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SELECTION OF MEDIA COMPONENTS FOR OPTIMIZATION IN THE SYNTHESIS OF ITACONICACID BY PLAKETTE –BURMANN DESIGN

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ABSTRACT:

The study of the screening of media components in the formation of Itaconic acid by shake flask fermentation from Glucose by *Ustlagomaydis* is reported from the Plakette- Burmann Design (P-BD). From the Design,it is found that the nutrients $(NH_4)_2SO_4$,$CaCl_2$, $MnSO_4$ H_2O and yeast extract apart from the sustrate Glucose have significant effect on the formation of Itaconic acid out of the total of 11 media componenets considered in the work . The maximum Itaconicacid formation obtained is 97.14g/L It is proposed further, to optimize these five media components by RSM .

Keywords: *Itaconic acid, Fermentation, Ustilagomaydis,Media components,Glucose, Pakette-Burmann design*

[I]INTRODUCTION

Itaconic acid is a promising organic acid. It is a white crystalline unsaturated di-carbonicacid in which one carboxyl group is conjugated to the methylene group. IA is used worldwide in the industrial synthesis of resins such as polyesters, plastics, and artificial glass and in the preparation of bioactive compounds in the agriculture, pharmacy, and medicine sectors coatings, and other industrial products, it is stable at acidic, neutral and middle basic conditions at moderate temperatures.

Many factors (physical and chemical) which were observed to have an effect on the *Ustilagomaydis* ability to produce the Itaconic acid such as temperature, pH, inoculums size, rate of mixing, aeration, moisture level, carbon source, nitrogen source and mineral salts in the media have been determined. Considering the chemical factors such as nitrogen source and mineral salts which they have strong effects on the rate of accumulation of Itaconicacid through the fermentation. Many researchers are encouraged to study the effect of these additives on the Itaconic acid production. The work has shown that the nitrogen and potassium concentrations should be limited. The excess of these two components would enhance the cell to consume the carbon source towards energy and biomass instead of other metabolic products [1]. Tran *et al.*found through their study the effects of metal components [2].

In this present work ability of *Ustilagomaydis.(*NCIM-983) to produce itaconic acid from glucose. Itaconic acid is now produced commercially by culture *Ustilagomaydis.* These are basidiomycetes and are believed to be closely related to *Pseudozyma* aantarctica[3]. *Ustilago maydis* appears to be a candidate for an alternative producer of Itaconicacid. This basidiomycetous fungus, that exhibits free-living yeast-like nonpathogenic and filamentous pathogenic forms, may produce high amounts of ITA up to 97.14 g/L under certain cultivation conditions with unlimited conditions cells from Glucose by Plakette

Burmann method [4]. The pH is held constant at about 3 cells stop growth and start ITA production.

[II] MATERIALS AND METHODS

Microorganism:

The fungal strain of *Ustilagomaydis* (NCIM no - 983) Culture is procured From NCL Pune. *Ustilagomaydis* is grown and cultured in PDA.(subculture medium).

2.1. Potato dextrose agar(PDA) medium

Two hundred grams of peeled potatoes are cut into small pieces and suspended in 1000 ml of distilled water and steamed for 30 min. Decant the extract or filter through maselin cloth and made the final volume to 1000 ml. Added 20 g of Dextrose, 0.1 g of yeast extract and 20.0 g of agar. And adjusted at certain pH-6 and sterilization was done in Autoclave at 15 Psia 121^0 C for 20 min.

2.2. Production medium and Effect of Parameters being studied

Glucose,$(NH_4)_2SO4$,KH_2PO_4,Na_2HPO_4,$MgSO_4 7H_2O$,$CaCl_2$,$ZnSO_4 7H_2O$,$FeSO_4 7H_2O$, $MnSO_4 H_2O$,Thiaminehydrochloride,Yeast extractusing different percentage component in the production medium with changing the concentrations. ions (Fe, Zn, Mn, Cu, and Mg) on the production of Itaconic acid [1]. This paper reported the screening of the media components to improve the Itaconicacid production. Statistical analysis through Plackett-Burman Design is used for screening the media components.

