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OPTIMIZING ETHANOL CONCENTRATION FROM ORANGE PEEL WASTES USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

This study was aimed at optimizing the concentration of bioethanol produced from orange peel using Response Surface Methodology (RSM). Ground orange peels were hydrolyzed using dilute sulphuric acid and Saccharomyces cerevisiae was used in the fermentation process. Central Composite Design (CCD) was used to analyze the effects of four variables, acid concentration (0.5 -1 v/v %), hydrolysis time (5 – 25 min), hydrolysis temperature (100 - 140°C) and pH (3 - 6) on the amount of ethanol produced from the fermented mixture. A total of 30 experimental runs which were generated by the CCD were carried out. Acid concentration, hydrolysis temperature and pH were significant factors affecting the concentration of ethanol. The quadratic model that described relationship between ethanol concentration and the variables had a coefficient of determination (R^2) of 0.976. The optimum conditions for the optimization process was found to be acid concentration of 0.743 v/v, hydrolysis time of 16.41 min, temperature of 116.54 °C and pH of 5.25 to give an ethanol concentration of 30.15%.

1. INTRODUCTION

Fossil fuels (coal, oil, and natural gas) are currently the world's primary energy source, formed from organic materials over the course of millions of years. Fossil fuels which are non-renewable have imparted negatively on the environment (Anand *et al.*, 2009). Hence, there is the need for alternatives source of fuels to replace fossil fuels. The use of these alternative fuels will not only reduce the negative impact of the use of fossil fuels but also boost economic development (Arndt *et al.* 2012). Biofuels are one of such alternative fuels

that are produced from living organisms or from their metabolic by- products (organic or food waste products). Currently, biofuels are produced from plants using microbes to facilitate the process.

The principle fuel used as a substitute to petrol for vehicles is bioethanol (Bata and Vermon, 1989; Wang *et al.* 1999). Bioethanol fuel is mainly produced by sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam (Morschbacker and Braskem, 2009). Ethanol is a versatile transportation fuel that offers high octane number, high heat of vaporization, and other characteristics that allow it to achieve higher efficiency. Ethanol has low toxicity, volatility, and photochemical activity, resulting in the reduction of ozone formation and smog compared to conventional petroleum-based fuels. Approximately, 80% of world supply of alcohol is produced by fermentation of sugar and starch containing crops or by-products from industries based on such crops (Aristidou and Pentila, 2008). The effect of ethanol blending into gasoline on the exhaust emission from a Ford 2.3 litre engine (model-1978) was studied by Bata and Vermon (1989). It was observed that there was a 40-50% decrease in the CO concentration and lower hydrocarbon emission as compared to the base fuel. In comparison to petrol, depending on the production method, ethanol releases less greenhouse gases (Wang *et al*., 1999).

Enormous quantities of agro-industrial residues are generated throughout the world from the processing of raw agriculture materials for foods. Also, fruit processing industries produce large amount of waste materials. These wastes and their disposal have become an environmental concern especially when they are not properly disposed (Sangeeta *et al*., 2013). Cellulolytic wastes from agricultural practices can be used to produce important compounds such as alcohol thereby assisting in controlling environmental pollution (Omojasola and Jilani, 2008). Orange peel wastes belong to cellulolytic group which is a valuable biomass wastes (Mrudula and Anitharaj, 2011). The peel contains various carbohydrate polymers, which makes it an interesting choice for the production of metabolites such as ethanol by appropriate microorganisms. The ability to produce ethanol from low cost biomass will be key to making it competitive with fuels obtained from fossil-based sources.

Bioethanol can be produced from many biomass materials, but the potential for use of these materials as feedstock, for large scale production especially in developing countries like Nigeria, depends on their cost, abundance, carbohydrate contents and the ease with which they can be converted to ethanol (Hajar *et al*., 2012; Irfan *et al.,* 2014; Khali *et al.,* 2015).

The main process variables which affect the yield of bioethanol are the fermentation conditions which include temperature, pH, time, acid concentration (Joshi *et al.* 2015). Thus, the aim of this study is to optimize the concentration of ethanol production from orange peels by optimizing the acid hydrolysis process using response surface methodology (RSM).

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

Orange (*Citrus sinensis*) peels were obtained from Uselu Market in Benin City, Nigeria. The chemicals used which include sulphuric acid (98.8%), sodium hydroxide, dextrose, beakers yeast (*Saccharomyces cerevisiae*), yeast extract, urea and magnesium sulphate were purchased from Mosdelic Scientific Global Services, Benin City. They were all of analytical grade.

