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Optimisation of Acid Hydrolysis of Grasses using Response Surface Methodology for the Preparation of Bioethanol

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The grasses–Chloris barbata Sw. and Ischaemum pilosum Klein ex Willd were chosen as sources of lignocellulosic material for the preparation of ethanol. Fresh stems of grass were processed into fermentable sugars by acid hydrolysis using sulfuric acid. Optimisation of cellulose hydrolysis was performed by using Central Composite design of response surface methodology (RSM). Three variables such as acid concentration, acid volume and hydrolysing time were considered as influencing factors on the yield of fermentable sugars during acid hydrolysis. Baker's yeast (Saccharomyces cerevisiae) was used in fermentation of the resulting sugars under anaerobic condition. The maximum yields of ethanol by volume were 24.88 ± 0.20 % and 6.01 ± 3.20 % with the yeast concentrations of 5 g/L and 4 g/L accordingly to their same reflux ratio of 1.01.

1. Introduction

Non-food plants of cellulosic materials become renewable feedstock for the production of ethanol. Cellulose in cellulosic biomass is usually organised into microfibrils, containing up to 36 glucan chains having thousands of glucose residues. According to the degree of crystallinity, cellulose is classified into crystalline and amorphous cellulose. It can be hydrolytically broken down into glucose either enzymatically by cellulytic enzymes or chemically by sulfuric or other acids. A key advantage of acid pre-treatment is that a subsequent enzymatic hydrolysis step is sometimes not required, as the acid itself hydrolyses the biomass to yield fermentable sugars (Yu et al., 2010). Production of ethanol from cellulosic biomass contains three main processes, including pre-treatment, hydrolysis, and fermentation. Pre-treatment facilitates the hydrolysis of cellulose to be rapid by altering the size and structure of biomass as well as its chemical composition. In the hydrolysis step, celluloses are converted into monomer sugars. The resulting fermentable sugars could be fermented into ethanol by ethanol producing microorganisms, which can be either naturally occurring or genetically modified microorganisms (Zheng et al., 2009).
The present study investigated the ethanol opportunity from the grasses (Chloris barbata and Ischaemum pilosum) through acid hydrolysis followed by fermentation. Response surface methodology (RSM) was applied to optimise the process variables during cellulosic hydrolysis. Central composite design was chosen for experimental design and a second order polynomial equation was developed by using Design Expert 7 software (Stat-Ease Inc., 2007).

2. Materials and Methodology

2.1 Materials

The grasses as shown in Figure 1 (Chloris barbata) and Figure 2 (Ischaemum pilosum) were harvested near Building 40, Campus of Dagon University (DU). Sulphuric acid (Sp-gr 1.84, Analar Grade) (Nice Chemicals Private, India) and Saccharomyces cerevisiae—baker's yeast-(La—Saf Instant, France) were purchased from Kemiko (Cosmetic and Chemical Dealers), 28th Street, Pabedan Township, Yangon.

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Figure 1: Grass (Chloris barbata Sw.)

Figure 2: Grass (Ischaemum pilosum Klein ex Willd.)

2.2 Methodology

Crushed fresh stems of grasses, 30 g was weighed and pre-treated with 125 mL of liquid hot water (LHW) at 100 °C for 10 min. Acid hydrolysis was then carried out with the acid concentration of 4.7 vol%, acid volume of 125 mL and hydrolysing time of 111 min at 100 °C in a reflux condenser. The hydrolysate was neutralised with 0.1 N NaOH solution and its pH was adjusted to 5.6. After filtration, the sugar solution was cooled to 32 °C and inoculated with yeast—Saccharomyces cerevisiae for three–day fermentation period under anaerobic condition. Finally, the ethanol was separated by distillation. The alcohol strength of ethanol was measured by distillation method. Ethanol content by volume from specific gravity at 20 °C was read from the table that tabulates the ethanol by volume at 15.56 °C from apparent specific gravity at 20 °C (Lees, 1975).

