International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:6, No:12, 2012

Optimization of Ethanol Fermentation from Pineapple Peel Extract Using Response Surface Methodology (RSM)

Nadya Hajar, Zainal, S., Atikah, O. and Tengku Elida, T. Z. M.

***Abstract*—**Ethanol has been known for a long time, being perhaps the oldest product obtained through traditional biotechnology fermentation. Agriculture waste as substrate in fermentation is vastly discussed as alternative to replace edible food and utilization of organic material. Pineapple peel, highly potential source as substrate is a by-product of the pineapple processing industry. Bio-ethanol from pineapple (*Ananas comosus*) peel extract was carried out by controlling fermentation without any treatment. *Saccharomyces ellipsoides* was used as inoculum in this fermentation process as it is naturally found at the pineapple skin. In this study, the capability of Response Surface Methodology (RSM) for optimization of ethanol production from pineapple peel extract using *Saccharomyces ellipsoideus* in batch fermentation process was investigated. Effect of five test variables in a defined range of inoculum concentration 6-14% (v/v), pH (4.0-6.0), sugar concentration (14-22°Brix), temperature (24-32°C) and time of incubation (30-54 hrs) on the ethanol production were evaluated. Data obtained from experiment were analyzed with RSM of MINITAB Software (Version 15) whereby optimum ethanol concentration of 8.637% (v/v) was determined. The optimum condition of 14% (v/v) inoculum concentration, pH 6, 22°Brix, 26°C and 30hours of incubation. The significant regression equation or model at the 5% level with correlation value of 99.96% was also obtained.

***Keywords*—**Bio-ethanol, pineapple peel extract, Response Surface Methodology (RSM), *Saccharomyces ellipsoideus*.

I. Introduction

TODAY, bio-ethanol production by fermentation is one of the popular subjects in the world with regards to the biological environment and economic challenges. Bio-ethanol fermentation process is usually done by species of the yeast *Saccharomyces*, whereby the sugars in the fruit juice are converted into alcohol and organic acid, that later react to form aldehydes, esters and other chemical components [1]. Sugar and starch based feedstocks are currently predominant at the industrial level and they are so far economically favourable. The current world bio-ethanol research is driven by the need to reduce the cost of production [2].

Inexpensive waste products from the forestry industry as well as agricultural residues can be utilized as raw material for biofuels [3]. More than 11,000 hectares of land in Malaysia currently planted with pineapples which generate 40 to 65 tons of waste per hectare, economical fermentation medium [4]. Pineapple waste is a material rich in sugars and lignocellulosic components [5].

Fermentation process has both the nonlinear and dynamic properties. Considerable attempts have been made by several researchers to propose a methodology based on mathematical models. Major problems of fermentation process are that they need a large number of experiments and often the models are very complicated to describe the experimental observation.

Optimization of process condition is one of the most critical stages in the development of an efficient and economic bioprocess. The classical method of studying on variable at a time can be effective in some cases, but it is useful consider the combined effects of the entire factor involved [3]. The conventional one-factor-at-a-time approach of optimization is not only tiresome but also ignores to merge interaction of each factor. One of the most common optimization used in last two decades is the Response Surface Methodology (RSM).

RSM is a powerful mathematical model with a collection of statistical techniques by which interaction between multiple processes variables can be identified with fewer experimental trials. It is widely used to examine and optimize the operational variables for experimental design, model developing, and test variable and condition optimization. There are various advantages in using statistical methodologies in terms of rapid and reliable short listing of process conditions, understanding interaction among them, and a tremendous reduction in total number of experiments, resulting in saving time, glassware, chemicals and manpower. In spite of various advantages, statistical designs have been applied to only limited number of aerobic submerged and solid state fermentation and anaerobic submerged fermentation processes deal with a large number of variables, and there are several reports on the application of RSM for the production of primary and secondary metabolites through microbial fermentation [3].

The present study was intended to determine the potential of the waste of pineapple peel for wine production. The outcome of this study may expand the utility of pineapple waste. This would not only ensure a cleaner environment but also create

Nadya Hajar is Masters Student at Department of Food Technology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia. (corresponding author e-mail: nadya0387@gmail.com).

Zainal, S. was with Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia. He is now Lecturer at Department of Food Technology (e-mail: zainal147@salam.uitm.edu.my).

