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Optimization of Process Variables for the Production of Oxalic Acid from Sweet Potato Starch Hydrolyzate.

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Abstract

In this study optimization of oxalic acid production from Sweet Potato Starch Hydrolyzate (SPSH) using *Aspergillus niger* was investigated. The effects of three independent variables (concentrations of SPSH, fermentation time and pH) on the response (oxalic acid yield) and their reciprocal interactions were established using Response Surface Methodology (RSM). The box behnken design (BBD) was used to generate a total of 17 fermentation runs, which were subsequently conducted. A second-order mathematical model was obtained to predict the oxalic acid yield. A statistical model predicted the highest conversion yield of oxalic acid to be 103.274 g/l, at the optimal condition of SPSH of 149.97 g /l, time of 9 days, and pH of 6. The optimized condition was validated with the actual oxalic acid yield of 103.26 g/l. This work revealed that sweet potato starch could serve as alternative carbon source for oxalic acid production and the results could be scaled up to industrial production.

Keywords: Sweet potato, Response Surface Methodology, Oxalic acid, Optimization, *Aspergillus niger.*

1. Introduction

Oxalic acid is also known as ethanedioic acid with I.U.P.A.C name of formula $H_2C_2O_4$. It is a naturally occurring acid that can be found in many plant species, such as Orange, rhubarb, spinach, tea, cocoa, nuts, berries and beans. Its compounds have widespread industrial applications in several fields such as textiles, tanning, oil refining, catalysts, pharmaceuticals, dyes, explosives, straw bleaching, printing, marble polishing, and metal and cloth cleaning. It is also a very important chemical in petroleum, rare-earth, ink, rust, corrosion inhibitor, and dental adhesive processing (Guru et al., 2000). Typically oxalic acid occurs as dihydrate with the formula $H_2C_2O_4 \cdot 2H_2O$. In its concentrated and pure form, oxalic acid is very toxic and needed to be handled with extreme care. However most of its products are in diluted forms and hence do not possess much danger.

There are mainly six methods for producing oxalic acid depending on the raw material selected. It can be prepared from various materials such as ethylene, glycol, propylene, lignin, molasses, sugarcane, sugars, flour, plant wastes, formate, and carbonate and bicarbonate salts etc. by using different methods. These methods are classified in six groups, namely: (i) fusion of sawdust with caustic soda, (ii) oxidation of olefines and glycols, (iii) radiation processing of carbonate solutions and molasses, (iv) fermentation of carbohydrates, (v) oxidation of carbohydrates by nitric acid, and (vi) decomposition of formates. Among these methods, the last three have been considered to be the most important of all (Guru et al., 2001). A variety of fungi, including saprophytic and phytopathogenic species can be used to synthesis this acid (Dutton and Evans, 1996). Saprophytic species, such as *Aspergillus niger*, remains the organism of choice for oxalic acid production due to ease of handling, its ability to ferment a variety of cheap raw materials, and high yields (Schuster et al., 2002). However, some authors have employed some microorganisms in the production of oxalic acid through fermentation. Rujiter et al. (1999) employed *Aspergillus niger* on a carbohydrate source, Hamel et al (1999) used *Pseudomonas fluorescens* on the same carbon source. None of these reports made use of design of experiment and optimization tool such as response surface methodology (RSM) for their studies. Response Surface Methodology (RSM) is a comprehensive experimental design and mathematical modeling, through the partial regression fitting of the experimental factors (Wang et al., 2011). It has the advantage of reducing number of experimental runs needed to give adequate information for statistically acceptable results. RSM has been applied in research for optimizing various processes including fermentation conditions, for example, in productions of citric acid (Imandi et al., 2008), ethanol (Wang et al., 2011), Scleroglucan (Desai et al., 2008) and

thermostable lipase (Ebrahimpour et al., 2008).

A cheap oxalic acid can be produced by using cheap agricultural products such as sweet potatoes. China accounts for 75-80% of worldwide sweet potato production with an annual production of 78.8 Tonnes followed by Nigeria with about 3.3 Tonnes which is the largest in africa. One of the challenges faced by developing countries such as Nigeria is the lack of storage facilities and a significant amount of the tubers are been destroyed due to improper storage management. In order to proffer solution to this wastage, value addition to these tubers to produce other useful products such as oxalic acid, is imperative (Betiku and Adesina, 2013)

In the development of any fermentation process, the optimization of the process variables, particularly physical and chemical parameters, is of primary importance due to its impact on the economy and feasibility of the process (Lie and Tzeng, 1998). Moreover, owing to the complexity of the metabolic state in fungus for the improved accumulation of desired product, there is an obvious need to optimize the important process variables, depending on the substrate (Dhillon, et al, 2008)

In this present study, oxalic acid production from sweet potato starch hydrolyzate (SPSH) using filamentous fungus, *Aspergillus niger*, in a submerged fermentation system was investigated. In order to optimize the fermentation variables of the process, Box Behnken design (BBD) and RSM were applied to determine the effects of three factors (carbon source, time and pH) and their reciprocal interactions on oxalic acid yield.

