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Kinetic study of growth for high biomass production using Chlorella Pyrenoidosa

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ABSTRACT

In this paper, biomass production parameter optimization was performed using Chlorella medium by design of experiment (DOE) with the help of Qualitek-4 software with bigger is better as quality characteristics with four media components and they are studied at three levels in submerged culture condition. I have studied four parameters like pH, Na_2CO_3, NH_4Cl, and $NaNO_3$ with three levels. These factors are optimized based on their S/N ratios are obtained from Qualitek-4 software and their significant individual interactions, and interactions with each other have been studied. During experimental studies, I had worked on the optimization of several parameters. It has found that there was an enhancement of chlorella pyrenoids by 6% in chlorella medium.

Keywords— *DOE (Design of Experiment), Chlorella Pyrenoidosa, Anova, S/N ratio, Taguchi methodology*

1. INTRODUCTION

There is an increasing demand for biofuel these days due to rapidly depleting fossil fuels. In this regards microalgae is important due to various reasons. Demibras, (2009), investigated that microalgae are quickly developing mammoths with an unquenchable longing for carbon dioxide. They can possibly deliver more oil per section of land than many feed stocks used in making biodiesel, and they can be developed ashore unacceptable sustenance crops. Ozkurt, (2009), studied that microalgae are unicellular photosynthetic microorganisms, living in saline or crisp water situations that change over daylight, water what's more, carbon dioxide to algal biomass. Algae show high potency in changing solar power to provide biodiesel than different crops. That is a result of Microalgae wants less space for cultivation just in case of indoors or outdoors system in comparative with crops. To provide a precise quantity of biodiesel in an indoor system Microalgae desires one thousand times water but crops. Chang et al (2007) examined that, there are two classes of algae: (1) Macroalgae (2) Microalgae. Macroalgae are the bloom (in inches or bigger than this inches) and they are multi cellular algae found in ponds. Microalgae are in smaller than Macroalgae. Microalgae are mostly grown in water bodies (size in micrometers).

Chisti (2007) examined that microalgae grow in ponds, or in PBR (photobioreactor). PBR's were distinctive sorts of tanks or shut frameworks in which green growth are developed. Open lake frameworks are shallow lakes in which green growth are developed. Supplements provided by spillover water by adjacent land zones or by directing from purified drainage water. Specialized organic impediments frameworks have offered to ascend the advancement of encased photoreactors. Microalgae development utilizing daylight vitality can be complete in open on the other hand secured lakes or shut photobioreactors, in light of tubular, level plate or different plans. A couple of open frameworks introduced for which especially dependable results are accessible. Microalgae generation in shut PBRs was profoundly costly. Shut frameworks are substantially more costly than lakes. Nevertheless, the shut frameworks require substantially less light and farming area to develop green growth. High oil types of microalgae refined in development streamlined states of photobioreactors can possibly yield (19,000–57,000 L) of microalgal oil per section of land and production of oil by microalgae was more than (200) times production.

Microalgae cells are kind of living cells and each cell contains equivalent internal organelles like chloroplasts, nuclei, and other parts. The biomass of algae was having by variety by different molecules like proteins and lipids algae contain lipid and protein which help in the production of diesel but some of the molecules, which did not help in the production of biodiesel.

Danielo et al, (2005), investigated that microalgae biomass made up of -1) sugars, 2), protein 3) Lipids. But lipids were the only help in the production of biodiesel in the form of TGA's. Currently, microalgae are the major focus of scientist to produce a higher amount of biodiesel. Chisti (2007) studied that microalgae are grown rapidly. It unremarkably twice every 24 hrs, whereas

some strains will reach as high as eightieth. Garcia et al. (2008) investigated that the F.A.'s converted to the tri-glyceraldehyde among Microalgae is each small and big chains of hydrocarbons. The smaller chain mainly used for the production of biodiesel.

