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Su Study On The Optimum Macro Nutritional Requirement For The Selected Strains Of Blue Green Algae

RUBI KUMARI

Research Scholar Dept of Botany J P University Chapra

Email ID- editorcassstudies@gmail.com

ABSTRACT:

Growth of the test material is adversely affected at the higher levels of ammoniacal nitrogen in comparison to the nitrite and nitrate nitrogen at similar level. N- fertilizer inhibition of growth of cyanobacteria in paddy fields have been reported by Singh, 1985, Roger and Kulasooriya, 1980. Negative correlation between biomass production of cyanobacteria and nitrogen fertilizer has now been established (Singh; 1989). However, Venkatraman (1979) reported no effect on N2 fixation by blue green algae in rice fields with less than 40 ppm ammonium nitrogen in stagnant flood water.

Calcium deficient medium supports very little or no growth of the-9 algae. Microscopic examination of calcium starved culture revealed slender and weak filaments. Biomass production is increased with the increase in levels of calcium. More calcium is required in N2 medium than N03~ medium.

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INTRODUCTION

Various culture media have been developed for the optimum utilization of the nutrients by Blue green algae under particular culture condition (Chu, 1942; Fogg, 1949, Gerloft *et al* 1950; Alien and Arnon, 1955). But no culture medium can be generalized due-to the heterogeneity of the group. Therefore, a suitable culture media should be decided for the selected test material under the prevailing culture condition for better growth. Experiments were designed with the instant aim and various nutrient compositions were modified qualitatively (different source of the nutrient) as well as quantitatively to ascertain the optimum nutritional requirements of the cyanobacterial strains *(Nostoc muscorum* and *Anabaena variabilis).*

2 Materials and Methods –

2.1 Selection of culture medium

Four culture media (Chu No.10, Chu No.10 modified by Gerloft *et al* 1940, Alien and Arnon, 1985 and Fogg 1949) were selected to find out

the most suitable one under the prevailing laboratory condition.

Equal amount of test alga grown in N_2 free medium was inoculated in 10 ml media of each selected culture medium and growth were recorded after 24 days of incubation in culture cabinet.

2.2 Growth curve

For determining the lag phase and log phase of growth of the two selected test algae, equal amount of N(V grown cultures were inoculated into test tubes containing 10 ml of the selected culture medium Chu No. 10 as modified by Gerloft *et al,* 1950 . Growth was recorded every alternate day till 30^{th} day of inoculation.

2.3 pH of the medium

pH of the culture medium has been found to affect the growth of the alga. Generally, the pH of paddy fields has been found to be slightly alkaline. Therefore, it became necessary to determine the pH of the culture medium at which the selected algae could grow suitably.

For this the pH of the medium was altered with the help of N/10 NaOH and bench HC1 which was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0. No change in pH of the medium so adjusted was found after autoclaving. Now. equal amount of unicelled, nitrate grown cultures of the two algae was added into 10 ml of culture media adjusted at different level of pH.Growth was recorded in terms of optical density of the extracted pigments from the exponentially growing cultures.

2.4 Growth under different nutrients modified qualitatively (different sources) arid quantitatively (different concentrations).

Different levels of various nutrient of the selected culture medium were used for determining their optimum utilization by the selected test algae. Different levels and sources of nutrients which were taken into consideration have been shown in table 1.

Table –1

Levels and sources of nutrients

Macronutrients :

Nutrients	Source	Level (ppm)
Nitrogen	KNO_3,KNO_2&NH4C1	3.39 to 135.84
Calcium	$CaCl_2$	0.102 to 4.1
IRON	Ferric citrate, Ferric Chloride	0.068 to 2.75

Phosphate	$K_2HPQ4_5Na_2HP0_4$	1.36 to 54.58

Different levels of the nutrients in culture medium were obtained where ever required, has been described along with the particular nutrient.

