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Short Communication

Optimization of sugar utilization by *Azotobacter chroococcum* in large scale fermentation for biomass production using response surface methodology

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ABSTRACT

A statistical model was developed in this study to describe biomass production through a fermentation process of *Azotobacter chroococcum*. Response surface methodology (RSM) based on central composite CCD was employed to statistically evaluate and optimize the conditions for maximum biomass production and study the significance and interaction of Sucrose, K_2HPO_4 and $FeSO_4$ concentration of Jensson's media on production of *Azotobacter chroococcum* biomass. In the current study *A* biomass optimize up to 13.4 g sucrose from 20g, K_2HPO_4 1.05g and $FeSO_4$ 0.14g which give same biomass of *A. chroococuum*. Thereby, sucrose cons different 6.6g in one liter, while compare with economic production cost at prize different Rs 4.3.00 for one liter. Also, for the production in e 300 liter fermenter Sucrose prize different was Rs 1292.00 per batch. Thereby, study has significantly optimize for quality purpose as well as prove to be economic.

1. Introduction

Azotobacter chroococum is a potent N_2 fixer broad spectrum inoculants useful for various leguminous crops such as pigeonpea (Patel *et al*., 2020),[1] mungbean (Yadav *et al*., 2019),[2] chickpea (Wani *et al*., 2007)[3] etc. and found to increase the percent of seed germination (Singh *et al*., 2014).[4] Jensen's medium is widely reported for the growth of nitrogen-fixing organisms are free-living bacteria. (Li *et al*., 2015).[5] Successful pipeline of fermentation project with industrial fermentation economics almost 30% dependence on their media and their associated ingredient cost. (Islam and Hossain., 2012).[6] Thereby, optimizing media and other associated fermentation parameter will lead us to durable sustenance for viable fermentation economics and efficient use of media resources. Previously, media optimization was successfully carried out for various bio fertilizers (Berruti *et al*., 2016).[7] However, systematic study for optimizing of media is lacking with *Azotobacter chroococum* for its applications at commercial level for biomass production. Additionally, optimization of Jenesen's medium is one of the prerequisite for its commercial exploitation of *A. chroococum.* Thereby, recent research efforts have focused on further process development or optimization and scale-up of *A. chroococum* biomass production for large scale multiplication and flourish viable growth of bio-fertilizer.

In such case, response surface methodology is a powerful and proficient mathematical approach widely applied in the optimization of fermentation process, e.g. media components on various bio-fertilizer production (Latha *et al*., 2017),[8] and production of other metabolites (Chuprom *et al*., 2016).[9]

In tabulated axial design of CCD in export design. The yield of *A. chroococcum* were determined for each set of

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Table 1: Level of the variable tested in central composite design

Variable	Unit	-1.682	-1	+1	1.682
Surose (X_1)	S	3.18207	10	30	36.8179
K_2HPO_4 (X_2)	K	0.494328	0.75	1.5	1.75567
$FeSO_4$ (X_3)	F	0.0494328	0.075	0.15	0.175567

Table 2: Experimental design matrix and results of central composite design

S. No	Type	Sucrose (X_1)	K_2HPO_4 (X_2)	$FeSO_4$ (X_3)
1	Fact	-1	-1	-1
2	Fact	1	-1	-1
3	Fact	-1	1	-1
4	Fact	1	1	-1
5	Fact	-1	-1	1
6	Fact	1	-1	1
7	Fact	-1	1	1
8	Fact	1	1	1
9	Axial	-1.68179	0	0
10	Axial	1.681793	0	0
11	Axial	0	-1.68179	0
12	Axial	0	1.681793	0
13	Axial	0	0	-1.68179
14	Axial	0	0	1.681793
15	Center	0	0	0
16	Center	0	0	0
17	Center	0	0	0
18	Center	0	0	0
19	Center	0	0	0
20	Center	0	0	0

