Bioresource Technology 231 (2017) 101–108

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Lignocellulosic butanol production from Napier grass using semi-simultaneous saccharification fermentation

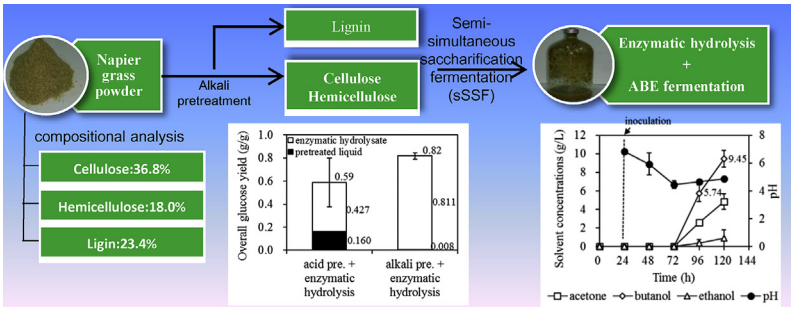
Chi-Ruei He, Yu-Yuan Kuo, Si-Yu Li *

*Department of Chemical Engineering, National Chung Hsing University, Taichung, Taiwan*

## HIGHLIGHTS

- An 0.81 g-glucose/g-glucose$_{total}$ is obtained in the enzymatic hydrolysate.
- Semi-simultaneous saccharification fermentation (sSSF) was employed.
- 9.45 g/L butanol is obtained after a 24-h hydrolysis and 96-h fermentation.
- The butanol yield reached 0.22 g/g-sugar$_{glucose+xylose}$.
- The efficiency of butanol production from Napier grass was 31%

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 22 November 2016
Received in revised form 19 January 2017
Accepted 20 January 2017
Available online 30 January 2017

*Keywords:*
Napier grass
*Pennisetum purpureum*
Alkali-pretreatment
Enzymatic hydrolysis
Semi-simultaneous saccharification fermentation (sSSF)
Butanol fermentation

## ABSTRACT

Napier grass is a potential feedstock for biofuel production because of its strong adaptability and wide availability. Compositional analysis has been done on Napier grass which was collected from a local area of Taiwan. By comparing acid- and alkali-pretreatment, it was found that the alkali-pretreatment process is favorable for Napier grass. An overall glucose yield of 0.82 g/g-glucose$_{total}$ can be obtained with the combination of alkali-pretreatment (2.5 wt% NaOH, 8 wt% sample loading, 121 °C, and a reaction time of 40 min) and enzymatic hydrolysis (40 FPU/g-substrate). Semi-simultaneous saccharification fermentation (sSSF) was carried out, where enzymatic hydrolysis and ABE fermentation were operated in the same batch. It was found that after 24-h hydrolysis, followed by 96-h fermentation, the butanol and acetone concentrations reached 9.45 and 4.85 g/L, respectively. The butanol yield reached 0.22 g/g-sugar$_{glucose+xylose}$. Finally, the efficiency of butanol production from Napier grass was calculated at 31%.



## 1. Introduction

Napier grass (*Pennisetum purpureum*) is a perennial grass having the properties of fast growth, high biomass yield, and impressive adaptability to the environment (Wen et al., 2015). These properties make Napier grass a competitive energy crop that can be a stable source for biofuels and bio-based chemical productions. According to statistical data from the Food and Agriculture Organization of the United Nations (FAO), the production of Napier grass in Tobago, Colombia, and Nigeria is 35.5, 32.4, and 20.8 tons-DM/ha/year, respectively.

Napier grass typically consists of 22.6–42.4 wt% of cellulose and 16.9–22.5 wt% of hemicellulose (Eliana et al., 2014; Wen et al., 2015; Yasuda et al., 2013). This is comparable to switchgrass, another energy crop that consists of 33.6–45.0 wt% of cellulose and 30.0–31.4 wt% of hemicellulose (Cateto et al., 2011; Sun and Cheng, 2002).

Various methods have been investigated to extract monosaccharides from Napier grass. Wen et al. combined biological pretreatment and enzymatic hydrolysis on Napier grass whereas the highest glucose yield (g-glucose/g-cellulose) of 79.4% was

* Corresponding author.
*E-mail address:* syli@dragon.nchu.edu.tw (S.-Y. Li).

http://dx.doi.org/10.1016/j.biortech.2017.01.039
0960-8524/

obtained in 13 days (Wen et al., 2015). Eliana et al. utilized 1% NaOH as a pretreated reagent followed by enzymatic hydrolysis to obtain a total sugar yield (g-sugar/g-sugar in biomass) of 36.8% (Eliana et al., 2014). Menegol et al. employed 3% NaOH as a pretreated solution and combined enzymatic hydrolysis to obtain a glucose yield of 70% (recalculated by the authors) (Menegol et al., 2014). Scholl et al. performed steam-explosion as a pretreatment on Napier grass under the conditions of 200 °C temperature and 10 min reaction time. After being enzymatically hydrolyzed for 48 h, the glucose yield reached about 0.25 g/g-biomass (or 0.68 g/g-glucose) (Scholl et al., 2015). Camesasca et al. combined an acid pretreatment followed by an alkali pretreatment on Napier grass, and then added the surfactant to promote the subsequent enzymatic hydrolysis process (Camesasca et al., 2015). After these processes, the cellulose hydrolysis reached 81% (Camesasca et al., 2015).

Cellulosic ethanol derived from Napier grass has been extensively investigated. Yasuda et al. employed Low-Moisture Anhydrous Ammonia Pretreatment (LMAA) combined with enzymatic hydrolysis on Napier grass to obtain a total sugar yield of 0.35 g-sugars/g-biomass (Yasuda et al., 2013). Besides, the use of two different strains of *Escherichia coli* KO11 and *Saccharomyces cerevisiae* enhanced the carbon utilization with an overall ethanol yield of 0.19 g-ethanol/g-biomass (Yasuda et al., 2013). Eliana et al. used an alkali pretreatment method combined with enzymatic hydrolysis on Napier grass. The total sugar yield was 0.42 g-sugar/g-biomass and the ethanol yield was 0.14 g-ethanol/g-biomass using *Saccharomyces cerevisiae* (Eliana et al., 2014). Camesasca et al. achieved a 0.13 g-ethanol/g-biomass by an acid pretreatment followed by an alkali-pretreatment as described above (Camesasca et al., 2015). The high conversion from biomass to biofuels demonstrates that Napier grass is a good lignocellulosic feedstock and this characteristic can be adopted by other fermentative biochemical productions.

Butanol is an alternative to ethanol as biofuels. It can be produced by Acetone-Butanol-Ethanol (ABE) fermentation using *Clostridium acetobutylicum*. Due to its high toxicity and separation cost, different fermentation/separation processes have been proposed (Li et al., 2016b; Lu et al., 2016; Lu and Li, 2014; Wang et al., 2016; Xue et al., 2016). Besides, various lignocellulosic biomasses, such as barley straw (Yang et al., 2015), sugarcane bagasse (Kong et al., 2016; Travaini et al., 2016), corn stalks (Cai et al., 2016), blended softwood (Yamamoto et al., 2014), rice straw (Rahnama et al., 2014), corncobs (Gao and Rehmann, 2014), cassava flour (Li et al., 2016a), and woody materials (Amiri and Karimi, 2015), have been examined to obtain sugars for ABE fermentation. Nevertheless, the production of butanol from Napier grass has not yet been reported.

