

Submission Confirmation

Dari: International Journal of Industrial Chemistry (IJIC) (em@editorialmanager.com)

Kepada: wenny_i_s@yahoo.com

Tanggal: Kamis, 19 Februari 2015 07.56 GMT+6

Dear Dr Wenny Irawaty,

Thank you for submitting your manuscript.

"Optimization of key variables of subcritical water hydrolysis and fermentation for the enhanced bioethanol production from durian seeds using response surface methodology", to International Journal of Industrial Chemistry.

During the review process, you can keep track of the status of your manuscript by accessing the following web site: <http://ijic.edmgr.com>

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Tanggal: Kamis, 19 Februari 2015 14.22 GMT+6

Dear Dr Wenny Irawaty,

We are pleased to inform you that your submission entitled: "Optimization of key variables of subcritical water hydrolysis and fermentation for the enhanced bioethanol production from durian seeds using response surface methodology" has been assigned to the Editor(s).

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Major Revisions requested IJIC-D-15-00023

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Tanggal: Senin, 4 Mei 2015 01.39 GMT+6

Dear Dr Irawaty,

We have received the reports from our advisors on your manuscript, "Optimization of key variables of subcritical water hydrolysis and fermentation for the enhanced bioethanol production from durian seeds using response surface methodology", which you submitted to International Journal of Industrial Chemistry.

Based on the advice received, I have decided that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments which can be found below, and submit a list of responses to the comments. You are kindly requested to also check the website for possible reviewer attachment(s).

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We look forward to receiving your revised manuscript before 02 Jun 2015.

With kind regards,

Hassan Ali Zamani

Editor in Chief

International Journal of Industrial Chemistry

COMMENTS FOR THE AUTHOR:

Reviewer #1: - Title of the manuscript too long and meaningless.

- It has not mentioned types of fermentation namely aerobic and anaerobic.
- Does Anaerobic fermentation occur in experimental conditions?
- Equation 1 was used for what purpose?
- References should be expanded.

Reviewer #2: The paper deals with recovery of reducing sugars from durian seeds by subcritical water hydrolysis. The paper presents interesting data. However, there are some points that need to be addressed before it can be accepted for publication.

The English is not acceptable for publication. There are several confusing sentences and agreement mistakes. It should be revised by a native speaker.

The authors do not present any discussion of the results comparing them to literature data. Discussion should be improved by comparing with literature data. This problem appears throughout the discussion.

Therefore, I recommend accepting the paper only after major revision.

Some more specific remarks follow.

Subcritical water hydrolysis of durian seeds waste for bioethanol production

**Adi Purnomo, Yulius Andy Wibowo
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Adi Tama Nugraha, Wenny Irawaty &
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Subcritical water hydrolysis of durian seeds waste for bioethanol production

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Abstract The feasibility of bioethanol production using durian seed waste was investigated in this study. The effects of hydrolysis parameters (temperature, time, pressure and solid to water ratio) on the yields of reducing sugars and bioethanol were also examined. Central composite design was used to determine the optimum conditions of both reducing sugars yields obtained from hydrolysis stage and ethanol from reducing sugars fermentation. The optimized values for subcritical water process of durian seeds to produce reducing sugars were achieved at temperature of 139.8 °C; solid to water ratio of 1:30; pressure of 30 bar; and reaction time of 3.58 h with 32.37 % yield of reducing sugars. The fermentation of 20 g L⁻¹ reducing sugars for 72 h gave the highest ethanol concentration, i.e., 9.85 g L⁻¹.

Keywords Durian seed · Bio-ethanol · Subcritical water · Hydrolysis

Introduction

Currently, the development of sustainable transportation fuels is a global challenge [1–3]. The burning of the fossil fuels produces many pollutant gases such as carbon dioxide, NO_x, and SO_x, causing severe environmental problems.

Long-term fossil fuel availability issues also become a big concern; therefore, studies on alternative fuels derived from biomass, called bio-fuels, have gained much attention [4–8]. Depending on the type of the process, feedstock and stage of development, the production of biofuel can be classified into primary (first generation) and secondary (second and third generation) [9].

