Journal of the Taiwan Institute of Chemical Engineers 000 (2017) 1–7

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

The utilization of sweet potato vines as carbon sources for fermenting bio-butanol

Chi-Ruei He[a], Che-Lun Huang[b], Yung-Chang Lai[b], Si-Yu Li[a,*]

[a] Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan
[b] Department of Agronomy, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan

ARTICLE INFO

Article history:
Received 26 October 2016
Revised 17 January 2017
Accepted 12 February 2017
Available online xxx

Keywords:
Ipomoea batatas Lam
Sweet potato vines
Clostridium acetobutylicum
Acetone-butanol-ethanol (ABE) fermentation
Lignocellulosic butanol
Semi-simultaneous saccharification fermentation (sSSF)

ABSTRACT

In this study, domestic sweet potato vines were selected as the lignocellulosic material for producing monosaccharides where monosaccharides as produced were used for biobutanol production by *Clostridium acetobutylicum*. First, the compositional analysis showed that dried sweet potato vines consisted of 17.7 ± 1.5 wt. % cellulose, 4.0 ± 1.4 wt. % hemicellulose, 17.2 ± 1.0 wt. % lignin, 1.5 ± 0.3 wt. % ash, and 8.5 ± 0.5 wt. % moisture. The combination of acid-pretreatment and enzymatic hydrolysis provided a good total glucose and xylose yields of 0.74 g/g-glucose$_{total}$ and 0.99 g/g-xylose$_{total}$. However, 0.25 g/g-glucose$_{total}$ and 0.68 g/g-xylose$_{total}$ were released during the acid pretreatment and therefore results in a low sugar concentration in the enzymatic hydrolysis liquid. On the other hand, the combination of alkali-pretreatment and enzymatic hydrolysis provided total glucose and xylose yields of 0.64 g/g-glucose$_{total}$ and 0.63 g/g-xylose$_{total}$ and most of released sugars were found in the enzymatic hydrolysate. The enzymatic hydrolysate of the alkali-pretreated sweet potato vines (1.5 wt. % NaOH, 12 wt. % sample loading, and a reaction time of 20 min) was used as feedstock for acetone-butanol-ethanol (ABE) fermentation and 6.4 ± 0.2 g/L of butanol was obtained in 72 h with the butanol yield of 0.18 g/g-sugar$_{total}$. In summary, the efficiency of converting dried sweet potato vines to bio-butanol was 23%.

1. Introduction

Sweet potato (*Ipomoea batatas* Lam.) is a herbaceous plant and an important food crop in the world. The root of sweet potato was the main part as the diet for the human society.

More than 105 million metric tons of sweet potatoes were harvested in the world each year. Sweet potato vines are the above ground part of sweet potato that is composed of leaves, stalks, and stems. They are the byproduct during the harvest and have the yield up to 2.6 metric tons of dry matter/ha [1]. Sweet potato vines have been shown to be nutritious and have abundant functionally valuable compositions such as antioxidants [2] and nitrogen/carbon source [3]. Some studies also proposed that the ingredients in sweet potato vines may have the antibacterial function and the efficacy on curing diabetes [4]. Therefore, sweet potato vines have been utilized as the supplement in some areas of South America and South-East Asia [5]. Nevertheless, large amounts of sweet potato vines are still abandoned as an agriculture waste around the world [3,6]. Typically, the production of sweet potato vines is higher than that of sweet potato tubers [7]. For example, while 230,284 metric tons of sweet potatoes were harvested in 2015 in Taiwan [8], more than 300,000 metric tons of sweet potato vines were simultaneously generated as the agro-waste (unpublished data).

Recently, attentions have been paid to utilizing agricultural wastes as carbon sources to produce cellulosic biofuels and bio-based chemicals [9-11]. This approach fits the concept of bio-refinery [12] and thus provides a sustainable and economical way for cellulosic biofuels and bio-based chemicals. Zhan et al. employed sweet potato vines as the carbon source for *Trichosporon fermentans* to produce bio-oils, which can be used as substitutes for value-added lipids and as a feedstock for biodiesel production [3].

Sweet potato vines are abundant lignocellulosic feedstock and its possibility for biofuel production has not been investigated. In order to fully explore the potential of sweet potato in the aspect of biorefinery, sweet potato vines were used as the feedstock for biobutanol production by *Clostridium acetobutylicum* in this study. *C. acetobutylicum* is a type strain that has been extensively used as the model microorganism for studying acetone-butanol-ethanol (ABE) fermentation. Its genome has been sequenced and published in 2001 [13]. To obtain monosaccharides from the hydrolysis of sweet potato vines, a process termed pretreatment is needed to loosen the recalcitrant structure. Currently, pretreatments can be

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

http://dx.doi.org/10.1016/j.jtice.2017.02.022
1876-1070/

categorized into physical, chemical, physico-chemical, and biological methods (see [14] for detail review). Among which, chemical pretreatment using H_2SO_4 or NaOH are promising and manageable techniques for industrial applications and these two acid- and alkali-pretreatments will be adopted and compared.

2. Material and methods

2.1. Materials

Sweet potato vines were the agro-wastes of Chia-Yi county, Taiwan. Sweet potato vines were dried in an oven at 102 °C until the weight maintained constant. Dried sweet potato vines were pulverized and sieved through a 20 mesh screen to obtain particle sizes that were below 0.84 mm. The collected powder was transferred into zipper bags and stored at room temperature.