In this study, Napier grass was converted to butanol by a pretreatment, enzymatic hydrolysis, and ABE fermentation. This study compared acid- and alkali-pretreatments on Napier grass. Furthermore, semi-simultaneous saccharification fermentation (sSSF) was carried out, where enzymatic hydrolysis and ABE fermentation were operated in the same batch. Overall, a feasible process of converting Napier grass to butanol was presented to provide a high sugar concentration, high butanol productivity, and high butanol yield. To the authors' knowledge, this is the first study to explore the use of Napier grass as a feedstock to produce butanol.

## 2. Materials and methods

### 2.1. Materials

Napier grass (*Pennisetum purpureum*, cultivated in a local area of Tainan City) was obtained from the Livestock Research Institute, Tainan City, Taiwan. Napier grass was first pulverized and then the Napier grass powder was sieved (mesh 20, 0.84 mm, Der Shuenn, Taiwan) to collect the particle size under 0.84 mm. The sieved Napier grass powder was dried in an oven at 45–60 °C until the weight remained constant. The sieved Napier grass powder was then sealed in zipper bags and stored at room temperature. The compositional analysis of Napier grass powder was conducted and more details can be found in Supplemental materials.

### 2.2. Chemical pretreatment on lignocellulosic material

Sulfuric acid was employed for the acid pretreatment and sodium hydroxide was utilized for the alkali pretreatment. The chemical concentration (wt%), sample loading (wt%), and reaction time (min) were examined during the pretreatment. The chemical concentrations of sulfuric acid were set at 2, 4, and 6 wt%. The concentrations of sodium hydroxide were set at 0.5, 1.5, and 2.5 wt%. The values for sample loading on pretreatment were 5, 8, and 11 wt%. Reaction times of 20, 40, 60, and 100 min were selected. The total amount of suspension including chemical solution and untreated biomass was 10 g for each pretreatment condition. Both acid- and alkali-pretreatments were carried out at 121 °C in test tubes.

After the acid/alkali pretreatment, each reaction mixture was transferred into a 15 mL centrifuge tube and centrifuged at 8500 rpm for 5 min. Then 1 mL of each supernatant liquid was collected to measure the monosaccharide in the liquid. The solid residues in the centrifuge tube was washed with deionized water to remove most of the residual chemicals. After freeze drying of solid residues, the pretreated solid was weighed to calculate the weight loss percentage, and then stored in a centrifuge tube at a room temperature.

### 2.3. Enzymatic hydrolysis

Novozymes Cellic® CTec2 was utilized for enzymatic hydrolysis in this study. The activity of Novozymes Cellic® CTec2 with the unit of the filter paper unit (FPU) was first quantified by the procedure of LAP-006 (issued by NREL) at 178 FPU/mL with the protein content of 0.29 mg/mL. To start the enzymatic hydrolysis of acid- or alkali-pretreated biomass, 0.05 g-pretreated substrate was mixed with 1 mL of 0.05 M citrate buffer (pH 5.0) containing 0.2 wt% sodium azide and Novozymes Cellic® CTec2. The reaction mixture was incubated at 50 °C for 96 h with a gentle shake. The monosaccharide concentration in the supernatant was determined by high-performance liquid chromatography (HPLC).

### 2.4. ABE fermentation with enzymatic hydrolysate using semi-simultaneous saccharification fermentation (sSSF)

*C. acetobutylicum* ATCC 824 stock in the endospore form was stored in LB broth in a serum bottle, which was filled with nitrogen to maintain the anaerobic environment. The pre-culture was anaerobically prepared by adding 5 v/v% of ATCC 824 stock in a serum bottle that contained 40 mL of Reinforced Clostridium Medium (RCM) containing 13 g/L yeast extract, 10 g/L peptone, 1 g/L soluble starch, 5 g/L sodium chloride, 3 g/L sodium acetate, 0.5 g/L L-cysteine-HCl, 0.5 g/L agar, and 5 g/L glucose. The initial pH of pre-culture was adjusted to 6.8. Then, the pre-culture solution was heat-shocked for 5 min, following which, the cultures were transferred into a shaking incubator controlled at 37 °C and 150 rpm for 18–24 h. The medium for main culture of *C. acetobutylicum* ATCC 824 was LB-s medium (Wang et al., 2016): 10 g/L tryptone, 10 g/L sodium chloride, 5 g/L yeast extract, 0.11 g/L $FeSO_4 \cdot 7H_2O$, 0.6 g/L $MgSO_4 \cdot 7H_2O$, and 0.008 g/L $CaCl_2$.

The alkali-pretreated Napier grass ($NG_{alkali\text{-}pretreated}$) was washed with tap water until the flow-through became neutral and transparent. The $NG_{alkali\text{-}pretreated}$ was then washed once with deionized water. The washed $NG_{alkali\text{-}pretreated}$ was dried in a drying oven until it reached a constant weight and stored in a Ziploc bag for sSSF.

The sSSF was started with the enzymatic hydrolysis reaction where 1.9 g $NG_{alkali\text{-}pretreated}$ was mixed with 15 mL sterilized deionized water, 3.75 mL of concentrated LB-s medium, and 0.43 mL of Novozymes Cellic® CTec2 enzyme solution. The enzymatic hydrolysis lasted for 24 h at 50 °C and 200 rpm. During the enzymatic hydrolysis, the sample and enzyme loadings were 99 g-$NG_{alkali\text{-}pretreated}$/L and 40 FPU/g-$NG_{alkali\text{-}pretreated}$, respectively. After then, 1.9 mL of pre-culture of *C. acetobutylicum* were added to the solution whereas pH was adjusted from around 5.0 to 6.8. ABE fermentation lasted for 96 h where samples were collected every 24 h. The samples were stored at −20 °C until needed.

The butanol efficiency, η, was calculated by:

$$\text{efficiency}\,\eta(\%) = \frac{\text{g-butanol}_{produced}/\text{g-substrate}}{\text{g-butanol}_{theoretical}/\text{g-substrate}} = \frac{Y_b \times (W_{glucose} \times Y_{glucose,EH} \times R_{glucose} + W_{xylose} \times Y_{xylose,EH} \times R_{xylose})}{0.41 \times W_{glucose} + 0.41 \times W_{xylose}} \times 100\% \quad (1)$$

with $Y_b$ (g-butanol/g-$sugar_{glucose+xylose}$) as the butanol yield with respect to the total glucose and xylose consumption. $W_{glucose}$ (g-glucose/g-substrate) and $W_{xylose}$ (g-xylose/g-substrate) are the glucose and xylose contents with respect to the un-pretreated biomass, which can be obtained by respectively multiplying the cellulose and xylose contents (Table S1) by 1.1. $Y_{glucose,EH}$ (g-glucose/g-$glucose_{total}$) and $Y_{xylose,EH}$ (g-xylose/g-$xylose_{total}$) are the glucose and xylose yields of the enzymatic hydrolysis reaction. $R_{glucose}$ ($glucose_{consumption}$/g-glucose) and $R_{xylose}$ ($xylose_{consumption}$/g-xylose) are the total glucose and xylose consumption proportions during ABE fermentation. Note that Eq. (1) is developed based on the situation that only sugar released from the enzymatic hydrolysis is used for ABE fermentation. This situation can be applied to most literature listed in Table 3. The coefficient of 0.41 (g-butanol/g-glucose) is a virtual butanol yield when 1 mol of glucose or 1 mol of xylose is consumed. The number was adopted for the purpose of comparison where more details about the theoretical butanol yield can be seen in (Jang et al., 2012; Papoutsakis, 1984). Note that the 1 mol of xylose is equivalent to 5/6 mol of glucose through the conversion of non-oxidative pentose phosphate pathway (Li et al., 2015; Yang et al., 2016).

