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Effect of salinity and pH on the growth and biomass production in the four species of estuarine cyanobacteria

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ABSTRACT

Four species of cyanobacteria isolated from estuarine habitats were studied in relation to salinity and pH on their growth rate and biomass production. The biochemicals like protein, carbohydrate and lipids were analyzed. The study has shown that although they could survive and grow at varying salinity, the range between 15 and 25 ppt was optimum for growth and biomass production in all the species. *Oscillatoria tenuis* and *Plectonema boryanum* showed maximum biomass of 2.2 mg l^{-1} and 2.4 mg l^{-1} at 25 ppt respectively, whereas *Oscillatoria splendida* showed good growth at 15 ppt (2.1mg l^{-1}). *Lyngbya aestuarii* grew well at 30 ppt to produce a biomass of 2.4 mg l^{-1}. The specific growth rate (μ) of each species ranged between 0.52 and 1.07 per day with varying salinity. All the species showed maximum biomass production between pH 6.5 and 7.5. The growth rates ranged between 0.37 and 1.02 per day for all the species with varying pH. Protein was the major component observed in all the four species and it was maximum in *Oscillatoria tenuis* (33.7% of dry weight). *Lyngbya aestuarii* showed higher concentration of total carbohydrate (18.4% of dry weight) and total lipid was highest in *Plectonema boryanum* (17.2% of dry weight). The species could be employed in aquaculture/ mariculture practices.

Key words: Estuarine cyanobacteria, Salinity, pH, Biochemical compounds, Growth rate, Biomass production.

INTRODUCTION

Cyanobacteria are a diverse group of photosynthetic prokaryotes found in almost all aquatic habitats. As a component of microalgae, cyanobacteria are proved to be the natural feed for aquatic animals (Brown and Farmer, 1994). They fix atmospheric nitrogen and are responsible for the fertility of the soil. In addition, they are employed in bioremediation, hydrogen gas production and in the production of secondary metabolites like exopolysaccharides, vitamins, enzymes and pharmaceuticals (Patterson, 1996; Dutta et al., 2005; Abed, *et al.* 2009). Recently, cyanobacteria gained much attention and explored widely because they are a rich source of biologically active compounds with antiviral, antibacterial, antifungal and anticancer activities (Priyadarshani, 2012). Therefore, they need to be isolated from the natural habitats systematically, purified and characterized by following optimal culture conditions.

Culture of cyanobacteria in the laboratory with suitable culture conditions can be achieved by knowing the eco-physiological requirements of a particular species. Formulating a suitable media and optimizing the cultural conditions is the prerequisite to achieve high biomass production and also to produce certain chemicals. Some studies have been carried out to optimize the media composition for marine cyanobacetria. The physiological factors like pH and salinity or chemical factors like media composition influence the growth rate, physiological status and biochemical composition of cyanobacteria in culture condition (Lee and Kim, 2002).

Measurement of chlorophyll-a can be used to monitor their physiological state and growth (Li, *et al.*1980, Morries, *et al.*, 1981). To know the nutritional status, evaluation of biochemical composition of the species is essential (Konopka, 1983) to determine the necessary nutritional requirements for the captive animals (Brown, *et al.*, 1997), to determine the biochemical variation with respect to the nutrients (Melo, *et al.* 1993) and also to study the physiology and growth rate (Anderson, 2005).

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Cyanobacteria exhibit a wide adaptability to pH and salinity, but for mass cultivation it is essential to determine optimal conditions (Nagle, *et al.* 2010). Although much work on screening of growth characteristics, biochemical and nutritional components of the efficient species like *Spirulina* are available (Soni, *et al.* 2012), much attention has not been given to the characterization of other species of cyanobacteria. They are abundant in open sea and estuarine habitats. Estuarine microalgae are proved to be an excellent source of protein, lipid, carbohydrates and vitamins and hence are employed in aquaculture studies. Salinity and pH have been shown to induce the characteristic of the nutritional properties in the phytoplankton. The characterization of these organisms to produce high quality and quantity biomass in laboratory condition, so as to utilize them in a better way has been little examined. Recently, Shruthi and Rajashekhar (2013) have reported 18 species of cyanobacteria from the Nethravathi-Gurupura estuary in the West Coast of India which has prompted the authors to take up the present study. In this study the optimum salinity and pH requirements for maximal growth and biomass production is determined in the four species of cyanobacteria isolated from some estuaries, West Coast of Karnataka. The data generated could be useful in the large-scale industrial production of the cyanobacterial products and fermentor designs in order to increase its production for industrial use.