2.2. The composition analysis of sweet potato vines

The composition of the untreated sweet potato vines was determined according to the Laboratory Analytical Procedures (LAPs) issued by National Renewable Energy Laboratory (NREL). The carbohydrate and lignin contents of sweet potato vines were determined as follows. 0.30 ± 0.01 g of dried powders of sweet potato vines were mixed with 3.00 ± 0.01 mL of 72 wt. % H_2SO_4 in a test tube until powders were thoroughly wetted. The test tube was then capped and placed in a water batch at 30 ± 1 °C for 2 h during which the suspension in the test tube were stirred every 15 min. Upon the completion of the 2 h hydrolysis, the suspension was transferred into a 250 mL serum bottle followed by the addition of 84.00 ± 0.04 mL deionized water to reach the final H_2SO_4 concentration of 4 wt. %. The suspension was then autoclaved at 121 ± 3 °C for 1 h followed by the centrifugation at 17,000 × g. The supernatant was neutralized to pH 7.0 with 5 N NaOH before the carbohydrate quantification using high-performance liquid chromatography (HPLC). Note that the sugar recovery standard (SRS) was considered and performed for the quantification of carbohydrates, as suggested by NREL.

The neutralized supernatant was used to determine the concentration of the acid-soluble lignin (ASL) and the solid residue was used to determine the content of acid-insoluble lignin (AIL). The total lignin content is defined as the summation of ASL and AIL contents. The ASL was quantified by measuring the absorbance at 205 nm, as suggested by NREL. The dynamic range between 0.2 and 0.7 was used and the absorptivity of ASL at 205 nm is 110 L/g cm. For the measurement of the AIL, the solid residue was washed and placed in a crucible to dry in an oven until a constant weight (W_1). The crucible containing the solid residue was then placed in a furnace at 575 ± 25 °C for 3 h whereas the heating rate was controlled at 10 °C/min to avoid flaming. After cooling down, weigh the remnant to obtain the weight of acid-insoluble ash (W_2) and the AIL can then be obtained by calculating the difference between W_1 and W_2.

The content of ash in biomass was estimated by weighing approximately 0.5–1.0 g of dried powders (W_3) and then heated in a furnace at 575 ± 25 °C for a minimum of 3 h Weighed the remnant (W_4) and the ash can then be obtained by calculating the difference between W_3 and W_4.

2.3. The acid- and alkali-pretreatments on sweet potato vines

The acid- and alkali-pretreatments were used in this study, where sulfuric acid and sodium hydroxide were employed, respectively. During which, the chemical concentration (wt. %), sample loading (wt. %), and reaction time (min), were selected to examine how these parameters effected on the pretreatment. The chemical concentrations of sulfuric acid were set at 2, 4, and 6 wt. % whereas the concentrations of sodium hydroxide were set at 0.5, 1.5, and 2.5 wt. %. The sample loadings were 6 wt. %, 9 wt. %, and 12 wt. % for both the acid- and alkali-pretreatments. The reaction times of 20, 40, 60, and 100 min were selected for both the acid- and alkali-pretreatments. The total amount of suspension, including the chemical solution and the untreated biomass, was 10 g for each pretreatment condition. All conditions tested were shown in Tables S-1 and S-2.

After the acid- or alkali-pretreatment, the suspension was transferred into a 15 mL centrifuge tube and centrifuged at 6900 × g for 5 min. Then the supernatant was subject to HPLC for the quantification of monosaccharides. The solid residue was washed with deionized water to remove the residual chemicals until the flow-through wash water is neutral and transparent. The washed solid residue was freeze-dried and weighed. The freeze-dried solid was then stored at room temperature for the following enzymatic hydrolysis.

Table 1
The composition of sweet potato vines.

Component[a]	Content in wt. %[d]
Cellulose	**17.7 ± 1.5**
Glucose[b]	19.5 ± 1.7
Hemicellulose	**4.0 ± 1.4**
Xylose[b]	4.5 ± 1.6
Arabinose	–
Galactose	–
Mannose	–
Lignin	**17.2 ± 1.0**
Acid soluble lignin	5.7 ± 0.5
Acid insoluble lignin	11.5 ± 0.9
Ash	**1.5 ± 0.3**
Water	**8.5 ± 0.5**
Others[c]	**51.2 ± 2.4**

[a] As defined in this study, cellulose consists of glucose whereas hemicellulose consists of xylose, arabinose, galactose, and mannose.
[b] Glucose and xylose contents are calculated by multiplying cellulose and xylose contents by 1.1 to take the hydration of polysaccharides into consideration.
[c] Unquantified components in biomass which are extractives, proteins, fats, and unknowns.
[d] The errors represent standard deviations with $n = 3$.

2.4. Enzymatic hydrolysis of acid- and alkali-pretreated sweet potato vines

Novozymes Cellic® CTec2 was utilized for the enzymatic hydrolysis of acid- or alkali-pretreated biomass 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). 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, where 20, 40, and 60 FPU/g-substrate of enzyme was used. The reaction mixture was incubated at 50 °C for 96 h with a gentle shake. The monosaccharide concentration in the supernatant was determined by HPLC.

2.5. The calculation of the monosaccharide yield

All glucose or xylose yields for each process was calculated with respect to the glucose or xylose contents of sweet potato vines as shown in Table 1.

Please cite this article as: C.-R. He et al., The utilization of sweet potato vines as carbon sources for fermenting bio-butanol, Journal of the Taiwan Institute of Chemical Engineers (2017), http://dx.doi.org/10.1016/j.jtice.2017.02.022