Optimization of Acid Hydrolysis Process on Macro-alga *Ulva lactuca* for Reducing Sugar Production as Feedstock of Bioethanol

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Abstract- The possibility of reducing sugar exploration as energy feedstock from *Ulva lactuca* through acid hydrolysis process was investigated in this study. In turn, reducing sugar can be fermented into ethanol as energy. The effects of hydrolysis time, sulfuric acid concentration, and sulfuric acid volume on the response of reducing sugar were also examined. Pretreatment procedure such as clean the *U. lactuca*, size reduction and drying process need to be done prior to the hydrolysis step. Analysis on reducing sugar concentration has been conducted by using dinitrosalicylic acid (DNS) method. The optimum condition of hydrolysis process determined by Response Surface Methodology (RSM) based on Central Composite Circumscribed (CCC) design as the original form of Central Composite Design (CCD). The analysis of variance (ANOVA) for response surface regression also has been observed. The optimum result of the hydrolysis process on *U. lactuca* to produce reducing sugar (29.050 mg/mL) were obtained at 87.4518 minutes of hydrolysis time, 3.72934% of sulfuric acid concentration, and 142.780 mL of sulfuric acid volume, with the desirability of 0.916242. The yield of reducing sugar from hydrolysis process of *U. lactuca* can be expressed by the equation of $Y = -59.04 + 0.4968 X_1 + 10.17 X_2 + 0.666 X_3 - 0.002853 X_1^2 - 1.0408 X_2^2 - 0.002118 X_3^2 - 0.01677 X_2X_3$. Where Y is yield of reducing sugar concentration, X_1 is hydrolysis time, X_2 is sulfuric acid concentration, and X_3 is sulfuric acid volume.

Keywords Alga, ANOVA, diluted acid hydrolysis, reducing sugar, *Ulva lactuca*.

1. Introduction

It has been for centuries that for their energy needs, people are depending on fossil fuels such as oil, coal and natural gas [1, 2, 3]. In addition to its increasingly depleted and non-renewable availability, burning of fossil fuels could lead to an accumulation of larger CO_2 [4] and the consequent worsening of global warming [5-6]. With these problems, it is necessary to find a solution by searching for alternative renewable energy sources [7], such as one derived from cellulosic biomass. Corn, wheat and sugarcane can be used as sources of alternative energy, but unfortunately, they compete with food.

Marine cellulosic biomass can be considered as a source of energy [8-9] that will not compete with food [10]; they are friendly and beneficial, and they have a simple cellular structure. Most compounds of marine biomass are carrageenan, cellulose and hemicellulose; their polymers can be broken down into molecules of an individual sugar by acid hydrolysis process [11]. One of the marine cellulosic biomasses is green macro-alga *U. lactuca* or sea lettuce that grows well in the coastal of the South Sea of Java, Indonesia; their numbers are abundant especially in the dry season [12]. Green macro-alga biomass *U. lactuca* has a good growth rate with high carbohydrate content and is considered a water plant that has potential as an energy crop, so it is important to be considered in bioenergy production [13]. The biochemical compositions contained in *U. lactuca* such as cellulose, hemicellulose and lignin depend on its growing condition. Cellulose and hemicellulose contained in biomass can be degraded into reducing sugar which is the source of energy because reducing sugar can be converted to ethanol chemically and biologically [14] through fermentation process and distillation process [15-16]. Concentration of reducing sugar can be measured by high-performance liquid chromatography (HPLC) [17-18] and DNS method [19-20].

The process of cellulose degradation into reducing sugar can be conducted in various ways. These are enzymatic hydrolysis processes [21-22] through three successive process stages: glycosidic bond breaking process, cellobiose forming process, and cellobiose conversion process to glucose [23]. Immobilization of cellulase enzyme is even capable of achieving hydrolysis conversion to *Chlorella sp.* microalgae by 62% [24]. According to Li, enzymatic hydrolysis is restricted by the enzyme activity and the cost of enzyme is expensive [25]. In addition to enzymatic hydrolysis, reducing sugar can also be obtained through subcritical water hydrolysis process under variety of temperature and acetic acid as catalyst [6, 17] and under diversity of hydrolysis temperature and time [25]. Another way to get the reducing sugar is by combining acid hydrolysis process and enzymatic hydrolysis process [26-27], through a solution plasma process [28], and a combination of hydrothermal and enzymatic hydrolysis processes [5]. Diluted acid such as sodium hydroxide also able to eliminate lignin and hemicellulose and increase the yield of sugar from cellulose during hydrolysis process [29].

