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Economic benefit from the optimization of citric acid production from rice straw through Plackett-Burman design and central composite design

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Abstract

An economic study was carried out to show the benefits of optimization during the production of citric acid from rice straw as a substrate by solid-state fermentation (SSF) using *Aspergillus niger*. The optimization procedure for the media components (NH_4NO_3, KH_2PO_4, $MgSO_4$, $CuSO_4$, $ZnSO_4$, $FeSO_4$, and $MnSO_4$) was carried out using Plackett-Burman design (PBD) for the screening and central composite design (CCD) for the optimization. The results obtained from PBD indicated that NH_4NO_3, KH_2PO_4, and $MgSO_4$ were the most significant components that affected the citric acid production. The maximum yield obtained by CCD was 50.23 g citric acid/kg rice straw. The rate of citric acid production by SSF after the optimization indicated a profit of about 65% for SSF without any nutrient addition and of about 27% for SSF without optimization.

Key Words: Citric acid, solid-state fermentation, rice straw, Plackett-Burman design, central composite design

1. Introduction

Citric acid is considered as one of the important organic acids that has a wide commercialization potential. Recently, major production of citric acid was conducted via microbial fermentation, as it was economical and easy to handle. Citric acid has multiple uses, particularly in food and beverage industries as a flavor enhancer and antioxidant agent. It also has other industrial uses, such as in pharmaceutical, cosmetic, and various chemical industries (Heinzle et al., 2007). *Aspergillus niger* was the most commonly used fungus for citric acid production due to the high yield and relatively high tolerance to acid accumulation (Shuler and Kargi, 2002). It has been used by many researchers and in many studies, mainly in solid-state fermentation (SSF), for its

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ability to live and grow in an environment similar to its natural habitat (Bari et al., 2009; Karthikeyan and Sivakumar, 2010; Dhillon et al., 2011).

In the last 3 decades, SSF has gained great interest from researchers and industries as an alternative technique to the traditional submerged fermentation (SmF). The unique characteristics of SSF, using solid materials, stimulated researchers to use waste such as agroresidual and agroindustrial wastes as an alternative to raw materials for the production of citric acid. Karthikeyan and Sivakumar (2010) used banana peel, and Khosravi-Darani and Zoghi (2008) used bagasse. Dhillon et al. (2011) used different kinds of agroindustrial waste, like apple pomace solid waste, brewery spent grain, citrus waste, and sphagnum peat moss. Imandi et al. (2008) used pineapple waste as a substrate for the production of citric acid. Several advantages of SSF over SmF have encouraged researchers to study and develop it, such as lower energy requirements, less risk of bacterial contamination, and fewer environmental concerns regarding the disposal of solid waste (Rao, 2005).

Since the Second World War, the production of citric acid has increased rapidly, reaching about 1.7 million tons per annum in 2008 with 5% predicted annual increase in the rate of production in order to meet the growing needs of the global market (Dhillon et al., 2011). The remarkable demand for citric acid stimulated researchers and industries to look for and modify an economic production process by reducing the cost of the raw materials and the additives. Rice straw is one of the agricultural residues selected as a solid substrate and alternative to the common raw materials due to its high rate of cellulose and hemicellulose, at 51%-64% (Reddy and Yang, 2005). It is considered one of the cheapest sources of cellulose and hemicellulose, and it is commonly left in the field to burn. This shows that the use of rice straw has 2 advantages: using it as a substrate costs almost nothing, and its usage also reduces the emission of heat and carbon dioxide. The cost estimate of the production requires knowing the amount of the nutrients that can be achieved with the higher rate of production. These nutrients affect the reproduction and growth of *A. niger*, which would lead to an effect on the production of citric acid. The nutrients have been of great interest for researchers due to the effect of these factors on the metabolic activity of *A. niger* and the rate of production of citric acid. Dhillon et al. (2011) showed the effect of ethanol and methanol addition on the rate of production, where the addition of 3% (v/w) ethanol and 4% (v/w) methanol increased the citric acid production from apple pomace solid waste 2-fold. Bari et al. (2009) studied the effect of the cosubstrate (sucrose), stimulator (methanol), and metal ions (Zn, Cu, Mn, and Mg). The optimization of the studied parameters increased the rate of production by 2.6 times.

The current study highlights the economic benefit of nutrient optimization for achieving a higher rate of citric acid production with the optimum amount of nutrients. The optimization procedure was carried out in 2 stages. First, the media components were screened by the Plackett-Burman design (PBD) to identify the significant components. Second, optimization of the selected media components was conducted according to the central composite design (CCD) to achieve a higher yield of citric acid.

