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Influence of Pretreatments on Sustainability of Bioethanol Production from Napier Grass

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Abstract: The influences of pretreatments and acid hydrolysis conditions in bioethanol production from Napier grass were investigated. The alkaline and acid pretreatments removed 6.63 % and 5.71 % of lignin more than the untreated grass. The recommended acid concentration in hydrolysis was 35% sulphuric acid for duration of 20 to 40 min. Fermentation of samples from acid and alkaline pretreatments for 48 h produced the highest ethanol concentration of 13 %v/v. However, when the sustainability was considered, these chemical pretreatments used chemical and required sample' neutralization prior to fermentation thus an additional chemical cost occurred and released more chemical to environments in comparison to steam pretreatments which produced slightly lower ethanol of 11 %v/v.

Keywords: sustainability; fermentation; steam explosion; yeast inhibitor

1. Introduction

The potential of energy shortages motivates the search for alternative energy sources. Bioethanol production from lignocellulose biomass has been extensively researched, examples of lignocellulose biomass used for bioethanol production include sweet sorghum bagasse, grasses, cotton stalk, and even agro-industrial wastes such as that from sugarcane [1-5]. Napier grass is abundant and can be found in many areas. It can be grown even at low nutrient conditions and is typically used as animal feed [6].

So far, technological performance has been the important selection criteria but recent studies have shifted interest to sustainable bioethanol production [7, 8]. R.H.W. Boyer et al. [9] described many sustainability models and each model consisted of economic, environmental and social sustainability. According to Scown et al. [8], the greenhouse gas emission scope for bioethanol life cycle analysis started from biomass production, feedstock transportation, biorefining, ethanol transportation and fuel combustion. This model is similar to system described by T. Hattori and S. Morita [10] who estimated the net energy balance of many cellulosic energy crops. Hattori and Morita [10] estimated the ethanol conversion efficiency to be 380 L t-1 of dried weight and the estimated net energy balance ratio of switchgrass to be 3.92 – 6.31. Both works from Scown et al.

[8] and T. Hattori and S. Morita [10] investigated the overall system so the technical bioethanol production data were taken from literatures. In contrast to this work, we focused on the influence of parameters on the sustainability of lignocellulosic crops.

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Napier grass was reported to contain 30.40% lignin, 36.34% cellulose, and 34.12% hemicellulose [11]. Bioethanol production of Napier grass consists of 4 necessary steps; delignification in pretreatment [12], hydrolysis, fermentation and ethanol purification [13]. B. Wen et al. [14] investigated the biological pretreatment of Napier grass by three microbial consortia. K.O. Reddy et al.

[15] studied the effects of a dilute alkali treatment (2% sodium hydroxide solution) on the chemical composition and structural characteristics of Napier grass fibers. Alkali pretreatment with a base is commonly used with other lignocellulose materials such as empty oil palm fruit bunches [16]. A. de Araújo Morandim-Giannetti et al. [11] compared delignification of Napier grass by calcium oxide and hydrogen peroxide. Apart from the chemical pretreatment, physical treatments such as steam explosion and ultrasound have also been applied in biomass pretreatments [2, 17]. However, results in the available literatures cannot be directly compared to identify the most effective pretreatment being due to different experimental parameters.

In the first stage of, this research aimed to find the optimum experimental parameters in 4 types of pretreatments; a distilled water and steam explosion pretreatment (W); an acid and steam explosion pretreatment (A); a base (alkali) and steam explosion pretreatment (B); and a control samples (C) were used as the baseline for comparisons. In the second stage, the technical and sustainability analysis based on the conditions of each pretreatment were carried out.

2. Materials and Methods

2.1 Materials and Chemicals

Napier grass (*Pennisetum purpureum*) was obtained from Sai Chai Field, Surat Thani province, Thailand. This grass is also known as elephant grass. The grass was chopped into small pieces, air-dried for 2 days, and milled to 0.5 mm size, before further drying in a hot air oven at 60 C for 48 h. All of the chemical reagents used were of analytical grade. All experiments were performed in triplicate.

