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BIOCONVERSION OF WASTE TO USEFUL PRODUCTS

WATER AND WASTEWATER TREATMENT TECHNOLOGIES

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CASSAVA LEAVES AND STEMS HYDROLYSIS FOR GLUCOSE PRODUCTION

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ABSTRACT

Cassava or tapioca leaves and stems are all year product that is far under researched and underutilized. It was reported that the leave contain essential minerals and nitrogen sources required by microorganisms such as yeast and other fermenting microbes for growth. In this study, cassava leave and stem were hydrolyzed using diluted acid concentration for glucose production. The research was conducted in shake flask and Taguchi's methodology was used to study significant parameters affecting hydrolysis process. Design expert v8.0 software aided the analysis for maximum glucose production. Validation result revealed that hydrolysis of 5% (w/v) mixture of cassava stems and leaves gave maximum of glucose yield of 0.991 g/g.

1.1 INTRODUCTION

Cassava can reach a height of 1-4 meters. It is easy to maintenance the live of cassava tree and very productive tree. Cassava can grow about 1200 meters above the sea surface. Cassava or tapioca leave are all year product that is far under researched and underutilized. The leave yield amounting to as much as 12 tons dry matter per hectare as a byproduct at root harvest (Lim 2011). It was reported

that the leave contain essential minerals and nitrogen sources required by microorganisms such as yeast and other fermenting microbes for growth (Ravindran 1992). Cassava stem mainly consist of lignocelluloses could potentially serve as a low cost feedstock to increase the production of fuel ethanol through proper pretreatment, hydrolysis and fermentation. Cassava stems skin contains tannin, peroxides enzyme, glycosides, and calcium oxalate (Wanapat, Pimpa et al. 1997, Fasuyi 2005).

These lignocellulose biomasses are most readily available and cheap feedstock for bioethanol production. It has been reported that cassava stems and leaves contained about 15% and 2% starch along with 23% and 17% cellulose (Pooja and Padmaja 2014). However, pretreatment or hydrolysis is required to release sugar that trap in the hemicellulose-lignin matrix is required. Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. Direct saccharafication at moderate temperature was reported to suffer from low yield because of sugar decomposition, while high temperature of dilute acid hydrolysis favor for cellulose hydrolysis (Sun and Cheng 2002).

In this paper, dilute acid hydrolysis of cassava stems and leaves using nitric acid (HNO_3) was studied. The aim was to determine parameters that significantly affect glucose yield released from the process.

1.2 MATERIALS AND METHOD

1.2.1 Substrates preparation

Cassava leaves and cassava stem cultivar from Thailand were cut into small pieces. A 500 g of cassava leaves and stem were weighed. Then they were dried in an oven around 100^{o}C for 24 hours and were weighed. The samples were dry again for couple hour until constant dry weight. The moisture content of the samples was calculated. Next, the samples were ground until powder form. Finally, the sample was transferred into close container and was kept in room temperature for further use.

1.2.2 Hydrolysis

Acid hydrolyses were carried out on triplicate by taking 2.5 g of cassava leaves and 2.5 g of cassava stem. A 100 ml of dilute acid in various concentrations HNO_3 (Table 1) was used to pretreat the samples at solid loading of 5% (w/v). Hydrolyses were performed at various temperature, pH and time. The reaction time was estimated after approaching a set temperature. After cooling the solid and liquid fraction of the treated biomass were collected by washing samples with distilled water to minimize the loss of biomass. Table 1 shows selected parameters and levels used in the experiment.

Table 1 Selected parameters and level for hydrolysis process

Parameter	Level		
	1	**2**	**3**
Temperature (^{O}C)	160	180	140
pH	4	5	6
Acid Concentration (M)	0.12	0.14	0.16
Time (min)	10	20	30

1.2.3 Glucose analysis

The concentration of glucose was analyzed using High Pressure Liquid Chromatography (HPLC). Ion exclusion and Sugar-D Waters column (7.8 x 150 mm) system were employed which equipped with a refractive index detector (RID), a guard column, an automated sampler and a gradient pump. The column was eluted at 75ºC with mobile phase 0.5 mM H_2SO_4 at flow rate of 1.0 ml min^{-1}. Prior to the HPLC injection, all of the samples were centrifuged at 9000 rpm for 20 minutes and filtered through 0.45 µm syringe filters. Each analysis was performed in triplicate.

1.3 RESULT

Nine runs of experiment were conducted as shown in Table 2. The maximum and minimum glucose yields are respectively equivalent to 0.911 g/g and 0.459 g/g. The yields are based on weight mixture of the stem and leave. From these nine runs of experiment using Taguchi's methodology, ANOVA analysis was performed and summarized in Table 3.