2. Materials and methods

2.1. Microorganism

The fungal strain of *A. niger* was obtained from the culture collection center (CCUniMAP) of the Bioprocess Engineering School, Universiti Malaysia Perlis. *A. niger* was grown and cultured in a selective agar containing NH_4NO_3 (2.5 g/L), KH_2PO_4 (1.0 g/L), $MgSO_4$ (0.25 g/L), $CuSO_4$ (0.0048 g/L), $ZnSO_4$ (0.0038 g/L), $FeSO_4$ (0.0022 g/L), and $MnSO_4$ (0.001 g/L) (Krishnan, 1999). Molasses was the carbon source (sugar) at 14% (w/v), and the strain was incubated for 7 days at 32 °C. The spore suspensions were collected and suspended using glass beads, and they were enumerated using a hemocytometer to maintain a density of (1-2) $\times 10^7$ spores/mL. The fungal strain was subcultured once every 20 days and stored at 4 °C.

2.2. Treatments of molasses

The molasses was treated with 1 N sulfuric acid at room temperature for 1.5 h and then centrifuged at 10,000 rpm for 10 min. It was neutralized with sodium carbonate and left overnight. The supernatant was diluted to the desired concentration (Lotfy et al., 2007).

2.3. Preparation for solid-state experiments

Into 500-mL Erlenmeyer flasks was placed 2 g of the treated rice straw with a particle size of >2 mm. The moisture level of the substrate was maintained at 80% by adding 8 mL of total liquid (molasses, mineral solution, and inoculum) based on the following relationship:

$$\text{Moisture Content} = \frac{\text{Weight of water}}{\text{Weight of water+Weight of Paddy}} \tag{1}$$

where 1 g of water was equal to 1 mL. The pH of the molasses was set to 5.5, and the concentration of reduced sugar in the molasses was 14% (w/v). The amount of spore suspension inoculated was 10% of the solid substrate (0.2 mL of distilled water with a spore density of (1-2) $\times 10^7$ spores/mL). The flasks were incubated at 31 °C for 8 days. The moisture content was maintained during the fermentation period. All of the experiments were performed in triplicate.

2.4. Analytical assay

The extraction of the citric acid from the SSF culture was carried out by adding 100 mL of sterilized water and shaking for 1 h at 150 rpm and room temperature. The extracted solution was made spore-free by filtering it through Whatman No. 1 filter paper, and an assay was carried out on the supernatant. The pH of the extracted solution was determined using a pH meter. The citric acid was determined spectrophotometrically by the acetic anhydride-pyridine method developed by Miller (1958). Total reducing sugars in the molasses were measured by the 3-5-dinitrosalicylic acid (DNS) method of Marier and Boulet (1958).

2.5. Experimental design

2.5.1. Plackett-Burman design

PBD was one of the statistical techniques used for the screening of the experimental factors. The purpose of using this technique was to identify the significant nutrients that had an impact on the amount of citric acid production in a minimum number of experiments. The statistical screening was based on the main effects of the experimental factors, but not on their interaction effects (Plackett and Burman, 1946). Each factor was represented within 2 levels, high (+) and low (-). Table 1 represents the 7 selected media components that were evaluated. The design of the 7 media components as evaluated in the 12 experimental runs is illustrated in Table 2. The factors that had a level above 95% ($P < 0.05$) were considered as significant and used for further optimization (Montgomery, 2005). The designing and analyzing of these experiments was carried out using Minitab Software v. 15.

Table 1. The actual values of the nutrients tested in PBD with P-values.

Variables		NH_4NO_3	KH_2PO_4	$MgSO_4$	$CuSO_4$	$ZnSO_4$	$FeSO_4$	$MnSO_4$
Coded		A	B	C	D	E	F	G
Low level (-)		0	0	0	0	0	0	0
High level* (+)		2.5	2.5	0.25	0.00006	0.001	0.00025	0.0013
P-value	Citric acid	***0.001***	***0.050***	0.418	0.093	0.089	0.818	0.553
	Reduced sugar	***0.001***	0.284	***0.039***	0.364	0.100	0.134	0.257
	pH	***0.003***	0.468	0.232	0.828	0.367	0.432	0.588
Significant sign		*	*	*				

*The concentration of the components in g/L.

Table 2. Plackett-Burman design matrix for seven media components with observed results.

