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Assessment of relative importance of Process variables in Citric Acid Fermentation by Plackett-Burman design

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Abstract

Citric acid fermentation process is controlled by different operating variables: Initial sucrose concentration, Initial pH, Stirrer speed, Incubation time, Fermentation temperature, O_2 flow rate, moisture content, particle size, inoculum density, methanol concentration, spore age etc. The present work reports the evaluation of the parameters by Plackett-Burman designs, one of the factorial design methods using design expert software to screen the strongly influencing variables for their subsequent optimization to improve the citric acid fermentation from Sucrose using Aspergillus Niger NCIM-705. Plackett-Burman is a specialized design for 2 to 31 variables where each variable can be varied over two levels. The lower and upper limits of eleven variables stated above were identified from the literature. The variables with their operational range were processed in Plackett-Burman design software until a point where the design matrix evaluation for factorial main effect model was obtained. All trials were performed in triplicate and citric acid yield hs been treated as response variable. The main effect of each variable was calculated as the differencce between the average of measurements made at the high setting(+) and at the low setting(-) of that factor. The six variables; Initial sucrose concentration, initial pH, stirrer speed, incubation time, fermentation temperature and O_2 flow rate were found to be the most influencing parameters on the yields of citric acid.

Keywords: Citric acid, *Aspergillus Niger*, fermentation, operating variables, factorial design and plackett-Burman design.

Introduction

A variety of carbon sources can be used for citric acid fermentation. Molasses, sucrose or glucose syrups are favored commercially. Citric acid is widely used in the food and beverage industries as an acidifying and flavor-enhancing agent, pharmaceutical industry and elsewhere[1]. The entire worldwide demand for citric acid is met by fermentation mainly by the process involving the filamentous fungus *A. niger* as it is the most commonly employed organism for citric acid fermentation[2]. For an efficient citric acid production, the growth of Aspergillus in pellet form is desirable and this can be achieved by process optimization[3]. A well defined statitistical experimental design is considered to be necessary for optimization of a fermentation process. The development of the fermentation process has got its impact on the economy and practability of the process. Hence, the optimization of operating conditions has become a challenging task for the researchers in the recent years. The diversity of combinational interactions of physical and chemical fermentation parameters and production do not permit satisfactory detailed modeling. In the present work, we report the optimization procedure for citric acid production by *A. niger* through response surface methodology, a statistical Optimization method as an effective tool for fermentation engineering[4]. The largest industrial consumers of Citric acid include food, beverage, chemical, pharmaceutical and other industries[5]. The effects of pH, temperature, time of fermentation, sugar concentration, nitrogen concentration and potassium ferrocyanide on citric acid production were investigated using response surface methodology. Regression equations were used to model the fermentation to determine the optimum fermentation conditions. Higher yields were obtained after optimizing media components and conditions of fermentation. Maximum citric acid production was obtained at pH 5.35, 29.76°C, 5.7 days of fermentation with 221.66 g of substrate/l, 0.479 g of ammonium nitrate/l and 2.33 g of potassium ferrocyanide/l[6]. Although many microorganisms can be used to produce citric acid, *A. niger* remains the main industrial producer. Specific strains that are capable of overproducing citric acid have been developed for various types of fermentation processes[7]. The Plackett-Burman designs (PBD) have been widely used for screening process variables that make the significant impact on a process[8]. PBD is a set of small and efficient experimental designs, which is very much reliable, widely applicable and especially well suited for bioresearch and development[9]. Recent reports on the use of PBD include its application toward improving antibiotic, *Saccharopolyspora spinosa* macrolide, *Colletotrichum coccodes*spores. Screening studies by PBD considers the ratio of the number of experiments to be conducted to the number of variables being studied. This design allows for the study of k= (N-1)/ (L-1) factors, each with L levels with N experimental trials. The usefulness of the design lies in the fact that in determining the effects of one variable, the net effects of changing other variables cancel out so that the effect of each variable on the system can be independently determined. The aim of the investigation was to study the application of PBD to assess the relative importance of process variables such as moisture, temperature, methanol concentration, fermentation time, age of spore, initial pH and sugar, inoculum size, steam time and type of solvent.

Material and Methods

A 1.2 liter capacity fermentor equipped with standard control and instrumentation as shown in figure1 was used for the citric acid fermentation. The 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. The power was switched on. The experimental conditions shown in table 1 were monitored. The Samples were collected from the fermentor and analyzed for citric acid production.

