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Potential Utilization of Low Quality Sweet Potato for Bioethanol Production by *Saccharomyces cerevisiae* TISTR5339

Supasit CHOOKLIN[1,*], Parichat NINUP-PATHAM[2] and Saovanee CHOOJIT[3]

[1]*Department of Food Science and Technology, Faculty of Agro-Industry,*
Rajamangala University of Technology Srivijaya, Thungyai, Nakhon Si Thamarat 80240, Thailand
[2]*Department of Biotechnology, Faculty of Agro-Industry,*
Rajamangala University of Technology Srivijaya, Thungyai, Nakhon Si Thamarat 80240, Thailand
[3]*Department of General Science, Faculty of Science and Technology,*
Muban Chombueng Rajabhat University, Chom Bueng, Ratchaburi 70150, Thailand

([*]Corresponding author's e-mail: supasit_c@yahoo.co.th, supasit.c2015@gmail.com)

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Abstract

This study was aimed to investigate the optimal condition of ethanol production that has 2 major stages: acid hydrolysis and fermentation processes. These processes came from low quality sweet potato (LQSP) which was destroyed by the sweet potato weevil. The main compositions of LQSP were starch and fiber which consist of 55.25 and 10.29 %, respectively. In this case, the starch can be hydrolyzed to reduce the sugar, followed by the fermentation of the reduced sugar to ethanol. For this experiment, the effecting factors on acid hydrolysis of LQSP and the ethanol fermentation condition were optimized by *S. cerevisiae* using Response Surface Methodology (RSM) with Box-Behnken design in order to maximize ethanol yield. It was found that the maximum reducing sugar concentration of 390.99 ± 5.35 g/L was obtained from the hydrolysis condition with 1 % (v/v) of sulfuric acid and 25 % (w/v) of LQSP. Accordingly, the effects of ammonium sulphate content (0.05 - 0.15 %), pH (4.5 - 5.5) and inoculum content (5 - 10 %) on ethanol production was determined by RSM using Box-Behnken experiment design with a total 17 sets of all trials. The results were found that the maximum experimental ethanol productivity of 5.98 g/L was obtained from the condition at 0.05 % of ammonium sulphate, pH 5.5 and 5.0 % of inoculum size to 90 mL LQSP based medium and incubated at 30 °C for 48 h. In addition, the scale-up of ethanol production was studied in 9 L fermenter which provided the maximum ethanol yield of 5.04 g/L. Therefore, it can be concluded that LQSP had a potential as a substrate for ethanol production.

Keywords: Low quality sweet potato, Acid hydrolysis, Ethanol, *Saccharomyces cerevisiae*, Response surface methodology

Introduction

Renewable and sustainable energy resources play a crucial role in the future of human life. As the demand for the limited global supply of non-renewable energy resources increases, the price of oil and natural gas also increases. Bioethanol is one of the most promising biofuels from renewable resources, since it has been blended with gasoline into gasohol to make E20 and E85 [1]. It has become an alternative renewable and sustainable energy source, which can be produced from agricultural crops or lignocellulosic biomass [2]. Most bioethanol production throughout the world, including Thailand that has a lot of resources of sugar and starch crops such as sugarcane and corn as sugar/starch based feedstocks are currently predominant at the industrial level. Such a production of bioethanol are

economically feasible. However, there are raising questions concerning the competition between food supply and arable land [3]. Thus, there is a growing interest to find alternative bioresources other than sugarcane/beet molassess and starchy crops such as cassava, sweet potato, and sweet sourghum for ethanol production [2].

Sweet potato (*Ipomoea batatas* L.) has been considered a promising substrate for the production of ethanol through fermentation since it has a higher starch yield per unit than grains [4]. Moreover, its average yielded carbohydrate is higher than cassava and corn, up to 80 %. This has greater potential as ethanol source [5]. Sweet potato is cheap, readily available in the local market, and offers ease in product processing. It contains starch (178 g/kg), total sugars (26 g/kg) and protein (3.2 g/kg) on fresh weight basis. The starch can be hydrolyzed to monomer units of carbohydrates and can be used by the microorganisms in fermentation process [6]. Industrial sweet potatoes are not intended for use as a food crop. They are bred to increase its starch content, significantly reducing its attractiveness as a food crop when compared to other conventional food cultivars. Therefore, they offer potentially greater fermentable sugar yields for industrial conversion processes, which implies an opportunity for an increase in planted acreage [4]. It has been reported that some industrial sweet potatoes breeding lines developed could produce ethanol yields of 4,500 - 6,500 L/ha compared to 2,800 - 3,800 L/ha for corn [7].

