EXTRACTION OF GLUCOSE FROM KENAF CORE USING MILD ACID TREATMENT

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ABSTRACT

Recently, many industries are aims to reduce the usage of petroleum based product or synthetic fibre due to the increment of the environmental consciousness. This has lead to the extensive research on the natural fibre in order to produce eco–friendly product that will replace the existing petroleum based products. Natural fibre can be derived from many sources such as plants, animals or minerals. However the plant fibre such as kenaf is more desirable by most of the researcher. Kenaf or scientifically known as *Hibiscus cannabinus. L* has a complex structure because it consists of lignin, cellulose and hemicelluloses. Due to this reason, kenaf need to undergo treatment process in order to remove lignin and hemicellulose, reduce crystallinity of cellulose and increase porosity. In this study, acid treatment method was used with several parameters process such as temperature and time for getting high yield of glucose conversion. The substantially highest glucose yield was 3.4 g/L produced at 200 ºC for 60 minutes.

INTRODUCTION

Lately, biopolymer industries are attempting to decrease the dependence on petroleum based fuels and products due to the increased environmental consciousness. This phenomenon leads to the need to replace synthetic fibre with environmental friendly and sustainable materials. The tremendous increase of production and use of plastics in every sector of our life led to huge plastic wastes. Disposal problems, as well as strong regulations and criteria for cleaner and safer environment, have directed scientific research towards eco-composite materials. Each individual among the different types of eco-composites which contain natural fibres and natural polymers have a key role. Currently, the most viable way towards eco-friendly composites is through the use of natural fibres as reinforcement.

Naturally, plants fibres such as from kenaf have three major components which consist of cellulose, lignin and hemicellulose (Maya and Sabu, 2008). These materials caused plants fibre to have a complex structure that cannot be directly converted into end products such as ethanol and lactic acid in biopolymer industry (Ren et al., 2009; Sun and Cheng, 2002). Due to this reason, plants fibres require treatment in order to remove lignin and hemicelluloses, to reduce cellulose crystallinity as well as to increase the porosity of the materials (McMillan, 1994; Sun and Cheng, 2002; Klinke et al., 2004). There are many types of treatment process such as physical, chemical (acid and alkaline) and biological treatment however acid treatment is commonly used by many researcher.

Sulphuric acid (H_2SO_4) or hydrochloric acid (HCl) is commonly utilized as the chemical for lignocellulosic material or plant fibre hydrolysis such as kenaf biomass. According to Xiang (2003) and Hamelinck (2005), there are two types of acid treatment which are mild acid treatment and concentrated acid treatment (Xiang et al., 2003; Hamelinck et al., 2005). In dilute acid treatment with concentration of 0.7 to 3.0 %, it requires high operating temperature around 160 to 250 ^oC. On top of that for concentrated acid treatment, it requires high amounts of acid about 34% and this can be considered as uneconomical since it will lead to high operating costs as well as various environmental problems such as corrosion and pollution (Banerjee et al., 2010).

Kenaf also known as *Hibiscus cannabinus* is biennial herbaceous plant that can grow up to 20 feet (Scott et al., 2007). It contains long soft base fibres, contributing 30 to 40% of the dry weight of the stem. The central core of the stem contains a weakly disbursed pith cells surrounded by a thick cylinder of short woody fibres.

The main objectives of this study is to establish analytical method of glucose content extraction from kenaf biomass as well as to decrease the crystallinity of cellulose in order to be used in producing various end products such as lactic acid and ethanol.

METHODOLOGY

Design of Experiment (DOE)

The experiment was developed and designed by Central Composite Design (CCD) using Design Expert v6.0.8 Software to determine the optimum yield of glucose content from kenaf biomass.

Sample Preparation

Kenaf biomass was obtained from Kenaf Fibre Industry Sdn Bhd, Kelantan, Malaysia and was stored in the room temperature upon arrival. Then, it was crushed and sieved using 100 μm nylon Mesh.

Hydrolysis Treatment Process

In this process, 2% (v/v) of sulphuric acid (H_2SO_4) was prepared in 100 ml working volume. The optimization process was conducted using hot plate with 100 rpm of agitation speed and 3 g of kenaf biomass in order to obtain optimum glucose content by varying two parameters with three levels which are mixing time and temperature. The experiments were carried out for 11 runs with two replications based on CCD method. For the analysis of the optimization results, regression analysis was done by developing the regression equation.

Total Glucose Content Analysis

Total glucose content was analyzed using high pressure liquid chromatography (HPLC) (Agilent model 1200) comprised of a quaternary pump with auto-sampler injector, micro-degassers, column compartment equipped with thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C_{18} end capped 5 μm, 4.6x150 mm reverse phase column (Agilent Technologies, USA) and UV detector at 210 nm. The conditions of HPLC were mobile phase acetonitrile/phosphate (1:9 v/v), flow rate 1.0 ml min^{-1} for 15 minutes, temperature 20^{o}C and injection volume 1 μL. Each injection was performed in duplicate.