Reijnders (2008) studied that the limitation of algal culture was costly and it required a high amount of energy in growing them. This was the main drawback of algal Biodiesel. The net cost of biodiesel production was higher than terrestrial. We are looking for the optimization of media, so it should grow rapidly at low cost.

2. MATERIAL AND METHODOLOGY

2.1 Media

We have used artificial media to grow Chlorella Pyrenoidosa recommended by NCIM, Pune.

Different media used for microalgae is AAF-6, Allen/6, MG, SOT, BG-11, CT, M11, MBM, P35Tre, TAP, MA etc. Nitrogen source is those which contain nitrogen like ammonium (NH^{4+}) and nitrate (NO^{3-}).The composition of chlorella medium recommended by NCIM, Pune as follows:

2.1.1 Composition of chlorella medium

Table 1: Composition of chlorella medium

$MgSO_4.7H_2O$	0.2g/L
K_2HPO_4	0.2g/L
Micronutrient (solution)	1.0 ml /L
$CaCl_2$	0.1 g /L
Fe-EDTA(Solution)	5.0 ml /L
Agar-Agar	12.0 g /L
Distill water	1000
pH	7.5

2.1.2 Fe-EDTA solution

Fe-EDTA has prepared by dissolving in boiling water, by taking 745mg /100mL of $FeSO4.7H_2O$. The solution has boiled again and final volume makes up 100 Ml.

2.1.3 Micronutrient solution

Table 2: Micronutrient solution

H_3BO_3	286.0 mg
$MnCl_2.4H_2O$	181.0 mg
$ZnSO_4$	22.0 mg
$NaMoO_4.2H_2O$	39.0 mg
$CuSO_4.5H_2O$	8.0 mg
Distilled water	100 ml

2.2 Algal culture

The algal culture has procured from NCIM, NCL Pune, 411008. Maharashtra, India, and this has been used for further study.

2.3 Inoculation preparation

Chlorella medium was prepared by using the above media content.100ml media in each Erlenmeyer flask of 250 ml from the stock of media. Then added 1ml algal culture to each flask with the help of Micropipette. Mixed well and note down the pH and OD of each flask for 10 days. Then the graph was plotted between Time and Absorbance for development of growth curve.

2.4 Reducing sugar test

(Gim G.H. et al., 2015) DNSA reagent. Prepared glucose concentration with different concentration from 1 mg to 10mg/ml. 500µL Chlorella Pyrenoidosa in each test-tube. Then DNSA solution (5 mL) as added in boiling water. Then the solution was cooled down and then add D.W. (8mL) and O.D. took at 540 nm. Performed the test twice.

2.5 Chlorophyll –a and Carotenoids Determination

(Thomas Z. and Maria A. 2015) Harvest 1ml of Chlorella Pyrenoidosa suspension culture. Centrifuged at 15000 g at laboratory temperature for 7 minutes and thoroughly discard the supernatant. Added 1ml methanol pre-cooled to +4ºC. Homogenized the sample by mixing by vortexing or by gentle mixing or gentle pipetting Covered the samples with aluminum foil incubate at 4ºC for 20 minutes. Centrifuged at 15000 g at 4ºC for 7 minutes visually check pellet. It should be ranging between bluish and purple with no green color. If the color is green repeat, 4-5th step. Calibrated spectrophotometer-using methanol as blank. Measured pigment concentration by a spectrophotometer with slit width 1nm. The absorbance of sample and black at 470 nm, 665 nm, and 720 nm. To calculate the chlorophyll contain by the following formula:-

2.6 Media optimization by changing the concentration of micronutrients

Chlorella medium was specialized for Chlorella Pyrenoidosa, which were freshwater algae. Chlorella Pyrenoidosa was cultured in chlorella medium containing 0.2g $MgSO_4.7H_2O$, O.2 g K_2HPO_4, 0.1 g ($CaCl_2.H_2O$), 5.0 ml (Fe-EDTA) solution, 1000 ml distilled water and different micronutrient solution. Photoautotrophic batch cultivation of Chlorella Pyrenoidosa has cultured in 100ml Erlenmeyer flask containing the 5ml culture of Chlorella Pyrenoidosa and 45 ml of media having different micronutrient solution

in chlorella medium as follows. Nine trails were having different pH ranges from 5 pH to 7 pH, with different micronutrient solution.