MATERIALS AND METHODS

Isolation and growth

The cyanobacteria were collected from various niches of estuarine regions of Karnataka coast. They were identified by microscopic examination referring standard manuals and research papers. (Desikachary, 1959, Anagnostidis & Komarek, 1985) Direct isolation of single species was carried out by micropipette method, which is done by picking up a single cell or a filament using a sterile micropipette, under a microscope and were cultured in the f/2 media (Guillard and Ryther, 1962)

Optimization of salinity and pH

Four isolates of cyanobacteria namely, *Oscillatoria tenuis, O. splendida, Lyngbya aestuarii* and *Plectonema boryanum* which showed fast growth under laboratory condition were selected to study their optimum salinity and pH requirement to get high biomass. To study the effect of pH, the f/2 media of varying pH (6, 6.5, 7, 7.5, and 8) was used. Similarly, salinity (10, 15, 20, 25, 30 ppt) was also adjusted by varying the amount of NaCl in the medium. All other components of the media and environmental conditions were kept constant. The N: P ratio of culture media was controlled as 14.5

A known volume of well homogenized culture of each isolate was inoculated in 50 ml of medium adjusted to respective pH and salinity levels and incubated under light/dark cycle (12h/12h) at 28º C. The cultures from the flasks were retrieved in triplicates at the beginning of the experiment and on every alternative day. The cells were then harvested by centrifugation and growth was estimated in terms of chlorophyll-a (mg Chl-a /L)

Growth rate measurement

The specific growth rate (μ) calculated from biomass increase in terms of chlorophyll-a content per unit time in this study by following the formula (Pirt 1975):

$\mu\ (day^{-1}) = \ln (X_1/X_0) / t_1 - t_0,$

where X_0 and X_1 are quantitative expression of the biomass of cells given in terms of chlorophyll-*a* concentration at beginning (t_0) and at end (t_1) of selected time interval during incubation.

Biochemical constituents

The major biochemical constituents namely, carbohydrate, lipid and protein were estimated using Standard Methods. Total carbohydrate was determined by phenol-sulfuric acid method (Dubois et al, 1956), Lipid extraction and estimation was done using Folch's method (Folch et al., 1957) and total protein was extracted and estimated by Lowry's method (Lowry et al., 1956).

RESULTS AND DISCUSSION

The characterization of growth at varying salinity was studied for four cyanobacteria species by growing them in standard f/2 media with different salinity levels (10-30ppt) and later by measuring their growth as chlorophyll-a content up to one month.

Growth of cyanobacteria species at different salinity range is shown in the Figure 1(a-d). All the four species of cyanobacteria showed similar range of salinity requirements for their growth and maximum biomass production. Although they could survive and grow in varying salinity, the range between 15 to 25 ppt was optimum for growth and biomass production of all the species. Similar pattern was reported earlier by Newby (2002). *Oscillatoria tenuis* and *Plectonema boryanum* showed maximum biomass of 2.2 mg $l^{-1}$and 2.4 mg l^{-1}at 25 ppt respectively, whereas *Oscillatoria splendida* showed good growth at 15 ppt (2.1mg l^{-1}). In the figure a and c it is clear that *Lyngbya aestuarii* and *Oscillatoria tenuis* continued in lag phase till ninth day at all the salinity range except at 25 ppt, where the organisms started growing exponentially on third day. *Lyngbya aestuarii* grew well at 30 ppt to produce a biomass of 2.4 mg l^{-1} and only this species showed a significant correlation (r=0.96) between salinity and biomass production. All the species except *Lyngbya aestuarii* showed lowest growth rate and biomass production at higher salinity. *Oscillatoria tenuis* and *O. splendida* proceeded to death phase after 21st and 23rd day respectively, at 30 ppt. However, higher salinity is unsuitable for certain species of cyanobacteria as it affects the protein content of the organism (Kaushik and Sharma, (1997). *Plectonema boryanum* did not show much difference in growth pattern with varying salinity, however produced highest biomass at 25 ppt. All the species showed exponential growth at 25 ppt, but it was observed that the initiation of exponential phase was differed among the species. It was reported earlier that the growth of cyanobacteria in the presence of sodium chloride depends on the availability of nutrients in the media and rate and mode of carbon fixation (Atre, 1998)