Atikah, O. was with Universiti Teknologi MARA, 26400 Bandar Tun Razak Jengka, Pahang, Malaysia. She is lecturer at Department of Biology (e-mail: atikah2198@gmail.com).

Tengku Elida, T. Z. M. was with Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia. She is lecturer at Department of Biology (email: tetzm@salam.uitm.edu.my).

more job opportunities and reduce seasonal losses of the fruits [1]. RSM was used for optimization of ethanol fermentation as a function of inoculum concentration, pH, sugar concentration, temperature and time of fermentation in a batch fermentation using limited experimental runs. The accuracy of the estimated data was defined and the overall prediction ability of this technique was assessed [6].

II. Materials and Methods

A. Pineapple Peel Extract

The harvested pineapples of N36 variety at index 2 ripening stage was obtained from Pineapple Estate, Lee Peninsular Plantation Sdn. Bhd. located at Simpang Renggam, Johor, Malaysia. The fruits were first removed the crown, peeled, and the central core. The peel was crushed in a blender (Waring, United States). Then, the pineapple peel extract was filtered using muslin cloth and kept frozen at -20°C before it was used for the further analysis. The pineapple peel extract was autoclaved prior used and freshly three replicates were prepared for fermentation process.

B. Microorganism and Media

Saccharomyces ellipsoideus was used for the alcoholic fermentation of pineapple *(Ananas comosus)* peel extract variety N36. The culture originated from the HAMBI Culture Collection (Department of Food and Environmental Sciences, University of Helsinki). It was maintained on malt Yeast Extract which consisted of malt extract (3g/L), yeast extract (3g/L), peptone water (5g/L) and distilled water (top up to 1L). The media was autoclaved at 121°C for 15min and added aseptically prior to fermentation. Before use as inoculum for the fermentation, the culture was aerobically propagated in 250 ml flasks in an incubator shaker at 24°C for 24 hours.

C. Fermentation Process

The fermentation media consisted of solely pineapple peel extracted. The batch fermentation was done in triplicate using 250ml Erlenmeyer flasks with 100ml working volume. The parameters were: inoculum concentration (%v/v), concentration of sugar (°Brix), pH, temperature (°C) and fermentation time (hour). The flasks were closed with gauze and aluminum foil. The temperature in the incubator shaker was maintained at appropriate temperature and agitated at 200rpm for respective time. All samples were stored at -20°C until further analysis.

D. Fermentation Analysis

Ethanol was determined by High-Performance Liquid Chromatography (HPLC) (Waters 2659 Alliance, Waters Assoc. Inc. Milford, MA, USA) with a refractive index detector and Aminex HPX-87°C, 250mm × 4.0mm column (Bio-Rad Corp., Richmond, CA, USA) with flow rate 0.3ml/min and back pressure/ temp: 35kg/cm^2 (497 psi) / 85°C. Filtered deionized water was used as the mobile phase. Standards for each sugar were made up in the range of 1 – 20 g/100ml (v/v %) and a correlation coefficient of >0.998 was accepted. All the standard solutions were dissolved in distilled water and filtered with 0.45 µm membrane filter (Millipore), respectively. The linearity on a five-point calibration curve for ethanol was determined.

E. Experimental Design and Statistical Analysis

A central composite design (CCD) was employed to study the response ethanol concentration (%). The settings for the test or test variables were (low/high value): inoculum concentration (8/12% v/v), pH (4.5/5.5), concentration of sugar (16/20°Brix), temperature (26/30°C) and fermentation time (36/48hours). Each test variable to be optimized was coded at five levels which gave range for inoculum concentration (6-14% v/v), pH (4-6), concentration of sugar (14-22°Brix), temperature (24-32°C) and fermentation time (30-54hours) as shown in Table I.

To identify optimum levels of these five test variables, the RSM was applied. Central composite design (CCD) in the experimental design consists of 2^3 factorial points, six axial points ($\alpha = 2.366$) and six replicates of the central point could be created. Table II was used to carry out the experimental with thirty two runs. The suggested optimized medium is shown at the last six rows of the table, which is the most probability fermentation condition to get the maximum sugar consumption.