The ethanol production process from starch has 2 major stages: hydrolysis of carbohydrate to produce fermentable sugars and fermentation of reducing sugars to ethanol [8]. Therefore, efficiency and cost-effectiveness of hydrolysis and fermentation are needed to maximize the reduction of sugar concentration and ethanol productivities [9]. In addition, the dilute acids have been successfully used in the hydrolysis of a wide range of feed stocks, ranging from hardwoods to grasses and agricultural residues. Furthermore, sulfuric acid (H_2SO_4) at low concentrations, has been widely studied because it is inexpensive, effective with low acid consumption, and gives high conversion of starch/cellulose to glucose [10,11]. While Baker's yeast *Saccharomyces cerevisiae* has been traditionally used in the brewing industry to produce ethanol from hexoses, it is one of the most importance microorganisms which is being widely used for the conversion of sugar to ethanol due to its high ethanol yield, high tolerance to ethanol concentration, high selectivity, low accumulation of by-products, high fermentation rate, good tolerance to substrate concentration and lower pH value [12].

Moreover, Response surface methodology (RSM) is a statistical and mathematical tool for designing experiments, building models, and searching for their optimal set-point for desirable response while reducing the number of required experiments [13]. The optimizing processes are based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical predictions. Moreover, it can be applied successfully when a response or a set of responses of interest are influenced by several variables [14].

Therefore, this study aimed to investigate the factors that affected the acid hydrolysis of LQSP and the optimization of the ethanol fermentation condition by *S. cerevisiae* using RSM with Box-Behnken design in order to maximize ethanol yield.

Materials and methods

Low quality sweet potato

The low quality sweet potato (LQSP) that was destroyed by sweet potato weevil was collected. The physical characteristics of the LQSP is mushy, shrivel, and its peel turns brown or black as shown in **Figure 1**. These LQSPs were supplied by the local sweet potato planting area in Nakhon Si Thammarat Province, Thailand.

Figure 1 The physical characteristics of Low quality sweet potato (LQSP).

Microorganism and culture conditions

S. cerevisiae TISTR 5339 was provided from the Culture Collection of the Microbiological Resources Center, Thailand Institute of Scientific and Technology Research (TISTR), Pathum Thani, Thailand. The culture of *S. cerevisiae* was maintained on YM agar slants (consisting of glucose, 20; yeast extract, 3; malt extract, 3; peptone, 5; and agar 15, all in g/L) at 4 °C. An inoculum was prepared by transferring a loopful of cells to 50 mL of YM medium broth, which was incubated and grown at 30 ± 2 °C on a shaker at 150 rpm before inoculating the reactor.

Experimental methods

LQSP preparation and composition analysis

LQSP samples were washed thoroughly to remove the dust and other debris, then peeled off and chopped into small pieces. After that, the LQSP pieces were dried in the oven at 55 °C for 24 h till the moisture content reduced to 8 % and grinded with mixture grinder into powder. Next, the LQSP grinded powder was sieved through a steel mesh to get 0.5 mm^3 diameter size and stored in aluminium foil bag for further use. Finally, the LQSP compositions were analyzed according to AOAC methods [15].

Acid hydrolysis of LQSP powder

The diluted sulfuric acid (H_2SO_4) hydrolysis of LQSP powder was operated in 250 mL round bottles. LQSP powder was used in various amounts to get the optimum ratio of sample to acid (5, 15 and 25 %w/v), in 100 mL H_2SO_4 which varied concentrations (1, 3 and 5 %v/v). Then, the suspension was performed in an autoclave at 121 °C, 15 psi for 30 min. After hydrolysis, the solid residue was separated from the diluted H_2SO_4 solution by using vacuum filtration with Whatman filter paper No. 4 and the filtrated solution from each experiments were collected and analyzed to determine the reducing sugar [16] and total sugar contents [17].

Optimization of fermentation variables using RSM

RSM is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical previsions. It can be well applied when a response or a set of responses of interest are influenced by several variables. Herein, Box-Behnken design is one of the most commonly used response surface designs to study the effects of variables on the response, and subsequently in optimization studies [14].

We expected that under optimum acid hydrolysis conditions of LQSP powder, there was a provided maximum glucose for ethanol fermentation by *S. cerevisiae*. In this study, the Optimization of ethanol production from LQSP powder was evaluated by using the Design expert software (Trial version 11.0,

Stat-Ease, Inc., Minneapolis, USA). 10 %v/v of inoculum size was transferred into a 250 mL Erlenmeyer flasks containing 90 mL of LQSP based culture medium and was subsequently incubated at 30 ± 2 °C for 48 h in triplicates. Samples were harvested at 48 h of fermentation to monitor ethanol productivity. Three independent variables, namely ammonium sulphate concentration (A, 0.05, 0.1 and 0.15 %w/v), pH (B, 4.5, 5.0 and 5.5) and inoculum size (C, 5, 7.5 and 10 %w/v) were used at 3 coded levels (-1, 0, +1), while the summarization of the range and levels of the variables was investigated with low, middle, and high levels of each variable. This is included as one of the 17 experimental designs shown in **Table 1**.

