Maejo Int. J. Sci. Technol. **2019**, *13*(02), 110-120

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Enzymatic hydrolysis of small-flowered nutsedge (*Cyperus difformis)* with alkaline pretreatment for bioethanol production

Rameshprabu Ramaraj [1,*] and Yuwalee Unpaprom [2]

[1] School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
[2] Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand
* Corresponding author, e-mail: rameshprabu@mju.ac.th ; rrameshprabu@gmail.com

Received: 2 August 2018 / Accepted: 23 May 2019 / Published: 30 May 2019

Abstract: The conversion of lignocellulosic biomass into a clean-burning fuel has recently drawn attention due to its advantages in terms of availability and non-competition with the food source. Small-flowered nutsedge (*Cyperus difformis*) is a well-known aquatic weed in the rice fields. Thus, this study aims to produce bioethanol from *C. difformis* through alkaline pretreatment. The *C. difformis* was pretreated with 1% NaOH and 1% H_2O_2 at different conditions. The hydrolysis process was carried out at 50°C for 24-72 hr with cellulase enzyme. The hydrolysate with the highest total sugar were inoculated with *Saccharomyces cerevisiae* and incubated for 9 days at 33°C. Total sugar and reducing sugars were also monitored simultaneously with ethanol production. The highest ethanol concentration (12.36 g/L) was observed on the 5th day of fermentation and declined afterwards. The study indicates that small-flowered nutsedge can be utilised as a promising feedstock for bioethanol production.

Keywords: *Cyperus difformis*, small-flowered nutsedge, alkaline pretreatment, immobilised yeast, bioethanol

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INTRODUCTION

Bioethanol is a good substitute for gasoline due to its renewable nature, high octane number and high energy content (Table 1). It can be produced from different kinds of biomass such as sugar-based and starch-based crops, lignocellulosic biomass and algal biomass [1]. One of the promising substrates for the production of bioethanol is lignocellulosic biomass due to its availability and short-cycle production from plants. Lignocellulose consists of cellulose, hemicellulose and lignin. Lignin is a large complex polymer of phenolic monomers, i.e. coniferyl alcohol, coumaryl alcohol and sinapyl alcohol. These aromatic alcohols are linked together and

function as structural support which provides resistance against microbial attack. Hemicellulose is composed mainly of pentose sugars such as ribose and arabinose. Compared with cellulose, hemicellulose has a shorter chain and lower molecular weight, which makes it easily break down by enzyme [2]. Moreover, cellulose is the most abundant polysaccharide, which is composed of β–D-glucose and the degree of polymerisation (DP) of cellulose plays an important role in the hydrolysis along with its crystallinity. A shorter cellulose chain (with lower DP) is more susceptible to hydrolysis than a longer one [3].

Table 1. Energy content and octane number of some fuels in comparison with ethanol [4]

Fuel type	MJ*/L	MJ/kg	RON**
Dry wood (20% moisture)		~19.5	
Methanol	17.9	19.9	108.7
Ethanol	21.2	26.8	108.6
E85 (85% ethanol, 15% gasoline)	25.2	33.2	105
Liquefied natural gas	25.3	~55	-
Autogas (LPG***) (60% propane, 40% butane)	26.8	50	-
Aviation gasoline (high-octane gasoline)	33.5	46.8	100/130 (lean/rich)
Gasohol (90% gasoline, 10% ethanol)	33.7	47.1	93/94
Regular gasoline/petrol	34.8	44.4	Min. 91
Premium gasoline/petrol	-	-	Max. 104
Diesel	38.6	45.4	25

*MJ = megajoule; **RON = research octane number; ***LPG = liquefied petroleum gas

The conversion process of lignocellulosic biomass into ethanol typically consists of four typical steps: pretreatment, saccharification, fermentation and purification. One of the challenges in using lignocellulosic biomass is the pretreatment process to access and break the cellulose chain into fermentable sugars with enzymes and microorganisms. The main purpose of the pretreatment is to remove lignin and disturb the cellulose structure in order to facilitate the enzymatic activity. The optimal conditions for pretreatment are mostly dependent on the type of biomass [5]. Many pretreatment methods have been proposed and developed to increase the sugar yield and consolidate the production of bioethanol from the lignocellulosic biomass. Currently, pretreatment methods can be grouped into physical, chemical and biological methods or a combination of these [1]. Physical pretreatment is used not only to increase accessible surface via size production process, e.g. milling, chipping and grinding, but also to change the biomass structure by treatment with high temperature and pressure, e.g. by microwave and pyrolysis [6]. However, these processes consume a high amount of energy and require a high cost of investment and maintenance.