The production of first generation biofuel is primarily from food crops such as starchy crops (wheat, barley, corn, cassava, and potatoes), sugar crops (sugarcane, sugar beet, and sweet sorghum) and oil seeds [2, 10]. In particular, the United States [11] and Brazil have commercially produced fuel ethanol from those kinds of biomasses [10]. However, the environmental issue and significant economic problems are tightly associated with the first generation of biofuel, the land area needed for growing the crops for bio-fuel production will be in competition with for food production, leading to severe food shortage problems [11, 12]. In addition, the increase in the crop harvesting rates for biofuel production has also raised the concerns about the fertilizer and pesticide pollution, eutrophication, and carbon debt [13–15]. Therefore, due to those limitations of the first generation of bio-fuels, the second and third generation of biofuels have also been developed [14]. Low-cost agricultural residues (corn Stover, wheat straw) and agricultural by-products (rice hulls, corn fibre) have been explored as the potential raw materials for the biofuel production [2].

The third generation of biofuels is made from the biomass from non-arable land or water based on integrated technologies that produce feedstock as well as fuels. As for third generation of biofuel, microalgae with short harvesting cycles and can produce more oil yields (15–300 times) than traditional crops on area bases is thought as a new alternative to biofuel production history [9]. However, scaling up the production of biofuel from microalgae can face

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unsustainable demands on energy, water (1 L biofuel: 3650 L water), and nutrients (nitrogen, phosphorus, and CO₂) required for cultivating this particular feedstock [16]. Thus, this option is not currently feasible. Whereas a second generation of biofuels is considered the most viable one considering that lignocellulosic biomass as the main source of this biofuel is abundantly available in most countries in the world and not competing with food production [17].

As an agricultural country, Indonesia produces different kinds of agricultural products such as rice, fruits, vegetables, cassava, sweet potato, corn, soy, and sugar cane. One of the famous agricultural fruit products of Indonesia is durian. Currently, the capacity of durian production of Indonesia reaches more than 880,000 tons/year. The edible part of durian only 10–30 %, and it depends on the durian variety, the rest are shell (50–60 %) and seeds (10–20 %) which is discharged as waste. Durian seeds contain 50–70 % carbohydrate, and currently have not been utilized as the source of carbohydrate in any food or starch production. Since it contains high amount of carbohydrate (amylose content is 20.8 %), in this study we utilized durian seeds as raw material for bioethanol production.

While attractive as an inexpensive and abundant feedstock, carbohydrate in durian seed must be converted into constituent sugar monomers prior to the bioethanol fermentation. So far, the conversion of carbohydrates into glucose can be achieved by acid hydrolysis as well as through enzymatic routes. However, the production cost is considered expensive since the methods require pretreatment, purification steps and often create environmental problems due to the use of acid catalyst and enzyme recovery [18, 19].

Subcritical water hydrolysis offers advantages to overcome the problems occur in acid and enzymatic hydrolysis by shortening the hydrolysis time and without using any catalyst. The subcritical water process has been widely used for hydrolyzing organic compounds [20–22], and in this paper we employed this method to hydrolyze carbohydrate from durian seed. To the best of our knowledge, there is no information about pretreatment of durian seeds using subcritical water process and subsequent used as precursor for bio-ethanol production. The objective of this study was to produce bioethanol from durian seeds. The effects of temperature, pressure, time, and ratio of durian seed to water on the yield of glucose and ethanol were studied.

Methods

Materials

Durian seeds were collected from local fruit markets in Surabaya. Prior to use, the durian seeds were repeatedly

washed, sliced, and dried in an oven until the moisture content was around 5 %. The durian seeds were then pulverized in hammer mill to pass through a 170–200 mesh screen and stored at ambient temperature in tightly closed containers for further use. The chemical composition of dried durian seeds powder (flour) consisted of 89.45 % carbohydrate, 5.32 % moisture content, 4.25 % protein, 0.68 % fat, and 0.30 % ash. The carbohydrate in the dried durian seeds powder was determined using enzymatic method (enzyme assay kit) [23]. The moisture content in the sample was analyzed by oven drying method at 105 °C. The protein content in the durian seeds powder was analyzed by micro Kjeldahl method [24]. The fat content was determined by Soxhlet extraction using petroleum ether at 65 °C, while the ash content was determined by burning of the durian seeds powder in muffle furnace at 800 °C.

Chemicals used in this study were Fehling A solution (>99 %, Merck), Fehling B solution (>99 %, Merck), glucose (>99 %, Sigma-Aldrich®), ethanol (96 %, w/w in water, Merck), 3,5-dinitrosalicylic acid (98 %, Sigma-Aldrich®), sodium potassium tartrate (99 %, Sigma-Aldrich®), phenol (99 %, Sigma-Aldrich®), sodium sulfite (98 %, Sigma-Aldrich®), sodium hydroxide (98 %, Merck), sulfuric acid (98 %, w/w in water, Merck), potassium dichromate (99.5 %, Sigma-Aldrich®), and instant dry yeast (Fermipan®). All chemicals were used without any further treatment or purification process. The high-purity nitrogen gas (99.9 %) was supplied by PT ANEKA GAS, Surabaya, Indonesia.

