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Characterization of growth of marine cyanobacterium *Phormidium fragile* JN 112 under different regimes of pH, light intensity and media composition

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Abstract
Optimization of culture collections for the mass cultivation of a fast growing filamentous marine cyanobacterium *phormidium fragile* JN112 was evaluated for pH, light intensity, nitrogen and phosphate concentration. Growth of the cyanobacterial isolate was measured at four different values each of pH (7.5, 8.0, 8.5 and 9.0) light intensity (30, 60, 90 and 120 µmol $m^{-2}s^{-1}$), nitrogen (0.075, 0.100, 0.125 and 0.150 g/L) and phosphate (0.02, 0.03 0.04 and 0.05 g/L). Growth determined in terms of protein and chlorophyll content for *P. fragile* JN112 indicated the preference of near neutral pH. Observation of cyanobacterial growth at higher pH values indicated the ability of *P. fragile* JN112 to tolerate high alkalinity. The maximum growth of *P. fragile* JN112 was determined at light intensity value of 30 µmol $m^{-2}s^{-1}$, nitrogen value of 0.1 g/L and phosphate value of 0.02 g/L.

Keywords: Cyanobacteria, pH, nutrients, light intensity, chlorophyll, protein.

Introduction
Marine cyanobacteria have emerged as an important source of high value products, as well as crucial bioactive and biotechnologically significant chemicals with potential biotechnological applications [1]. Biotechnological application of cyanobacteria, besides other parameters, is achieved only on having more of its biomass. The growth and biomass production of marine cyanobacteria are influenced by number of factors and for the successful cultivation and growth of cyanobacteria it is necessary to optimize the culture media composition and other physico-chemical parameters [2, 3]. The growth, biochemical composition and pigments of marine cyanobacteria vary from species to species and are affected by various physicochemical parameters [4].
Nitrogen is considered as one of the most important macronutrients for cyanobacterial growth and pigments synthesis since it is a fundamental element for the proteins and chlorophyll [5]. Both nitrogen source and its concentration in the media can be responsible for the changes in chlorophyll contents, proteins, lipids, carbohydrates and biomass productivity [6]. On the other hand, oversupplying the culture with nutrients may have a negative impact on the biomass, especially under un-controlled pH condition, since the pH determines the availability and solubility of the nutrients [7].
Phosphorus is an important component required for normal growth and development of algal cells [8]. Environmental factors such as temperature, light, pH, and nutrients not only affect photosynthesis and the growth rate of the algae, but also influence the activity of cellular metabolism and composition. [9] have made a detailed survey of the Southern east coast of India and reported a number of marine cyanobacterial species.
In this context, understanding the chemical and abiotic variables that influence the growth of marine cyanobacteria is important. In this study, pH, light intensity and media composition requirement of the marine cyanobacterium *Phormidium fragile* JN112 was characterized for its ability to produce chlorophyll and protein to evaluate the growth.

Materials and methods
Experimental organism and culture conditions
A fast growing, filamentous marine cyanobacterium *Phormidium fragile* JN112 was obtained from the germplasm collections of Department of Botany, Jamal Mohamed College, Tiruchirappalli, India. The cyanobacterial isolate was cultivated and maintained in ANS-III

growth medium [2] at 25 ± 2°C, 14/10-h light/dark cycle, with illumination of 20.15 µmol m^{-2} s^{-1} under cool white fluorescent lamps (Philips).

Growth characterization of cyanobacteria

The growth of the chosen cyanobacterial isolate was characterized with ASNIII medium. The pH, light intensity and concentrations of nitrogen and phosphate were manipulated keeping all other conditions the same. The pH of the growth medium was adjusted to 7.5, 8.0, 8.5 and 9.0; and light intensity to 30, 60, 90 and 120 µmol/$m^{-1}s^{-1}$. Nitrogen as sodium nitrate was provided at 0.075, 0.100, 0.125 and 0.150 g/L) and phosphate as K_2HPO_4 was supplied at 0.02, 0.03, 0.04 and 0.05 g/L. A known amount of inoculum was added to a 250 ml Erlenmeyer flask containing 100 ml of medium adjusted to respective pH, light intensity, nitrate and phosphate concentrations. The cultures were manually stirred every day. Cultures were maintained in triplicates. On 7th day, the cultures were harvested by centrifugation to measure the growth as increment in chlorophyll and total protein contents. Chlorophyll *a* was determined following the method of [10] and total protein by [11].