On the other hand, chemical pretreatment includes the use of acids or bases to eliminate lignin and hemicellulose, decrease DP, and break the crystalline structure of cellulose into an amorphous shape which provides access to enzyme and microorganism [7]. Some of the regular acids and bases are sulfuric acid, sodium hydroxide, potassium hydroxide and calcium hydroxide

(lime). Even though the processes are effective in increasing sugar yield, the major disadvantages of diluted/concentrated acid methods are the production of some possible inhibitors such as 5-hydroxymethylfurfural and 2-furfuraldehyde, the necessity of neutralisation, corrosion of equipment and long retention time [7, 8]. Among those methods, alkaline pretreatment of lignocellulosic materials can increase the internal surface area, remove lignin layer, disturb crystalline structures, and decrease the DP without producing harmful by-products and corrosion of equipment [5]. Treatment with sodium hydroxide and hydrogen peroxide is considered as an environment-friendly and effective method for various types of lignocellulosic biomass including water hyacinth and wheat straw [9, 10].

Small-flowered nutsedge (*Cyperus difformis*) (Figure 1) is listed in the Holm's list of the world's worst weeds [11]. It is distributed worldwide and grows in several parts of Thailand [12]. It is an invasive plant which grows on wetlands and considered as a problematic weed in rice fields [13]. Mongkolchaiarunya et al. [14] reported a preliminary study of ethanol production from this plant pretreated by steam explosion at 198ºC and with different nitrogen sources. An optimisation of the process is further investigated in our study, which focuses on the bioconversion of the plant into ethanol using alkaline pretreatment and saccharification process.

Figure 1. Aerial photograph of small-flowered nutsedge

MATERIALS AND METHODS

Plant Collection and Physicochemical Analysis

Small-flowered nutsedge growing in the rice fields was obtained from Ban Cho Lae, Chiang Mai, Thailand (19° 8' 32" N; 99° 0' 51" E) during September – October 2016. Fresh whole plant samples were washed and air dried at ambient temperature (38 – 40°C) for three days. They were then continually dried at 50°C in an air oven overnight. Finally, they were ground to a powder that could pass through a 1-mm mesh by a high-speed blender and stored in a desiccator. The moisture content, volatile matter, ash content and fixed solid were determined [10, 15], and the data were

recorded as means of triplicate. The sample composition (cellulose, hemicellulose and lignin) were determined by the methods of van Soest et al. [16].

Pretreatment and Sugar Analysis

The pretreatment method was adopted and modified from Mishima et al. [17]. The powdered sample (5 g) was soaked with 1% sodium hydroxide (100 mL) for a specified time period and at a specified temperature. After that 1% hydrogen peroxide (100 mL) was added and the mixture was left for another specified time period at the same temperature (Table 2). The supernatant was obtained by filtering through one layer of cotton cloth in order to eliminate large particles. The filtrate was then examined for total sugar and reducing sugar by phenol-sulfuric acid procedure [18] and dinitrosalicylic acid (DNS) method [19]. Standard curves were made using D-Glucose (Merck, USA). The filtrate with the highest total sugar concentration was selected for further hydrolysis and fermentation. Sugar and acetic acid concentrations of the liquid phase from the pretreatment were analysed by high-performance liquid chromatography (HPLC, Shimadzu, Japan) (condition: mobile phase=5 mM H_2SO_4; flow rate=0.7 mL/min.; temperature of column = 60°C; column = Hi-Plex H).

