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PRODUCTION AND OPTIMIZATION OF CITRIC ACIDS FROM CASSAVA PEELS USING ASPERGILLUS NIGER

*Ajala A. S., Olaniyan S. A., Fasonyin O. T. and Adeoye A. O.

Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State.

*Corresponding Author
Ajala A. S.
Department of Food
Science and Engineering,
Ladoke Akintola University
of Technology, Ogbomoso,
Oyo State.

ABSTRACT

In this study, cassava peels were hydrolyzed using Hydrochloric acid (HCl) and fermented with *Aspergillus niger* (LAU111) to get citric acid. The optimization of the citric acid was carried out using Central Composite Experimental Design (CCED). Design Expert 6.8 version was used as software. The variables used for the optimization include: A- inoculum concentration (7-11 %), B- fermentation time (1-4 days), C- temperature (23-39 ^{o} C) and D-ethanol concentration (0-4 %),. The optimization results showed that the linear model had good fit with experimental data. Analysis of variance results showed that the model had R^2, adjusted R^2 and predicted R^2 value of 0.8733, 0.8521 and 0.8280, respectively. F-value of the model was 41.34 and this was significant because the model had the probability chance of 0.0001 of noise. Adequate precision value was as high as 24.37; this was greater than 4 which is the minimum required value. The lowest and highest values of citric acid recorded were 2.5 and 7.6 g/l. The optimum value of citric acid recorded was obtainable through the combination factors of inoculum concentration and fermentation time. Therefore, cassava peels have a good potential to produce enough citric acid needed for local consumption if properly harnessed. This will reduce the cost of importation into the country and boost local economy.

KEYWORDS: *Cassava-peel, citric acid, energy, cost, enzyme, acid.*

1. INTRODUCTION

Nigeria is the highest producer of cassava worldwide (FAO, 2008) with approximation of more than 45 million metric ton yearly (Egesi et al., 2006). Cassava is a root crop which has peels as the outer cover of the roots and is often removed manually using knives or can be mechanically peeled by machines. These peels constitute about 15-25% of the roots (Aro *et al.,* 2010, Fakir *et al.,* 2012). By this estimate, over 9 million metric tons of peels are annually generated as wastes from processing cassava roots. Most often, these wastes have constituted an environmental problem and posed challenges to the health of millions of people. The peels are frequently left to litter the ground or are sometimes spread on the roadside fermenting making the roads impassable. The wastes are sometimes heaped on dung hills and usually left to rot away or burnt to create space for the accumulation of more wastes. The wastes emit carbon dioxide and produce a strong offensive smell (Aro *et al.,* 2008; Adebayo, 2008). Besides, the wastes constitute a breeding ground for flies and mosquitoes which are carriers of diseases (Omotosho and Sangodoyin, 2013) and Eze (2010) has linked adverse health respiratory problems with offensive gas smell emanated from the heaps of cassava peels. This challenge needs to be overcome by creating a means through which such cassava peels can be turned into useful products and thereby reducing various cassava waste centres across the country.

Nigeria is a major utiliser of citric acid in Africa. The production of citric acid in Nigeria is still at the primitive level and has to depend on importation from other countries. The soared exchange rate from naira to dollar has brought unpleasant experience to the importers of the product to the country. Therefore, conversion of cassava peels could be considered a better alternative because the materials are abundantly available and considered as wastes. Secondly, local production will go a long way in improving the local technology for industrial use and economy will also be improved. Therefore, the main objective of this paper is to produce and optimize citric acids from cassava peels using *Aspergillus niger*

2. MATERIALS AND METHODS

The following materials were used for the production of citric acid: cassava peels, Sodium Hydroxide, D-glucose, Calcium chloride, distilled water, Benedict reagent, Sodium carbonate and Hydrochloric acid.

2.1 Hydrolysis of Cassava Peel Substrates

Fresh cassava peels were obtained from Ladoke Akintola University of Technology Ogbomoso, Nigeria processing factory. The peels were fermented for three days and dried in a tunnel dryer at temperature of 60 ^{o} C. The peels were then milled into flour and packaged for further analysis.

HCl concentrations range from 0.5- 1.5% were prepared and were mixed with 100 ml distilled water to form solution. About 20 g of cassava peels' substrates were mixed with the solution containing the acid. The mixtures were allowed for 2 hrs. for proper mixing to take place. The substrate obtained was heated at temperature range of 50-100 ^{o} C in 20 minutes.