2. Materials and Methods

2.1 Sweet Potato Starch Hydrolyzate (SPSH) Production

Freshly harvested matured sweet potato (Ipomoea bantatas) tubers were sourced from a local market in Ogbomoso, Oyo State, Nigeria. The tubers were washed to remove adhering latex and dirt, it was then peeled and afterward milled. Water was added to the slurry followed by filtration using muslin cloth. The filtrate was allowed to settle and then the top water was decanted (Betiku and Adesina, 2013). The starch obtained was sun-dried to constant weight and then packed in a container. To make 25 % slurry, 20 g of starch was weighed into 80 ml distilled water to make slurry. The solution of 40 ppm Ca^{2+} was added for the stability of the enzyme. The pH was adjusted to 6.5 with Citrate-phosphate buffer. Gelatinization was carried out by heating the mixture to 97 ^{o}C and was held at this temperature for 10 min. For the hydrolysis of the starch, two-step enzymatic hydrolysis method was applied. For the first step, the slurry was cooled to 60 ^{o}C and the gelatinized starch slurry was liquefied by adding 1% (w/v) of partially purified α-amylases at 60 ^{o}C for 1 h. for the second step, the liquefied starch was buffered to pH of 4.5 and was subsequently saccharified with partially purified glucoamylase (1 % w/v) at 50 ^{o}C for 40 min. The enzyme activity was stopped by heating the mixture for 15 min. The mixture was centrifuged to remove the residue.

2.2 Microorganism

Aspergillus niger used in this study was obtained from Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The microorganism was maintained on PDA (Potato-Dextrose) agar. Subsequent sub culturing of microorganism was done by heating 39 g of PDA agar in 1 liter of distilled water. It was then transferred into sterile plates and autoclaved where it was allowed to cool at room temperature. The solidified medium was later inoculated with the microorganism and was incubated at 30 ^{o}C for 5 – 7 days. The microorganism was kept in the refrigerator at temperature of 4 ^{o}C with monthly sub-culture.

2.3 Inoculum preparation

Cultures grown on PDA medium in petri dishes was transferred into Duran flask (250 ml) containing 100 ml of sterile distilled water. The inoculated flasks were shaken continuously on an environment-controlled incubator shaker (New Brunswick Scientific Co., USA) at 200 rpm and 30 ^{o}C for 1 h before it was used for the fermentation.

2.4 Media composition

Fermentation medium used for this study was composed of carbon source, (sweet potato starch hydrolysate, SPSH), $NaNO_3$ (1.5 g/l); KH_2PO_4 (0.5 g/l); $MgSO_4.7H_2O$ (0.025 g/l); KCl (0.025 g/l); yeast extract (1.6 g/l), (Strasser et al., 1994). The agitation speed was maintained at 200 rpm. All media and flasks were sterilized using an autoclave at 121 ^{o}C for 15 min.

2.5 Submerged Fermentation procedure

Fifty milliliter of the hydrolysate was measured into 250-ml of Duran flask and various nutrients were added into the

hydrolyzate.. The pH of each medium was adjusted using 1 N of HCl and 2 M of NaOH according to the experimental design. Thereafter, 5% (v/v) of inoculum size was added aseptically then covered with cotton plug. The inoculated flasks were then shaken continuously on an environment-controlled incubator shaker (model G25-R, New Brunswick Scientific, Edison, N.J., USA) at 200 rpm. Samples were withdrawn at the interval according to the experimental design.

2.6 Analytical techniques

The concentration of Oxalic acid in the fermentation medium was determined using catalytic kinetic spectrophotometric method described by Jiang et al (1996). This was based on catalytic effect on the redox reaction between dichromate and rhodamine B in sulphuric acid which was measured at wavelength of 555 nm. 10 ml of the sample was withdrawn from the fermentation medium and filtered with Whatman No 1 filter paper. Subsequently, 1 ml from the filtrate was mixed thoroughly with 100 ml of distilled water and the resulting solution was used for oxalic acid analysis. The analysis was done under the condition of 0.05 mol l^{-1} of sulphuric acid, 0.03 mol l^{-1} potassium dichromate and $3.28*10^{-6}$ of rhodamine B at 90^{o} for 8 min after which the calibration graph of oxalic acid had been obtained.

