Journal of Applied Biology & Biotechnology Vol. 3 (04), pp. 038-042, July-Aug, 2015
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2015.3408

Effect of external pH on cyanobacterial phycobiliproteins production and ammonium excretion

Ojit Singh Keithellakpam[1*], Tiwari Onkar Nath[1], Avijeet Singh Oinam[1], Indrama Thingujam[1], Gunapati Oinam[1], Sharma Gauri Dutt[2]

[1]Freshwater Cyanobacterial and Microalgal Repository, Microbial Resources Division, Institute of Bioresources and Sustainable Development (A National Institute of DBT, Govt. of India) Takyelpat, Imphal-795001, Manipur, India. [2]Department of Life Science and Bio-informatics, Hargobind Khurana School of Life Sciences, Assam University, Silchar-788011, Assam, India.

ARTICLE INFO

Article history:
Received on: 06/07/2015
Revised on: 16/07/2015
Accepted on: 02/08/2015
Available online: 24/08/2015

Key words:
Cyanobacteria, Loktak Lake, pH, Phycobiliproteins, Ammonium excretion

ABSTRACT

In the present study, cyanobacterial strains isolated from Loktak Lake were investigated for their possibility of increasing the content of phycobiliproteins and ammonium excretion under different levels of pH. At pH 5.0, maximum amount of PE was observed in *Nostoc* sp. BTA-61 (111.10±9.43 µgmg^{-1}). Maximum PC was expressed in *Phormidium* sp. BTA-1048 (155.17±10.15 µgmg^{-1}). APC content was observed maximum in *Nostoc commune* BTA-67 (81.45±7.68 µgmg^{-1}). At pH 6.0, high PE was observed in *Nostoc* sp. BTA-61 (125.11±11.60 µgmg^{-1}). PC was maximum in *Phormidium* sp. BTA-1048 (168.15±9.58 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (91.41±2.88 µgmg^{-1}). Regarding at pH 7.0, PE was found in high quantity in *Nostoc* sp. BTA-61 (127.91±14.91 µgmg^{-1}). PC was maximum in *Nostoc commune* BTA-67 (128.52±5.66 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (96.40±3.52 µgmg^{-1}). pH 8.0 showed high PE content in *Nostoc* sp. BTA-61 (115.75±6.27 µgmg^{-1}). PC was highest in *Phormidium* sp. BTA-1048 (160.09±10.56 µgmg^{-1}). APC was maximum in *Nostoc commune* BTA-67 (84.15±5.30 µgmg^{-1}). pH 9.0 showed maximum PE content in *Nostoc* sp. BTA-61 (95.39±4.87 µgmg^{-1}). PC was high in *Phormidium* sp. BTA-1048 (148.13±9.20 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (77.43±5.78 µgmg^{-1}). PE, PC and APC concentration was significantly higher (LSD test, p<0.05) in pH 7.0 when compared with the other pH 5.0, 6.0, 8.0 and 9.0. In this study, maximum ammonium excretion was observed in *Nostoc muscorum* BTA-950 in all the different pH and minimum in *Anabaena* sp. BTA-964. The findings indicates that these cyanobacterial strains may be proved as promising microorganism for the phycobiliproteins production and ammonium excretion which could be exploited biotechnologically for the benefit of commercial applications.

1. INTRODUCTION

Cyanobacteria are photoautrophic prokaryotes which are known for their remarkable ability to grow and survive in varying environmental conditions [1]. They are known to produce a variety of water soluble photosynthetic pigments. These pigments could be used as natural colorants for food and cosmetic products as well as molecular fluorescent markers, depending on their purity grade [2]. The use of phycobiliproteins as non-toxic and non-carcinogenic natural food colorants is gaining importance because of the potential toxicity and carcinogenicity of the currently used synthetic food colourants or additives [3]; [4]. The prices of phycobiliproteins vary from US$ 3-25 mg^{-1} for food/cosmetic grade pigments but they can reach US$ 1500 mg^{-1} for highly purified molecular markers (with antibodies or other fluorescent molecules). In 1997, the value of these pigments in the commercial sector was estimated to be US$ 50 million worldwide [5]. Currently, nutraceuticals segment of food industry is exploring. This market is growing at 5% per annum and is estimated at between $6 billion USD and $60 billion USD in today's global market [6]. The utilizing cyanobacteria as an efficient source of biofertilizer for rice field has been practised and adopted in India [7]. Among the different soil factors affecting the survival of cyanobacteria, pH is particularly important directly influencing cyanobacterial distribution and abundance in soil [8]. Use of biofertilizers is cost-effective, cheap and renewable source to supplement the chemical fertilizers. pH plays a very important role in the metabolic and physiological activities of cyanobacteria. It strongly affects biomass production, chemicals dissociation and cell physiology. As with any other living cells, cyanobacterial cell function depends on the maintenance of a narrow range of survivable intracellular pH [9]. Cyanobacteria are capable of maintaining a constant internal pH of 7.1-7.5 in the range of external pH from 5.0 to 10 [10].

