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Optimization of Lactic Acid Fermentation from Overriped Plantain Using Response Surface Methodology (RSM)

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Abstract: Lactic acid is a building block chemical used by many manufacturing industries. The search for cheap biochemical feedstock and process is of great concern to lactic acid producing industries. Plantain is a rich source of carbohydrate but the overriped plantains are thrown away as waste because of their non-firmness. This study evaluates the utilization of overripe plantain as biochemical feedstock for optimum production of lactic acid. Optimization of lactic acid production with overriped plantain was studied using Response Surface Methodology. The initial pH and reducing sugar content of the plantain hydrolysate were 4.89 and 166.05 g/l respectively. The response of lactic acid concentration to four factors: substrate concentration (138.25 – 166.05 g/L), initial pH (4 – 8), fermentation temperature (30 – 50°C) and time (24 – 168 h) was studied. The lactic acid concentration ranged from 123.50 – 163.00 g/l. A statistically significant [(P_{model}>F) < 0.0001] second order quadratic polynomial regression model was obtained for lactic acid production; the R^2 and adjusted-R^2 were 0.9935 and 0.9896 respectively. Numerical optimization was used to obtain optimum lactic acid production (157.53 g/ L) at glucose concentration, pH, temperature and time of 159.10 g/l, 7.0, 42.3°C and 60 h respectively. Overriped plantain was a good biochemical feedstock for lactic acid production.

Keywords: Lactic Acid, Optimization, Overripe Plantain, Fermentation, Response Surface Methodology

1. Introduction

Lactic acid is considered a very important chemical compound with numerous applications in food, pharmaceutical and packaging industries [1-3]. The economics of pure lactic acid production depends on the raw material (or substrate) and microbial strain used for fermentation and the post-production purification method employed [4-7]. Refined sucrose, lactose, maltose and glucose had been evaluated as possible substrates for commercial lactic acid production. The associated disadvantages with these refined sugars are their high cost and the need for nutrient supplement while multi-stage production process that involves conversion of the non-reducing sugars to reducing sugars before fermentation are the bane of the agricultural residues [8, 9].

Many researchers have sourced their raw materials for lactic acid production from crude agricultural products and by-products of food processing operations like cellulose [10], xylose [11], cassava bagasse [12, 13], carrot processing waste [14], corn fibre hydrolysate [15], wheat bran [16, 17], sugarcane bagasse [18], broken rice [19], corn stover [20], Jerusalem artichoke tuber extract [21], liquid distillery stillage [22], oil palm empty fruit bunch hydrolysate [23], liquefied sago starch [24]. The disadvantages associated with these crude and byproduct materials are laborious purification process, low lactic acid yield and high purification cost [9]. In addition, there is saccharification cost incurred and elongated production time. Thus, there is need to study the use of hydrolyzed starchy materials, rich in reducing sugars. Enzymatic hydrolyzed materials like overripe plantain (regarded as waste) may give better option in terms of production cost and time. This would reduce environmental problem

caused by plantain loss (35 – 60 %) and increase the potential of plantain [25]. In this research, overripe plantain was used to optimize lactic acid production using *Streptococcus thermophilus* strain.

2. Materials and Methods

2.1. Materials

Plantain (*Musa paradisiaca*, AAB genome) was procured from the Teaching and Research Farm of The Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. A false horn cultivar also known as "Agbagba" was used throughout the study. The plantain was allowed to ripe to ripening stage 7 with black peel and very soft pulp [26; 27]; the pH and reducing sugar content of the hydrolysate were determined. A homofermentative lactic acid bacteria (LAB), *Streptococcus thermophilus* procured from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria was used for the study.

Figure 1. *Different ripening stages of plantain (stages 5 – 7 are overriped).*

2.2. Preparation of Plantain Hydrolyzate (Substrate)

300 g of overripe plantain was mashed and filtered with 1.5 litres of distilled water. The particulates was allowed to settle down and separated out by sedimentation. The clear supernatant was filtered using Whatman no 54 filter paper under vacuum. The reducing sugar (glucose) concentration of the hydrolyzate was determined.

2.3. pH Determination

The pH of the samples were measured with calibrated pH meter. The calibration was performed with standard pH 4.00, 7.00 and 9.00 buffers. Ten milliliters of samples was measured and the pH probe was immersed in the solution to a sufficient depth. While using the pH meter, the probe was carefully rinsed with the test solution. The pH reading was then taken after a minimum of 5 minutes [28].