2.2 Pretreatments

There were 3 pretreatments (W, A and B) and 3 replications were carried out of each procedure. Lignin content was determined by the method developed by NREL, which is described in the determination of structural carbohydrates and lignin in biomass [18]. 15 g of milled and dried Napier grass powder was soaked in 750 mL of distilled water at room temperature for 6 h. The mixture was autoclaved at 121 ℃, 15 psi for 15 min, and cooled. The mixture was vacuum filtered and the remaining solids were washed thoroughly. The grass powder was dried at 60 ℃ before storage, and was later used in the acid hydrolysis. 15 g of the milled and dried Napier grass powder was soaked in 750 mL of 2% H_2SO_4 at room temperature for 6 h. The acidic suspension was vacuum filtered and the remaining solids were washed with distilled water, then 750 mL of distilled water was added.

The water/grass suspension was autoclaved at ℃, 15 psi for 15 min, and cooled. The suspension was vacuum filtered and the remaining solids were washed thoroughly to pH 7. The grass powder was dried at 60 ℃ before storage, and was later used in the acid hydrolysis. 15 g of the milled and dried Napier grass powder was soaked in 750 mL of 2% NaOH at room temperature for 6 h. The basic (alkaline) suspension was vacuum filtered and the remaining solids were washed with distilled water, then 750 mL of distilled water was added. The suspension was autoclaved at 121 ℃, 15 psi for 15 min, and cooled. It was then vacuum filtered and the remaining solids were washed thoroughly to pH 7. The grass powder was dried at 60 ℃ before storage, and was later used in the acid hydrolysis

2.3 Acid Hydrolysis

The variables in acid hydrolysis were the sulphuric acid concentration and the hydrolysis duration. The sulphuric acid concentrations tested were 10, 20, and 35%. The hydrolysis durations were 10, 20, 40, and 60 min. Concentration of reducing sugar in hydrolysate was determined using the 3,5–dinitrosalicylic acid (DNS) method developed by G.L. Miller [19]. The highest sugar condensate from each pretreatment was neutralized to pH 5.0-5.5 and then fermented.

2.4 Fermentation

The preparation of the yeast inoculum was carried out aseptically in an Erlenmeyer flask containing 15 %w/v sucrose, 1% peptone, and 10% extract of *Saccharomyces cerevisiae* yeast, at room temperature for 48 h. The medium was stirred at 8,000 rpm for 10 min before the liquid medium was removed, and the yeast inoculum was washed thoroughly with distilled water. The obtained inoculum was stirred for another 10 min and washed again to remove the residual medium from the inoculum. The fermentation substrate after hydrolysis was fermented with the prepared inoculum of *Saccharomyces cerevisiae* (10% inoculum in the fermentation media) in an Erlenmeyer flask. The experiments were carried out at room temperature for various fermentation times (24, 48, 72, and 96 h). Aliquots were extracted after the filtration of fermentation broth and the centrifuge of filtrate at 8,000 rpm for 10 min to remove solid particles from broth. Ethanol percentages were measured by using an alcohol refractometer.

2.5 Technical and Sustainability Analysis

The technical and sustainability analysis covered the economic, environmental, social impacts [9] and technical performance of the pretreatment procedures. The bioethanol procedures giving the highest ethanol content from W, A, B and C procedures were marked as W*, A*, B* and C*, respectively. The technical and sustainability impacts of the selected W*, A*, B* and C* procedures were analyzed here. The impacts of W*, A*, B* and C* procedures were analyzed, discussed and later evaluated their suitable scores. The analysis was scaled from 1 to 5; 1 indicates strong negative impact, 2 indicates mild negative impact and 3 indicates neutral, 4 indicates mild positive impact and 5 indicates strong positive impact.

