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# Evaluation of Process Parameters and Media Components by Plackett-Burman Design for Enhancement of Biomass using Cyanobacteria (*Anabaena ambigua*)

**M.B.Venkata Ramana Reddy*, C.S.Rao, S.S.Lakshmana Rao**

**Department of Biotechnology,Sreenidhi Institute of Science and Technology(An Autonomous Institution) Ghatkesar, Yamnampet, Hyderabad-501301, AndhraPradesh, India.**

****Corres. author: mbv.235@gmail.com,***
***Cell No:9912038795.***

**Abstract :** Cyanobacteria (*Anabaena ambigua*) has diverse applications in the field of agriculture as biofertilizer, in removal of $CO_2$ from atmosphere, in waste water treatment etc. So in the present study the effect of process parameters like pH, Light intensity, temperature, r.p.m and constituents of media on the growth of *Anabaena ambigua* are studied. Student's t-test, and Pareto chart of Plackett- Burman design revealed that pH (confidence level=98.84%), Light Intensity (confidence level=92.23%) are very significant variables, sodium Nitrate (confidence level=71.44%) is an important variable and calcium chloride (confidence level=38.74%) is not so significant Variable, but influences the growth of *Anabaena ambigua*. The higher value coefficient of Determination ($R^2$=0.93), justified an excellent correlation between the factors and biomass formation. The model fitted well with high statistical reliability and significance.
**Keywords**: *Anabaena ambigua ,* Biomass, Plackett Burman design, Paretochart.

## INTRODUCTION

Fossil fuels are the major source of energy all over the world. It is their combustion that has largely resulted in an increase in atmospheric $CO_2$ [1]. During the last few years there has been an growing interest in using microalgae for reducing the $CO_2$ burden on atmosphere[2].Microalgae produce oxygen during photosynthesis process and bacteria utilize oxygen to oxidize substances thereby releasing inorganic nutrients and $CO_2$ required for growth of microalgae[3,4,5,6,7,].Microalgae are potentially applied in various aspects like agriculture products eg. biofertilizers, soil conditioners and animal feed, Human food supplements, medicinal products eg. antimicrobial, anticancer etc. Different varieties of cyanobacterial species growing in tropical soils serve as major sources of fixed N for rice cultivation[8] The Cyanobacteria (*Anabaena ambigua*) also constitutes as a source of valuable products such as phycobiliproteins, polysaccharides, protein for feed and food[9]. Heterocysts are the primary sites of nitrogen fixation in *Anabaena ambigua* and many other filamentous blue-green algae (cyanobacteria) under aerobic conditions.The nitrogen fixed by these cyanobacteria is immediately available to the crop. Hence the biomass of heterocystous cyanobacteria is used as nitrogen source in paddy fields[10].Due to diversed applications of Cyanobacteria ( *Anabaena ambigua* ) ,many studies were made for its better growth . In this study process parameters like pH, temperature, r.p.m, Light intensity, and constituents of media are studied to enhance the biomass. Optimization technique Plackett-Burman design is applied to screen the important process parameters and media constituents that effects the growth of the species (*Anabaena ambigua*)

## MATERIALS AND METHODS

Cyanobacteria (*Anabaena ambigua*) is brought from NCIM, Pune with accession number 2785.*Anabaena ambigua* is cultured in BG-11 media[11,12] in 250ml flasks. This cultured cyanobacteria is used for further experiments,where the effect of process parameters like pH,temperature,light intensity, r.p.m and media constituents like sodium Nitrate,dipotassium hydrogen phosphate, magnesium sulphate(hydrated),calcium chloride(dihydrate) are studied at different combinations based on the design of Plackett Burman .After 15 days of growth, the resultant mixture is filtered by Whatman filter paper. It is dried in hotair oven for two days at a temperature of 62°c and the weight of obtained biomass is estimated.