Table 2. Pretreatment conditions

Treatment	Chemical	Temperature	Time (hr)
Control (untreated)	H_2O	Ambient	24
T1	1% NaOH	Ambient	24
T2	1% NaOH	Ambient	48
T3	1% NaOH, 1% H_2O_2	99°C*	1.5+1.5
T4	1% NaOH, 1% H_2O_2	Ambient	12+12
T5	1% NaOH, 1% H_2O_2	Ambient	24+24
T6	1% NaOH, 1% H_2O_2	Ambient	48+48

*Boiling in water bath

Morphological and Structural Changes

The morphology of small-flowered nutsedge before and after pretreatment was studied using a scanning electron microscope (SEM) (JSM-5410LV, JEOL Ltd., Tokyo, Japan). Powdered biomass and residue after pretreatment were coated in pure gold to create conductivity properties and dried by a dryer (CPO 7501 Critical Point Dryer, USA) at 15 mA for 150 sec. Both gold and sample were then attached inside the specimen chamber and shot by an electron beam at 15000 kV. The secondary electron detector caught the signal and presented an enlarged image of the sample surface on the monitor screen.

Saccharification by Cellulase

Enzymatic saccharification process was carried out with commercial cellulase (2398 units/g, beta-glucosidase 577 units/g, pH4) (Sigma-Aldrich, USA). The pretreated supernatant (200 mL) from the pretreatment step was adjusted to pH 5.0 by addition of hydrochloric acid and 2% (v/v) of cellulase was added. The reaction was performed in a shaking water bath at 50°C and 150 rpm for 24, 48 and 72 hr (separately) and sugar analysis was carried out.

Immobilised Yeast Preparation

Saccharomyces cerevisiae TISTR 5020 was obtained from the Faculty of Science, Maejo University, Chiang Mai, Thailand. This yeast was cultivated in liquid YPD medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} dextrose) at 33°C and 150 rpm for 24 hr. Then the broth culture was centrifuged at 7000 rpm, 4°C for 10 min. The same volume of 2% sodium alginate was added to the yeast cell pellet and mixed properly. Immobilised yeast cells were then washed with sterile distilled water and stored at 4°C for further use.

Fermentation and Ethanol Yield Determination

After hydrolysis process, the hydrolysate solution was fermented with 2% (w/v) of immobilised yeast (*S. cerevisiae*) beads in a fermenter (200-mL working volume), and incubated at 33°C for 9 days in an incubator (Gallenkamp, Germany). Aliquots of fermented samples (50 mL) were taken after 3, 5, 7 and 9 days to determine the percentage of ethanol with an ebulliometer (Dujardin-Salleron, France). The sample solution was centrifuged to separate suspended solids and the ethanol concentration and temperature were measured. The percentage of ethanol was also determined using a calculating disk with comparison at two temperatures. Total and reducing sugars were also determined.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA), IBM SPSS statistical package version 22.0 (IBM Corp., USA). Differences between means were paralleled by LSD and Tukey's B. Statistical significances were reached when $p < 0.05$).

RESULTS AND DISCUSSION

Characteristics of Small-flowered Nutsedge

In this study the percentage of moisture of *C. difformis* was quite low, i.e. 5.61 ± 0.22%, similar to the moisture content of dry rice straw (5.65%) [20]. Moisture affects the storage condition, handling, feeding facilities and conversion processes of biomass [21, 22]. High moisture content may lead to fungal contamination and give an adverse effect in the overall process. Other physical parameters are shown in Table 3. Cellulose and hemicellulose content is high while lignin content is quite low compared with those of water hyacinth, which contains 8.3% cellulose, 23.3% hemicellulose and 17.7% lignin [23]. On the other hand, rice straw contains a high 44.3% cellulose, 33.5% hemicellulose and 20.4% lignin [24].

Pretreatment of Small-flowered Nutsedge

Pretreatment plays a crucial step in disrupting the crystalline structure of cellulose and removing lignin for the production of bioethanol from lignocellulosic biomass [25]. Pretreated biomass is more accessible to enzymes or microorganisms than untreated biomass [26]. The alkaline pretreatment with NaOH and H_2O_2 has been proven to be an efficient method for removal of the lignin layer and interrupting the regular shape of cellulose [5, 9, 10, 27]. The total sugar (bound and free) and free reducing sugar after different pretreatments are presented in Table 4. The

Table 3. Proximate analysis and composition of small-flowered nutsedge

Constituent	Weight %
Total solid	94.39 ± 0.22
Moisture	5.61 ± 0.22
Fixed carbon	2.72 ± 0.05
Volatile matter	82.42 ± 0.17
Ash	9.25 ± 0.09
Cellulose	22.05 ± 0.11
Hemicellulose	30.20 ± 1.06
Lignin	2.78 ± 0.09

highest total available sugar of 0.199 g/g and 0.194 g/g dry biomass is achieved by treating with NaOH and H_2O_2 at 99°C for 3 hr and at room temperature for 96 hr respectively. The results of the two treatments are not significantly different. In this study the latter treatment was selected for preparing the biomass for the hydrolysis and fermentation steps since it gave the highest total sugar concentration needed for the production of bioethanol at room temperature.