2.4. Reducing and Total Sugar Determination

The quantitative determination of reducing sugar was carried out using dinitrosalicylic acid (DNS). A set of eleven glucose standard ranging from 0.0 to 1.0 mg/ ml (total sample volume 1 ml) were prepared into different sample tubes. 1.0 ml DNS reagent and 2 ml water was added to each tube using pipettes. All the tubes were heated in boiling water bath for 5 minutes to allow reaction between glucose and DNS. Each volume was cooled and adjusted to 10 ml with distilled water using pipette. The absorbance of each resulting solution was read at 540 nm after each had been thoroughly mixed. A standard curve of absorbance against standard glucose concentration was plotted and hydrolyzate sugar concentration was estimated from the standard curve after its absorbance had been read [27].

In order to determine the total sugar content, all non-reducing sugar (sucrose) was first hydrolyzed to reducing sugars (glucose and fructose) by adding 2.5 ml of 2M HCl into 25 ml sample and boiling for 5 minutes. The solution was allowed to cool, neutralized with phenolphthalein containing 10% NaOH and then made up to 50 ml with distilled water. The resulting reducing sugar concentration was determined with DNS reagent [29].

2.5. Statistical Experimental Design for Lactic Acid Fermentation

The number of experimental runs was determined from response surface design using central composite design (CCD). Temperature, initial pH, substrate concentration (glucose) and time were considered as input factors in the design. The efficiency of *Streptococcus thermophilus* was considered and centre points for fermentable variables were chosen as 64.7 g/l, 6.00, 45°C and 60 hr, for glucose concentration, initial pH, fermentation temperature and time, respectively [30, 31, 32, 33]. The range values of variables in the experimental design with coded factors are shown in Table 1. The variables of the experiments were coded according to the following equation:

$$x_i = \frac{(X_i \quad X_{cp})}{\Delta X_i} \tag{1}$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_{cp} is the value of an independent variable at the centre point, and ΔX_i is the step change value.

The behavior of the system was explained by the following quadratic equation:

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j \tag{2}$$

where Y is the predicted response, i. e. lactic acid concentration; b_0 is the offset term; b_i is the linear effect; b_{ii} is the squared effect; b_{ij} is the interaction effect and x_i is ith independent variable.

Table 1. *Real value of variables used in central composite design.*

Variables		Range and levels		
		–1	0	+1
Glucose concentration (g/l)	X_1	145.2	152.15	159.1
Initial pH	X_2	5	6	7
Temperature (° C)	X_3	35	40	45
Fermentation time (hr)	X_4	60	96	132

2.6. Substrate Fermentation: Batch Anaerobic Fermentation Experiment

This was achieved by introducing 200 ml of deMan-

Rigosa-Sharpe (MRS) medium which had been sterilized in an autoclave (model YX-280DG-18L) for 15 minutes at 121°C and cooled at 47°C into a 1000 ml flask. 600 ml of sterilized plantain hydrolyzate was added to make up 800 ml nutrient medium [34, 35, 36]. 0.3 g (about 7.5 ml) of LAB broth was introduced into100 ml of the sterile nutrient medium, to give cell concentration of about 1.62 x 10^8 cfu/ml. The incubation was carried out for 12 hours, agitated at 200 rpm and at a temperature of about 37 °C. The culture was aerated at a rate of 0.06 m^3/ hr [30].

The fermentation was carried out in a shaker incubator (SEARCHTECH THZ model) at each designed condition by transferring 2 ml of bacterium medium to a 30 ml experimental vial containing 20 ml of sterile plantain hydrolysate. Acetic acid and NaOH, as design required, were used for pH adjustment of the mixtures before fermentation. The vial was flushed with nitrogen gas, sealed with stopper and covered with aluminum foil to create anaerobic condition. This was placed in the incubator after the initial temperature required for the fermentation has been set. The agitation speed for fermentation was 150 rpm [30, 35]. The lactic acid concentration of the samples was then determined after the designed time.