2.3. Analytical assay:

The sample is collected at an interval of 24 hours (Walinky, 1984).The collected sample is used for the determination of Itaconic acid and qualitative analysis of Itaconic acid is measured by UV visible spectrophotometer at 210 nm.The Itaconicacid sample is determined by UV Visible spectroscopy at 210 nm by the by Miller [5].

2.4. Experimental Design:

The Plackett-Burman design is used for screening of the factors (media components) that Significantly influenced Itaconicacid production. The design considers the main effect of these variables but not their interaction effects [6]. It can represent by first-order polynomial equation. Where

Y represents the response,$\beta_\circ$ is the model coefficient,β_i is the liner coefficient, x_i is the Variables, and *n* is the number of parameters (variables. Each variable is represented in two levels, i.e. high (+) and low (-).

The effect of each variable is determined by the following equation

$$Y = \beta_o + \sum^{n}_{i=1}\beta_i x_i \quad \text{..... (1)}$$

Where *Y* represents the response$_\circ$ is the model coefficient,β_i is the liner coefficient, x_i is the Variables, and*n* is the number of parameters (variables). Each variableis represented in two levels, i.e. high (+) and low (-). The effect of each variable is determined by the following equation

$$E_{(xi)} = (\sum_{mi+} - \sum_{mi-}) /N \quad \text{....(2)}$$

Where $E_{(xi)}$ tested variable; is the response value effect of the tested variable . $\sum_{mi+}$ is the summation of the response value at high level, $\sum_{mi+}$ is the summation of the response value at low level, and *N* is the number of experiments. Table 3 represented the selected variables to be evaluated, whereas, Table 4 showed the design matrix; eleven assigned variables are screened in the 12 experimental runs. The Itaconicacid, The factors significant at 90% level (*p*-value 0.336) are considered reliable [7]. The statistical analyses are performed by use of multiple regression and ANOVA with the Minitab V.15 so.

[III] RESULTS

3.1.Plakette Burmann design:

Response1R ANOVA for selected factorial model

Table 1 -Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	means quare	F-value	p-value prob>F
Model 12.73	5	2.55	58.13	<0.0001	Significant
A-Glucose	*12.40*	1	12.40	282.96	<0.0001
B-(NH4)SO4	*0.20*	1	0.20	4.57	0.0764
F-CaCl2	0.18	1	0.18	4.18	0.0869
J-MnSO47H2O	0.099	1	0.099	2.26	0.1834
L-Yeast extract	0.28	1	0.28	6.42	0.0445
Residual	0.26	6	0.044		
Cor Total	13.00	11			

Table 2-Standard error and Coefficient estimate

Factor	Coefficimet Estimate	df	Standard Error	Low	95%cl High	95% VIF
Intercept	8.10	1	0.063	7.95	8.25	
A-Glucose	1.05	1	0.063	0.90	1.21	1.07
B-(NH4)SO4	0.13	1	0.063	-0.019	0.29	1.07
F-CaCl2	0.13	1	0.063	-0.025	0.28	1.07
J-MnSO47H2O	0.097	1	0.065	-0.061	0.26	1.11
L-Yeast extract	0.16	1	0.065	5.554E-003	0.32	1.11

The "Pred R-Squared" of 0.9045 is in reasonable agreement with the "Adj R-Squared" of 0.9629. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 18.234 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

R1 = +8.10+1.05 * A+0.1 * B+0.1 * F+0.09 * J+0.16 * L

Final Equation in Terms of Actual Factors:

R1 =-1.87087 +0.7514* Glucose+13.3702 *(NH4)SO4+255.73810 CaCl2+194.21429* MnSO47H2O+16.36071* Yeast extract

Production medium of Itaconic acid is carried out in a medium with the followingcomposition(g/100ml);

Table 3: The table showed the levels, actual values of the factors tested in Plackett-Burman design