The amounts of sugars after pretreatment with 1% NaOH and 1% H_2O_2 are illustrated in Figure 2. The main compounds in the liquid phase are inulin (14.3 mg/g) and hexose sugars, i.e. fructose (12.1 mg/g) and glucose (4.7 mg/g). Other sugars per g of dry biomass are maltotriose (2.4 mg), cellobiose (1.1 mg), xylose (2.02 mg) and arabinose (1.2 mg).

Table 4. Total available sugar (bound and free) and free reducing sugar of small-flowered nutsedge after different pretreatments

Reagent	Temp. (°C)	Time (hr)	Total sugar (g/g dry biomass)	Reducing sugar (g/g dry biomass)
Control (untreated)	Ambient	24	0.104 ± 0.002^{a}	0.020 ± 0.003^{a}
1%NaOH	Ambient	24	0.162 ± 0.004^{c}	0.036 ± 0.003^{a}
1%NaOH	Ambient	48	0.134 ± 0.007^{b}	0.037 ± 0.002^{a}
1%NaOH, 1%H_2O_2	99°C	1.5	0.199 ± 0.007^{d}	0.022 ± 0.002^{a}
1%NaOH, 1%H_2O_2	Ambient	12+12	0.157 ± 0.016^{c}	0.033 ± 0.008^{a}
1%NaOH, 1%H_2O_2	Ambient	24+24	0.168 ± 0.001^{c}	0.022 ± 0.002^{a}
1%NaOH, 1%H_2O_2	Ambient	48+48	0.194 ± 0.003^{d}	0.024 ± 0.002^{a}

Note: Standard deviation is less than 10%. Means with the same alphabetic label in the same column are not significantly different ($p < 0.05$). The test is based on Tukey B' test at 95% confidence interval.

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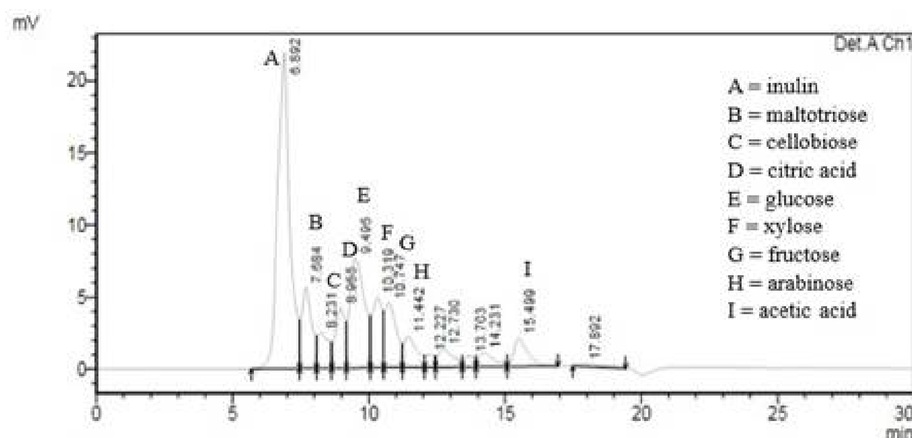

Figure 2. High-performance liquid chromatogram of mono-sugars released after pretreatment with $NaOH/H_2O_2$

Acetic acid, which is a result of degradation of acetyl groups of hemicellulose under pretreatment condition, was also found at 0.5 g/L. Acetic acid is an inhibitor that can disturb the growth of yeast in the fermenter [28, 29]. However, the acetic acid level in this study did not affect the hydrolysis and fermentation process since the metabolism of *S. cerevisiae* is affected only when the acetic acid concentration exceeds 2.0 g/L [7]. Some inhibitors for example furfural and hydroxymethylfurfural, at a lower concentration may not at all have any impact on those processes due to pretreatment with alkali [7, 29].