2.7. Determination of Lactic Acid Concentration, Yield and Productivity

The percentage of lactic acid in the fermentation product was determined using High Performance Liquid Chromatography (HPLC) according to Akyol *et al.* [37]. 1 ml of the fermented medium was centrifuged at 14,000 × g at 4°C for 5 min. The supernatant was filtered and placed in a fresh Appendorf tube. The sample was diluted 1-fold in 0.5% meta-phosphoric acid (Sigma-Aldrich), and 20 µL of the solution was injected onto the HPLC. Separation was performed on a 150 × 4.6-mm Capcell Pak 5 µM C18 MG column (Shiseido Co. Ltd., Tokyo, Japan) by using continuous gradient elution with phosphate buffer solution (mobile phase A; Sigma-Aldrich) and acetonitrile (mobile phase B; Sigma-Aldrich) at 40°C. The total separation time was 5 min, and the gradient was run for 6 min to ensure full separation. Calibration graphs for absorbance against changing concentration of the metabolite were drawn to calculate lactic acid amounts in the sample. L-lactic acid, which absorbs the light at 210 nm, was used as the standard for lactic acid. Consequently, lactic acid concentrations were represented chiefly by the L-lactic acid produced.

The lactic acid concentration was determined using titration methods [28]. 1.5 ml of each fermented broth was filled into Appendorf tube and centrifuged at 5000 rpm for 3 min. The supernatant from the fermented broth was withdrawn using 25 µl pipette from which lactic acid concentrations was subsequently determined via titration with NaOH solution. The lactic acid yield for each run was determined by finding the ratio of lactic acid concentration to initial substrate concentration. The production rate (productivity) was determined as the ratio of yield to the production time [9].

2.8. Statistical Analysis and Optimization of Fermentation Results

The experimental data from the fermentation process was analyzed using the Stat-Ease statistical package from which response surface curves and corresponding contour plots were drawn. The analysis of variance (ANOVA) technique was used to determine the relationship of response (lactic acid concentration) to the process variables in the design. The relationships and interrelationships of each of the process variables was determined by fitting a model to the data obtained from the experiment using mean value of triplicates for each experimental run. The coefficients and t-values of the independent variables, as well as the level of significance of the variables were determined. The ANOVA for each of the dependent variables was obtained and the significance or fitness of the model was determined. The insignificant terms, based on *p*-values, was discarded from the model [30, 31, 35, 36].

The optimum production of lactic acid was determined from the response surface analyses based on the dependence of lactic acid concentration on the four chosen fermentation variables [30, 31, 35, 36]. An experiment was conducted to validate the statistical model using the statistically derived optimum condition. The deviation of the optimum lactic acid concentration obtained statistically from the experimentally determined optimum lactic acid concentration was evaluated.

3. Results and Discussion

3.1. Proximate and Chemical Properties of Plantain Substrate

The proximate and chemical properties of overriped plantain are presented in Table 2. Broth pH, carbohydrate (or reducing sugars) and protein contents are some of the significant properties that affect lactic acid fermentation. The carbohydrate of the overriped plantain was 87 % while the reducing sugars concentration of the plantain extract was 166.05 g/l. The reducing sugar concentration obtained from overriped plantain is better than the concentration of starch obtained from other raw materials: xylose – 103.2 g/l [11], broken rice - 64 g/l [19], corn stover – 14.7 g/l [20], Jerusalem artichoke tuber extract – 91 g/l [21], liquid distillery stillage– 13.6 g/l [22], liquefied sago starch – 20.7 g/l [24]. Also, the required processing time and cost from starch to reducing sugars associated with starchy raw materials are excluded in the case of using overriped plantain as lactic acid fermentation substrate. Therefore, the relatively high value of the reducing sugar present in the overriped plantain indicates the viability and reliability of overriped plantain as feedstock for lactic acid production.

The pH of the overriped plantain was 4.9. This makes the overriped plantain acidic and unfavourable for many LABs, thus reducing the performance of the microorganisms in the cause of fermenting the overriped plantain extract. Most LABs; *L. delbrueckii* [19], *L. rhamnosus* [23], *L. paracasei* [31], *L. plantarum* [38], *Bacillus sp.*-Na-2 [39], *Sporolactobacillus sp.*-CASD [40], *Bacillus sp.*-WL-S20 [41] were reported to thrive

at pH in the range between 5.5 and 7.0. However, the lower pH observed can be surmounted by using NaOH or $CaCO_3$ to correct the pH of the fermentation broth [42].

