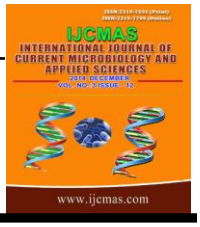
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 12 (2014) pp. 173-182**
http://www.ijcmas.com



Original Research Article

Influence of aeration and light on biomass production and protein content of four species of marine Cyanobacteria

Indira Priyadarshani[1], Nooruddin Thajuddin[2] and Biswajit Rath[1]*

[1]P.G.Department of Biotechnology, North Orissa University, Takatpur, Baripada, Mayurbhanj, Odisha, India, 757003
[2]Department of Microbiology,Bharathidasan University,Tiruchirapalli-620024
**Corresponding author*

A B S T R A C T

The present investigation depicts the influence of aeration and light intensity on biomass production and protein biosynthesis in four species of marine cyanobacteria isolated from Odisha coast. The biomass and protein content of aerated cultures of the organism were compared with non-aerated cultures after 7 days and 14 days incubation period supplemented with moderate light intensity. The results as obtained from the present investigation infers that all the test species showed increase in biomass and protein content in aerated culture condition as compared to non aerated cultures. However, supplementing the cultures with artificial illumination provides a promising result on both aerated and non aerated cultures. The aeration and artificial illumination (medium light intensity) together significantly increases the biomass and protein content of all the test species. Thus the present results indicates that not only aeration or illumination alone is suitable for mass culture of the test organism, rather the combination of both these factors found to be suitable for mass culture of the organisms for isolation of novel bioactive compounds.

Keywords

Aeration, Biomass, Cyanobacteria, Light, Protein

Introduction

Microalgae are free-living photoautotrophic microorganisms that can derive energy from sunlight and carbon from the air. Microalgae including cyanobacteria are wide spread in many ecosystems including polluted ecosystems. Cyanobacteria usually only used as natural feed, single cell protein, pharmacies and also used as an alternative energy source producing biofuel. Cyanobacteria have been found in various aquatic habitats including marine, fresh water, hot spring, hypersaline environments and ice (Castenholz, 2001), and play an ecological role as major contributors to photosynthesis in most environments (Whitton and Potts, 2000). Some important factors affecting the growth of algae are light, temperature, pH, buoyancy, nutrients and biological factors.

Aeration is a management technique that involves introducing dissolved oxygen into a

lake. In some lakes, aeration can help increase dissolved oxygen content, control algae growth, decrease internal recycling of phosphorus and convert ammonium to nitrate (Cooke *et al.,* 2005). Aeration can reduce internal phosphorus release by creating conditions that promote the precipitation of phosphorus from the water column. In theory, aeration should create conditions within a lake that promote the precipitation of phosphorus. However, in practice, this is not always the case. In some instances, declines in nuisance blue-green algae have been reported as the result of aeration, but this effect appears to be more the result of the physical mixing of the water column than reduced phosphorus levels (Jungo *et al.,* 2001). Mixing is necessary to prevent sedimentation of the microalgae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (e.g. in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, Erlenmeyers), aerating (bags, tanks), or using paddle wheels and jet pumps in open ponds. However, it should be noted that not all algal species can tolerate vigorous mixing and you will need to know or experiment to create the best algae growing conditions. Aeration, which could be achieved by rotators, and which provides agitation of growing cells to maintain the cells in suspension, has been described as very necessary in getting good quality and better yields of *Spirulina* species (Dubey, 2006). Light intensity and quality are the most significant environmental factors influencing the photosynthetic pigments in Cyanobacteria. Algal cultures are influenced by a variety of environmental factors and they play a significant role in the production and composition of the photosynthetic pigments. Most cyanobacteria are shade-adapted organisms, possessing efficient mechanisms to counteract the harmful effects of solar radiation, especially freshwater forms exposed to high tropical irradiances. Under natural growth conditions phototrophic microalgae absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the aquatic habitats. Therefore, as far as possible, artificial production should attempt to replicate and enhance the optimum natural growth conditions. The use of natural conditions for commercial algae production has the advantage of using sunlight as a free natural resource (Janssen *et al.,* 2003). However, this may be limited by available sunlight due to diurnal cycles and the seasonal variations; thereby limiting the viability of commercial production to areas with high solar radiation. For outdoor algae production systems, light is generally the limiting factor (Pulz and Scheinbenbogan, 1998). To address the limitations in natural growth conditions with sunlight, artificial means employing fluorescent lamps are almost exclusively used for the cultivation of phototrophic algae at pilot scale stages (Muller-Feuga *et al.,* 1998). Light quality has a strong influence in light harvesting system of the cyanobacterium (Korbee *et al.,* 2005). The objective of this work was to evaluate the growth of the four cyanobacteria of Odisha coastline i.e. *Oscillatoria boryana, Oscillatoria pseudogeminata, Phormidium tenue* and *L. majuscule* in different light and aeration conditions seeking to reduce the labor for the cyanobacterial production.