Run	A	B	C	D	E	F	G	Citric acid[a]	Reduced sugar[b]	pH[c]
1	+	-	+	-	-	-	+	4.44	83.86	33.89
2	-	+	+	+	-	+	+	4.36	85.46	37.13
3	+	-	-	-	+	+	+	7.47	62.93	46.53
4	-	-	+	+	+	-	+	4.63	82.14	37.59
5	+	-	+	+	-	+	-	5.20	85.46	37.59
6	+	+	+	-	+	+	-	4.32	85.46	34.82
7	-	+	+	-	+	-	-	6.70	63.05	46.53
8	-	-	-	+	+	+	-	7.28	44.82	47.30
9	+	+	-	+	+	-	+	8.47	52.58	47.61
10	+	+	-	+	-	-	-	6.74	82.01	43.29
11	-	+	-	-	-	+	+	8.39	64.77	46.68
12	-	-	-	-	-	-	-	7.12	64.16	46.53

[a]Final conc. of citric acid, g/L; [b]amount of consumed sugar, %; [c]reduction of acidity, %.

2.5.2. Central composite design

After evaluation and screening of the media components by the PBD method, a statistically based optimization method was carried out using CCD under response surface methodology (RSM). The statistical method has been widely used in biotechnology experiments. The primary interest was in modeling and ultimately developing a production model that could explain the relationships between a number of experimental factors and the response. Furthermore, the optimum conditions of the experimental factors that would achieve higher yields for the response could be identified (Box et al., 1978). CCD can decrease the required experiments, time, materials, and resources, which would lead to effectiveness and reduced research costs. Furthermore, the analysis performed on the results can be realized and the experimental errors are thus minimized (Cox, 1958).

CCD consists of a 2^n factorial point with 2n axial points and C_o center points, as explained in the following equation:

$$A = 2^n + 2n + C_o \tag{2}$$

where A is the total number of experiments, n is the number of factors studied for the optimization, and C_o is the number of the repetitions of experiments at the center points (Ali and Al-Azzawi, 2010).

The current equation could explain the relationship between the actual and coded value:

$$X_{coded} = \frac{[X_{act} - X_{cen}]}{\left[\frac{X_{cen} - X_{\min}}{\sqrt{n}}\right]} \tag{3}$$

where X_{act} represents the actual value of the factor, X_{cen} represents the center value, and X_{min} represents the minimum value.

The second-order polynomial equation describing the relationships between the experimental factors and the response that gives all of the information about the interaction between factors in their relationship to the response is:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_{ij} \tag{4}$$

where Y represents the predicted response, X_i through X_n the input factors, β_0 the intercept term, β_i the linear effects, β_{ii} the squared effects, and β_{ij} the interaction term. Design Expert Software v. 7 was used for the regression and graphical analysis of the data obtained.

3. Results and discussion

It was essential to determine the fermentation period. The increased amount of citric acid accumulated over time would be toxic to *A. niger* and would start to affect its metabolic activity. The *A. niger* would start to oxidize the citric acid if the fermentation were carried out for a long time. The citric acid concentration and the sugar concentration in molasses were recorded (Figures 1 and 2).

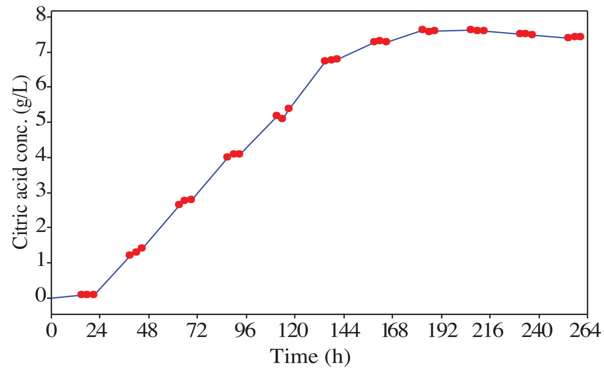


Figure 1. Citric acid concentration during solid state fermentation.

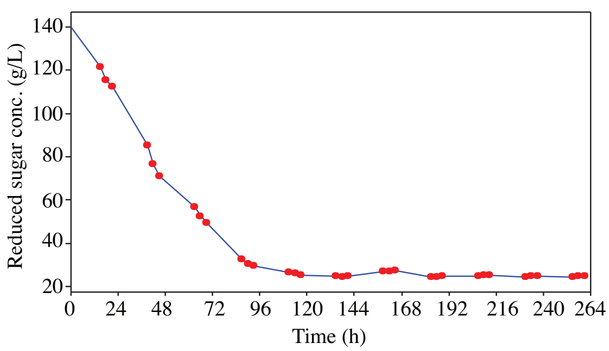


Figure 2. Reduced sugar concentration during solid state fermentation.