Table 3: Analysis of variance for fitted quadric polynomial model

Source	Sum of Squares	DF	R^2 Mean squire	F Value	p-value Prob > F
Model	0.017556	9	0.001951	1.579702	0.2430
X_1-Sucrose	0.000531	1	0.000531	0.430252	0.5267
X_2- K_2HPO_4	0.000147	1	0.000147	0.118858	0.7374
X_3- $FeSO_4$	0.003215	1	0.003215	2.603741	0.1377
X_1X_2	0.000181	1	0.000181	0.146173	0.7102
X_1X_3	0.00045	1	0.00045	0.364419	0.5595
X_2X_3	0.004141	1	0.004141	3.353062	0.0970
X_1^2	1.04E-05	1	1.04E-05	0.008453	0.9286
X_2^2	0.007825	1	0.007825	6.33676	0.0305
X_3^2	0.000558	1	0.000558	0.451515	0.5168

Std. Dev. Mean and C.V. analysis

Table 4: Std. Dev. Mean and C.V. analysis

Std. Dev.	0.03514	R-Squared	0.587072
Mean	0.21035	Adj R-Squared	0.215437
C.V. %	16.70564	Pred R-Squared	-2.20503
PRESS	0.095845	Adeq Precision	4.620833

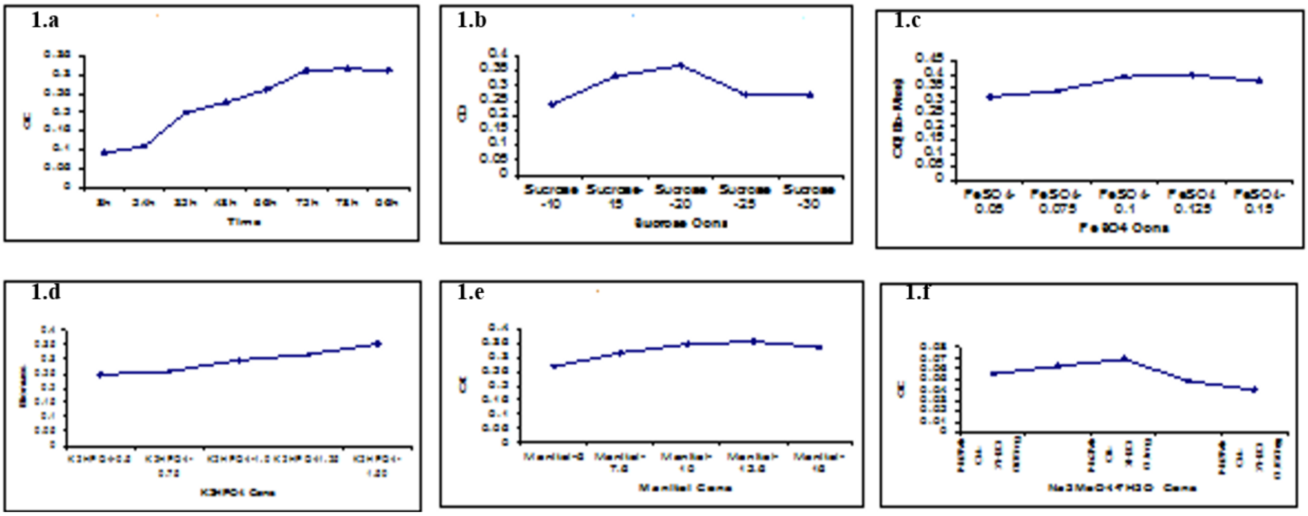

Fig. 1: Growth curve and association analysis; **a:** Explained the association between biomass (OD) or period of time; **b:** Corellation of sucrose cons and biomass (OD; **c:** Correlation of FeSo4. cons and biomass (OD); **d:** Corellation of K2HPO4 and biomass (OD); **e:** Corellation of manitol cons and biomass (OD); **f:** Corellation of Na2Mo4-7H2O cons and biomass (OD)

experiments which were shown in Figure 2, among sets no 11 give high biomass and microscopy count (Figure 2 a). However, selected six sets form CCD were performed and among these sets no 1,11,12,16,17,18 give the high yields of *A. chroococuum* (Figure 2) there, these six sets were analysis in 3 liter Jar Fermenter, which mansion as 1,2,3,4,5,6 the high yields biomass of *A. chroococuum* in sets no 4 and 6 higher (Figure 3) in which amount of Sucrose 13.4 g, K_2HPO_4 1.05 gm and $FeSO_4$ 0.14 g.