Morphological Changes

The morphological features of raw and pretreated small-flowered nutsedge biomass samples are shown in Figure 3. Raw biomass shows a regular and compact surface structure with fibres arranged in bundles, which impedes access by cellulose [30, 31]. Also, the surface of the pretreated biomass shows bundles of fibres clearly but the untreated biomass has no visible microfibre. Most of the soluble fraction of the biomass enters the liquid phase during the pretreatment process and the microfibre is left behind.

Hydrolysis/Saccharification

Enzyme saccharification is the next essential step required for the conversion of biomass into bioethanol. The primary goal of this process is to hydrolyse polysaccharides to fermentable sugars, notably glucose and fructose. Cellulase, a group of enzymes including glucosidase, was used to break polysaccharides released from the pretreatment process into fermentable sugars. Table 5 shows amounts of total sugar and reducing sugar from the hydrolysis process at different times. The means of total sugar after 24, 48 and 72 hr are not significantly different.

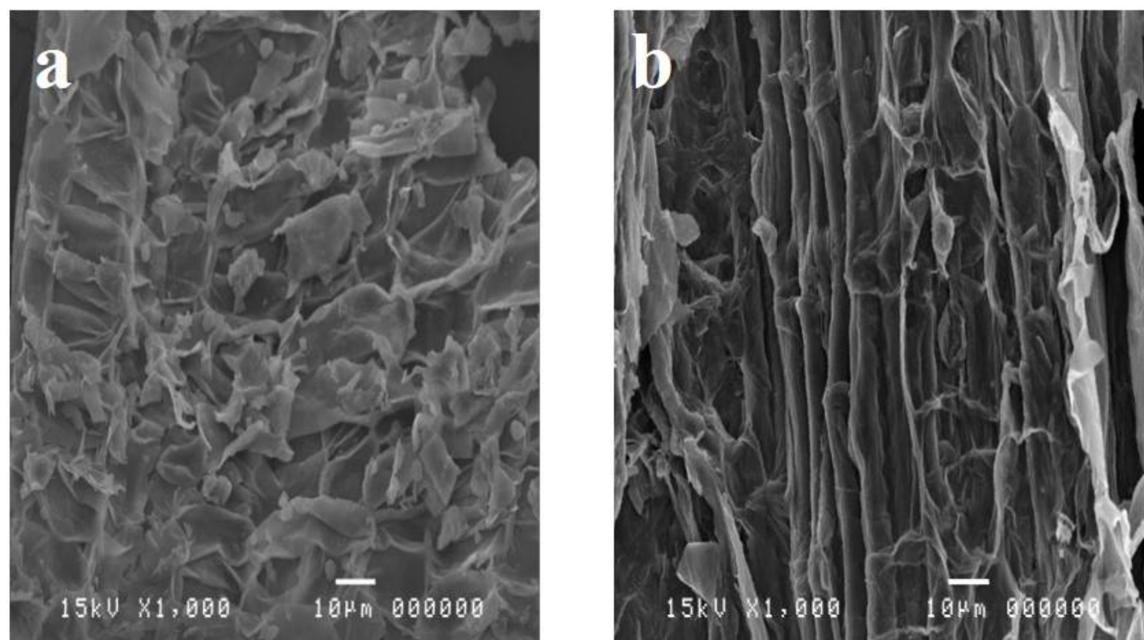

Figure 3. SEM images of small-flowered nutsedge biomass before (a) and after pretreatment by NaOH (1%) and H_2O_2 (1%) at ambient temperature for 96 hr (b)

Table 5. Total sugar and reducing sugar from saccharification process

Sugar	Hydrolysis yield (g/g dry biomass)			
	0 hr	**24 hr**	**48 hr**	**72 hr**
Total sugar	0.199 ± 0.003^{a}	0.196 ± 0.006^{ab}	0.188 ± 0.003^{b}	0.192 ± 0.004^{ab}
Reducing sugar	0.020 ± 0.000^{a}	0.094 ± 0.001^{b}	0.079 ± 0.000^{c}	0.089 ± 0.002^{d}

Note: Standard deviation is less than 10%. Means with the same letter in the same row are not significantly different ($p < 0.05$). The test was based on Tukey's B test at the 95% confident interval.