The butanol productivity ($P_{BuOH,two\text{-}step}$) is defined as Eq. (2):

$$P_{BuOH,\ two\text{-}step} = \frac{C_{butanol}}{t_{enz} + t_{fer}} \quad (2)$$

$C_{butanol}$ is the butanol titer (g/L) whereas $t_{enz}$ (h) and $t_{fer}$ (h) are time required for enzymatic hydrolysis and ABE fermentation, respectively.

### 2.5. Analytical methods

Monosaccharides, D-glucose, D-xylose, L-arabinose, D-galactose, and D-mannose were quantified by the Thermo Dionex ultimate 3000 HPLC system, equipped with a Transgenomic CHO-682 Lead column (7.8 i.d. × 300 mm, particle size: 7 μm, Transgenomic Inc, USA) where deionized water was used as the mobile phase at the flow rate of 0.4 mL/min at 80 °C. All monosaccharides were monitored by a refractive index (RI) detector. Samples were diluted with deionized water and filtered through a 0.22 μm filter.

Samples collected from ABE fermentation culture were centrifuged at 17,000×*g* for 3 min and the supernatants were filtered through a 0.22 μm filter. The fermentation products: acetic acid, butyric acid, acetone, butanol, ethanol, residual glucose, and residual xylose were quantified using the Thermo Dionex ultimate 3000 HPLC system, equipped with a Transgenomic ICSep ORH-801 column (6.5 i.d. × 300 mm, particle size of 9 μm, Transgenomic Inc, USA) at 45 °C and operated with 5 mM $H_2SO_4$ as the mobile phase at the flow rate of 0.6 mL/min. The glucose, xylose, acetic acid, ethanol, and butanol were analyzed with a RI detector. Butyric acid and acetone were analyzed using a UV–VIS spectrophotometer at the wavelengths of 210 nm and 258 nm.

## 3. Results and discussion

### 3.1. The sugar release of Napier grass after acid- and alkali-pretreatments

The composition of dried Napier grass derived from a local area in Taiwan was first characterized as follows: 36.8 wt% cellulose, 18.0 wt% hemicellulose, 23.4 wt% lignin, 7.3 wt% ash, 7.5 wt% water, and 7.0 wt% uncharacterized remnant (see Table S1). Table S2 compares the composition of Napier grass from difference areas where variations in composition of Napier grass do not significantly affect the sugar extraction. This phenomenon justifies that the composition variation through the year will not affect the efficiency of the pretreatment. This Napier grass was subject to acid- and alkali-pretreatments and the yields of glucose and xylose are shown in Table 1. In general, glucose and xylose can be released to the supernatant during acid pretreatment; however, no significant glucose and xylose were found in the supernatant during the alkali pretreatment. The glucose and xylose yields of acid-pretreatment were 0.18 g/g-$glucose_{total}$ and 0.73 g/g-$xylose_{total}$, respectively (see batch #7 in Table 1). On the other hand, the alkali-pretreatment can only release glucose up to a yield of 0.03 g/g-$glucose_{total}$, while no xylose was found in the supernatant. It has been proposed that the alkali-pretreatment has the effect of dissolving lignin rather than cellulose or hemicellulose (Kumar et al., 2015; Pedersen and Meyer, 2010), and the proposed mechanism can also be applied to other biomasses, utilizing sodium hydroxide as a reagent and in pretreating sugarcane bagasse and corncob (Gao and Rehmann, 2014; van der Pol et al., 2015). Table 1 also demonstrates that the cellulose was not readily hydrolyzed by both the acid- and alkali-pretreatments compared to hemicellulose. This can be attributed to its high crystallinity (Menon and Rao, 2012).

### 3.2. The determination of monosaccharides in the enzymatic hydrolysate of acid- and alkali-pretreated Napier grass

The characteristics of acid-pretreated Napier grass ($NG_{acid\text{-}pretreated}$) and alkali-pretreated Napier grass ($NG_{alkali\text{-}pretreated}$) can be distinguished by examining their accessibility to enzyme hydrolysis. The optimal enzyme loadings were first investigated by examining the kinetics of enzymatic hydrolysis with different enzyme loadings (Fig. 1). Sugars can be fast released in 2 h when $NG_{alkali\text{-}pretreated}$ was subject to enzymatic hydrolysis. The highest total sugar concentration of 34.9 g/L was obtained in 72 h with an enzyme loading of 40 FPU/g-$NG_{alkali\text{-}pretreated}$.

$NG_{acid\text{-}pretreated}$ and $NG_{alkali\text{-}pretreated}$ obtained from conditions earlier tested were all subject to enzymatic hydrolysis (40 FPU/g-$NG_{pretreated}$) in this study whereas the glucose and xylose yields of enzymatic hydrolysis were shown in Table 2. While glucose and xylose were released after enzymatic hydrolysis from both $NG_{acid\text{-}pretreated}$ and $NG_{alkali\text{-}pretreated}$, the glucose yield from $NG_{acid\text{-}}$