## EXPERIMENTAL DESIGN

This study was undertaken using Plackett Burman design for screening the process parameters and media components which have more effect on the growth of *Anabaena ambigua*.The above said eight variables affecting the growth were screened at two levels, maximum(+) and minimum(-) [Table1]. Now experiments are carried out as per the design [Table 2].They are performed in duplicates and the average biomass produced is taken as the response(Y)

Plackett-Burman experimental design which is based on the linear first order regression model,offers a good and fast screening procedures and gives the effect of change of more than one factors in single experiment.

$$Y=\alpha +\Sigma\beta_i X_i$$

Where Y is the biomass formed in g/l. α is the intercept, $\beta_i$ is the coefficient of the variable "i" estimates, $X_i$ is the independent variable and "i" is the variable number. In the first step of Plackett-Burman Design 12 experimental runs were carried out to evaluate the effect of 8 factors of the medium components and operating conditions on biomass production.Dummy variables are used to estimate experimental errors in data analysis.The effect of each variable was calculated using the following equation.

$$E=(\sum M_{+} - \sum M_{-}) / N$$

Where E is the effect of tested variable, $M_+$ and $M_-$ are responses (Biomass formation) of trials at which the parameter was at its higher and lower levels respectively and N is half the number of trials carried out,SE(Standard error) is calculated by using student's t-test.

## RESULTS AND DISCUSSION

The data on biomass formation was subjected to multiple linear regression analysis using Sigma XL 6.11 software to estimate t-value, p-value and confidence level [Tables 3 & 4].The data on biomass formation using Plackett-Burman experiments showed a variation from 0.01 to 0.135 g/L. The student's t-test for any individual effect allows an evaluation of probability of finding the observed effect purley by a chance. Hence variables with higher confidence level (pH=99.8%,Light intensity=92.8%) are considered as highly significant variables ,Sodium Nitrate (Confidence level=71.3%) is an important variable,Finally Chloride with confidence level=39.8% is not so significant variable ,among those that influences the growth of *Anabaena ambigua*. In PB design [13], effect of independent variables on biomass production is given by first order linear model[ equation-1].The coefficient of determination of model ($R^2$ =0.931),( $R^2$Adjusted=74.32%)[ Table 5] validates that the model is well suited to experimental results.

**Table 1. Coded and actual values of the variables.**

| Process Variables & media constituents | Low level (-1) | High level (+1) |
|---|---|---|
| pH(X1) | 5 | 9 |
| Light Intensity(X2) | 20μmol/$m^2$s | 80 μmol/$m^2$s |
| R.P.M.(X3) | 50 | 150 |
| Temp(X4) | 20°c | 40°c |
| Nitrate(X5) | 0.75 g/L | 2.25 g/L |
| Sulphate(X6) | 0.0375 g/L | 0.1125 g/L |
| Phosphate(X7) | 0.02 g/L | 0.06 g/L |
| Chloride(X8) | 0.018 g/L | 0.054 g/L |

**Table 2. Two-level Plackett-Burman design for Biomass formation.**

| Experiment No | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 | Biomass(g/L) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | +1 | -1 | +1 | +1 | -1 | -1 | -1 | +1 | 0.06 |
| 2 | +1 | +1 | -1 | -1 | -1 | -1 | -1 | -1 | 0.09 |
| 3 | -1 | +1 | +1 | +1 | +1 | -1 | -1 | -1 | 0.02 |
| 4 | +1 | -1 | +1 | +1 | -1 | +1 | +1 | -1 | 0.055 |
| 5 | +1 | +1 | -1 | -1 | +1 | -1 | -1 | +1 | 0.115 |
| 6 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | -1 | 0.135 |
| 7 | -1 | +1 | +1 | +1 | -1 | +1 | +1 | +1 | 0.03 |
| 8 | -1 | -1 | +1 | +1 | +1 | -1 | -1 | +1 | 0.015 |
| 9 | -1 | -1 | -1 | -1 | +1 | +1 | +1 | -1 | 0.0135 |
| 10 | +1 | -1 | -1 | -1 | +1 | +1 | +1 | +1 | 0.07 |
| 11 | -1 | +1 | -1 | -1 | -1 | +1 | +1 | +1 | 0.028 |
| 12 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 0.01 |