On the other hand, the means of reducing sugar are significantly different at different times. After 24 hr, the amount of reducing sugar is highest at 0.094 g/g biomass. The results also indicate that nearly 50% of the total sugar is not degraded to fermentable sugars, which might be due to some types of polysaccharides that are not completely degraded by enzymes. Thus, there is a need for further study to optimise the hydrolysis by using a co-enzyme which can break all the available polysaccharide chains to fermentable sugars.

Ethanol Production

Fermentation time is one of the most significant parameters in the production of bioethanol as it determines the viability of fermentative microorganisms employed as well as the economical part of the whole production process. Ethanol production results are presented in Figure 4. The maximum ethanol yield reaches 12.36 g/L on the 5th day of fermentation and declines rapidly afterwards. The same trend was observed and reported [33]. Senac and Hahn-Hägerdal [34] reported that different types of free reducing sugars have different optimum fermentation time due to distinct metabolic regulation for each of them. As for comparison, glucose consumption by

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S. cerevisiae was faster than other types of sugars. The ethanol yield from small-flowered nutsedge was apparently higher than that obtained from water hyacinth at 9.6 ± 1.1 g/L [35, 36].

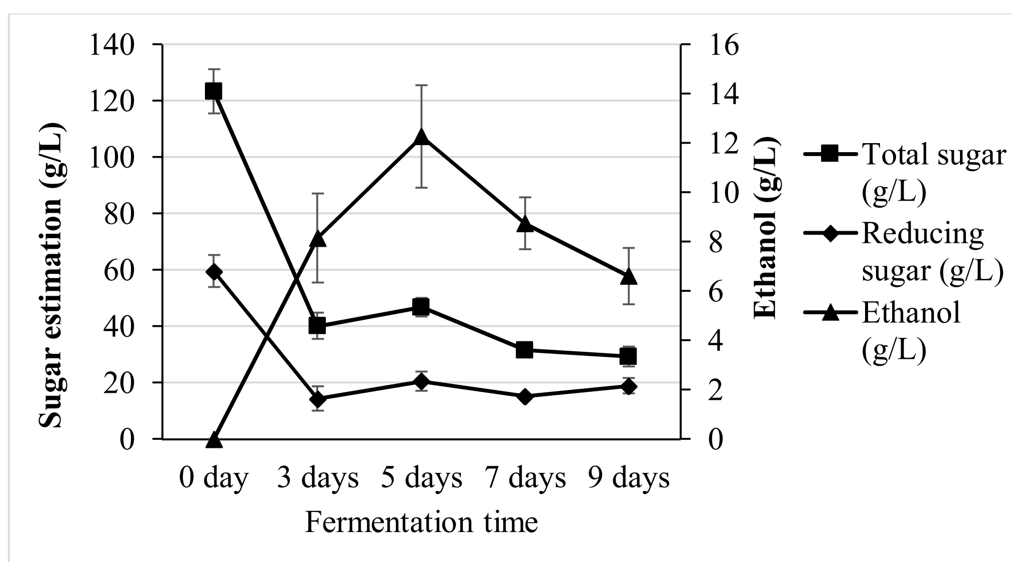

Figure 4. Ethanol, total sugar and reducing sugar concentrations during fermentation

CONCLUSIONS

Small-flowered nutsedge, a weed freely available from the rice field, can be used as a raw material for bioethanol production. Alkaline pretreatment, which is a low cost process, can be applied on this renewable feedstock. The highest ethanol yield was obtained at 12.36 g/L after 5 days of fermentation with immobilised yeast (*S. cerevisiae* TISTR) 5020.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the School of Renewable Energy, Energy Research Center and Program in Biotechnology Laboratory (Faculty of Science). Also, special thanks are expressed to Ms. Phuong Thi Vu for substrate and chemical preparation, and to Associate Professor Dr. Niwooti Wangchai for his valuable suggestion and equipment given to accomplish this study.