Materials and Methods

Test Organism

The test organisms (*Oscillatoria boryana, O. pseudogeminata, Phormidium tenue* and *L. majuscule*) were taken from the culture collection unit of Marine algae collected from Coastal areas of Orissa which were maintained in unialgal condition in the P. G. Department of Biotechnology of North Orissa University, Baripada.

Treatment of aeration

Six 100 ml conical flasks each containing 50 ml of the growth medium were set up. Cultivation volume being used in this research is 50ml, using 100ml conical flask, first conical flask is the cultivation without aeration as the control, and the second flask was using aeration with the help of controlled aerator. Each flask was inoculated with 0.3g weight of organism. One set of three flasks was aerated using an aquarium pump which pumped air at *ca* 180 bubbles per min through a drip set (plastic tubing) fitted with a regulator (Anaga and Abu, 1996). This set-up also provided agitation. Three sets were taken for experiment under different light intensity (high, medium, low).

Culture condition

The cultures were incubated in the culture room for 7 and 14 days under different light intensity i.e. high (4500 lux), medium (3000 lux) and low (1500lux), at 26 ± 1^0C temperature and in non aerated (Control) and aerated condition.

Biomass analysis

Biomass concentrations in the cultures were determined by cell dry weight by the method of Vonshak *et al.* (1982).

Protein estimation

The protein was estimated at 700 nm spectrophotometrically following the method of Lowry *et al.* (1951).

Statistical analysis

The observation was taken in triplicate. The mean data of triplicate value was put in statistical analysis by taking their standard deviation.

Results and Discussion

In the present experiment attempt has been made to find out the effect of two important factors i.e. aeration and light on changes in the growth of the four test organisms (*Oscillatiria boryana*, *Oscillatoria pseudogeminata*, *Phormidium tenue* and *Lyngbya majuscule*) (Table 1). When the test organism *O. boryana* grown in different light intensity (low, medium and high) in aerated and non aerated condition for 7 and 14 days, it was observed that biomass of O. *boryana* was reduced from 0.3 g to 0.248 g in 7 days old culture but when given aerated the biomass content increased to 0.290 g under low light condition. On the other hand when the organism was grown for further period of 14 days then the biomass content was further decreased from 0.3 g to 0.207 g in low light non aerated condition but in the same low light and aerated condition the biomass content increased from 0.3 g to 0.389 g (Figure 1). This implies that aeration or light does not independently accelerate the increase in biomass. The test organism *O. pseudogeminata* in low light showed decrease in growth from 0.3g to 0.281 g in 7 days old culture in non aerated condition but when kept in aerated condition the biomass content increased to 0.298 g. Further the test organism *O. pseudogeminata* when grown for 14 days in non aerated condition biomass was increased less as that of aerated low

light condition where it increase from 0.3 g to 0.386 g (Figure 1).

In *P. tenue* and *L. majuscule* the biomass reduced from 0.3 g to 0.225 g and 0.3 g to 0.249 g in 7 days old culture under low light condition. In same condition when kept aerated condition the biomass also reduced in case of *P. tenue* whereas in *L. majuscule* the biomass remain static i.e. 0.306 g after 7 days. When both organism *P. tenue* and *L. majuscule* grown in same condition for further 14 days it was found that in *P. tenue* the biomass increased from 0.3g to 0.408 g in non aerated condition whereas in aerated condition it increase little bit i.e. 0.292 g that that of 7 days old culture, whereas in case of *L. majuscule* the biomass increased significantly in non aerated condition than that of aerated condition where it slightly increase from 0.3g to 0.316g under low light condition (Figure 1).