$$\text{Chlorophylls (mg/L)} = 8.02 \times A_{663} + 20.21 \times A_{720}$$

3. RESULT

3.1 Biomass production determination test

Biomass (dry) has been establishing by the 10mL culture of Microalgae, Centrifuge the sample at 4000rpm for 20 minutes, then it shows to layer in which supernatant was discarded from the test-tube centrifuge. Then by using the electronic weighing balance. This experiment performed in duplicates.

Test sample =0.33 g/10 mL of microalgae

3.2 Standard graph biomass

To know the concentration of unknown sample we plot the standard graph. For that, centrifuged the 10 ml culture of Chlorella Pyrenoidosa. Discard the supernatant, then add 10 ml of Phosphate buffer in the palate and homogeneously mix them. Then make the different concentration of culture 3mg to 27 mg/ml. and add 2ml distilled water. Then the samples detected by UV-visible spectrophotometer at 680 nm. All test-tube the samples tested in duplicate.

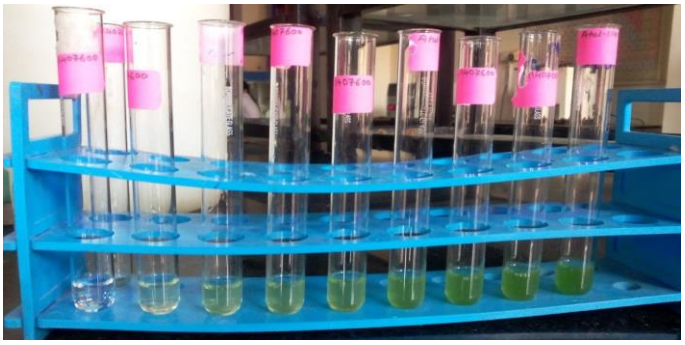

Fig. 1: Different concentration of culture for the plotting of a standard graph

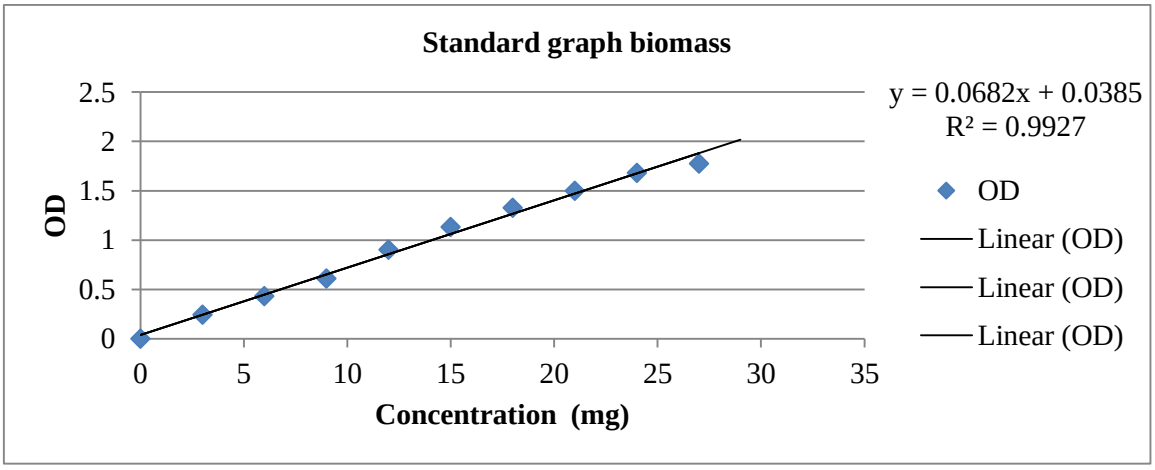

Fig. 2: Standard graph biomass

3.3 Reducing sugar test

By using DNSA (3, 5-Dinitrosalicylic Acid) method given by Miller, quantify the concentration of reducing sugar. Prepared glucose solution with different concentration, ranges from 1mg/ml to 10mg/ml for the plotting of a standard graph. To examine the concentration of glucose in Chlorella Pyrenoidosa. We have taken 500 µl sample of Chlorella Pyrenoidosa from the culture and add in glucose solution of different concentration. Then add 3ml of DNS solution and keep it in water bath at 100ºC for 10 minutes. Wait for cooled down then add 5 ml of D.W. for dilution of the concentration of DNS reagent. Take absorbance at 540 nm.