**Table 3. Estimated effects and coefficients for analysis of PB design on Biomass production**.

| Variable | Main effect | Coefficients | t-value | p-value | SE coefficient | Confidence level |
|---|---|---|---|---|---|---|
| Constant | | 0.05345 | 8.707 | 0.0032 | 0.006139 | 99.68% |
| X1 | 0.068 | 0.03404 | 5.545 | 0.0116 | 0.006139 | 98.84% |
| X2 | 0.0324 | 0.01620 | 2.640 | 0.0777 | 0.006139 | 92.23% |
| X3 | -0.00191 | -0.000958 | -0.156 | 0.8859 | 0.006139 | 11.41% |
| X4 | -0.00191 | -0.000375 | -0.06108 | 0.9551 | 0.006139 | 4.49% |
| X5 | 0.0159 | 0.007958 | 1.296 | 0.2856 | 0.006139 | 71.44% |
| X6 | 0.00358 | 0.001791 | 0.29183 | 0.7894 | 0.006139 | 21.06% |
| X7 | 0.00358 | -0.000458 | -0.07466 | 0.9452 | 0.006139 | 5.48% |
| X8 | -0.00091 | 0.003458 | 0.5633 | 0.6126 | 0.006139 | 38.74% |

**Table 4. Analysis of variance for model.**

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Model | 8 | 0.018015 | 0.00225 | 4.979 | 0.1071 |
| Error | 3 | 0.001356 | 0.000452 | | |
| Total | 11 | 0.019372 | 0.001761 | | |

**Table 5 . Model Summary.**

| | |
|---|---|
| R-Square | 93.00% |
| R-Square(Adjusted) | 74.32% |
| S(Root mean) | 0.021267313 |

**The Linear multiple regression equation:**

Y=0.05345+ 0.03404*pH + 0.0162*Light Intensity - 0.000958*R.P.M. -0.000375*Temp + 0.00795*Nitrate + 0.001791*Sulphate -0.000458*phosphate + 0.003458*Chloride……..[Equation-1]

Pareto plot shows the main effect of all medium components and operating conditions[ **Chart 1]** **.**It provides the facts needed for setting priorities on media components and process variables, concentrating on improvement efforts on these variables it has greater impact and is cost effective for process development .Hence the effect of process variables and media components on biomass formation are studied graphically using Pareto chart.From the chart pH,Light intensity,are highly significant variables. Sodium Nitrate is an important variable, Calcium chloride is not so significant variable. Similar results were obtained by Nelson's method. In this method mean square of the variables is calculated, Mean square of errors introduced by the dummy variables were calculated. The effect of each variable is calculated by dividing the mean square of the variable with mean square of error due to dummy variables.

Graphs were plotted between the average biomass formed vs. pH [ Graph 1], average biomass formed vs light intensity[ Graph 2], average biomass formed vs Nitrate[Graph 3] and finally average biomass formed vs. chloride[ Graph 4].

## CONCLUSION:

Among the variables (pH, Light Intensity, Temperature, r.p.m, sodium nitrate, dipotassium hydrogen phosphate,magnesium sulphate,calcium chloride) studied for biomass formation of cyanobacteria (*Anabaena ambigua*) four variables (pH, Light Intensity, sodium nitrate, calcium chloride) were screened out using Plackett-Burman design. These variables are further studied in detail and can be optimized using Response Surface Methodology to get much better values where the formation of biomass is further increased.