Both species of *Oscillatoria* showed differential result in medium light in both aerated and non aerated condition (Table 1). In non aerated condition the biomass decreased from initial weight in *O. boryana* after both 7 days and 14 days incubation, whereas in same medium light condition when kept in aerated condition the biomass is same as that of initial weight after 7 days and increased from 0.3 g to 0.389 g after 14 days (Figure 2). In case of *O. pseudogeminata* it was found that better growth in both aerated and non aerated condition after 7 and 14 days of incubation in medium light. The biomass was almost same after 7 days i.e. 0.305 g and increased from 0.3 g to 0.356 g in non aerated condition after 14 days in *O. pseudogeminata*. In aerated condition under medium light the biomass increased from 0.3 g to 0.321 g and from 0.3 g to 0.386 g after 7 and 14 days of incubation in *O. pseudogeminata* (Figure 2). This refer that aeration have some role in increase of biomass in *O. pseudogeminata*. The biomass in case of *P. tenue* reduced to 0.180 g from 0.3 g in aerated condition under medium light after 7 days, but under non aerated condition biomass was 0.280 g after 7 days. In *P. tenue* biomass was increased from 0.3 g to 0.473 g in non aerated culture and the biomass was 0.3 g to 0.355 g in aerated cultures after 14 days (Figure 2). Thus the aeration has no such significant effect on medium light cultures. Similar result found in *L. majuscule* like that of *O. pseudogeminata* where better growth showed in both aerated and non aerated condition under medium light condition. Biomass was increased from 0.3 g to 0.421 g in aerated condition which was higher than that of non aerated condition where biomass also increased from 0.3g to 0.365g (Table 1, Figure 2). This showed that aeration have some role in medium light condition to increase the biomass.

In high light all test organisms showed better growth in aerated condition. The biomass in aerated condition was highest in *P.tenue* where it increased from 0.3 g to 0.480 g and from 0.3 g to 0.528 g after 7 and 14 days, respectively whereas in non aerated condition it reduced from 0.3 g to 0.168 g and as same that of initial weight (i.e., 0.3 g) after 7 and 14 days of incubation (Figure 3). In both species of *Oscillatoria* showed the biomass increased in aerated condition in both 7 and 14 days than that of non aerated condition where biomass increased little bit (Figure 3). On the other hand in *L. majuscule* the biomass reduced in non aerated condition to 0.189 g and 0.280 g from initial weight (i.e., 0.3 g) after 7 and 14 days incubated under high light. When *L. majuscule* kept in aerated condition under high light the biomass decreased after 7 days whereas increased little bit after 14 days from 0.3 g to 0.310 g (Figure 3).

In addition to biomass the protein content was also analysed in both the test organism for 7 and 14 days old culture. In both species of *Oscillatoria* the protein content was higher in aerated culture condition than that of nonaerated culture after both 7 days and 14 days old culture. The protein content in *O. boryana* at low light non aerated condition was 1188.8 µg/50 ml of culture and 1033.338 µg/50 ml of culture after 7 days and 14 days of incubation which increased to 1644.448 µg/50 ml of culture and 2892.598 µg/50 ml of culture after 7 and 14 days of incubation respectively when aerated (Figure 4).

In *O. boryana* protein content in medium light was 1188.88 µg/50 ml of culture and 1444.4 µg/50ml of culture in non aerated culture and was increased to 1262.96 µg/50 ml of culture and 2925.5 µg/50 ml of culture at aerated culture condition after 7 days and 14 days incubation (Figure 5). Similarly in higher light intensity the protein content was 1522.22 µg/50 ml of culture in non aerated condition after 7 days and was decreased to 1274.04 µg/50 ml of culture when aerated after 7 days in *O.boryana*. However after 14 days protein content was 1485.18 µg/50 ml of culture in non aerated condition which was increased to 2514.8 µg/50 ml of culture in aerated culture (Figure 6).

Similarly in *O. pseudogeminata* the protein content in nonaerated low light condition was 1307.69 µg/50 ml of culture and 1435.8 µg/50 ml of culture after 7 days and 14 days of incubation respectively which was increased to 1738.46 µg/50 ml of culture and 3010.25 µg/50 ml of culture when aerated after 7 days and 14 days, (Figure 4).

The *O. speudogeminata* under medium light non aerated condition the protein content was found to be 1902.56 µg/50 ml of culture and 2010.256 µg/50 ml of culture after 7 days and 14 days of incubation. When this organism grown under medium light aerated condition the protein, content increased to 1974.358 µg/50 ml of culture and 3235.89 µg/50 ml of culture after 7 days and 14 days of incubation (Figure 5). Similar trend in protein content was found at high light condition (Figure 6).