Run	Glucose	$(NH_4)_2SO_4$	KH_2PO_4	Na_2HPO_4	$MgSO_47H_2O$	$CaCl_2$	$ZnSO_47H_2O$	$FeSO_47H_2O$	$MnSO_4H_2O$	THC	Yeast extract
1	10.8	0.07	0.24	1.68	0.20	0.003	0.003	0.07	0.003	0.00084	0.07
2	8	0.07	0.17	1.68	0.20	0.003	0.002	0.05	0.002	0.00084	0.05
3	8	0.05	0.24	1.20	0.20	0.003	0.003	0.05	0.002	0.0006	0.07
4	10.8	0.05	0.17	1.68	0.14	0.003	0.003	0.07	0.002	0.0006	0.05
5	8	0.07	0.17	1.20	0.20	0.002	0.003	0.07	0.003	0.0006	0.05
6	8	0.05	0.24	1.20	0.14	0.003	0.002	0.07	0.003	0.00084	0.05
7	8	0.05	0.17	1.68	0.14	0.002	0.003	0.05	0.003	0.00084	0.07
8	10.8	0.05	0.17	1.20	0.20	0.002	0.002	0.07	0.002	0.00084	0.07
9	10.8	0.07	0.17	1.20	0.14	0.002	0.002	0.05	0.002	0.00084	0.05
10	10.8	0.07	0.24	1.20	0.14	0.003	0.003	0.05	0.002	0.00084	0.05
11	8	0.07	0.24	1.68	0.14	0.002	0.002	0.07	0.002	0.0006	0.07
12	10.8	0.05	0.24	1.68	0.20	0.002	0.002	0.05	0.003	0.0006	0.05

Table 4: Twelve trial Plackett-Burman Design with the responses

Run	Glucose	$(NH_4)_2SO_4$	KH_2PO_4	Na_2HPO_4	$MgSO_47H_2O$	$CaCl_2$	$ZnSO_47H_2O$	$FeSO_47H_2O$	$MnSO_4H_2O$	THC	Yeaste xtract	Resnse (g/100ml)
1	+	+	+	+	+	+	+	+	+	+	+	9.714
2	-	+	-	+	+	+	-	-	-	+	-	6.88
3	-	-	+	-	+	+	+	-	-	-	+	7.201
4	+	-	-	+	-	+	+	+	-	-	-	8.96
5	-	+	-	-	+	-	+	+	+	-	-	6.896
6	-	-	+	-	-	+	-	+	+	+	-	7.06
7	-	-	-	+	-	-	+	-	+	+	+	6.86
8	+	-	-	-	+	-	-	+	-	+	+	8.74
9	+	+	-	-	-	-	-	_	-	+	-	8.96
10	+	+	+	-	-	+	+	-	-	+	-	9.04
11	-	+	+	+	-	-	-	+	-	-	+	7.4
12	+	+	+	+	+	-	-	-	+	-	-	8.986

[IV] DISCUSSION

4.1. Conversion of Glucose

The fermentation of Glucose to Itaconicacid was shown in Fig. 1, which shown the concentration of Itaconic acid with time. The fungus consuming almost all the nutrients in 5 days (90%) convert it to Itaconic acid by using *Ustilagomaydis* that able to convert the Glucose to Itaconicacid.. Itaconicacid rather than to grow and produce biomass. The concentration of Itaconicacid appear to rise after the second day (g/L) and keep rising till it reach to a constant value in the 6^{th} day (g/L), this value kept constant for 6 days then the concentration start to decrease because of oxidize of Itaconicacid, that mean the maximum concentration for Itaconicacid is reached in the 6^{th} day and no need to make the fermentation time more than 5 days. As it shown in Fig. 1, *Ustilagomaydis* is able to consume 89% of the Glucose that used during the fermentation time. Conversion of Itaconic acid is 90% from Glucose.

4.2. Screening of Media Components:

The nutrients concentrations have shown an important role in the fermentation of Itaconicacid from Glucose. The significant parameter (nutrients) is chosen due to their effect on the final concentration of Itaconicacid, effect different component concentrations. The Plakett-Burman Design is used for screening the parameters (nutrients) to find the significant one. The *p*-value is considered as a tool for evaluating the significance of each of the coefficients. The parameters with confidence levels greater than 95% are considered as influencing the response significantly.