2.2 Solid State Fermentation of Cassava Peels and Inoculation of *Aspergillus niger* into Substrates

Conical flask method was used for the solid-state fermentation. Standard experiments was conducted in 250ml sterilized flask, each containing 20g of treated cassava peels with 60% moisture level. Samples from previous hydrolyses were used to prepare the citric acid production experiments. In Central Research Laboratory of Ladoke Akintola University of Technology, Ogbomoso, a full grown *Aspergillus niger* was collected. This was further sub cultured at interval of 7 weeks to obtain pure culture of microbes on potato dextrose agar. These cultures were incubated at 32 ^{o} C for 72 hours and were then stored on agar slant at 4 ^{o} C until needed. The culture of *Aspergillus niger* (LAU 111) was added to the substrate within the range of 7-11 % and ethanol at the range of 0-4 %. The substrate was fermented within 1-4 days at temperatures 23-39 ^{o} C. Citric acid production of each of the samples was done using titrimetric method and the process was as follows: 10 g of the mixture was dissolved in 100 ml of distilled water and was titrated with NaOH to determine the acidity level using phenolphthalein as indicator. Table 1 shows the experimental design used for the process. Design Expert 6.8 was used as software and central composite experimental design was used for all the experiments. The equation to determine the citric acid concentration in the substrate is as given in Equation 1

$$citric\ acid\ \left(\frac{g}{l}\right) = \frac{titre\ valu\ x\ acid\ factor}{10\ ml\ of\ filtrate} x 1000 \quad 1$$

Where acid factor =0.061.

Table 1: Design Summary: Production of Citric acid Hydrolsed by Acid (HCl) Using *A. niger*.

Factors	Name	Units	Observation	Minimum	Maximum
A	Inoculum concentration	%	29	8.00	10.00
B	Fermentation Time	Days	29	1	3
C	Temperature	^{o}C	29	27	39
D	Ethanol concentration	%	29	1	4

2.3 Statistical Analysis of the Samples

The SPSS 20.0 version (2015), a software package was used for statistical analysis. Analysis of variance (ANOVA) was carried out on the data obtained from citric acid production earlier described. All the experimental treatments were conducted in three replicates. The experimental data were analysed by using one-way ANOVA and means separation/comparison using Duncan's multiple range test at 95% confidence level.

3 RESULT AND DISCUSSION

3.1 Proof of Reliability of Optimization Model

Table 2 shows the fit summary of the model's experiment applicable to determine the yield of citric acid. Four types of model were generated namely linear, 2FI, quadratic and cubic. The linear and quadratic models have the least of degree of freedom with value of 4 whereas cubic model has the highest value of 8. Linear model has the least F-value of 0.29 whereas 2FI has the highest F-value of 1.6. Furthermore, the linear model has probability< 0.0001 chance for noise to occur in the model. Therefore was recommended as suitable for the experiments. The low F-value of 0.29 shows that there was less relative variance among the group means.

Table 2: Fit Summary of Different Models Applicable to Yield of Citric Acid (g/l)

Source	Sum of square	DF	Mean square	F-value	Prob > F	Remarks
Mean	1125.95	1	1125.95	41.34		
Linear	36.33	4	9.08	0.29	< 0.0001	Suggested
I	0.46	6	0.077	1.60	0.9340	
Quadratic	1.51	4	0.38	0.51	0.2295	Aliased
Cubic	1.33	8	0.17			
Residual	1.97	6	0.33			
Total	1167.55	29	40.26			

The analysis of variance for the model of citric acid produced with F-value of 41.34 is as shown in Table 3. This was significant because the model had the probability chance of

0.0001 of noise. Therefore, the model in Equation 2 can represent the data well in 95% confidence limit. The lack of fit was not significant, meaning that, the outliers had little effect on the model. It also shows that the experimental and predicted values have good correlation as shown in Table 5. Moreover, the high values of R^2, adjusted R^2 and predictive R^2 of 0.8733, 0.8521 and 0.8280, respectively, are evidences of reliability of the model. The high value of adequate precision of 24.37 which is greater than 4 proves further the reliability of the model. Furthermore, the low value of 7.6 of CV means that the size of the standard deviation is as low as 7.6% of the size of the mean. This implies that there were small differences among data. Also, the value of 7.15 for Predicted Residual Error Sum of Squares (PRESS) in the table is satisfactory with the model. PRESS value is to cross-validate data for regression analysis to provide a summary measure of the fit of a model to a sample of observations (Shu-Ping, 2016). Since lack of fit is not significant, then the model is reliable.