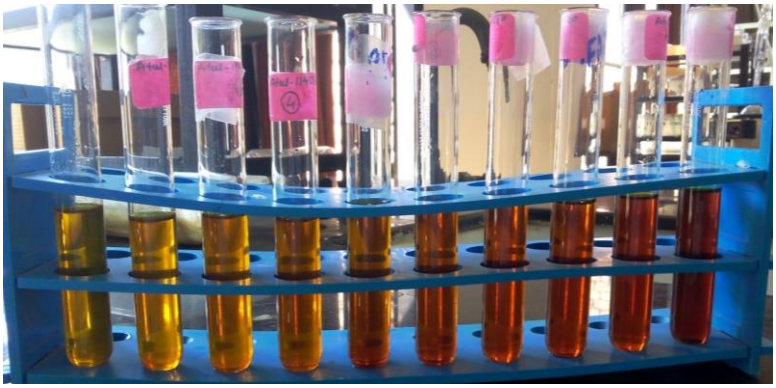

Fig. 3: DNS test

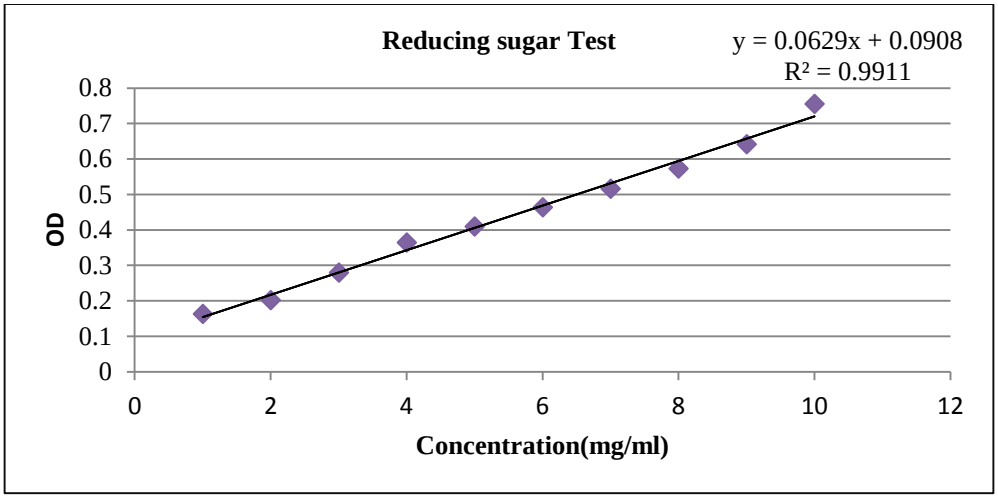

Fig. 4: Graph of reducing sugar test

3.4 Chlorophyll determination

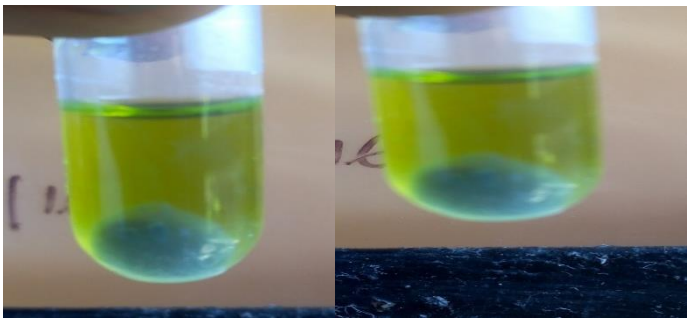

Fig. 5: Chlorophyll a determination

3.5 Effect of different parameters on the growth of chlorella pyrenoidosa

Factor and their level set for the optimization media for the growth of Chlorella Pyrenoidosa microalgae. The designed experimental condition L9 with their response and obtained S/N ratio. Optimization of media is significantly effected by the culture conditions parameters like pH, Na_2CO_3, NH_4Cl, and $NaNO_3$.

The main effects of the factors along with the interactions at the assigned levels on the biomass production by Chlorella Pyrenoidosa. The difference between the average value of each factor at levels 1 and 2 indicates the relative influence of the effect; the larger the difference, the stronger the influence. The sign of the difference (+ or -) indicates whether the change from level 1 to level 2 increased or decreased the result and thus the main influencing factor can be determined. Based on this data, we conclude that pH shows a strong positive value −3.445, $NaNO_3$ with positive value −1.188, NH_4Cl with negative value -0.761, $NaNO_3$ with negative value −0.354. Hence, the main influencing factors in biomass production were pH, NH_4Cl, Na_2CO_3, and $NaNO_3$.

Table 3: The main effects of the factors along with the interactions

Column#/ Factor	Level 1	Level 2	Level 3	L2-L1
pH	12.902	16.348	21.205	3.445
Na_2CO_3	15.566	16.755	18.135	1.188
NH_4Cl	17.431	16.671	16.355	-0.761
$NaNO_3$	17.225	16.872	16.359	-0.354

3.6 S/N ratio