Table 2. *Proximate and chemical properties of overriped plantain substrate.*

Properties	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate	pH	Reducing sugar
Values (%)	87.00[a]	8.98[b]	3.25[b]	0.34[b]	0.56[b]	86.87[b]	4.9	166.05[c]

[a] (% composition on wet basis), [b] (% composition on dry basis), [c] (g/l)

The protein content of the overriped plantain is 8.98 %. This increases the availability of nutrient to the LABs, when compared with starchy raw materials with low or no protein content [19, 20, 21, 23].

3.2. Fermentation Product and Analyses

3.2.1. Lactic Acid Concentration, Yield and Productivity

The percentage of lactic acid in the fermented product obtained at optimum condition is reported in Figure 2. There is 93.35% of lactic acid in the fermentation product as shown in Table 3. This shows *Streptococcus thermophilus* is a good LAB and will thrive very well at the condition used to maximize lactic acid production.

The design matrix of the variables in coded units with the experimental results was presented in Table 4. The lactic acid concentration (C) obtained from fermentation of overriped plantain ranges from 123.5 – 163.0 g/l, more than that of cassava bagasse – 83.8 g/l [11, 12], broken rice – 79.0 g/l [19], corn stover – 21.0 g/l [20], corn starch – 73.2 g/l [38], obtained corncob – 39.1 g/l [43]. This could be as a result of high content of reducing sugar present in overriped plantain and low starch content of other raw materials.

Table 3. *Concentration (in %) of lactic acid in the fermentation product.*

Peak No.	Peak ID	Retention Time	Height	Area	Concentration %
1	Unidentified	0.190	5808.923	116023.406	1.9868
2	Lactic Acid	1.207	196460.813	5451263.500	93.3490
3	Unidentified	2.548	13212.470	257909.359	4.4165
4	Unidentified	3.190	109.500	180.600	0.0031
5	Unidentified	3.248	180.857	385.150	0.0066
6	Unidentified	3.540	108.905	233.814	0.0040
7	Unidentified	3.782	934.651	13665.586	0.2340

Table 4. *Experimental design and results of lactic acid fermentation.*

Run	x_1	x_2	x_3	x_4	C (g/l)	Y (g/g substrate)	P (g/l/h)
1	159.10	7	45	132	159.1	1.0000	1.21
2	152.15	6	40	96	150.1	0.9865	1.56
3	152.15	6	40	24	129.0	0.8478	5.38
4	145.20	7	45	132	145.1	0.9993	1.10
5	152.15	6	30	96	133.2	0.8755	1.39
6	145.20	7	45	60	139.1	0.9580	2.32
7	145.20	5	45	60	127.9	0.8809	2.13
8	159.10	5	45	60	140.1	0.8806	2.34
9	159.10	5	45	132	141.5	0.8894	1.07
10	152.15	6	40	96	151.6	0.9964	1.58
11	145.20	5	45	132	134.2	0.9255	1.02
12	159.10	5	35	132	135.7	0.8529	1.03
13	152.15	8	40	96	152.0	0.9990	1.58
14	152.15	6	40	96	151.6	0.9964	1.58
15	166.05	6	40	96	163.0	0.9816	1.70
16	145.20	5	35	60	125.6	0.8650	2.09
17	159.10	7	35	132	154.2	0.9692	1.17
18	152.15	4	40	96	123.5	0.8117	1.29
19	159.10	5	35	60	134.3	0.8441	2.24
20	138.25	6	40	96	135.7	0.9816	1.41
21	145.20	7	35	60	136.4	0.9394	2.27
22	152.15	6	40	96	150.6	0.9898	1.57
23	152.15	6	40	96	151.6	0.9964	1.58
24	152.15	6	40	168	142.2	0.9346	0.85
25	152.15	6	40	96	151.6	0.9964	1.58
26	159.10	7	35	60	150.1	0.9434	2.50
27	145.20	5	35	132	133.8	0.9215	1.01
28	159.10	7	45	60	158.5	0.9962	2.64
29	145.20	7	35	132	144.9	0.9979	1.10
30	152.15	6	50	96	141.7	0.9313	1.48