**Table 1**
Glucose and xylose yields of acid- and alkali-pretreatments.

| Batch # | Condition | | | Acid-pretreated liquid | | Alkali-pretreated liquid | |
|---|---|---|---|---|---|---|---|
| | Chemical conc. (wt%)* | Sample loading (wt%) | Reaction time (min) | Glucose yield (g/g) | Xylose yield (g/g) | Glucose yield (g/g) | Xylose yield (g/g) |
| 1 | 2/0.5 | 5 | 20 | 0.14 | 0.69 | 0.02 | 0.01 |
| 2 | 2/0.5 | 8 | 20 | 0.12 | 0.69 | 0.00 | 0.01 |
| 3 | 2/0.5 | 11 | 20 | 0.11 | 0.82 | 0.03 | 0.00 |
| 4 | 4/1.5 | 5 | 20 | 0.10 | 0.68 | 0.00 | 0.00 |
| 5 | 4/1.5 | 8 | 20 | 0.12 | 0.59 | 0.00 | 0.00 |
| 6 | 4/1.5 | 11 | 20 | 0.14 | 0.69 | 0.00 | 0.00 |
| 7 | 6/2.5 | 5 | 20 | 0.18 | 0.73 | 0.00 | 0.01 |
| 8 | 6/2.5 | 8 | 20 | 0.13 | 0.51 | 0.00 | 0.00 |
| 9 | 6/2.5 | 11 | 20 | 0.17 | 0.87 | 0.00 | 0.01 |
| 10 | 2/0.5 | 5 | 40 | 0.05 | 0.73 | 0.00 | 0.00 |
| 11 | 2/0.5 | 8 | 40 | 0.09 | 0.85 | 0.00 | 0.00 |
| 12 | 2/0.5 | 11 | 40 | 0.14 | 0.75 | 0.00 | 0.00 |
| 13 | 4/1.5 | 5 | 40 | 0.08 | 0.66 | 0.00 | 0.02 |
| 14 | 4/1.5 | 8 | 40 | 0.06 | 0.72 | 0.00 | 0.00 |
| 15 | 4/1.5 | 11 | 40 | 0.15 | 0.75 | 0.00 | 0.00 |
| 16 | 6/2.5 | 5 | 40 | 0.09 | 0.61 | 0.01 | 0.00 |
| 17 | 6/2.5 | 8 | 40 | 0.10 | 0.60 | 0.00 | 0.02 |
| 18 | 6/2.5 | 11 | 40 | 0.15 | 0.59 | 0.00 | 0.01 |
| 19 | 2/0.5 | 5 | 60 | 0.09 | 0.74 | 0.00 | 0.03 |
| 20 | 2/0.5 | 8 | 60 | 0.08 | 0.76 | 0.00 | 0.01 |
| 21 | 2/0.5 | 11 | 60 | 0.10 | 0.82 | 0.01 | 0.01 |
| 22 | 4/1.5 | 5 | 60 | 0.10 | 0.67 | 0.00 | 0.02 |
| 23 | 4/1.5 | 8 | 60 | 0.11 | 0.73 | 0.00 | 0.02 |
| 24 | 4/1.5 | 11 | 60 | 0.10 | 0.70 | 0.00 | 0.01 |
| 25 | 6/2.5 | 5 | 60 | 0.10 | 0.52 | 0.00 | 0.03 |
| 26 | 6/2.5 | 8 | 60 | 0.13 | 0.57 | 0.00 | 0.02 |
| 27 | 6/2.5 | 11 | 60 | 0.11 | 0.56 | 0.00 | 0.01 |
| 28 | 2/0.5 | 5 | 100 | 0.14 | 0.72 | 0.00 | 0.03 |
| 29 | 2/0.5 | 8 | 100 | 0.16 | 0.73 | 0.00 | 0.02 |
| 30 | 2/0.5 | 11 | 100 | 0.16 | 0.76 | 0.01 | 0.00 |
| 31 | 4/1.5 | 5 | 100 | 0.15 | 0.52 | 0.00 | 0.01 |
| 32 | 4/1.5 | 8 | 100 | 0.18 | 0.61 | 0.00 | 0.01 |
| 33 | 4/1.5 | 11 | 100 | 0.18 | 0.60 | 0.00 | 0.00 |
| 34 | 6/2.5 | 5 | 100 | 0.17 | 0.37 | 0.00 | 0.02 |
| 35 | 6/2.5 | 8 | 100 | 0.17 | 0.35 | 0.00 | 0.01 |
| 36 | 6/2.5 | 11 | 100 | 0.17 | 0.66 | 0.00 | 0.01 |

* Chemical concentration: 2, 4, 6 wt% for $H_2SO_4$ and 0.5 1.5 2.5 wt% for NaOH.

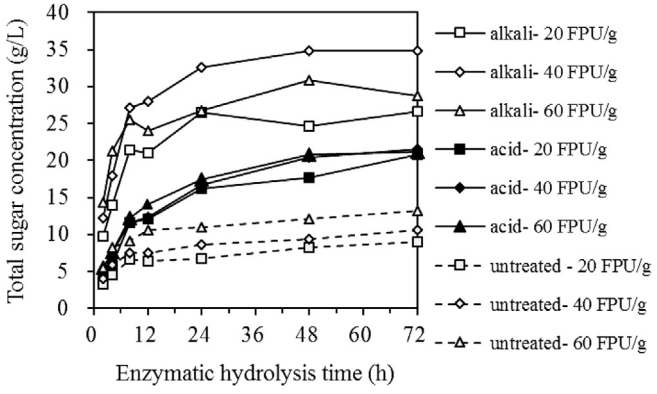



**Fig. 1.** The enzymatic hydrolysis of alkali-pretreated, acid-pretreated, and untreated Napier grass using enzyme loadings of 20, 40, and 60 FPU/g-substrate.

$_{pretreated}$ was generally lower than that from $NG_{alkali\text{-}pretreated}$. Moreover, when the acid concentration exceeded 4%, the glucose concentration in the enzymatic hydrolysate was low. This is because significant amount of glucose was released during the acid pretreatment. On the other hand, while limited glucose and xylose were released during the alkali-pretreatment process as shown in Table 1, almost all glucose and xylose can be released from $NG_{alkali\text{-}pretreated}$ after enzymatic hydrolysis. This indicates that alkali can effectively loosen the recalcitrant structure of Napier grass by removing lignin without hydrolyzing cellulose and hemicellulose so that an enzymatic hydrolysate with high sugar concentrations can be obtained. The high sugar concentrations are essential for the subsequent fermentation process.

The above description can be quantitatively described by showing the maximum overall glucose and xylose yields from a combination of pretreatment and enzymatic reaction (Fig. 2). While the overall glucose and xylose yields for acid-pretreatment + enzymatic hydrolysis were 0.59 g-glucose/g-$glucose_{total}$ and 0.76 g-xylose/g-$xylose_{total}$, respectively, the overall glucose and xylose yields for alkali-pretreatment + enzymatic hydrolysis were 0.82 g-glucose/g-$glucose_{total}$ and 0.44 g-xylose/g-$xylose_{total}$, respectively. For the overall glucose yield of 0.59 g-glucose/g-$glucose_{total}$, 0.16 g-glucose/g-$glucose_{total}$ was released during the acid-pretreatment and the remaining 0.43 g-glucose/g-$glucose_{total}$ was released during enzymatic hydrolysis. Meanwhile, the acid-pretreatment provided a high yield of 0.73 g-xylose/g-$xylose_{total}$, indicating that the hemicellulose of Napier grass is more accessible to acids, which is consistent with previous studies where a significant amount of glucose and xylose were released with 0.5–2.0% $H_2SO_4$ (Chen et al., 2009; Kootstra et al., 2009; Saha et al., 2005). On the other hand, not only a high overall glucose yield of 0.82 g-glucose/g-$glucose_{total}$ was obtained by alkali-pretreatment + enzymatic hydrolysis, 0.81 g-glucose/g-$glucose_{total}$ was found in the enzymatic hydrolysate.

Alkali-pretreatment typically provides a good efficacy to break up the structure of lignocellulosic materials and thus facilitate