REFERENCES

1. S. S. S. Abdullah, Y. Shirai, A. A. M. Ali, M. Mustapha and M. A. Hassan, "Case study: Preliminary assessment of integrated palm biomass biorefinery for bioethanol production utilizing non-food sugars from oil palm frond petiole", *Energy Convers. Manage.,* **2016**, *108*, 233-242.
2. W. H. Chen, Y. S. Chu and W. J. Lee, "Influence of bio-solution pretreatment on the structure, reactivity and torrefaction of bamboo", *Energy Convers. Manage.,* **2017**, *141*, 244-253.
3. Y. H. Zhang and L. R. Lynd, "Determination of the number-average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis", *Biomacromolec.*, **2005**, *6*, 1510-1515.
4. S. H. Ho, S. W. Huang, C. Y. Chen, T. Hasunuma, A. Kondo and J. S. Chang, "Bioethanol production using carbohydrate-rich microalgae biomass as feedstock", *Bioresour. Technol.*, **2013**, *135*, 191-198.

5. D. M. de Carvalho, O. Sevastyanova, J. H. de Queiroz and J. L. Colodette, "Cold alkaline extraction as a pretreatment for bioethanol production from eucalyptus, sugarcane bagasse and sugarcane straw", *Energy Convers. Manage.*, **2016**, *124*, 315-324.
6. M. A. Ahmed, M. S. U. Rehman, R. Terán-Hilares, S. Khalid and J. I. Han, "Optimization of twin gear-based pretreatment of rice straw for bioethanol production", *Energy Convers. Manage.,* **2017**, *141*, 120-125.
7. A. Iwaki, T. Kawai, Y. Yamamoto and S. Izawa, "Biomass conversion inhibitors furfural and 5-hydroxymethylfurfural induce formation of messenger RNP granules and attenuate translation activity in *Saccharomyces cerevisiae*", *Appl. Environ. Microbiol.*, **2013**, *79*, 1661-1667.
8. M. F. Li, Y. M. Fan, F. Xu, R. C. Sun and X. L. Zhang, "Cold sodium hydroxide/urea based pretreatment of bamboo for bioethanol production: Characterization of the cellulose rich fraction", *Ind. Crops Prod.*, **2010**, *32*, 551-559.
9. P. Moodley and E. B. G. Kana, "Development of a steam or microwave-assisted sequential salt-alkali pretreatment for lignocellulosic waste: Effect on delignification and enzymatic hydrolysis", *Energy Convers. Manage.,* **2017**, *148*, 801-808.
10. D. Mishima, M. Kuniki, K. Sei, S. Soda, M. Ike and M. Fujita, "Ethanol production from candidate energy crops: Water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes* L.)", *Bioresour. Technol.*, **2008**, *99*, 2495-2500.
11. L. Holm, "Weeds problems in developing countries", *Weed Sci.*, **1969,** *17*, 113-118.
12. CABI, "*Cyperus difformis* (small-flowered nutsedge)", **2015**, www.cabi.org/isc (Accessed: June 2016).
13. A. K. Gupta, "*Cyperus difformis*", **2011**, http://www.iucnredlist.org/details/164294/0 (Accessed: June 2016).
14. S. Mongkolchaiarunya, P. Vaithanomsat and S. Chuntranuluck, "Effect of nitrogen source on ethanol production from weeds by a simultaneous saccharification and fermentation process", *KKU Res. J.,* **2016**, *21*, 210-213.
15. A. Sluitor, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter and D. Templeton, "Determination of ash in biomass", Technical Report, **2008**, National Renewable Energy Laboratory, Colorado, USA.
16. P. J. van Soest, J. B. Robertson and B. A. Lewis, "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition", *J. Dairy Sci.*, **1991**, *74*, 3583-3597.
17. D. Mishima, M. Tateda, M. Ike and M. Fujita, "Comparative study on chemical pretreatments to accelerate enzymatic hydrolysis of aquatic macrophyte biomass used in water purification processes", *Bioresour. Technol.*, **2006**, *97*, 2166-2172.
18. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, "Colorimetric method for determination of sugars and related substances", *Anal. Chem.*, **1956**, *28*, 350-356.
19. G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar", *Anal. Chem.*, **1959**, *31*, 426-428.
20. N. Akhtar, D. Goyal and A. Goyal, "Characterization of microwave-alkali-acid pre-treated rice straw for optimization of ethanol production via simultaneous saccharification and fermentation (SSF)", *Energy Convers. Manage.,* **2017**, *141*, 133-144.
21. A. A. Rentizelas, A. J. Tolis and I. P. Tatsiopoulos, "Logistics issues of biomass: The storage problem and the multi-biomass supply chain", *Renew. Sust. Energy Rev.,* **2009**, *13*, 887-894.