The result obtained after performing experiments was entered in the result column of software and analysis was done further for significant factor determination, Analysis of Variance (ANOVA), Pooled ANOVA, and optimum condition and for expected result based on the Signal-to-Noise (S/N) ratio. The experiment was performed again based on obtained optimum results for determination of actual yield of lactic acid. The average of S/N ratio is 16.819.

Table 4: S/N Ratio

Conditions	Sample #1	Sample #2	Sample #3	S/N Ratio
Trail#	4.286	4.342	4.271	12.668
Trail#	4.500	4.486	4.070	12.745
Trail#	4.928	5.214	4.000	13.295
Trail#	5.243	5.857	4.928	14.489
Trail#	5.857	7.314	6.500	16.227
Trail#	8.786	8.343	7.728	18.330

Trail#	9.629	9.685	9.171	19.542
Trail#	12.01	11.414	11.428	21.294
				16.819

*Mean of three responses with SD not more than ±4.475

3.7 Interacting factors pairs

Table 5: Interacting factors pairs (order based on SI)

S no.	Interacting factors pairs(order based on SI)	Columns	SI (%)
1	NH_4Cl x $NaNO_3$	3 x 4	77.61
2	Na_2CO_3 x NH_4Cl	2 x 3	51.27
3	Na_2CO_3 x $NaNO_3$	2 x 4	51.2
4	pH x NH_4Cl	1 x 3	19.37
5	pH x $NaNO_3$	1 x 4	10.01
6	pH x Na_2CO_3	1 x 2	8.21

3.8 Main effects of the factors at their individual level

In the current experiment, pH was in the range of 5-7. The following Figure shows the individual effects of pH, Na_2CO_3, NH_4Cl, $NaNO_3$. Following Figure also shows the increase in pH from 7 to increases lactic acid production, while further increase in pH from 5 to 6 decreases biomass production. In S/N ratio pH shows a strong positive influence on biomass production. This result is supported by the data obtained from ANOVA. It can be seen that pH have a maximum % of contribution and shows the relative influence of the factor in the pie chart. In this chart, pH covered the maximum area. Shows optimum performance of the major factor contribution before and after pooled ANOVA. pH shows contribution with all the factors in biomass production. Table 4 shows the interaction between each factor. Interaction of pH with CaCO3 shows SI percentage.