In *P. tenue* protein content at low light non aerated condition was found to be 522.2 µg/50 ml in 7 days old culture and was increased to 1485.18 µg/50 ml in 14 days old culture (Table 1). Along with low light when aeration was provided the protein content was found to increase in both the day of culture and was significantly high too (Figure 4). In medium light protein content in aerated culture was more than double then that of non aerated culture. In 7 days old culture at medium light non aerated culture the protein content was 600 µg/50 ml and in aerated culture it was 1248 µg/50 ml.

Similarly in 14 days old culture the protein content was 1281 µg/50 ml and 2722 µg/50 ml for non aerated and aerated culture, respectively (Figure 5). In high light non aerated culture the protein content was reduced to 400 µg/50 ml in 7 days old culture but was increased significantly to 1822 µg/50 ml in 14 days old culture. On the other hand in aeration supplemented culture the protein content was significantly higher and was became 1485 µg/50 ml and 2840 µg/50 ml, respectively (Figure 6).

The protein content in *L. majuscule* under aerated low light condition was found to be 1482.051 µg/50 ml of culture and 2143.58 µg/50 ml of culture after 7 days and 14 days which was higher than that of non aerated condition i.e. 1010.25 µg/50 ml of culture and 1866.66 µg/50 ml of culture after 7 days and 14 days (Table 1, Figure 4).

Table.1 Influence of aeration and different light intensities on biomass production and protein content of two species of cyanobacteria after 7 and 14 days of incubation

Organisms name	Different Light intensity (in lux)	Initial biomass weight (in g)	Aerated				Nonaerated			
			Biomass weight (after 7days) (in g)	Biomass weight (after 14days) (in g)	Protein content (after 7days) (µg/50ml of culture)	Protein content(after 14 days) (µg/50ml of culture)	Biomass weight (after 7 days) (in g)	Biomass weight(after 14days) (in g)	Protein content (after 7days) (µg/50ml of culture)	Protein content (after 14 days) (µg/50ml of culture)
O.boryana	Low	0.300	0.290± 0.003	0.389±0. 010	1644.444±157 .413	2892.592±14. 615	0.248± 0.007	0.207± 0.002	1188.88±257 .422	1033.333±38 .904
	Medium	0.300	0.300± 0.002	0.389±0. 003	1262.962±29. 63	2925.925±17 1.070	0.280± 0.006	0.279± 0.003	1188.888±80 .109	1444.444±18 7.043
	High	0.300	0.304± 0.003	0.400±0. 002	1274.074±181 .48	2514.814±12 0.374	0.200± 0.003	0.310± 0.0025	1522.222±37 0.37	1485.185±18 1.48
O. pseudogeminata	Low	0.300	0.298± 0.003	0.386±0. 003	1738.461±104 .091	3010.256±38. 830	0.281± 0.006	0.292± 0.002	1307.692±18 .489	1435.897±54 .028
	Medium	0.300	0.321± 0.001	0.386±0. 002	1974.358±20. 725	3235.897±45. 580	0.305± 0.003	0.356± 0.002	1902.564±51 .367	2010.256±15 .667
	High	0.300	0.301± 0.004	0.401±0. 007	1973.589±81. 407	2707.692±20 0.547	0.302± 0.003	0.311± 0.004	1799.999±51 .367	2071.794±69 .625
P. tenue	Low	0.300	0.260± 0.004	0.292±0. 003	1270.37±224. 076	2185.185±59. 26	0.225± 0.004	0.408± 0.003	522.222± 42.605	1485.185±15 9.262
	Medium	0.300	0.180± 0.005	0.355±0. 003	1248.148±246 .298	2722.222±25. 925	0.280±0. 003	0.473± 0.002	600.00±128. 788	1281.481±81 .48
	High	0.300	0.480± 0.003	0.528±0. 002	1485.185±255 .577	2840.740±62. 965	0.168±0. 003	0.300± 0.002	400.00±77.7 77	1822.222±88 .89
L.majuscule	Low	0.300	0.306± 0.005	0.316±0. 004	1482.051± 40.703	2143.589±31. 333	0.249± 0.003	0.282± 0.003	1010.256±74 .491	1866.666±14 .803
	Medium	0.300	0.301±0. 002	0.421±0. 003	1656.410±34. 906	3348.717±28. 553	0.308±0. 003	0.365±0. 002	1287.179±51 .367	1907.692±60 .097
	High	0.300	0.286±0. 002	0.310±0. 002	2097.435±11. 843	3297.435±40. 052	0.189±0. 002	0.280±0. 001	733.333±23. 500	1728.205±74 .726