2.7 Optimization studies of oxalic acid production

The Box behnken design (BBD) was employed to generate 17 experimental run by considering three factors, viz. SPSH concentration, fermentation time and pH. Response surface methodology (RSM) was used to optimize the process and regression equation analysis was used to evaluate the response surface model. The three different factors chosen as main fermentation media variables were designated as X_1, X_2, and X_3, in Table 1. The low, middle, and high levels of each variable were designated as -1, 0, +1, respectively. Table 2 shows the 17 experimental runs generated in term of coded variables, the observed oxalic acid yield, the predicted oxalic acid yield and the residual value. The independent variables that were used were coded according to Eq. (1).

$$x_i = \frac{x_i - x_o}{\Delta x} \qquad i = 1, 2, \ldots, k, \tag{1}$$

Where, X_i and x_i are the actual value and coded value, respectively, X_o is the value of X_i at center point, and Δx is the step change value (Myers, 2003). To correlate the response variable to the independent factors, multiple regressions was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA). The generalized response surface model for describing the variation in response variable is given below.

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

where Y is the predicted response by RSM, i and j are the linear and quadratic coefficients, respectively, β is the regression coefficient, k is the number of factors studied and optimized in the experiment, and ε represents the random error.

3. Results and discussion

3.1 Optimization of oxalic acid production from SPSH

This study was aimed at finding the optimal condition for oxalic acid production. Table 2 depicts the coded factors considered in the study with the observed, predicted and the residual values obtained. Figure 1 depicts the Predicted and actual yield of oxalic acid. Design Expert 8.0.3.1 software was used to evaluate and determine the coefficients of the full regression model equation and their statistical significance. Table 3 shows the results of test of significance for every regression coefficient. The results showed that the p-values of the model terms were significant, i.e. $P<0.05$. In this case, the three linear terms (X_1, X_2, X_3), cross-products (X_1X_2, X_2X_3) and the quadratic terms (X_1^2 and X_2^2) were all significant model terms at 95% confidence level (Table 3). Results of the analysis of variance of regression equation model are presesented in Table 4. The model F-value of 83.98 with corresponding low p-value ($p < 0.0001$)

implied the model obtained was significant. The data obtained fitted best to a quadratic model and it exhibited low standard deviation. The value of coefficient of determination (R^2) gives an indication of consistency between the experimental values and the predicted values. Guan and Yao (2008) suggested that R^2 should be at least 0.80 for the good fit of a model. In this case, R^2 of the model obtained was 0.9908, which indicated that the sample variation of 99.08 % for oxalic acid yield was attributed to the independent factors and only 0.92 % of the total variation are not explained by the model. This obeservation implied that the model proved suitable for the adequate representation of the actual relationship among the selected factors. The lack-of-fit term greater than 0.05 was not significant, which revealed that the model was significant for the response. Table 5 depicts regression coefficients and significance of response surface quadratic. Therefore, the quadratic model obtained in this study could be used in theoretical prediction of oxalic acid production from *A. niger* grown of SPSH.The final equation in terms of coded factors for the BBD response surface quadratic model is expressed in Eq. (3).

$$Y = +46.97 + 3.24X_1 + 21.76X_2 + 16.79X_3 - 4.33X_1X_2 + 2.86X_1X_3 + 10.56X_2X_3 - 5.73X_1^2 + 4.43X_2^2 + 2.67X_3^2 \quad (3)$$

The nature of 3-D surfaces indicated the mutual interaction of SPSH concentration with fermentation time and pH. Figures (2-4) describe the surface plots for the conversion of SPSH into oxalic acid. The optimal condition values of the independent variables selected for the production of oxalic acid was obtained by solving the regression equation (Eq. 3) using the Design-Expert software. The values obtained were pH of 6.2, time of 9 days and SPSH concentration of 149.97 g/l. The predicted oxalic acid concentration under the above set of values was Y =103.274 g/l. To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average oxalic acid yield obtained was 103.26 g/l, which was well within the estimated value of the model equation.