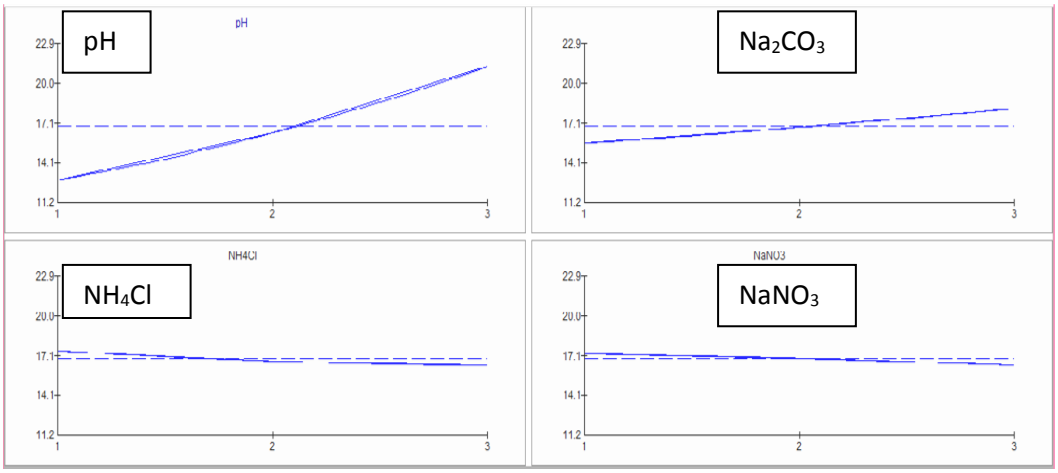

Fig. 6: Main effects of the factors at their individual level

3.9 Determination of significant factors

The factors showing significant influence on biomass production were determined based on the level difference. Understanding the interaction between two factors gives a better insight into the overall process analysis. Any individual factor may interact with any or all others factors creating the possibility of the presence of a large number of interactions. This kind of interaction is possible in Taguchi DOE methodology. Estimated interaction severity index (SI) of the factors are shown in Table 4 in decreasing order. Interactions under study help to know the influence of two individual factors at various levels of the interactions. In this table, the column represents the locations to which the interacting factors were assigned. Interaction SI presents 100% of SI for a 90-degree angle between the lines while 0% SI is for parallel lines. The reversed column should be reserved if this interaction effect has to be studied. "Levels" indicate the factor levels desirable for the optimum condition (based on the first two levels).

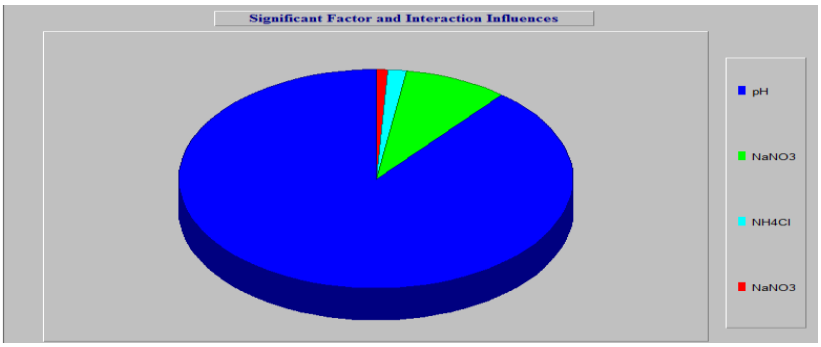

Fig. 7: Significant factor and interaction influences

3.10 ANOVA

Col # / Factor	DOF (f)	Sum of Sqrs. (S)	Variance (V)	F - Ratio (F)	Pure Sum (S')	Percent P(%)
1 pH	2	104.405	52.202	-----	104.405	89.01
2 NaNO3	2	9.918	4.959	-----	9.918	8.456
3 NH4Cl	2	1.834	.917	-----	1.834	1.564
4 NaNO3	2	1.136	.568	-----	1.136	.968
Other/Error	0					
Total:	8	117.295				100.00%