Table 1 Experimental range and levels of the independent variables on the fermentation of LQSP.

Variables	Symbols	Coded levels		
		-1	0	+1
Ammonium sulphate	A	0.05	0.1	0.15
pH	B	4.5	5.0	5.5
Inoculum size	C	5	7.5	10

The significance of each variable, interactions, and fitting factors are based on the following 2nd order polynomial that coded according to Eq. (1);

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

where Y_i is the predicted response (ethanol content), β_0 is the intercept coefficient, β_1, β_2, β_3 are the linear coefficient, β_{11}, β_{22}, β_{33} are the quadratic coefficients, β_{12}, β_{13}, β_{23} are the cross-product coefficients and A, B, C are the independent variables studied.

Model fitting and statistical analysis

All fermentation experiments were carried out in triplicate and results expressed as mean values. The results obtained from Box-Behnken Design were used to determine the regression coefficients of the 2nd order multi-regression model. The analysis of variance (ANOVA) was evaluated using Design-Expert 11.0. The quality of the fit of the polynomial model equation was assessed by determining the R^2 coefficient; its statistical and regression coefficient significance were checked with *F*-test and *P*-value, respectively. Three dimensional (3D) surface plot and corresponding contour plots were drawn to illustrate the effect of the independent variables on the response (ethanol content). Finally, the optimum values for the selected variables were obtained by solving the regression equation.

Analytical methods

The reduction of sugar was measured by dinitrosalicylic colorimetric method (DNS). The standard glucose stock solution 10 g/L was prepared by dissolving 0.20 g of D-(+)-Glucose anhydrous ($C_6H_{12}O_6$) in 20 mL of DI water. Working solutions were daily prepared by appropriate dilution of the stock solution in DI water. After that 3,5-dinitrosalicylic acid reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid in 20 mL of 2 M NaOH. It was then mixed with potassium sodium tartrate ($C_4H_4KNaO_6$) solution (30 g of $C_4H_4KNaO_6$ in 50 mL of DI water) on a magnetic stirrer hot plate and diluted to 100 mL with DI water. Finally, Calibration curve for estimation of reducing sugar yield was obtained by plotting the absorbance (at 520 nm) vs. concentrations of standard glucose in the range of 0.20 - 1.00 g/L. The concentrations of glucose were daily prepared by dilution of the stock solution ($y = 1.0303x + 0.0225$; $R^2 = 0.995$) [16].

Total sugar was measured by phenol-sulphuric method. Standard curve of sugar was prepared using the serial concentration of glucose solution (10 - 100 µg/mL) in DI water. The 1 mL of each

concentration was transferred to test tube and added with 1 mL of 5 % phenol solution. The mixtures were shaken and followed by the addition of 5 mL conc. sulphuric acid. All mixtures were homogenized by vortex and stand for 10 min. The absorbance (488 nm) of the reaction mixture was measured. Finally, the relation between absorbance and glucose concentration was plotted ($y = 0.0094 \times -0.0207$; $R^2 = 0.993$) [17].

Ethanol content was measured by flash distillation method. The 1 mL of diluted sample was transferred to screw cape tube and added with 2 mL of 0.1 M potassium dichromate in 0.5 M sulphuric acid. After that, DI water was added and the screw cape tube was closed. It was boiled for 5 min and cooled in ice water immediately. Finally, the relation between absorbance and ethanol concentration was plotted ($y = 0.0309x$; $R^2 = 0.991$) [18].

Results and discussions

LQSP compositions

To identify the compositions of the LQSP powder before acid hydrolysis, there were moisture, starch, protein, fat, ash and fiber contents which were analysed according to the standard AOAC methods [15]. The chemical compositions of LQSP powder are listed in **Table 2**. The main compositions were starch and fiber that consist of 55.25 and 10.29 %, respectively. Moreover, the composition of LQSP was as same as fresh sweet potato (FSP) that reported by [19,20]. Therefore, LQSP is suitable to use as raw material for ethanol production.

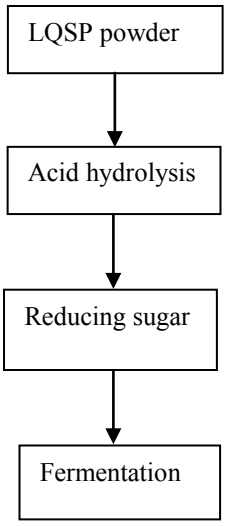
Table 2 LQSP compositions.

