OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF RAMIE DECORTICATION WASTE-BASED CELLULOSE USING RESPONSE SURFACE METHODOLOGY

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Abstract

Ramie is a herbaceous species which is usually used to produce fibre. The decortication process of ramie will leave the waste that still contain high cellulose. The high cellulose content of ramie decortication waste is a potential raw material for producing bioethanol.Cellulose conversion into bioethanol need four consecutive process e.g. pretreatment, hydrolysis, fermentation and purification. This research is combining microwave heating in the alkaline pretreatment toward the ramie decortication waste and followed by investigating the optimization of enzymatic hydrolysis of ramie decortication waste-based cellulose. The cellulase used in the hydrolysis process was isolated from cow rumen fluids. Response Surface Methodology was used for the optimization of hydrolysis process. A 2^3Central Composite Design (CCD) was used to develop statistical model and analyze the effect of each variables, which are hydrolysis time (36-60 hr), solid liquid rasio (2-4%) and temperature (35-45^{0}C). The optimum condition obtained for the hydrolysis of RDW was temperature 41^{0}C, solid liquid rasio 3,4% and time 48,9 hr with glucose content was 3,51 mg/g.

Keywords:*cow rumen, enzymatic hydrolysis, ramie decortication waste*

Introduction

Ramie (Boehmeria nivea) is a herbaceous perennial monoecious species belonging to the family Urticaceae. Ramie commonly known as China grass is considered to be originated and domesticated in China. It is mainly grown in temperate and tropical areas. Ramie is grown in China, Brazil, Indonesia, Philippines, Korea, Vietnam, Japan, India and other South Asian countries. While the main producers of ramie today are China, Philippines and Brazil. As the major producer of ramie fibre, China is contributing to 96.3% of the ramie global production (Romanzini et al., 2012; Mitra et al., 2013).

In the production of ramie fibre, stems of ramie was harvested, decorticated, cortex scrapped, degummed and bleached. The decortication process was tended to removed the cortex and bark of ramie. Commonly from around 800-1000 kg cane of ramie stems will produces approximately 35-45 kg fibre (Mitra et al., 2013). Waste of the decortication process, which up to 75% of the cane was usually returned to the field for enriching the organic matter content of the soil. Purwati (2010) stated that cellulose content of the waste of decortication process was up to 63.27%. The high content of cellulose of ramie decortication waste has made it as a potential raw material for various chemicals of cellulose derivatives. One of them was bio-ethanol.

Cellulose conversion into bioethanol need four consecutive process e.g. pretreatment, hydrolysis, fermentation and purification (Taherzadeh et al., 2007). The hydrolysis process can be carried out chemically by applied acid solution or enzymatically. Enzymatic hydrolysis of cellulose offers many advantages over acid hydrolysis, such as mild hydrolysis condition, high yield of hydrolysis and there is no formation of inhibitory product (Taherzadeh et al., 2007; Wahlstrom and Suurnakki, 2015). Enzymatic hydrolysis of cellulose is performed in synergy by different cellulolytic enzymes (Wahlstrom and Suurnakki, 2015). It was well known that cellulases constitute the second largest cost factor in the bioethanol production. Thus, the search of the possibility for the utilization of various source of enzyme for cellulose conversion is continously researched and investigated.

In the present work, we carried out an combination of microwave heating in the alkaline pretreatment toward the waste of decortication process of ramie and followed by investigating the optimization of enzymatic hydrolysis of ramie decortication waste-based cellulose. The cellulase used in the hydrolysis

process was isolated from cow rumen fluids. Response surface methodology (RSM) was used to determine the optimum condition of the hydrolysis process.

MATERIALS AND METHODS

A. Materials

Ramie decortication waste (RDW) as lignocellulosic material was gained from ramie processing industry in Wonosobo, Central Java Indonesia. The waste choosen is the fresh waste that is obtained after the decortication process running. The waste is washed with distilled water and dried for 24 hours in 105^0C. Dried ramie decortication waste than mashed and sieved in 18 mesh. The fine grained waste is stored in sealed container in room temperature and called 'sample'. The alkaline used for pretreatment is calcium hydroxide/lime.

B. Method of Pretreatment

Ten gr of sample was added in 6% $Ca(OH)_2$ solution with solid liquid ratio 1 : 20. The solution is then pretreated using microwave (power 30% from the maximum power 399W) for 15 minutes. After pretreatment, solution was filtered. The solid part is washed until pH 7 and dried for 4 hours.