3. Results and Discussion

3.1 Lignin Removal

The measured lignin contents were compared and analyzed by using the statistical program SPSS, One-Way ANOVA (Tukey) at $\alpha\alpha = 0.05$. The control sample (C) contained 29.75% lignin, which is significantly higher than the lignin content in A, B, or W pretreated grass. The W did not significantly reduce lignin, but the cell structure was damaged, which exposed cellulose to the chemical reactions in hydrolysis and fermentation. Although the non-chemical pretreatment could not significantly remove lignin, its advantage is the absent of toxic chemical derivatives such as carboxylic acid, phenolic acid, and furan acid, which inhibit fermentation by the yeast [20].

Table 1 - Lignin contents in the control and the treated Napier grass samples

Samples	% remained lignin		
	Acid soluble	Non-acid soluble	Total
"C"	12.97 ± 0.26^{c}	16.78 ± 0.26^{c}	29.75 ± 0.52^{c}
"W"	12.44 ± 0.45^{c}	16.14 ± 0.05^{b}	28.58 ± 0.50^{b}
"A"	7.47 ± 0.33^{a}	16.57 ± 0.33^{bc}	24.04 ± 0.64^{a}
"B"	9.57 ± 0.15^{b}	13.55 ± 0.15^{a}	23.12 ± 0.30^{a}

The "A" and "B" pretreatments dissolved acid soluble lignin by 5.5% and 6.63%, respectively, more than the control "C". This dissolved lignin exposed cellulose/hemicellulose [21] to acid hydrolysis. R. Gupta and Y.Y. Lee [6] also found that sodium hydroxide pretreatment removed a great portion of the lignin in biomass. Sodium hydroxide reacted with ester functional groups of lignin chains, cellulose swelling and partially solubilized of hemicelluloses [2, 22]. Once the lignin was removed, hemicellulose and cellulose were in direct contact with sodium hydroxide, which caused them to swell and split away from the covering lignin layer [21].

3.2 Reducing Sugar Production

From Fig. 1, the maximum (denoted by *) concentrations of reducing sugar prepared from "A" and "B" pretreated hydrolysates were the top values while the "C" hydrolysate gave the minimum sugar concentration. Without pretreatment, the hemicelluloses and lignin would cover cellulose, which then cannot be hydrolyzed to sugars. The steam pretreatment gave a moderate sugar concentration of 0.8% because although steam could not dissolve hemicelluloses/lignin but steam could effectively fractionated cellulose, hemicellulose, and lignin [23] so a portion of cellulose would still be uncovered from lignin. These data showed that chemical pretreatment was necessary for bioethanol production from cellulosic biomass and the statement agreed well with work from Suthkamol Suttikul *et al.* [5] who successfully pretreated the sugarcane trash with a dilute alkaline method.

Referring to Fig. 1, the average reducing sugar concentration increased with acid concentration: 35% sulphuric acid produced the highest reducing sugar concentration. Similar conclusions were reported by E. Palmqvist and B. Hahn-Hägerdal [24] who hydrolyzed spruce wood. Hemicellulose was hydrolysed to xylose [25, 26] and the hydrogen bonds between hemicellulose and cellulose were destroyed, so the cellulose chains became free to react with sulphuric acid. Sulphuric acid broke chemical bonds in the cellulose chains and produced short-chained molecules. These C5 and C6 short chains were reducing sugars, such as glucose [25], and can be fermented by *S.cerevisiae* to ethanol [27].

From Fig. 1, the optimum time were observed to be 20 and 40 min depending on hydrolysate's pretreatment and acid concentrations. When the hydrolysis period was as short as 10 min, the reaction did not reach its full extent. In contrast, if the grass were hydrolyzed for too long (i.e. 60 min), the acid reacted with the produced reducing sugar and undesirably created yeast inhibiting derivatives such as furfural. The adverse effects of excessive hydrolysis period are also reported by E. Takata *et al.* [28]. They hydrolyzed Napier grass with phosphoric acid and when hydrolysis period was changed from 4 min to 8 min, they observed increased furfural and decreased xylose. In conclusion, acid hydrolysis for 20-40 min was recommended in our study.