TABLE I
Coded and Actual Levels of the Test Variables for Design of Experiment

Test variables	Symbols	Coded levels				
		-α (-2.366)	-1	0	+1	+α (+2.366)
Inoculum (%)	X_1	6	8	10	12	14
pH	X_2	4	4.5	4.8	5.5	6
Sugar conc. (°Brix)	X_3	14	16	18	20	22
Temp (°C)	X_4	24	26	28	30	32
Time (hrs)	X_5	30	36	42	48	54

The second order model was selected for predicting the optimal point and was expressed as:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{55}X_5^2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{15}X_1X_5 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{25}X_2X_5 + \beta_{34}X_3X_4 + \beta_{35}X_3X_5 + \beta_{45}X_4X_5 \quad (1)$$

where Y represents response variable sugars concentration, β_1, β_2, β_3 β_4 and β_5 are linear terms, β_{11}, β_{22}, β_{33}, β_{44} and β_{55} are quadratic terms, β_{13}, β_{14}, β_{15}, β_{23}, β_{24}, β_{25}, β_{34}, β_{35} and β_{45} are interaction terms and X1, X_2, X_3, X_4 and X_5 are test variables studied. Regression analysis, analysis of variance (ANOVA) and response optimizer were performed by MINITAB software (Version 15) for determination of regression equation or model to determine the optimized condition [7].

III. Results and Discussion

The averages of the triplicate measurements of the ethanol concentration are shown in Table II. Optimum ethanol

concentration 8.637% (v/v) was determined at the optimum condition of 14% (v/v) inoculum concentration, pH 6, 22°Brix, 26°C and 30hours. The significant regression equation or model at the 5% level with correlation value 99.96% was also obtained. Table V shows that the highest predicted response was 8.698%. Values for actual and predicted responses were very close because the correlation value, $R^2 = 99.96\%$ that means the experimental data could be accepted [7] indicates that only 0.04% of the total variations are not explained by the model.

TABLE II
FIVE-LEVEL CENTRAL COMPOSITE DESIGN AND THE EXPERIMENTAL RESPONSE OF DEPENDENT VARIABLES

Run Order	X_1	X_2	X_3	X_4	X_5	Ethanol conc. (%)	
						Observed	Predicted
1	8	4.5	16	26	48	6.1002611	6.0637429
2	12	4.5	16	26	36	6.2326240	6.2172807
3	8	5.5	16	26	36	5.9749649	5.9387291
4	12	5.5	16	26	48	5.4016387	5.3673762
5	8	4.5	20	26	36	7.1999486	7.1824204
6	12	4.5	20	26	48	6.039758	6.0242030
7	8	5.5	20	26	48	7.1746004	7.1381530
8	12	5.5	20	26	36	6.0888357	6.0735633
9	8	4.5	16	30	36	5.5599137	5.5483416
10	12	4.5	16	30	48	5.4729663	5.4633674
11	8	5.5	16	30	48	5.4151114	5.3846201
12	12	5.5	16	30	36	5.6431639	5.6338475
13	8	4.5	20	30	48	7.2155610	7.2037773
14	12	4.5	20	30	36	7.0509383	7.0603295
15	8	5.5	20	30	36	6.7782220	6.7667208
16	12	5.5	20	30	48	7.3804673	7.3709393
17	6	5	18	28	42	8.6379659	8.6975594
18	14	5	18	28	42	8.1803626	8.1936598
19	10	4	18	28	42	7.3361571	7.3539659
20	10	6	18	28	42	7.0265054	7.0815875
21	10	5	14	28	42	5.2288853	5.2841091
22	10	5	22	28	42	7.5671423	7.5848093
23	10	5	18	24	42	1.2834286	1.3505646
24	10	5	18	32	42	1.4514286	1.4571833
25	10	5	18	28	30	8.370667	8.3879107
26	10	5	18	28	54	8.2310001	8.2866473
27	10	5	18	28	42	6.7322110	6.7296454
28	10	5	18	28	42	6.7170552	6.7296454
29	10	5	18	28	42	6.7061721	6.7296454
30	10	5	18	28	42	6.7671479	6.7296454
31	10	5	18	28	42	6.7846521	6.7296454
32	10	5	18	28	42	6.7435249	6.7296454