Statistical analysis

All the experiments were conducted in triplicate and the data calculated were expressed as mean± standard error using Origin pro software version 8.1.

Results

Growth of marine cyanobacterium at different pH conditions, determined in terms of protein and chlorophyll, are shown in Fig. 1. The marine cyanobacterium *Phormidium fragile* JN112 showed more growth near neutral pH both in terms of proteins and chlorophyll *a* content. Growth at other pH conditions viz. 8.0, 8.5 and 9.0 were found to be less for proteins and chlorophyll.

Light intensity variation on the growth of *Phormidium fragile* JN112 measured in terms of protein and chlorophyll content exhibited higher values at 30 µmol $m^{-2}s^{-1}$ while at other light intensity values such as 60, 90 and 120 µmol $m^{-2}s^{-1}$, a lower level of growth was noticed. The level of protein and chlorophyll content was observed at 120 µmol $m^{-2}s^{-1}$ (Fig. 2).

Variation of concentration of sodium nitrate as a source of nitrogen to *phormidium fragile* JN112 revealed more protein content at 0.1 and 0.125 g/L compared to other concentrations used. The variation of protein content between 0.1 and 0.125 g/L was found to negligible. The lowest level of protein content was recorded at 0.15 g/L $NaNO_3$. In terms of chlorophyll, the growth of *Phormidium fragile* JN112 did not vary between the nitrogen concentrations used. However, the maximum chlorophyll content was recorded at 0.075 g/L and the minimum at 0.15 g $NaNO_3$/L (Fig. 3).

Variation of phosphate concentration exhibited a differential response of growth in *P. fragile* JN112. In terms of protein, the marine cyanobacterium *P. fragile* JN112 showed more growth at 0.02 g/L and lesser level of growth at 0.04 and 0.05 g/L. While the growth level was moderate at 0.03 g K_2HPO_4/L. In terms of chlorophyll, maximum growth was recorded at 0.02 g/L and minimum growth at other concentrations used. Growth of *P. fragile* JN112, at 0.03, 0.04 and 0.05 g K_2HPO_4/L was more or less the same.

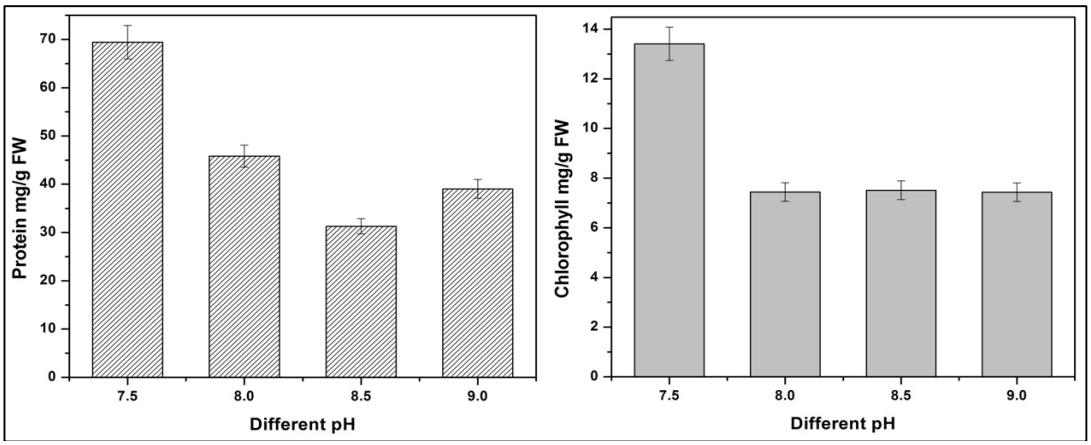

Fig 1: Effect of pH variation on the growth of *Phormidium fragile* JN112, measured in terms of protein and chlorophyll *a* contents.