**Table 2**
Glucose and xylose yields of enzymatic hydrolysis.

| Batch # | Condition | | | Enzymatic hydrolysis of acid-pretreated Napier grass | | | | Enzymatic hydrolysis of alkali-pretreated Napier grass | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chemical conc. (wt%)* | Sample loading (wt%) | Reaction time (min) | Glucose yield (g/g) | SD | Xylose yield (g/g) | SD | Glucose yield (g/g) | SD | Xylose yield (g/g) | SD |
| 1 | 2/0.5 | 5 | 20 | 0.30 | 0.11 | 0.05 | 0.04 | 0.66 | 0.11 | 0.50 | 0.08 |
| 2 | 2/0.5 | 8 | 20 | 0.39 | 0.20 | 0.06 | 0.01 | 0.59 | 0.04 | 0.40 | 0.10 |
| 3 | 2/0.5 | 11 | 20 | 0.41 | 0.26 | 0.15 | 0.12 | 0.42 | 0.04 | 0.24 | 0.03 |
| 4 | 4/1.5 | 5 | 20 | 0.22 | 0.01 | 0.01 | 0.01 | 0.67 | 0.00 | 0.46 | 0.03 |
| 5 | 4/1.5 | 8 | 20 | 0.21 | 0.01 | 0.01 | 0.01 | 0.67 | 0.13 | 0.50 | 0.19 |
| 6 | 4/1.5 | 11 | 20 | 0.23 | 0.08 | 0.01 | 0.02 | 0.59 | 0.00 | 0.46 | 0.13 |
| 7 | 6/2.5 | 5 | 20 | 0.15 | 0.03 | 0.00 | 0.00 | 0.75 | 0.15 | 0.38 | 0.16 |
| 8 | 6/2.5 | 8 | 20 | 0.17 | 0.05 | 0.00 | 0.00 | 0.75 | 0.04 | 0.47 | 0.02 |
| 9 | 6/2.5 | 11 | 20 | 0.15 | 0.04 | 0.00 | 0.01 | 0.52 | 0.03 | 0.39 | 0.09 |
| 10 | 2/0.5 | 5 | 40 | 0.29 | 0.07 | 0.01 | 0.02 | 0.78 | 0.04 | 0.59 | 0.10 |
| 11 | 2/0.5 | 8 | 40 | 0.40 | 0.13 | 0.05 | 0.01 | 0.61 | 0.03 | 0.45 | 0.04 |
| 12 | 2/0.5 | 11 | 40 | 0.35 | 0.11 | 0.04 | 0.02 | 0.52 | 0.07 | 0.35 | 0.15 |
| 13 | 4/1.5 | 5 | 40 | 0.15 | 0.05 | 0.00 | 0.00 | 0.75 | 0.08 | 0.47 | 0.05 |
| 14 | 4/1.5 | 8 | 40 | 0.18 | 0.02 | 0.01 | 0.01 | 0.74 | 0.08 | 0.55 | 0.16 |
| 15 | 4/1.5 | 11 | 40 | 0.16 | 0.03 | 0.01 | 0.01 | 0.77 | 0.13 | 0.59 | 0.21 |
| 16 | 6/2.5 | 5 | 40 | 0.10 | 0.03 | 0.00 | 0.00 | 0.81 | 0.03 | 0.44 | 0.04 |
| 17 | 6/2.5 | 8 | 40 | 0.10 | 0.07 | 0.00 | 0.00 | 0.81 | 0.06 | 0.46 | 0.06 |
| 18 | 6/2.5 | 11 | 40 | 0.13 | 0.04 | 0.00 | 0.00 | 0.74 | 0.06 | 0.52 | 0.17 |
| 19 | 2/0.5 | 5 | 60 | 0.29 | 0.03 | 0.02 | 0.03 | 0.64 | 0.11 | 0.48 | 0.05 |
| 20 | 2/0.5 | 8 | 60 | 0.31 | 0.04 | 0.02 | 0.02 | 0.53 | 0.08 | 0.43 | 0.16 |
| 21 | 2/0.5 | 11 | 60 | 0.38 | 0.10 | 0.04 | 0.04 | 0.45 | 0.05 | 0.34 | 0.04 |
| 22 | 4/1.5 | 5 | 60 | 0.19 | 0.05 | 0.00 | 0.00 | 0.80 | 0.12 | 0.47 | 0.10 |
| 23 | 4/1.5 | 8 | 60 | 0.21 | 0.01 | 0.01 | 0.01 | 0.63 | 0.08 | 0.43 | 0.08 |
| 24 | 4/1.5 | 11 | 60 | 0.20 | 0.02 | 0.01 | 0.01 | 0.65 | 0.10 | 0.49 | 0.10 |
| 25 | 6/2.5 | 5 | 60 | 0.12 | 0.06 | 0.01 | 0.01 | 0.57 | 0.10 | 0.22 | 0.06 |
| 26 | 6/2.5 | 8 | 60 | 0.16 | 0.05 | 0.00 | 0.00 | 0.72 | 0.06 | 0.40 | 0.05 |
| 27 | 6/2.5 | 11 | 60 | 0.13 | 0.05 | 0.01 | 0.02 | 0.50 | 0.01 | 0.34 | 0.00 |
| 28 | 2/0.5 | 5 | 100 | 0.40 | 0.11 | 0.01 | 0.02 | 0.62 | 0.00 | 0.45 | 0.02 |
| 29 | 2/0.5 | 8 | 100 | 0.43 | 0.21 | 0.04 | 0.01 | 0.49 | 0.01 | 0.34 | 0.03 |
| 30 | 2/0.5 | 11 | 100 | 0.37 | 0.23 | 0.09 | 0.06 | 0.51 | 0.09 | 0.33 | 0.07 |
| 31 | 4/1.5 | 5 | 100 | 0.18 | 0.05 | 0.03 | 0.04 | 0.78 | 0.05 | 0.48 | 0.05 |
| 32 | 4/1.5 | 8 | 100 | 0.17 | 0.02 | 0.00 | 0.00 | 0.62 | 0.07 | 0.45 | 0.05 |
| 33 | 4/1.5 | 11 | 100 | 0.24 | 0.06 | 0.01 | 0.01 | 0.61 | 0.07 | 0.48 | 0.02 |
| 34 | 6/2.5 | 5 | 100 | 0.13 | 0.11 | 0.00 | 0.00 | 0.50 | 0.05 | 0.24 | 0.01 |
| 35 | 6/2.5 | 8 | 100 | 0.23 | 0.26 | 0.02 | 0.03 | 0.67 | 0.03 | 0.44 | 0.10 |
| 36 | 6/2.5 | 11 | 100 | 0.17 | 0.05 | 0.00 | 0.00 | 0.68 | 0.04 | 0.52 | 0.11 |

* Chemical concentration: 2, 4, 6 wt% for $H_2SO_4$ and 0.5 1.5 2.5 wt% for NaOH.

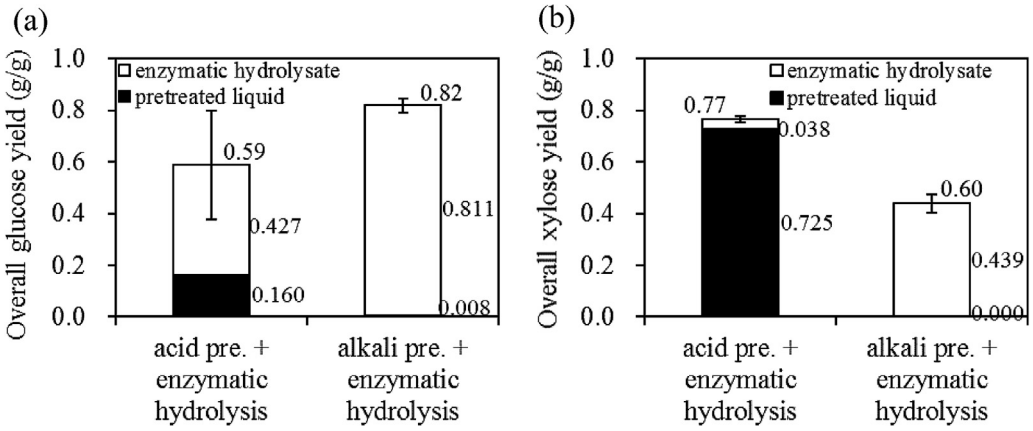



**Fig. 2.** (a) The overall glucose yield and (b) overall xylose yield of the pretreatment and enzymatic hydrolysis process. The conditions for acid-pretreatment were 2 wt% $H_2SO_4$, 8 wt% sample loading, and reaction time of 100 min. The conditions for alkali-pretreatment were 2.5 wt% NaOH, 5 wt% sample loading, and reaction time of 40 min. The enzyme loading for both acid- and alkali-processes was 40 FPU/g. The data was chosen based on the conditions that can achieve the maximum glucose yield.

