanwhile, the two PIs ET1 and Kenaf with the lowest acetate concentrations (2.3–2.4 g/L) compared to the other cultivars (2.7–5.1 g/L) had the lowest residual xylose (Figure 3a). These results suggest that acetate might be a primary inhibitor of fermentation in kenaf hydrolysates.

**Figure 3** Effect of acetate concentration on xylose fermentation. (a) Correlation between initial acetate concentrations of kenaf hydrolysates before saccharification and fermentation and the residual xylose concentration after fermentation for 24 hr. (b) Changes in xylose consumption rates by the addition of acetate at pH 5 and at pH 6, tested in complex media by engineered yeast *Saccharomyces cerevisiae*. pH was not controlled at 0 g/L acetate sample.
When the xylose-fermenting strain SR8 was tested in a kenaf-like medium (20 g/L glucose and 10 g/L xylose) under various acetate concentrations (Figure 3b), the presence of 2.0 g/L acetate delayed xylose consumption but not glucose use. When the pH of the medium was then adjusted from 5 to 6, there was no effect on xylose consumption rate—even at 5 g/L acetate. These results suggest that the controlling pH above the pKₐ value of acetate (pKₐ = 4.75) can be enough to overcome the acetic acid toxicity on xylose fermentation, and this observation was also noted previously (Kim, Skerker, et al., 2013).

When the pH control strategy was applied to the two kenaf hydrolysates WIR275 and Local that had the highest acetate and xylose concentrations (4.4–4.5 g/L acetate and 12.5–12.7 g/L xylose), xylose consumption and ethanol productivity increased by over 66% and 64%, respectively (Table 3). Although cellulosic biomass hydrolysates are known to contain various fermentation inhibitors such as furan derivatives (5-hydroxymethylfurfural and furfural) and phenolic compounds (syringaldehyde and vanillin; Zhang et al., 2018), the primary factor inhibiting the fermentation of the kenaf hydrolysates was high concentration of acetate.

### DISCUSSION

In many studies, acetate has been identified as the most critical factor inhibiting the fermentation of cellulosic hydrolysates, especially xylose (Bellissimi et al., 2009; Ko et al., 2020; Matsushika & Sawayama, 2012). There are three known strategies to avoid acetate toxicity, as summarized in Table 3. First, choose a lignocellulosic biomass with natural or genetically engineered low acetylation (Brandon & Scheller, 2020). Among the 10 kenaf PIs tested in this study, the ET1 and Kenaf hydrolysates had the lowest acetate concentration and might be more suitable as cellulosic biomass. Second, fermentation processes can be modified to reduce the acetate toxicity by adjusting the pH (as shown here) or removing acetate from the hydrolysates. Recently, deacetylation using dilute alkaline solution has been effective in removing acetate from intact hemi-cellulose (Castro et al., 2017; Wang et al., 2019). Lastly, yeast can be engineered to tolerate or assimilate acetate. Inverse metabolic engineering represented by adaptive evolution is the most common and effective approach to develop acetate-resistant yeast (González-Ramos et al., 2016; Ko et al., 2020; Oh et al., 2019). In addition, the introduction of a heterologous acetate consumption pathway can allow the conversion of acetate into ethanol (Wei et al., 2013; Zhang, Kong, et al., 2016).
This study evaluated 10 different PIs of kenaf as a potential feedstock for cellulosic ethanol production. Fermentation both of glucose and xylose and under elevated pH conditions, each contributed to 12% and 47% increase in ethanol productivity, respectively. Therefore, cellulosic ethanol processing needs to either account for the structural and compositional variations in different kenaf PI or select the most suitable cultivars based on their fermentation potential.