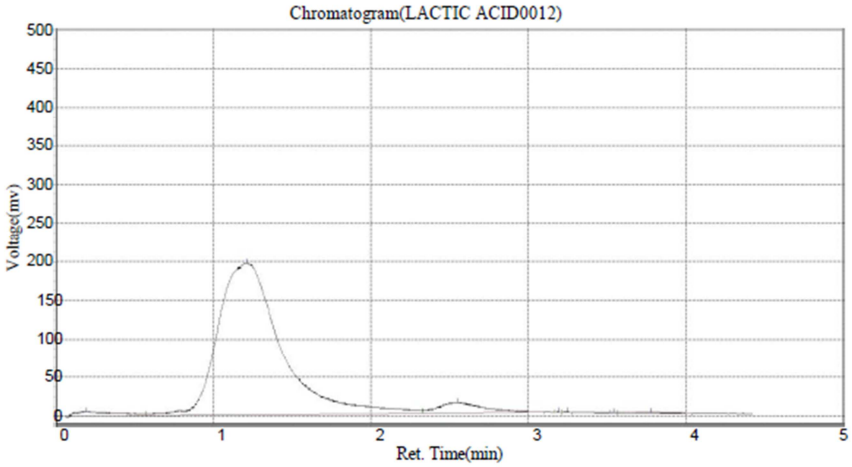

Figure 2. *HPLC expression of lactic acid percentage in the fermentation product.*

The lactic acid yield (Y) and productivity (P) obtained in this work range from 0.43 – 0.99 g/g and 0.31 – 6.42 g/l/h, respectively. The obtained parameters are within the range reported for some agro wastes like broken rice – 0.81 g/g, 3.58 [19], corncob – 0.70, 0.81 [43], corn starch – 0.85, 3.86 [38], corn stover – 0.70, 0.58 [20], cassava bagasse – 0.96, 1.40 [11, 12]. The lowest yield (0.8117) was observed at the lowest initial pH of 4 while the highest yield was observed at highest substrate concentration. However, the highest productivity (5.38 g/ l/ h) was observed at a lower fermentation time and reduced substrate concentration level. This shows that the pH and temperature of the medium play significant roles in successful lactic acid fermentation, and not really the high concentration of the substrate. Lactic acid fermentation does not solely depend on the quantity of the substrate but how the microorganism can effectively utilize and convert the substrate to lactic acid. As a matter of fact, a very high sugar concentration can inhibit the growth rate of the microorganism, which would eventually decrease the lactic acid production [44, 45].

The observation on the effect of pH follows the trend reported by Tamakawa *et al*. [42] that observed a high yield of 0.91 g/g, productivity 2.18 g/l/h, with an initial pH of 6.8, using calcium carbonate to neutralize the low pH of the fermenting medium. Also, a better yield (0.96 g/ g) and productivity (3.99 g/l/h) was observed by Moon *et al.* [33] at a controlled pH of 6.5. Lactic acid yield (0.70 g/ g) and productivity (0.58 g/l/h) was also observed by Cui *et al.* [20] at initial pH of 5. These show that most lactic acid bacteria favour a pH between 6 and 7 for their optimum performance.

There was an increase in lactic acid concentration (125.6 to 159.1 g/l) and yield (0.86 to 1.00 g/g) as temperature increases from 35 to 45°C. A decrease in the concentration and yield was observed as the temperature exceeds 45°C. However, a decrease in initial pH did not favour lactic acid production and yield as initial pH of 4 gave lactic acid concentration of 123.5 g/l and yield of 0.81 g/g despite the fermentation taken place at temperature of 40°C. The observation is similar to that reported by Beal *et al.* [46] where optimal performance of *Streptococcus thermophilus* for lactic acid production was observed to be 45°C.

3.2.2. Experimental Model and Statistical Analysis for Lactic Acid Fermentation

The application of multiple regression analysis was performed on the experimental data. A quadratic model was modified to fit the experimental data. The result of the analysis of variance performed to obtain the model is given in Table 5. The "model F-value" of 252.01 implies the model is significant and there is only 0.01% chance that a "model F-value" this large could occur due to noise. Also, the "Prob>F" values of the model is less than 0.0500 which indicates that the model is significant to fit the experimental data. The significant experimental terms of the model are substrate concentration (x_1), initial pH (x_2), fermentation temperature (x_3) and fermentation time (x_4). The other significant terms of the model are the interactive terms (x_1x_2), (x_1x_3), (x_1x_4) and the quadratic terms $(x_2)^2$, $(x_3)^2$, $(x_4)^2$, based on their "Prob>F" values that are less than 0.0500. The "lack of fit" value obtained implies the lack of fit is not significance relative to the pure error. There is a 10.87% chance that a "lack of fit" F-value this large could occur due to noise. A large non-significant value is desirable for the fitness of a model, and this implies this model will adequately fit the experimental data.