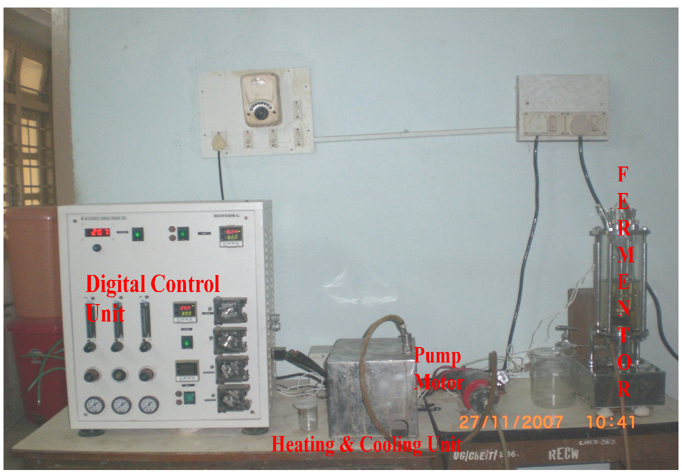

Figure-1
Experimental setup

Table-1
Variables monitored in Citric acid fermentation from Sucrose

Variable	Low level (-)	High level (+)
Initial sucrose concentration (g/l)	80	240
Initial p^H	5.0	7.0
Stirrer speed (rpm)	170	310
Incubation time (days)	2	10
Fermentation temperature (^{0}C)	28	32
O_2 flow rate (lpm)	0.5	2.5
Moisture content (v/w %)	60	85
Particle size(mm)	0.4	1.0
Inoculum density (Spore/ml)	10000	1E+009
Methanol concentration (v/w %)	3	4
Spore age(days)	4	6

Results and Discussion

Plackett-Burman Designs: The first screening step was to identify the variables which have significant effects on citric acid production by *A. niger*. In order to maximize *A. niger* growth and citric acid production, the effective factors and their levels were selected based on literature review. The important criteria to choose each factor settings for any two-levels screening design have been mentioned elsewhere [Khosravi Darani, K. Vasheghani Farahani, E, 2003]. In the experiment, elven variables between their low and high concentrations were tested. Twelve runs were performed according to plackett burman design. All trials were performed in triplicate and citric acid yield is treated as response. The main effect of each variable was simply calculated as the differencce between the average of measurements made at the high setting(+) and the average of measurements observed at low setting(-) of that factor. Plackett burman experimental design is based on the first order model.

$$Z= b_0+\sum b_i x_i$$

Where Z is the response (citric acid yield), b_0 is the model intercept and b_i is the linear coefficient and x_i is the level of the independent variable.The trial results by PBD are shown table 2.

Identification of contributing factors: Eleven factors were studied in this design for the increment of citric acid production. As it can be observed from table, the production of citric acid varies from 35 to 57 g/l. The main effects of factors on citric acid yield from the Plackett-Burman experimental results are shown in Pareto chart in figure 2. From figure 2, it was observed that Initial sucrose concentration, Initial p^H, Stirrer speed, Incubation time, Fermentation temperature and O_2 flow rate gave the positive effect in contributing to the higher citric acid yield. The highest effect would be from initial sucrose concentration and pH most likely. It is observed that higher concentration of sucrose would result in higher yield of citric acid.

Conclusion

Among the variables, Initial sucrose concentration, Initial p^H, Stirrer speed, Incubation time, Fermentation temperature and O_2 flow rate were found to be the most significant variables affecting citric acid yields from sucrose. However the response to any variable depends on the selected range for it. If the difference between two levels of each variable is not large enough to ensure a measurable response, sometimes dummy or null variables may occur in PBD or other screening designs,. Some sensitive variables on the other hand may have their high and low levels chosen such that the size of their differential response is so great as to mask the effect of other variables. More accurate quantitative analysis of the effect of these variables for citric acid production is required as PBD is typically used as a preliminary optimization technique. Further studies have been planned based on the use of different strategies such as central composite design to study the effect of screened variables.

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Table-2
Twelve trial PBD to study eleven factors in Citric acid fermentation from Sucrose

Run	Factor 1 A:Sucrose, g/l	Factor 2 B:P^H	Factor 3 C:Speed,rpm	Factor 4 D:Time day	Factor 5 E:Temp ^{0}C	Factor 6 F:O_2 flow rate, lpm	Factor 7 G:Moisture, v/w%	Factor 8 H:Particle size, mm	Factor 9 J:Inoculum d, Spores/ml	Factor 10 K:Methanol, v/w%	Factor 11 L; Spore age, day	Response R1, g/l
1	80	7.0	310	2	32	2.5	85.0	0.4	10000.0	3.0	6.0	35
2	80.00	5.00	170.00	2.00	28.00	0.50	60.00	0.40	10000.0	3.00	4.00	25
3	240.00	5.00	310.00	10.00	32.00	0.50	60.00	0.40	10^9.00	3.00	6.00	61
4	80.00	5.00	170.00	10.00	28.00	2.50	85.00	0.40	10^9.00	4.00	6.00	38
5	240.00	7.00	170.00	2.00	28.00	2.50	60.00	1.00	10^9.00	3.00	6.00	40
6	80.00	7.00	170.00	10.00	32.00	0.50	85.00	1.00	10^9.00	3.00	4.00	43
7	240.00	5.00	310.00	10.00	28.00	2.50	85.00	1.00	10000.00	3.00	4.00	68
8	240.00	5.00	170.00	2.00	32.00	0.50	85.00	1.00	10000.00	4.00	6.00	39
9	80.00	7.00	310.00	10.00	28.00	0.50	60.00	1.00	10000.00	4.00	6.00	45
10	80.00	5.00	310.00	2.00	32.00	2.50	60.00	1.00	10^9.00	4.00	4.00	38
11	240.00	7.00	310.00	2.00	28.00	0.50	85.00	0.40	10^9.00	4.00	4.00	42
12	240.00	7.00	170.00	10.00	32.00	2.50	60.00	0.40	10000.00	4.00	4.00	57