RESULTS AND DISCUSSION

This study investigates the potential of chemical treatment which is mild acid treatment in extracting the glucose from the kenaf biomass which used as the raw material. Two factors have been chosen for the optimization of chemical treatment which are mixing time and temperature in order to maximize the amount of glucose conversion. In order to determine the maximum glucose conversion from kenaf biomass with optimum condition, the experiments were designed based on Central Composite Design (CCD) by Design Expert v6.0.8 software. The polynomial regression model relating the percentage of conversion (Y) with independent variables, mixing time (A) and temperature (B) is as follows:

$$Y = -13.9001 + 2.1586\,A - 0.0526\,B - 0.0282\,A^2 + 7.8599 \times 10^{-4}\,B^2 - 0.0126\,AB + 1.6667 \times 10^{-4}\,A^2B$$

where Y= the predicted of glucose content

The average amount of glucose content of duplicate values obtained was taken as observed amount of glucose content. All the variables, observed and predicted results are recorded in Table 1.

Table 1: Values of observed and predicted of response

Run	Mixing Time (min)	Temperature (°C)	Glucose (g/L)	
			Actual	Predicted
1	30.00	180.00	0.366	0.555176
2	60.00	180.00	0.754	0.944114
3	30.00	160.00	0.334	0.238706
4	45.00	200.00	0.457	0.64638
5	45.00	180.00	0.388	0.355657
6	60.00	200.00	3.397	3.30231
7	45.00	180.00	0.439	0.355657
8	30.00	200.00	0.374	0.27843
9	60.00	160.00	0.531	0.436694
10	45.00	180.00	0.620	0.355657
11	45.00	160.00	0.504	0.693713

The determination coefficient (R^2 = 0.904) in this study specifies high correlation between the observed and predicted values and indicates the level of precision with which the glucose content is attributed to the independent variables, mixing time and temperature. This sample of variation implies that only 90.4% of glucose content was contributed to the independent variables meanwhile another 9.6% of the total variable cannot be explained by the model. The analysis of variance (ANOVA) is presented in Table 2. From this table, it shows that the model is significant (p value is 0.0480) and the Lack of Fit is also significant which is 0.0394 and the R^2 for this analysis is 90.45%.

Table 2: ANOVA for response surface quadratic model

Sources	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-Value (Prob> F)
Model	7.15	6	1.19	6.31	0.0480*
A	2.17	1	2.17	11.49	0.0275*
B	1.105E-003	1	1.105E-003	5.848E-003	0.9427
A^2	0.39	1	0.39	2.08	0.2226
B^2	0.25	1	0.25	1.33	0.3137
AB	2.00	1	2.00	10.57	0.0313*
A^2B	0.75	1	0.75	3.97	0.1171
Residual	0.76	4	0.19		
Lack of Fit	0.73	2	0.36	24.41	0.0394
Pure Error	0.030	2	0.015		
Cor Total	7.91	10			

S = 0.43 R-Sq = 90.45% R-Sq(adj) = 76.11% * significant

From the statistical analysis which is in Table 2, the variable that give significant model or results are the linear effect of mixing time (A) and also the interaction between mixing time and temperature (AB). Since the linear and the interaction of mixing time gives significant results so it can be considered as the limiting factors and a little variation in this mixing time value, it will give great affect to entire results which is in glucose production. From Table 1, it shows the maximum glucose content is produced in Run 6 which is 3.397 g/L (observed value) and it also shows the predicted value is quite similar withe the predicted value which is 3.30231 g/L. The Run 6 shows optimum condition when the mixing time is 60 minutes and the temperature is at 200 ^oC. Chemically, the treatment process using dilute H_2SO_4 allow the microorganism exhibit the complex structure of kenaf biomass as lignocellulosic materials and break the complex structure into the simple sugars which can be consumed by microorganisms. By referring to the optimization chemical treatment's results, the higher the heating rate (mixing time and temperature), the easiest the separation of the carbohydrates from the lignin matrix of kenaf biomass. Thus, it will lead to the lowest chemical destruction of fermentation sugars which required for any end products like ethanol or lactic acid production.

