

STUDY ON THE SELECTION OF CULTURE MEDIUM, GROWTH CURVE, PH OF THE MEDIUM FOR THE SELECTED STRAINS OF BLUE GREEN ALGAE

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ABSTRACT:

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

INTRODUCTION:

The 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*).

MATERIALS AND METHODS:

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 N₂ 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 N₂ 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 30th 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.

Results

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 al., 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

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 muscorum* appeared to attain log phase earlier than *Anabaena variabilis*. However, for both the algae growth appeared to be stationary 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 *N. muscorum*. The two algae being N₂-fixers grew even in the absence of combined inorganic nitrogen and they form heterocyst in such medium for carrying the process of nitrogen fixation

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 N₂ & N₀₃ medium containing calcium at different levels. The source of calcium was Calcium chloride.

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 N₂ as well as NO_a 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 K₂HP₀₄ is the source of potassium as well as phosphate in the original ChuNo.10 culture medium. For the study of the effect of phosphate at various levels from different sources, K₂HP₀₄ was replaced with Na₂HP₀₄ and potassium was supplemented in from of potassium chloride in one set of experiment and in other set of experiment the culture medium contained K₂HP₀₄ as source of P₀₄ and potassium. Different levels of P₀₄ were obtained by appropriately diluting the stock soln. of both K₂HP₀₄ and Na₂HP₀₄. Nitrate grown cultures of the two algae was allowed to grow in nitrate free medium i.e. N₂ medium for 24 hrs and washed thoroughly. The cultures were then transferred into minimal medium i.e. without K₂HP₀₄ but with KC₁ for obtaining P₀₄ 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 P₀₄ from either K₂HP₀₄ or Na₂HP₀₄ as the source. So far as the utilization of the combined inorganic nitrogen source (KNO₃, KN₀₂ & NH₄C₁) 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 as the 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 N₂ medium than N₀₃~ 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 N₂ 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 N₂ 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. Moreover, 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 Gerloff et al, 1950

and accordingly modified the Chu No. 10 culture medium for culture of blue green algae.

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