Table 1: Coding of experiment factor and levels

Variable	Unit	Symbol	Coded levels		
			-1	0	1
pH		X_1	5	6	7
Time	days	X_2	5	7	9
Glucose Conc.	g/l	X_3	50	100	150

Table 2: Data for experimental, predicted and residual oxalic acid yield

Std run	X_1	X_2 (days)	X_3 (g/l)	Experimental (g/l)	RSM Predicted(g/l)	Residual
1	-1	-1	0	15.79	16.34	-0.55
2	1	-1	0	30.00	31.47	-1.47
3	-1	1	0	70.00	68.53	1.47
4	1	1	0	66.89	66.34	0.55
5	-1	0	-1	27.37	26.74	0.63
6	1	0	-1	29.04	27.49	1.55
7	-1	0	1	53.05	54.60	-1.55
8	1	0	1	66.16	66.79	-0.63
9	0	-1	-1	26.00	26.08	-0.08
10	0	1	-1	46.38	48.48	-2.10
11	0	-1	1	40.63	38.53	2.10
12	0	1	1	103.26	103.18	0.078
13	0	0	0	47.79	46.97	0.82
14	0	0	0	41.74	46.97	-5.23
15	0	0	0	49.95	46.97	2.98
16	0	0	0	49.53	46.97	2.56
17	0	0	0	45.84	46.97	-1.13

Table 3: Test of significance for every regression coefficient

Source	Sum of Squares	df	Mean Square	F- Value	p-value
X_1	83.72	1	83.72	9.14	0.0193
X_2	3789.29	1	3789.29	413.85	< 0.0001
X_3	2254.90	1	2254.90	246.27	< 0.0001
X_1X_2	75.00	1	75.00	8.19	0.0243
X_1X_3	32.72	1	32.72	3.57	0.1006
X_2X_3	446.27	1	446.27	48.74	0.0002
X_1^2	138.30	1	138.30	15.11	0.0060
X_2^2	82.68	1	82.68	9.03	0.0198
X_3^2	29.93	1	29.93	3.27	0.1135

Table 4: Analysis of variance (ANOVA) of regression equation

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	6920.69	9	768.97	83.98	<0.0001
Residual	64.09	7	9.16		
Lack of Fit	19.36	3	6.45	0.58	0.6603
Pure Error	44.74	4	11.18		
Cor Total	6984.79	16		R sq = 0.9908	

Table 5: Regression coefficients and significance of response surface quadratic

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	46.97	1	1.35	43.77	50.17	
X_1	3.24	1	1.07	0.71	5.76	1.00
X_2	21.76	1	1.07	19.23	24.29	1.00
X_3	16.79	1	1.07	14.26	19.32	1.00
X_1X_2	-4.33	1	1.51	-7.91	-0.75	1.00
X_1X_3	2.86	1	1.51	-0.72	6.44	1.00
X_2X_3	10.56	1	1.51	6.98	14.14	1.00
X_1^2	-5.73	1	1.47	-9.22	-2.24	1.00
X_2^2	4.43	1	1.47	0.94	7.92	1.00
X_3^2	2.67	1	1.47	-0.82	6.15	1.00

