ASSESSMENT OF ETHANOL PREPARATION BY ACID HYDROLYSIS AND FERMENTATION OF GRASSES

Soe Soe Than

Abstract

Two different grasses, *Pennisetum hordeoridas* Lam. and *Chloris barbata* Sw., were chosen as sources of cellulosic materials for the preparation of ethanol. Fresh stems of grasses were processed for conversion of cellulose into fermentable sugars by acid hydrolysis using sulphuric acid (H_2SO_4). Response Surface Methodology (RSM) was applied to optimize the process variables such as acid concentration, acid volume and hydrolyzing time for the yield of fermentable sugars during acid hydrolysis. The maximum yield of fermentable sugar of 55 mg/g was obtained at the acid concentration of 3.4% (V/V), acid volume of 135 ml and hydrolyzing time of 86 min for 30 g of freshly crushed stems of grasses. Baker's yeast (*Saccharomyces cerevisiae*) was used in fermentation of the resulting sugars under anaerobic condition. The maximum yields of ethanol by volume were 3.83% and 24.88% with the yeast concentrations of 3g/L and 5g/L respectively.

Keywords: grass, acid hydrolysis, RSM, fermentation

Introduction

The utilization of renewable and sustainable resources for the production of fuels, chemicals and materials has become a global research theme and biofuels produced from biomass have taken a leading position as a viable option to petroleum-derived fuels. The production of ethanol from non-food cellulosic biomass has been extensive interest over the past decade.

Cellulosic biomass consists of three major structural biopolymers, namely cellulose, hemicellulose, and lignin. Cellulose, a polysaccharide, consists of a linear chain of several hundred to over ten thousand β (1→4) linked D-glucose units. Meanwhile, hemicelluloses consist of a broad class of mixed heteroglycans of pentoses and hexanoses (mainly xylose and mannose) which link together and frequently have branching and substitution groups. Lignin is an irregular polyphenolic biopolymer constructed of phenylpropanoid monomers with various degrees of methoxylation that are biosynthesized into a complex and highly heterogeneous aromatic macromolecule (Pu et al., 2013).

Lecturer, Department of Industrial Chemistry, Dagon University

The conversion of plant polysaccharides to monosaccharides usually involves three steps:

pretreatment, acid or enzymatic hydrolysis, and fermentation. pretreatment technologies are

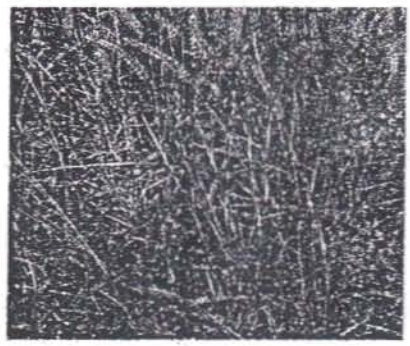
usually classified into physical, chemical, physicochemical, and biological treatments. Liquid hot water (LHW) pretreatment uses water at elevated temperatures and high pressures to maintain its liquid form in order to promote disintegration and separation of the lignocellulosic matrix. Temperatures can range from 160°C to 240°C and over lengths of time ranging from a few minutes up to an hour with temperatures dominating the types of sugar formation. There are two products at the outlet of pretreatment: the solubilized hemicellulose-rich slurry and the cellulose-rich solid fraction that are separated from each other. The solubilized product, consisting primarily of oligosaccharides derived from hemicellulose (nearly complete removal from solid fraction) and lignin (35–60% of total starting material) and a minor amount of cellulose (4–15%), is the primary focus for this particular pretreatment. Hydrolysis of the cellulosic fraction to glucose for subsequent fermentation to ethanol can be accomplished using either acid or enzyme. Acid treatment involves the use of concentrated and diluted acids to break the rigid structure of the cellulosic material. The most commonly used acid is sulphuric acid (H_2SO_4), which has been commercially used to treat a wide variety of biomass types—switchgrass, corn stover, spruce (softwood), and poplar. A key advantage of acid pretreatment 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).

The present research investigated the ethanol opportunity from two different grasses, *Pennisetum hordeorides* Lam. and *Chloris barbata* Sw. The conversion of cellulosic materials to fermentable sugars was carried out by LHW pretreatment, followed by acid hydrolysis using H_2SO_4 and subsequently, fermentation for ethanol. Design Expert 8 software was used to estimate the optimum variables such as acid concentration, acid volume and hydrolyzing time during acid hydrolysis. Fermentation was conducted with yeast *Saccharomyces cerevisiae*.