3.3 Ethanol Production

The W*, A*, B* and C* solutions were then fermented by *Saccharomyces cerevisiae*. The measured ethanol concentrations at select fermentation durations are reported in the following table.

Table 2 - The measured ethanol concentrations during fermentation

Samples	Ethanol contents (% v/v) after			
	24 h	48 h	72 h	96 h
"C*"	8.1 ± 0.8^{a}	11.1 ± 0.1^{a}	10.7 ± 0.1^{a}	10.0 ± 0.2^{a}
"W*"	11.4 ± 0.2^{bc}	13.0 ± 0.3^{b}	12.2 ± 0.1^{b}	12.0 ± 0.1^{b}
"A*"	10.6 ± 0.2^{b}	13.2 ± 0.1^{b}	12.8 ± 0.1^{c}	12.4 ± 0.1^{c}
"B*"	12.0 ± 0.0^{c}	13.4 ± 0.2^{b}	12.8 ± 0.1^{c}	12.4 ± 0.1^{c}

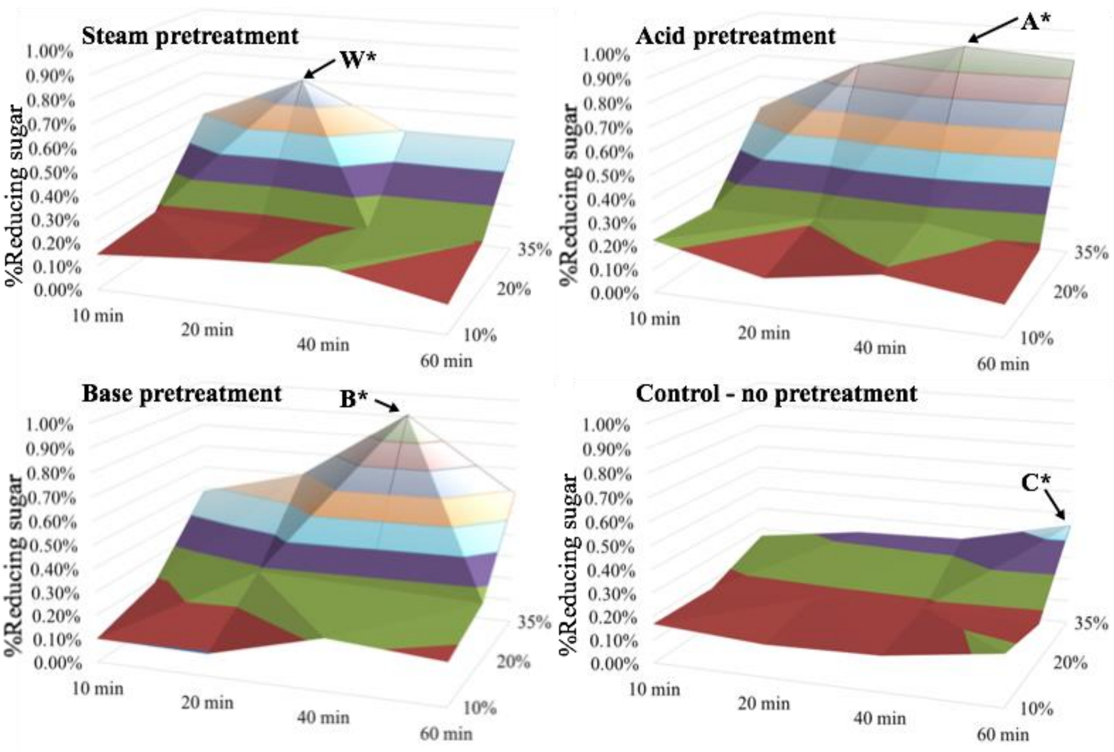

Fig. 1 - Concentration of reducing sugar

The ethanol contents were analyzed by using the statistical program SPSS, One-Way ANOVA (Tukey) at $\alpha\alpha = 0.05$. The ethanol concentration in all broths initially increased, reached its maximum in 48 hours, and then declined towards the end of fermentation. At 48 h, ethanol concentrations from "B" and "A" were similar with values of 13.4 and 13.2 %v/v. These samples had similar lignin content, so it was reasonable to produce the similar ethanol concentrations. However, "A" gave slightly lower ethanol concentration than "B" being due to the yeast inhibitors created during "A" pretreatment.

3.4 Technical and Sustainability Evaluations

The technical evaluation based on the bioethanol production performance of the procedure W*, A*, B* and C*. The procedures W*, A* and B* gave the highest bioethanol contents of approximately 13 %v/v so their scores were 5. The procedure C* produced a lower content (11.1 %v/v) so it received score of 4. In economic aspect; C* involved no pretreatment cost thus its economic score was 5 and W* used water and electricity to autoclave solution thus its economic score was 4. The pretreatment A* and B* used electricity during autoclave, chemicals in pretreatment and chemical in neutralization steps so they received the economic scores as 2. The social impacts of all procedures were determined