Table 1 showed the ANOVA for the three responses. It is obviously seen that $(NH_4)_2SO_4$ 3 is the most significant substrate due to the low *p*-value in all the responses where it noted (Itaconicacid) response, respectively. $(NH_4)_2SO_4$ is observed to effect the Itaconicacid fermentation indirectly through the effect on the final concentration of glucose response where its *p*-value is, Yeast extract,$MnSO_4H_2O$,also a significant nutrients and $CaCl_2$ where it effect on the final concentration of Itaconicacid response which its *p*-value is the main effect of each response is evaluated according to Eq. 2, as the difference between both averages of measurement made at the high level (+1) and the low level (-1) of that factor.

A large contrast coefficient either positive or negative indicates that a factor has a large impact on fermentation; while a coefficient close to zero means that a factor has little or no effect. It is another indicator to confirm the chosen of the significant nutrients which is selected according to the *p*-value. As it seen in table3 all the insignificant parameters are close to zero. For the model can be check by the determination of R^2(regression coefficient) which it provides a measure of how much variability in the observed response values can be explained by the experimental factors, the closer R^2 to 1, the better the model predicts the response[8,9].The following first-order polynomial equation for Itaconicacid concentration: R1 = +8.10+1.05 * A+0.1 * B+0.1 * F+0.09 * J+0.16 * L Here the R^2value was 90.45%, which indicating that 90.45% of the variability in the response could be explained by the model, and it indicates an acceptable agreement between experimental and predicted values and implies that the mathematical model is very reliable for Itaconic acid production in the present study. The model R^2 (96.29%)

For consuming reduces glucose and 92.32% for reducing in suggested that the fitted liner models could explain 96.29% and 90.45% of the total variation. Nitrogen plays a major role in the metabolism of Itaconic acid. The cell needs nitrogen in form of ammonium to build up cell substances. On the other side too much nitrogen inhibits the production of Itaconic acid due to it will enhance the cell to grow and produce

biomass [10]. Manganese and Calcium in ($(NH_4)_2SO_4$) considered also a growth enhanced nutrient so it must be limited to enhance the cell to produce Itaconic acid rather than growth and produce biomass. Effects the rate of Glucose utilization by the cell that relates to biomass rise [11].

[V] CONCLUSION)

Five components Glucose substrate, $(NH,)_2$ SO4,, $CaCl_2$, $MnSO_4$ H_2O, and Yeast extraxt are significant for Itaconicacid production. The maximum Itaconicacid concentration obtained is 97.14g/L by Plakette-Burmann design.

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REFERENCES

[1] Dawson M.W., Madox I.S. and Brooks J.D.,Evidence for nitrogen catabolite repression during citric acid production by Aspergillus niger underphosphatelimitedgrowth conditions, Biotechnol. Bioeng., 1989, 33, 1500–1504.

[2] Tran C.T., Sly L.I. and Mitchell D.A., Selection of a strain of Aspergillus for the production of citric acid from pineapple waste in solid-state fermentation, World Journal of Microbiology &Biotechnology, 1998, 14, 399–404.

[3] WilliaE.Levinson,CletusP.Kurtzman,TsungMinK "Production of itaconicacid byPseudozym aantarctica Enzymeand Microbial echnology39(2006):824–827&Biotechnology, 1998, 14, 399–404.

[4]Walinky SW(1984) US-Patent4 485223(to Pfizer)Meth)acrylicacid/itaconicacid biosynthesis by *Aspergillus terreus.*

[5] Miller G.L., Use of dinitrosalycilic acid reagent for determination of reducingsugars, Anal Chem, 1958, 31, 426–429.

[6] Plackett R.L. and Burman J.P., The design of optimum multifactorial experiments, Biometrica,1946, 33, 305–325.

[7] Montgomery D.C., Design and Analysis of Experiment, John Wiley and Sons, England, 2005.

[8]Aghaiea E., Pazoukib M., Hosseinia M.R.,Ranjbara, M. and Ghavipanjehb F., Response surface methodology (RSM) analysis of organic acid production for Kaolin beneficiation by Aspergillus niger, Chemical Engineering Journal,2009, 147, 245–251.

[9] Alam M.Z., Jamal P. and Nadzir M.M., Bioconversion of palm oil mill effluent for citric acid production: statistical optimization of fermentation media and time by central composite design, World J Microbiol Biotechnol,2008, 24, 1177–1185.