C. Method of enzyme isolation from cow rumen fluids

Cow rumen fluids is taken from the fresh cow rumen and filtered in cold condition. The filtrate is then centrifuged 10000 rpm for 10 min in 4^0C to separate the supernatant from the cells. Supernantant is used as raw enzyme source. Supernatant was then reacted with 60% ammonium sulfat and agitated used stirer for 1 hr. The fluids is then stored for 24 hr in 4^0C. After taht, supernatant is centrifuged 10000 rpm for 15 min in 4^0C. The enzyme produced is collected and suspended in buffer phosphat pH 7,0.

D. Method of hydrolysis

Pretreated sample was added with 50 ml buffer phosphat pH 5. The rasio is based on research design. The mixture is then agitated using shaker for 15 min with 150 rpm. One ml enzyme is added to the mixture and the mixture is incubated in 150 rpm mechanical shaker for spesific time and temperature. After incubation time, sample is filtered, the supernatant is then analyzed its glucose content.

E. Analysis of glucose content

Glucose content was analyzed using Luft Scoorl methods.

F. Experimental design and statistical analysis

Experimental design use Central Composite Design (CCD) to investigate the effects of different factors on hydrolysis process. The software used was STATISTICA 8,0 to obtain the analysis of variance (ANOVA) and polynomial regression equation. The CCD contains 16 experiments with dependent variable is glucose content, while the three independent variables are temperature, time and solid liquid ratio. The range and levels of variables optimized are given in Table 1. A mathematical model that describing relationship between dependent and independent variable will follow equation (1) below :

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 \quad \text{..... (1)}$$

The quality of fit of the second order equation was expressed by the coefficient of determination R^2 and its statistical significance was determined by the F-test. The p value (probability value) were used as a tool to check the significance of the interaction effects (S. Ezhumalai, and V. Thangavelu, 2010)

Table 1. Range and levels of independent variables

Variable	Range and levels				
	Star point (-α/-1,682)	Low level (-1)	Center level (0)	High level (+1)	Star point (+α/1,682)
Temperature (^{0}C)	31,6	35	40	45	48,4
Time (hr)	27,8	36	48	60	68,2
Solid liquid rasio (% b/v)	1,3	2	3	4	4,7

RESULT AND DISCUSSION

The experimental results together with each independent variables are listed in Table 2, while Table 3 showed the analysis of variance (ANOVA).

Table 2. Experimental Response

NO	X_1	X_2	X_3	Y
1	35	36	2	1.728
2	35	36	4	2.448
3	35	60	2	1.728
4	35	60	4	2.448
5	45	36	2	1.248
6	45	36	4	2.928
7	45	60	2	2.448
8	45	60	4	2.928
9	31,6	48	3	3.168
10	48,4	48	3	2.928
11	40	27,8	3	2.688
12	40	68,2	3	1.448
13	40	48	1,3	2.448
14	40	48	4,7	2.688
15	40	48	3	3.408
16	40	48	3	3.408

X_1 = temperature (^{0}C)
X_2 = time (hr)
X_3 = solid liquid rasio (%b/v)
Y = glucose content (mg/g)

Table 3. Analysis of ANOVA

Factor	SS	DF	Mean square	F	p
X_1	0,046	1	0,046	0,143	0,718
X_2	0,057	1	0,057	0,177	0,688
X_3	1,174	1	1,174	3,622	0,106
X_1X_2	0,180	1	0,180	0,555	0,484
X_1X_3	0,065	1	0,065	0,199	0,670
X_2X_3	0,180	1	0,180	0,555	0,484
X_1X_1	0,336	1	0,336	1,039	0,347
X_2X_2	2,673	1	2,673	8,248	0,028
X_3X_3	1,203	1	1,203	3,712	0,102
Error	1,944	6	0,324		
Total SS	6,592	15			

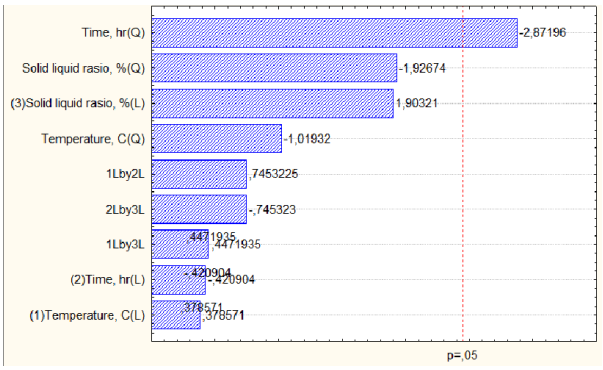

Figure 1. Pareto chart diagram

From the Table 3, we can conclude that only X_2X_2 variabel is significant in this hydrolysis process, since it has the highest score of F and p<0,05. Another variabel, including interaction factor is not significant for

the process. The result also shown on the Figure 1, in which X_2X_2 give the highest score.