2.2. Sample Preparation

The orange peels were subjected to pretreatment, hydrolysis, fermentation and distillation. Before the pretreatment was done, the peels were reduced to size between 3- 4 cm in length using a laboratory knife and thereafter sundried for three days. The dried peels were grinded with a locally fabricated grinding

machine and the resulting particles were sieved. Maximum particle size of 1 mm was used for further analysis. The samples were kept in an air tight container and at low temperature until the next stage of the experiment.

2.3. Pretreatment of Sample

The pre-treatment of the ground orange peel was carried out to remove the lignin. This was done to reduce the crystallinity of the cellulose and also to increase the porosity of the materials. The pre-treatment of the samples was carried out according to the method described by Mekonnen (2012). The grinded peels were fed in batches of 50 g each with 5:1 v/w ratio of water to peels in a 500 ml conical flask and placed in an autoclave. The temperature of the autoclave was set at 121°C and the pressure was gradually released until it reached 0 bar. The retention time for each batch was 15 min. At the end of each pre-treatment, the samples were allowed to cool and the soluble and the non-soluble portion were separated. The non-soluble portion was used for the acid hydrolysis

2.4. Experimental Design of Acid Hydrolysis

The central composite design (CCD) was employed to optimize the acid hydrolysis process for maximum concentration of bioethanol from the orange peels. Five-level-four variable design was used to generate 30 experimental runs. The variables selected that influence the acid hydrolysis process were acid concentration (X_1), temperature (X_2), pH (X_3) and time (X_4). The coded and actual levels of these four independent variables are shown in Table 1.

Table 1. Coded and actual levels for the four independent variables in the CCD

Variable	Symbol	Range and their levels				
		-2	1	0	1	+2
Acid Conc. (v/v)	X_1	0.5	0.63	0.75	0.88	1.0
Temperature (°C)	X_2	100	110	120	130	140
pH	X_3	3	3.75	4.50	5.25	6.00
Time (min)	X_4	5	10	15	20	25

The acid hydrolysis experiment for ethanol production and optimization was carried out in batches using 50 g of the pre-treated sample. Dilute sulphuric acid concentration between 0.5 - 1% v/v was added to the non-soluble component of the pre-treated sample. The temperature for hydrolysis was varied from 100 - 140°C for 5 - 25 min and was neutralized to pH of 6.7 ± 0.5. The solid particles were separated from the sugar-rich liquid in the hydrolysate by centrifugation to remove the non-fermentable lignin. The pH of the hydrolysate was adjusted within the range shown in Table 1. Bioethanol production was performed by adding the adjusted sample to the fermentation media to ferment released sugar into ethanol. The experimental results obtained from the use of the CCD were analyzed by response surface methodology (RSM) using the second order polynomial equation (Equation 1)

$$Y = b_o + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{ii>j}^{n} \sum_{j}^{n} b_{ij} X_i X_j + e \quad (1)$$

where Y is the predicted response (Ethanol concentration), b_o, b_i, b_{ii}, b_{ij} are intercept, linear, quadratic and interaction constant coefficient respectively, n is the number of independent variables studied and optimized in the experimental work. X_i and X_j are the actual independent variables and e is the error term. The RSM was applied to the experimental data using a commercial statistical package, Design Expert software version 10 (STAT-EASE Inc., Minneapolis, USA). The experiments were carried out in a random order to minimize effects of explained variability in the observed responses due to extraneous factors. Coefficient of

determination (R^2) and analysis of variance (ANOVA) were used to evaluate the quality of the model. The mathematical model was only considered satisfactory when the ANOVA data showed a high level of statistical significance.

2.5. Bioethanol Production

The fermentation process was carried out in an anaerobic condition at room temperature (27 $\pm$ 2°C) for three days. Before undergoing fermentation, the fermentation media was prepared from the yeast. For bioethanol production, 100 ml of media was prepared by adding 10g of sucrose, 0.2g of yeast extract, 1g of urea, 1g of $MgSO_4.7H_2O$, and 0.5g of beakers yeast *saccharomyces cerevisiae* (Mekonnen, 2012). The sample was inoculated with the prepared media (at 1:10 ratio) and flask was incubated in an anaerobic incubator at 30°C (Khali *et al.,* 2015).