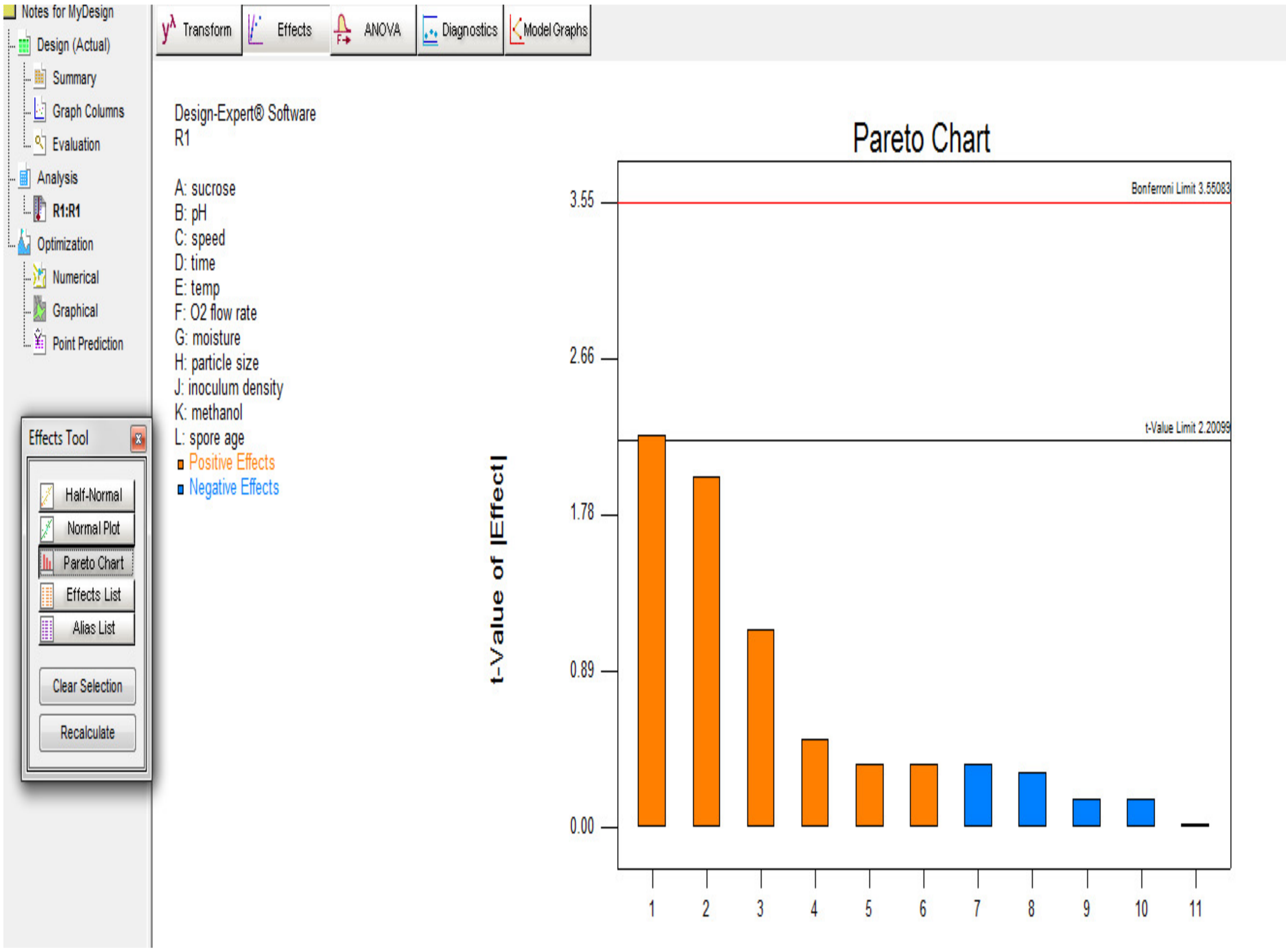

Figure-2
Effects of factors on citric acid production according to plackett burman design experimental results