[10]Wieczorek S. and Brauer, H., Continuous production of citric acid with recirculation of the fermentation broth after product recovery, Bioprocess Engineering, 1998, 18, 1–5.

[11] SHU P. and Jonhnson M.J., The interdependence of medium constituents in citric acid production by submerged fermentation, J. of bacteriology, 1948, 56, 577–585.

Fig: 1. Effect of componets on production of Itaconicacid: Concentration of itaconicacid is changed due to different reactants concentrations and five component are selected by Plakette –burmann design.

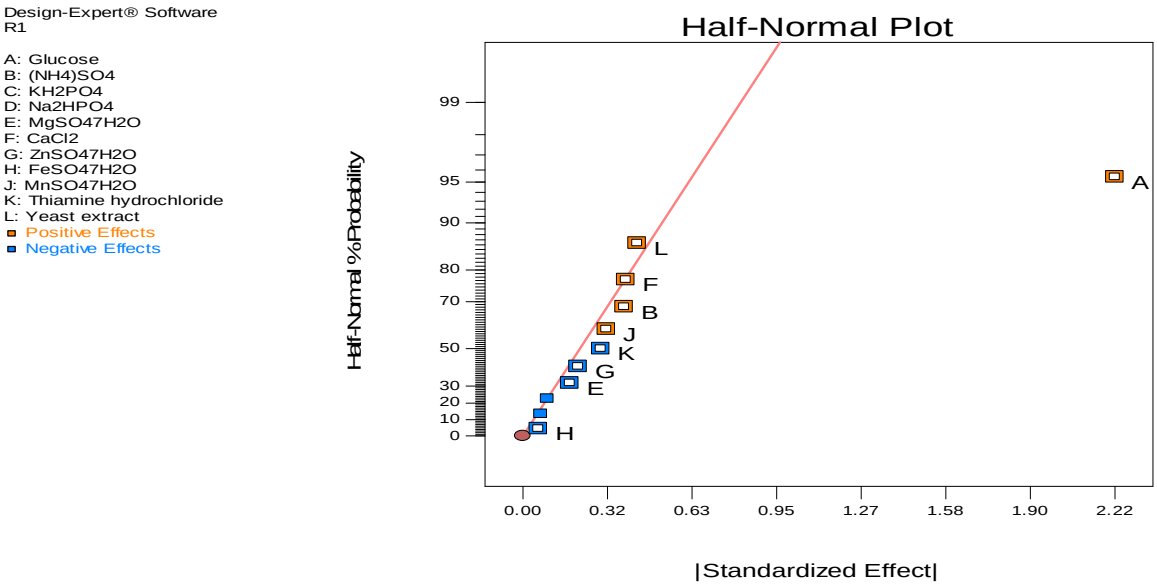

Fig: 2. Effect of componets on production of Itaconicacid: Concentration of itaconicacid is changed due to different reactants concentrations and five component are selected by Plakette –burmann design.
Positive effect of components are selective components from reactant componets.