the following enzymatic reaction (Kumar et al., 2013). In this study, it can be concluded that the alkali-pretreatment using NaOH is favored for the treatment of Napier grass. Compared to mild alkali reagents of $Ca(OH)_2$ and $H_2O_2$ where long reaction times of 4 h to 16 weeks and high temperatures of 121–125 °C were needed (Chen et al., 2009; Kim and Holtzapple, 2005), the advantage of using NaOH as alkali reagent is that the reaction time and temperature for the pretreatment is significantly undemanding (Chen et al., 2008a; Saha and Cotta, 2006).where typically 80–121 °C and 60–20 min were needed (Chen et al., 2009, 2008b; Kapoor

et al., 2015; Michalska et al., 2015). The enzymatic hydrolysate with high glucose and xylose concentrations can be obtained by the combination of alkali-pretreatment and enzymatic hydrolysis. This saves any possible separation cost to pull together sugars from the pretreatment and enzymatic processes for the subsequent fermentation process. Besides, the use of alkali-pretreatment is free from the formation of furfural and 5-hydroxymethyl furfural (HMF), well-known fermentation inhibitors that are generated during the acid-pretreatment process. Consequently, an additional removal process is needed. In this study, furfural or HMF was not found during alkali-pretreatments (data not shown). The high glucose yield demonstrates that a simple alkali-pretreatment is sufficient to treat crop/herb biomass with low lignin content such as Napier grass.

### 3.3. ABE fermentation with enzymatic hydrolysate using semi-simultaneous saccharification fermentation (sSSF)

The separate hydrolysis and fermentation (SHF) is typically used since the dilute-acid pretreatment is a widely used method. Between the hydrolysis and fermentation, an additional process is often needed for the separation of furfural and HMF. During which, the residual pellets from the enzymatic hydrolysis was simultaneously removed. In this study, alkali-pretreatment was found to be the superior method compared to acid-pretreatment in terms of sugar yield and sugar concentration of enzymatic hydrolysate. Besides, no inhibitors to the fermentation process were found. Therefore, semi-simultaneous saccharification fermentation (sSSF) (Shen and Agblevor, 2010) was employed for the conversion of $NG_{alkali\text{-}pretreated}$ into bio-butanol to avoid the additional process for removing the residual pellets. A concentration of 99 g-$NG_{alkali\text{-}pretreated}$/L was first subject to enzymatic hydrolysis (40 FPU/g) for 24 h to release the glucose and xylose while decreasing the viscosity of the suspension. It can be seen in Fig. 3a that the glucose and xylose concentration reached 34.8 g/L and 16.4 g/L at 24 h, respectively, which is in great agreement with data shown in Table 2 (Batch #10). The inoculation of *C. acetobutylicum* was achieved at 24 h and the butanol and acetone concentrations reached 9.45 and 4.85 g/L in the following 96 h (Fig. 3b). With the glucose consumption of 34.8 g/L and xylose consumption of 8.2 g/L, the butanol yield ($Y_b$) can be calculated to be 0.22 g/g-$sugar_{glucose+xylose}$ (Table 3). This is a competitive performance since batch ABE fermentation that has a butanol yield higher than 0.2 g/g-glucose is considered to be efficient (Chen et al., 2013; Li et al., 2016b). Note that both acetic acid and butyric acid concentrations were below 2 g/L at the fermentation time of 96 h. Overall, $NG_{alkali\text{-}pretreated}$ can be converted into bio-butanol by sSSF. Moreover, the advantage of sSSF can be demonstrated by the high butanol productivity as shown in Table 3. sSSF provided a high butanol productivity of 0.08 g $L^{-1}$ $h^{-1}$ which is higher than most studies. The difference in productivity is significant since butanol productivities of all other studies presented in Table 3 are overestimated but presented for the comparison purpose. This is because studies listed in Table 3 employed SHF where the times between hydrolysis and fermentation are not reported. Moreover, the purification of hydrolysate may also needed for removing inhibitors. This not only decreases butanol productivity but also increases the capitol cost. For example, although a butanol productivity of 0.15 g $L^{-1}$ $h^{-1}$ (Kong et al.) is presented in Table 3, process time of recovering pretreatment enhancer from the hydrolysate was not included in the calculation. Overall, with no additional process between hydrolysis and fermentation, this study presents a manageable process that can achieved a competitive butanol productivity by sSSF. Table 3 compares the butanol yield and productivity of ABE fermentation that are derived from various biomasses.

Table 3 also compares the butanol efficiency, an index that consolidates performances of pretreatment, enzymatic hydrolysis, and butanol fermentation. It can be seen that while a high productivity of 0.08 g $L^{-1}$ $h^{-1}$ can be achieved as discussed above, the overall butanol efficiency derived from Napier grass has a high efficiency of 31%. Note that the butanol efficiency is calculated according to Eq. (1) where $Y_b$ is 0.22 g-butanol/g-$sugar_{glucose+xylose}$, $W_{glucose}$ is 0.405 g-glucose/g-substrate, $Y_{glucose,EH}$ is 0.78 g-glucose/g-$glucose_{total}$, $R_{glucose}$ is 1.00 g-$glucose_{consumption}$/g-glucose), $W_{xylose}$ is 0.109 g-xylose/g-substrate, $Y_{xylose,EH}$ is 0.59 g-xylose/g-$xylose_{total}$, and $R_{xylose}$ is 0.50 g-$glucose_{consumption}$/g-xylose. The high butanol efficiency reflects that alkali-pretreatment is suitable for Napier grass since a high sugar yield without the formation of furfural and HMF can be followed by the subsequent ABE fermentation achieved by sSSF. By contrast, when inhibitors were generated during the pretreatment of sugarcane bagasse, a worse butanol yield and thus a low butanol efficiency of 12% was reported (Travaini et al., 2016). Moreover, it can be also deduced that the crop/herb biomass had the potential to perform butanol fermentation compared to elm wood and pinewood. The high butanol efficiency of Napier grass is also contributed by the high butanol yield. The butanol efficiency of Napier grass can be further elevated by solving the xylose consumption ($R_{xylose}$, Eq. (1)) of *C. acetobutylicum* ATCC824 where only 50% of xylose in the broth was consumed (see Table 3). It is believed that by overexpressing proteins that are associated with xylose uptake and consumption, both glucose and xylose can be simultaneously consumed and

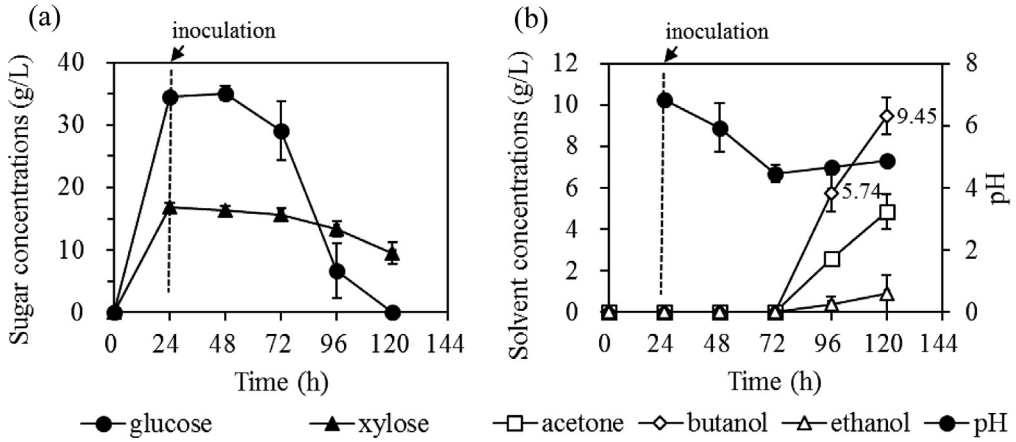



**Fig. 3.** Profiles of (a) sugar concentration and (b) solvent concentration of ABE fermentation using semi-simultaneous saccharification fermentation (sSSF). The enzymatic hydrolysis of alkali-pretreated Napier grass was achieved in the first 24 h followed by the inoculation of *C. acetobutylicum* at 24 h.