In present study, optimization of fermentation media of *A. chroococuum* was investigated using RSM to increase the biomass production, based on optimal medium, scale-up was carried out in a 3-L jar fermenter.

The strains of *A. chroococcum* used in the present study collected from bio-fertilizer production unit Cordet, IFFCO, Kalol, Gujarat, India. Medium mainly used for *A. chroococuum* optimization is Jenson' medium. A loopful strain of *A. chroococcum* from the slant were transferred into a 250-ml conical flask and incubated for 24 h on a rotary shaker operating at 200 rpm at 30ºC. It was then inoculated into 3-L jar fermenter (Biostat M.B. Braun Co., Germany) in shake flask culture, fermenter culture, it was incubated at 30ºC, the agitation speed was controlled at 400 rpm.

After 12, 24, 36, 48, 60, 74 h, fermentation broth was collected and cells were removed by centrifugation at 12000 rpm for 10 min; the supernatant was collected and further diluted by a factor of 10 with Mili-Q water, this diluents sample was checked for microscopic and spectrophotometer analysis.

Central Composite design (CCD) is one of the response surface methodologies (Ghelich *et al*., 2019)[10] for identification of the components affecting the biomass production of *A. chroococcum* yield significantly, a CCD was adopted to optimize the major variables (Sucrose, K_2HPO_4 and $FeSO_4$) (Table 1). A 2^3 - factorial CCD, with six axial points (a= 1.682) and six replications at the centre points (n_0=6) leading to a total number of 20 experiments was employed (Table 2) for the optimization of the three chosen medium variables. *A. chroococcum* yield was used as the dependent output variable.

The experiments were performed in duplicate with the mean values taken for analysis. Design Expert trial Software was used for multiple regression analysis of the experimental data obtained. *F*-test was employed to evaluate the statistical significance of the quadric polynomial, and multiple coefficients of correlation R and the determination coefficient of correlation R_2were calculated to evaluate the performance of the regression equation.

In the growth curve increase time interval growth of *A. chroococcum* biomass increase up to 72 hour after time interval it can achieve stationary phage (Figure 1 a). The different concentration of Sucrose, $FeSO_4$, K_2HPO_4, Manitol, $Na_2MoO_4.7H_2O$, where check in which 15 gm sucrose give high biomass of the *A. chroococcum* (Figure 1 b) while incase of the K_2HPO_4, if we increase K_2HPO_4, cons biomass *of A. chroococcum* also increase (Figure 1 d) and in case of$FeSO_4$ ManitolandNa$_2$MoO$_{4-}$7H$_2$O biomass of *A. choococcum* is increase up to certain level as in $FeSO_4$ up to 0.1 gm Manitol up to 12.5 gm and $Na_2MoO_4.7H_2O$ is up to 0.1 mg, (Figure 1 .c, 1.e, 1.f) and above this level biomass of *A. choococcum* decrease.

The model's goodness of fit was checked by determination coefficient (R^2), in this case, the value of the determination coefficient (*R*=0.59) (Table 4) indicated that only 16.70% of the total variations were not explained by the model. The value of the adjusted