2.6. Purification of Bioethanol

Distillation which is a purification step was carried out on the fermented solution after incubation. It is the last step involved in the production of ethanol from the orange peel. It was carried out using Soxhlet apparatus at a temperature of 85°C and a distillation time of 3 h. The ethanol concentration of the distilled sample was measure according to the procedure by Geirwyr (1995). Briefly, the specific gravity of the produced alcohol was determined and alcohol concentration was obtained from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20°C. The percentage of ethanol by weight in the mixture was calculated from alcohol-water mixture data (Perry and Green, 1999).

3. RESULTS AND DISCUSSION

3.1. Model Fitting and Analysis of Variance

The experimental design matrix and results obtained are shown in Table 2. Thirty experiments with different experimental parameters, ranges and level of independent variables were performed. The responses obtained were correlated using the second order polynomial equation (Equation 1) in terms of the both the coded (Equation 2) and actual factor (Equation 3).

$$\begin{aligned} Y_{coded} = {} & 27.50 - 1.28X_1 - 0.54X_2 + 2.98X_3 + 0.11X_4 + 0.22X_2 + 1.05X_1X_3 - 0.45X_1X_4 \\ & - 0.17X_2X_3 - 0..43X_2X_4 + 0.80X_3X_4 - 3.79{X_1}^2 - 1.23{X_2}^2 - 1.16{X_3}^2 \\ & + 1.92{X_4}^2 \end{aligned} \quad (2)$$

$$\begin{aligned} Y_{actual} = {} & -316.05 + 292.13X_1 + 2.983X_2 + 13.78X_3 + 2.932X_4 + 0.180X_1X_2 + 11.20X_1X_3 \\ & - 0.720X_1X_4 - -0.0233X_2X_3 - 0.0085X_2X_4 + 0.213X_3X_4 - 242.40{X_1}^2 \\ & - 0.0123{X_2}^2 - 2.067{X_3}^2 - 0.0770{X_4}^2 \end{aligned} \quad (3)$$

Where X_1, X_2, X_3 and X_4 are the acid concentration (v/v%), hydrolysis temperature (°C), pH and hyrolysis time (minutes) respectively.

The validity of the models and their statistical significance and fitness were evaluated using analysis of variance (ANOVA). The results of the ANOVA for the second order response surface model are shown in Table 3. The model F-value of 43.51 with a low probabaility value (<0.0001) is an indication of the sigificance of the fitted model. Each term in the model was also checked for significance. The most significant term in the model is pH (X_3) with a very high F-value of 172.42, followed by acid concentration (X_1) and temperature (X_2). Based on the p-value, the hydrolysis time (X_4) was not a significant term in the model. For a term to be significant, the p-value has to be less than 0.05. The significance of the interacting terms was also evaluated. The interacting terms between the acid concentration and pH (X_1X_3) and that

between pH and time (X_3X_4) were found to be slightly significant based on their p-values of 0.0018 and 0.0116 respectively. The quadratic terms were all significant. The adequacy of the model was further checked using the "Lack of fit" F-value.

Table 2. Experimental Design Matrix and Results obtained from CCD

Run	Coded values				Actual values				Bioethanol conc. (%)	
no.	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Experimental	Predicted
1	-1	-1	1	1	0.63	110	5.25	20	25.20	25.34
2	-1	1	-1	1	0.63	130	3.75	20	19.20	17.49
3	-1	-1	1	-1	0.63	110	5.25	10	20.90	21.78
4	1	1	1	-1	0.88	130	5.25	10	21.30	21.63
5	0	-2	0	0	0.75	100	4.50	15	24.20	23.68
6	-1	1	1	-1	0.63	130	5.25	10	21.40	20.74
7	0	0	0	2	0.75	120	4.50	25	20.20	20.02
8	1	1	-1	1	0.88	130	3.75	20	12.20	12.38
9	-1	-1	-1	1	0.63	110	3.75	20	18.80	19.53
10	-1	-1	-1	-1	0.63	110	3.75	10	20.00	19.16
11	-1	1	-1	-1	0.63	130	3.75	10	17.20	18.83
12	0	2	0	0	0.63	140	4.50	15	21.80	21.52
13	1	-1	1	-1	0.88	110	5.25	10	20.30	21.76
14	0	0	0	0	0.75	120	4.50	15	28.50	27.50
15	2	0	0	0	1.00	120	4.50	15	10.20	9.78
16	1	1	-1	-1	0.88	130	3.75	10	15.90	15.51
17	1	-1	1	1	0.88	110	5.25	20	24.10	23.53
18	0	0	2	0	0.75	120	6.00	15	30.20	28.82
19	1	-1	-1	-1	0.88	110	3.75	10	15.30	14.94
20	0	0	0	0	0.75	120	4.50	15	27.30	27.50
21	-2	0	0	0	0.50	120	4.50	15	15.30	14.92
22	1	1	1	1	0.88	130	5.25	20	21.10	21.69
23	0	0	0	0	0.75	120	4.50	15	27.10	27.50
24	0	0	-2	0	0.75	120	3.00	15	16.30	16.88
25	0	0	0	0	0.75	120	4.50	15	27.90	27.50
26	0	0	0	0	0.75	120	4.50	15	26.80	27.50
27	0	0	0	0	0.75	120	4.50	15	27.40	27.50
28	-1	1	1	1	0.63	130	5.25	20	21.20	22.61
29	1	-1	-1	1	0.88	110	3.75	20	13.10	13.51
30	0	0	0	-2	0.75	120	4.50	5	20.20	19.58