Type	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Starch (%)
LQSP	7.35 ± 1.61	3.89 ± 0.16	1.34 ± 0.21	3.94 ± 0.03	10.29 ± 2.16	55.25 ± 5.37
FSP [1]	7.34 ± 0.77	3.31 ± 0.42	0.29 ± 0.08	-	-	51.89 ± 8.97
FSP [2]	8.06 ± 1.13					55.76 ± 6.82

[1] Fresh sweet potato, [19]; [2] [20]

Acid hydrolysis of LQSP powder

LQSP is a starchy material and needs to be hydrolyzed before fermentation by following this flowchart.



From **Table 2**, it was found that the main LQSP composition was starch; therefore, hydrolysis was needed before the fermentation of starchy material. Starch is a polysaccharide comprising of glucose monomers linked by alpha - glycosidic bonds [21]. Acid hydrolysis actually will break down the starch molecules at random and sugar is mainly produced. The acid disrupts the hydrogen bonding between starch chains, converting it to a completely amorphous state, thereby forming a homogenous gelatin [22]. Starch hydrolysis is expressed as;

$$(C_6H_{10}O_5)_n + nH2O = nC_6H_{12}O_6$$
Starch D - glucose

The effected parameters for acid hydrolysis of LQSP via LQSP powder to acid and H_2SO_4 concentrations were investigated and evaluated in order to obtain the maximum reducing sugar concentration of LQSP. This was, then sequentially utilized for ethanol fermentation. It was found that the maximum reducing sugar concentration of 390.99 ± 5.35 g/L was obtained in the hydrolysis condition with 1 % (v/v) of sulfuric acid and 25 % (w/v) of LQSP. The effect of acid concentrations and LQSP starch concentration on reducing sugar and total sugar concentrations after acid hydrolysis of LQSP can be seen in **Figure 2**. It was found that increasing acid concentration caused to decrease reducing sugar and total sugar concentrations. Research by [23] studied the glucose production from hydrolysate pineapple residue. It was found that reducing sugar can be decreased in an increasing sulfuric concentration. Yoonan *et al*. [24] demonstrated that optimal % carbohydrate conversion could be obtained at 60.74 % from hydrolysis at 135 °C for 90 min with 0.1 M sulfuric acid. Increasing the acid concentration resulted in marginal improvements in cassava peel conversion with sulfuric acid, and a decrease of about 16 % when the acid level was raised more than 0.1 M. At the higher acid concentration, a dark colored hydrolysate, along with conversion by-products, was observed, thus suggesting sugars degradation. Maxwell *et al*. [25] reported the highest reducing sugar yield obtained in this study condition of 3 g of sweet potato peels hydrolyzed with 0.6 M sulphuric acid at 30 °C for 16 h is 0.141 g/100 mL. Above 0.6 M acid concentration, it was observed that the monomer sugar yields decreased as the acid concentration increased. This could be due to the degradation of product monomers.

Moreover, these results were in agreement with the results obtained by research [26] found that the decreasing trends in the reducing sugar at too high acid concentration are due to the occurrence of the decomposition of sugars to form inhibitory compounds such as hydroxymethylfurfural, furfural, levulinic acid and acetic acid. Moreover, this study found that reducing sugar and total sugar increased remarkably with increasing LQSP starch concentration and the conversion of 5, 15 and 25 %w/v LQSP with 1 %v/v sulfuric acid was 37.30, 69.39 and 69.99 %, respectively (data not showed).