Table 5. *Analysis of variance for the quadratic model in lactic acid fermentation.*

Source	Sum of squares	Degree of freedom	Mean square	F-value	P>F
Model	3079.10	11	279.92	251.001	< 0.0001
Residual	20.07	18	1.12		
Lack of Fit	17.86	13	1.37	3.11	0.1087
Pure Error	2.21	5	0.44		
Cor Total	3079.17	29			

R^2 = 0.9935; Adjusted R^2 = 0.9896; Predicted R^2 = 0.9748; CV = 0.74%; Adeq Precision = 57.10

The coefficient of determination value (R = 0.9935) suggests that more than 99.35 per cent of the variance is attributable to the variables and indicated the high significance of the model; only 0.65 per cent of the variance cannot be explained by the model. The 'Pred R^2' of 0.9778 obtained is in a reasonable agreement with the 'Adj R^2' of 0.9896 and close to the value of R^2. This implies that the model terms obtained can be used to predict the the value of any term in the model. The 'Adequate Precision' measures the signal to noise ratio, with a value greater than 4. The ratio of 57.10 obtained from the analysis indicates an adequate signal and shows that this model can be used to navigate the design space used in the experiment. The reduced model (coded) terms of lactic acid concentration (C), and based on the significance of each term is therefore:

$$(C) = 151.8 + 5.59(x_1) + 7.05\,(x_2) + 1.90\,(x_3) + 2.70\,(x_4) + 1.52\,(x_1x_2) + 1.08\,(x_1x_3) - 1.22\,(x_1x_4) - 3.22\,(x_2)^2 - 3.30\,(x_3)^2 - 3.76(x_4)^2 \quad (3)$$

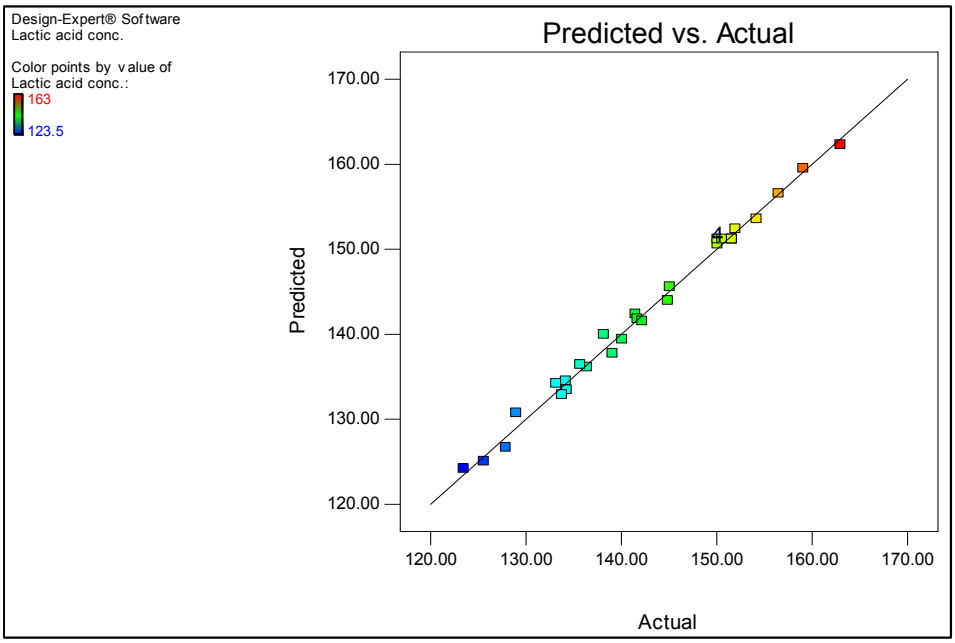

Figure 3. *Predicted vs experimental values for lactic acid concentration.*

The closeness of the predicted values from the model to the actual lactic acid experimental data is shown on Figure 3. The closeness of each of the data points to the model line shows that there is minor deviation for each of predicted value from the corresponding experimental value.