The coefficient determination of R^2 was found only 70,5%. This number shows overall variation in the data accounted by the model. The regression model with R^2 higher than 0,90 is considered to have very high correlation. Since the R^2 resulted only 70,5% thus mean around 29,5% total variation is not explained by the model.

Enzymatic hydrolysis is one method of hydrolysis which is reported has many advantages. It uses mild condition and can lead to 100% conversion of cellulose (Taherzadeh and Karimi, 2007). In this method, pretreatment plays an important role, since without any pretreatment, the conversion of native cellulose to sugar is extremely low. Without any pretreatment, cellulose is well protected by the matrix of lignin in macrofibrils. Therefore, the reduction of lignin content in pretreatment process will impact on the hydrolysis step. When the lignin content is low, then the hydrolysis rate will increase and produce more sugar.

Another important factor affecting the enzymatic hydrolysis result is the enzym used. This research use the enzyme isolated from cow rumen. The enzyme activities inthe rumen are diverse, including plant cell wall polymers, (e.g. cellulases, xylanases), amylases and also those that degrade specific plant toxins (Wang and McAllister, 2002). The diversity of this enzyme activities will affect the hydrolysis process and make the conversion lower. Moreover, Wang and MaAllister (2002) stated that although there are many microbial in the rumenof ruminant, but the cell wall polysaccharides are rarely completely degraded. The reason of this incomplete degradation can be because of biochemical and physical barriers of substrateor limits on the retention time.

Figure 2 until 4 give information about the contour plot of two variables toward glucose content, while another one variable kept constant. From the result of hydrolysis, the significant variable is time (X_2). Time is known as a significant factor because enzymatic hydrolysis is a biological reaction catalyzed by cellulase. This process needs longer time than the known routes of acid hydrolysis (Fang et al, 2010). The optimum condition obtained for the hydrolysis of RDW was temperature 41^0C, solid liquid rasio 3,4% and time 48,9 hr with glucose content was 3,51 mg/g.

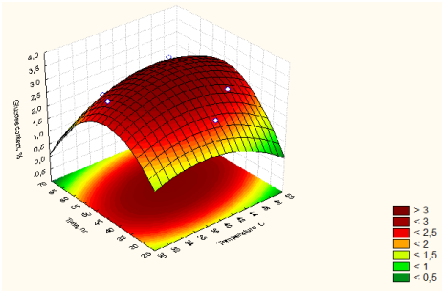

Figure 2. Contour plot of time and temperature on solid liquid rasio 3%

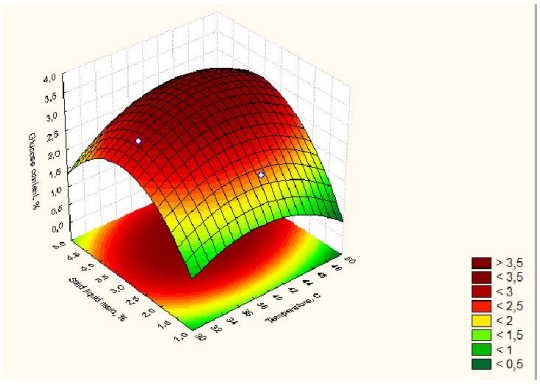

Figure 3. Contour plot of solid liquid rasio and temperature on time 48 hr

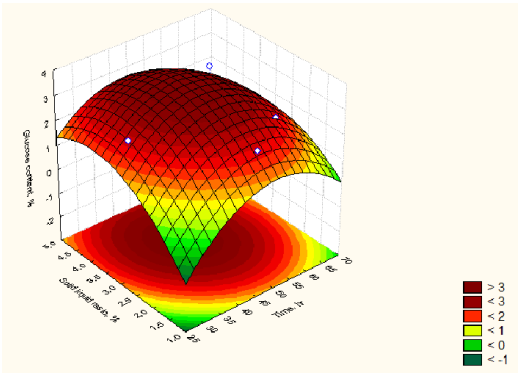

Figure 4. Contour plot of solid liquid rasio and time on temperature 40^0C

CONCLUSION

Cow rumen has been isolated its enzyme and used to hydrolyze pretreated of ramie decortication waste. The optimization was done using response surface methodology with three independent variables, they are temperature, time and solid liquid rasio. From the ANOVA analysis, the significant factor for hydrolysis process is time. The optimum condition obtained for the hydrolysis of RDW was temperature 41^0C, solid liquid rasio 3,4% and time 48,9 hr with glucose content was 3,51 mg/g.

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