Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN 0976-9595
Short Communication

OPTIMIZATION OF CULTURE MEDIA FOR THE GROWTH OF *ANABAENA PCC550, ANABAENA PCC 574,* AND *CYLINDROSPERMUM PCC518, CYLINDROSPERMUM PCC 567*

Keerthi Patil*, Doris M. Singh[1]
Department of Botany, Karnatak Science College, Dharwad, Karnataka, India
**Corresponding author: keerthipatil9945@gmail.com*

Received: 11-03-2022; Revised: 19-06-2022; Accepted: 02-07-2022; Published: 31-07-2022

 https://doi.org/10.55218/JASR.202213619

ABSTRACT

In the present study, *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567* have been subjected to 7 different inorganic culture media. In order to identify the best growth medium i.e.; optimized medium, the nutrient requirement of these two algae have been evaluated as prime requisite. The present investigation analyzed the growth of wet biomass of the four microalgae. In order to attain optimal growth of Anabaena and Cylindrospermum species, the 7 culture media employed in the current study were (i) BG11 medium (ii) Knoops medium (iii) Cyanophycean agar medium (iv) Modified Bristols medium (v) Prigsheims Medium (vi) Foggs Medium and (vii) Algal culture medium. Highest growth on 60th day was seen in *Cylindrospermum PCC 518* (0.134mg/100ml), *Anabaena PCC 574* (0123mg/100ml), *Cylindrospermum PCC 567* (0.098mg/100ml), *Anabaena PCC* 550 (0.094mg/100ml) in Algal culture media which shows luxurient growth when compared to BG11, Foggs Media, Modified Bristols media, Knoops media. While, Prigsheims medium did not show any growth of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567*.

Keywords: Culture Media, *Anabaena PCC 550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC567*.

1. INTRODUCTION

Cyanobacteria are ancient photosynthetic prokaryotes that are the progenitors of the higher plant chloroplast. They inhabit virtually any environment that contains water and can grow under diverse conditions [1]. These organisms are the originators of photosynthesis and are responsible for generating the planet's original oxygen supply [2, 3]. The order Nostocales includes filamentous cyanobacteria that are capable of cell differentiation in heterocysts, akinetes or reproductive trichomes (hormogonia).

Cyanobacteria (blue-green algae) are a group of photosynthetic prokaryotes, with an oxygenic photosynthesis like plants [4] and a cellular organization similar to that of gram-negative bacteria (Stanier, 1988). Cyanobacteria are a group photosynthetic and nitrogen fixing organisms. They evolved during protozoic era and consisting of nearly 2000 species. They are cosmopolitan in distribution, found in aquatic, terrestrial habitat and even in extreme and unfavorable places like glaciers, desert and hot springs. Some members show symbiotic association with, algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms. They are morpho-logically and physiologically diverse organisms, showed wide range of organization from unicellular forms, colonial forms true branched filamentous forms [5]. Cyanobacteria are found in fresh and marine waters. They produce a diversity of secondary metabolites having potential activity as antimicrobials, antivirals, and as other pharmacologically active compounds [6]. Marine algae are one of the largest producers of biomass in the marine environments. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids, are produced by several species of marine macro and microalgae and have antibacterial, antialgal and antifungal properties which are effective in the prevention of biofouling and have therapeutics uses [7, 8].

The diversity of their specialised metabolites is due to the ability of cyanobacteria to combine genes encoding nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKSs) through enzymatic reactions such as methylations, oxidations, reductions and other chemical

modifications [9, 10]. Cyanobacteria have gained significance as sources of wholesome food materials, nitrogen fixers, natural colorants, bio plastics, biofuels, fine chemicals, bioactive substances, common and fine chemicals like lipids, pigments, enzymes, poly-saccharides, glycerol and other novel biologically active compounds [11, 12]. Cyanobacteria produce different types of pigments, amongst them chlorophyll, phycobiliproteins (c-phycocyanin, callophycocyanin and c-phycoerythrin) and carotenoids are the main pigments. These pigments have commercial value as natural coloring agents, drugs, antioxidant in cosmetic industries, to improve health and fertility of cattle and used in the biomedical research [13, 14].