** Corresponding Author*
Ojit Singh Keithellakpam, Microbial Resources Division Institute of Bioresources and Sustainable Development (A National Institute of DBT, Govt. of India) Takyelpat, Imphal-795001, Manipur, INDIA
Email: ojit1999@gmail.com

Research on cyanobacterial pigment production originating in different habitats and effects of environmental factors on its content could contribute on the understanding of cyanobacteria-specific acclimation processes and responses to changes of environmental conditions. On the other hand, it could be very important for optimization of cyanobacterial production of biotechnologically valuable chemicals, such as pigments [11]; [12]. In the work presented here, we analyse the effect of different levels of pH in phycobiliproteins production and ammonium excretion of the cyanobacterial strains isolated from freshwater habitats of Loktak Lake.

2. MATERIALS AND METHODS

2.1 Cyanobacterial strains

Cyanobacterial strains used in the present study were isolated from freshwater habitats of Loktak Lake located in Manipur, India and is the largest freshwater wetland in the North-Eastern region of India. They were identified referring to monograph [13] and were deposited to Freshwater Cyanobacterial and Microalgal Repository (National facility created by the Department of Biotechnology, Government of India with reference No. BT/PR 11323/PBD/26/171/2008 dated 31-03-2009), Institute of Bioresources and Sustainable Development (IBSD), Imphal, Manipur, India.

2.2 Growth conditions

Cyanobacterial strains were optimized for its growth and high yield production of phycobiliproteins and ammonium excretion from it. In order to assess the influence of pH on the phycobiliprotein production and ammonium excretion, the pH of the BG-11 medium [14] was adjusted at 5.0, 6.0, 7.0, 8.0 and 9.0 using 1N HCl and 1N NaOH. Dried cyanobacterial biomass of 10 mg was inoculated into 100 ml of sterile BG-11 medium in 150 ml cotton-plugged Erlenmeyer flask. Experimental strains were maintained at 28±2ºC under illumination by cool white fluorescent light of intensity 54-67 µmol photons $m^{-2}s^{-1}$ with a 14:12 h light:dark conditions.

2.3 Phycobiliproteins estimation

The content of phycobiliproteins (PC, PE, and APC) was determined using a spectrophotometric method [15].

2.4 Ammonium determination

The amount of ammonium concentration in the medium was measured by Solorzano's phenol-hypochlorite method [16].
All the experiments were carried out in triplicates and the data were presented as mean values ± SD of three replicates.

3. RESULTS AND DISCUSSION

Quantitative analysis of PE, PC and APC against different levels of pH was presented (Fig 1a-1j). At pH 5.0, maximum amount of PE was observed in *Nostoc* sp. BTA-61 (111.10±9.43 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA950 (0.13±0.09 µgmg^{-1}). Maximum PC was expressed in *Phormidium* sp. BTA-1048 (155.17±10.15 µgmg^{-1}) whereas minimum was observed in *Nostoc muscorum* BTA950 (0.75±0.03 µgmg^{-1}). APC content was observed maximum in *Nostoc commune* BTA-67 (81.45±7.68 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (0.32±0.07 µgmg^{-1})

Analysis in pH 6.0, high PE was observed in *Nostoc* sp. BTA-61 (125.11±11.60 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (1.00±0.11 µgmg^{-1}). PC was maximum in *Phormidium* sp. BTA-1048 (168.15±9.58 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (4.29±0.65 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (91.41±2.88 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (2.17±0.12 µgmg^{-1}).