Citric acid =6.22+1.19 A+0.19B -0.056C+ 0.17D 2

Table 3: Analysis of Variance for Yield of Citric Acid Using Linear Model.

Source	Sum of square	DF	Mean square	F-value	Prob > F	Remarks	
Model	36.33	4	9.08	41.34	< 0.0001	Significant	
A	33.50	1	33.50	152.51	< 0.0001		
B	0.90	1	0.90	4.08	0.0546		
C	0.075	1	0.075	0.34	0.5655		
D-	0.69	1	0.69	3.12	0.0898		
Residual	5.27	24	0.22				
Lack of fit	3.96	19	0.21	0.80	0.6762	Not Significant	
Pure Error	1.31	5	0.26				
Cor. Total	41.60	28					
Std Dev	Mean	CV	Press	R^2	Adj-R^2	Pre R^2	Adequate Precision
0.47	6.23	7.52	7.15	0.8733	0.8521	0.8280	24.37

Table 4 shows the coefficient of the models for the yield of citric acid, factor A (inoculum concentration) has the highest coefficient with the value of 1.19 while C has the lowest value of -0.056. This was translated into equation and is as written in Equation 2. The values of Variance Inflation Factor (VIF) are as presented in the table. The minimum and maximum value are 1.01 and 1.02, respectively. The low value of VIF indicates good multicollinearity in the model.

Table 4: Estimated Coefficients for the Models for the Yield of Citric Acid (g/l).

Factor	Coefficient Estimate	Standard error	95% C.I Low	95% C.I High	VIF
Intercept	6.22	0.087	6.04	6.40	-
A	1.19	0.096	0.99	1.38	1.01
B	0.19	0.096	-4.1E-03	0.39	1.01
C	-0.056	0.096	-0.25	0.14	1.01
D	0.17	0.097	-0.029	0.37	1.02

Table 5: Diagnostics Case Statistics for Citric Acid Experiements.

Runs	Actual value	Predicted values	Residuals	Outliers
1	5.10	4.72	0.38	0.886
2	7.70	7.10	0.60	1.478
3	5.30	5.11	0.19	0.441
4	7.80	7.48	0.32	0.756
5	5.20	4.61	0.59	1.434
6	7.10	6.98	0.12	0.275
7	5.10	5.00	0.100	0.236
8	7.90	7.71	0.19	0.430
9	5.10	5.07	0.033	0.078
10	7.80	7.44	0.36	0.860
11	5.40	5.45	-0.055	-0.129
12	8.10	7.83	0.27	0.641
13	5.10	4.95	0.15	0.340
14	7.70	7.33	0.37	0.882
15	5.10	5.34	-0.24	-0.568
16	8.10	7.71	0.39	0.905
17	3.80	3.85	-0.048	-0.112
18	7.80	8.59	-0.79	-2.006
19	5.30	5.83	-0.53	-1.285
20	6.80	6.61	0.19	0.453
21	6.40	6.33	0.069	0.161
22	6.40	6.11	0.29	0.692
23	5.30	5.88	-0.58	-1.419
24	6.40	6.56	-0.16	-0.378
25	5.40	6.22	-0.82	-1.869
26	5.30	6.22	-0.92	-2.140
27	5.40	6.22	-0.82	-1.869
28	6.40	6.22	0.18	0.386
29	6.40	6.22	0.18	0.386

3.2 Effect of Input Parameters on the Yield of Citric Acid Using Interactive Graphs

The yield of citric acid as affected by fermentation time and innoculum concentration is as shown in Figure 1. Inoculum concentration which ranged from 8 to 10 % of the broth played a significant role in the production of citric acid than fermentation time as displayed by the slope of the figure. The values of citric acid produced ranged from 3.8 to 7.6 g/l and are