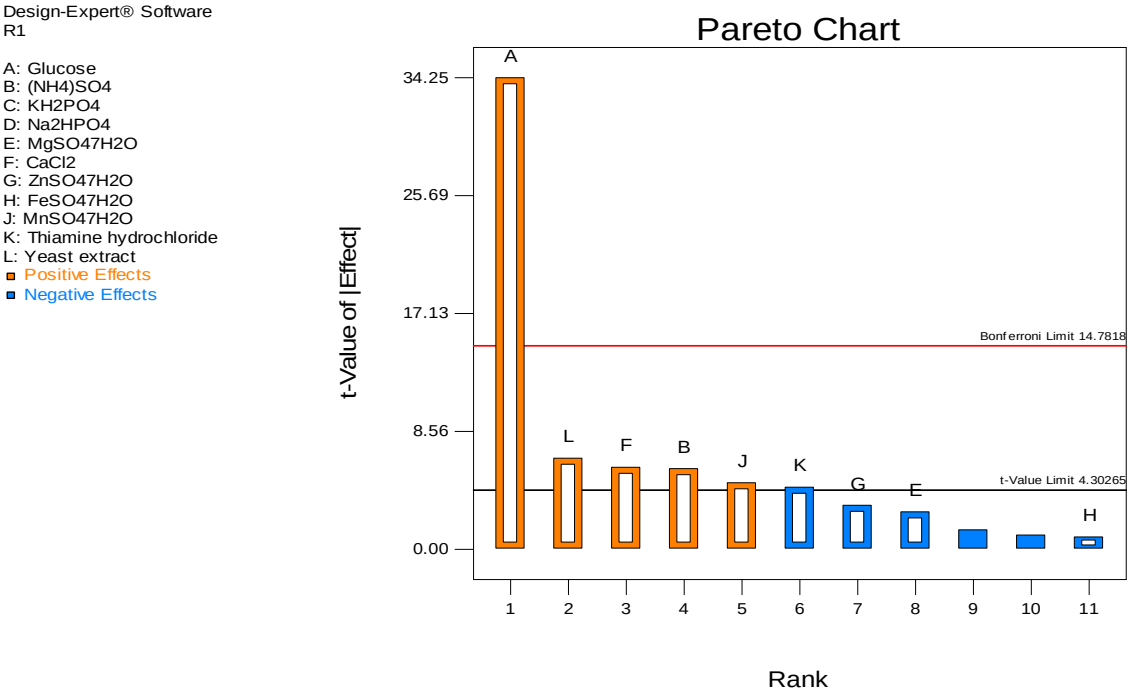

Fig: 3. Effect of componets on production of Itaconicacid: Concentration of itaconicacid is changed due to different reactants concentration and standard deviation of error is determined.

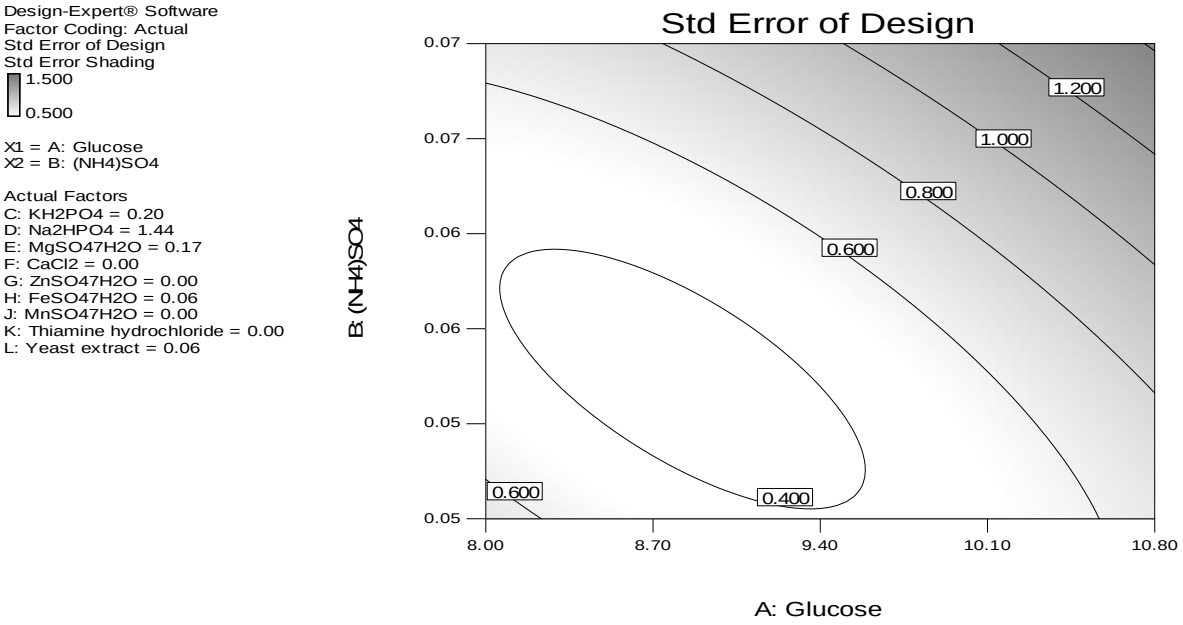

Fig: 4. Effect of componets on production of Itaconicacid: 3D –surface is created by Plakette-burmann design are glucose and $(NH_4)_2SO_4$ is oberverd and they are showing major effect on Itaconic acid production.