Lignocellulosic biomass such as agricultural residue with significant lignin content is also capable of producing less sugar but its procedure is more complex since lignin in cell walls inhibits hydrolysis process [29]; otherwise the process of exploitation reducing sugar from cellulosic biomass such as *U. lactuca* is simpler because it does not contain lignin. Surprisingly acid hydrolysis is suitable for production of reducing sugar from macro-algae, whereas acidity and volume of acid actuated during hydrolysis process will have a significant role to limit the hydrolysis time [15]. Acid hydrolysis process has been widely used for sugar exploration from organic materials, and this study applied this method in order to hydrolyze *U. lactuca* for reducing sugar exploration [27].

The study aimed to explore the reducing sugar content of *U. lactuca* by diluted acid hydrolysis at different hydrolysis times and with varying acid concentration and acid volume. From the results obtained it is expected that *U. lactuca* can have good prospect to be used as feedstock of ethanol as energy. Other objectives were to optimize the free variables of hydrolysis time, sulfuric acid concentration, and sulfuric acid volume on reducing sugar obtained by RSM with CCD concept [30-32]. RSM is chosen because it has advantages compared with conventional methods, it is more accurate and less required treatment, therefore more efficient time wise and cost lesser. Data analysis was solved by ANOVA, using Minitab 17 software application.

2. Materials and Methods

2.1. Materials

The main material used during experiments was green macro-alga of *U. lactuca* obtained from Kondangmerak Beach, Malang-Indonesia. The analysis result of that Indonesian *U. lactuca* contains concentrations of HWS, hemicellulose, cellulose, and lignin around 50%, 27%, 20%, and 1.3% respectively. As in general, lignin content of *U. lactuca* relatively small because this biomass is categorized as cellulosic biomass, not as lignocellulosic biomass with significant concentration of lignin. This *U. lactuca* has a very minor ash content, therefore it can be ignored as its sample was completely free from sand. Because algae with sand pollutant can cause high content of ash in its structure.

Supporting chemicals such as sulfuric acid, sodium hydroxide, phenol, sodium sulfite, and potassium sodium tetrahydrate are purchased from Merck; and dinitrosalicylic acid is purchased from Sigma. Processing of cellulosic to reducing sugar consist of two main processes, they were pretreatment process and hydrolysis process.

2.2. Pretreatment Process

Pretreatment is required to alter *U. lactuca* purity and size in order to achieve high yield of reducing sugar. In this step, *U. lactuca* first washed and rinsed with aquadest to avoid pollutants such as sand which can cause high ash content. The wet alga material then dried for 2-3 days under the sun and followed by drying in an oven at 40-50^{o}C, until a stable weight was obtained. Moreover, dry alga was grinded and sifted to obtain alga powder with a particle size of about 100 mesh. Initial analysis was performed on alga for reducing sugar content with DNS method [33] and for concentrations of hot water soluble (HWS), hemicellulose, cellulose and lignin by using Chesson method [34].

2.3. Hydrolysis Process

Hydrolysis step is an important process to convert cellulose consists in *U. lactuca* into reducing sugar. Experiments were conducted in an autoclave at 121^{o}C of temperature and under variations of hydrolysis times (60, 90, and 120 minutes), sulfuric acid volumes (125, 150, and 175 mL) and concentrations of sulfuric acid (2%, 3.5% and 5%). This hydrolysis temperature is well below the auto-ignition temperature of cellulosic material. It was therefore safe because in general cellulose material contains combustible oil that can burn by itself at its auto-ignition temperature [35]. After experiments, reducing sugar concentrations were analyzed by using DNS method with glucose as a standard, and absorbance level at 575 nm was measured with a spectrophotometer. Concentrations of HWS, hemicellulose and cellulose were investigated by using Chesson method.