3.3. Effects of Fermentation Variables on Lactic Acid Concentration

The probability values of the regression coefficient, before the modification of the quadratic model are presented in Table 6. The P-values of the regression coefficients suggest that the linear terms of all the variables, the quadratic terms $(x_2)^2$, $(x_3)^2$ and $(x_4)^2$ and the interactive terms (x_1x_2), (x_1x_3), (x_1x_3) significant terms for the model. The terms' coefficients show that (x_2x_3) had the least effect on the response (C) while (x_2) had the biggest effect on the response. This implies the pH of the medium for lactic acid fermentation must be properly controlled as bad management of the factor could lead to low production and yield. The insignificant terms were removed to obtain Equation 3.

Table 6. *Probability values of the regression coefficients of lactic acid fermentation (before model modification).*

Factor	Coefficient Estimate	Standard Error	F-Value	P-value	Remarks
Intercept	151.18	0.43	194.88	< 0.0001	Significant
x_1-Substrate conc.	5.59	0.22	663.25	< 0.0001	Significant
x_2-pH	7.05	0.22	1057.14	< 0.0001	Significant
x_3-Fermentation temp	1.90	0.22	776.63	< 0.0001	Significant
x_4-Fermentation time	2.70	0.22	155.35	< 0.0001	Significant
x_1x_2	1.52	0.27	32.67	< 0.0001	Significant
x_1x_3	1.08	0.27	16.56	0.0001	Significant
x_1x_4	-1.22	0.27	21.04	0.0004	Significant
x_2x_3	-0.0063	0.27	0.0006	0.9815	Insignificant
x_2x_4	0.24	0.27	0.84	0.3735	Insignificant
x_3x_4	-0.37	0.27	1.93	0.1855	Insignificant

Factor	Coefficient Estimate	Standard Error	F-Value	P-value	Remarks
x_1^2	-0.011	0.20	0.0032	0.9557	Insignificant
x_2^2	-3.22	0.20	252.35	< 0.0001	Significant
x_3^2	-3.30	0.20	264.23	< 0.0001	Significant
x_4^2	-3.76	0.20	343.51	< 0.0001	Significant

The interactive terms with good significance, as shown in Table 6 are (x_1x_2), (x_1x_3) and (x_1x_4). This implies that pH, fermentation temperature and time will independently combined with substrate concentration to affect lactic acid production. The response surface plots; Figures 4 – 6 were quadratic in terms of shape, indicating a quadratic model would best fit the empirical data. The figures show how the other three factors affect the depletion of substrate. In Figure 3, the interaction of x_1 and x_2 was positive for lactic acid production, that is, lactic acid increased in quadratic form as substrate concentration and initial pH of the system simultaneously increased. However, lactic acid was less produced at lower pH(s), as a result of inhibition experienced by the microorganism from the acidic fermenting medium. This is similar to the observations of Oshiro *et al.* [44] and Abdel-Rahman *et al.* [45] on substrate and end-product inhibitions.

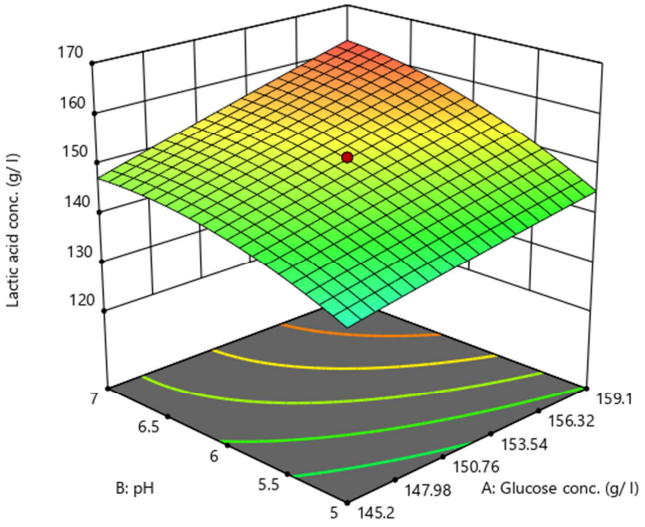

Figure 4. *Response surface plot showing the interactive effect of substrate concentration and pH on lactic acid concentration.*

In lactic acid concentration as substrate concentration and temperature increase from their initial values of 145.20 g/l and 35°C. However, there was reduction in lactic acid concentration as the temperature reached 43°C despite increase in substrate concentration. An optimum temperature of 44°C for lactic acid fermentation of whey-based medium using *Lactobacillus bulgaricus* was also reported by Aghababaie *et al.* [47] while an optimum temperature of 43°C for lactic acid production using *Streptococcus thermophilus* was reported by Beal *et al.* [46]. This implies that *Streptococcus thermophilus* would performed optimally for lactic acid production at temperature approximately 43°C. Thus, the pH and temperature must be properly studied for each microorganism for effective utilization of substrate by the microorganism.