Fig.1 Influence of aeration on biomass production of four species of marine cyanobacteria kept under low light intensity after 7 and 14 days

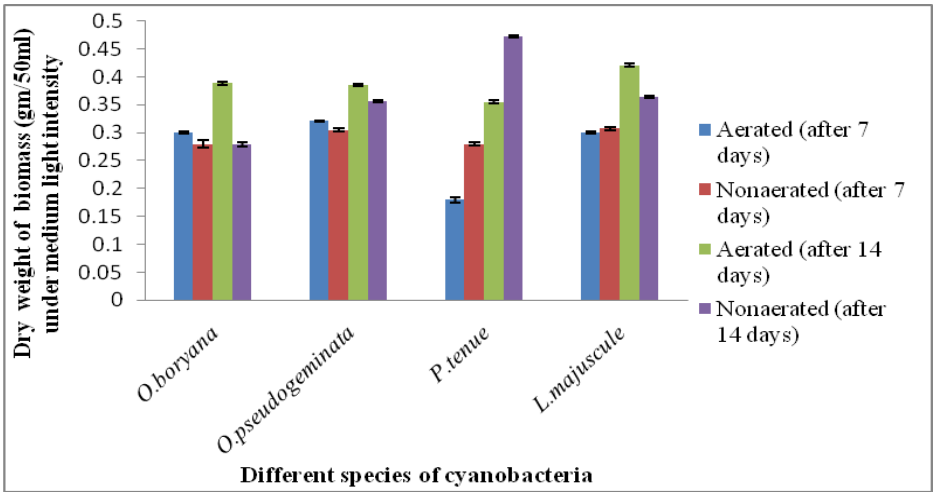


Fig.2 Influence of aeration on biomass production of four species of marine cyanobacteria kept under medium light intensity after 7 and 14 days

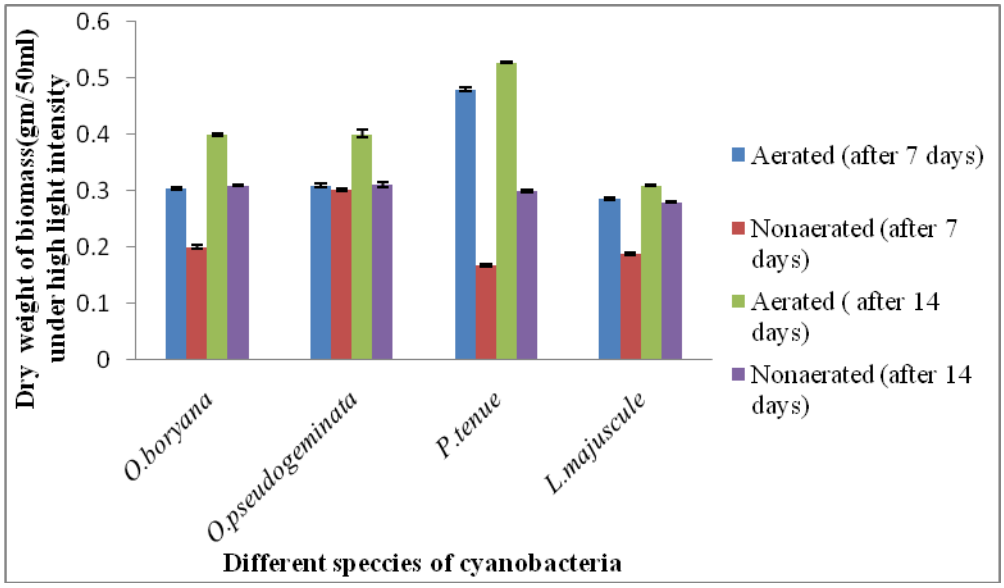


Fig.3 Influence of aeration on biomass production of four species of marine cyanobacteria kept under high light intensity after 7 and 14 days