2.4. RSM for Statistical Analysis

Identification of correlation between research variables to reducing sugar response was studied by RSM using Minitab 17 software. At first it was tracking the test point with zero order model to get the peak test points (0), (-1) and (+1). Next, first order regression model was applied for the relationship between factor and response in the form of equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \varepsilon \qquad (1)$$

At first order, to determine the optimum conditions, the experimental design and the statistical analysis were applied by the factorial design of 2^k+6, where k is the number of factors and 6 is the number of center point repetitions. From the three zero phase test points used, it will be known whether there is a significant curvature or not. If the first order regression model proved to be unsuitable, then the polynomial approach will be applied with regression of order-2 model by using RSM in the form of equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

Where Y is the responds of reducing sugar (mg/mL), β_0 is a constant coefficient, β_i is a linear coefficient, β_{ii} is the quadratic or square coefficient, β_{ij} is the two-way interaction coefficient, X_i is the factor where i = 1,2,3,…k, X_j is the factor where j = 2,3,…k, and ε is an error. In the present study, as independent variables were hydrolysis time (X_1, minute), sulfuric acid concentration (X_2, %), and sulfuric acid volume (X_3, mL).

In order-2, the quadratic model test was performed using CCC design, which is the original form of CCD. Comparison of the accuracy between the prediction and results of the study was conducted with ANOVA. Furthermore, from the 2nd order, model will be observed on stationary point, surface response characteristics and optimization model.

3. Results and Discussion

The preliminary analysis of *U. lactuca* on the reducing sugar content before hydrolysis was 5.233 mg/mL. While the levels of HWS, hemicellulose, cellulose, and lignin before and after the hydrolysis process (averagely) is given in Fig. 1 as follow.

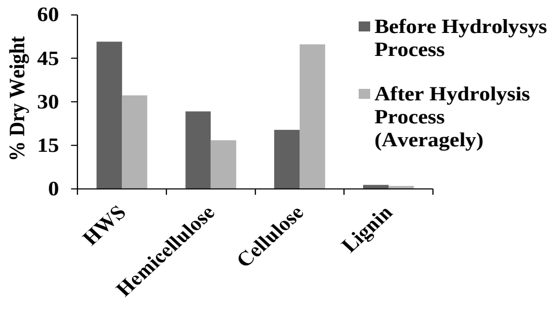

Fig. 1. Percentage of HWS, hemicellulose, cellulose, and lignin before and after hydrolysis process

It can be seen from Fig. 1, after the hydrolysis process, all the concentrations of HWS, hemicellulose and lignin became lower. HWS levels fell from 50.76% to 32.19%, the hemicellulose level decreased from 26.67% to 16.78% and the lignin level slightly dropped from 1.33% to 1.04%. Meanwhile, the cellulose levels increased significantly from 20.33% to 49.82%, indicating that the hydrolysis process using sulfuric acid successfully converts cellulose into reducing sugar which is a mixture of carbohydrate polymers of cellulose [15, 27].

The cellulose concentration mentioned above, increases more than 100%, which is slightly higher than the results of a study conducted by Sugiharto who was using enzymatic hydrolysis on the empty fruit bunch of palm [21]. Under acidic conditions and high temperatures, hemicellulose will be degraded into xylose, mannose, acidic acid, galactose, glucose. Cellulose is degraded into glucose only and lignin becomes a phenolic component. Meanwhile at high pressure and temperature, xylose will be degraded into furfural and hexose degraded to HMF (5-hydroximethyl furfural) [15].

3.1. Tracking of optimum points

During the study, the free variables or factors of hydrolysis time (X_1, minutes), acid concentration (X_2, %), and acid volume (X_3, mL) were applied to conversion process of *U. lactuca* into reducing sugar (Y, mg/mL) in response. A few points have been observed and with tracking studied through the order-0, the experimental design points are derived as summarized in Table 1 below.

Table 1. Factors and experimental design levels

No	Factor	Symbol	Level		
			-1	0	+1
1	Hydrolysis time, minutes	X_1	60	90	120
2	Acid concentration, %	X_2	2	3.5	5
3	Acid volume, mL	X_3	125	150	175

Table 1 identify the three independent factors namely hydrolysis time (minutes), acid concentration (%), and acid volume (mL). The levels were selected based on preliminary study results using order-0 tracking worked. The design factors with low (-1) and high (+1) levels are hydrolysis time (60 and 120 minutes), acid concentration (2 and 5 %), and acid volume (125 and 175 mL). The zero levels or central values were 90 minutes, 3.5 %, and 150 mL for hydrolysis time, acid concentration, and acid volume, respectively.