significantly different as shown in Table 6. The values were lesser than those reported by Adeoye *et al*. (2015) but in the same range with those reported by Rivas *et al.,* (2008). Furthermore, Adeoye *et al*. (2015) reported the influence of fermenting time on the production of citric acid, the ferment times used were between 84 and 114 hours to get considerable amout of citric acid range 16.1-88.73 g/l. However, in the present study, the maximum time used was 72 hours. Also, Bekir *et al*. (2009) observed that incraese in fermentation period up to 7 days increased citric acid production and then decreased afterward. The reason for the decrease in citric acid after 7 day was due to the depletion in sugar content of the substrates (Ali *et al.,* 2002).
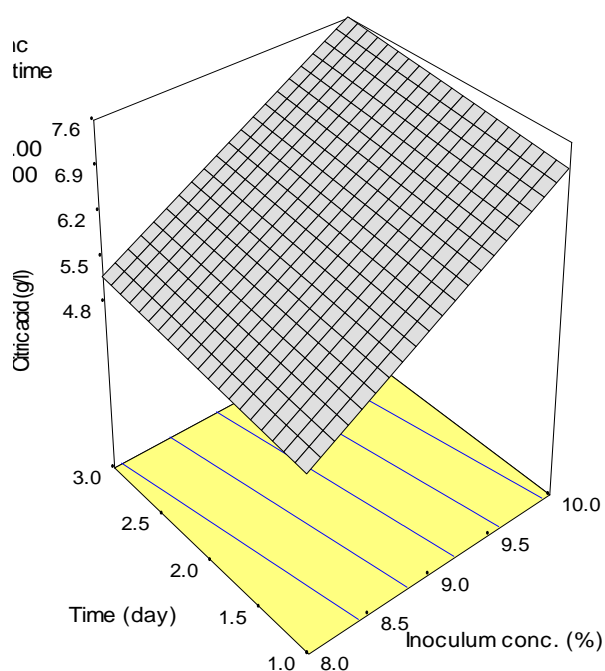

Figure 1: Effect of Fermentation Time and Inoculum Concentration on the Yield of Citric Acid.

The yield of citric acid as affected by temperature and fermentation time is as shown in Figure 2. The maximum value of citric acid produced was 6.5 g/l while the minimum was 5.8 g/l and were significantly different as shown in Table 6. The value is greater than the values reported by Anbuselvi (2015) which was 0.76 g/l. The reason for the lower value in his work could be due to different carbon source the author used. The increase in temperature did not significantly increased the production of citric acid in this study as shown in the figure. This was earlier observed by Bekir *et al.,* (2009), that increase in temperature above 30 ^{o} C would not increase the production of citric acid but rather decrease it.

The effect of ethanol and inoculum concentration on the production of citric acid is as displayed in Figure 3. The values ranged from 3.32 to 7.57 g/l and significantly different as shown in Table 6. The values were greater than those values reported by Mansor *et al.,* (2017) in which date-extract byproduct was used to produce citric acid. The effect of ethanol on citric acid production has been studied by researchers; Hauka *et al.,* (2005) reported that addition of ethanol slightly boosted citric acid production up to 33.61g/l whereas the experiment without ethanol was 33.05 g/l. Rugsaseel *et al.* (1995a) discovered that addition of methanol changed the activity of some enzyme relating to citric acid cycle and thereby increased the production of citric acid. Ethanol also prevents spore formation on the cell thereby allowing cell to secret citrate into the solution which favoured the production of citric acid (Hauka *et al.* , 2005). Also, Ana *et al.* (2011) also observed that adddition of methanol was detrimental to the production of citric acid in solid state fermentation of orange peels. Mannomani and Sreekantiah (1987) observed that addition of ethanol to substrate in citric acid production provided two fold activities; it doubles citrate activities and reduces aconitase activity by 75%. Furthermore Ogbonnaya (2015) observed that addition of methanol up to 3% produced 7.8 g/l whereas 4% methanol addition decreased the value to 7.1 g/l. Further increase of methanol to 5% dropped the citric acid vale to 4.9 g/l. Therefore, it could be deduced that addition of methanol/ethanol must not exceed 4% to get sufficient citric acid.
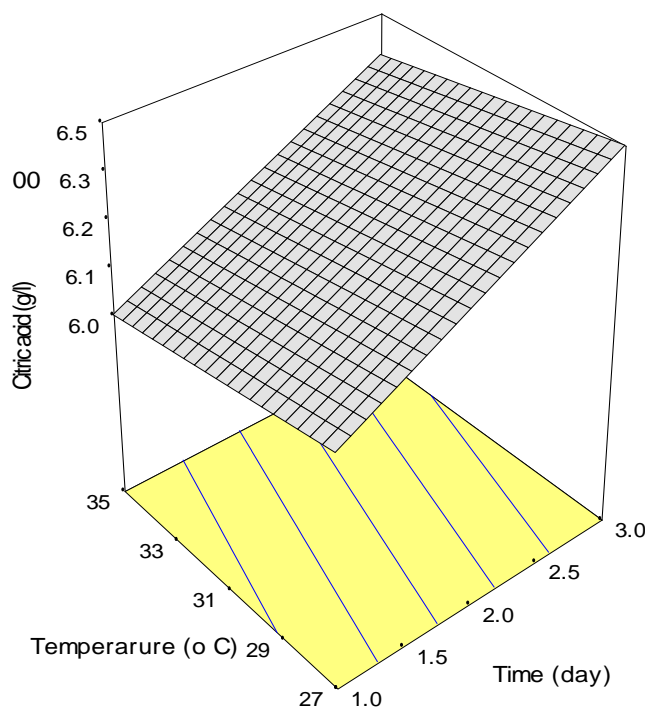