Cylindrospermum usually inhabits soft, acidic freshwater lakes and is one of the filamentous, heterocystous and non-branched cyanobacteria, classified traditionally in Nostocaceae family. The filaments form fine or compact benthic mats or colonies and may be epiphytic or metaphytic [15]. *Cylindrospermum sp* are employed in agriculture as biofertilizers and soil conditioners. They are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers its application is useful for the reclamation of soils [16]. Cyanobacteria provide a powerful platform for the development of green catalysts that utilize renewable feed stock in the form of atmospheric carbon dioxide (CO_2) and convert it into fuels, commodity chemicals, and value-added products using (sun)light as the energy source [17]. The role of nitrogen fixing cyanobacteria in enhancing soil fertility has been long known [18, 19]. The choice of the medium mainly depends on several factors that include chemical composition of the medium [20]. Various workers have developed different nutrient media to culture *Anabaena and Nostoc*. These two algae are heterocystous cyanobacteria. Demand for replacement of synthetic substances by natural ones has emerged huge opening for study of biopigments. Many bacterial pathogens have acquired resistance against antibiotics hence discovery of new bactericidal agents is growing trend in the field of pharmacology. Bacterial pigments can become the new potent drug in treating life threatening microbial diseases as alternative of synthetic drugs which have many adverse effects on living beings [21]. Nitrogen is one of the major components in every organism body forming components, which are proteins. Cyanobacteria are rich source of structurally novel and biologically active compound. BGA play a potential role in crop protection as herbicides, algaecides, nematicides, fungicides, bactericides and insecticides [22, 23] and release growth promoting substance like Auxins, Gibberlines, Cytokinens and Abscisic acid [24].

2. MATERIAL AND METHODS

The strain of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC567* species were procured from Indian Agricultural Research Institute (IARI), New Delhi.

For the evaluation of suitable media, 07 different media were experimented out on Anabaena PCC550, Anabaena PCC574 and Cylindrospermum PCC518, Cylindro-spermum PCC 567 species i.e, (i) BG11 medium, (ii) Knoops medium (iii) Cyanophycean agar medium (iv) Modified Bristols medium (v) Prigsheims soil water medium (vi) Foggs medium (vii) Algal culture medium. Culture was done according to Thakare et al 2018 [25]. Biomass Estimation was performed according to Richmond and Gobbellar 1986 [26].

Trace metal mix (A5solution):

1. Boric acid H_3BO_3-2.86gm
2. Manganese II chloride tetrahydrate$MnCl_2.4H_2O$-1.81 gm
3. Zinc sulphate heptahydrate $ZnSO_4.7H_2O$-0.31 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 $Co(NO_3)_2.6H_2O$- 49.4mg

3. RESULTS

Anabaena PCC550, Anabaena PCC574 and *Cylindrospermum PCC518, Cylindrospermum PCC567* are very common in the rice field 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 4 strains in different quantity; but better growth was exhibited by algal culture media which shows growth 0.021mg/100ml, 0.015mg /100ml, 0.033mg/ 100ml, 0.029mg/100ml after 15 days in *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC567* respectively. Growth was continuously incresesing i.e, 0.094mg/100ml, 0.138mg /100ml, after 60^{th} day 0.094mg/100ml, 0.123/100ml, 0.134mg/100ml, 0.098mg/100ml and shows comparatively different and less growth rate in BG11 medium i.e, 0.028mg/ml, 0.031mg/ml, 0.036mg /100ml, 0.033mg/100ml in *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC567* respectively. Knoops, Cyanophycean agar, Modified Bristols and Foggs media shows comparatively very less growth. No growth in Prigsheims Medium.

Table 1: Media composition of different culture media (Weight in grams)

Chemical	BG11	Knoops media	Cyanophycean agar media	Modified Bristols media	Prignsheims Media	Foggs Media	Algal culture Media
$NaNO_3$	1.5	-	-	10	-	1.5	1
K_2HPO_4	0.04	-	-	3	-	0.2	0.250
$MgSO_4.7H_2O$	0.075	1	0.100	3	0.010	0.2	0.513
$CaCl_2.2H_2O$	0.036	-	-	1	0.005	0.1	0.058
Citric acid	0.006	-	-	-	-	-	-
Ferric ammonium citrate	0.006	-	1%	-	-	-	-
EDTA	0.001	-	-	-	-	-	-
Na_2CO_3	0.02	-	-	-	-	-	-
Agar	10	-	15	-	-	-	-
Distilled water	1 litre	1 litre	1 litre	1 litre	1 litre	1 litre	-
Calcium nitrate	-	3	-	-	-	-	-
Potassium nitrate	-	1	5	-	0.200	-	-
Potassium phosphate, Dibasic	-	1	-	-	-	-	-
sucrose	-	50	-	-	-	-	-
K_2HPO_4	-	-	-	-	-	-	-
Nacl	-	-	-	1	-	-	-
1% Iron III chloride	-	-	-	1 drop	-	-	-
Prigsheims soil water extract	-	-	-	40ml	-	-	-
Iron 2 chloride	-	-	-	-	0.0005	-	-
Dipotassium hydrogen phosphate	-	-	0.200	-	-	-	-
Ammonium hydrogen phosphate	-	-	-	-	0.020	-	-
Fe-EDTA stock solution	-	-	-	-	-	1ml	-
Ammonium chloride	-	-	-	-	-	-	0.050
Ferric chloride	-	-	-	-	-	-	0.003
Trace metal mix A5solution	1ml	-	-	-	-	1ml	-