3.2. Experimental Design Order-1

From the points of experimental design level, a first order simulation process with ANOVA of regression CCC model on concentration of reducing sugar was performed. The model of linear regression equation obtained in uncoded units was:

$$Y = 24.6 - 0.062 X_1 + 2.18 X_2 + 0.022 X_3 + 0.0071 X_1X_2 + 0.00015 X_1X_3 - 0.0168 X_2X_3 \qquad (3)$$

The simulation result shows that the order-1 model was not significant because P-value of linear model was 0.995, this number is much bigger than 0.05. And the value of P-value of curvature was 0.000, this value was very less than 0.05 with a very small R-sq (7.18%), meaning that there was an indication of the curvature leading to the order-2 model. Therefore, the linear model was not suitable for the model design of the effect of hydrolysis time, acid concentration, and acid volume on reducing sugar response during hydrolysis process.

3.3. Experimental Design Order-2

For the experimental design model of second order, values of factors and response needed is tabulated in Table 2. While the results of ANOVA of regression CCC model on reducing sugar concentration response are given in Table 3 for all interaction factors and in Table 4 for selected interaction factors.

Table 2. Independent variables for regression on CCC model of reducing sugar response

Run	Factors/Variables			Coded Levels			Response
	Hydrolysis time (min), X_1	Acid concentration (%), X_2	Acid volume (mL), X_3	X_1	X_2	X_3	Reducing sugar (mg/mL), Y
1	60	2	125	-1	-1	-1	22.88
2	120	2	125	1	-1	-1	21.89
3	60	5	125	-1	1	-1	25.14
4	120	5	125	1	1	-1	24.01
5	60	2	175	-1	-1	1	23.46
6	120	2	175	1	-1	1	21.51
7	60	5	175	-1	1	1	21.78
8	120	5	175	1	1	1	22.54
9	39.546	3.5	150	-1.682	0	0	22.32
10	140.454	3.5	150	1.682	0	0	20.19
11	90	0.97731	150	0	-1.682	0	20.78
12	90	6.02269	150	0	1.682	0	23.01
13	90	3.5	107.955	0	0	-1.682	26.21
14	90	3.5	192.045	0	0	1.682	23.34
15	90	3.5	150	0	0	0	27.78
16	90	3.5	150	0	0	0	29.01
17	90	3.5	150	0	0	0	28.97
18	90	3.5	150	0	0	0	28.11
19	90	3.5	150	0	0	0	29.86
20	90	3.5	150	0	0	0	29.67

Tabel 3. ANOVA of regression CCC model on reducing sugar concentration (order 2), all interaction factors

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	188.004	20.8893	33.33	0.000
Linear	3	14.124	4.7080	7.51	0.006
X_1	1	3.478	3.4783	5.55	0.040
X_2	1	4.097	4.0973	6.54	0.029
X_3	1	6.548	6.5484	10.45	0.009
Square	3	169.784	56.5946	90.29	0.000
X_1*X_1	1	95.039	95.0390	151.63	0.000
X_2*X_2	1	79.029	79.0287	126.09	0.000
X_3*X_3	1	25.244	25.2443	40.28	0.000
2-Way Interaction	3	4.096	1.3654	2.18	0.154
X_1*X_2	1	0.826	0.8256	1.32	0.279
X_1*X_3	1	0.108	0.1081	0.17	0.687
X_2*X_3	1	3.163	3.1626	5.05	0.048
Error	10	6.268	0.6268	0.84	0.574
Lack-of-Fit	5	2.858	0.5716		
Pure Error	5	3.410	0.6820		
Total	19	194.272			
Model Summary					
S	R-sq		R-sq(adj)	R-sq(pred)	
0.791699	96.77%		93.87%	85.51%	

It can be seen from Table 2, the total number of experiments (runs) was 20 which formulated from $2^k + 2k + 6$, where k is the number of independent variables (k = 3) and 6 is the number of replicates at the centre point. The hydrolysis conditions were based on CCC design