2.3 Optimisation of Process Variables by RSM

The process variables such as acid concentration, acid volume and hydrolysing time which influenced the yield of fermentable sugar were optimised by using RSM. Table 1 presents the level of process variables chosen. The low and high values were chosen based on the previous experiment of cellulytic process of grass in which the maximum yield of fermentable sugar was 55 mg/g at the acid concentration of 3.4 vol%, acid volume of 135 mL and hydrolysing time of 86 min for 30 g of freshly crushed stems of grasses. For fitting a second–order model Central Composite design was used for acid hydrolysis of grasses. The design allowed a minimal number of experimental runs, 17 runs (Montgomery, 2001). The analysis of variance (ANOVA) and regression analysis were performed with the aid of Design Expert 7 software (Stat-Ease Inc., 2007).

Table 1: Level of variables chosen

Variables	Level Chosen	
	Low	High
Acid concentration (vol%)	1.4	5.4
Acid volume (mL)	120	140
Hydrolysing time (min)	80	120

3. Results and Discussion

The harvest period of grasses was between November 2013 and February 2014, with lignin contents of 12.76 ± 0.20 and 13.06 ± 0.27 for grasses (Chloris barbata and Ischaemum pilosum). Lignin content was determined by using 72 % sulfuric acid method. As stated by Brodeur (2011), cellulosic materials, especially grasses usually comprise 10 - 30 % of lignin which has no sugars and lignin entraps cellulose and hemicelluloses molecules. Chemically, lignin is an irregular polyphenyl polymer constructed of phenylpropanoid monomers with various degree of methoxylation. Deposition of lignin in cellulosic material is necessary in order to soften the biomass as well as to cleave the biomass. Pretreatment of crushed grasses by using liquid hot water (LHW) was therefore carried out at 100 °C before cellulose hydrolysis for its porosity for further acid hydrolysis.

Sulfuric acid was used in cellulosic hydrolysis. Cellulosic hydrolysis of 17 experimental runs was conducted according to the experimental design. The suggested model is shown in Table 2 and the second-order polynomial quadratic regression equation is stated in Eq(1). The ANOVA of the regression model (Table 3) demonstrated that the model F value of 5.52 implied that the model was significant because mean square of the model as regression was greater than mean square of residual. It was greater than F critical value ($F_{critical}$ or $F_{0.05,9,6}$) of 4.10 that was obtained from the F-distribution table (Montgomery, 2001). There was only a 2.5 % chance that a large model F value could occur due to noise.

By solving Eq(1) in matrix notation, the estimated values for the optimum variables were 4.7 % of acid concentration, 125 mL of acid volume and 111 min of hydrolysing time for 97 mg/g of the yield of fermentable sugar over 30 g of crushed fresh stems of grass. The 3-D graphs of the regression equation called the response surfaces are shown in Figure 3. It was apparent that the maximum yield of fermentable sugar was achieved when two influencing factors increased and decreased at the same time. Cellulose fraction may be hydrolysed with increase in two variables and converted into the maximum yield of fermentable sugar, however, decreased value of fermentable sugar could be obtained by larger values of two variables. Aside from fermentable sugar, undesired products such as furfural and hydroxymethyl furfural may be found during acid hydrolysis according to Yang and Wyman (2009).

$$\hat{y} = 70.90 + 15.32\, x_1 - 2.99\, x_2 + 7.03\, x_3 + 13\, x_1^2 - 3.67\, x_2^2 - 1.83\, x_3^2 + 3.83\, x_1 x_2 - 4.81\, x_1 x_3 - 2.78\, x_2 x_3 \quad (1)$$

Table 2: Model summary statistics

Source	Standard deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	16.03	0.5650	0.4563	0.1740	5,854.51	
2FI	17.38	0.6164	0.3607	-0.2021	8,520.40	
Quadratic	11.28	0.8922	0.7305	0.3875	4,340.99	Suggested
Cubic	17.47	0.9139	0.3542	-116.6494	8.339×10^5	Aliased

Table 3: Results of ANOVA analysis

	df	SS	MS	F	Significance F
Regression	9	6,323.78	702.64	5.52	0.025
Residual	6	763.95	127.33		
Total	15	7,179.78			

Anaerobic fermentation of sugar solution using yeast Saccharomyces cerevisiae was then conducted at room temperature of 37 °C and the fermentation period was limited for three days. The pH of culture medium was 5.6. The effect of concentration of yeast on the yield of ethanol was observed by varying amount of yeast of 2 g/L, 3 g/L, 4 g/L, 5 g/L and 6 g/L. From Table 4 and Figure 4, high yield of ethanol - 24.88 ± 0.20 % by volume has resulted with the yeast of 5 g/L for grass (Chloris barbata), while 6.01 ± 3.20 % ethanol by volume was obtained for grass (Ischaemum pilosum) using yeast of 4 g/L with the same reflux ratio of 1.01.