Estimated regression for the amount of sugar content determined is shown in Table III. Table III shows that when $p \leq 0.05$, this indicate that the test variables (X_1, X_2, X_3, X_4, X_5) gave significant effect on the response (ethanol concentration (%)). Equation 2 shows that the response dependent variable or ethanol concentration has a complex relationship with the test variables that encompass both first and second order polynomials. By considering the significant effect of linear, square or interaction of test variables (Table III) and Analysis of variance (ANOVA) (Table IV) , the more specific and significant regression equation model at the 5% level for the actual ethanol concentration is still same as equation 2 [7]. Thus, the significant regression equation or model at the 5% level after considering Table III and Table IV was created as shown below:

$$Y = -3.11 X_1 - 9.578 X_2 - 1.001 X_3 + 16.612 X_4 - 1.443 X_5 + 0.107 X_1X_1 + 0.488 X_2X_2 - 0.018 X_3X_3 - 0.33 X_4X_4 + 0.011 X_5X_5 - 0.024 X_1X_3 + 0.051 X_1X_4 - 0.006 X_1X_5 + 0.053 X_2X_3 + 0.053 X_2X_4 + 0.044 X_2X_5 + 0.055 X_3X_4 + 0.009 X_3X_5 + 0.006 X_4X_5 \quad (2)$$

TABLE III
RESULTS OF REGRESSION ANALYSIS OF OPTIMIZATION OF ETHANOL PRODUCTION USING SECOND-ORDER POLYNOMIAL MODEL

Model term	Regression coefficient	Std deviation	T-statistic	P-value
X_1	-3.11	0.14691	-21.167	<0.0001
X_2	-9.578	0.63949	-14.978	<0.0001
X_3	-1.001	0.15667	-6.378	<0.0001
X_4	16.612	0.17506	94.893	<0.0001
X_5	-1.443	0.05039	-28.639	<0.0001
$X_1 X_1$	0.107	0.00247	43.366	<0.0001
$X_2 X_2$	0.488	0.03957	12.336	<0.0001
$X_3 X_3$	-0.018	0.00247	-7.46	<0.0001
$X_4 X_4$	-0.33	0.00247	-134.593	<0.0001
$X_5 X_5$	0.011	0.00027	40.628	<0.0001
$X_1 X_2$	0.028	0.01339	2.103	0.059
$X_1 X_3$	-0.024	0.00335	-7.039	<0.0001
$X_1 X_4$	0.051	0.00335	15.238	<0.0001
$X_1 X_5$	-0.006	0.00112	-5.194	<0.0001
$X_2 X_3$	0.053	0.01339	3.951	0.002
$X_2 X_4$	0.053	0.01339	3.967	<0.0001
$X_2 X_5$	0.044	0.0046	9.806	<0.0001
$X_3 X_4$	0.055	0.00335	16.52	<0.0001
$X_3 X_5$	0.009	0.00112	7.994	<0.0001
$X_4 X_5$	0.006	0.0112	5.749	<0.0001

$R^2 = 99.96\%$
X_1 = inoculum concentration (% v/v), X_2 = pH,
X_3 = concentration of sugar (° Brix), X_4 = fermentation temperature (° C),
X_5 = fermentation time (hour)

It was found that linear, square and interaction of test variables gave significant effect in the determination of ethanol concentration because $p \leq 0.05$ as shown in Table IV.

TABLE IV
ANALYSIS OF VARIANCE (ANOVA) FOR THE QUADRATIC POLYNOMIAL MODEL ON THE ETHANOL PRODUCTION

Source	Sum of squares	Degrees of freedom (DF)	Mean square (MS)	F-value	P-value
Regression	78.8184	20	3.9409	1372.9	0
Linear	8.4644	5	8.0129	2791.4	0
Square	68.0274	5	13.6055	4739.7	0
Interaction	2.3266	10	0.2327	81.05	0
Residual error	0.0316	11	0.0029		
Lack-of-fit	0.0271	6	0.0045	5.07	0.048
Pure error	0.0045	5	0.0009		
Total	78.8499	31			

Table V indicates that the experiment at optimum condition for three goals (Target, Maximum and Minimum) is feasible and not feasible. From the response optimizer, the optimum conditions for five test variables of experimental and predicted response were found to be, as shown in the Table V. From Table V, the optimum condition of 14% inoculum concentration, pH 6, 22°Brix, 26°C and 30hours was selected because the difference between the value of the target response and the value of the predicted response for the same goal target was closest compared to that between other values of target response (maximum and minimum) and others values of predicted response for the same goal by considering overlaid contour plot as shown in Figs. 1, 2 and 3 for three optimum conditions. Fig. 1 shows that the optimum condition 14% (v/v) inoculum concentration, pH 6, 22°Brix, 26°C and 30hours is feasible to carry out as compared to others two goals where Figs. 2 and 3 indicate that the experiment at optimum conditions for the goals of maximum and minimum are not feasible to carry out. Fig. 1 shows the optimum condition is in the feasible region, which is white area.