Table 2: Wet Biomass estimation in algal culture media (Volume in mg/100ml)

Species	Day 15	Day 30	Day 45	Day 60
Anabaena PCC 550	0.021	0.036	0.062	0.094
Anabaena PCC 574	0.015	0.033	0.057	0.123
Cylindrospermum PCC 518	0.033	0.044	0.068	0.134
Cylindrospermum PCC 567	0.029	0.047	0.073	0.098

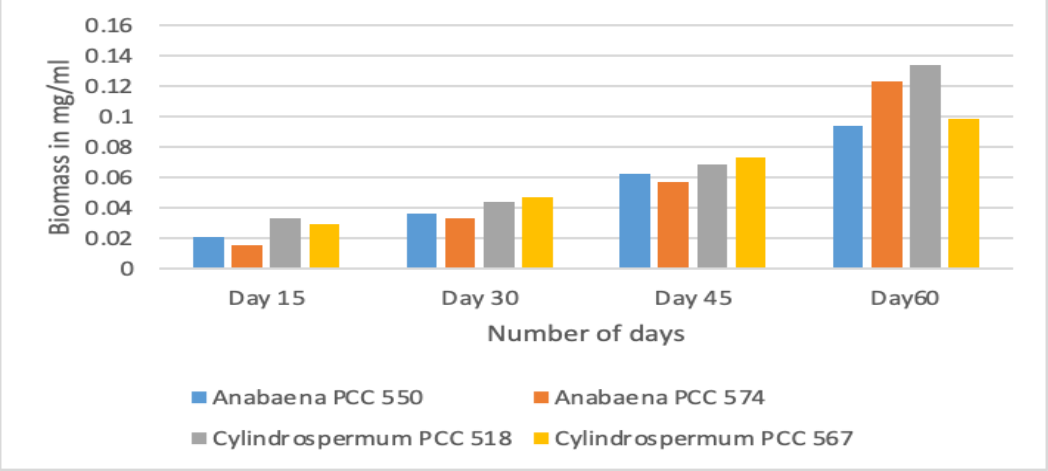

Table 3: Wet Biomass estimation in BG11, Knoops, Cyanophycean agar, Modified Bristols, Foggs, Prigsheims and Algal culture medium

Medium	Growth period in Days															
	15 days				30 days				45days				60days			
	A1	A2	C1	C2	A1	A2	C1	C2	A1	A2	C1	C2	A1	A2	C1	C2
BG11 mg/100ml	0.004	0.003	0.006	0.005	0.010	0.012	0.015	0.014	0.024	0.027	0.032	0.033	0.028	0.031	0.036	0.033
Knoops mg/100ml	-	-	-	-	-	-	-	-	0.002	0.003	0.006	0.005	0.005	0.007	0.010	0.012
Cyanophycean agar mg/100ml	-	-	-	-	-	-	0.002	0.004	0.003	0.002	0.005	0.007	0.006	0.005	0.008	0.010
Modified Bristols mg/100ml	0.002	0.002	0.003	0.004	0.005	0.006	0.007	0.009	0.010	0.013	0.015	0.017	0.015	0.016	0.020	0.021
Foggs mg/100ml	0.003	0.004	0.004	0.006	0.007	0.009	0.010	0.011	0.012	0.014	0.016	0.018	0.016	0.018	0.020	0.022
Prigsheims mg/100ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Algal culture mg/100ml	0.021	0.015	0.033	0.029	0.036	0.033	0.044	0.047	0.062	0.057	0.068	0.073	0.094	0.123	0.134	0.098

4. DISCUSSION

Cyanobacteria have long been used as biofertilizer, so it is necessary to find the optimal medium for their biomass accumulation. Optimization of media for the growth of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567* and culture condition to obtain maximum yield require repeated experiments. 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 [27][28], the concentration of these inorganic elements may vary from one medium to another. In the present study algal culture media nitrate is present in the form of $NaNO_3$ as well as NH_4Cl, this source of nitrogen may be the cause of high biomass in the 4 species studied, *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567*. In algal culture media along with increase in nitrogen, the source of phosphate and potassium was in the element K_2HPO_4 and iron in the form of ferric chloride, this could be cause of high biomass. Phosphorous was reported to be essential element for pigment development [29][30]. In algal culture medium the amount of biomass was maximum in *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567*. The increased nitrate may have resulted in better growth and higher biomass of blue green algae which is in accordance to Miller et al, 1999 [31]. Maximum growth of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC567* I in algal culture media which supplies nutrients like N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn from its high composition of compounds when compared to the BG11, Knoops, Cyanophycean agar, Modified Bristols, Foggs, Prigsheims Media. The optimized culture media is used for mass culture of algae and their potential biochemicals are studied. In future chemical constituents and Phyto-components of *Anabaena PCC550, Anabaena PCC574* and *Cylindrospermum PCC518, Cylindrospermum PCC 567* will be determined and separation, isolation, and characterisation of individual phytocomponents from cyanobacteria could be done in order to find novel medications and their therapeutic effects to cure a variety of illnesses.

