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Optimization of culture media for the growth of *Anabaena spiroides and Nostoc punctiformae* of Jorhat district, Assam

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Abstract: *Blue green algae are very common in Indian rice fields .The present study is focused towards developing ecofriendly technique for mass production and suitable bio-inoculum development for field application of BGA strains. For that purpose two BGA strain namely, Anabaena spiroides and Nostoc punctiformae were isolated from selected rice field of Jorhat district Assam, India. The purpose of this research was to determine the optimized media for Biomass production and chlorophyll-a concentrations in the cyanobacteria Anabaena spiroides and Nostoc punctiformae, which were maintained in eight different modified culture media.*

Keywords: *Blue green algae, Anabaena spiroides and Nostoc punctiformae, modified media, Biomass production.*

I. Introduction

Blue green algae are diverse group of prokaryotic photosynthetic microorganisms. They can grow rapidly in almost all habitats due to their simple structure. Now a days blue green algal biomass are widely used for different purpose like in cosmetic ,bio-fuel, pharmaceuticals biofertilizer etc. Amongst all these uses of BGA, biofertilizer is most prominent one in agricultural based country like India. Anabaena and Nostoc are the two most important genus available in natural condition is used as biofertilizer. These photosynthetic microorganisms can be cultured, harvested, processed and used as a natural source of biofertilizer. Blue green algal biomass is generally used in dried algal flakes forms. They are widely used in rice field as they fix atmospheric nitrogen to the soil. In rice cultivation nitrogen is the second limiting factor after water. In rice cultivation it is quite impossible to ignore the role played by chemical fertilizer but yet at least it is becoming essential for the alternative like blue green algae which would add fertility to the soil, improve the soil health cheap and eco-friendly to the environment. There are so many different standard culture media developed by different workers. To produce high amount of biomass attention should be always given to the culture component. Large scale production of microalgal biomass depend on different factors, the most important of which are nutrient availability, temperature and light (Shay et al 1987). The choice of the medium mainly depend on several factors that include chemical composition of the medium (Borowitzka 2005).Various workers have develop different nutrient media to culture Anabaena and Nostoc. These two algae are heterocystous cyanobacteria.

In the present study two genus *Anabaena* and *Nostoc* have been subjected to 8 different inorganic culture media both standard and modified culture media differing in chemical composition. In order to identify the best growth medium i.e.; optimized medium the nutrient requirement of these two algae have been evaluated. The main objective of the present investigation is to analyze the growth (dry biomass) and chlorophyll-a of the above said microalgae in order to find out best growing inorganic media.

II. Materials And Methods:

Sampling Sites: The Blue Green Algal samples were randomly collected from the rice field of Jorhat district, Assam.

Isolation and Identification: The collected samples were enriched initially in BG-11 medium in conical flask at 24+2°c under light intensity (3200 lux) and photoperiod of 16:8 hour for 10 days. Then the enriched culture samples were spread on algal broth agar plates and incubated at the above mentioned condition. After the incubation period time freshly grown individual colonies were picked out and transferred to BG-11 medium for purification in 250 ml conical flask. These culture flasks were shaken manually for 3 to 4 times in a day. The purity of the culture were monitored by regular observation under microscope. The isolated microalgae were identified with standard manual for algae (Desikachary,1959).

Test Medium: The blue green algal sample *Anabaena spiroides* and *Nostoc punctiformae* had been raised into unialgal culture by plate culture technique. They were further cultured separately in liquid medium for the next

process. In order to attain optimal growth of these two algae, the evaluation of suitable media was felt to be the prime requisite. For and this purpose 8 different media both standard and modified were experimented out on these two algal sample. The modified culture media were prepared keeping on the mind on nutritional requirement of blue green algae. Different cultured media employed in the current project were (i)BG11, (ii) BG11 Modified 1 (As per SHUKLA,)(iii) BG11 Modified 2, (iv)) BG11 Modified 3, (v) BG11 Modified 4, (vi)BG11 Modified 5, (vii) BG11 Modified 6 and (viii)) BG11 Modified 7 .

The individual composition of all the 8 culture media have been listed in Table1. All these growth media were transferred to 250ml conical flask and sterilized at 121˚c for 15 minutes at 15lbs.The Ph of all the prepared media were adjusted to 7.5. The blue green algal species *Anabaena spiroides* and *Nostoc punctiformae* were inoculated in the 8different culture media and allowed to grow. The inoculated cultures were shaken regularly to accelerate the algal growth. All the experiments were done in triplicates.