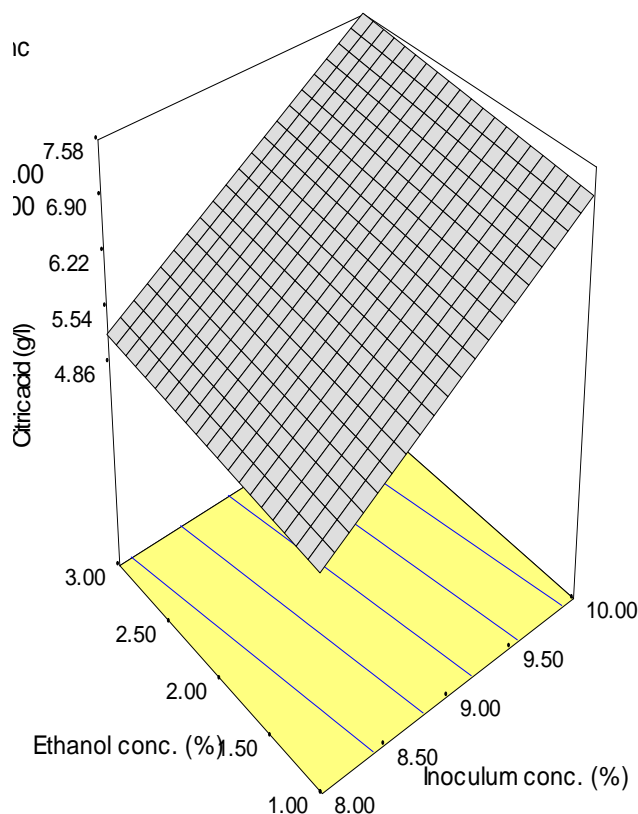**Figure 2: Effect of Temperature and Fermentation Time on the Yield of Citric Acid.**

Figure 3: Effect of Ethanol and Inoculum Concentration on the Yield of Citric Acid.

Factors of temperature and inoculum concentration were used to produce citric acid as shown in Figure 4. The minimum and maximum values of citric acid produced were 2.5 and 7.5 g/l, respectively and were significantly different as shown in Table 6. These values were greater than those reported by Chirova *et al*., (2016) using potato and rice extracts. Increase in inoculum concentration was observed to have produced more citric acid as shown in the inclined sharp slope of the figure than increase in temperature with a mild slope in the figure. This suggests that inoculum concentration had a predominant effect on the production of citric acid in this study. As inoculum concentration increased up to 10%, the value of citric acid increased, but further increase in inoculum concentration would not further increase the citric acid as Adeoye *et al*. (2015) noted that increase in inoculum size above 8% decreased the production of citric acid. This is because increase in population of *Aspergillus niger* led to competition due to insufficient substrate from the organisms thus led to the reduction in citric acid.
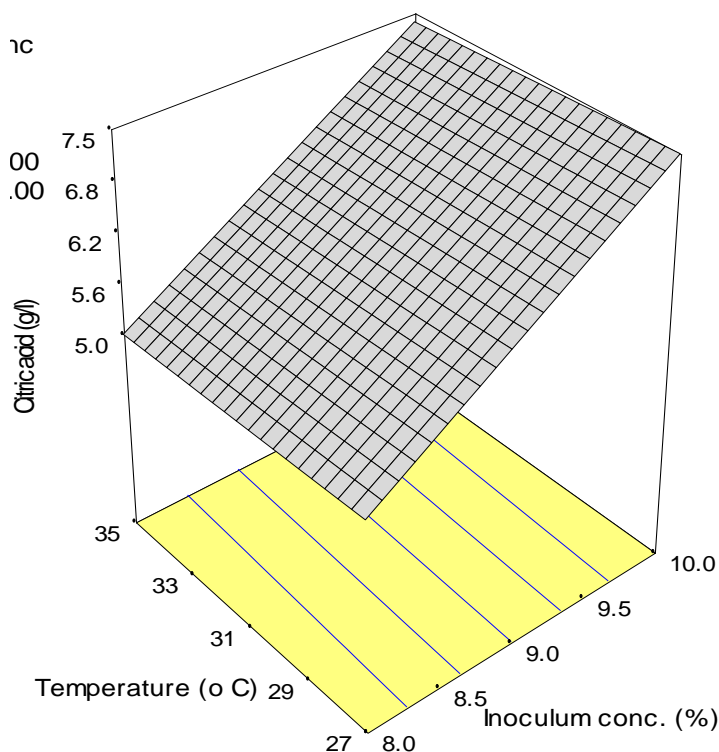
Figure 4: Effect of Temperature and Inoculum Concentration on the Yield of Citric Acid.