The Regression equation model of order-2 in uncoded units for all interaction factors obtained was:

$$Y = -54.7 + 0.4485 X_1 + 9.52 X_2 + 0.652 X_3 - 0.002853 X_1X_1 - 1.0408 X_2X_2 - 0.002118 X_3X_3 + 0.00714 X_1X_2 + 0.000155 X_1X_3 - 0.01677 X_2X_3 \quad (4)$$

The ANOVA analysis of equation (4) for all interaction factors presented the results as tabulated in Table 3. From the surface response analysis of order-2, both the linear model and square model of the main simulation influence the reducing sugar concentration response, while the 2-way interaction model has no effect on the response except the interaction between acid concentration (X_2) and acid volume (X_3). While the P-value of lack-of-fit is not significant because of its value > 0.05, then the order regression model 2 is feasible to use. Since the interactions of hydrolysis time (X_1) to acid concentration (X_2) and hydrolysis time (X_1) to acid volume (X_3) have no effect on the response, then these two interactions were eliminated and re-arrangement of order-2 model need to be carried out with results as given in Table 4 as follow:

Tabel 4. ANOVA of regression CCC model on reducing sugar concentration (order 2), selected interaction factors

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	187.070	26.7243	44.53	0.000
Linear	3	14.124	4.7080	7841	0.004
X_1	1	3.478	3.4783	5.80	0.033
X_2	1	4.097	4.0973	6.83	0.023
X_3	1	6.548	6.5484	10.91	0.006
Square	3	169.784	56.5946	94.30	0.000
X_1*X_1	1	95.039	95.0390	158.36	0.000
X_2*X_2	1	79.029	79.0287	131.69	0.000
X_3*X_3	1	25.244	25.2443	42.06	0.000
2-Way Interaction	1	3.163	3.1626	5.27	0.041
X_2*X_3	1	3.163	3.1626	5.27	0.041
Error	12	7.202	0.6001		
Lack-of-Fit	7	3.792	0.5417	0.79	0.624
Pure Error	5	3.410	0.6820		
Total	19	194.272			
Model Summary					
S	R-sq		R-sq(adj)	R-sq(pred)	
0.774682	96.29%		94.13%	89.70%	

The Regression equation model of order-2 in uncoded units for selected interaction factors obtained was:

$$Y = -59.04 + 0.4968 X_1 + 10.17 X_2 + 0.666 X_3 - 0.002853 X_1X_1 - 1.0408 X_2X_2 - 0.002118 X_3X_3 - 0.01677 X_2X_3 \quad (5)$$

The ANOVA analysis of equation (5) for selected interaction factors presented the results as summarized in Table 4. As can be seen from Table 4, the result of the second ANOVA order-2 is suitable because all linear interactions, square and inter-factor selected are significant with all p-value less than 0.05 and the lack-of-fit value bigger than 0.05. Therefore, the order-2 model is in accordance with the above equation with R-sq of 96.29%.

The following Fig. 2 is the result of the hydrolysis process to obtain the reducing sugar of U. lactuca shown in the form of contours. As can be seen from Fig. 2, the hydrolysis process that has been carried out on U. lactuca alga produces the highest reducing sugar with hydrolysis time between of 70-110 minutes, the addition of sulfuric acid with a concentration of about 2.6-4.5% and the application of sulfuric acid with volume about 120-160 mL. The results of these contours if performed in response surface plot will also give the same trend result in form of three dimensional parabolic curves.

To optimize the response, response surface optimizer was implemented with the “maximize goal” to be selected. The result of this optimizing work gives the important points to figure out an efficient process that can be obtained through the conditioning of all parameters at optimum conditions. This response optimization provides in Fig. 3 and Table 5.