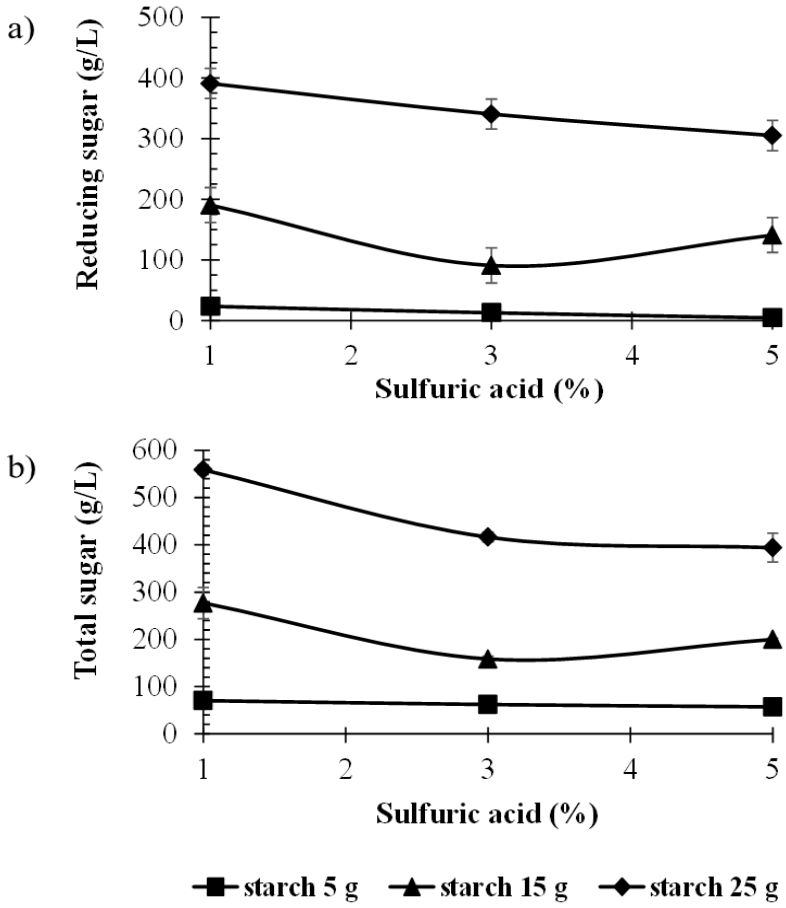


Figure 2 Effects of various acid concentrations and ratio of sample to acid on (a) reducing sugar and (b) total sugar after acid hydrolysis of LQSP.

RSM optimization of the fermentation conditions

The Box-Behnken experiment design led to a total 17 sets of experiments. The low, middle, and high levels of each variable and the experimental design and respective experimental results are given in **Table 3**. The maximum experimental ethanol production of 5.98 g/L was obtained with 0.05 % of ammonium sulphate, pH 5.5 and 5.0 % of inoculum size (Trial 2).

Table 3 Experimental range and levels of the 3 independent variables used in RSM with terms of coded, actual factors and the ethanol production results from each experimental trial for the 3 factor with response surface analysis.

Trial	Independent variables			Ethanol (g/L)
	Ammonium sulphate (%w/v)	pH	Inoculum size (%v/v)	
1	-1(0.05)	1(5.5)	0(7.5)	4.18
2	-1(0.05)	1(5.5)	0(5.0)	5.98
3	1(0.15)	1(5.5)	0(7.5)	5.49
4	1(0.15)	-1(4.5)	0(7.5)	1.85
5	0(0.10)	1(5.0)	0(7.5)	2.27
6	0(0.10)	-1(4.5)	-1(5.0)	0.56
7	0(0.10)	1(5.0)	0(7.5)	2.53
8	0(0.05)	1(5.0)	-1(5.0)	3.13
9	0(0.10)	0(5.0)	0(7.5)	3.84
10	-1(0.05)	0(4.5)	0(7.5)	1.42
11	-1(0.10)	-1(4.5)	1(10)	2.64
12	0(0.10)	0(5.0)	0(7.5)	1.99
13	0(0.05)	0(5.0)	1(10)	3.96
14	1(0.15)	0(5.0)	1(10)	2.84
15	0(0.10)	0(5.0)	0(7.5)	3.84
16	1(0.15)	0(5.0)	-1(5.0)	4.84
17	0(0.10)	1(5.5)	1(10)	3.98

Table 4 Regression of coefficients and analysis of variance of the 2nd order polynomial using quadratic model for response variables.

Term	SS	DF	*F value*	*p*-value
A-Ammonium sulphate	0.0351	9	0.0453	0.8408
B-pH	21.65	1	26.79	0.0013*
C-Inoculum size	0.0630	1	0.0780	0.7881
AB	0.2116	1	0.2618	0.6246
AC	2.00	1	2.48	0.1595
BC	1.30	1	1.6100	0.2453
A^2	2.84	1	3.5200	0.1028
B^2	0.0040	1	0.0049	0.9460
C^2	0.0023	1	0.0028	0.9592
Residual	5.66	7		
Lack of Fit	2.53	3	1.08	0.4534
Pure Error	3.13	4		
Core Total	33.76	16		

R^2 = 0.8325; Predicted R^2 = 0.8318; *CV* (%) = 27.64; Adequate Precision = 22.49; *SS*, Sum of squares; *DF*, degrees of freedom, * Significant at < 0.01.

Model fitting and statistical analysis

The 2nd order regression fit to the experimental data. The following 2nd order polynomial model describes the ethanol production from LQSP-reducing sugar concentration following by Eq. (2);

$$Y = 2.89 + 0.0677A + 1.65B + 0.0891C + 0.8189A^2 - 0.0292B^2 - 0.0221C^2 - 0.2281AB - 0.7056AC - 0.5702BC \quad (2)$$