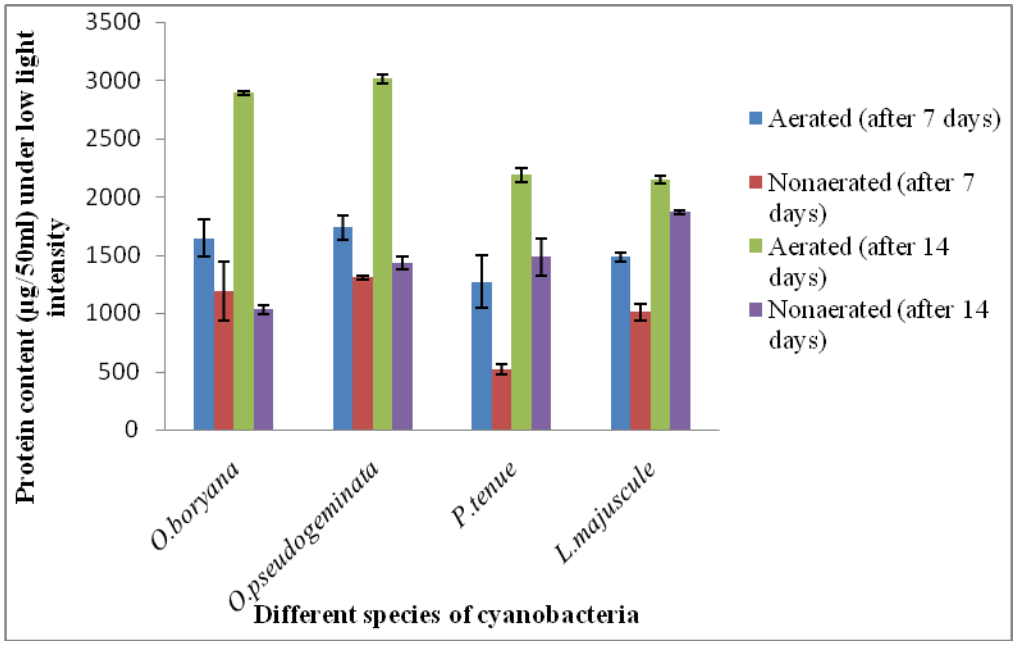


Fig.4 Influence of aeration on protein content (µg/50ml) of four species of marine cyanobacteria kept under low light intensity after 7 and 14 days

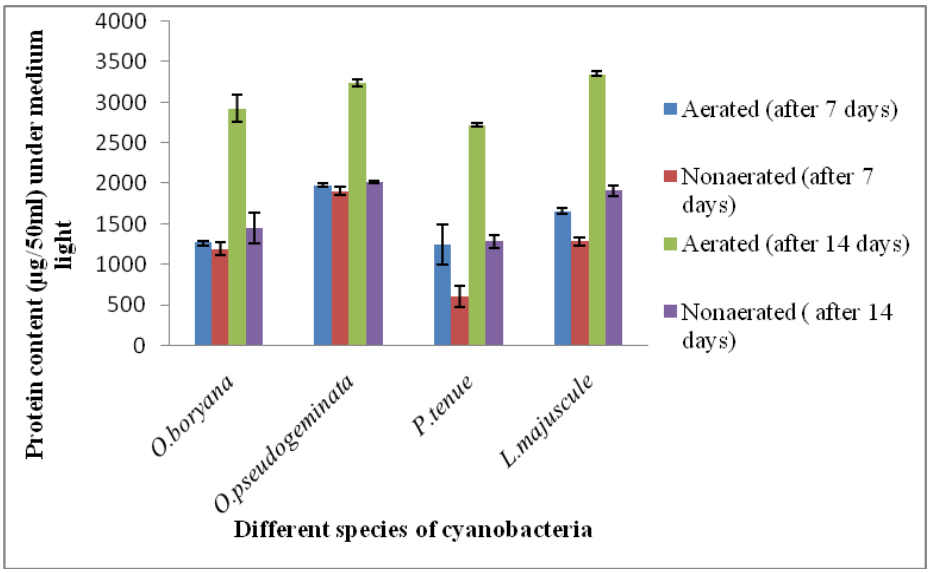
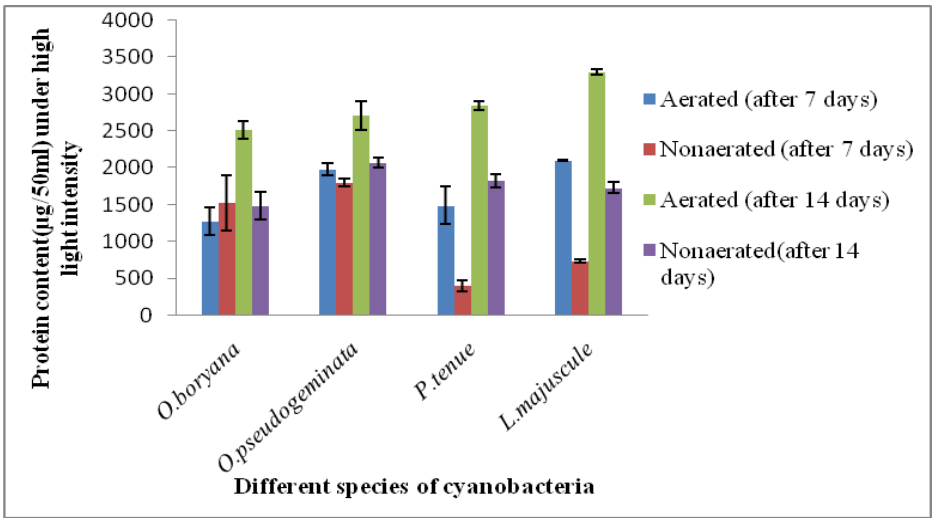