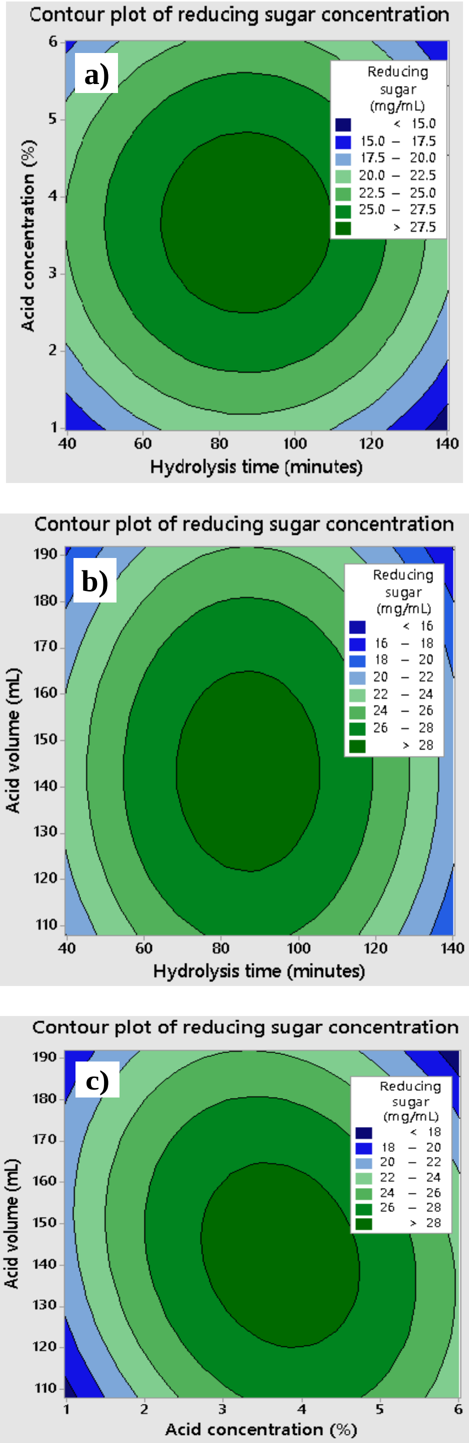

Fig. 2. Contour plot of the influence of variables on reducing sugar concentration. a) Hydrolysis time versus Acid concentration, b) Hydrolysis time versus Acid volume, c) Acid concentration versus Acid volume

The optimum condition of independent parameters in acid hydrolysis process on *U. lactuca* is shown in Fig. 3 and Table 5 respectively. As can be seen from Fig. 3 and Table 5, the smallest and largest responses were 20.19 mg/mL and 29.86 mg/mL respectively. The solution resulted in 29.050 mg/mL of reducing sugar response by using hydrolysis time of 87.4518 min, sulfuric acid concentration of 3.72934%, and sulfuric acid volume of 142.780 mL. With desirability of 0.916242 or close to 1. Therefore, this model can be assumed to be close to perfection

Reducing sugar concentration during hydrolysis process can be observed as the functions of independent variables hydrolysis time, sulfuric acid concentration and sulfuric acid volume as illustrated in Fig. 4. All calculation of reducing sugar concentrations at the optimum points were using equation (5) stated above. As figured out in Fig. 4, with the hydrolysis time of 90 minutes, 3.5% of sulfuric acid concentration and 150 mL of sulfuric acid volume, the resulting of reducing sugar gave the highest value of approximately 29 mg/mL. Meanwhile, the lowest reducing sugar yield of 21 mg/ml was obtained from the variable treatment of hydrolysis time of 120 min, 5% of sulfuric acid concentration and 175 mL of sulfuric acid volume.

In the experimental of hydrolysis process on *G. verrucosa* conducted by Kim, the result of total reducing sugar (TRS) can be increased through the further process by application of enzyme hydrolysis process after acid hydrolysis process, results in the increase of TRS yield from 34.9% to 46.5% [27]. Referring to the Kim's experiment, further treatment after sulfuric acid hydrolysis using enzymatic hydrolysis from hydrolysis process on *U. lactuca* may also able to increase the yield of the sugar reduction.