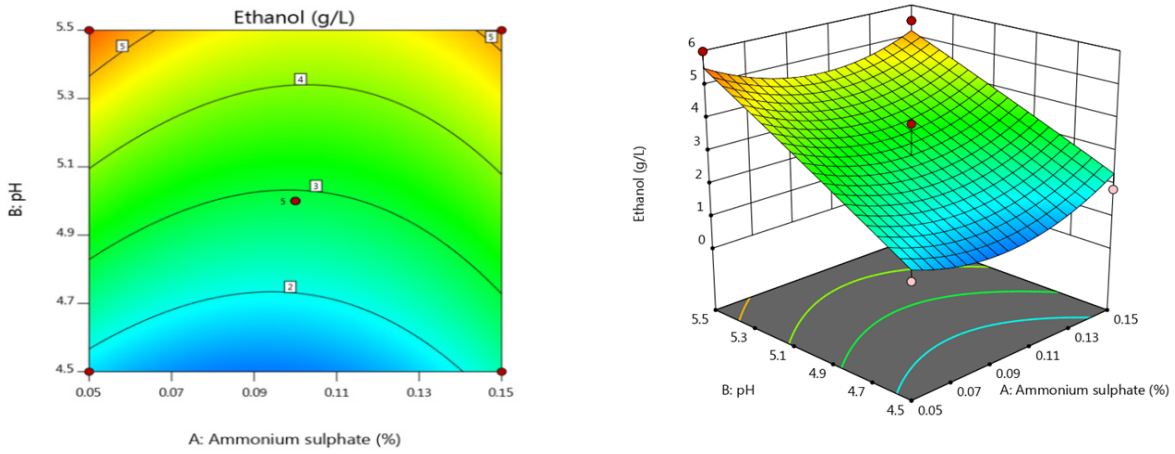
Here A, B and C are ammonium sulphate, pH, and inoculum size, respectively. The models fitted satisfactorily with the experimental data as indicated by their goodness of fit expressed by R^2 and P values.

The significance and effects of each variable on ethanol production from LQSP-reducing sugar are presented in **Table 4**. The value of $R^2 = 0.8325$. This indicated that 83 % of the variations in ammonium sulphate, pH, and inoculum can be explained by this equation. Furthermore, it has only 16.75 % of the total variation which is not explained by the model. Thus, the correlation of experimental and fitted values is accepted. The predicted determination coefficient $R^2_{Pred} = 0.8318$ points to the good agreement of the experimental and the predicted values for ethanol production. The coefficient of variation (*CV*) value indicates the degree of precision with which the experiments are compared. The lower reliability of the experiment is usually indicated by a higher value of *CV* (>15). In the present case, acceptable *CV* values were observed for the model of ethanol production (27.64). This denotes that the experiments performed were reliable. The lack of fit measures means the failure of the model to represent the experimental data. Therefore, the lack of fit of regression Eq. (2) is not significant ($P = 0.4534$). This indicates that the model equation was adequate for experimental data on the ethanol production. In this study, pH (B) was highly significant in their individual effect. Representative response surface plots are shown in **Figures 3(a) - 3(c)**.

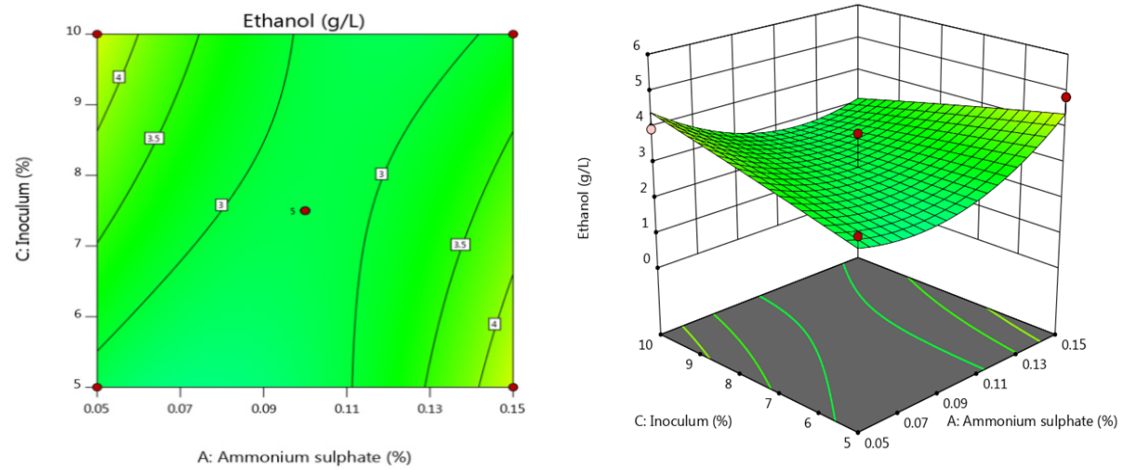
Response surface was generated by plotting the response (ethanol production) on the y-axis against any 2 independent variables on the x-axis, while keeping the other independent variables at zero level. The effects of variables and their interactions on reducing sugar yield are described by the 3D response surface plots and 2D contour plots. Therefore, 3 response surfaces were obtained by considering the possible combination. **Figures 3(a) - 3(c)** represents the 3-dimensional surface plots for the optimization conditions. The plot illustrates the main and the interactive effect of the independent variables on the dependent ones. The response surface plots were generated by plotting the response on the y-axis. In **Figure 3a,** the interaction plot of pH and ammonium sulfate concentration shows that the ethanol production increased remarkably with pH and ammonium sulfate concentration. On the other hand, the ethanol production increased with the increase of an ammonium sulfate concentration. However, the inoculum decreased in size (**Figure 3b**). The decreasing level of inoculum size and the increasing level of pH, causes to increase in ethanol production (**Figure 3c**). The research conducted by [27] which reported the effect of inoculum size and pH found that ethanol productivity by baker yeast decreased as yeast concentration increased from 3 to 4 and 5 g/L in coffee husk based substrate. Moreover, the effect of different sunflower head waste inoculum size viz., 2, 4, 6, 8 and 10 % on the ethanol production from unspecialized juice of sweet sorghum obtained a maximum alcohol concentration of 12.45 and 12.23 % (v/v) at inoculum sizes of 6 and 2 %, respectively. pH is one of the important factors that affect the bioethanol production through SHF (separate hydrolysis and fermentation). The rate of ethanol production by yeast cells is highly affected by the pH of the fermentation medium. The acidic condition hinders the growth of harmful bacteria and enhances yeast growth. However, more acidic and basic conditions retard the yeast metabolic pathways and the growth of the cells. So, optimum pH is required for growth of the yeast and ethanol yield. When the pH was lower than 4.0, the incubation time for maximum ethanol concentration was prolonged and the maximum concentration was not very low. When the pH value was above 5.0, the quantity of ethanol produced substantially decreased. Therefore, a pH range of 4.0 - 5.0 may be regarded as the operational limit for the anaerobic ethanol production process. Previous studies showed that high ethanol production was obtained using pH of 5.0 to 6.0. It was also shown that no ethanol production exists lower than pH of 4.0. Optimum pH for *S. cerevisiae* BY4742 was