Subcritical water hydrolysis

The hydrolysis of durian seed flour in subcritical water was conducted in a high-pressure reactor system. The high-pressure reactor system consists of 150 cm³ of stainless steel reactor (SS-316) with maximum temperature and pressure of 250 °C and 100 bar, respectively. The reactor was equipped with an external heater (ceramic band heater Type CF400, Thermotech Co., Ltd), a pressure gauge, a Type K thermocouple and M8 screws for tightening the reactor with its cap. A pre-determined amount of durian seed powder and distilled water were mixed (1:10, 1:20, and 1:30) and charged into the reactor. Subsequently, nitrogen gas was then flowed to the reactor to remove air and build a bit of pressure prior to heating. The reactor then was heated from room to the desired temperature (120, 140, and 160 °C) at heating rate of 20 °C/min and kept at the final temperature for 1, 3, and 5 h. The pressure of the system was kept at 20, 30, and 40 bar. Following the hydrolysis, the solid material was separated using a centrifuge (Hettich, EBA 20) and the amount of reducing sugar in the supernatant was determined colorimetrically at 508 nm [25] by a spectrophotometer (Shimadzu, UV-VIS



Table 1 Independent variables of CCD employed for durian seed powder hydrolysis in subcritical conditions

Run no.	Variables				Coded levels				Response
	Time (h) <i>t</i>	Temperature (°C) <i>T</i>	Pressure (atm) <i>P</i>	Solid to water ratio (–) SW	<i>t</i>	<i>T</i>	<i>P</i>	SW	Yield (%)
1	1	120	20	1:10	–1	–1	–1	–1	2.49
2	5	120	20	1:10	+1	–1	–1	–1	9.65
3	1	160	20	1:10	–1	+1	–1	–1	1.52
4	1	120	40	1:10	–1	–1	+1	–1	2.49
5	1	120	20	1:30	–1	–1	–1	+1	3.34
6	5	160	20	1:10	+1	+1	–1	–1	10.23
7	1	160	40	1:10	–1	+1	+1	–1	1.52
8	1	120	40	1:30	–1	–1	+1	+1	3.34
9	5	120	40	1:10	+1	–1	+1	–1	9.65
10	5	120	20	1:30	+1	–1	–1	+1	17.11
11	1	160	20	1:30	–1	+1	–1	+1	2.27
12	5	160	40	1:10	+1	+1	+1	–1	10.23
13	1	160	40	1:30	–1	+1	+1	+1	2.27
14	5	120	40	1:30	+1	–1	+1	+1	17.11
15	5	160	20	1:30	+1	+1	–1	+1	16.42
16	5	160	40	1:30	+1	+1	+1	+1	16.42
17	5	140	30	1:20	+1	0	0	0	25.27
18	3	160	30	1:20	0	+1	0	0	20.52
19	3	140	40	1:20	0	0	+1	0	30.87
20	3	140	30	1:30	0	0	0	+1	31.88
21	1	140	30	1:20	–1	0	0	0	3.24
22	3	120	30	1:20	0	–1	0	0	17.23
23	3	140	20	1:20	0	0	–1	0	30.87
24	3	140	30	1:10	0	0	0	–1	20.74
25	3	140	30	1:20	0	0	0	0	30.87
26	3	140	30	1:20	0	0	0	0	30.87
27	3	140	30	1:20	0	0	0	0	30.87
28	3	140	30	1:20	0	0	0	0	30.87
29	3	140	30	1:20	0	0	0	0	30.87
30	3	140	30	1:20	0	0	0	0	30.87
31	3	140	30	1:20	0	0	0	0	30.87

1201). The yield of reducing sugars was defined as the amount of reducing sugar obtained after the hydrolysis divided by the amount of durian seed flour used in the hydrolysis experiment (dry basis). The hydrolysis conditions were based on central composite design (CCD) with total of 31 experiments as shown in Table 1.