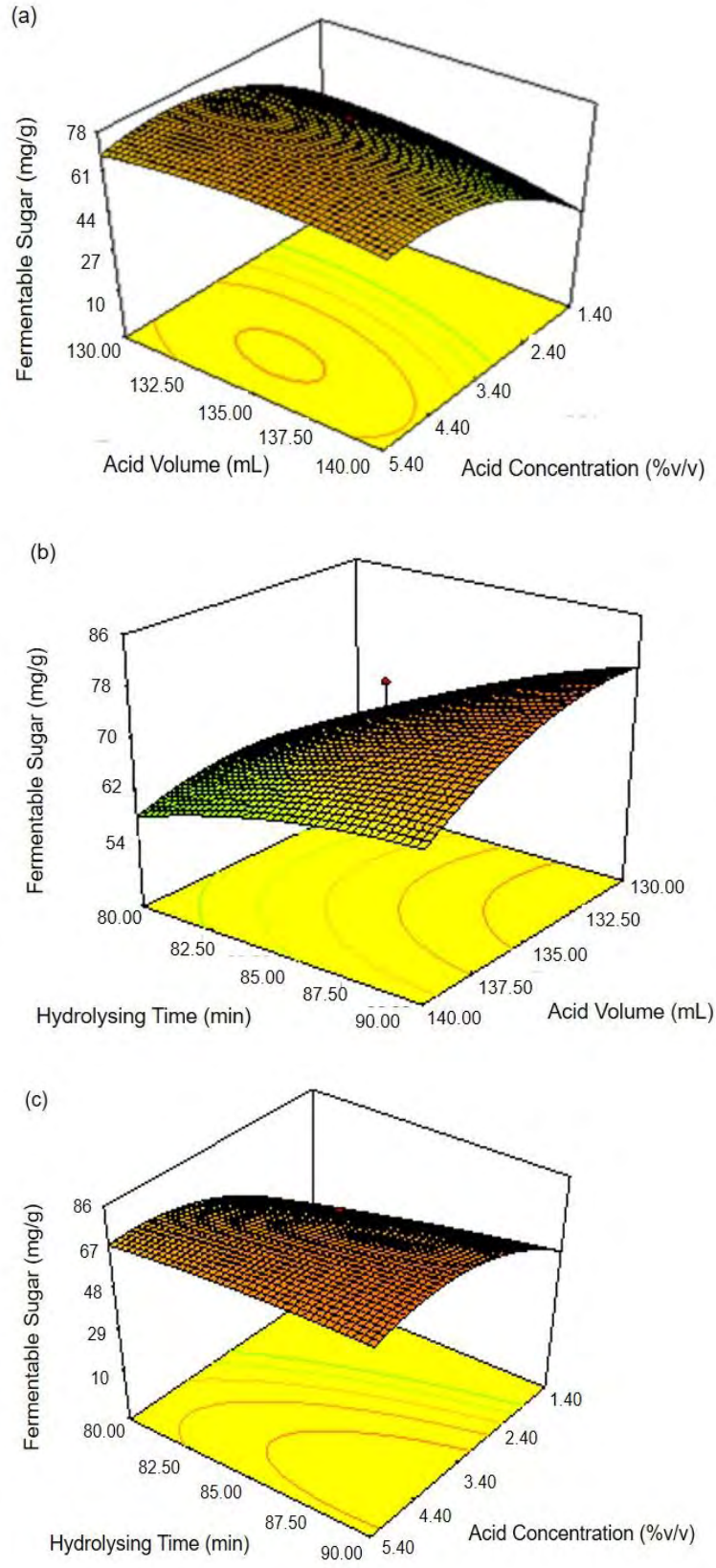

Figure 3: 3-D graph of the effect of (a) acid concentration (x_1) and acid volume (x_2), (b) acid volume (x_2) and hydrolysing time (x_3), and (c) acid concentration (x_1) and hydrolysing time (x_3) on the yield of fermentable sugar