The citric acid was produced from the second day of fermentation until it reached the highest value (7.6 g/L) on the eighth day. The acid concentration remained roughly constant despite the passage of time. The concentration started to decrease because of the oxidation of the citric acid. The maximum concentration for citric acid was reached on the eighth day; thus, there was no need to let the fermentation period exceed 8 days. The higher yield of citric acid achieved without the addition of any nutrients to the fermentation culture was 30.4 g citric acid/kg rice straw. The citric acid was considered as a secondary metabolic product, so it was important to stimulate the cell to produce citric acid rather than to grow and produce biomass. The low concentration of reduced sugar in the molasses would enhance the efforts of *A. niger* to spread on and penetrate into the substrate for germination, and to build up the hyphae. The fungus had consumed almost all of the reduced sugar in the first 4 days (79%) (Figure 2). Consequently, the fungus started to consume the cellulose and convert it to citric acid.

3.1. Screening of media components

The nutrient concentration seemed to have an important role in the fermentation of citric acid. PBD was used for the screening of the parameters (nutrients) that had significant implications. The P-value was considered as a tool for evaluating the significant nutrients. Parameters with confidence levels greater than 95% were considered to significantly influence the response. The analysis of variance (ANOVA) of the measured responses (citric acid concentration, concentration of reduced sugar in molasses, and pH) is given in Table 1. The ammonium (NH_4NO_3) showed a significant influence on all of the responses based on the P-value, which was 0.001, 0.001, and 0.003 for citric acid, reduced sugar, and pH, respectively. KH_2PO_4 was observed to affect the citric acid fermentation through the effect of the citric acid concentration, where the P-value was 0.05. $MgSO_4$ was also considered to be a significant nutrient, as it affected the reduced sugar response with a P-value of 0.04. The maximum yield obtained from this set of experiments was 33.88 g citric acid/kg rice straw (experiment no. 9).

Table 3. The CCD matrix of selected variables in coded and actual values, with their corresponding responses.

Exp. no	Coded and real values						Responses		Yield[a]
	$NH_4NO_3^*$		$KH_2PO_4^*$		$MgSO_4^*$		Citric acid*	Reduced sugar*	
1	**(−1)**	*0.528*	**(−1)**	*0.528*	**(−1)**	*0.052*	8.714	47.32	35.0
2	**(1)**	*1.972*	**(−1)**	*0.528*	**(−1)**	*0.052*	2.677	41.88	10.5
3	**(−1)**	*0.528*	**(1)**	*1.972*	**(−1)**	*0.052*	9.292	50.20	37.0
4	**(1)**	*1.972*	**(1)**	*1.972*	**(−1)**	*0.052*	3.228	42.59	13.0
5	**(−1)**	*0.528*	**(−1)**	*0.528*	**(1)**	*0.197*	8.970	46.72	36.0
6	**(1)**	*1.972*	**(−1)**	*0.528*	**(1)**	*0.197*	5.218	45.37	21.0
7	**(−1)**	*0.528*	**(1)**	*1.972*	**(1)**	*0.197*	8.929	50.00	35.5
8	**(1)**	*1.972*	**(1)**	*1.972*	**(1)**	*0.197*	3.242	41.17	13.0
9	**(−1.681)**	*0*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	9.817	71.53	39.5
10	**(1.681)**	*2.5*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	2.112	42.27	8.50
11	**(0.0)**	*1.25*	**(−1.681)**	*0*	**(0.0)**	*0.125*	5.823	44.59	23.5
12	**(0.0)**	*1.25*	**(1.681)**	*2.5*	**(0.0)**	*0.125*	5.390	44.59	21.5
13	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(−1.681)**	*0*	5.702	50.00	28.0
14	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(1.681)**	*0.25*	5.662	43.24	22.5
15	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	5.46	42.46	22.0
16	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	5.299	47.88	21.0
17	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	5.702	48.65	23.0
18	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	5.702	54.07	23.0
19	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	6.186	47.49	24.5
20	**(0.0)**	*1.25*	**(0.0)**	*1.25*	**(0.0)**	*0.125*	5.944	58.71	24.0

Values within parentheses indicate the coded values. *Concentration measured in g/L;
[a]yield in g citric acid/kg rice straw used.

3.2. Optimization of significant selected media components

The selected media components that had a significant effect on citric acid production were optimized by using CCD to achieve the maximum yield. The CCD matrix of the 3 selected media components in the form of coded and actual values, along with measured and calculated responses of each experimental trail, is shown in Table 3. The results were analyzed with Design Expert Software and are summarized in Table 4. The ANOVA of the quadratic regression model indicates that the model was significant, at $P < 0.001$.

Table 4. ANOVA for response surface quadratic model.