### Pareto Chart

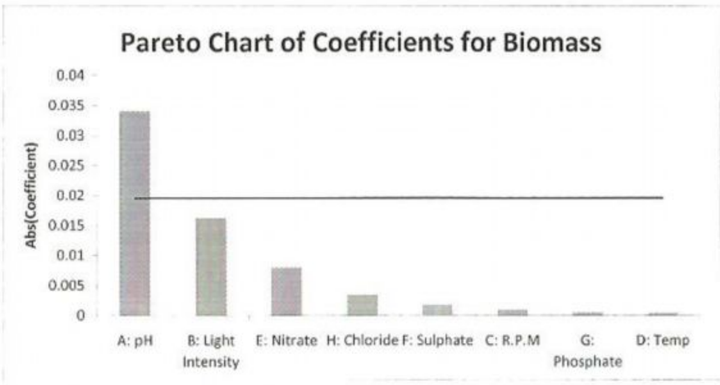
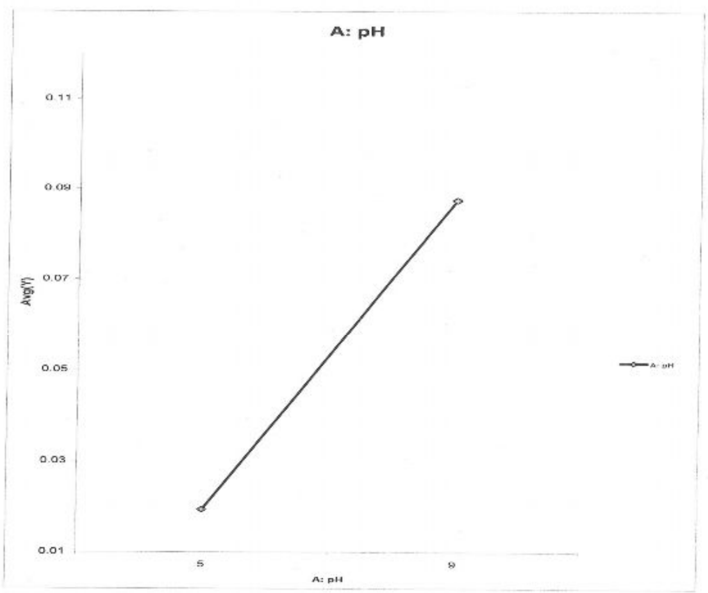
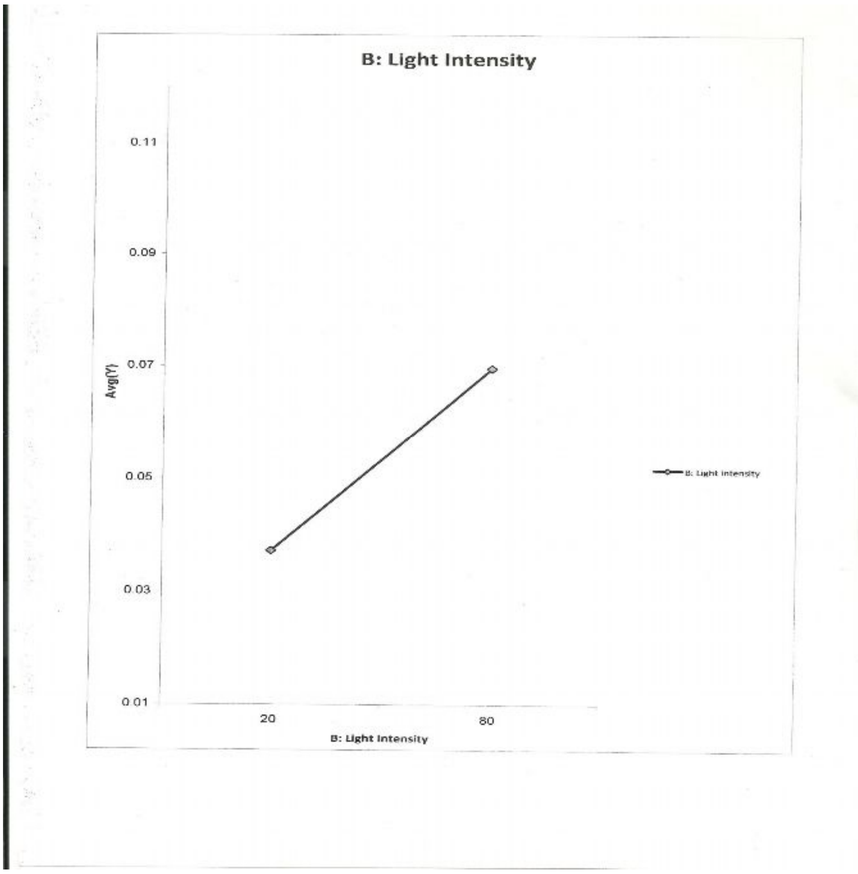