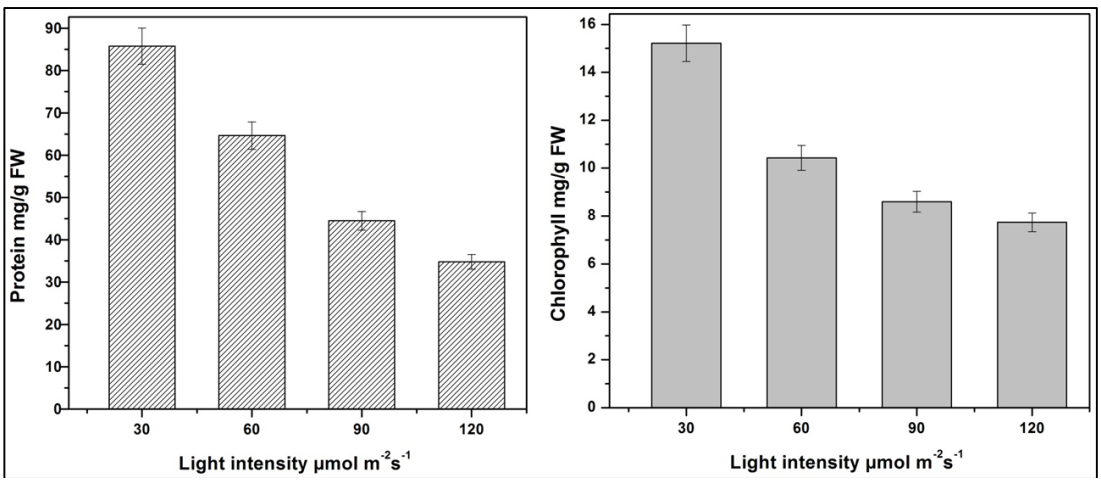

Fig 2: Effect of light intensity variation on the growth of *Phormidium fragile* JN112, measured in terms of protein and chlorophyll *a* contents.