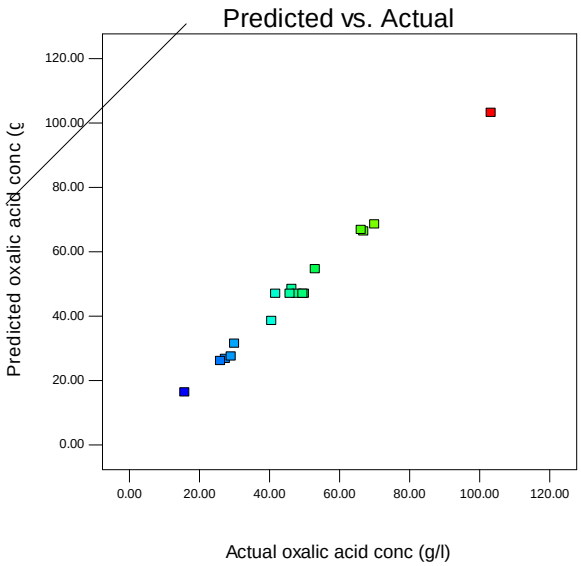

Figure 1: The predicted and actual yield of oxalic acid

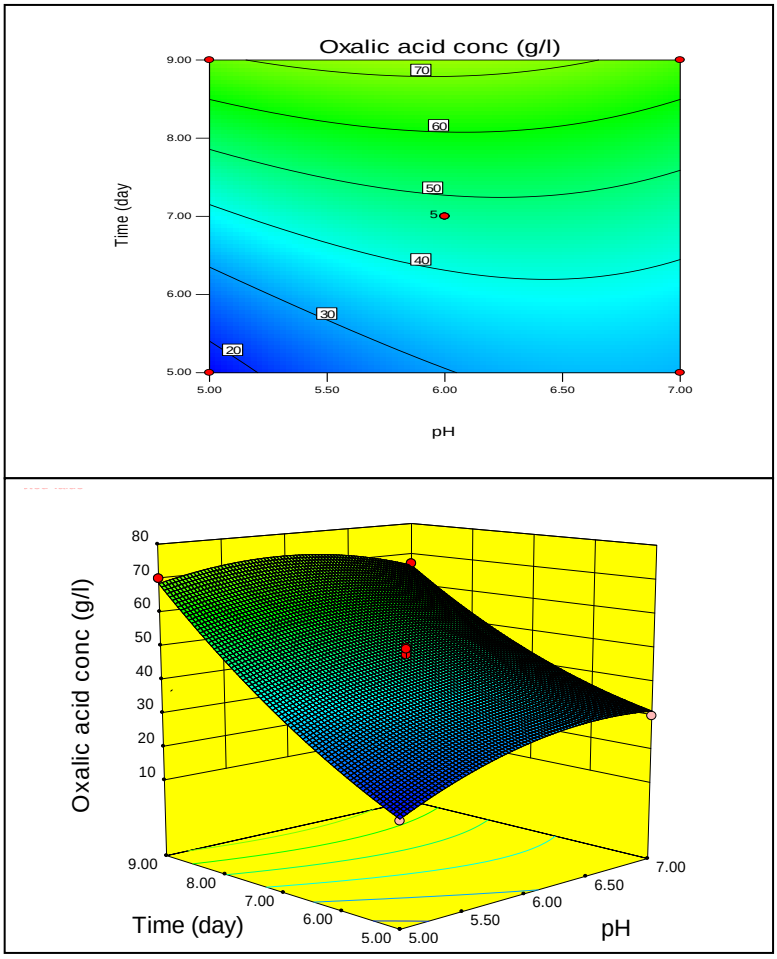

Figure 2: The contour and response surface plots of the effect of time, pH and their reciprocal interaction on oxalic acid yield.

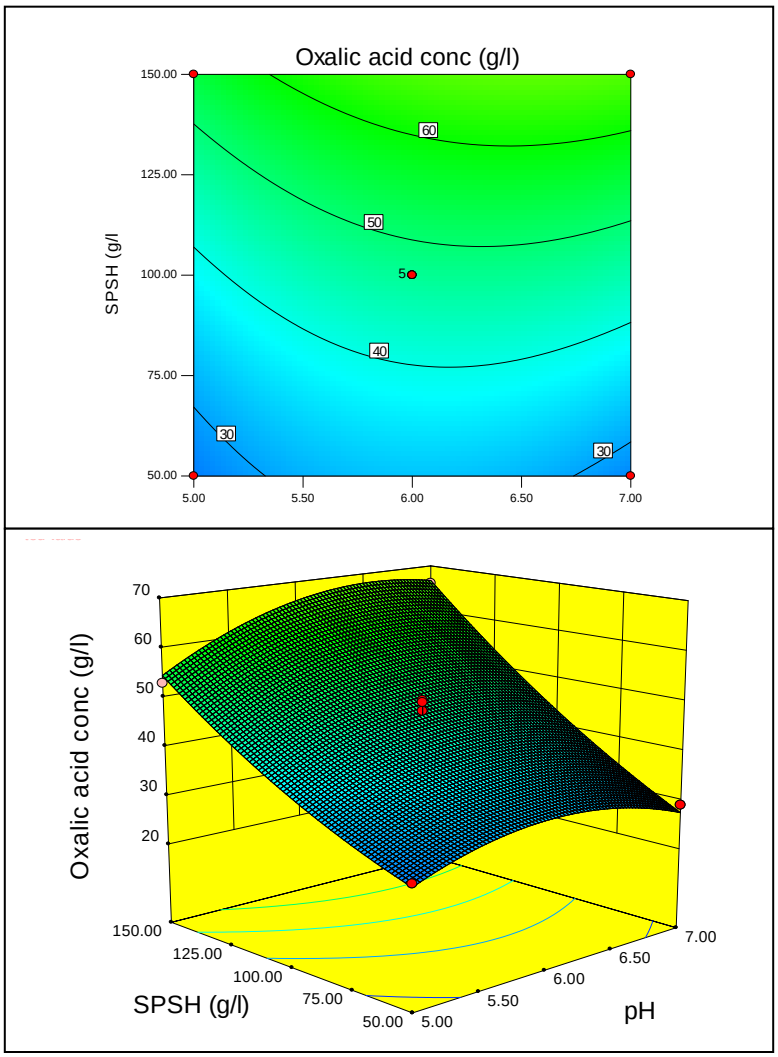

Figure 3: The contour and response surface plots of the effect of glucose concentration, pH and their reciprocal interaction on oxalic acid yield keeping time constant