Table 2 Glucose yield from 9 runs of hydrolysis process

Run	Temperature (ºC)	pH	Acid Conc. (M)	Time (min)	Max glucose yield (g/g)
1	160	4	0.12	10	0.459
2	180	4	0.14	30	0.816
3	140	5	0.12	30	0.489
4	180	5	0.16	10	0.650
5	160	6	0.16	30	0.505
6	160	5	0.14	20	0.644
7	180	6	0.12	20	0.543
8	140	6	0.14	10	0.911
9	140	4	0.16	20	0.547

Table 3 ANOVA analysis for the process

Source	Sum of square	DF	Mean Square	F-value	Prob>F	
Model	23.75	4	5.94	24.50	0.0045	significant
A (Temp)	5.35	2	2.67	11.03	0.0236	
C (Acid conc)	18.41	2	9.20	37.97	0.0025	
Residual	0.97	4	0.24			
Cor Total	24.72	8				

$R^2 = 0.9608$ Adj-R^2=0.9216 Pred-$R^2 = 0.8015$

Table 3 showed that the model F-value of 24.50 which implies that the model is significant. There is only a 0.45% chance that a "Model F-Value" this large could occur due to noise. The Pred-R^2 of 0.8015 is in reasonable agreement with the Adj-R^2 of 0.9216. In this case only temperature and acid concentration are the significant model term for the hydrolysis process. Final equation in terms of coded factors represented by Equation (1) where the power number of the model equation is -2.38.

$$(\text{Glucose yield})^{-2.38} = +3.77 + 1.00 * A[1] - 0.88 * A[2] + 1.61 * C[1] - 1.86 * C[2] \quad (1)$$

Figures 1 and 2 showed one factor plot for maximum glucose yield which is the inverse of model equation term. In another word, the lowest value is the maximum yield. Figure 1 showed highest yield is obtained at 180^{o}C of temperature while Figure 2 showed highest yield was achieved at 0.14 M of HNO_3 concentration.

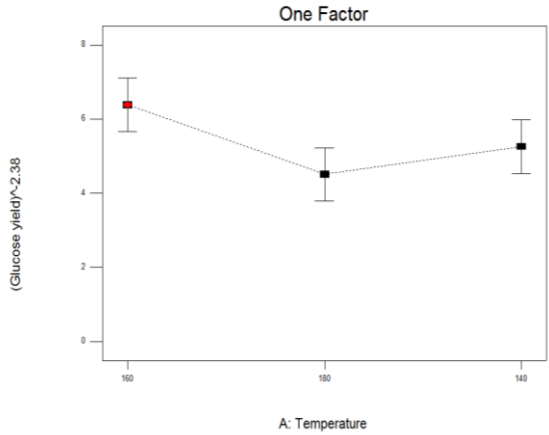

Figure 1 One factor plot for maximum glucose yield based on temperature

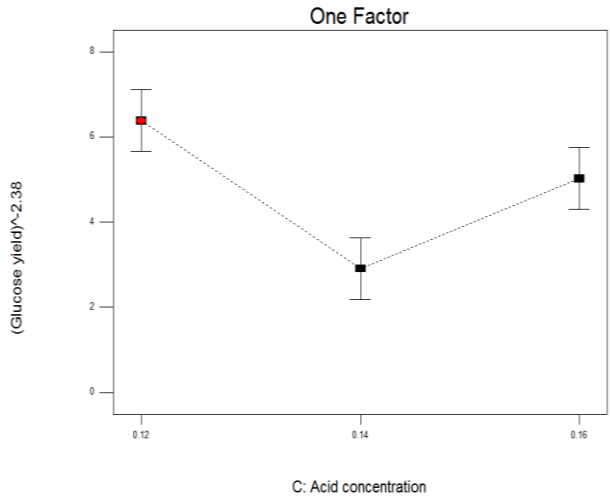

Figure 2 One factor plot for maximum total sugar based on acid concentration.

Table 4 suggested conditions for maximum glucose yield at desirability of 1. Time and pH are insignificant to the yield, thus they varies between 4 to 6 and 10 to 30, respectively. The glucose yield is equivalent to 0.929 g/g. A validation run was conducted to check yield at suggested conditions at temperature of 180^{o}C, pH of 4, acid concentration of 0.14 M and hydrolysis duration in 10 minutes. The glucose yield obtained was 0.991 g/g which was 6.67% higher than predicted.

Table 4 Suggested condition for maximum total sugar based on Taguchi's design

No	Temperature	pH*	Acid concentration	Time*	(Glucose yield)$^{-2.38}$	Desirability	
1	180	4	0.14	30	1.03149	1	Selected
2	180	4	0.14	10	1.03149	1	
3	180	5	0.14	10	1.03149	1	
4	180	6	0.14	10	1.03149	1	
5	180	4	0.14	20	1.03149	1	
6	180	5	0.14	20	1.03149	1	
7	180	6	0.14	20	1.03149	1	
8	180	5	0.14	30	1.03149	1	
9	180	6	0.14	30	1.03149	1	

1.4 CONCLUSION

Hydrolysis process using diluted HNO_3 acid was successfully conducted. The maximum yield was obtained at 0.911 g/g using Taguchi's methodology. ANOVA analysis showed that only two factors significantly affect the yields which were temperature and acid concentration. From the modeling equation, the software suggested several conditions for maximum glucose yield. One of the runs was selected and repeated. The yield obtained was 6.67% higher than predicted which was at yield of 0.991 g/g. The glucose is essential for fermentation process especially in production of biofuel.

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