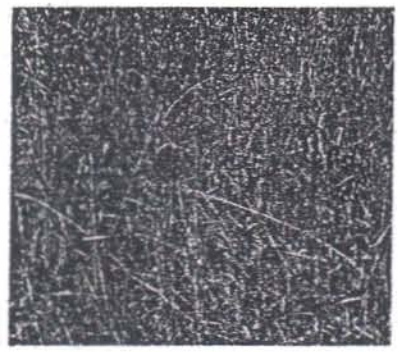
Materials and Methodology

Materials

Grasses were harvested near building 40, Department of Industrial Chemistry, campus of Dagon University. 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.



Grass (*Pennisetum hordeorides* Lam.)



Grass (*Chloris barbata* Sw.)

Methodology

Preparation of Ethanol by Acid Hydrolysis Followed by Fermentation

Crushed fresh stems of grasses, 30 g was weighed and pretreated 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 3.4% (V/V), acid volume of 135 ml and hydrolyzing time of 86 min at 100 °C. The hydrolysate was neutralized 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.

Experimental Design

The process variables such as acid concentration, acid volume and hydrolyzing time which influenced the yield of fermentable sugar were optimized using Response Surface Methodology (RSM). Box-Behnken design was chosen for experimental design. The range of influence factors (including the acid concentration, acid volume and hydrolyzing time) and response

function was listed in Table 1. According to Box-Behnken design, 17 experimental runs were conducted. Regression analysis of the data was then carried out by using Design Expert 8 software, statistical design software (trial version 8.0.7.1, Stat Ease Inc. USA).

Determination of Ethanol Content

The alcohol strength of ethanol was measured by distillation method. Ethanol sample 100 g was weighed and filled into a 500 ml round bottom flask. 50 ml of distilled water was added into it. The liquid was distilled until approximately 100 ml of solution was obtained. The solution was then cooled to 15 °C and the specific gravity of the solution was measured at 20 °C using a specific gravity bottle (capacity of 50 ml). A clean, dried and previously weighed specific gravity bottle (S.G bottle) was used for this purpose. The specific gravity of the distilled water was also measured using a specific gravity bottle. After that density of the solution was determined from the ratio of the weight of liquid held in S.G. bottle and the weight of water held in S.G. bottle. 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).

Table 1 Level of Variables Chosen and Response Function

Variables	Symbol		Level		
	uncoded	coded	Lower -1	Basis 0	Upper 1
Acid concentration (%) (V/V)	X_1	x_1	1	7	13
Acid volume (ml)	X_2	x_2	50	150	250
Hydrolyzing time (hr)	X_3	x_3	2	6	10
Response (Reducing sugar (mg/g))	y				

Results and Discussion

The collection period of both grasses was from November, 2012 to February, 2013. Lwin Mar Saing (2010) confirmed the name of grasses as *Pennisetum hordeorides* Lam. and *Chloris barbata* Sw. The pretreatment of crushed grasses with LHW was firstly conducted for its porosity for further acid hydrolysis. The regression equation obtained using the design expert 8 software for the response function of fermentable sugar was as follows:

$$\hat{y} = 46.314 + 8.475\,x_1 - 8.94\,x_2 + 1.1025\,x_3 - 3.54x_1^2 - 2.48\,x_2^2 - 10.652\,x_3^2$$
$$- 1.63\,x_1x_2 - 1.055\,x_1x_3 + 3.895\,x_2x_3$$

The estimated values for the optimum variables were acid concentration of 3.4%, acid volume of 135 ml and the hydrolyzing time of 86 min for the maximum fermentable sugar of 55 mg/g based on 30 g of crushed grasses. The 3-D graphs and contour graphs of the regression equation called the response surfaces are shown in Figures 1, 2 and 3. It was apparent that the maximum yield of fermentable sugar was achieved when two influence factors increased and decreased at the same time. Cellulose fraction may be hydrolyzed 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 occur during acid hydrolysis according to Yang & Wyman (2009).

The contents of two different grasses are shown in Table 2. Grass (*Chloris barbata* Sw) consisted of higher amount of fermentable sugar and lesser moisture content than the grass (*Pennisetum hordeorides* Lam). During 86 min of acid hydrolysis both grasses could be hydrolyzed efficiently to maximum yields of sugar. Chen et al (2010) presented that hemicellulose and lignin are solubilized with minimal degradation, and the hemicelluloses are converted to sugars with acid treatment.