Source	Sum of squares	DF	F-value	P-value
Model	1463.98	9	29.14	< 0.001
NH_4NO_3	1397.21	1	250.29	< 0.001
KH_2PO_4	3.97	1	0.71	0.4187
$MgSO_4$	0.041	1	7.3E-003	0.9332
$NH_4NO_3 \times KH_2PO_4$	6.13	1	1.10	0.3195
$NH_4NO_3 \times MgSO_4$	15.13	1	2.71	0.1308
$KH_2PO_4 \times MgSO_4$	21.13	1	3.78	0.0804
$(NH_4NO_3)^2$	5.63	1	1.01	0.3388
$(KH_2PO_4)^2$	0.13	1	0.023	0.8819
$(MgSO_4)^2$	16.41	1	2.94	0.1172
R^2	0.9633			
Adj. R^2	0.9302			
Adequate precision	20.364			

The determination coefficient (R^2) provides an indicator for the variability of the predicted response with the experimental results. The closer R^2 is to 1, the better able the model is to predict the response (Wang, 2006; Aghaie et al., 2009). The R^2 of the following model was 0.9633, indicating that 96.33% of the variability in the experimental results could be explained by the estimated model. Furthermore, the adjusted determination coefficient (adj. R^2) was very high (93.02%), which confirmed that the model was highly significant. The adequate precision gives a value measuring the signal-to-noise ratio. A ratio greater than 4 is desirable. The adequate precision value of 20.364 was very high compared to the desirable ratio. This confirms the possible use of the developed polynomial model to navigate the design space.

A second-order polynomial equation was developed to fit and describe the citric acid yield for the experimental results. The developed model, in terms of real values, can be illustrated as follows:

$$\begin{aligned} Yield \quad = \quad & 42.58 - 17.57 \times NH_4NO_3 + 4.5 \times KH_2PO_4 - 41.9 \times MgSO_4 + 1.13 \times NH_4NO_3^2 \\ & +0.17 \times KH_2PO_4^2 + 193.17 \times MgSO_4^2 - 1.58 \times NH_4NO_3 \times KH_2PO_4 \\ & +24.89 \times NH_4NO_3 \times MgSO_4 - 29.41 \times KH_2PO_4 \times MgSO_4. \end{aligned} \tag{5}$$

The variability between the values predicted by the above model versus the observed data obtained from the experimental work is illustrated in Figure 3, which shows the experimental results clustered around the diagonal line, where (R^2) was 96.33%, indicating a reasonable fit of the model to the experimental data.

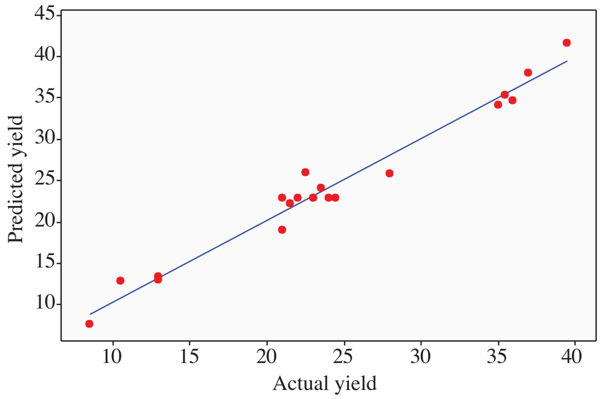


Figure 3. Compression between predicted and observed citric acid yield.

In observing the ANOVA for the results given in Table 4, the significance for each of the model parameter coefficients was evaluated using Student's F-test and P-values. It was seen that the variables with highly significant effects were the linear term of the nitrogen source (A) and the interactive term between potassium and magnesium (BC); these 2 terms also had the highest F-values.