The "Lack of fit" F-value of 4.50 implies that the model lack of fit is not significant. According to Montgomery (2001), the "Lack of fit" is an indication of that the model was adequate in prediction the ethanol concentration within the ragne of study of the various factors.

The sutability of the model was tested using the coefficient of determination (R^2). The coefficient of determination (R^2) is the proportion of variation in the response that is attributed to the model. For a good fitted model, R^2 should not be less than 0.9. Values close to unity signify the suitability of the fitting emperical model to the actual model. From the analysis, the R^2 value was 0.9760. However, a large value of R^2 does not always imply the adequatacy of the model. Thus, an adjusted R^2 value of over 0.9 more appropriately evaluates the model adequacy. The adjusted R^2 value of 0.9535 was close to the R^2 value. The coefficient of variation (CV=5.30%) was less than 10% which indicates a high degree of precision and relability of the model. The high Adequate Precision value of 24.183 was greater than the critical value of 4, an indication that the signal was adequate.

This model could therefore be use to navigate the design space. The data was analyzed to check the normality of the residuals. The normal probability plot shown in Figure 1 is a graphical technique for assessing whether or not a data is approximately distributed. The normality plot shows a linear pattern which indicates the normal distribution of the residuals. Residuals are the difference between the observed and predicted responses. They are estimates of experimental error obtained by subtracting the observed responses from the

predicted responses. A closer examination of this plot shows most of the points are closer to the line, having some points also scattered. This was expected from normal data. From the observed distribution, it can thus be concluded that the values were normally distributed.

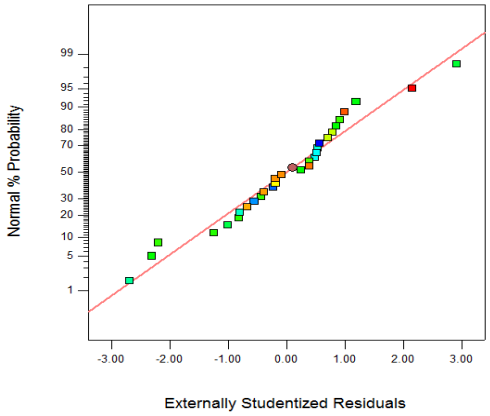

Figure 1: Normal probability plot

Table 3. ANOVA results for the second-order model

Source	Sum of squares	df	Mean Square	F value	p-value Prob.> F
Model	754.70	14	53.91	43.51	<0.0001 significant
X_1	39.53	1	39.53	31.90	<0.0001
X_2	7.04	1	7.04	5.68	0.0308
X_3	213.61	1	213.61	172.42	<0.0001
X_4	0.28	1	0.28	0.23	0.6404
X_1X_2	0.81	1	0.81	0.65	0.4314
X_1X_3	17.64	1	17.64	14.24	0.0018
X_1X_4	3.24	1	3.24	2.62	0.1267
X_2X_3	0.49	1	0.49	0.40	0.5389
X_2X_4	2.89	1	2.89	2.33	0.1475
X_3X_4	10.24	1	10.24	8.27	0.0116
X_1^2	393.47	1	393.47	317.60	<0.0001
X_2^2	41.16	1	41.16	33.22	<0.0001
X_3^2	37.07	1	37.07	29.92	<0.0001
X_4^2	101.64	1	101.64	81.04	<0.0001
Residual	18.58	15	1.24		
Lack of fit	16.72	10	1.67	4.50	0.0554 not significant
Pure error	1.86	5	0.37		
Cor Total	773.29	29			
C.V. = 5.30%	$R^2 = 0.9760$	Adjusted $R^2 = 0.9535$		Pred. $R^2 = 0.8720$	$Adeq.Preci. = 24.183$