Table 2 Analysis of Grasses

Content	Grass (*Pennisetum hordeorides* Lam.)	Grass (*Chloris barbata* Sw.)
Moisture (%) (w/w)	63.90 ± 0.2	56.13 ± 0.15
Ash (%) (w/w)	12.46 ± 0.17	13.88 ± 0.19
Fermentable sugar after acid hydrolysis (mg/g)	61.08 ± 7.84	74.96 ± 7.07

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. 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 Figure 4 high yield of ethanol- 24.88% by volume has resulted with the yeast of 5 g/L for grass (*Chloris barbata* Sw), while 3.83% ethanol by volume was obtained for grass (*Pennisetum hordeorides* Lam) using yeast of 3 g/L.

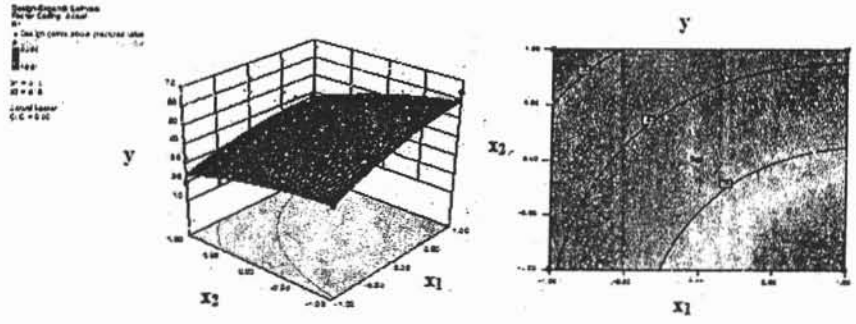


Figure 1 The Effect of Acid Concentration and Acid Volume on the Yield of Fermentable Sugar at Hydrolyzing Time of 86 min

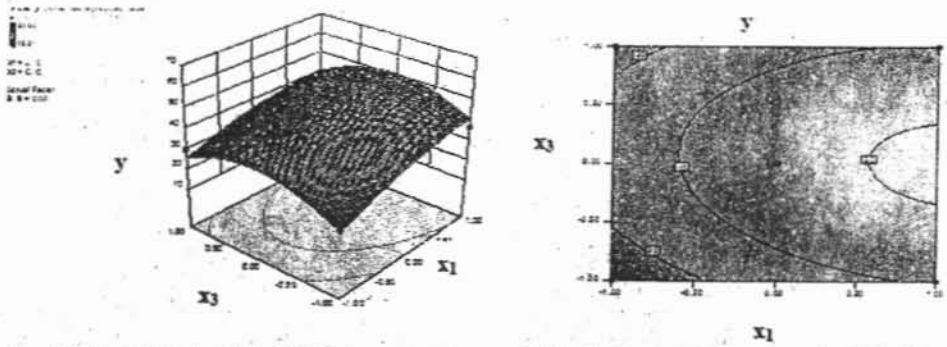


Figure 2 The Effect of Acid Concentration and Hydrolyzing Time on the Yield of Fermentable Sugar at Acid Volume of 135 ml

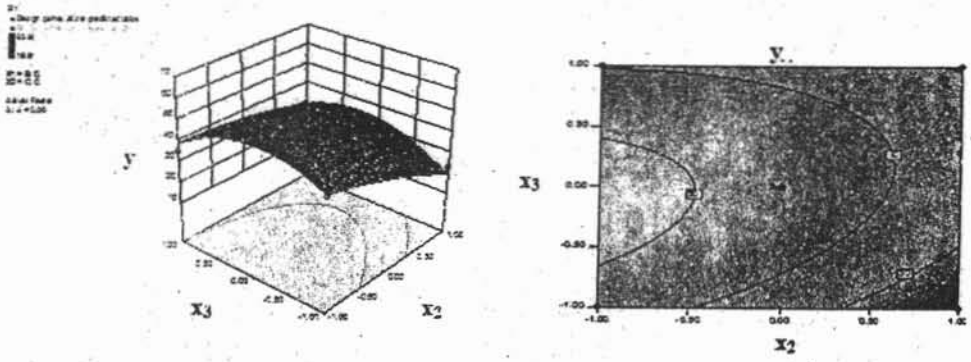


Figure 3 The Effect of Acid Volume and Hydrolyzing Time on the Yield of Fermentable Sugar at Acid Concentration of 3.4%

Cellulose and hemicellulose would be the primary source of fermentable sugars for ethanol production from cellulosic material. Liu and Wyman (2005) stated that cellulose and hemicelluloses can be broken down into simple sugars either enzymatically or by acid hydrolysis and the resulting hydrolysis product, six carbon sugars (hexoses), can easily be fermented to ethanol, but only a few microorganism strains can ferment the five carbon sugars (pentoses). However, washing and/or a detoxification step is required to remove the acid before a fermentation step. In the present work washing, neutralization with 0.1N NaOH solution and vaporization of remaining acid in sugar solution were intensively and carefully carried out before fermentation after neutralization of grass (*Pennisetum hordeorides* Lam.) has been experienced.