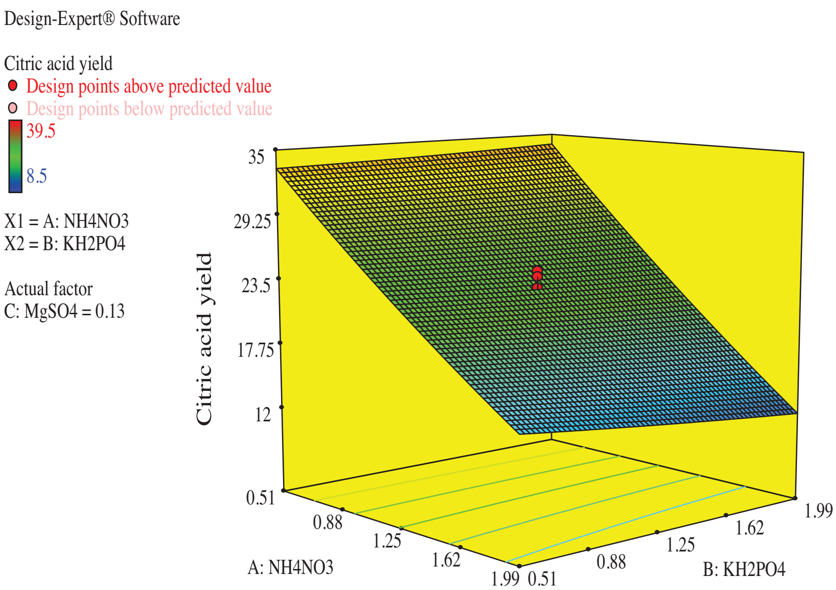
The 2D counterplot, along with the 3D response surface plots, was considered as the graphical representation of the regression equation to determine the optimum concentration for the selected media components within the considered ranges (Tanyildizi et al., 2005).

The interaction response was illustrated by changing the values of 2 parameters and fixing the others at a central level (0 level). The interaction effect of NH_4NO_3 and KH_2PO_4 on citric acid production was recorded; it seemed that the production increased with a decrease in NH_4NO_3 and an increase in KH_2PO_4 (Figure 4). The decreasing of NH_4NO_3 and $MgSO_4$ would increase the rate of citric acid production (Figure 5). The yield of citric acid production was increased in 2 predicted directions, either by increasing the KH_2PO_4 in the range of 1.6-2.0 g/L and decreasing the $MgSO_4$ in the range of 0.05-0.07 g/L, or by decreasing the KH_2PO_4 in the range of 0.5-0.7 g/L and increasing the $MgSO_4$ in the range of 0.18-0.2 g/L (Figure 6).

Nitrogen seemed to play a major role in the metabolism of citric acid. The cell needed nitrogen in the form of ammonium to build up cell substances. On the other hand, a high nitrogen concentration seemed to inhibit the production of citric acid, as it would enhance the cell to grow and produce biomass rather than produce citric acid (Wieczorek and Brauer, 1998). This is shown in Figures 4 and 5, as the yield of the citric acid increases with the decreasing of the NH_4NO_3 concentration. Potassium and phosphate in KH_2PO_4 were also considered as growth-enhancing nutrients, and they seemed to have had a noticeable effect during the growth of *A. niger* and its metabolic activity during fermentation (Imandi et al., 2008; Singh and Dikshit, 2010). Furthermore, it is considered as a buffering agent, as it acts to keep the pH at a desired value, so it must be used in a reasonable amount to keep the pH of the substrate within the desired range. This is reflected in the predicted results in Figures 4 and 6. Magnesium affects the rate of sugar utilization by the cell, related to the rise in mycelia weight (biomass rise) (Shu, 1948), so this parameter must be limited in order to stimulate the fungus to produce citric acid rather than growth. The results of magnesium incorporation are shown in Figures 5 and 6.

Accounting for the observed results from Figures 4-6 and analyzing the developed model to achieve the highest yield and the optimal concentration for the selected nutrients, the 5 best predicted citric acid yields with nutrient concentrations are given in Table 5. The 5 suggested optimum concentrations of the nutrients somewhat converged. The experimental trail was performed for each suggested optimum case (Table 5). From the validation experiments, the highest citric acid yield of 50.23 g/kg rice straw (experiment no. 4) was obtained with nutrient concentrations as follows: 0.05 g/L of NH_4NO_3, 2.46 g/L of KH_2PO_4, and 0.03 g/L of $MgSO_4$. Thus, the predicted values from the fitted equation and the experimental values were in very good agreement.

The optimization procedure for the media components was determined with an increase in citric acid fermentation of 19.83 g citric acid/kg rice straw upon citric acid fermentation without the addition of nutrients to the culture. The calculations of the gain in production and the cost of the nutrients added are given in Table 6. Those calculations show the benefit from the addition of the nutrients in fermentation and the improvement