in the range of 4.0 - 5.0. The mutual interactions of the factors can also be assessed from contour plots. There are some studies that reported on the optimization of ethanol production from sweet potato. For example, the study by [28] reported on ethanol production from sweet potato flour using co-culture of *Trichoderma* sp. and *S. cerevisiae* in solid-state fermentation which was composed of ammonium sulphate 0.2 %, pH 5.0, inoculated with 10 % inoculum size at 30 °C for 72 h with highest ethanol concentration, maximum ethanol productivity (2.8 g/kg substrate/h), microbial biomass (2.3×10^9 CFU/ g substrate), ethanol yield (47 g/100g sugar consumed). While the study by [29] reported the ethanol production from sweet potato by *S. cerevisiae* and investigated the effect of inoculum size, temperature, pH and nitrogen in nutrient, they found that the maximum ethanol was 7.59 %(v/v) which was obtained with 10 % inoculum size, peptone 1.5 g/L, pH 6 after 48 h at 30 °C.

(a)



(b)



(c)

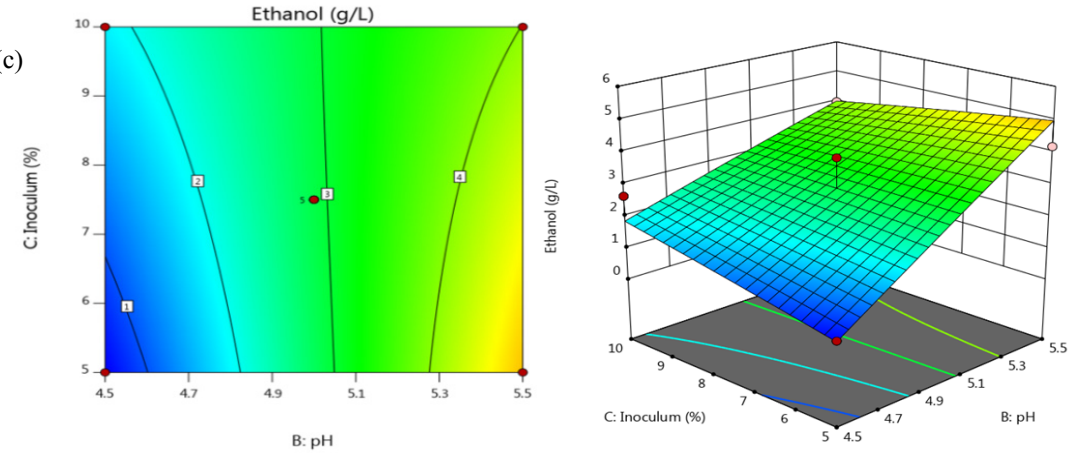


Figure 3 Response surface plot and the corresponding contour plot showing the effect of ammonium sulphate and pH (a), ammonium sulphate and inoculum (b), pH and inoculum (c) on ethanol production.