TABLE V
COMPARISON VALUES OF TARGET AND PREDICTED RESPONSE FOR ETHANOL PRODUCTION AT DIFFERENT OPTIMUM CONDITIONS AND FEASIBILITY OF EXPERIMENT

Goal		Lower	Target	Upper	X_1	X_2	X_3	X_4	X_5	Predicted	F/NF
Target	EtOH	1.283	8.637	8.638	14	6	22	26	30	8.637	F
	FITS	1.351	8.697	8.698							
Min	EtOH	1.283	8.638	8.638	9	5	14	32	42	13.1887	NF
	FITS	1.351	8.698	8.698							
Max	EtOH	1.283	1.283	8.638	6	6	22	28	54	1.01662	NF
	FITS	1.351	1.351	8.698							

X_1 = inoculum concentration (% v/v), X_2 = pH, X_3 = concentration of sugar (° Brix), X_4 = fermentation temperature (° C), X_5 = fermentation time (hour), F = Feasible, NF = Not Feasible

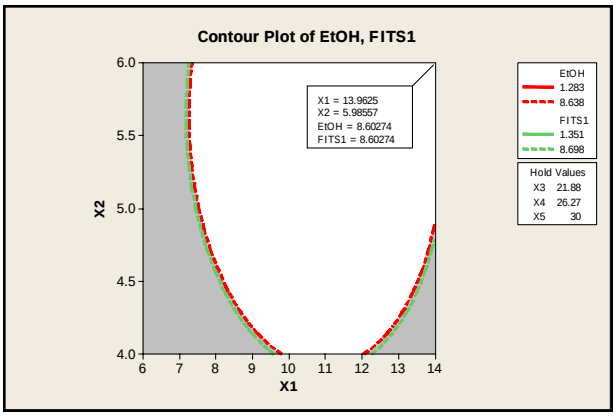


Fig. 1 Overlaid contour plot for the ethanol concentration at the optimum condition (Goal: Target)

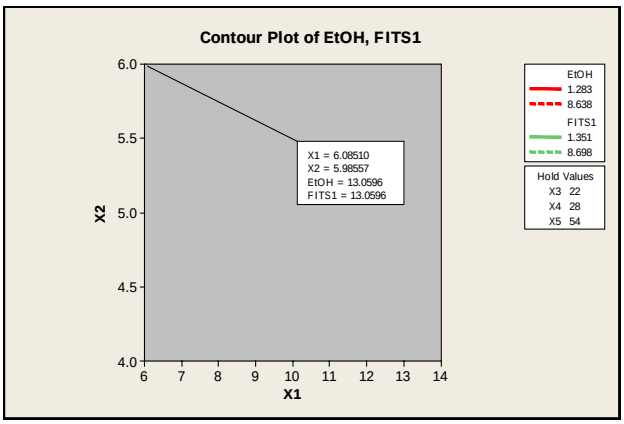


Fig. 2 Overlaid contour plot for the ethanol concentration at the optimum condition (Goal: Max)

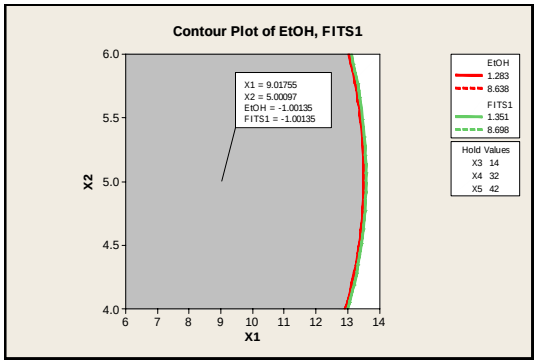
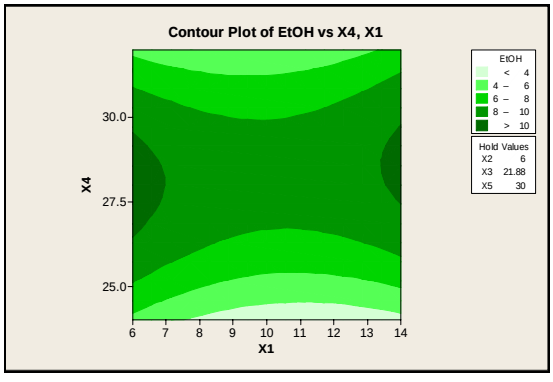