Fermentation experiment

Dry yeast (*Saccharomyces cerevisiae* from common baker's yeast) was employed in the fermentation studies and was routinely cultured on yeast extract peptone dextrose (YPD) agar plates (20 g L^{–1} glucose, 20 g L^{–1} peptone, 10 g L^{–1} yeast extract, and 16 g L^{–1} agar) at 30 °C. A small-scale

culture was prepared by inoculating a single colony of *S. cerevisiae* into a medium containing 20 g L^{–1} glucose, 20 g L^{–1} peptone, and 10 g L^{–1} yeast extract. After 24 h, the culture was removed into fermentation media, the ratio of the culture with fermentation media was 1:10 (v/v). Fermentation experiment was carried out in micro-aerobic and aerobic conditions in a 250 mL flask at pH 5.0 ± 0.5 and 30 °C under slow and constant agitation (100 rpm). The concentrations of the reducing sugars used in the fermentation experiments were 10, 15, and 20 g L^{–1}, while the time for fermentation experiments were 24, 48, and 72 h. The concentration of reducing sugars in the fermentation experiments was adjusted to the desired concentration (20 g L^{–1}) by addition of glucose. Two variables CCD was



Table 2 Independent variables of CCD employed of ethanol production

Run no.	Variables		Coded levels		Response Ethanol (g L ⁻¹)
	Reducing sugars concentration (g L ⁻¹) <i>S</i>	Time (h) <i>t_F</i>	<i>S</i>	<i>t_F</i>	
1	20	72	+1	+1	11.76
2	20	24	+1	-1	6.86
3	10	72	-1	+1	5.37
4	10	24	-1	-1	3.87
5	10	48	-1	0	5.44
6	15	24	0	-1	4.81
7	20	48	+1	0	11.76
8	15	72	0	+1	7.89
9	15	48	0	0	7.97
10	15	48	0	0	7.97
11	15	48	0	0	7.97
12	15	48	0	0	7.97
13	15	48	0	0	7.97

used to optimize the fermentation conditions (Table 2). Samples were taken periodically to determine the ethanol concentration (g L⁻¹). The amount of ethanol produced was determined by dichromate method [26].

Experimental design and statistical analysis

Central composite design was employed to determine the optimum conditions of both reducing sugars yields obtained from hydrolysis stage and ethanol production from reducing sugars fermentation. The following regression equation was fitted to the response resulted from CCD by the least square method (LSM):

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

where Y is the yield of reducing sugars or ethanol, x_i and x_j the coded values of the variables, β_0 a constant coefficient, β_i the linier coefficients, β_{ij} the interaction coefficients, and β_{ii} are the quadratic coefficients. For hydrolysis stage, the independent variables were time (t , h), temperature (T , °C), pressure (P , atm), and solid to water ratio (SW, -). The yield of reducing sugars (%) was selected as the dependent output variable at the hydrolysis stage. For fermentation stage, the independent variables were reducing sugars concentration (S , g L⁻¹), and time (t_F , h). The concentration of ethanol (g L⁻¹) was selected as the dependent output variable. The regression model was calculated with Minitab 16.1.1 to estimate the response of the dependent variables. Adequacy of the parameters in the model was confirmed by analysis of variance (ANOVA). The fit of the model was evaluated by the R^2 value. Three-dimensional surface plots were also drawn based on the final equation.

Results and discussion

Hydrolysis of durian seed powder in subcritical conditions

Model for hydrolysis

Time, temperature, pressure, and solid to water ratio were examined as factors that might affect the yield of reducing sugars. From the general analysis, it is possible to select variables and interactions that are significant in the confidence range of 90–95 %. The significant values from Student's t distribution (obtained from ANOVA) were employed to determine the significance of the regression model. The linear and full quadratic models are given as follow:

$$Y = 16.91 + 6.10t - 0.11T - 0P + 2.34SW \quad (2)$$

and

$$S = 29.54 + 6.09t - 0.06T + 0P + 2.31SW - 13.79t^2 - 9.17T^2 + 2.83P^2 - 1.74SW^2 + 0.24t \cdot T - 0T \cdot P + 0t \cdot P + 1.51t \cdot SW - 0.17T \cdot SW + 0P \cdot SW \quad (3)$$

where S is the reducing sugars yield, t , T , P , and SW are time, temperature, pressure, and solid to water ratio, respectively. The analysis of the experimental data using linear model gave poor R^2 (0.1844).

The p value of the quadratic model (<0.0001) was quite significant at the probability level of 5 % ($R^2 = 0.9602$). In this study, the first-order effects of hydrolysis time, temperature, and solid to water ratio were significant at the confidence level of 95 %. However, the interactions between pressures, solid to water ratios, time and pressure,

temperature and pressure, and pressure and solid to water ratio were insignificant. Re-arrangement of Eq. (2) with the inclusion only the significant parameters give the following result:

$$S = 29.74 + 6.09t - 0.06T + 2.31SW - 12.95t^2 - 8.33T^2 - 0.89SW^2 + 1.51t \cdot SW \quad (4)$$

The ANOVA analysis of Eq. (3) gave the results as summarized in Table 3. The results show that the p value of the model is significant (<0.0001) and a good fitting of the model with the experimental data is also observed ($R^2 = 0.9564$).