Also, in order to overcome the inhibition of pentose consumption by acetate and to increase the ethanol production significantly, SSF could be performed with the fed-batch method (Figure S1). In fed-batch fermentation, the initial solid loading was increased to 14.3% (w/w) adjusted to pH 6.0, and after starting the fermentation 16.7% (w/w) solid loading of acid pretreated slurry was fed twice. As a result, ethanol production increased up to 17.10 g/L, which was 37% and 18.8% higher than in the initial pH 5.0 batch fermentation and pH 6.0 batch fermentation (ethanol production; 10.75 and 13.88 g/L respectively). Therefore, since the ethanol production can be greatly increased by overcoming acetate inhibition, ethanol production can be significantly increased through various methods introduced in Table 3 such as deacetylation. In particular, since the SSF and fed-batch methods were able to effectively increase the production of ethanol by controlling other inhibitory factors such as feedback inhibition, as well as acetate, there is sufficient potential for development on an industrial scale through these methods.

Kenaf has a higher content of non-cellulose (e.g., hemi-cellulose and pectin) compared to other fibers, making the fiber texture rough and limited for industrial applications, but very suitable for lignocellulosic biomass feedstock(Azelee et al., 2016). With the stem of kenaf used as a biomass material, the other parts are edible and rich in pharmacological ingredients, which is an added bonus when compared to other energy-dedicated crops (Giwa et al., 2019). As kenaf fibre is more robust than conventional biomass for mass production and has a fast growth rate (~4 months), making the plant more robust than conventional biomass for mass production (Islam, 2019; Ramesh et al., 2018). This are the few studies that tested different cultivars from one crop, and even for the same crop, the SSF results were significantly different depending on the cultivars, and it was confirmed that such sugar content and acetate content greatly influence the fermentation. In addition, it was analyzed that acetate is one of the major inhibitory factors in microbial fermentation. In this study, the pentose fermentation was more facilitated by controlling the pH level and other various methods to overcome acetate inhibition were suggested. Through our investigation of SSF profiles and the desirable agronomic properties of 10 different kenaf PIs, we see kenaf as a potential primary crop for the production of cellulosic ethanol and other biochemicals.

ACKNOWLEDGEMENTS
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2019R1F1A1061916 and 2019R1A6A1A11052070). Also, we thank Sustainable Agriculture Research Institute (SARI) in Jeju National University for providing the experimental facilities.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Yong Suk Chung https://orcid.org/0000-0003-3121-7600

REFERENCES
Abraham, A., Mathew, A. K., Sindhu, R., Pandey, A., & Binod, P. (2016). Potential of rice straw for bio-refining: An overview. Bioresource Technology, 215, 29–36. https://doi.org/10.1016/j.biortech.2016.04.011
Akil, H., Omar, M., Mazuki, A., Safiee, S., Ishak, Z. M., & Bakar, A. A. (2011). Kenaf fiber reinforced composites: A review. Materials & Design, 32(8–9), 4107–4121. https://doi.org/10.1016/j.matdes.2011.04.008
Albers, S. C., Berkland, A. M., & Graff, G. D. (2016). The rise and fall of innovation in biofuels. Nature Biotechnology, 34(8), 814–821. https://doi.org/10.1038/nbt.3644
Ayadi, R., Hanana, M., Mzid, R., Hamrouni, L., Khouja, M. L., & Salhi Hanachi, A. (2017). Hibiscus cannabinus L. – Kenaf: A review paper. Journal of Natural Fibers, 14(4), 466–484. https://doi.org/10.1080/15440478.2016.1240639
Azelee, N. I. W., Jahim, J. M., Ismail, A. F., Fusli, S. F. Z. M., Rahman, R. A., & Illias, R. M. (2016). High xylooligosaccharides (XOS) production from pretreated kenaf stem by enzyme mixture hydrolysis. Industrial Crops and Products, 81, 11–19. https://doi.org/10.1016/j.indcrop.2015.11.038
Baghban, M. H., & Mahjoub, R. (2020). Natural Kenaf fiber and LC3 binder for sustainable fiber-reinforced cementitious composite: A review. Applied Sciences, 10(1), 357. https://doi.org/10.3390/app10010357
Bellissimi, E., Van Dijken, J. P., Pronk, J. T., & Van Maris, A. J. A. (2009). Effects of acetic acid on the kinetics of xylose fermentation by an engineered, xylose-isomerase-based Saccharomyces cerevisiae strain. FEMS Yeast Research, 9(3), 358–364. https://doi.org/10.1111/j.1567-1364.2009.00487.x
Brandon, A. G., & Scheller, H. V. (2020). Engineering of bioenergy crops: Dominant genetic approaches to improve polysaccharide properties and composition in biomass. Frontiers in Plant Science, 11(282). https://doi.org/10.3389/fpls.2020.00282
Castro, R. C. D. A., Fonseca, B. G., dos Santos, H. T. L., Ferreira, I. S., Mussatto, S. I., & Roberto, I. C. (2017). Alkaline deacetylation as a strategy to improve sugars recovery and ethanol production from rice straw hemi cellulose and cellulose. Industrial Crops and Products, 106, 65–73. https://doi.org/10.1016/j.indcrop.2016.08.053
de Assis Castro, R. C., Fonseca, B. G., dos Santos, H. T. L., Ferreira, I. S., Mussatto, S. I., & Roberto, I. C. (2017). Alkaline deacetylation as a strategy to improve sugars recovery and ethanol production from rice straw hemi cellulose and cellulose. Industrial Crops and Products, 106, 65–73. https://doi.org/10.1016/j.indcrop.2016.08.053
deacetylation as a strategy to improve sugars recovery and ethanol production from rice straw hemi cellulose and cellulose. Industrial Crops and Products, 106, 65–73. https://doi.org/10.1016/j.indcrop.2016.08.053