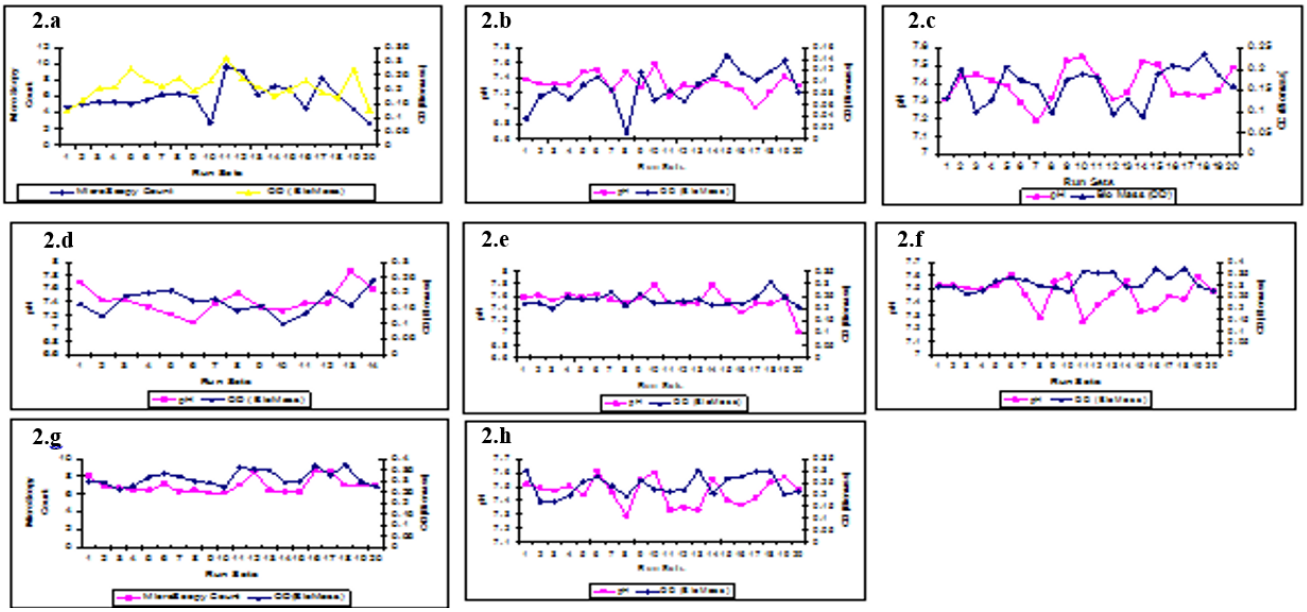

Fig. 2: Central composite design experimental set analysis; **a:** Central composite design set analysis for microscopy count and biomass (OD); **b:** Central composite design set analysis at 12 h for pH and biomass (OD); **c**: Central composite design set analysis at 36 h for pH and biomass (OD); **d:** Central composite design set analysis at 44 h for pH and biomass (OD); **e:** Central composite design set analysis at 56 h for pH and biomass; **f:** Central composite design set analysis at 64 h for pH and biomass (OD); **g:** Central composite design set analysis at 64 h for microscopy count and biomass (OD); **h:** Central composite design set analysis at 76 h for pH and biomass(OD)