Biomass Estimation (Richmond and Gobbellar, 1986): Whatman filter paper were soaked in water to saturate and dried overnight. Next day the weight of the filter paper were noted as the initial reading. The culture of Anabaena and Nostoc in the conical flask were homogenized by vigorous shaking by adding oven dried glass beads of 4-5mm.100ml of culture was taken and filtered through previously dried filter paper using vaccum filtration assembly. These were kept for drying and transferred to hot air woven and maintained at about 60 degree Celsius. Samples should be weighted quickly after drying and cooled in a desiccators to avoid absorption of moisture. The difference in initial and final reading of the weight gave the dry biomass in mg/ml

Pigment Analysis (Mckinney1942)**:** Growth of algal culture does not necessarily imply cell division, but cell division usually accompanies it. Growth, which is the addition of organic material to the cell can be measured directly by analyzing the standard stock or biomass of the culture. Several cellular components can be used as a measure of biomass, such as carbon, lipids, proteins and plant pigments. Out of the cellular components the pigment analysis especially Chlorophyll-a is most widely accepted (BGA-A manual for their production, evaluation and utilization, IARI). Chlorophyll-a can be extracted by hot extraction method (Mckinney1942). The growth of the algal biomass were assessed by the means of optical density (OD) with 7 days intervals from 7^{th} day up to 35^{th} day at 650nm to 665nm (Mckinney 1942) using U-3210 HITACHI-Spectrophotometer.

Table 1: Media Compositions Of Different Culture Media (Chemical component weight is in Gram)

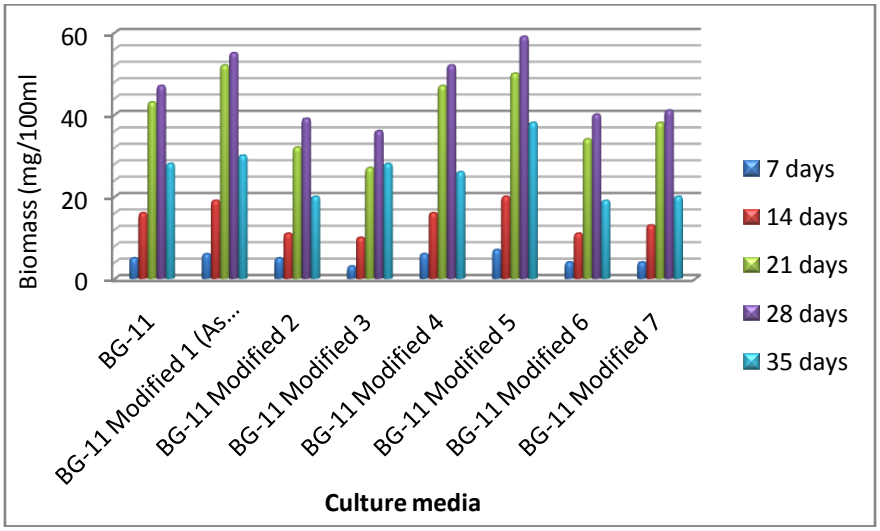
Chemical	BG11	BG11 Modified 1 (As per SHUKLA)	BG11 Modified 2	BG11 Modified 3	BG11 Modified 4	BG11 Modified 5	BG11 Modified 6	BG11 Modified 7
$NaNO_3$	1.5	0.9	00	1.5	3	2.5	1.5	1.5
K_2HPO_4	0.04	0.02	0.04	00	0.04	0.08	0.04	0.04
$MgSO_4.7H_20$	0.075	----	0.075	0.075	0.075	0.095	0.075	0.075
$CaCl_2.2H_2O$	0.036	0.036	0.036	0.036	0.036	0.036	0.036	0.050
Citric Acid	0.006	0.012	0.006	0.006	0.006	0.006	0.006	0.006
Ferric Ammonium Citrate	0.006	0.002	0.006	0.006	0.006	0.006	0.008	0.008
Na_2CO_3	0.02	----	0.02	0.02	0.02	0.02	0.02	0.02
Na EDTA	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
$MgCl_2$	----	0.10	----	----	-----	----	----	----
$NaHCO_3$	----	0.05	----	----	-----	----	----	----
NH_4Cl	----	0.026	----	----	-----	0.015	----	----
Trace metal mix (A4 solution)*	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml

***Trace metal mix(A4 solution):**

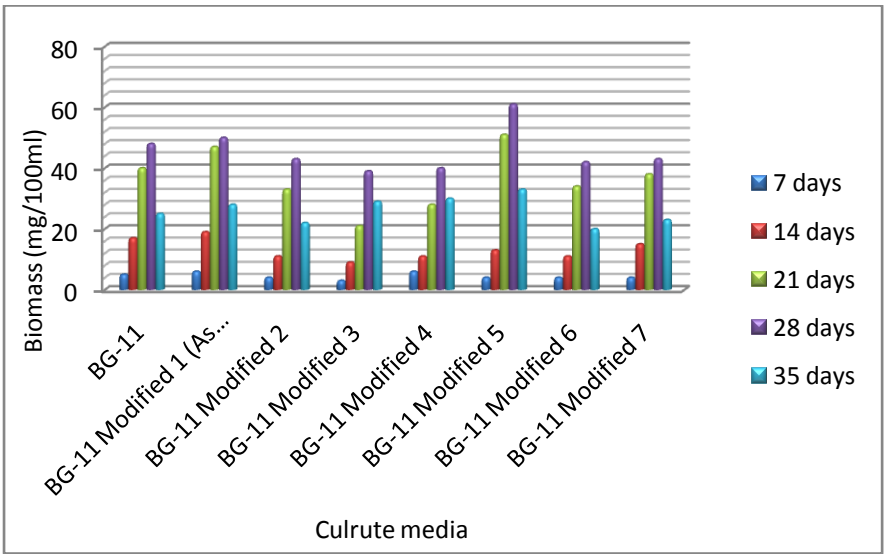
1.Boric acid H_3BO_3-2.86gm
2.Manganese II chloride tetrahydrate$MnCl_2.4H_2O$-1.81 gm
3. Zinc sulphate heptahydrate $ZnSO_4.7H_2O$-0.222 gm
4. Sodium Molybdate Dihydrate $Na_2MoO_4.2H_2O$-0.39 gm
5. Coper Sulphate $CuSO_4$. $5H_2O$- 0.079 gm
6. Cobalt Nitrate $Co(NO3)_2.6H_2O$-0.0494 gm

III. Result

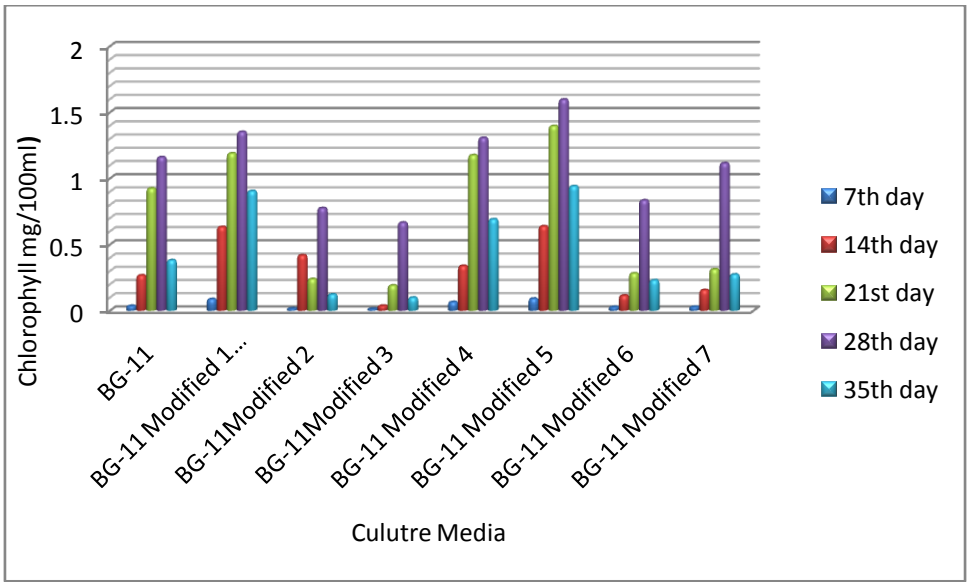
Anabaena spiroides and *Nostoc punctiformae* are very common in the ricefield of Jorhat district as both have the evidence of supplying huge amount of nitrogen in rice field. During the present work it was noticed that different media supported growth of these two strains in varying quantum; however better growth was exhibited by BG11 Modified 5 which showed growth 59mg/100ml (DW) on 28th day in *Anabaena spiroides* and 61mg/100ml (DW) on 28th day in *Nostoc punctiformae*. Both these two species show different growth rate in different culture media. But in general, the optimum growth s of these two strains occurred in between the day period of 14-28th days of culture ,thereafter the growth declined. In case of chl-a the maximum value was shown by *Nostoc punctiformae* 1.6 mg/100ml on 28th day and Anabaena spiroides showed 1.5 mg/100ml on 28th day. These two maximum results were showed on BG11 Modified 5 culture medium. This same medium also showed maximum biomass on dry weight (DW) measurement.