De Bhowmick, G., Sarmah, A. K., & Sen, R. (2018). Lignocellulosic biorrefinery as a model for sustainable development of biofuels and value added products. Bioresource Technology, 247, 1144–1154. https://doi.org/10.1016/j.biortech.2017.09.163

Giwa Ibrahim, S. A., Karim, R., Saari, N., Wan Abdullah, W. Z., Zawawi, N., Ab Razak, A. F., & Umar, R. U. A. (2019). Kenaf (Hibiscus cannabinus L.) seed and its potential food applications: A review. Journal of Food Science, 84(8), 2015–2023. https://doi.org/10.1111/1750-3841.14714

González-Ramos, D., Gorter de Vries, A. R., Grijseels, S. S., van Harsanti, E. S., Kusnoputranto, H., Suparmoko, M., Ardiwinata, A. P., & Jin, Y.-S. (2013). Rational and evolutionary engineering of a recombinant flocculent Saccharomyces cerevisiae strain that co-ferments glucose and xylose: II. Influence of pH and acetic acid on ethanol production. Applied Biochemistry and Biotechnology, 168(8), 2094–2104. https://doi.org/10.1007/s12010-012-9920-4

Guo, F., Sun, W., Li, X., Zhao, J., & Qu, Y. (2014). Pretreatment of ramie and kenaf stalk for bioethanol production. Shengwu Gongcheng Xuebao/Chinese Journal of Biotechnology, 30(5), 774–783. https://doi.org/10.13345/j.cjb.140037

Islam, M. M. (2019). Varietal advances of jute, kenaf and mesta crops in Bangladesh: A review. International Journal of Bifurcation and Chaos, 4, 24.

Johnson, A. M., Kim, H., Ralph, J., & Mansfield, S. D. (2017). Natural acetylation impacts carbohydrate recovery during deconstruction of Populus trichocarpa wood. Biotechnology for Biofuels, 10(1), 48. https://doi.org/10.1186/s13068-017-0734-z

Kamaruddin, N., & Othman, M. S. H. (2012). Quantifying of farmers’ acceptance and perception in developing kenaf. Hibiscus cannabinus industry in Malaysia. International Journal of Green Economics, 6(4), 401–416. https://doi.org/10.1504/ijge.2012.051491

Kim, J.-W., Jang, J. H., Yeo, H. J., Seol, J., Kim, S. R., & Jung, Y. H. (2019). Lactic acid production from a whole slurry of acid-pretreated spent coffee grounds by engineered Saccharomyces cerevisiae. Applied Biochemistry and Biotechnology, 189(1), 206–216. https://doi.org/10.1007/s12257-019-03000-6