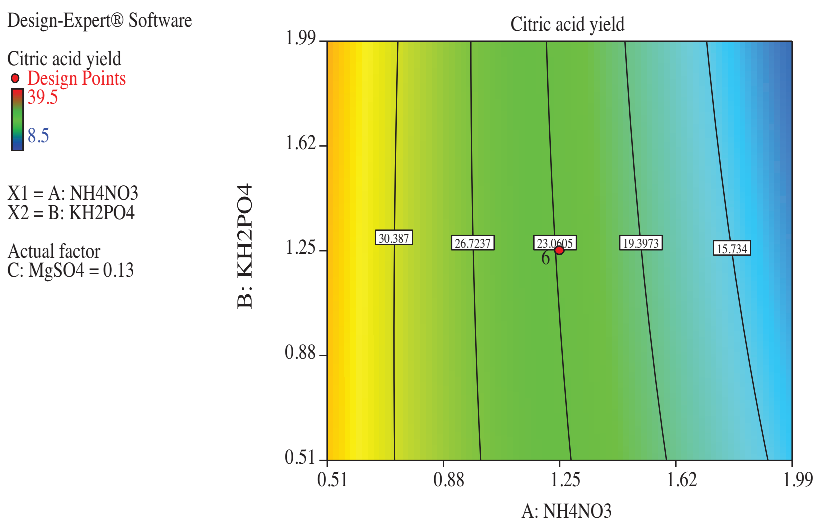


Figure 4. Response surface curve and counterplot for the yield of citric acid as a function of NH_4NO_3 and KH_2PO_4.

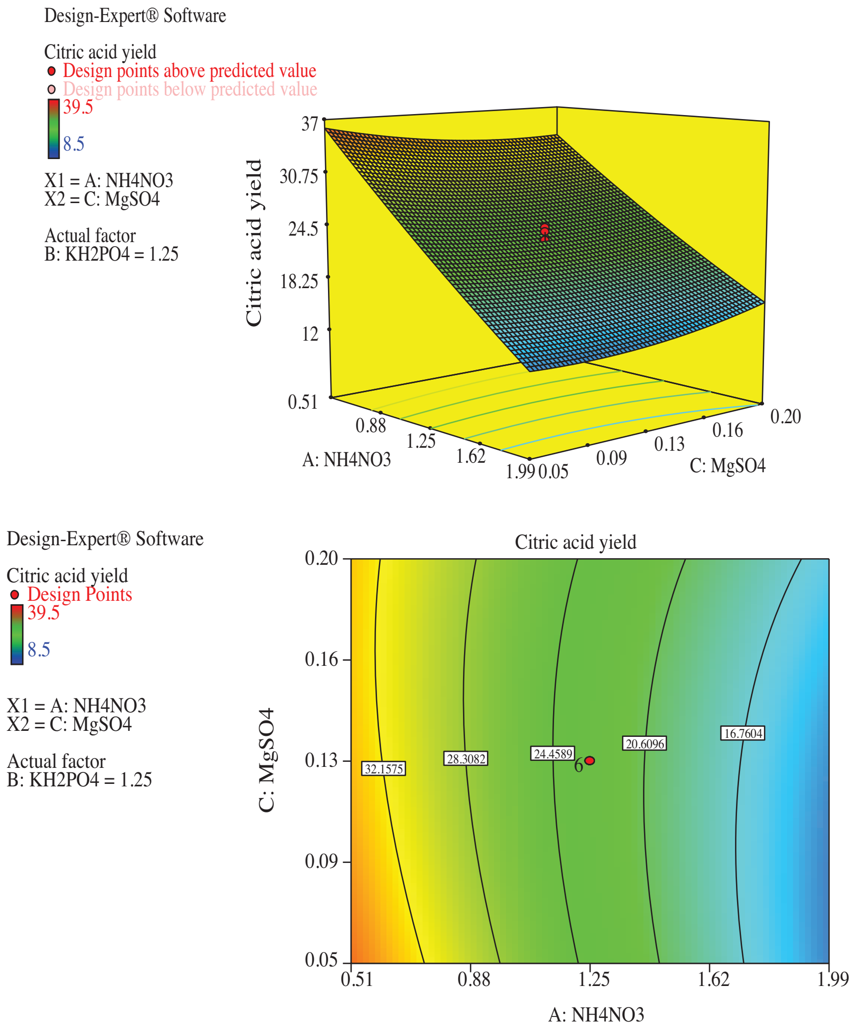


Figure 5. Response surface curve and counterplot for the yield of citric acid as a function of NH_4NO_3 and $MgSO_4$.

through the optimization. The addition of the nutrients made for an increase in profit of 27%, and, after the optimization procedure, the improved production had a profit increase reaching 65%. This led to a final profit of 0.000107 US$ based on the laboratory scale and 2 g of rice straw.

Table 5. Validation and confirmation of the developed second-order polynomial model and the optimum media component concentration.

No.	NH_4NO_3	KH_2PO_4	$MgSO_4$	Predicted	Experimental
1	0.12	2.36	0.01	50.9025	50.14
2	0.02	2.25	0.02	50.7224	49.93
3	0.02	2.38	0.03	50.3061	49.36
4	0.05	2.46	0.03	50.982	50.23
5	0.02	2.44	0.03	50.8771	49.97

Table 6. The profit gain from nutrient optimization through SSF.