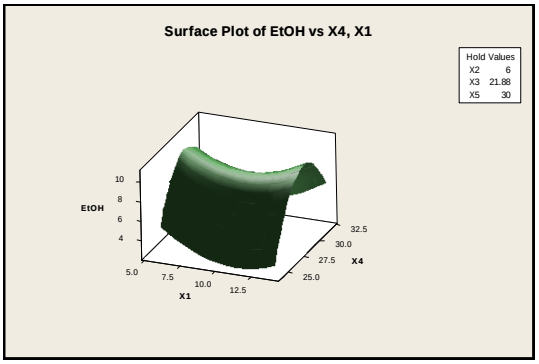
Fig. 3 Overlaid contour plot for the ethanol concentration at the optimum condition (Goal:Min)

Three dimensional surface plots and two dimensional contour plots show the effect of the feasible optimum on the amount of sugar consumption determined in the ethanol fermentation. Three dimensional graphs were generated for the pair-wise combination of the five test variables. Figures 4, 5, 6, 7 and 8 shows highlight the roles played by various test variables and comparison between test variables. All the figures are saddle shape which shows the probability of the variables at either the maximum or minimum point. The system of the contours is called a saddle or minimax system [8].

The wine yeast *Saccharomyces ellipsoideus* naturally accumulates on the skins as grapes or other citrus fruits when mature. It is found in low numbers on the grape bloom but proliferates rapidly to dominate the main fermentation. In commercial operation special strains of *Saccharomyces ellipsoideus* may be used to supplement the natural inoculum and better control fermentation. Jay (1987) reported that wine yeast is relatively resistant to sulfur dioxide and so this agent is commonly added to the grapes or must to help control undesirable microorganisms. In this fermentation study, *Saccharomyces ellipsoideus* was used as solely inoculum and the results showed that *Saccharomyces ellipsoideus* can be used as inoculum instead of using *Saccharomyces cerevisiae*.



(a)



(b)

Fig. 4 Contour plot (a) and surface plot (b) showing the effect of inoculum concentration and temperature of fermentation on ethanol production for the feasible optimum condition

The pH range normally found in juice and must has little effect on the rate of fermentation, or on the synthesis and release of aromatic compounds by yeast. Only at abnormally low pH values (<3.0) is fermented impeded. Low pH may assist the uptake of some amino acids, by supplying protons used in activating transport cross the cell membrane [9].

In this controlled fermentation using pineapple peel extract without any treatment, the ethanol produced can be achieved to the optimum value (8.637%) which is higher value than be reported by Isitua and Ibeh (2010). Ethanol from banana waste without any treatment other than controlled fermentation had alcoholic content of 0.035% (w/v), while pineapple waste yielded wine with alcoholic content of 0.21% (w/v). This finding suggests that pineapple waste had more sugar content than the banana waste. This result agrees with the report of Igue (1995) which showed that pineapple waste contains almost twice as much sugar as plantain peels [1].

Fermentation is slow in a medium containing low sugar, whereas its speed increases in must which have 15-20 g of sugar per litre and remain stable until about 200 g/L. Above this concentration, fermentation slows. Thus, an elevated amount of sugar hinders yeast growth and decreases both the maximum population and the ethanol concentration [10]. It is known that the high substrate concentrations may cause osmotic shock of the yeast cells and slow down the mass and heat transfer. A decline of the ethanol concentration could be noticed because of the exhaustion of the release glucose and the transition of the yeast metabolism towards utilization of ethanol as a carbon source. Glucose utilization was almost completed within 38hours of fermentation time. The glucose consumption was in accordance with the results of ethanol concentration since the glucose was consumed as a carbon source by the yeast. Substrate inhibition significantly effect on ethanol and biomass yield and their results concerning the substrate inhibition were in agreement with the results in this study [2].