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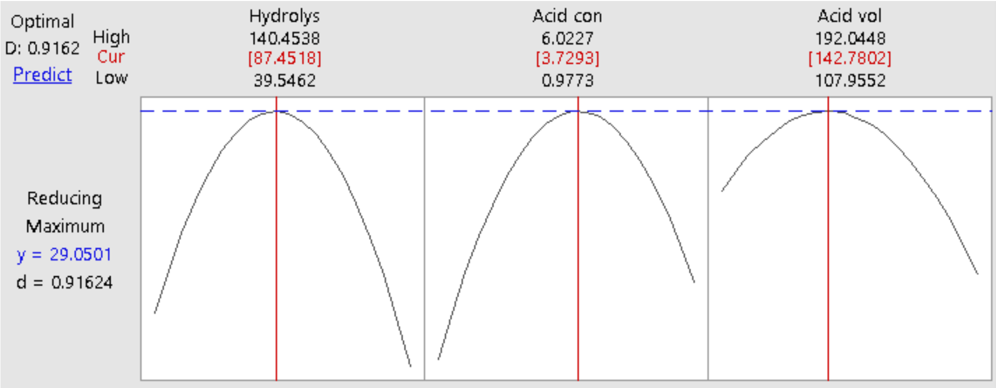


Fig. 3. Independent parameters optimization

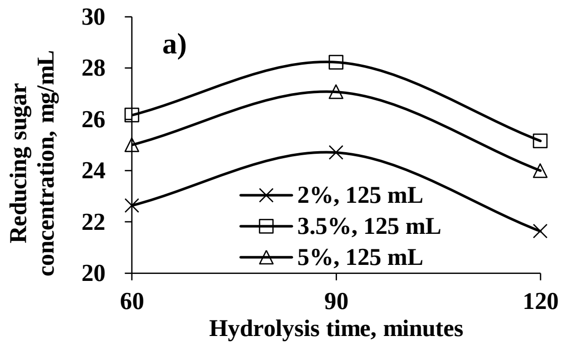
Table 5. Response Optimization: Reducing sugar (mg/mL)

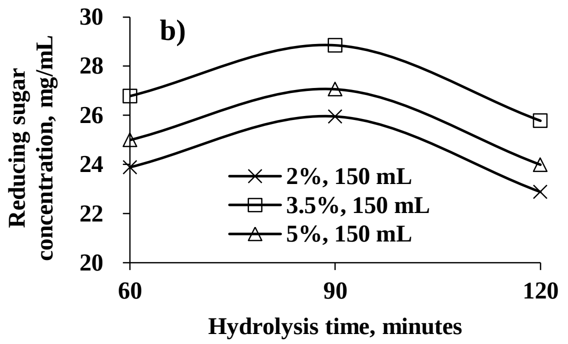
Parameters response	Goal	Lower	Target	Upper	Weight	Importance
Reducing sugar solution (mg/mL)	Maximum	20.19	29.86		1	1

Solution	Hydrolysis time (minutes)	Acid concen tration (%)	Acid volume (mL)	Reducing sugar (mg/mL) Fit	Composite Desirability
1	87.4518	3.72934	142.790	29.0501	0.916242

Multiple Response Prediction Variables	Setting
Hydrolysis time (minutes)	87.4518
Acid concentration (%)	3.72934
Acid volume (mL)	142.780

Response	Fit	SE Fit	95% CI	95% PI
Reducing sugar (mg/mL)	29.060	0.312	(28.370, 29.731)	(27.230, 30.870)





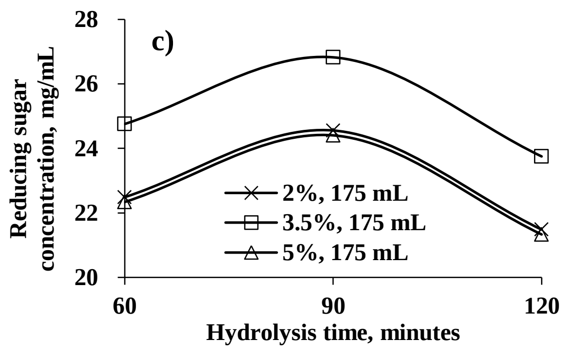


Fig. 4. Effect of hydrolysis time and acid concentration on reducing sugar concentration corresponds to acid volume, a) 125 mL, b) 150 mL, c) 175 mL

In this study, as stated by El-Khair there is a tendency that the concentration of reducing sugar production is decreasing [36], as the declining usage of sulfuric acid concentration. This is because the higher acid concentration will speed up the recovery process of glucose from cellulose. Unfortunately the application of acid with concentrations higher than 3.5% tend to decrease glucose content, it is in accordance with the statement of Ge and Meinita that the high acid concentration will cause the formation of by products such as hydroxymethylfurfural (HMF) during the hydrolysis process and potential to inhibit the glucose recovery process from cellulose [10, 37]. Hydrolysis time is also an important factor for the reducing sugar yield, this phenomenon has in general matching with those reported by Ge [10].