3 Results

3.1 Selection of culture medium

Table - 1 represents the growth of the selected test algae in different culture media. Altogether four culture media (Chu No. 10, Chu No 10 as modified by the Gerloff *et al.* 1950, Fogg: 1949, and Allan & Arnon 1950) were considered. Maximum growth of the two algae was recorded in Chu No.10 as modified by Gerloff *et a!.,* 1950 (Table-4.). Growth was recorded after 24 day of inoculation in terms of optical density of the acetone extracted pigments of the harvested culture. On the basis of the findings modified Chu No. 10 culture medium was selected for further studies.

Table-.2

End point growth of the cultures in different culture media.

Organism	N_2-free medium			
	ChuNo.10 (1942)	Mod. Chu No.lO(1950)	Fogg (1949)	Alien & Arnon(1950)
N.muscorum	0.46 ±0.009	0.50 ±0.009	0.44 ±0,009	0.42 ±0.009

A.variabilis	0.48 ±0.009	0.56 ±0.009	0.44 ±0.009	0.40 ±0.009
Combined Nitrogen (NO_3-) Culture medium				
N.muscorum	0.49 ±0.009	0.62 ±0.009	0.52 ± 0.009	0.48 ±0.009
A.variabilis	0.47 ±0.009	0.61 ±0.009	0.48 ±0.009	0.44 ± 0.009

3.2 Growth Curve

Nitrate grown cultures of the two cyanobacterial strains were broken into unicells and homogenized. Equal amount of the cultures was inoculated in test tubes containing 10 ml liquid media and incubated in growth chamber for 30 days. Growth was recorded alternate day and it was found that the two algae have almost identical log and lag phase of growth. Lag phase of the growth was found to be 2-4 days whereas log phase period of the growth was achieved on 24 - 28th day of inoculation.

Nostoc musocorum appeared to attain log phase earlier than *Anabaena variabilis.* However, for both the algae growth appeared to be stationaiy or declining after 24th day of inoculation. Therefore, 24th day was taken as the log period of the growth for the two algae.

Comparatively at lower levels suppressed the heterocyst formation in both the algae. A lower level of nitrogen is required to stop heterocyst formation in A *variabilis* than *mN. muscorum.* The two algae being N_2-fixers grew even in the absence of combined inorganic nitrogen and they form heterocyst in such medium for carrying the process of nitrogen fixation.

3.4.2 Calcium

Calcium starved cultures were used for this experiment i.e. grown culture was grown for 24 hrs. in medium devoid of calcium in any form. Homogenous suspension of such culture was prepared and equal amount of algae was inoculated in 10 ml liquid N_2 & $N0_3$ medium containing calcium at different levels. The source of calcium was Calcium chloride.

3.4.3 Iron

Nitrate grown culture of the two algae were washed thoroughly and allowed to grow in iron unsupplemented Chu 10 medium for 24 hrs to obtain iron starved cultures. Homogenous suspension of these cultures was prepared and equal amount of aliquot was added into 10 ml N_2 as well as NOa culture medium containing iron from two different sources (Ferric citrate- citric acid and Ferric chloride) at different levels. Different levels of iron were obtained by appropriately diluting the stock solution.

3.4.7 Phosphate

K_2HP04 is the source of potassium as well as phosphate in the original Chu-No.10 culture medium. For the study of the effect of phosphate at various levels from different sources, K_2HP0_4 was replaced with Na_2HP0_4 and potassium was supplemented in from of potassium chloride in one set of experiment and in other set of experiment the culture medium contained K_2HP0_4 as source of $P0_4$ and potassium. Different levels of $P0_4$ were obtained by appropriately diluting the stock soln. of both K_2HP0_4 and Na_2HP0_4.

Nitrate grown cultures of the two algae was allowed to grow in nitrate free medium i.e. N_2 medium for 24 hrs and washed thoroughly. The cultures were then transferred into minimal medium i.e. without K_2HP0_4 but with KC1 for obtaining $P0_4$ starved culture. Such cultures were homogenized and equal amount of aliquot was added into test tube containing 10 ml culture medium at different levels of $P0_4$ from either K_2HP0_4 or Na_2HP0_4 as the source.