3.2. Effect of Variables on Ethanol Concentration

The model generated by the software was used to obtain response surface plots which are graphical representation of the model. These response surface plots were generated by keeping two independent variables constant at their central level, while varying the other two variables within experimental ranges. Figure 2 shows the response surface plots which describe the interactive effect of variables and indicate the optimal level of each parameters for attaining ethanol concentration. Figure 2a shows the combined effect of acid concentration and temperature on the ethanol concentration at constant pH of 4.5 and time of 15 min.

From the plot, increasing the acid concentration, while keeping temperature constant leads to an initial increase in ethanol concentration. However, further increase in acid concentration beyond 0.75 v/v leads to a decrease in the ethanol concentration. At 0.75 v/v acid concentration a temperature of 100°C, the maximum ethanol concentration of 24.2 wt.% was obtained. On the other hand, increasing hydrolysis temperature from the experimental range of 100 – 140 °C at a fixed acid concentration of 0.50 v/v, the ethanol concentration slightly increased from 12.14 – 14.92 wt.% and then decreased to 8.37 wt.%.

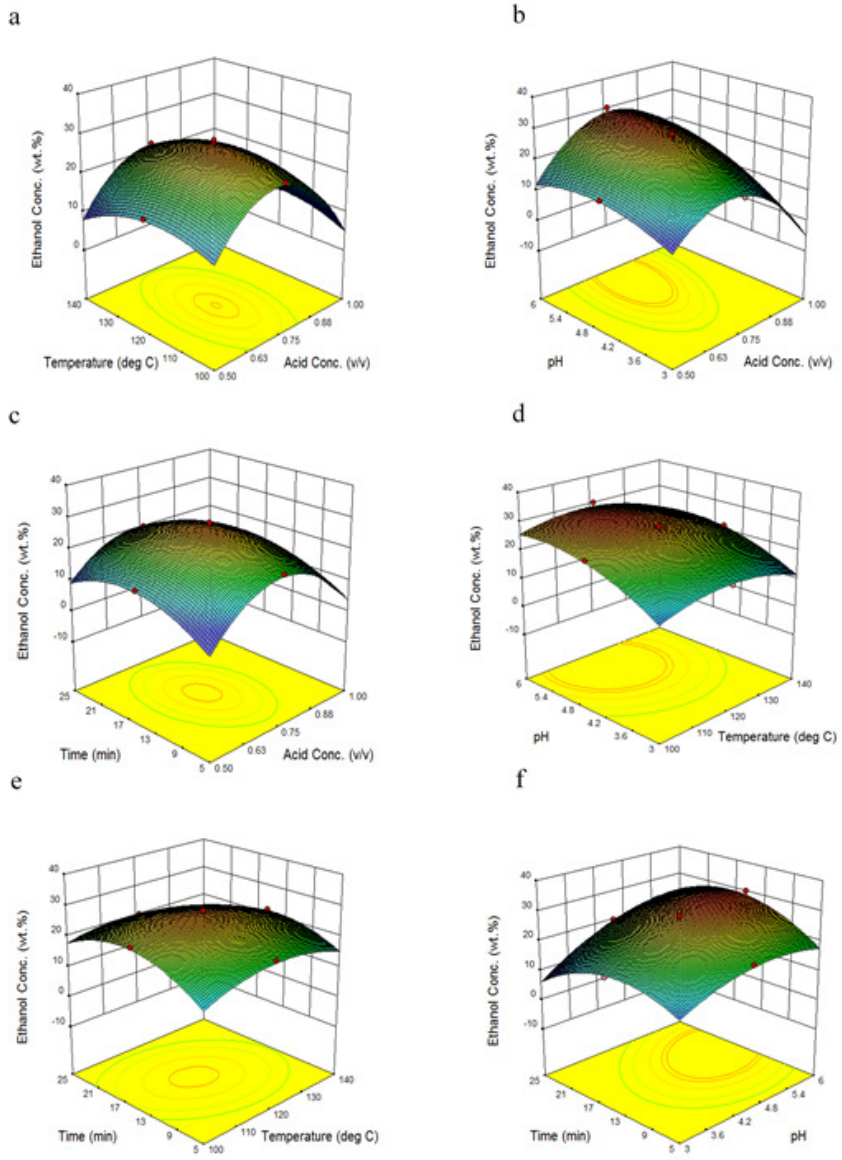

Figure 2: Response surface plots (a) acid concentration and temperature (b) acid concentration and pH (c) acid concentration and time (d) temperature and pH (e) temperature and time (f) temperature and time (f) pH and time

This observation may be due to the fact that at lower acid concentration and hydrolysis temperature the cellulose of the orange peel might not hydrolysate to simple sugar (glucose) and at higher acid concentration and hydrolysis temperature, the cellulose might convert to other sugar molecules that are not fermentable (Irfan *et al.*, 2014).