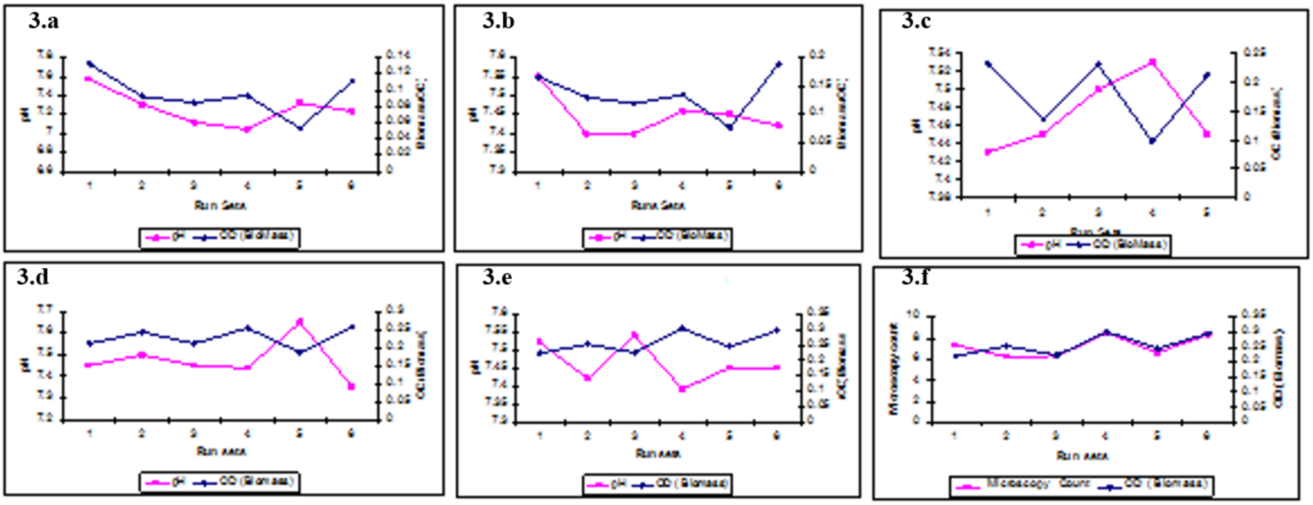

Fig. 3: Explanation of selected six set from central composite design; **a:** Selected set analysis at 12h for biomass and pH; **b:** Selected set analysis at 24h for biomass and pH; **c:** Selected set analysis at 48h for biomass and pH; **d:** Selected set analysis at 56h for biomass and pHc- Selected set analysis at 48h for biomass and pH; **e:** Selected set analysis at 72h for biomass and pH; **f:** Selected set analysis at 72h for microscopy count and biomass

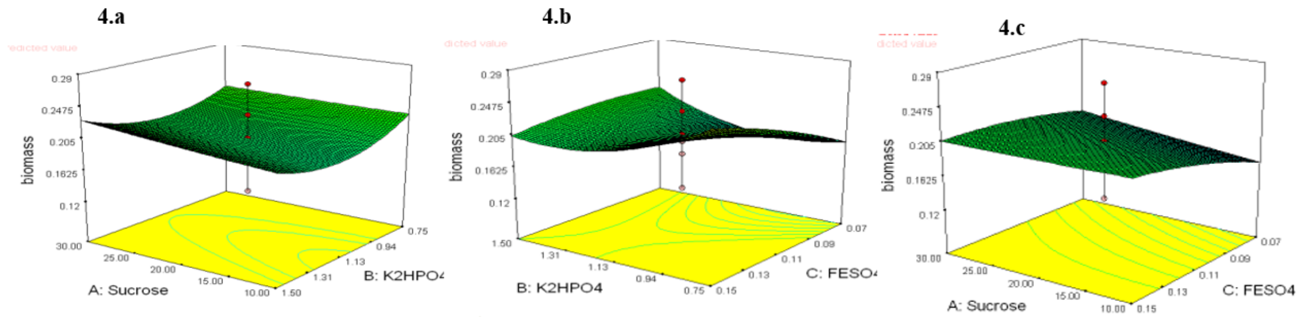

Fig. 4: a: 3-D curve analysis for sucrose, K2HPO4 and FeSo4; **b:** 3-D curve analysis for FeSo4 and K2HPO4 for biomass production; **c:** 3-D curve analysis for sucrose and FeSo4 for biomass production

determination coefficient [Adj(R2)= 0.22] (Table 4) was also very high in supporting the high significance of the model. Among the model terms, X_1, X_2, X_3 and X were significant with a probability of 99%; X_2X_1 were significant with a probability of 95% (Table 3). The fitted response for the above regression model was plotted in (Figure 4) 3D graphs were generated for the pair-wise combination of the three factors while keeping the other one at its optimum levels for *A. chroococcum* production graphs are given here to highlight the roles played by various factors and also to emphasize the roles played by the physical constraints vis-à-vis the biosynthetic aspect in the final yield of the *A. chroococcum*.

2. Conflict of Interest

None.

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