Table 4: Data sheet of grasses

Content	Grass (Chloris barbata)	Grass (Ischaemum pilosum)
Moisture (wt%)	56.13 ± 0.15	74.45 ± 0.50
Ash (wt%)	13.88 ± 0.19	8.64 ± 0.26
Lignin content (wt%)	12.76 ± 0.20	13.06 ± 0.27
Fermentable sugar after acid hydrolysis (mg/g)	74.96 ± 7.07	86.28 ± 6.00
Ethanol strength (vol%)	24.88 ± 0.20	6.01 ± 3.20
Ethanol yield (g of ethanol/g of crushed fresh stem of grass)	0.30	0.20

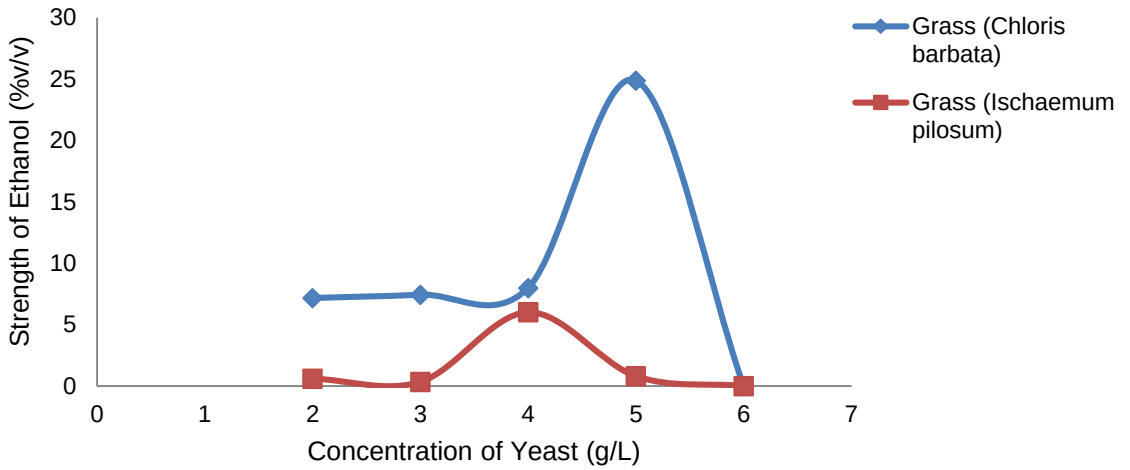

Figure 4: Strengths of ethanol obtained at different dosage of yeast in fermentation

The carbohydrates present in cellulosic material are cellulose and hemicelluloses that are the primary source of fermentable sugars for ethanol production. Hydrolysis of cellulose produces glucose that can readily be fermented by the existing strain and hydrolysis of hemicellulose produces both hexose and pentose (6-carbon and 5-carbon) sugars that are not all fermented with existing strains (Liu and Wyman, 2005). Demirbaş (2005) also stated that grasses composed of 25 - 40 % of cellulose and 25 - 50 % of hemicelluloses. The efficiency of the fermentation of 5-carbon sugars has been important to recover the high yield of ethanol and the baker's yeast used in this research could not ferment all the available sugars. When compared to ethanol strength of two grasses, lower strength of ethanol 6.01 ± 3.20 % has resulted for grass (Ischaemum pilosum).

4. Conclusion

This study investigated ethanol availability from the grasses (Chloris barbata and Ischaemum pilosum). The maximum yields of fermentable sugar were obtained as 74.96 ± 7.07 mg/g and 86.28 ± 6.00 mg/g for the two grasses by optimization of process variables using RSM. The grass Chloris barbata has resulted higher ethanol strength than the grass Ischaemum pilosum that contained higher fermentable sugar content. It was observed for low strength of ethanol that the washing and/or vaporisation step before fermentation was important for the removal of the acid after acid hydrolysis. After neutralisation of hydrolysate obtained from cellulose hydrolysis, the residual acid could contaminate the fermentation process. The other factor was due to the strain of yeast used in fermentation that may ferment only for C6 sugars and the grass Ischaemum pilosum may comprise of more C5 sugars.

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