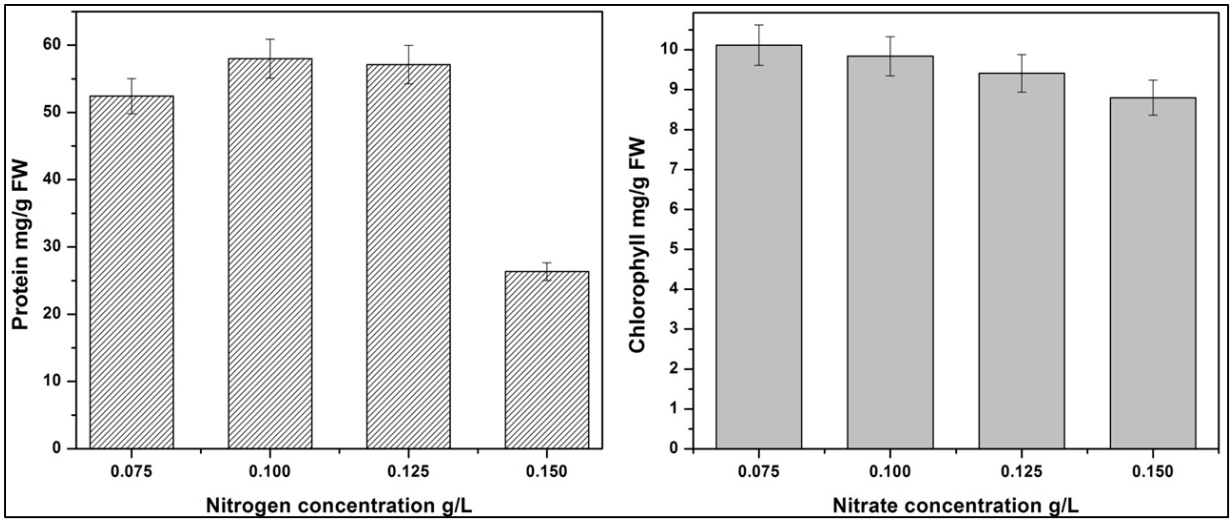

Fig 3: Effect of variation of nitrate nitrogen concentration on the growth of *Phormidium fragile* JN112, measured in terms of protein and chlorophyll *a* contents.

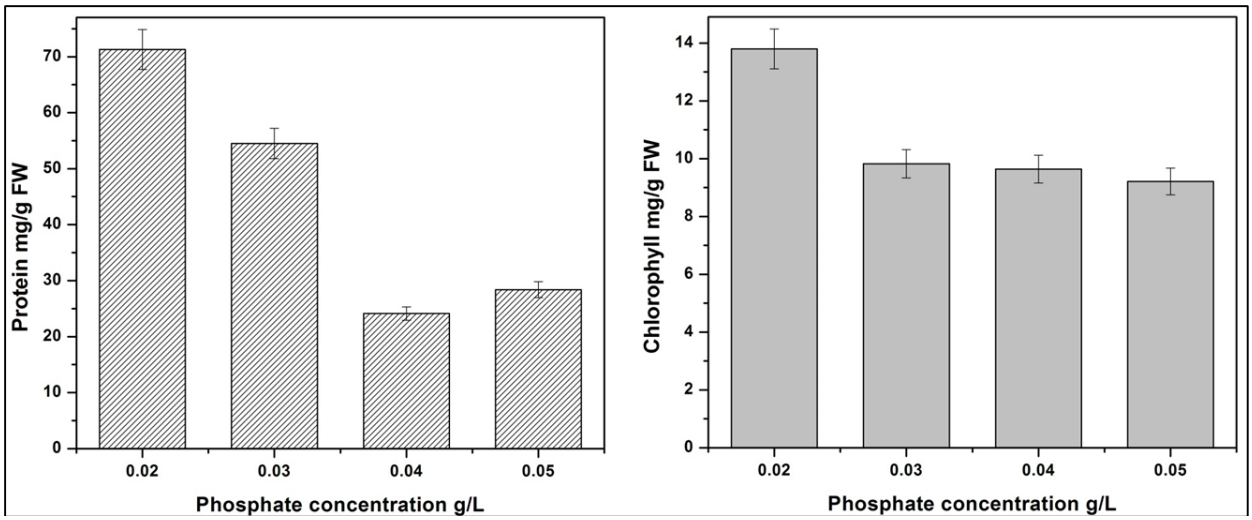

Fig 4: Effect of variation of phosphate concentration on the growth of *Phormidium fragile* JN112, measured in terms of protein and chlorophyll *a* contents.

Discussion

High biomass production of microalgae or cyanobacteria could be achieved through the formulation of a suitable culture medium [12] as the non-optimized media could not support. To begin with, information on pH requirement of cyanobacteria is very important for mass culture for industrial purposes. In the present investigation, the favoured pH for the growth of *Phormidium fragile* JN112 was found to 7.5. Earlier literature indicated that pH value lower or higher than 7.5 was associated with decreased growth [13]. Light intensity is the major factor of photosynthetic organisms [14]. Results of this study indicated that light intensity at 30 µmol $m^{-2}s^{-1}$, supported more growth both in terms of protein and chlorophyll content in *P. fragile* JN112, compared to other high light intensity levels studied. Earlier reports indicate that in algal cultures, high cell density decreases the efficiency of light energy utilization due to mutual shading effect [15]. Light delivery and distribution in high-density cultures are always a challenge. Nitrogen is an important element required for the growth and synthesis of proteins and other cellular components.