	Items	Price	Amount	Total	Ref.
Increase in product	Citric acid	1.3 US$/kg	0.1 g	0.00013 US$	Dhillon et al., 2011
Cost	Ammonium nitrate	0.22 US$/kg	0.0004 g	88×10^{-9} US$	LookChem
	Potassium phosphate	1.2 US$/kg	0.0196 g	23×10^{-6}US$	Made-in-China
	Magnesium sulfate	66.0 US$/kg	0.00024 g	24×10^{-8} US$	bio-World
Profit				0.000107 US$	

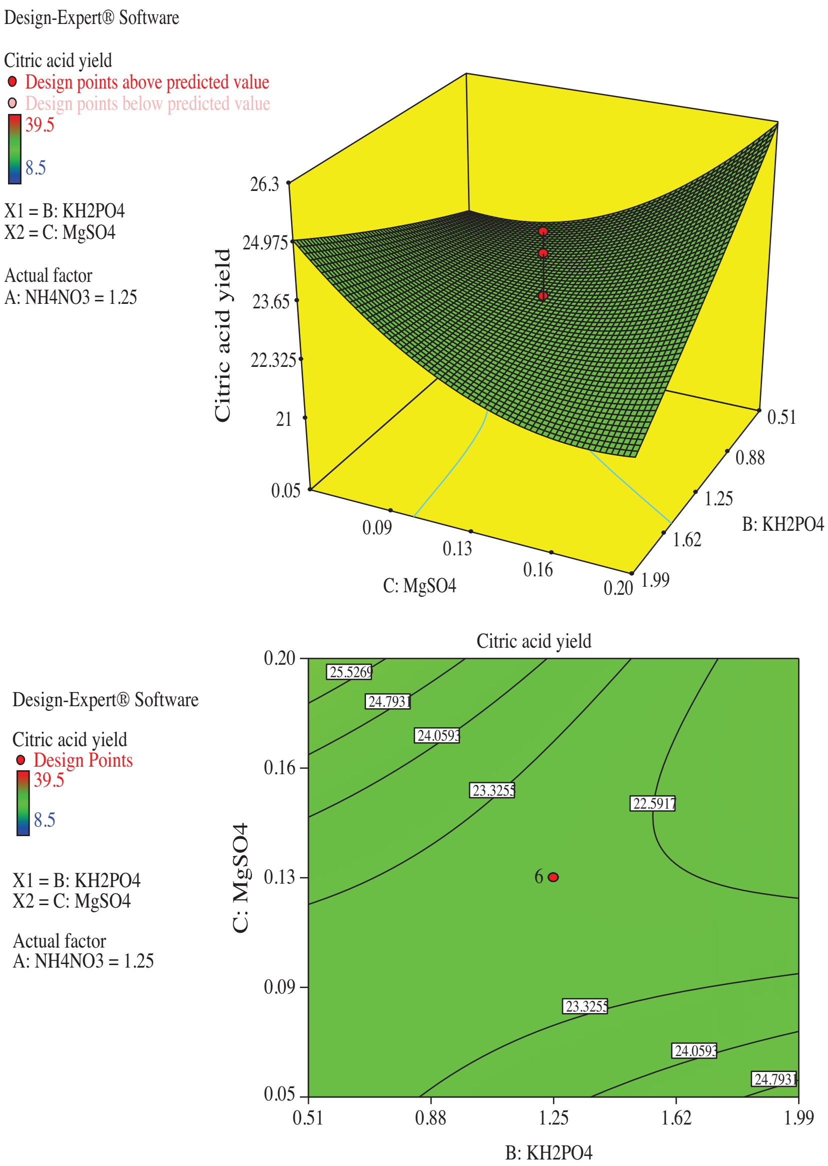


Figure 6. Response surface curve and counterplot for the citric acid yield as a function of NH_4NO_3 and $MgSO_4$.

4. Conclusion

The 3 media components NH_4NO_3, KH_2PO_4, and $MgSO_4$ were selected by PBD as significant parameters for citric acid production from rice straw. The selected parameters were optimized using CCD under RSM. The maximum yield obtained after the optimization of the selected parameters was 50.23 g citric acid/kg rice straw, for a 1.65-fold increase. The optimization of citric acid production led to a net profit rate of 0.000107 US$/2 g rice straw. The results indicate that the optimization proved to be a successful tool for the improvement of the solid-state fermentation process by reducing operation costs and increasing production.

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