Fig.5 Influence of aeration on protein content (µg/50ml) of four species of marine cyanobacteria kept under medium light intensity after 7 and 14 days



Similarly after 7 days in medium light non aerated condition the protein content was found to be 1287.17 µg/50 ml of culture which increased to 1656.41 µg/50 ml of culture when kept in aerated condition. In medium light after 14 days the protein content of *L. majuscule* was 1907.69 µg/50ml of culture and 3348.71 µg/50 ml of culture in non aerated and aerated condition respectively (Figure 5). In high light non aerated condition the protein content was 733.33 µg/50 ml of culture and 1728.20 µg/50 ml of culture after 7 days and 14 days. But when aerated the protein content increased to 2097.43 µg/50

ml of culture and 3297.43 µg/50 ml of culture after 7 days and 14 days incubation (Figure 6).

Aeration of cultures serves to keep microalgae in suspension, to supply the carbon needed for plant growth and p^H control and to strip O_2 from the culture media, preventing super saturation. Aeration agitates the growth medium and this gives a homogenous distribution of algal cells throughout the vessel for adequate exposure to illumination. It also helps to distribute oxygen concentration uniformly and removes some inhibitory substances produced such as carbon dioxide (Richmond and Vonshak, 1978; Famelart *et al.,* 1987). Microalgae can grow almost anywhere, requiring sunlight and some simple nutrients, although the growth rates can be accelerated by the addition of specific nutrients and sufficient aeration (Pratoomyot *et al.,* 2005; Asian and Kaplan, 2006). Several studies have characterized the optimized aeration by carbon dioxide gas for microalgal production in a photobioreactor. Air enriched with 5% or 10% (v/v) CO_2 at rates of 0.025–1 vvm (volume of air/ medium/time) is found to be cost-effective for mass culture (Zhang *et al.,* 2002). In flat panel photobioreactors, an optimum aeration rate of 0.05 v/v/min has been proposed sufficient to improve the mixing and mass transfer (Sierra *et al.,* 2008). Higher growth was observed in culture with constant aeration under artificial light used for *Spirulina maxima* (Maria *et al.,* 2010). Similarly results showed that both aeration and exposing the cultures of the organism to artificial illumination improved biomass production and protein biosynthesis in the *Spirulina* sp. compared to sunlight illuminated and non aerated cultures (Kemka *et al.,* 2007). Anaga and Abu (1996) have observed that artificial illumination resulted in higher biomass production in *Spirulina* species. Another factor that favorably affected the microalgal growth was the illumination, since constant circulation kept the algal cells in suspension, thus allowing them to receive the same amount of light exposing as much surface area as possible to the light, promoting the photosynthesis, and consequently, better growth and characteristic green coloration. Mixing is the most practical way to dilute radiation evenly to all cells in the culture, improving the light regime (Richmond and Cohen, 1999). This can be done by supplying an adequate air flow rate.

In conclusion, the result of this investigation shows that agitation (by aeration) and adequate lighting are very important factors in biomass production and protein biosynthesis in the all four species isolated from Odisha coastline. In controlled cultivation of these organism adequate aeration, agitation and lighting are necessary for increased yield of cell mass and cell protein.