In general, this study shows that the longer the hydrolysis time, the higher the reducing sugar yield. However, after 90 minutes, the reducing sugar yield decreases, which is reasonable because the longer hydrolysis time causes the faster glucose degradation which can result in loss of sugar content.

4. Conclusion

Experiment of the potential of *U. lactuca* as feedstock of energy was conducted in this study. Acid Hydrolysis Process was employed to convert the cellulose on Macro-alga *U. lactuca* into reducing sugar. Hydrolysis time, acid concentration, and acid volume were examined as variables that might affect reducing sugar by using Central Composite Circumscribed (CCC) design as the original form of Central Composite Design (CCD). By using the developed quadratic model, contour plots, and independent parameters optimization, the investigation for the reducing sugar concentration depending on the changes of variables was found to be easily predicted in this study. Second order model on reducing sugar concentration for selected interaction factors is appropriate because all linear interactions, square and inter-factor selected of acid concentration–acid volume are significant with all p-value less than 0.05 and the lack-of-fit value bigger than 0.05. Therefore, the orde-2 model is in accordance with the above equation with R-sq of 96.29%. Result of reducing sugar was in the range of 20.19 mg/mL and 29.86 mg/mL. From this range, the best result based on the model was 29.050 mg/mL of reducing sugar under the applications of 87.4518 min of hydrolysis time, 3.72934% of sulfuric acid concentration and sulfuric acid volume of 142.780 mL. With the desirability of 0.916242 or close to 1 as figured out in Fig. 3. Therefore, this model can be assumed to be close to perfection. From the results obtained, it is evident that the cellulose contained in *U. lactuca* biomass can be degraded into reducing sugar, which in turn can be converted to ethanol. Therefore, research on this subject needs to be continuously developed.

The causes of low reducing sugar content may be due to inhibitors that may occur during hydrolysis processes such as weak acid, furfural, 5-hydroxy methyfurfural (HMF) furan derivatives and phenolic components. This requires further research. Besides, acid hydrolysis process should be continued by enzyme hydrolysis process to increase the yield of reducing sugar content. Although the resulting sugar reduction content is relatively small, green macro-alga *U. lactuca* can be considered as an alternative energy feedstock.

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International Journal of Renewable Energy Research-IJRER

Optimization of Acid Hydrolysis Process on Macro-alga Ulva lactuca for Reducing Sugar Production as Feedstock of Bioethanol

Tri Poespowati

Abstract

The possibility of reducing sugar exploration as energy feedstock from Ulva lactuca through acid hydrolysis process was investigated in this study. In turn, reducing sugar can be fermented into ethanol as energy. The effects of hydrolysis time, sulfuric acid concentration, and sulfuric acid volume on the response of reducing sugar were also examined. Pretreatment procedure such as clean the U. lactuca, size reduction and drying process need to be done prior to the hydrolysis step. Analysis on reducing sugar concentration has been conducted by using dinitrosalicylic acid (DNS) method. The optimum condition of hydrolysis process determined by Response Surface Methodology (RSM) based on Central Composite Circumscribed (CCC) design as the original form of Central Composite Design (CCD). The analysis of variance (ANOVA) for response surface regression also has been observed. The optimum result of the hydrolysis process on U. lactuca to produce reducing sugar (29.050 mg/mL) were obtained at 87.4518 minutes of hydrolysis time, 3.72934% of sulfuric acid concentration, and 142.780 mL of sulfuric acid volume, with the desirability of 0.916242. The yield of reducing sugar from hydrolysis process of U. lactuca can be expressed by the equation of $Y = -59.04 + 0.4968 X_1 + 10.17 X_2 + 0.666 X_3 - 0.002853 X_1^2 - 1.0408 X_2^2 - 0.002118 X_3^2 - 0.01677 X_2X_3$. Where Y is yield of reducing sugar concentration, X_1 is hydrolysis time, X_2 is sulfuric acid concentration, and X_3 is sulfuric acid volume.

Keywords

Alga, ANOVA, diluted acid hydrolysis, reducing sugar, Ulva lactuca.

Full Text:

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