Kim, S. R., Kwee, N. R., Kim, H., & Jin, Y.-S. (2013). Feasibility of xylose fermentation by engineered Saccharomyces cerevisiae over-expressing endogenous aldose reductase (GRE3), xyitol dehydrogenase (XYL2), and xylulokinase (XYL3) from Schefteromyces stipitis. FEMS Yeast Research, 13(3), 312–321. https://doi.org/10.1111/1567-1364.12036

Kim, S. R., Skerker, J. M., Kang, W., Lesmana, A., Wei, N., Arkin, A. P., & Jin, Y.-S. (2013). Rational and evolutionary engineering approaches uncover a small set of genetic changes efficient for rapid xylose fermentation in Saccharomyces cerevisiae. PLoS One, 8(2), e57048. https://doi.org/10.1371/journal.pone.0057048

Ko, J. K., Enkh-Aamgalan, T., Gong, G., Um, Y., & Lee, S.-M. (2020). Improved bioconversion of lignocellulosic biomass by Saccharomyces cerevisiae engineered for tolerance to acetic acid. GCB Bioenergy, 12(1), 90–100. https://doi.org/10.1111/gcbb.12656

Lynd, L. R. (2017). The grand challenge of cellulosic biofuels. Nature Biotechnology, 35(10), 912–915. https://doi.org/10.1038/nbt.3976

Malddie, S., & Sudip, R. (2020). Utilization of microbial oil from poplar wood hemicellulose prehydrolysat for the production of polyol using chemo-enzymatic epoxidation. Biotechnology and Bioprocess Engineering, 25(2), 327–335. https://doi.org/10.1007/s12257-019-0416-8

Matsushika, A., & Sawayama, S. (2012). Characterization of a recombinant flocculent Saccharomyces cerevisiae strain that co-ferments glucose and xylose: II. Influence of pH and acetic acid on ethanol production. Applied Biochemistry and Biotechnology, 168(8), 2094–2104. https://doi.org/10.1007/s12010-012-9920-4

Nguyen Duc, H., Thiyagarajan, S., Sang Hoon, H., & Seung-Moon, P. (2020). Enhanced enzymatic saccharification of wheat flour arabinoxylan and barley straw using recombinant hemicellulases. Biotechnology and Bioprocess Engineering, 25(3), 431–441. https://doi.org/10.1007/s12257-019-0231-2

Oh, E. J., Wei, N., Kwak, S., Kim, H., & Jin, Y.-S. (2019). Overexpression of RCK1 improves acetic acid tolerance in Saccharomyces cerevisiae. Journal of Biotechnology, 292, 1–4. https://doi.org/10.1016/j.jbiotec.2018.12.013

Park, H., Jeong, D., Shin, M., Kwak, S., Oh, E. J., Ko, J. K., & Kim, S. R. (2020). Xylose utilization in Saccharomyces cerevisiae during conversion of hydrothermally pretreated lignocellulosic biomass to ethanol. Applied Microbiology and Biotechnology, 104(8), 3245–3252. https://doi.org/10.1007/s00253-020-10427-z

Ramesh, P., Durga Prasad, B., & Narayana, K. (2018). Characterization of kenaf fiber and its composites: A review. Journal of Reinforced Plastics and Composites, 37(11), 731–737. https://doi.org/10.1177/0731684418760206

Ruan, Q., Qi, J., Hu, K., Fang, P., Lin, H., Xu, J., & Yi, L. (2011). Effects of microbial pretreatment of kenaf stalk by the white-rot fungus Pleurotus sajor-caju on bioconversion of fuel ethanol production. Shengwu Gongcheng Xuebao/Chinese Journal of Biotechnology, 27(10), 1464–1471.