So far as the utilization of the combined inorganic nitrogen source (KNO_3, $KN0_2$ & NH_4C1) is concerned the two algae did not differ. Ammoniacal nitrogen (NE^Cl) is utilized in preference to nitrate and nitrite source. This is in agreement with energy economy of the cell asjhe required energy is in

decreasing order from ammonia to nitrite to nitrate and to elemental nitrogen. More energy is required for conversion of elemental nitrogen into its utilizable form where as less energy is required for the ammoniacal nitrogen where it is in readymade form. The instant findings point the existence of regulatory interactions between the involved assimilatory processes (Guerrero and Cataline, 1987).

Growth of the test material is adversely affected at the higher levels of ammoniacal nitrogen in comparison to the nitrite and nitrate nitrogen at similar level. N- fertilizer inhibition of growth of cyanobacteria in paddy fields have been reported by Singh, 1985, Roger and Kulasooriya, 1980. Negative correlation between biomass production of cyanobacteria and nitrogen fertilizer has now been established (Singh; 1989). However, Venkatraman (1979) reported no effect on N2 fixation by blue green algae in rice fields with less than 40 ppm ammonium nitrogen in stagnant flood water.

Calcium deficient medium supports very little or no growth of the-*9* algae. Microscopic examination of calcium starved culture revealed slender and weak filaments. Biomass production is increased with the increase in levels of calcium. More calcium is required in N_2 medium than $N0_3$~ medium. Natarajan (1959) in *Selenastrum wastii,* Verma (1980 and 1989) in *Draparwldiopsis indica* and *Chaetophora passiformis* have found almost similar effect of calcium. Alien (1952) has reported that more calcium is required in elemental nitrogen medium than in combined source of nitrogen.

Iron is one of the essential elements required for the growth of cyanobacteria especially for the N_2 fixer. Less growth has been observed in iron deficient culture medium. Growth increases with increase in the levels of iron. More iron is required in N_2 medium than nitrate medium because iron is one of the constituent of nitrogen fixing enzyme nitrogenase and in absence of combined inorganic source of nitrogen the nitrogen fixer will have to remain more active for fixation of elemental nitrogen for their metabolic needs. Morever, it has been observed that iron in form of ferric nitrate along with citric acid is utilized well than in form of ferric chloride. Similar effect have been found by Gerlofft *et al,* 1950 and accordingly modified the Chu No. 10 culture medium for culture of blue green algae. Rodhe (1948) has reported that ferric citrate with

citric acid stabilizes the concentration of reactive iron in the nutrient solution. The chelating action of citric acid has been found by Kratz and Myers(1955).

REFERENCES

Adhikary, S.P., 1989 : Effect of Pesticides on growth, photosynthetic oxygenEvolution and Nitrogen fixation of *Westiellopsis prolific,* **L. Gen. apl. Microbiol. 35; 319-325.**

Adhikary, S.P., and Pattanaik, H., 1984: Effect of the carbamate insecticide Sevin on *Anabaena sp.* And *Westiellopsis prolific.* **Acta. Microbiol. Hung., 31; 335-338.**

Ahmad, S.U., Kalita, M.C., Deka, K. and Hazarika, S.B.M., 1999 : Distrbutional pattern of blue-green algae in rice field soil of nagaon sub division. **Phykos, 38 (1 & 2), 101-107.**

Ahmad, M.H. and Venkatraman, G.S., 1973 : Tolerance of Aulosira fertilissima to pesticides. **Curr. Sci. 42;** 108

Aiyer, R.S., 1965 : Comparative algological studies in rice fields in Kerala State. **Agric. Res. Kerala 3; 100-104.**

Allen, M.B., 1952 : The cultivation of Myxophyceae. **Arch. Mikrobiol, 17; 34-53**