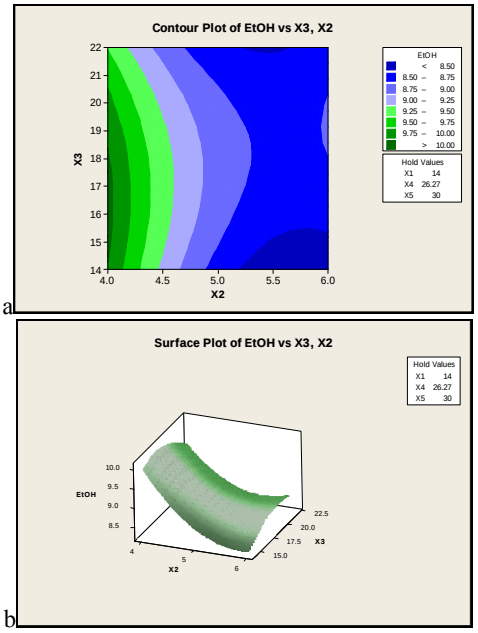


Fig. 5 Contour plot (a) and surface plot (b) showing the effect of pH and concentration of sugar (°Brix) on ethanol production for the feasible optimum condition

In term of effect temperature, it is known that this parameter influences yeast activity. In fact, the alcohol yield is generally lower at elevated temperature. In addition temperature affects fermentation speed and limits; between 15 to 35°C, the duration of the latent phase and the delay before the beginning of fermentation become shorter as temperature decrease.

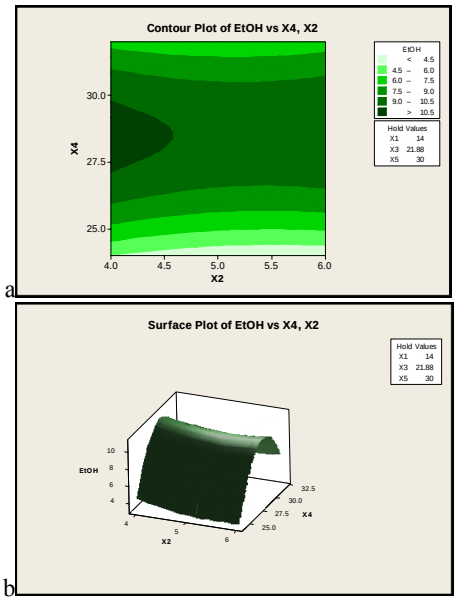


Fig. 6 Contour plot (a) and surface plot (b) showing the effect of pH and temperature of fermentation on ethanol production for the feasible optimum condition

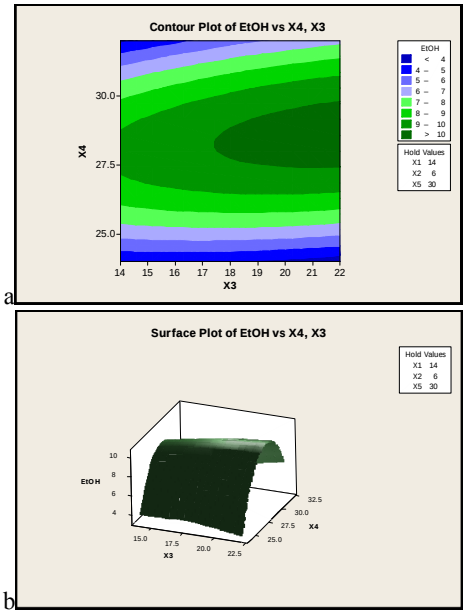


Fig. 7 Contour plot (a) and surface plot (b) showing the effect of concentration of sugar (°Brix) and temperature of fermentation on ethanol production for the feasible optimum condition

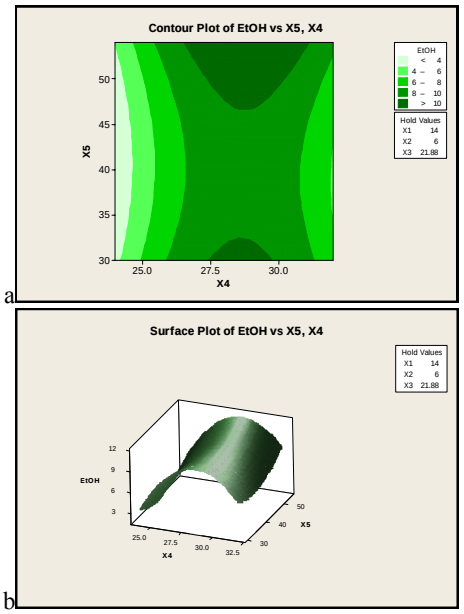


Fig. 8 Contour plot (a) and surface plot (b) showing the effect of temperature and time of fermentation on ethanol production for the feasible optimum condition

Table VI shows the difference between the predicted value from RSM of MINITAB software version 15 and the values determined by the experiment at the feasible optimal condition for ethanol concentration. The difference was 0.061. This demonstrates that the response model is a suitable tool to display the prediction.

