Design and Optimization of Citric Acid Fermentation Process by Statistical Method

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Abstract—Citric acid fermentation is constrained by the operating variables: Initial sucrose concentration, Initial pH, Stirrer speed, Incubation time, Fermentation temperature and O2 flow rate. The present work reports the statistical design of experiments using Central composite design (CCD) for submerged fermentation of Citric acid and optimization of Incubation time, Fermentation temperature and O2 flow rate by Response surface methodology (RSM). For the three variables, CCD had designed fifteen different combinations of experiments. From the experiments, the concentrations of citric acid produced were estimated and from the results a second order polynomial equation was developed with the coefficients in a mathematical model and predicting the response variable. The optimization involves three steps: Performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model [8].

Corn cobs were used as a substrate for citric acid production by Aspergillus Niger. Methanol had a significant effect on fungal production of citric acid from corncobs. Of the four cultures examined, A. niger NRRL 2001 was found to produce the highest amount of citric acid (250 g/kg dry matter of corncobs) after 72 h of growth at 30°C in the presence of 3% methanol. The yield of citric acid was over 50% based on the amount of sugar consumed [9].

Evaluated three different agro-industrial wastes, sugar cane bagasse, coffee husk and cassava bagasse for their efficiency in production of citric acid by a culture of Aspergillus Niger. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. Results showed the fungal strain had good adaptation to the substrate (cassava bagasse) and increased the protein content (23 g/kg) in the fermented matter. Citric acid production reached a maximum (88-g/kg dry matter) when fermentation was carried out with cassava bagasse having initial moisture of 62% at 26°C for 120 h [6].

Observed that Natural oils with high unsaturated fatty acids content when added at concentrations of 2% and 4% (v/v) to beet molasses (BM) medium caused a considerable increase in citric acid yield from Aspergillus niger. The fermentation capacities were also examined for production of citric acid using BM-oil media under different fermentation conditions. Maximum citric acid yield was achieved in surface culture in the presence of 4% olive oil after 12 days incubation [1].

Studied on Citric acid (CA) production by Aspergillus Niger ATCC9642 from whey with different concentrations of sucrose, glucose, fructose, galactose riboflavin, tricalcium phosphate and methanol in surface culture process was studied. It was found that whey with 15% (w/v) sucrose with or without 1% methanol was the most favourable medium producing the highest amount (106.5 g/l) of citric acid. Lower CA was produced from whey with other concentrations of sugars and other additives used. Highest biomass of A. niger was produced with the addition of riboflavin. In general, extension of the fermentation (up to 20 days) resulted in an increase in CA and biomass, and decrease in both residual sucrose and pH values [2].

Reported the effects of various nutrients (glucose, (NH4)2SO4, KH2PO4 and NaCl), fermentation parameters (moisture content, temperature, inoculum density, composition of solid substrate and particle size) and of initial level of potential stimulators (ethanol, methanol, phytate and surfactant) were evaluated with respect to citric acid production by A. niger grown on damp peat moss. Three variables including aeration, thickness of solid substrate bed
and incubation temperature were optimized using a 23 full factorial design (FFD). Under optimum, the total citric acid production and yield were 120.6 g/kg DPM and 18.5% respectively. A third experiment (study 3) compared the production of citric acid by A. niger in submerged fermentation using cheese whey, as opposed to batch and semi-continuous fermentation using peat moss. Various fermentation conditions such as nutrients (glucose, (NH4)2SO4 and KH2PO4), stimulators (methanol, olive oil and phytate) and fermentation parameters (pH, fermentation time and inoculum density) were optimized using a central composite design (CCD). Citric acid production improved citric acid production by a factor of 13.3 when compared to the production of citric acid by A. niger NRRL 567 using whey-based medium (50 g/l) alone [7].

II. MATERIALS AND METHODS

A 1.2 liter capacity glass fermentor equipped with standard control and instrumentation as shown in Fig.1 was used for the citric acid fermentation. Fermentor was thoroughly cleaned with water and sterilized in an autoclave for 20 minutes. The sterilized fermentor was placed in the main assembly and tube connections were given for water and air supply. Then the sterilized medium containing vegetative inoculums was transferred to the fermentor from the conical flask after 24 hours of incubation. Thus the system was ready for fermentation. The power was switched on. Fifteen different combinations of experiments designed using CCD (2k +2k+1, where k = no. of variables) were conducted. The samples were collected for every 24 hours from the fermentor and analyzed titrimatically for citric acid produced.