The effects of hydrolysis operating parameters on the reducing sugars yield are plotted as contour plots as shown in Fig. 1. By comparing the generated plots in Fig. 1, it can be seen that the highest value of solid to water ratio (+1) had a tendency to increase the yield of reducing sugars. Higher solid to water value increase the yield due to more carbohydrate is available and the breakdown of carbohydrate will produce reducing sugars. On the other hand, by increasing the temperature (0) and time (0) of hydrolysis of durian seed until certain values (140 °C and 4 h), has the tendency to enhance the reducing sugars yield.

The effect of solid to water ratio on the yield of reducing sugars can be observed as a function of time and temperature as shown in Fig. 2. At hydrolysis time of 1 h, the reducing sugars yield was observed around 2.9 %. By increasing the hydrolysis time to 3 and 5 h, the reducing sugars yield increased up to around 31 %. With the increase of hydrolysis time, the contact between the carbohydrate molecules and the ionic product of water (H_3O^+ and OH^-) become more intense and longer, and more of the carbohydrate molecules were hydrolyzed and converted

into monomeric sugars. At subcritical condition, water acts as an acid or base catalyst because of the presence of H_3O^+ and OH^- at higher concentration than in ambient temperature [27]. Subcritical water, therefore, has better catalytic activity to breakdown the complex carbohydrate molecules into simple sugar molecules. Further increase of hydrolysis time to 5 h, the reducing sugars yield decreased as indicated in Fig. 2. The decrease of the yield of reducing sugars mainly due to the dehydration of reducing sugars into other products such as humins, furfural, hydroxymethyl furan (HMF), and levulinic acid [28].

It has been known that the temperature has the positive effect on the hydrolysis process. By increasing temperature, the breakdown of water molecules into the ionic products also increase leading to the increase of the H_3O^+ concentration. The water become more reactive and more carbohydrate molecules were converted into monomeric sugars. However, at temperature higher than 140 °C, the dehydration reaction of monomeric sugars into 5-(hydroxymethyl)furfural and levoglucosan also increase; these side reactions decreased the yield of monomeric sugars.

Based on the experimental results as well as ANOVA analysis, the pressure was found to have insignificant effect on the yield of reducing sugars. In general, the yield of reducing sugars was not affected by the change of pressure. In the subcritical process, the pressure has a role to maintain water at its liquid state, since the hydrolysis process occurs in liquid phase [29].

Maximizing the product yield is an important point to establish an efficient process, and it can be achieved through the setting of all significant parameters at optimum conditions. Figure 3 depicts the optimum condition of each significant parameter in the durian seed hydrolysis in subcritical conditions. The optimum conditions for hydrolysis of durian seed were 0.2929, -0.0101 , and 1.0

Table 3 Analysis of variance for regression on CCD model of reducing sugars production

Statistical parameter	Sum of squares	Degrees of freedom	Mean square	F ratio	p value
Regression model of CCD					
Model	4028.04	14	287.72	39.560	<0.0001
Residual	116.33	16	7.27		
Lack-of-fit	116.33	10	11.63	0.050	0.050
Pure error	0.00	6	0.00		
Total		30			
R^2	0.9474				
Screening regression model of CCD					
Model	4005.92	7	572.27	95.070	<0.0001
Residual	138.45	23	6.02		
Lack-of-fit	138.45	7	19.78	0.050	0.050
Pure error	0.00	16	0.00		
Total		30			
R^2	0.9564				