At pH 7.0, PE was found in high quantity in *Nostoc* sp. BTA-61 (127.91±14.91 µgmg^{-1}) and minimum in *Phormidium* sp. BTA-52 (2.11±0.43 µgmg^{-1}). PC was maximum in *Nostoc commune* BTA-67 (128.52±5.66 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (8.79±0.19 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (96.40±3.52 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (5.90±0.69 µgmg^{-1}).

At pH 8.0, high PE content was expressed in *Nostoc* sp. BTA-61 (115.75±6.27 µgmg^{-1}) and minimum in *Phormidium* sp. BTA-52 (1.05±0.26 µg mg^{-1}). PC was highest in *Phormidium* sp. BTA-1048 (160.09±10.56 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (3.85±0.97 µgmg^{-1}). APC was maximum in *Nostoc commune* BTA-67 (84.15±5.30 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (1.25±0.15 µgmg^{-1}).

At pH 9.0, maximum PE was observed in *Nostoc* sp. BTA-61 (95.39±4.87 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (0.08 ± 0.02 µgmg^{-1}). PC was high in *Phormidium* sp. BTA-1048 (148.13±9.20 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (0.63±0.08 µgmg^{-1}). APC was highest in *Nostoc commune* BTA-67 (77.43±5.78 µgmg^{-1}) and minimum in *Nostoc muscorum* BTA-950 (0.11±0.09 µgmg^{-1}). PE, PC and APC concentration was significantly higher (LSD test, $p<0.05$) in pH 7.0 when compared with the other pH 5.0, 6.0, 8.0 and 9.0.

In this study, maximum ammonium excretion was observed in *Nostoc muscorum* BTA-950 in all the different pH and minimum in *Anabaena* sp. BTA-964 when compared to all the other cyanobacterial strains and were presented (Fig 2). In order to determine the impact of pH on phycobiliproteins production and ammonium excretion, cyanobacterial strains were exposed to different levels of pH 5.0-9.0. Among the different levels of pH applied, pH 7.0 was found to be the best when compared to all the other four pH. At pH-5.0 and 9.0, the strains did not show proper growth. The increase in external pH 7.0-9.0 significantly increased the total phycobiliprotein content in *Nostoc* sp. UAM 206 [17]. The content of pigments varied to a great extent depending on the strain and also on growth conditions. Although *Nostoc* and *Anabaena* are phylogenetically very close in origin [18], differences in their pigment content between the strains of both are evident, indicating a strain-specific property. Responses of microalgae to variation of environmental conditions could be done through cell changes or modulations of metabolism, and cellular

compositions (acclimation response) or responses could be expressed through maintaining a balanced cell composition (homeostatic responses) [19]. Actually extreme buffer's pH cause internal electrostatic attraction by changing the charge on protein giving net positive charge and at this stage protein open up and bound solvent is lost, resulting denaturation of protein [20].

Accumulation of ammonia by living cells is highly dependent on pH. However, it could comfortably grow over the pH range of 6.0-8.0. The data was in good agreement with the previous reports [21]; [22]. However, when the pHex (external pH) fell below a certain value there was a significant decline in pHi (internal pH), suggesting that acidic sensitivity might be