References

Anaga, A., Abu, G.O. 1996. A laboratory-scale cultivation of *Chlorella* and *Spirulina* using waste effluent from a fertilizer company in Nigeria. *Bioresour. Technol.,* 58: 93–95.

Asian, K., Kapdan, I.K. 2006. Kinetics of nitrogen and phosphorus removal from synthetic waste water by algae. *Ecol. Eng.,* 28(1): 64-7

Castenholz, R.W. 2001. General characteristics of the cyanobacteria. In: Bergey's manual of systematic bacteriology, 2nd edn, Vol.1. Boone, D.R. and Castenholz, R.W., (Eds.), Springer, New York, Pp. 474–487.

Cooke, G.D., Welch, E.B., Peterson, S., Nichols, S.A. 2005. Restoration and

management of lakes and reservoirs, 3rd edn. Taylor and Francis, New York.

Dubey, R.C. 2006. A textbook of biotechnology. 4th rev. edn. S. Chand and Company Limited, New Delhi. Pp. 419–421.

Famelart, M., Kobilinsky, A., Bouillamnne, C., Desmazeaud, M.J. 1987. Influence of temperature, pH and dissolved oxygen on growth of *Brevibacterium linen* in a fermentor. *Appl. Microbiol. Biotechnol.*, 25: 442–448.

Janssen, M., Tramper, J., Mur, L.R., Wijffels, R.H. 2003. Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnol. Bioeng.*, 81(2): 193–210.

Jungo, E., Visser, P.M., Stroom, J., Mur, L.R. 2001. Artificial mixing to reduce the growth of blue-green alga Microcystis in Lake Nieuwe Meer, Amsterdam: an evaluation of 7 years of experience. *Water Sci. Technol. Water Supply*, 1(1): 17–23.

Kemka, H.O., Rebecca, E.A., Gideon, O.A. 2007. Influence of aeration and lighting on biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted brackish water marsh in the Niger Delta, Nigeria. *Afr. J. Biotechnol.*, 6(22): 2596–2600.

Korbee, N., Figueroa, F.L., Aguilera, J. 2005. Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga Porphyra leucosticta (Bangiales, Rhodophyta). *J. Photochem. Photobiol. B.*, 80: 71–78.

Lowry, O., Rosenbrough, H., Farr, A., Rondall, R. 1951. Protein measurement with the folin-phenol reagent. *J. Biol. Biochem.*, 193: 265–275.

Maria Paula da, C.M., Rosa, H.L., Theresinha, M.A. 2010. Effect of three different types of culture conditions on *Spirulina maxima g*rowth. *Braz. Arch. Biol. Technol.*, 53(2): 369–373.

Muller-Feuga, A., Le Gue´des, R., Herve´, A., Durand, P. 1998. Comparison of artificial light photobioreactors and other production systems using *Porphyridium cruentum*. *J. Appl. Phycol.*, 10(1): 83–90.

Pratoomyot, J., Srivilas, P., Noivaksar, T. 2005. Fatty acid composition of ten microalgal species. *Songklanakavin J. Sci. Technol.*, 27(6): 1179–1187.

Pulz, O., Scheinbenbogan, K. 1998. Photobioreactors: design and performance with respect to light energy input. *Adv. Biochem. Eng. Biotechnol.*, 59: 123–52.

Richmond, A., Cohen, Z., (Eds). 1999. Chemicals from microalgae. Capt. 15, Taylor & Francis.

Richmond, A., Vonshak, A. 1978. *Spirulina* culture in Israel. *Arch. Hydrobiol. Bein. Ergebn. Limnol.*, 11: 274–280.

Sierra, E., Acién, F.G., Fernández, J.M., García, J.L., González, C., Molina, E. 2008. Characterization of flat plate photobioreactors for the production of microalgae. *Chem. Eng. J.*, 138: 136–147.

Vonshak, A., Abeliovich, A., Boussiba, S., Arad, S., Richmond, A. 1982. Production of *Spirulina* biomass: effects of environmental factors and population density. *Biomass*, 2: 175–185.

Whitton, B.A., Potts, M. (Eds.), 2000. The ecology of cyanobacteria: Their diversity in time and space. Dordrecht: Kluwer Academic.

Zhang, L., Happe, T., Melis, A. 2002. Biochemical and morphological characterization of sulfur-deprived and H_2-producing *Chlamydomonas reinhardtii* (green alga). *Planta*, 214: 552–561.