Allen, M.B., 1956 : Photosynthetic nitrogen fixation by blue-green algae, **Sci. Month, 83; 100-106**

Allen, M.B. and Arnon, D.I., 1955 : Studies on nitrogen fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrical* Lemm. **Plant Physiol, 30 : 366-372.**

Amma, P.A., Aiyer, R.S. and Subramoney, 1966 : Occurrence of blue green algae in acid soil of Kerala. **Agril. Res. J. Kerala. 4:141.**

Armstrong, T.F., Williams, F.M. and Donald, P., 197 : Yellow nutsedge control with alachlor. **Weed Sci. 21; 354-357.**

Arvik, J.H., Wilson, D.L. and Darlington, L.C., 1971 : Response of soil algae to picloram – 2, 4 – D mixtures. **Weed Sci. 19 (3) : 276-278.**

Austin, S; handerson, n. and Dixor, R., 1990 : Characterization of *Kebsiela pneumonia* nitrogen fixation regulatory proteins Nif. A and Nif. L in vitro. **Europ J. biochem. 187, 353-360.**

Batterton, J.C.; Bousch, G.M. and Matsmura, F., 1971 : Growth responses of blue-green algae to aldrin, dieldrin, endrin and their metabolities. **Bull. Environ. Contam. Toxicol., 6; 589-594.**

Brill, W.J., 1980 : Biochemical genetics of nitrogen fixation. **Microbiol Rev. 44;** 449-467

Bisoyi, R.N. and Singh, P.K., 1988a : Effect of phosphorous fertilization in blue green algal inoculums production and nitrogen yield under field conditions. **Biol. Fert. Soils 5 : 338-343.**

Bisoyi, R.N. and Singh, P.K., 1988b : Effect of seasonal changes on cyanobacterial production and nitrogen yeld. Microbiol. Ecol. 16 : 149-154.

Brusslan, J. and Haselkorn, R. 1988 : Molecular genetics of herbicide – resistance in cyanobacteria. **Photosynthesis Research 17; 115-124.**

Bunt, J.S., 1961 : Nitrogen fixing blue-green algae in Australian rice soils. **Nature, 192; 479-480**

Brownell, P.F. and Nicholas, D.J.D. 1967; Some effects of sodium on nitrate assimilation and N2 fixation in Anabaena cylindrical. **Plant Physiol. 42; 915-921.**

Carpenter, E.J., and price, C.C., 1976 : Science, 191; 1278-80 – cited by Stewart, 191; 1278-80 – cited by Stewart, 1980.

Carr, N.G. and Bradley, S., 1973 : Aspects of development in blue-green algae. **Symp. Soc. Gen. Microbiol, 23; 161-188.**

Choudhary, K.K., 2009 : Occurrence of Chroococcaceae during rice cultivation in northern Bihar, India, **Bagladesh J. plant Taxon. 16(1) 73-76.**

Choudhary, K.K., 2011 : Occurrence of nitrogen fixing cyanobacteria during different stages of paddy cultivation. **Bagladesh J. plant taxon. 18(1) 73-76.**

Prasanna, R. jaiswal, P. and Kaushik, B.D., 2008 : Cyanobacteria as potential options for environmental sustainability – promises and challenges. **Indian J. Microbiol. 48, 89-94.**

Prasad, A.B., Samanta, R., Vishwakarma, M.L. and Vaishampayan, A. (1986) : Biological effects of a mercury fungicide on a nitrogen fixing blue green alga *Nostoc muscorum.* Isolation and preliminary characterization of Hg-resistant mutant. **New phytologist. 102 ; 45-50.**

Rai, M.K., 2006 : Handbook of Microbial Biofertilizers. **Howarth Press, New York.**

Rao, Digamber, Srinivas, B.D., Padmaja, O. and Rani, K. 2008 : Blue-green algae of South Telangana rehion, Andhra Pradesh. **Indian Hydrobiology. 11 (1) : 79-83.**