**Chart-1**

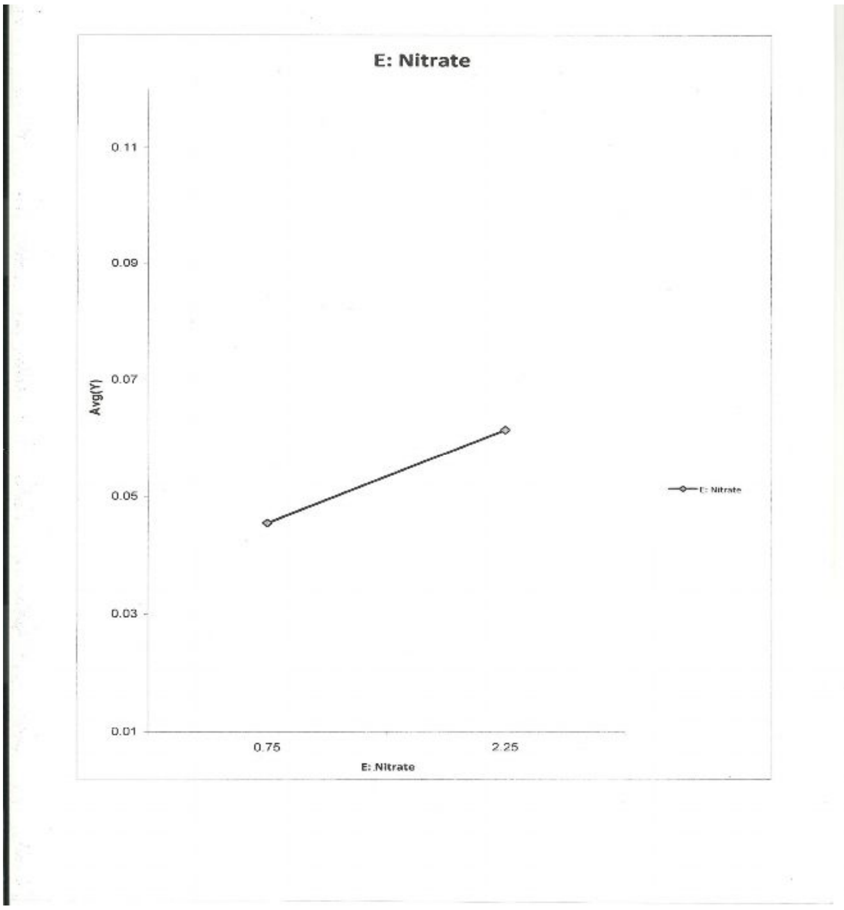
### Graphs



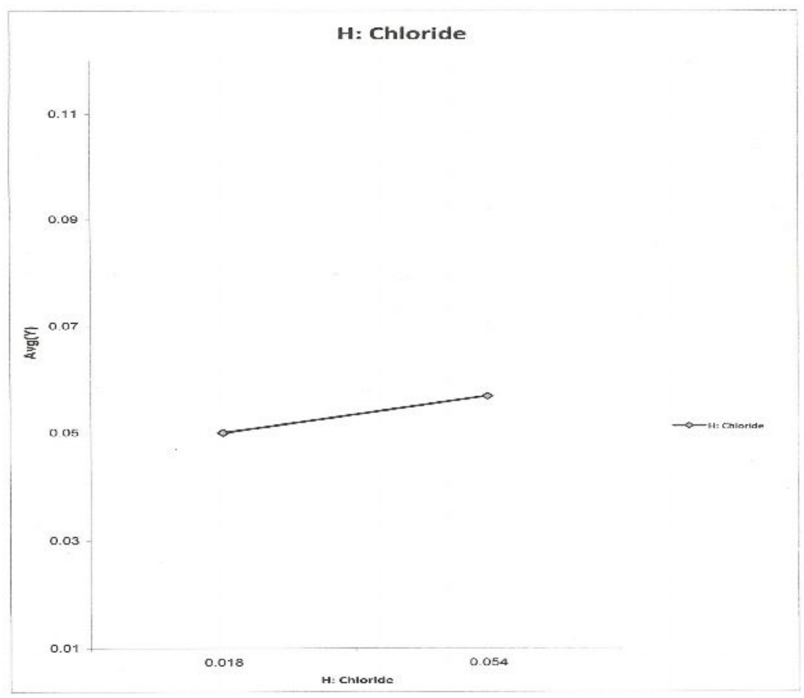

**Graph-1**




**Graph-2**




**Graph-3**




**Graph-4**

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