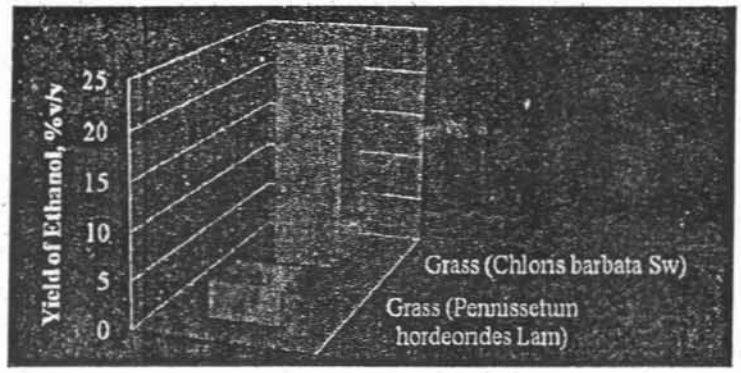


Figure 4 Maximum Yield of Ethanol at Respective Yeast Concentration

The furfural and other degradation products can be poisonous to the fermentation microorganisms. On the other hand, detoxification is a crucial step for further process. These fermentation inhibitors may accompany with sugar solutions, especially in the sugar solution of grass (*Pennisetum hordeorides* Lam.) due to poor handling in neutralization and filtration step that reduce the yield of ethanol. In addition, the yeast that is used to ferment the glucose molecules to ethanol may only ferment the 6-carbon cellulose-derived glucose molecules, and 5-carbon hemicellulose-derived xylose molecules may not be fermented and are wasted (Liu & Wyman, 2005). Therefore, existing yeast strain cannot withstand highly toxic hydrolyzates or ferment 5- carbon sugars and minor 6-carbon sugars efficiently for grass (*Pennisetum hordeorides* Lam.).

Conclusion

In this study, ethanol was prepared from the second generation raw materials–two different grasses– *Pennisetum hordeorides* Lam. and *Chloris barbata* Sw. The presence of high fermentable sugar content was found in grass (*Chloris barbata* Sw) and consequently, high yield of ethanol was obtained. Neutralization and detoxification steps were crucial for further fermentation. Thus further research for ethanol preparation from grass ((*Pennisetum hordeorides* Lam.) needs to conduct based on its preliminary results.

References

Chen XW, M Lawoko, and A van Heiningen, (2010), Kinetics and Mechanism of Autohydrolysis of Hardwoods, *Bioresource Technology, Vol 101*, 7812–7819.

Lees R, (1975), Food Analysis: Analytical and Quality Control Methods for the Food Manufacturer and Buyer, 3rd Edit, Leonard Hill Books, a Division of Textbook Company Limited, London, 51,52,163.

Liu C, and CE Wyman, (2005), Partial Flow of Compressed-Hot Water Through Corn Stover to Enhance Hemicellulose Sugar Recovery and Enzymatic Digestibility of Cellulose. *Bioresource Technology, Vol 96,* 1978–1985.

Lwin Mar Saing, (2010), Taxonomic Study of Family Poaceae (Gramineae) in Meik Area of Taninthayi Division, Ph.D Dissertation, Botany Department, University of Yangon, Myanmar (unpublished).

Pu Y, F. Hu, F. Huang, B. H. Davison and A. J. Ragauskas, (2013), Assessing the Molecular Structure Basis for Biomass Recalcitrance During Dilute Acid and Hydrothermal Pretreatments, *Biotechnology for Biofuels*, Retrieved April 20, 2013, from http://www. biotechnology for biofuels.com/content/6/1/15.

Yang B, and CE Wyman, (2009), Dilute Acid and Autohydrolysis Pretreatment. *Methodology of Molecular Biology, vol 581,* 103–114.

Yu G, S. Yano, H. Inoue, S. Inoue, T. Endo, and S. Sawayama, (2010), Pretreatment of Rice Straw by a Hot-Compressed Water Process for Enzymatic Hydrolysis, *Applied Biochemistry and Biotechnology*, vol. 160, No. 2, 539–551.