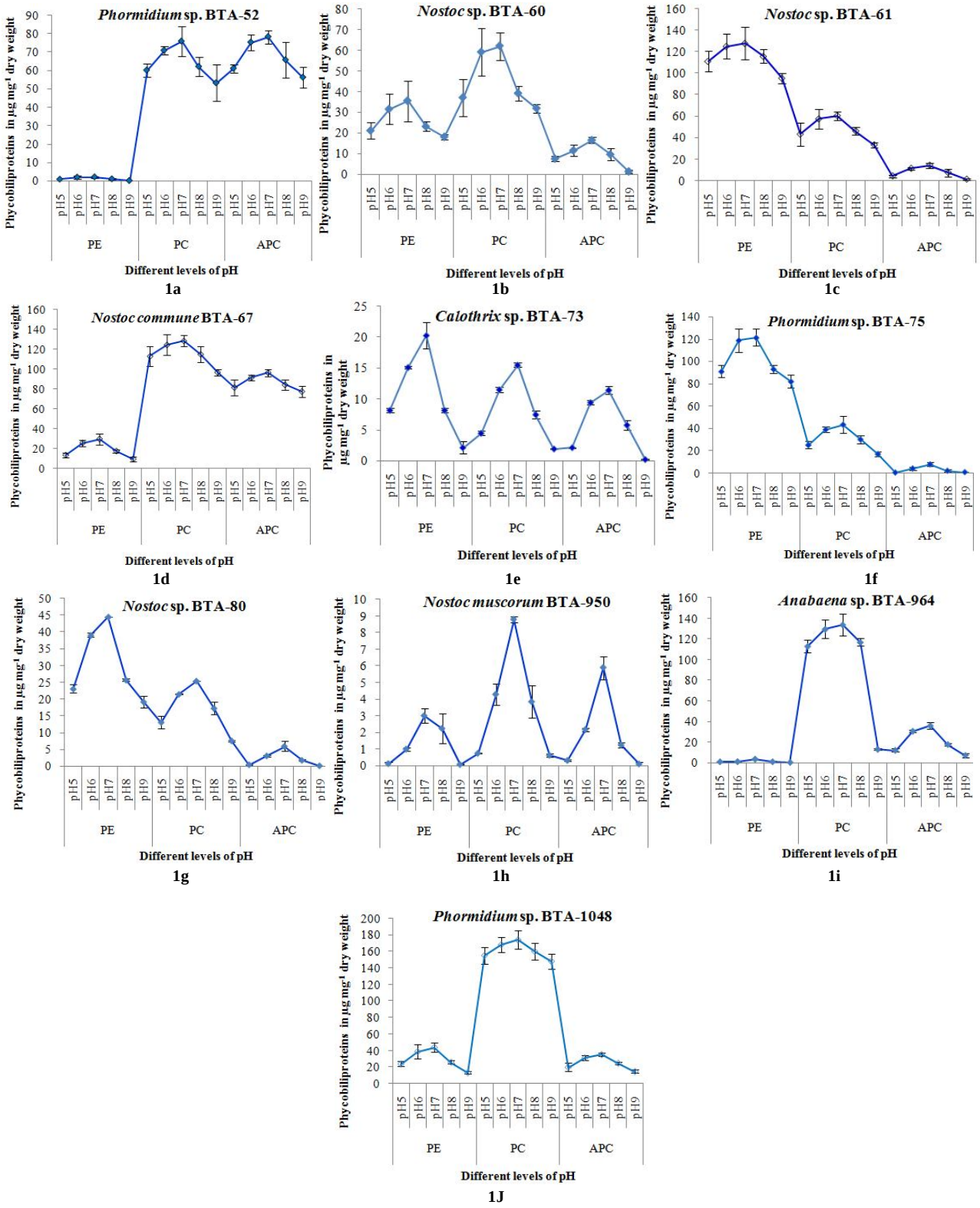

Fig. 1a-1j: Effect of different pH levels on phycobiliproteins production.