4. CONCLUSION

From the study, linear model has good fit for the modelling of citric acid; the values of R^2 is as high as 0.8733 and the lack of fit is not significant. Inoculum concentration played a major role in the conversion of cassava peels to citric acid more than other factors. The high value of adequate precision also proves the reliability of model. Inoculum concentration has the highest coefficient in the model. The actual values are close to predicted values in the diagnostic case statistics. The highest value of 7.6 g/l citric acid was achievable using combination factors of inoculum concentration and time while the lowest value was 2.5 g/l citric acid was achieved using combination factors of inoculum concentration and temperature.

Table 6: Production of citric acid.

Runs	Coded Factors				Actual Factors				Citric acid (g/l)
	A (%)	B (days)	C (°C)	D (%)	A (%)	B (days)	C (°C)	D (%)	
1	1	-1	-1	-1	10.00	1	27	1.00	4.7±0.91[b]
2	-2	0	0	0	7.00	2	31	2.00	3.8±0.61[a]
3	2	0	0	0	11.00	2	31	2.00	7.8±2.01[g]
4	-1	-1	1	1	8.00	1	35	3.00	5.1±0.71[b]
5	0	0	0	-2	9.00	2	31	0.00	5.3±0.15[c]
6	1	1	-1	1	10.00	3	27	3.00	4.3±0.73[b]
7	-1	1	1	1	8.00	3	35	3.00	5.1±0.83[b]
8	0	0	0	0	9.00	2	31	2.00	5.3±0.83[c]
9	1	1	1	1	10.00	3	35	3.00	8.1±1.11[i]
10	-1	-1	1	-1	8.00	1	35	1.00	5.2±0.13[c]
11	-1	-1	-1	-1	8.00	1	27	1.00	5.11±0.33[b]
12	0	0	0	0	9.00	2	31	2.00	5.42±0.89[c]
13	1	1	1	1	10.00	3	35	3.00	7.91±0.97[g]
14	-1	1	-1	1	8.00	3	27	3.00	5.42±0.66[c]
15	0	0	0	2	9.00	2	31	4.00	6.41±0.56[d]
16	1	-1	-1	1	10.00	1	27	3.00	7.80±0.67[g]
17	0	0	0	0	9.00	2	31	2.00	5.47±0.81[i]
18	1	1	-1	-1	10.00	3	27	1.00	7.85±1.03[g]
19	0	-2	0	0	9.00	1	31	2.00	5.31±0.33[h]
20	1	-1	1	-1	10.00	1	35	1.00	7.14±0.56[f]
21	-1	-1	-1	1	8.00	1	27	3.00	5.13±0.46[h]
22	1	-1	1	1	1.00	1	35	3.00	7.72±0.51[g]
23	0	0	2	0	9.00	2	39	2.00	6.43±0.71[d]
24	0	0	0	0	9.00	2	31	2.00	6.43±0.69[d]
25	0	0	0	0	9.00	2	31	2.00	6.42±0.48[d]
26	-1	1	1	-1	8.00	3	35	1.00	5.15±0.51[b]
27	0	2	0	0	9.00	4	31	2.00	6.82±0.54[e]
28	0	0	-2	0	9.00	2	23	2.00	6.44±0.54[d]
29	-1	1	-1	-1	8.00	3	27	1.00	5.32±0.84[c]

Key: A – Inoculum concentration (%); B- Fermentation time (days); C- Fermentation temperature (^{o} C), D- Ethanol concentration (%).

5. ACKNOWLEDGMENT

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