Schmer, M. R., Vogel, K. P., Mitchell, R. B., & Perrin, R. K. (2008). Net energy of cellulosic ethanol from switchgrass. Proceedings of the National Academy of Sciences of the United States of America, 105(2), 464–469. https://doi.org/10.1073/pnas.0704767105

Searchinger, T. D., Wirsenius, S., Beringer, T., & Dumas, P. (2018). Assessing the efficiency of changes in land use for mitigating climate change. Nature, 564(7735), 249–253. https://doi.org/10.1038/s41586-018-0757-z

Shah, S. S. M., Luthfi, A. A. I., Low, K. O., Harun, S., Manaf, S. F. A., Illias, R. M., & Jahim, J. M. (2019). Preparation of kenaf stem hemicellulosic hydrolysate and its fermentability in microbial production of xylitol by Escherichia coli BI216. Scientific Reports, 9(1), 4080. https://doi.org/10.1038/s41598-019-40807-z

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10), 3583–3597. https://doi.org/10.3168/jds.s0022-0302(91)78551-2

Wan Azeele, N. I., Md Jahim, J., Rabu, A., Abdul Murad, A. M., Abu Bakar, F. D., & Md Illias, R. (2014). Efficient removal of lignin with the maintenance of hemicellulose from kenaf by two-stage pretreatment process. Carbohydrate Polymers, 99, 447–453. https://doi.org/10.1016/j.carbpol.2013.08.043

Wang, Z., Dien, B. S., Rausch, K. D., Tumbleson, M. E., & Singh, V. (2019). Improving ethanol yields with deacetylated and two-stage pretreated corn stover and sugarcane bagasse by blending...
commercial xylose-fermenting and wild type *Saccharomyces* yeast. *Bioresource Technology*, 282, 103–109. https://doi.org/10.1016/j.biortech.2019.02.123

Wei, N., Quarterman, J., Kim, S. R., Cate, J. H. D., & Jin, Y.-S. (2013). Enhanced biofuel production through coupled acetic acid and xylose consumption by engineered yeast. *Nature Communications*, 4(1), 2580. https://doi.org/10.1038/ncomms3580

Yang, Z., Qian, K., Zhang, X., Lei, H., Xin, C., Zhang, Y., Qian, M., & Villota, E. (2018). Process design and economics for the conversion of lignocellulosic biomass into jet fuel range cycloalkanes. *Energy*, 154, 289–297. https://doi.org/10.1016/j.energy.2018.04.126

Zhang, G.-C., Kong, I. I., Wei, N. A., Peng, D., Turner, T. L., Sung, B. H., Sohn, J.-H., & Jin, Y.-S. (2016). Optimization of an acetate reduction pathway for producing cellulosic ethanol by engineered yeast. *Biotechnology and Bioengineering*, 113(12), 2587–2596. https://doi.org/10.1002/bit.26021

Zhang, Y., Kumar, A., Hardwidge, P. R., Tanaka, T., Kondo, A., & Vadlani, P. V. (2016). d-lactic acid production from renewable lignocellulosic biomass via genetically modified *Lactobacillus plantarum*. *Biotechnology Progress*, 32(2), 271–278.

Zhang, Y., Xia, C., Lu, M., & Tu, M. (2018). Effect of overliming and activated carbon detoxification on inhibitors removal and butanol fermentation of poplar prehydrolysates. *Biotechnology for Biofuels*, 11(1), 178. https://doi.org/10.1186/s13068-018-1182-0

Zhang, Z., Vancov, T., Mackintosh, S., Basu, B., Lali, A., Qian, G., Hobson, P., & Doherty, W. O. S. (2016). Assessing dilute acid pretreatment of different lignocellulosic biomasses for enhanced sugar production. *Cellulose*, 23(6), 3771–3783. https://doi.org/10.1007/s10570-016-1043-6

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Park H, Park SU, Jang B-K, Lee JJ, Chung YS. Germplasm evaluation of Kenaf (*Hibiscus cannabinus*) for alternative biomass for cellulosic ethanol production. *GCB Bioenergy*. 2021;13:201–210. https://doi.org/10.1111/gcbb.12758