22. J. Cai, Y. He, X. Yu, S. W. Banks, Y. Yang, X. Zhang, Y. Yu, R. Liu and A. V. Bridgwater, "Review of physicochemical properties and analytical characterization of lignocellulosic biomass", *Renew. Sust. Energy Rev.,* **2017**, *76*, 309-322.
23. J. Cheng, A. Xia, H. Su, W. Song, J. Zhou and K. Cen, "Promotion of H_2 production by microwave-assisted treatment of water hyacinth with dilute H_2SO_4 through combined dark fermentation and photofermentation", *Energy Convers. Manage.,* **2013**, *73*, 329-334.
24. X. L. Chen, J. Yu, Z. B. Zhang and C. H. Lu, "Study on structure and thermal stability properties of cellulose fibers from rice straw", *Carbohydr. Polym*., **2011**, *85*, 245-250.
25. K. Özdenkçi, C. de Blasio, H. R. Muddassar, K. Melin, P. Oinas, J. Koskinen, G. Sarwar and M. Järvinen, "A novel biorefinery integration concept for lignocellulosic biomass", *Energy Convers. Manage.,* **2017**, *149*, 974-987.
26. M. Balat, "Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review", *Energy Convers. Manage.,* **2011**, *52*, 858-875.
27. K. K. Cheng, J. A. Zhang, W. X. Ping, J. P. Ge, Y. J. Zhou, H. Z. Ling and J. M. Xu, "Sugarcane bagasse mild alkaline/oxidative pretreatment for ethanol production by alkaline recycle process", *Appl. Biochem. Biotechnol.*, **2008**, *151*, 43-50.
28. H. B. Klinke, A. B. Thomsen and B. K. Ahring, "Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass", *Appl. Microbiol. Biotechnol.*, **2004**, *66*, 10-26.
29. M. F. Li, P. Yu, S. X. Li, X. F. Wu, X. Xiao and J. Bian, "Sequential two-step fractionation of lignocellulose with formic acid organosolv followed by alkaline hydrogen peroxide under mild conditions to prepare easily saccharified cellulose and value-added lignin", *Energy Convers. Manage.,* **2017**, *148*, 1426-1437.
30. H. Amiri and K. Karimi, "Improvement of acetone, butanol, and ethanol production from woody biomass using organosolv pretreatment", *Bioprocess Biosyst. Eng.*, **2015**, *38*, 1959-1972.
31. J. Shadbahr, F. Khan and Y. Zhang, "Kinetic modeling and dynamic analysis of simultaneous saccharification and fermentation of cellulose to bioethanol", *Energy Convers. Manage.,* **2017**, *141*, 236-243.
32. J. J. Liu and X. J. Cao, "Biodegradation of cellulose by beta-glucosidase and cellulase immobilized on a pH-responsive copolymer", *Biotechnol. Bioprocess Eng.,* **2014**, *19*, 829-837.
33. S. Fan, Z. Xiao, X. Tang, C. Chen, Y. Zhang, Q. Deng, P. Yao and W. Li, "Inhibition effect of secondary metabolites accumulated in a pervaporation membrane bioreactor on ethanol fermentation of *Saccharomyces cerevisiae*", *Bioresour. Technol.*, **2014**, *162*, 8-13.
34. T. Senac and B. Hahn-Hagerdal, "Intermediary metabolite concentrations in xylulose- and glucose-fermenting *Saccharomyces cerevisiae* cells", *Appl. Environ. Microbiol*., **1990**, *56*, 120-126.
35. T. Takagi, M. Uchida, R. Matsushima, M. Ishida and N. Urano, "Efficient bioethanol production from water hyacinth *Eichhornia crassipes* by both preparation of the saccharified solution and selection of fermenting yeasts", *Fish. Sci.*, **2012**, *78*, 905-910.
36. M. X. He, Q. Hu, Q. Zhu, K. Pan and Q. Li, "The feasibility of using constructed wetlands plants to produce bioethanol", *Environ. Prog. Sustain. Energy*, **2015**, *34*, 276-281.

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