In the culture media optimization strategy, N and P concentrations are optimized before all other nutrients because when N and P are available in the culture media so that no other nutrient would become limiting for the growth [12]. Phosphate is also essential to algal growth because they are the sources for phosphorous that is required in almost all cellular processes. In the present investigation, N showed more growth at 0.1 g/L and P exhibited more growth at 0.02 g/L while, the other P concentration showed reduced growth. The results of reduced level of growth of *P. fragile* JN112 at elevated concentrations of P corroborates with the result of high levels of phosphorous inhibiting the growth of Anacysts variabilis [16].

Conclusion

In conclusion, the present study investigated the effect of pH, light intensity, nitrate and phosphate concentrations of ASNIII culture medium on the growth of the marine cyanobacterial species *phormidium fragile* JN 112 for optimizing the culture media. On the basis of our findings, it is concluded that the lower values of pH, light intensity, nitrogen and phosphate concentrations used, exhibited maximum growth of *P. fragile* JN112 in terms of chlorophyll and protein contents.

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References

1. Begum H, Yusoff FM, Banerjee S, Khatoon H, Shariff M. Availability and utilization of pigments from microalgae. Critical reviews in food science and nutrition. 2016; 56(13):2209-22.
2. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology. 1979; 111(1):1-61.
3. Allen MB, Arnon DI. Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. Plant Physiology. 1955; 30(4):366.
4. Manirafasha E, Ndikubwimana T, Zeng X, Lu Y, Jing K. Phycobiliprotein: potential microalgae derived pharmaceutical and biological reagent. Biochemical Engineering Journal. 2016; 109:282-96.
5. Khazi MI, Demirel Z, Dalay MC. Evaluation of growth and phycobiliprotein composition of cyanobacteria isolates cultivated in different nitrogen sources. Journal of applied phycology. 2018; 30(3):1513-1523.
6. Fábregas J, Abalde J, Herrero C. Biochemical composition and growth of the marine microalga *Dunaliella tertiolecta* (Butcher) with different ammonium nitrogen concentrations as chloride, sulphate, nitrate and carbonate. Aquaculture. 1989; 83(3-4):289-304.
7. Mahmood HM, Abed IJ, Mahmood KH. Al-Mashhadani. Evaluating harvesting of Chlorella sp. biomass and chemical composition under the influence of different concentrations of nutrients. Current Research in Microbiology and Biotechnology. 2017; 5(6)1:375-1379
8. Hu Q. Environmental effects on cell composition. Handbook of microalgal culture: biotechnology and applied phycology. 2004; 1:83-93.
9. Thajuddin N, Subramanian G. Survey of cyanobacterial flora of the southern east coast of India. Botanica Marina. 1992; 35(4):305-14.
10. Mackinney G. Absorption of light by chlorophyll solutions. J biol. Chem. 1941; 140(2):315-22.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry. 1951; 193:265-75.
12. Fábregas J, Domínguez A, Regueiro M, Maseda A, Otero A. Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. Applied Microbiology and Biotechnology. 2000; 53(5):530-5.
13. Rai SV, Rajashekhar M. Effect of pH, salinity and temperature on the growth of six species of marine phytoplankton. Journal of Algal Biomass Utilization. 2014; 5(4):55-9.
14. Hong SJ, Lee CG. Statistical optimization of culture media for production of phycobiliprotein by *Synechocystis* sp. PCC 6701. Biotechnology and Bioprocess Engineering. 2008; 13(4):491-8.
15. Lee CG. Calculation of light penetration depth in photobioreactors. Biotechnology and bioprocess engineering. 1999; 4(1):78-81.
16. Battah MG, Shabana EF, Kobbia IA, Eladel HM. Differential effects of thiobencarb toxicity on growth and photosynthesis of Anabaena variabilis with changes in phosphate level. Ecotoxicology and environmental safety. 2001; 49(3):235-9.