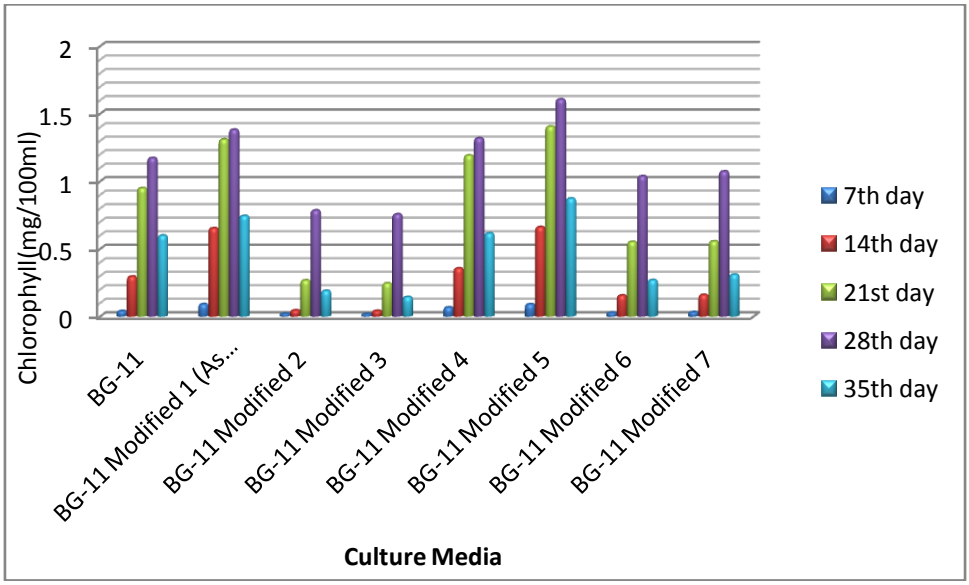
Graph 1: Dry Biomass estimation of *Anabaena spiroides* under different culture media at different intervals of time (mg/100ml).



Graph 2 :Dry Biomass estimation of *Nostoc punctiformae* under different culture media at different intervals of time (mg/100ml).



Graph 3: Chlorophyll estimation of *Anabaena spiroides* under different culture media at different intervals of time (mg/100ml)



Graph 4: Chlorophyll estimation of *Nostoc punctiformae* under different culture media at different intervals of time (mg/100ml)

IV. Discussion

Both the algae *Anabaena spiroides* and *Nostoc punctiformae* have long been used as biofertilizer so it is necessary to find the optimal medium for their biomass accumulation. Optimization of media and culture condition to obtain maximum yield require a large number of experiment. A large number of macro-nutrients including sulphur (S),calcium (Ca), magnesium (Mg) and potassium (K) is essential for algal growth . Micronutrients requirement includes molybdenum (Mo), iron (Fe) nickel (Ni) , copper (Cu), zinc (Zn), cobalt (Co), boron (B), manganese (Mn) and chloride (Cl).The elements like N,P,K, Mg, Ca, S, Fe, Cu, Mn and Zn are essential for algal growth and these elements are added in the form of salts (Oh-Hama and Miyachi,1988; Kaplan et al.,1986) the concentration of these inorganic elements may vary from one medium to another. But the growth of algae, in general depends upon the availability of nitrogen and/or phosphate (Lapointe 1989, Larned 1998, Russ et. al.,1999). In standard BG11 medium the nitrate is present in the form of NaNO3 and nitrate is very much essential for algal growth. In the present study BG11 modified No.5 media the nitrate is present in the form of NaNO3 as well as NH4Cl,this double source of nitrogen may be the cause of high biomass in both the blue green algae *Anabaena spiroides* and *Nostoc punctiformae*. This fact is supported by the study of Singh and Srivastava (1968). In BG11 modified No.5 media along with increase in nitrogen , the

source of phosphate and potassium was also doubled in the element K2HPO4 and this could be cause of high biomass and chlorophyll-a in these two algae. Phosphorous was reported to be essential element for pigment development (Subramanian1982, Subramanian 1989). This fact is also supported by Alahari and Apte where the photosynthetic, nitrogen-fixing cyanobacterium *Anabaena torulosa*, K+ starvation caused impairments in photosynthesis, nitrogen fixation, protein synthesis and consequently inhibited growth (Alahari and Apte, 1998).In BG11 Modified 1(As per Shukla) the source of Mg was replaced by MgCl2 in place of MgSO4 .7H2O and its amount was increased by 10 times than that of normal BG11 media. In this media the amount of chlorophyll was maximum in both the BGA Anabaena spiroides and Nostoc punctiformae. This result must be due to increase amount of Mg, because magnesium is very much essential element of chlorophyll and contributed towards the brilliant blue green colour of the algae.

The increased nutrient resulted in better growth and higher biomass of blue green algae (Miller et. al, 1999). Algae are nutrient limited, their biomass will be increased by nutrient addition. My research concludes that nutrient alone N and P not in combination have different effect on algal biomass.

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