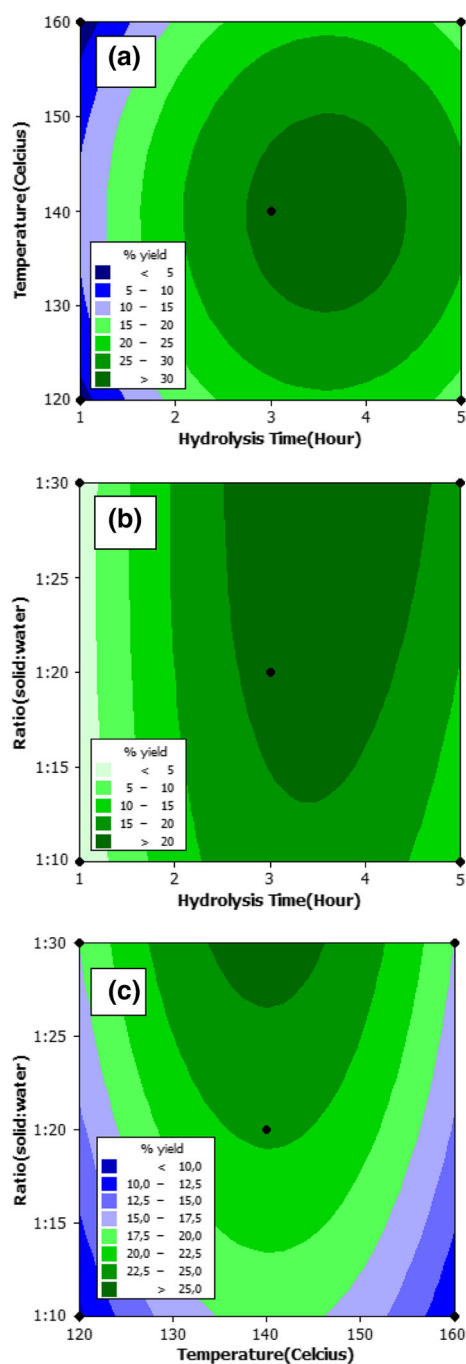


Fig. 1 Contour plots of selected independent variables of durian seed hydrolysis on the reducing sugars yield. **a** Hydrolysis time versus temperature, **b** hydrolysis time versus ratio, and **c** temperature versus ratio

coded unit for parameters of time, temperature, and solid to water ratio, respectively.

These units correlate to solid to water ratio of 1:30, hydrolysis temperature of 139.8 °C, and hydrolysis time of 3.58 h. The optimum sugar yield is 32.37 % with 0.978 precision.

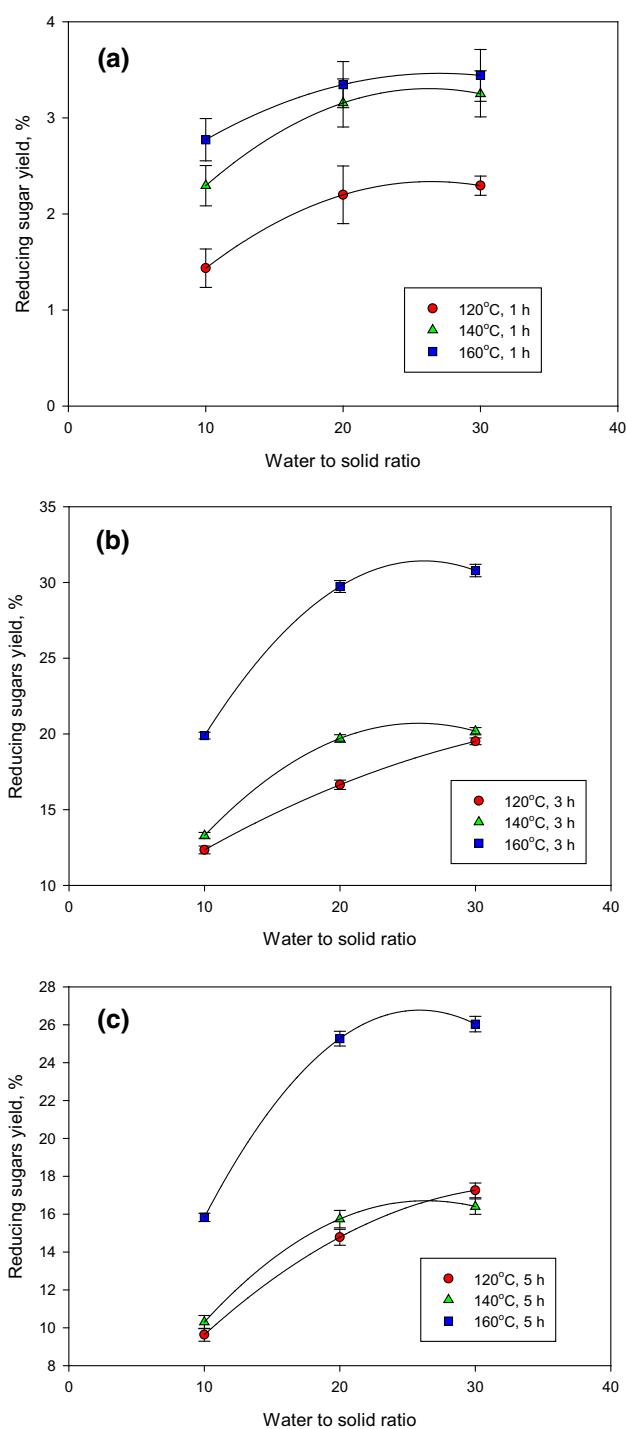
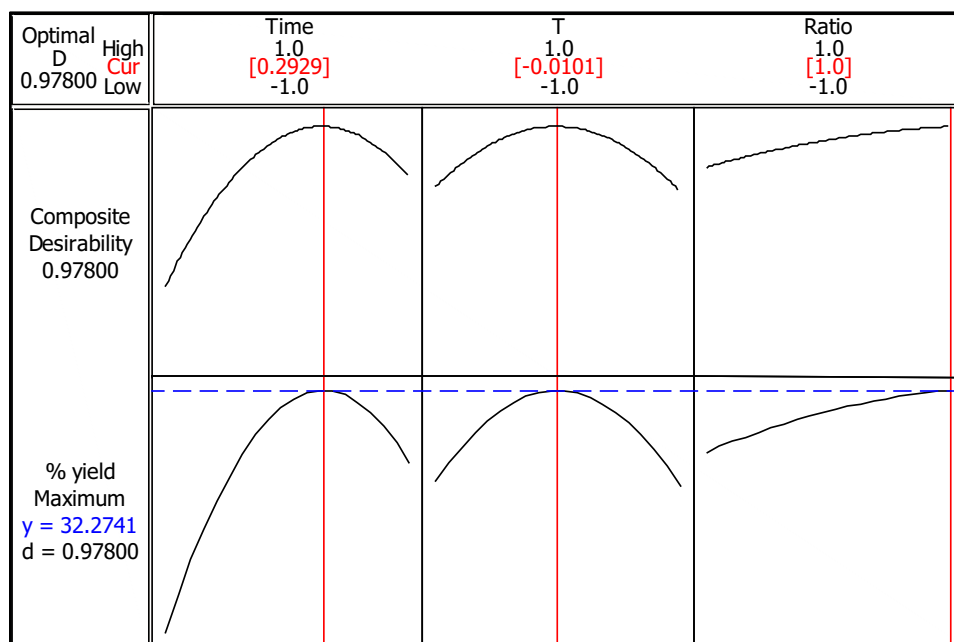