TABLE VI
FEASIBLE OPTIMUM TEST VARIABLES FOR ETHANOL PRODUCTION AND THE PREDICTED AND EXPERIMENTAL VALUES FOR ETHANOL CONCENTRATION (%)

Feasible optimum condition		Ethanol concentration (%)		
Test variables	Values	Actual value	Predicted value	Difference
X_1	14	8.637	8.698	0.061
X_2	6			
X_3	22			
X_4	26			
X_5	30			

X_1 = inoculum concentration (% v/v), X_2 = pH,
X_3 = concentration of sugar (° Brix), X_4 = fermentation temperature (° C), X_5 = fermentation time (hour)

IV. CONCLUSION

This study investigated ethanol production using *Saccharomyces ellipsoideus* from pineapple peel extract. The pineapple peel extract was used as main substrate for ethanol production, and it was very suitable for ethanol fermentation because of the high content of fermentable sugars and *Saccharomyces ellipsoideus* can be used as solely inoculum for ethanol fermentation. Data obtained from experiment were analysed with RSM of MINITAB Software (Version 15) gave the optimum ethanol concentration 8.637% (v/v) was determined at the optimum condition of 14% (v/v) inoculum concentration, pH 6, 22°Brix, 26°C and 30hours. The significant regression equation or model at the 5% level with correlation value 99.96% was also obtained.

ACKNOWLEDGMENT

The authors would like to express our gratitude to Universiti Teknologi MARA (UiTM) for the financial support and thanks to Pineapple Estate, Lee Peninsular Plantation Sdn. Bhd. located at Simpang Renggam, Johor, Malaysia for the pineapple supplies and HAMBI Culture Collection (Department of Food and Environmental Sciences, University of Helsinki) for *Saccharomyces ellipsoideus* culture stock.

REFERENCES

[1] C. C. Isitua and I. N. Ibeh, "Novel method of wine production from banana (Musa acuminata) and pineapple (Ananas comosus) wastes," African Journal of Biotechnology, vol. 9, pp. 7521-7524, 2010.
[2] S. Nikolic´, L. Mojovic´, M. Rakin, and D. Pejin, "Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of Saccharomyces cerevisiae var. ellipsoideus," Fuel, vol. 88, pp. 1602–1607, 2009.
[3] M. Karuppaiya, E. Sasikumar, T. Viruthagiri, and V. Vijayagopal, "Optimization of process conditions using Response Surface Methodology (RSM) for ethanol production from waste cashew apple juice by Zymomonas mobilis," Chemical Engineering Communications, vol. 196, pp. 1425-1435, 2009.
[4] A. Rosma, M. T. Liong, M. N. Mohd. Azemi, and W. A. Wan Nadiah, "Optimization of Single Cell Protein Production by Candida utilis using juice extracted from pineapple wastee through Response Surface Methodology," Malaysian Journal of Microbiology, vol. 1, pp. 18-24, 2005.
[5] D. Prados, S. M., and P. L. Fito, "Industrial pineapple waste as a feasible source to produce bioethanol," in International Conference on Food Innovation. universidad polinecnica de valencia, 2010.
[6] M. Esfahanian, M. Nikzad, G. Najafpour, and A. A. Ghoreyshi, "Modeling and optimization of ethanol fermentation using Saccharomyces cerevisiae: Response surface methodology and artificial neural network," Scientific paper 2012.
[7] A. G. A. Samah, "Modification of Formaldehyde Method, Optimisation of Formaldehyde Content in Rastrelliger Faughni and Euthynnus Affinis and Storage Studies," vol. Master Degree. Malaysia: Universiti Teknologi MARA 2008.
[8] W. S. A. W. Omar, "Development of Fed-Batch Cultivation Process for Escherichia Coli Harbouring Superoxide Dismutase," vol. Master Degree. Malaysia: Universiti Putra Malaysia, 2009.
[9] R. S. Jackson, Wine Science: Principles and applications: Academic Press, 2008.
[10] D. D'Amato, M. R. Corbo, M. A. D. Nobile, and M. Sinigaglia, "Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system," International Journal of Food Science and Technology, vol. 41, pp. 1152-1157, 2006.