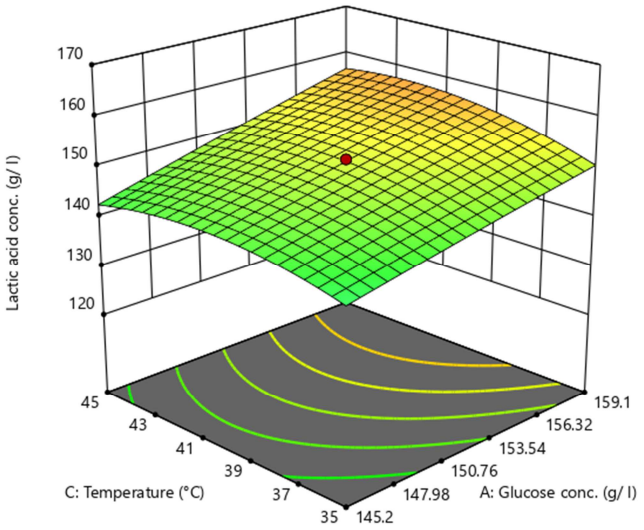

Figure 5. *Response surface plot showing the interactive effect of substrate concentration and temperature on lactic acid concentration.*

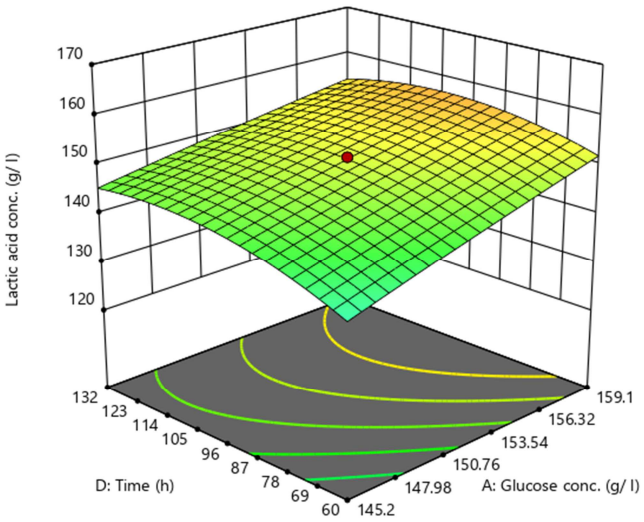

Figure 6. *Response surface plot showing the interactive effect of substrate concentration and fermentation time on lactic acid concentration.*

The same trend was observed with interaction of substrate concentration and fermentation time, as shown in Figure 5, as more lactic acid was produced up to 105 h, before a decrease in lactic acid concentration was observed. However, 158.5 g/l of lactic acid was able to be produced in 60 h at substrate concentration 159.1 g/l, initial pH 7 and temperature 45°C, indicating that 60 h is sufficient for conversion of the substrate to lactic acid by *Streptococcus thermophilus.*

3.4. Optimization and Validation of Fermentation Process

Numerical optimization was used from the package, based on desirability function to optimize lactic acid production.

The optimum combinations needed to achieve a predicted maximum lactic acid concentration and yield of 157.53 g/l and 0.99 g/g substrate were found to be as follows; glucose concentration, 159.1 g/l; pH, 7.0; fermentation temperature, 42.3 °C and fermentation time, 60 h, with desirability factor 0.93. The lactic acid concentration obtained at the optimum condition was found to be 151.6 g/l, with a variation of 0.04% from the predicted data. The results clearly showed high degree of reliability of model data obtained for optimization of lactic acid production from overriped plantain.

4. Conclusion

Overriped plantain was successfully used to produce lactic acid using *Streptococcus thermophilus* at pH of 7.0 and temperature of 42.3°C. A conversion time of 60 h without saccharification was used. The high reducing content of overriped plantain was significant for optimum production of lactic acid. Thus, the cost of lactic acid production and plantain wastage are reduced. The process could be scaled up to determine the feasibility of industrial production of lactic acid from overriped plantain.

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