to be neutral because the bioethanol production from Napier grass could increase product value from agricultural crops such as Napier grass and could be beneficial to farmers. The social scores of 3 were determined to be appropriate for all procedures. Lastly, the environmental scores of all procedures were low being due to the hydrolyzation of grass with concentrated acid and due to the chemical neutralization of processing solutions before discharging it to environment. The non- chemical pretreatments (C* and W*) received environmental score of 2 while the chemical pretreatments (A* and B*) received environmental score of 1 due to the use of chemical in pretreatment processes. In overall, the chemical treatments (A* and B*) gave the highest technical performance but the procedures had negative impacts on economic and environmental aspects. Replacing of chemical pretreatment with steam pretreatment reduced the potential ethanol content from 13 %v/v to 11 %v/v but effectively improved the economic and environmental impacts.

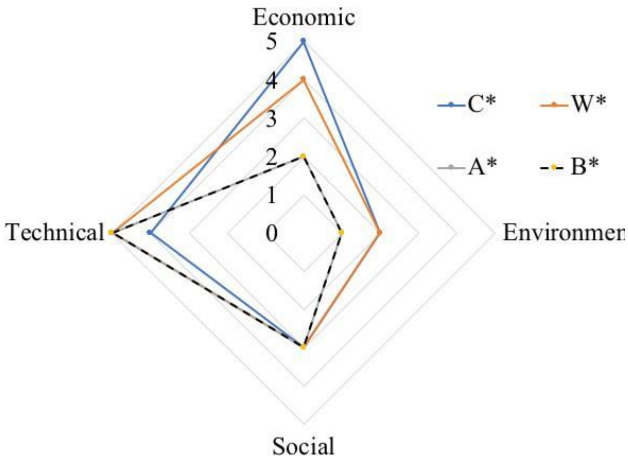

Fig. 2 - Sustainability evaluation of bioethanol productions from selected pretreatments

4. Conclusions

The acid (A) and alkaline (B) pretreatments satisfactorily dissolve lignin from Napier grass by 5.71% and 6.63%, respectively. The distilled water and steam explosion pretreatment (W) damaged the cell walls and exposed cellulose/hemicellulose to acid hydrolysis. The optimum hydrolysis conditions used 35% sulphuric acid for 20 to 40 min. Fermentation with *Saccharomyces cerevisiae* for 48 h was suitable for all selected pretreatments. The Acid and alkaline pretreated samples gave approximately 13 %v/v. The combined technical and sustainability evaluation shows that the chemical pretreatments had negative impacts on environmental and economic aspects. The steam pretreatment had slightly lower technical performance but gave better influence on environmental and economic aspects.

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