Obtaining of Reducing Sugars from Kikuyu Grass (*Pennisetum Clandestinum*)

Obtención de Azúcares Reductores a partir de Pasto Kikuyo (*Pennisetum Clandestinum*)

Andrés Felipe López Vásquez[1]*, Gabriel Alberto Ortegón Rey[2], Fredy Antonio Robles Rodríguez[3]

[1] *Chemical Engineering, MSc, Department of Environmental Engineering, Universidad Libre, Av Cr 70 # 53-40, Sede Campus Bosque, Bogotá, Colombia. * andresf.lopez@ unilibrebog.edu.co.*
[2] *Department of Chemical Engineering, Universidad de La Sabana, Campus Universitario del Puente del Común, Km 7, Autopista Norte, Chía, Cundinamarca, Colombia. gabrielorre@unisabana.edu.co.*
[3] *Departament of Agroindustrial Production, Universidad de La Sabana, Campus Universitario del Puente del Común, Km 7, Autopista Norte, Chía, Cundinamarca, Colombia. fredy.robles@ unisabana.edu.co.*

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Abstract

The production of reducing sugars by acid and enzymatic hydrolysis of *Kikuyu* grass was performed in order to study reducing sugar production. The study involved hydrolysis of treated grass by immersed in water at environmental temperatures. Acid hydrolysis was carried out through reflux boiling with different concentrations of diluted sulphuric acid (2%, 4%, 6% v/v) with in a liquid/solid ratio of 30/1, while enzymatic hydrolysis was carried out with stirring in a water/enzyme (celluclast 1,5 LFG) solution. Both procedures were developed to different reaction times (2, 4 and 6 hrs). Reducing sugars concentrations were determined by UV-Vis spectrophotometry using the 3,5 dinitrosalisilic acid (DNS) method. Also, Benedict qualitative test confirmed reducing sugars presence in the samples. The greatest reducing sugars concentration was 2.5 g/l when using grass with aqueous treatment, with a 4 % sulfuric acid solution and 6 hours of reaction time. In general, results showed that greatest reducing sugars concentration were to high acid concentrations and reaction times.

Keywords

Hydrolysis, Kikuyu grass, Reducing sugars.

Introduction

Lignocelluloses are the most abundant organic compounds in nature and represent an important resource for producing valuable products [1]. Lignocellulosic biomass consists of cellulose, hemicellulose, lignin, organic extractives (mixture of different organic compounds) and some organic components, which turn into ash following combustion [2]. Cellulose, hemicellulose and lignin, constitute more than 75% of the vegetal material, and are composed of organic polymers of high molecular weight. Due to its chemical composition, lignocellulosic biomass differs greatly from products with high sugar or starch content.

Cellulose and hemicelluloses must first be hydrolyzed to simple sugars and then fermented to ethanol. Fermentation of hydrolyzed glucose from cellulose is an established process. However, fermentation of hydrolyzed hemicellulose pentoses currently presents some technical and economic difficulties [3].

Lignin introduces a negative effect on the fermentative processes; therefore, any viable process for obtaining ethanol from lignocellulosic biomass must include lignin extraction and its exploitation in obtaining valued products and/or energy generation [4].

Lignocellulosic materials require a particular combination of the pretreatment methods to optimize the yields of the feedstock, minimize the degradation of substrate, and maximize the sugar yield. One of the most thoroughly investigated methods is steam pretreatment using an acid catalyst [5]. The general method used to obtain ethanol from lignocellulosic biomass consists of the following steps: pre-treatment of the biomass, hydrolysis, fermentation and distillation [6].

Colombia is an agricultural country and a lot of agricultural wastes are available in every year. Millions of tons of pastures are produced every year due to ecosystemic diversity of country (7.7 GHa) [7]. For example, in Bogota's savannah, one of the most popular pastures mixes is composed for of kikuyu, rye grass and natives (about 90 per cent kikuyu content).

Previous researches indicate that kikuyu grass contains an appreciable quantity of starch. While starch will not contribute directly to the silage fermentation, as silage bacteria cannot ferment starch, hydrolysis of starch to sugars during wilting and prior to the establishment of anaerobic conditions in the silo could boost the supply of sugars available for fermentation, provided there are not significant losses due to respiration [8].

Typical composition of Kikuyu grass in Colombian pastures is listed in Table 1 [9].

Table 1. Cellulose, Hemicellulose and Lignin Content in Kikuyu Grass (Dry Basis Percentage).

Component	Mean
Hemicellulose	26.2
Cellulose	26.9
Lignin	5.88

In present work, the acid and enzymatic hydrolysis of lignocellulosic biomass as well as possibilities for obtaining reducing sugars from KG were investigated.

Materials and Methods

Raw Materials

Kikuyu Grass (KG) was harvested in early 2010 from Universidad de la Sabana (Chía, Cundinamarca, Colombia) surroundings. The waste material with initial moisture content of 10% was shredded to 2–4 inches size and dried to 50°C. The feedstock for pretreatment was further ground and passed through a 20 mesh screen [10]. NaOH (Merck), H_2SO_4 (Sigma), DNS reactive (Fluka) and glucose patron were used according described methodology to sugars determination.