**Table 3**
Performance of ABE fermentation derived from various biomasses.

| Biomass | Pretreatment, enzyme loadings, and butanol producing strain | Butanol productivity ($g\ L^{-1}\ h^{-1}$)[*1] | Butanol yield (g/g-$sugar_{glucose+xylose}$) | Butanol efficiency | Ref. |
|---|---|---|---|---|---|
| Napier grass | 0.5%w/w NaOH, 40 FPU Novozymes Cellic® CTec2, *C. acetobutylicum* ATCC824 | 0.08 | 0.22 | 31% | This study |
| Barley straw | 2%w/w $H_2SO_4$, Celluclast 1.5 L and Novozyme 188, *C. acetobutylicum* DSM 1731 | 0.05 | 0.20 | 36% | Yang et al. (2015) |
| Corn stalk leaf | 0.5%w/w NaOH, 40 FPU Cellulase, *C. acetobutylicum* ABE 1301 | 0.05 | 0.24 | 31% | Cai et al. (2016) |
| pinewood | Autohydrolysis method, 25 FPU cellulose and 40 IU β-glucosidase, *C. acetobutylicum* NRRL B-591 | 0.05 | 0.20 | 19% | Amiri and Karimi (2015) |
| Rice straw | 2%w/v NaOH, *Trichoderma harzianum* SNRS3, *C. acetobutylicum* ATCC824 | 0.01 | 0.14 | 13% | Rahnama et al. (2014) |
| Sugarcane bagasse | Ozonolysis method, 30 CBU Novozymes® 188,Cellulast, *C. acetobutylicum* DSM 792 | 0.01 | 0.07 | 12% | Travaini et al. (2016) |
| Elmwood | Autohydrolysis method, 25 FPU cellulose and 40 IU β-glucosidase, *C. acetobutylicum* NRRL B-591 | 0.03 | 0.22 | 2% | Amiri and Karimi (2015) |
| Sugarcane bagasse | 1%w/w NaOH, 50 mM $H_2SO_4$ for the hydrolysis of cellulose, *C. acetobutylicum* XY16 | 0.15 | 0.23 | N/A | Kong et al. (2016) |
| Blended softwood | 0.1 M HCl, Novozymes Cellic® CTec2 and Novozymes Cellic® HTec2, *C. acetobutylicum* B 5313 | 0.04 | 0.15 | N/A | Yamamoto et al. (2014) |

[*1] The butanol productivity was calculated with respect to the time required for enzymatic hydrolysis and ABE fermentation.

further enhance butanol fermentation (Yu et al., 2015). Besides, mild ozonation pretreatment may facilitate the release of xylose ($Y_{xylose,EH}$) and thus enhance the butanol efficiency (Cardeña et al., 2017).

## 4. Conclusions

Napier grass has the overall 54% carbohydrate content which results in the potential to be a carbon source. The 82% glucose yield obtained from the alkali-pretreatment combined with enzymatic hydrolysis showed that Napier grass could efficiently transfer polysaccharides into monosaccharides in this process. The alkali-pretreatment facilitates the employment of sSSF which provides a high butanol productivity of 0.08 $g\ L^{-1}\ h^{-1}$. The butanol yield reached 0.22 g/g-$sugar_{glucose+xylose}$ and the efficiency of the butanol production from Napier grass was calculated to be 31%. The high efficiency of butanol production demonstrated that Napier grass is feasible for cellulosic butanol production.

## Acknowledgements

This work is supported by Aph Co. Ltd., and the Ministry of Science and Technology Taiwan, MOST-103-2221-E-005-072-MY3 and MOST-104-2621-M-005-004-MY3.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.01.039.

## References

Amiri, H., Karimi, K., 2015. Autohydrolysis: a promising pretreatment for the improvement of acetone, butanol, and ethanol production from woody materials. Chem. Eng. Sci. 137, 722–729.
Cai, D., Li, P., Luo, Z., Qin, P., Chen, C., Wang, Y., Wang, Z., Tan, T., 2016. Effect of dilute alkaline pretreatment on the conversion of different parts of corn stalk to fermentable sugars and its application in acetone–butanol–ethanol fermentation. Bioresour. Technol. 211, 117–124.
Camesasca, L., Ramírez, M.B., Guigou, M., Ferrari, M.D., Lareo, C., 2015. Evaluation of dilute acid and alkaline pretreatments, enzymatic hydrolysis and fermentation of Napier grass for fuel ethanol production. Biomass Bioenergy 74, 193–201.
Cardeña, R., Moreno, G., Bakonyi, P., Buitrón, G., 2017. Enhancement of methane production from various microalgae cultures via novel ozonation pretreatment. Chem. Eng. J. 307, 948–954.
Cateto, C., Hu, G., Ragauskas, A., 2011. Enzymatic hydrolysis of organosolv Kanlow switchgrass and its impact on cellulose crystallinity and degree of polymerization. Energy Environ. Sci. 4, 1516–1521.
Chen, H., Han, Y., Xu, J., 2008a. Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide. Process Biochem. 43, 1462–1466.
Chen, M., Zhao, J., Xia, L., 2008b. Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. Carbohydr. Polym. 71, 411–415.
Chen, M., Zhao, J., Xia, L., 2009. Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. Biomass Bioenergy 33, 1381–1385.
Chen, Y., Zhou, T., Liu, D., Li, A., Xu, S., Liu, Q., Li, B., Ying, H., 2013. Production of butanol from glucose and xylose with immobilized cells of Clostridium acetobutylicum. Biotechnol. Bioprocess Eng. 18, 234–241.
Eliana, C., Jorge, R., Juan, P., Luis, R., 2014. Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass. Fuel 118, 41–47.
Gao, K., Rehmann, L., 2014. ABE fermentation from enzymatic hydrolysate of NaOH-pretreated corncobs. Biomass Bioenergy 66, 110–115.
Jang, Y.-S., Lee, J.Y., Lee, J., Park, J.H., Im, J.A., Eom, M.-H., Lee, J., Lee, S.-H., Song, H., Cho, J.-H., Seung, D.Y., Lee, S.Y., 2012. Enhanced butanol production obtained by reinforcing the direct butanol-forming route in Clostridium acetobutylicum. mBio 3.
Kapoor, M., Raj, T., Vijayaraj, M., Chopra, A., Gupta, R.P., Tuli, D.K., Kumar, R., 2015. Structural features of dilute acid, steam exploded, and alkali pretreated mustard stalk and their impact on enzymatic hydrolysis. Carbohydr. Polym. 124, 265–273.
Kim, S., Holtzapple, M.T., 2005. Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresour. Technol. 96, 1994–2006.
Kong, X., Xu, H., Wu, H., Wang, C., He, A., Ma, J., Ren, X., Jia, H., Wei, C., Jiang, M., Ouyang, P., 2016. Biobutanol production from sugarcane bagasse hydrolysate generated with the assistance of gamma-valerolactone. Process Biochem. 51, 1538–1543.
Kootstra, A.M.J., Beeftink, H.H., Scott, E.L., Sanders, J.P., 2009. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochem. Eng. J. 46, 126–131.
Kumar, G., Sen, B., Lin, C.-Y., 2013. Pretreatment and hydrolysis methods for recovery of fermentable sugars from de-oiled Jatropha waste. Bioresour. Technol. 145, 275–279.
Kumar, G., Bakonyi, P., Periyasamy, S., Kim, S.H., Nemestóthy, N., Bélafi-Bakó, K., 2015. Lignocellulose biohydrogen: practical challenges and recent progress. Renewable Sustainable Energy Rev. 44, 728–737.
Li, Y.-H., Ou-Yang, F.-Y., Yang, C.-H., Li, S.-Y., 2015. The coupling of glycolysis and the Rubisco-based pathway through the non-oxidative pentose phosphate pathway to achieve low carbon dioxide emission fermentation. Bioresour. Technol. 187, 189–197.
Li, H.-G., Zhang, Q.-H., Yu, X.-B., Wei, L., Wang, Q., 2016a. Enhancement of butanol production in Clostridium acetobutylicum SE25 through accelerating phase shift by different phases pH regulation from cassava flour. Bioresour. Technol. 201, 148–155.
Li, S.-Y., Chiang, C.-J., Tseng, I.T., He, C.-R., Chao, Y.-P., 2016b. Bioreactors and in situ product recovery techniques for acetone–butanol–ethanol fermentation. FEMS Microbiol. Lett. 363.
Lu, K.-M., Li, S.-Y., 2014. An integrated *in situ* extraction-gas stripping process for Acetone–Butanol–Ethanol (ABE) fermentation. J. Taiwan Inst. Chem. Eng. 45, 2106–2110.