Fig. 2 Effect of time on the yield of reducing sugars, **a** 1 h, **b** 3 h, and **c** 5 h

Fermentation of sugars to ethanol

The fermentation of reducing sugars into bio-ethanol was modeled using full quadratic polynomial model with the independent variables: reducing sugars concentration and fermentation time. Table 4 shows the

Fig. 3 Independent factor optimization during subcritical water hydrolysis of durian seed**Table 4** Analysis of variance for ethanol production (E) as a function of reducing sugars concentration (S) and time of fermentation (t_F), and regression model of CCD

Effects	Sum of squares	Degrees of freedom	Mean square	F ratio	p value
Analysis of variance for ethanol production					
S	29.6148	1	29.6148	764.06	0.000
t_F	6.5731	1	6.5731	169.59	0.000
$S \cdot S$	0.7315	1	0.7315	18.87	0.003
$t_F \cdot t_F$	2.9321	1	2.9321	75.65	0.000
$S \cdot t_F$	0.7482	1	0.7482	19.30	0.003
Total	40.3211	12	$R^2 = 0.9932$		
Regression model of CCD					
Model	39.9164	5	7.9833	205.97	<0.0001
Residual	0.2713	7	0.0388		
Lack-of-fit	0.2324	3	0.0776	7.97	0.037
Pure error	0.0389	4	0.097		
R^2	0.9932				

result of ANOVA analysis of the significant values obtained from the Student's t distribution. The results show that the main significant factors at a 95 % confidence level are both reducing sugars concentration and fermentation time. From this table, it can be seen that the quadratic relation between the independent variables was statistically significant with a good confidence level.

The results of the adjustment of ANOVA analysis of the quadratic model to the experimental data are summarized in Table 4. The F values estimated with the experimental data and corresponded to total residual and lack-of-fit were lower than the tabular F values, indicating that the model was significant in the region studied. The yield of ethanol

from the fermentation process of reducing sugars from durian seed can be written by the following equations (linear and quadratic forms):