Fig. 8: ANOVA

3.11 Optimum conditions and performance

Column # / Factor	Level Description	Level	Contribution
1 pH	7	3	4.386
2 NaNO3	0.004	3	1.316
3 NH4Cl	0	1	.611
4 NaNO3	0.05	1	.406
Total Contribution From All Factors.....			6.718
Current Grand Average Of Performance...			16.819
Expected Result At Optimum Condition...			23.538

Fig. 9: Optimum conditions and performance

3.12 Variation Reduction Plot

The variation reduction plot shows that the current condition shows S/N ratio is 16.819, the average is 7.605 and the standard deviation of the current condition is 3.322. This was shown by qualitek-4 software, which was automatic and by bigger is a better method. However, the improved condition shows that the S/N ratio is 23.538 and the average is 7.605 and standard deviation of improved is 1.532.

Therefore, the improved condition is better for Chlorella Pyrenoidosa to produce higher biomass.

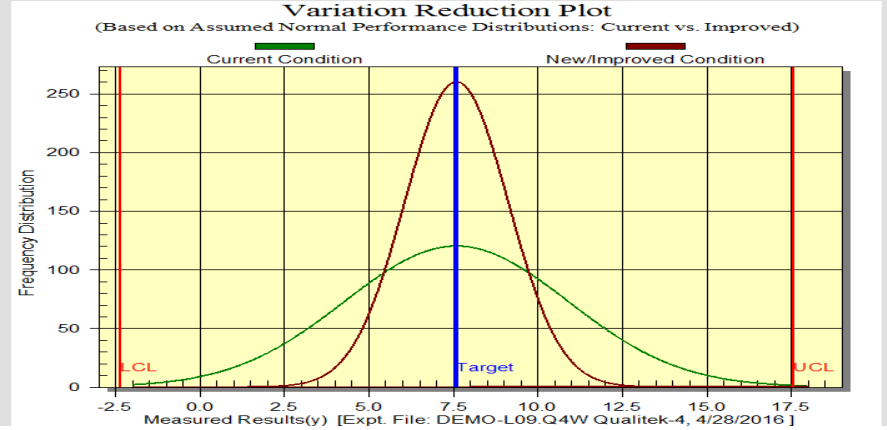

Fig. 10: Variation reduction plot

4. CONCLUSION

Various factors of media, components were optimized and validated for biomass production from Chlorella Pyrenoidosa *NCIM* 2738 by using Taguchi DOE Methodology. Four factors with three levels chosen were pH, Na_2CO_3, NH_4Cl and $NaNO_3$. Significant factors were pH, Na_2CO_3 and $NaNO_3$. Biomass production has found significantly affected, also by the interaction of factors like NH_4Cl x $NaNO_3$, Na_2CO_3 x NH_4Cl, Na_2CO_3 x $NaNO_3$, together, but individually they have minimum impact on biomass production. Individually NH_4Cl and $NaNO_3$ are the most significant factors in biomass production in descending order. pH and Na_2CO_3 along with $NaNO_3$were the most significant factors and determine the overall production.

Optimum value obtained was, pH 7 (level 3), Na_2CO_3 0.4 %(Level 3), NH_4Cl 0 % (Level 1) and $NaNO_3$ 5% (Level 1).This optimal condition actually resulted in the growth of Chlorella Pyrenoidosa 0.33 g/10 mL of microalgae with SD }1.5 of biomass. The growth of Chlorella Pyrenoidosa was improved by about 6% with the same species with improved conditions.

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