Lu, K.-M., Chiang, Y.-S., Wang, Y.-R., Chein, R.-Y., Li, S.-Y., 2016. Performance of fed-batch acetone–butanol–ethanol (ABE) fermentation coupled with the integrated in situ extraction-gas stripping process and the fractional condensation. J. Taiwan Inst. Chem. Eng. 60, 119–123.
Menegol, D., Luisi Scholl, A., Claudete Fontana, R., Pinheiro Dillon, A.J., Camassola, M., 2014. Potential of a Penicillium echinulatum enzymatic complex produced in either submerged or solid-state cultures for enzymatic hydrolysis of elephant grass. Fuel 133, 232–240.
Menon, V., Rao, M., 2012. Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog. Energy Combust. Sci. 38, 522–550.
Michalska, K., Bizukojć, M., Ledakowicz, S., 2015. Pretreatment of energy crops with sodium hydroxide and cellulolytic enzymes to increase biogas production. Biomass Bioenergy 80, 213–221.
Papoutsakis, E.T., 1984. Equations and calculations for fermentations of butyric acid bacteria. Biotechnol. Bioeng. 26, 174–187.
Pedersen, M., Meyer, A.S., 2010. Lignocellulose pretreatment severity–relating pH to biomatrix opening. New Biotechnol. 27, 739–750.
Rahnama, N., Foo, H.L., Abdul Rahman, N.A., Ariff, A., Md Shah, U.K., 2014. Saccharification of rice straw by cellulase from a local Trichoderma harzianum SNRS3 for biobutanol production. BMC Biotechnol. 14, 103.
Saha, B.C., Cotta, M.A., 2006. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. Biotechnol. Prog. 22, 449–453.
Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochem. 40, 3693–3700.
Scholl, A.L., Menegol, D., Pitarelo, A.P., Fontana, R.C., Filho, A.Z., Ramos, L.P., Dillon, A. J.P., Camassola, M., 2015. Ethanol production from sugars obtained during enzymatic hydrolysis of elephant grass (Pennisetum purpureum, Schum.) pretreated by steam explosion. Bioresour. Technol. 192, 228–237.
Shen, J., Agblevor, F.A., 2010. Modeling semi-simultaneous saccharification and fermentation of ethanol production from cellulose. Biomass Bioenergy 34, 1098–1107.
Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol. 83, 1–11.
Travaini, R., Barrado, E., Bolado-Rodríguez, S., 2016. Effect of ozonolysis parameters on the inhibitory compound generation and on the production of ethanol by Pichia stipitis and acetone-butanol-ethanol by Clostridium from ozonated and water washed sugarcane bagasse. Bioresour. Technol. 218, 850–858.
van der Pol, E., Bakker, R., van Zeeland, A., Sanchez Garcia, D., Punt, A., Eggink, G., 2015. Analysis of by-product formation and sugar monomerization in sugarcane bagasse pretreated at pilot plant scale: differences between autohydrolysis, alkaline and acid pretreatment. Bioresour. Technol. 181, 114–123.
Wang, Y.-R., Chiang, Y.-S., Chuang, P.-J., Chao, Y.-P., Li, S.-Y., 2016. Direct in situ butanol recovery inside the packed bed during continuous acetone-butanol-ethanol (ABE) fermentation. Appl. Microbiol. Biotechnol. 100, 7449–7456.
Wen, B., Yuan, X., Li, Q.X., Liu, J., Ren, J., Wang, X., Cui, Z., 2015. Comparison and evaluation of concurrent saccharification and anaerobic digestion of Napier grass after pretreatment by three microbial consortia. Bioresour. Technol. 175, 102–111.
Xue, C., Liu, F., Xu, M., Zhao, J., Chen, L., Ren, J., Bai, F., Yang, S.-T., 2016. A novel in situ gas stripping-pervaporation process integrated with acetone-butanol-ethanol fermentation for hyper n-butanol production. Biotechnol. Bioeng. 113, 120–129.
Yamamoto, M., Iakovlev, M., Bankar, S., Tunc, M.S., van Heiningen, A., 2014. Enzymatic hydrolysis of hardwood and softwood harvest residue fibers released by sulfur dioxide–ethanol–water fractionation. Bioresour. Technol. 167, 530–538.
Yang, M., Zhang, J., Kuittinen, S., Vepsäläinen, J., Soininen, P., Keinänen, M., Pappinen, A., 2015. Enhanced sugar production from pretreated barley straw by additive xylanase and surfactants in enzymatic hydrolysis for acetone–butanol–ethanol fermentation. Bioresour. Technol. 189, 131–137.
Yang, C.-H., Liu, E.-J., Chen, Y.-L., Ou-Yang, F.-Y., Li, S.-Y., 2016. The comprehensive profile of fermentation products during in situ $CO_2$ recycling by Rubisco-based engineered Escherichia coli. Microb. Cell Fact. 15, 1–10.
Yasuda, M., Takeo, K., Nagai, H., Uto, T., Yui, T., Matsumoto, T., Ishii, Y., Ohta, K., 2013. Enhancement of ethanol production from Napiergrass (Pennisetum purpureum Schumach) by a low-moisture anhydrous ammonia pretreatment. J. Sustainable Bioenergy Syst. 3, 179–185.
Yu, L., Xu, M., Tang, I.C., Yang, S.-T., 2015. Metabolic engineering of Clostridium tyrobutyricum for n-butanol production through co-utilization of glucose and xylose. Biotechnol. Bioeng. 112, 2134–2141.