Hydrolysis

The hydrolysis of cellulose to glucose only occurs at economically viable yields when a catalyst is used. The three main catalyst classifications are: enzymatic, concentrated acid and dilute acid catalysts. The main advantages in using enzymatic catalysts are the high specific characteristic of enzymes (i.e., no by–products), enzymes operate under mild conditions, are environmentally friendly and a small amount of enzyme results in high yields. In using enzymatic hydrolysis however, pre–treatment is necessary to open up the structure and to provide access for the enzyme to the active sites. Pre–treatment is usually preformed by energy intensive physical methods, high temperature and pressure or the uses of a chemical solvent e.g., dilute acid [11].

KG were pretreated by immersion in distilled water (solid to liquid ratio of 5:1) for 1 h. at ambient temperature and dried at 80°C during 24 hours. Acid hydrolysis at reflux boiling of pretreated materials was carried out to laboratory scale. Diluted sulphuric acid was used to different concentrations (2.0, 4.0, and 6.0 %v/v) with a liquid solid ratio of 30:1 to several reaction times (2.0, 4.0, and 6.0 h.) and the reaction was interrupted by cooling. Enzyme hydrolysis was performed with water–cellulose mixture (90 ml–0.03 ml CELLUCLAST 1,5 LFG) at ambient temperature in stirring for 2.0, 4.0, and 6.0 hours respectively.

The hydrolyzed materials were filtered through preweight filter paper (Whatman N° 1) and the pH was adjusted to 4.5 using 2N NaOH solution. Lately, the solution was discolored by activated carbon and the supernatant was recovered for sugar analysis by DNS method with glucose as standard [12]. Absorbance were measure at 540 nm. An additional qualitative test (Benedict) was used to confirm reducing sugars presence. The residual solids were dried in a convection oven at 105°C and weighed to calculate percentage weight loss.

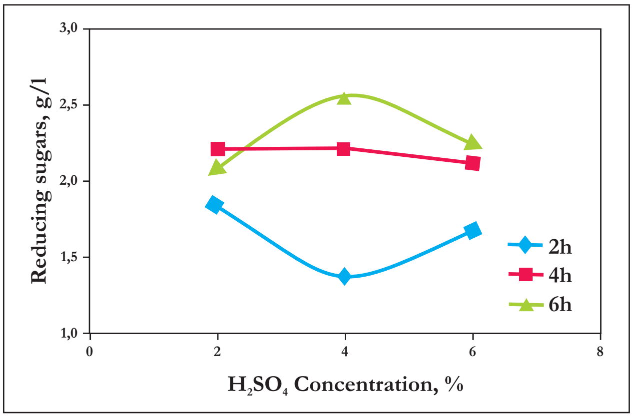

Figure 1. Kikuyu grass hydrolysis at different sulphuric acid concentration.

Results and discussion

Reducing sugars concentration (g/l) determinate during experiments is presented in Table 2.

In general during acid hydrolysis, an increase of time reaction improves reducing sugars production, on the contrary increasing acid concentration its content decrease. Through enzymatic hydrolysis of Kikuyu grass reducing sugars production it was not significant (0.032 g/l). The maximum sugar content occurred at 4.0 % of sulphuric, with a total reducing sugar of approximately 2.533 g/l.

Figure 1 illustrates a plot of reducing sugars content versus sulphuric acid concentration for the hydrolysis of Kikuyu grass cuttings.

Analyzing the relationship between sugar degradation and acid concentration, suggests that treatment are prone to degradation with increased acid concentration, which may indicate that the acid also acts as a catalyst for sugar decomposition.

To 4 and 6 h. treatment, an increase in the acid concentration decreased the sugar yield due to possible sugar degradation. However, an additional increase in acid content in 2 h. treatment, it seems re–activate reducing sugars generation. Possibly, at experimental conditions still it exist starch available to degradation.

Conclusions

According revealed color during Benedict test, it confirms presence of sugar in samples. Kikuyu grass was analyzed to determine its carbohydrate content. The use of diluted sulphuric acid as well as the enzymatic digestion did not provide reliable results in the case of raw material. Better performances of both methods were achieved after 6 h. of treatment, but only the acid method led to a realistic value of reducing sugars of 2.533 g/l.

Total amount of carbohydrate in the filtrates decreased with increasing acid concentration,

Table 2. Experimental Results for Kikuyu Grass Hydrolysis. Reducing Sugars Content, g/l.

Time, h	Acid (H_2SO_4)			Enzymatic
	2 %	4 %	6 %	
2	1.844	1.373	1.678	0.0
4	2.210	2.204	2.120	0.0
6	2.081	2.533	2.250	0.053

suggest that high H_2SO_4 content severity augmented degradation of reducing sugars to other by-products, leading to low sugar recovery from hydrolysis filtrate.

The sugar conversion efficiency was more sensitively affected by the variation of H_2SO_4 treatment conditions than enzymatic efficiency. However, the study of experimental conditions, enzyme loading test, variables relationship, scale-up and economic analysis of the overall conversion process is needed for further conclusions.

Behavior processes suggest a strong interaction between studied variables, supposing the existence of optimal conditions in analyzed region.

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