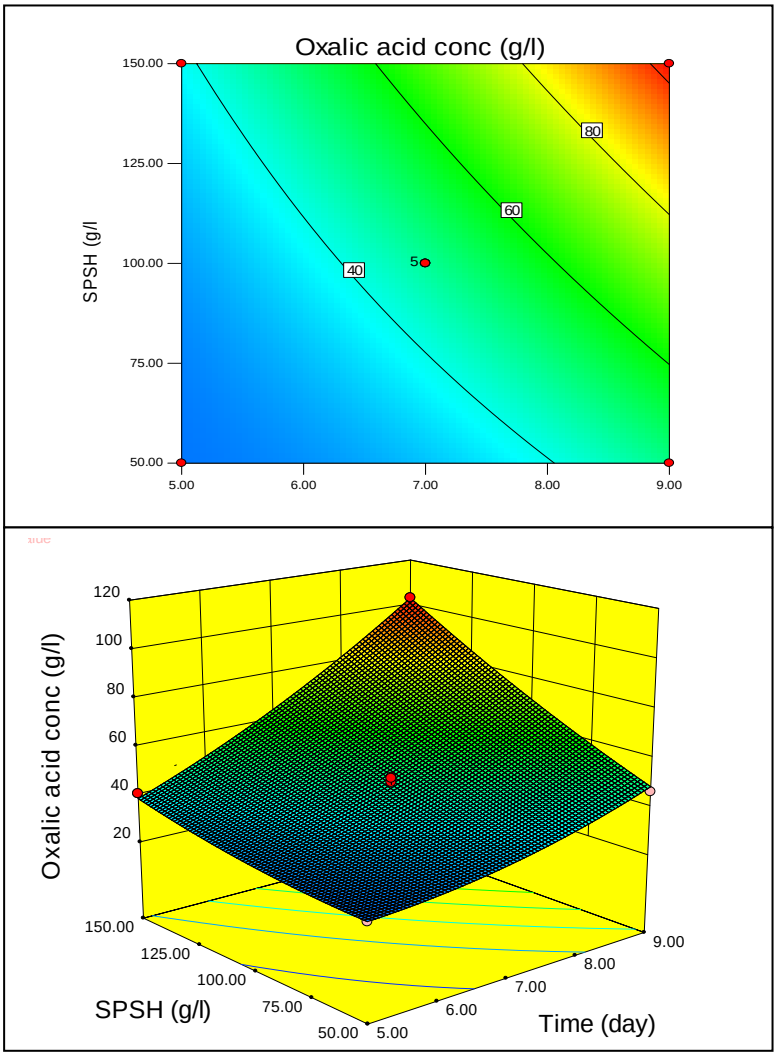

Figure 4: The contour and response surface plots of the effect of glucose concentration, time and their reciprocal interaction on oxalic acid yield keeping pH constant.

The results of this study revealed that RSM with appropriate experimental design can be effectively applied to the optimization of the process variables in oxalic acid fermentation using *A. niger* and SPSH. This may provide useful information regarding the development of economic and efficient fermentation processes.

4. Conclusion

This work focused on optimization of process variables for the production of oxalic acid production from Sweet Potato Starch Hydrolyzate (SPSH) using A. niger. The effects of three independent variables viz. SPSH conc, time and pH on the oxalic acid concentration and their reciprocal interactions were established using Response Surface Methodology (RSM). All the three variables showed significant influence on the production of the organic acid and significant interactions were also observed from the 3-dimensional profiles obtained. A second-order mathematical model was obtained to predict the oxalic acid. The optimal concentration of the oxalic acid produced was 103.26 g/l at optimal condition of 6.2, time 9 days and SPSH concentration of 149.97 g/l, this showed that SPSH could serve as carbon source for oxalic acid production. This work may provide useful information on the development of economic and efficient fermentation processes for the production of oxalic acid and could be scaled up to pilot

production.

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