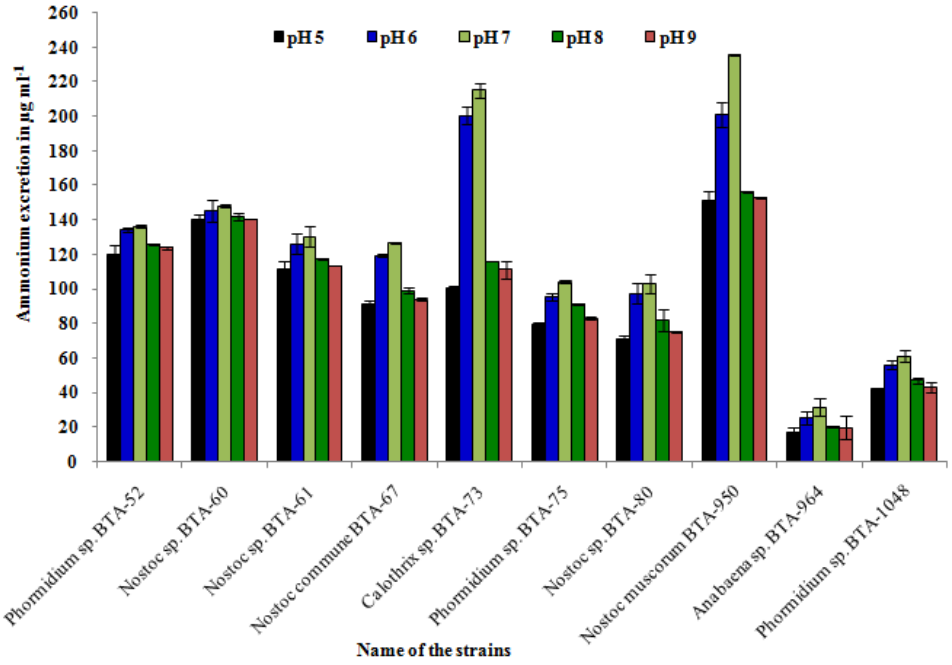

Fig. 2: Effect of different levels of pH on extracellular ammonium excretion.

related to the limited ability of the cyanobacterium to regulate the internal pH as the external pH decreases. Changes in pH affect various processes such as solubility and bioavailability of nutrients [23], transport of substances across the cytoplasmic membranes, activity of both intra and extracellular enzymes, photosynthetic electron transport and the osmotic potential of the cytoplasm [24]. Low external pH values may therefore limit cyanobacterial growth by lowering the intracellular pH, by increasing the maintenance energy requirement, by affecting solute transport, or by affecting cell membrane or cell wall biosynthesis [25]. In this study, the observed pH optimum value for the maximum production of phycobiliprotein is consistent with the values reported for the cyanobacteria *Synechocystis* [26] and *Anabaena* NCCU-9 [27]. Among other consequences, an internal pH drop could lead to the pheophytinization (the displacement of Mg^{2+} by $2H^{+}$) of chlorophyll [28]. The phycobiliproteins produced in the present was found comparable with other reported cyanobacterial strains by [29].

4. CONCLUSIONS

The findings indicates that cyanobacterial strains of the present study may be proved as promising microorganism for the industrial applications as source of natural biopigments and biofertilizers. Changes in growth conditions could influence secondary metabolites content in these strains, and a high amount of phycobiliproteins and ammonium excretion can be achieved by combining appropriate factors. Cyanobacteria are imperative source of biopigments that may be exploited biotechnologically for the pharmaceutical industries. From this study, it can be concluded that these cyanobacterial strains from Loktak Lake could be led to further additional research at the genetic level as a potential platform which can be exploited at commercial purposes.

5. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support by Indian Council of Agricultural Research (ICAR), Govt. of India. Thanks to Director, IBSD-DBT, for providing required infrastructure. The authors thank the labmates for the kind help during this research work.

6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7. REFERENCES

1. Bhaya D, Schwarz R, Grossman AR. Molecular responses to environmental stress. In: Whitton BA, Potts M, editors. The ecology of cyanobacteria: their diversity in time and space, New York: Springer; 2000, p. 397-442.
2. Chu WL. Biotechnological applications of microalgae. International e-Journal of Science, Medicine and Education. 2012; 6:24-37.
3. Cohen Z. Products from microalgae. In: Richmond A, editor. CRC handbook of microalgae mass culture, Boca Raton, Florida, USA: CRC Press; 1986, p. 421-454.
4. Chaneva G, Furnadzhieva S, Minkova K, Lukavsky J. Effect of light and temperature on the cyanobacterium *Arthronema africanum*- a prospective phycobiliprotein-producing strain. Journal of Applied Phycology. 2007; 19:537-544.
5. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. Journal of Bioscience and Bioengineering. 2006; 101:87-96.