5. CONCLUSION

Algal cultue media shows maximum growth of *Anabaena PCC550, Anabaena PCC574 and Cylindrospermum PCC518, Cylindrospermum PCC 567* than BG11 than Prigsheims Media than modified bristol's media than Knoop's media and Foggs media does not show any kind of growth of Anabaena species and Cylindrospermum species.

Conflicts of interest

The authors declare that there is no conflicts of interest.

6. REFERENCES

1. Whitton BA, PottsM. *Springer*, 2002.
2. HamiltonTL, Bryant DA, Macalady JL. *Environ. Microbiol,* 2016; **18:**325-340.
3. 3.Castenholz RW, Waterbury JB. *Bergey's manual of systematic bacteriology*, 1989; 1710-1728.
4. Adams DG, Duggan PS. *New Phytol*, 1999; **144**:3-33.
5. Geethu V, Shamina M. *J. Algal Biomass Utln,* 2018; **9(1)**:26-30.
6. Rao M, Malhotra1 S, Fatma T, Rattan A. *Pharmaceutical Biology,* 2007; **45(2)**:88-93.
7. Bhadury P, Wright PC. *Planta,* 2007; **219**:561-578.
8. Bhagavathy S,Sumathi, Sherene J. *Asian Pacific Journal of Tropical Biomedicine,* 2011; **1(1)**:S1-S7.

9. Gademann K, Portmann C. *Curr. Org. Chem,* 2008; **12:**326-341.
10. Welker M. *Taylor & Francis Group;* Boca Raton, FL, USA, 2008, 825-843.
11. Thajuddin N, Subramanian G. *Current Science Association,* 2005; **89(1):**47-57.
12. Mishra SK, Shrivastav A, Pancha I, Jain D, Mishra S. *International journal of biological macromolecules,* 2010; **47(5)**:597-602.
13. Uzair B, Tabassum S, Rasheed M, Rehman SF. *The Scientific World Journal*, 2012; 1-10.
14. Pandey VD, Pandey A, Sharma V. *Int J Curr Microbiol App Sci.*, 2013; **2**:89-97.
15. Hijova E, Chmelarova A. *Bratisl Lek Listy*, 2007; **108(8)**:354-358.
16. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman, London, 1973.
17. Knoot C, Ungerer J, Wangikar P, Pakrasi H. *Journal of Biological Chemistry,* 2017; **293(14):**1-18.
18. Singh RN. Role of blue-green algae in nitrogen economy of Indian agriculture. New Delhi: Indian Council of Agricultural Research, 1961.
19. Venkataraman GS. *Current Science,* 1981; **50(6):**253-256.
20. Borowitzka MA. Culturing microalgae in outdoor ponds. In: Andersen, R. A. (ed.) Algal culturingtechniques. Oxford, Elsevier Academic Press, 2005, 205-218.
21. Shetti AA, Patil K, Potadar RR, KulkarniBB, Hiremath GB, Hoskeri J, et al. *IOSR-JBB*, 2017; **3(4):**49-52.
22. Deviram GVNS, Pant SG, Prasuna RG. *European J. Exp. Bio*, 2011; **1(4)**:50-54.
23. Biondi NR, Piccardi M, Margheri L, Rodolfi GD, Smith, Tredici MR. *Appl. Environ. Biotechnol,* 2004; **70:**3313-3320.
24. Gupta AB, Agarwal PR. *Ann. Bot.,* 1973; **37(152)**:737-741.
25. Thakare KT, Nadare M, Parbat JM. *Int. J. Curr. Microbiol. App. Sci*, 2018; **7(10)**:2685-2690.
26. Richmond A, Grobbalaar JU. *Biomass,* 1986; **10(4)**:253-264.
27. Oh-Hama T, Miyachi S. *Microalgal biotechnology;* 1990; **47(2):**181-182.
28. Kaplan D, Richman A, Boussiba S, Vonshak A, Cohen Z, Abeliovich A. Solar Energy Research Institute;1986, 184-198.
29. Subramanian G. Proceedings of National Symposium on Biological Nitrogen Fixation: IARI New Delhi,1982,567-587;
30. Subramaniyam MNV, Bhabanarayana PV. *Indian Journal of Marine Science,* 1981; **8:**251-259.
31. Miller MJ, Critchley MM, Hutson J, Fallowfield HJ. *Wat. Res,* 2001; **35(6):**1461-1468.