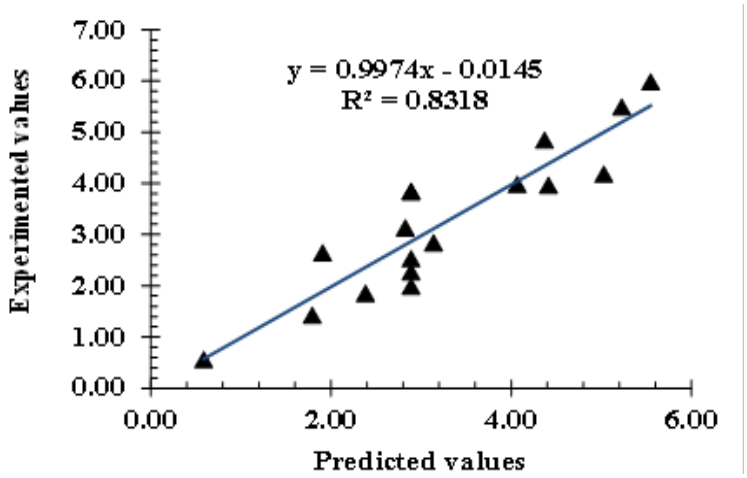


Figure 4 The predicted values and experimental values on ethanol production from the quadratic model.

The observed and model-predicted values of ethanol production after 48 h are shown in **Figure 4**. It was found that the predicted data of the response from the quadratic model agrees well with the experimental results in the range of the operating variables.

Ethanol production in 9 L bio-fermenter

Figure 5 demonstrates the time-course profile of ethanol production in 9 L bio-fermenter from LQSP using optimized fermentation conditions above (0.05 % of ammonium sulphate, pH 5.5 and 5.0 % of inoculum size). Ethanol yield, reducing sugar, cell dry weight, and pH were investigated. Ethanol yield dramatically increased in the first fermentation period of 6 h with 4.0 g/L and then slightly increased until 36 h with 5.04 g/L. While reducing sugar dramatically decreased in 6 h and was constant at 36 h of

fermentation time, yeast growth (cell dry weight) slightly decreased in 6 h and then was at constant the whole fermentation period.

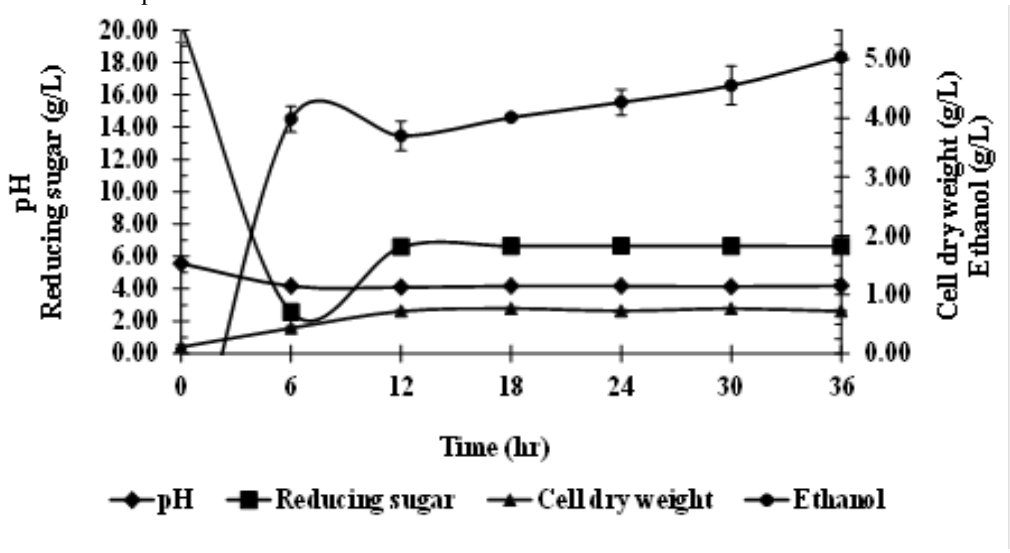


Figure 5 Time-course profile of ethanol production in 9 L bio-fermenter from LQSP under optimized conditions.

Conclusions

The results can be concluded that reducing sugar concentration would only be increased when there is an increase of LQSP. However, when acid concentration is increased, the reducing sugar decreased in acid hydrolysis with sulfuric acid. In addition, Ammonium sulphate content, pH and inoculum content had effected on ethanol production corresponding to the response surface methodology (RSM) using Box-Benhken design. The results were found that the optimum conditions were 0.05 % of ammonium sulphate, pH 5.5 and 5.0 % of inoculum which can be produced by 5.98 g/L of ethanol. Moreover, the maximum ethanol was 5.04 g/L in 9 L fermenter using the optimum conditions. Therefore, LQSP had a potential as a substrate for ethanol production.

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