$$E = 7.51 + 2.62S + 1.58t_F \quad (5)$$

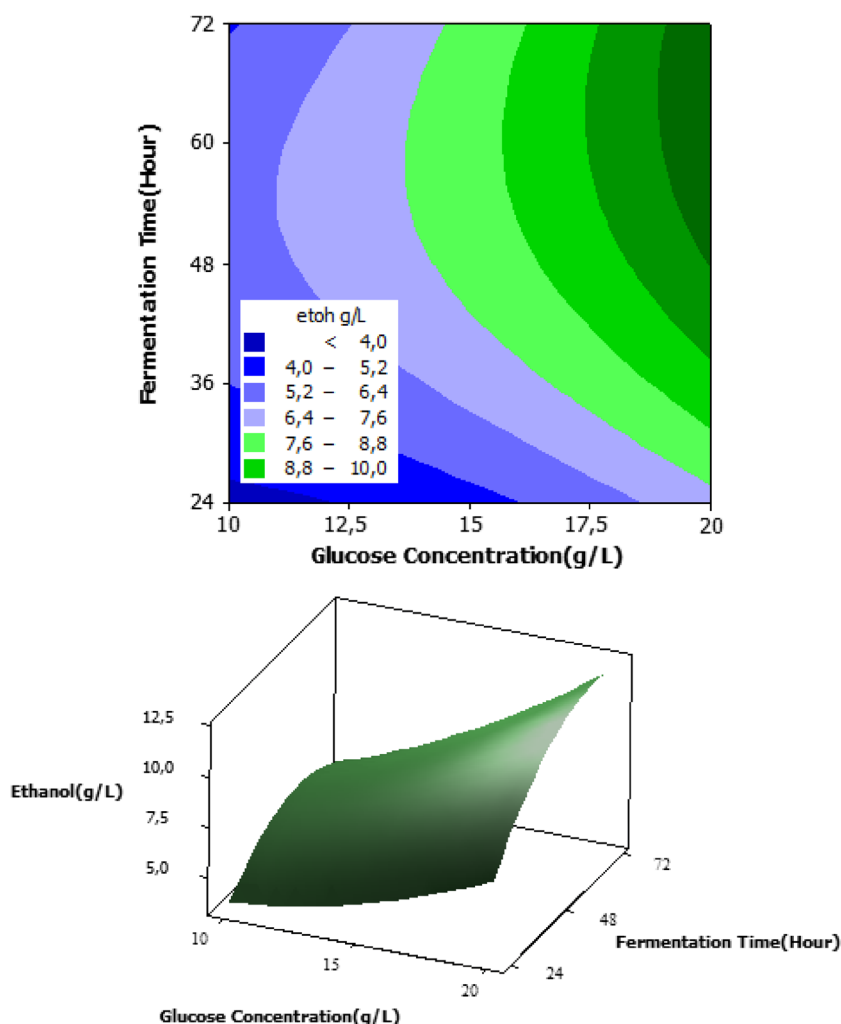
and

$$E = 7.97 + 2.62S + 1.58t_F + 0.62S^2 - 1.62t_F^2 + 0.85S \cdot t_F \quad (6)$$

where E is the ethanol concentration, S and t_F are reducing sugars concentration and fermentation time, respectively. The linear form model gave low value of R^2 (0.8351), a strong indication that the linear form could not represent the experimental data well.



Fig. 4 Contour plot (*top*) and response surface (*bottom*) of the reducing sugars concentration and fermentation time on the yield of ethanol



The response surface of the ethanol yield obtained from the quadratic form is given in Fig. 4. The response surfaces as indicated in Fig. 4 reveals the high levels of reducing sugars concentration and fermentation time tend to augment the ethanol production. The increase of reducing sugars concentration at the highest value (20 g L^{-1}) and fermentation time (72 h) enhances the ethanol production from 3.55 to 9.85 g L^{-1} . The increase of initial reducing sugars concentration to enhance ethanol production can be explained by the availability of more carbon source, i.e., glucose, to be utilized by yeast (*S. cerevisiae*) to produce ethanol.

Conclusion

The potential application of durian seed was as a new resource for bioethanol production was explored in this study. Subcritical water process was employed to convert the durian seed starch into glucose. Time, temperature, pressure, and solid to water ratio were examined as factors

that might affect yield of glucose using CCD. Pressure has no significant effect on the yield of glucose. The increase of glucose concentration and fermentation time enhanced the bioethanol production. The optimized values for subcritical water process to produce reducing sugars were achieved at 139.8°C ; 1:30 solid to water ratio; and reaction time of 3.58 h with 32.37 % reducing sugars yield. The fermentation of 20 g L^{-1} reducing sugars for 72 h results the highest ethanol concentration, i.e., 9.85 g L^{-1} .

Authors' contributions AP and YAWY conducted the hydrolysis and fermentation experiments and performed the statistical analysis, JNP and ATN conducted the revision of manuscript, WI drafted the manuscript, SI performed the experiment design and corrected the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.



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