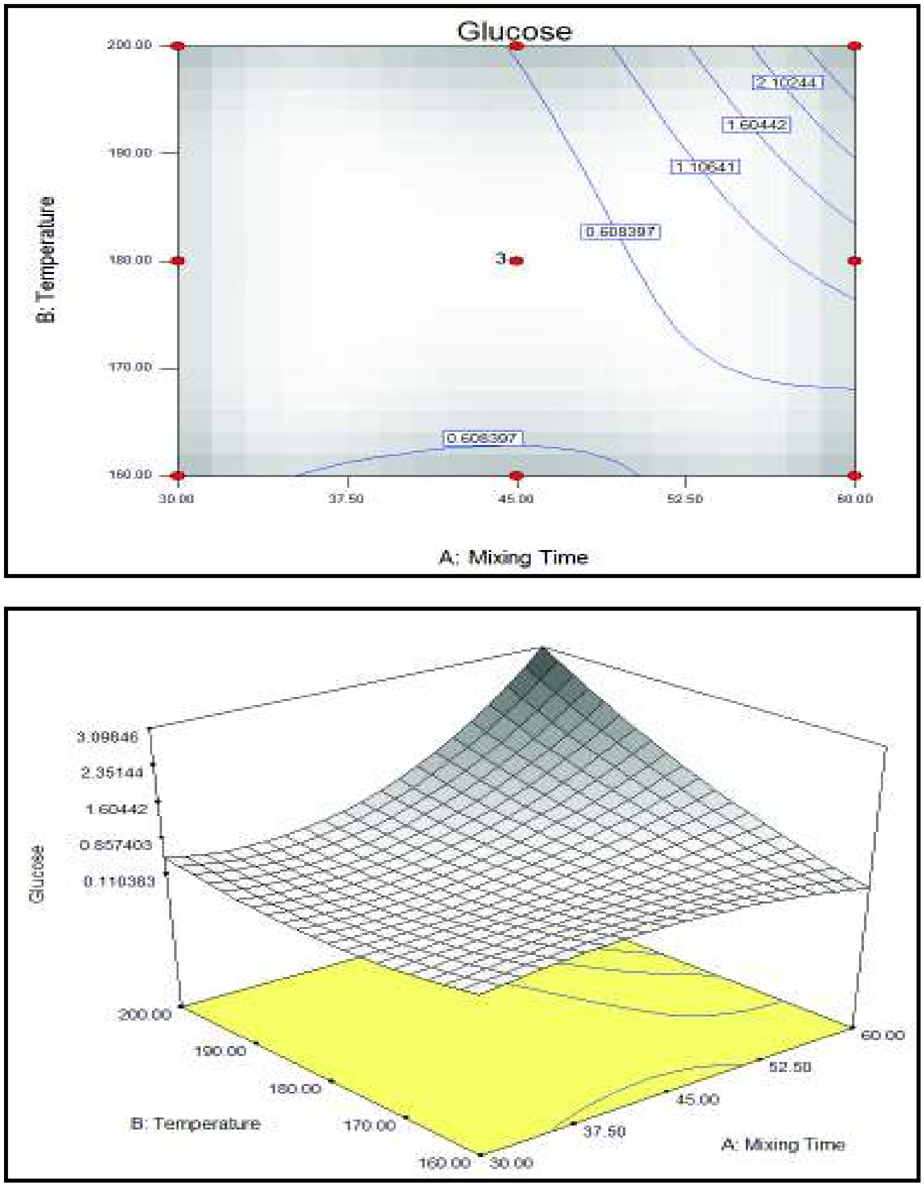

Figure 1: 2D contour plots and 3D response surface shows the effect of time (min) and temperature (°C) on the glucose concentration (mass of fiber was 3 g and agitation speed was 100 rpm).

In order to determine the optimum values of variables, 3D surface and 2D contour plots are used as the graphical representation of the regression equation (Tanyildizi et al., 2005) as shown in Figure 1. The ultimate objective of the response surface is to investigate the optimum values of the variables such as the response is maximized (Tanyildizi et al., 2005).

In 2D plot, each contour curve signify the infinity number of two variables with the other three remain at their respective zero level. The smallest ellipse from the confined surface in the contour plot are used to indicate the maximum predicted value. The perfect interaction between the independent variable will results an elliptical contour (Muralidhar et al., 2001). Figure 1 illustrate the response surface of the model equation in order to estimate glucose conversion over mixing time time and temperature. The 3D surface plot was described by the regression model in order to illustrate the effects of the independent variables and the combined effects of each independent variable upon the response variable (Duta et al., 2006). It can be observed that the high percent conversion was achieved at highest time and temperature. According to Xiang et al.(2003), the hydrogen bonding in the hemicellulose and cellulose fraction are easily break down at high temperature compare to low temperature which results to high yield of glucose.

CONCLUSIONS

This study established that the best chemical treatment condition for optimization of high yield total glucose content from kenaf biomass was obtained at 200 ^{o}C temperature and 60 minutes mixing time with total glucose content of 3.4 g/L.

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