![Figure 1 Photograph of Experimental setup](image)

III. RESULTS AND DISCUSSION

Using CCD different combinations of experiments were designed. The maximum and minimum values were denoted as (+1) and (-1) respectively. The center point was the average of maximum and minimum values. The alpha values (-1.68 and +1.68) are default values taken in the design expert software. The effects of Incubation time, Fermentation temperature and O2 flow rate on citric acid production were experimentally studied and were optimized using RSM. The range of values selected for the variables under study were as shown in table 2. The statistical software Design-Expert ® 8.0 was used to generate a regression model for predicting the effect of combined parameters (Incubation time, Fermentation temperature and O2 flow rate) on the responses (citric acid yield). To construct the response surface model, a second-order polynomial equation was fitted to the experimental data obtained using multiple regressions [12]. The response of tested variables was predicted by the quadratic polynomial equation [10]. The second-order polynomial equation for predicting the yield of citric acid (Y) as a function of the three variables generated by CCD was obtained as

\[ Y = -1463.44326 + 1.99043 x_1 + 98.25444 x_2 + 12.47096 x_3 + 0.35126 x_1 x_2 + 0.029100 x_1 x_3 - 0.053142 x_2 x_3 - 1.16895 x_1^2 - 1.63925 x_2^2 - 2.87816 x_3^2 \]

Table II

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Units</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>x_1</td>
<td>Incubation time</td>
<td>days</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>x_2</td>
<td>Fermentation temperature</td>
<td>°C</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>x_3</td>
<td>O2 flow rate</td>
<td>lpm</td>
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<td>2.5</td>
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</table>

The goodness of fit of the equation was determined by computing predicted citric acid yields and correlating them with those measured. At medium pH of 6.0, R^2 value for the citric acid production was obtained as 0.90. With the citric acid yields computed using equation (1), the significance of each parameter was analyzed using ANOVA table. For citric acid production at medium pH of 6.0, Incubation time, Fermentation temperature and O2 flow rate were observed to exert a significant effect. Thus all the three variables found to have significant impact on citric acid production.

Three-dimensional response surface curves were generated using citric acid production values predicted by the CCD second order equations. For each curve, two variables were varied while the other variable was fixed.

Figs. 2 & 3 indicate that at initial medium pH of 6.0, citric acid production was increased with increasing levels of incubation time and as well as with increasing levels of fermentation temperature. A slight decrease in citric acid production was observed for O2 flow rate above 1.0 lpm. When O2 flow rate was increased from 1.0 – 2.5 lpm, citric acid production dropped for levels of incubation period. A maximum citric acid concentration of 52.49 g/l was achieved with incubation time of 5 days and fermentation temperature of 30°C.
Fig. 2 Response surface curve for citric acid production as a function of Incubation time and Fermentation temperature while O₂ flow rate was fixed at 1.0 lpm.

Fig. 3 Response surface curve for citric acid production as a function of Incubation time and O₂ flow rate, while Fermentation temperature was fixed at 30°C.

Fig. 4 Response surface curve for citric acid production as a function of Fermentation temperature and O₂ flow rate, while the other variable Incubation time was fixed at 5 days.

Table III

<table>
<thead>
<tr>
<th>Variable</th>
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<tr>
<td>Fermentation temperature</td>
<td>°C</td>
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<tr>
<td>O₂ flow rate</td>
<td>lpm</td>
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<tr>
<td>Citric acid yield</td>
<td>g/l</td>
<td>52.49</td>
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</table>

IV. CONCLUSIONS

Based on the examinations, it can be concluded that for high productivity of citric acid, optimization of its process variables is required. It was noticed that, Incubation time, Fermentation temperature and O₂ flow rate have lot of impact on citric acid production and found that maximum yield of citric acid (52.49 g/l) was possible at the RSM optimized values of 5 days of Incubation time, 30°C of Fermentation temperature and 1.0 lpm of O₂ flow rate.

REFERENCES