6. Rodriguez-Sanchez R, Ortiz-Butron R, Blas-Valdivia V, Hernandez-Garcia A, Cano-Europa E. Phycobiliproteins or C-phycocyanin of *Arthrospira* (*Spirulina*) *maxima* protect against $HgCl_2$-caused oxidative stress and renal damage. Food Chemistry. 2012; 135(4):2359-2365.
7. Venkataraman GS. Blue green algae: a possible remedy to nitrogen scarcity. Current Science. 1981; 50:253-256.
8. Sardeshpande JS, Goyal SK. Effect of pH on growth and nitrogen fixation by blue green algae. Phykos. 1981; 20:107-113.
9. Giraldez-Ruiz N, Mateo P, Bonila I, Fernandez-Pinas F. The relationship between intracellular pH, growth characteristics and calcium in the cyanobacterium *Anabaena* sp. strain PCC7120 exposed to low pH. New Phytologist. 1997; 137:599-605.
10. Ritchie RJ. Membrane potential and pH control in the cyanobacterium *Synechococcus* R- 2 PCC7242. Journal of Plant Physiology. 1991; 137:409-418.
11. Eriksen NT. Production of phycocyanin-a pigment with applications in biology, biotechnology, foods and medicine. Applied Microbiology and Biotechnology. 2008; 80:1-14.
12. Gupta A, Sainis JK. Isolation of C-phycocyanin from *Synechococcus* sp. (*Anacystis nidulans* BD1). Journal of Applied Phycology. 2010; 22:231-233.
13. Desikachary TV. Cyanophyta. Monograph on Algae. New Delhi, India: ICAR; 1959.
14. Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriology Reviews. 1971; 35(2):171-205.
15. Bennett A, Bogorad L. Complementary chromatic adaptation in a filamentous blue-green alga. Journal of Cell Biology. 1973; 58(2):419-435.
16. Solorzano L. Determination of ammonia in natural waters by the phenol hypochlorite method. Limnology and Oceanography. 1969; 4:799-801.
17. Poza-Carrion C, Fernadez-Valiente E, Fernadez-Pinas F, Leganes F. Acclimation of photosynthetic pigments and photosynthesis of the cyanobacterium *Nostoc* sp. Strain UAM 206 to combined fluctuations of irradiance, pH and inorganic carbon availability. Plant Physiology. 2001; 158:1455-1461.
18. Schirrmeister BE, Antonelli A, Bagheri CH. The origin of multicellularity in cyanobacteria. BMC Evolutionary Biology. 2011; 11:45.
19. Montechiaro F, Hirschmugl CJ, Raven JA, Giordano M. Homeostasis of cell composition during prolonged darkness. Plant, Cell and Environment. 2006; 29:2198-2204.
20. Roe S. Protein purification techniques: A practical approach. Oxford, UK: Oxford University Press; 2001.
21. Ripkka R, Dereulles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology. 1979; 111:1-61.
22. Rippka R. Provisional generic assignments of cyanobacteria in pure cultures. In: Starr MP, Stolp H, Truper HC, Balows-Stolp H, Schlegel HG, editors. The prokaryotes, Verlag, Berlin, Heidelberg, New York: Springer; 1981, p. 247-256.
23. Stumn W, Morgan JJ. Aquatic chemistry. 2nd ed. New York: Wiley; 1981.
24. Walsby AE. Cell-water and cell-solute relations. In: Carr NG, Whitton BA, editors. The biology of cyanobacteria, Oxford: Blackwell Science Publications; 1982; p. 237-262.
25. Kallas T, Castenholz RW. Internal pH and ATP-ADP pools in the cyanobacterium *Synechococcus* sp. during exposure to growth-inhibiting low pH. Journal of Bacteriology. 1982a; 149(1):229-236
26. Hong SJ, Lee CG. Statistical optimization of culture media for production of phycobiliprotein by *Synechocystis* sp. PCC6701. Biotechnology and Bioprocess Engineering. 2008; 13:491-498.
27. Hemlata, Fatma T. Screening of cyanobacteria for phycobiliproteins and effect of different environmental stress on its yield. Bulletin of Environmental Contamination and Toxicology. 2009; 83:509-515.
28. Brock TD. Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science. 1973; 179:480-483.
29. Ranjitha K, Kaushik BD. Purifications of phycobiliproteins from *Nostoc muscorum*. Journal of Scientific and Industrial Research. 2005; 64:372-375.

How to cite this article:

Ojit S.K., Tiwari O.N., Avijeet S.O, Indrama Th., Gunapati O., Sharma G.D. Effect of external pH on cyanobacterial phycobiliproteins production and ammonium excretion. J App Biol Biotech. 2015; 3 (04): 038-042.