The variation of ethanol concentration with acid concentration and pH at a fixed hydrolysis temperature of 120^{o}C and hydrolysis time of 15min is shown in Figure 2b. From the figure, it was observed that when acid concentration is kept constant at 0.50 v/v and pH is increased from 3 - 6 which is an acidic medium, the yield increased from 8.647 – 12.17 wt.%. At constant pH of 3, increasing acid concentration from 0.5 – 0.75 v/v, ethanol concentration increased from 8.647 – 16.26 wt.%. Further increase in acid concentration beyond 0.75 v/v resulted to a decrease in the ethanol concentration. Increasing acid concentration beyond the optimum concentration of 0.75 v/v, has a negative effect on the ethanol concentration. The combined effect of acid concentration and time on the ethanol concentration is shown in Figure 2c. The plot was similar to that of the combined effect of acid concentration and pH. Maximum ethanol concentration (27.5 wt.%) is obtained at a time of 15 min and acid concentration of 0.75 v/v. At a fixed acid concentration of 0.5 v/v and increasing time from 5 – 25 min, the ethanol concentration increases from 5.76 – 12.09 wt.%.

On the contrary, at a fixed time of 5 min, with increase in acid concentration of 0.5 – 1 v/v, ethanol concentration increased from 3.857 – 9.78 wt.% at 0.75 v/v. Further increase in the acid concentration resulted to a decrease in the ethanol concentration (1.44 wt.%) The response surface plot for the combined effect of pH and temperature is shown in Figure 2d. There was a mild increase in ethanol concentration, when the temperature was increased at low pH. There was not much increase in ethanol concentration as a result of a low value of the pH that is, an acidic solution, which will have a negative effect on the micro-organism during fermentation. Figure 2e shows a variation of ethanol concentration with hydrolysis time and hydrolysis temperature at constant acid concentration of 0.5 v/v and pH of 4.5. It was observed that as temperature is kept constant at 100^{o}C and time increased from 5 – 10 min, the ethanol concentration increased from 15.36 – 23.68 wt.% and then made a little decrease to 18.07 wt.%. The effect of hydrolysis time and pH at constant hydrolysis temperature of 120^{o}C and acid concentration of 0.75v/v is shown in Figure 2f. At fixed pH of 3, varying hydrolysis time from 5 – 25 min, there is a slight increase in ethanol concentration. This is due to the fact that pH of 3 is acidic and the growth of microorganism for fermentation is negatively affected. With this poor condition for the growth of microorganism, this will lead to a decrease in the concentration of ethanol. On the contrary, at a constant hydrolysis time of 5 min and varying pH values of 3 – 6, there was an increase in ethanol concentration from 12.56 – 18.28 wt.%, which indicated that a non-acidic medium is favourable for fermentation which in turn will lead to increase in ethanol concentration.

3.3. Optimization of Ethanol Concentration

Numerical optimization was used to optimize the ethanol yield. The optimal factors given by the software are acid concentration of 0.743 v/v, temperature of 116.54^{o}C, pH of 5.25 and hydrolysis time of 16.41 min to give an ethanol concentration of 29.58%. The optimal hydrolysis values obtained from this study was thereafter verified. The mean average value of ethanol concentration that was obtained was 30.15 %, an error of 1.927% from that predicted by the model.

4. CONCLUSION

The concentration of ethanol production from orange peel was optimized in this study. Four variables involved in the optimization process were acid concentration, temperature, pH and time. The optimum conditions for ethanol concentration are; acid concentration of 0.743 v/v, hydrolysis temperature of 116.54^{o}C, pH of 5.25 and hydrolysis time 16.40 min. Under these conditions, the experimental yield was obtained to be 30.15% as against the predicted yield of 29.58 %, which was verified by carrying out three

experimental replicates to validate the model. This study demonstrated that orange peels could serve as a feedstock for ethanol production.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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