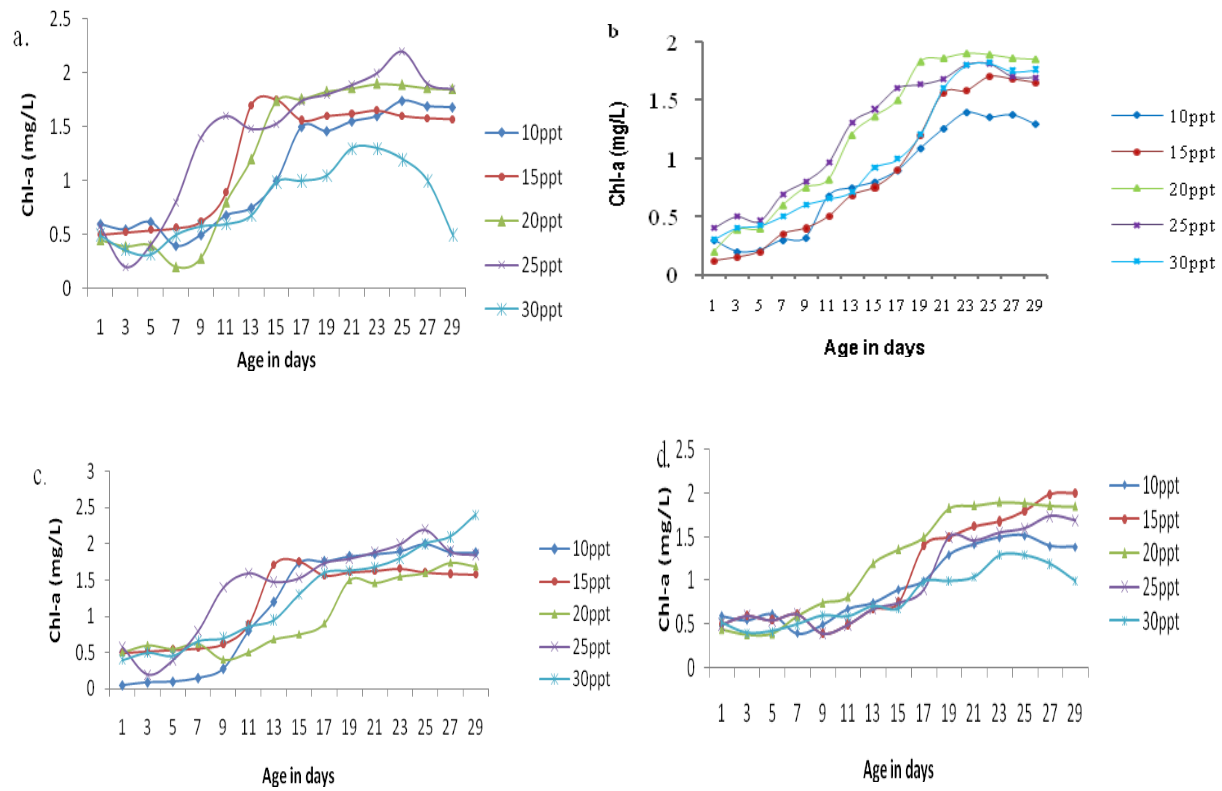

Figure 1(a-d): Effect of different concentration of salinity on the growth curve of four cyanobacteria species: a. *Oscillatoria tenuis,* b. *Plectonema boryanum*, c. *Lyngbya aestuarii*, d. *Oscillatoria splendida*

The growth rate of all the species varied with varying salinities (Fig. 2). The specific growth rate (μ) of each species ranged between 0.52 to 1.07 per day. *Oscilatoria tenuis* and *Lyngbya aestuarii* showed highest growth rate at 25 ppt (Fig. 2 a and 2 c), whereas *Plectonema boryanum* (Fig. 2b) had highest growth rate at 20 ppt. *O. splendida* (Fig. 2d) showed no much variation in growth rates along salinity changes.

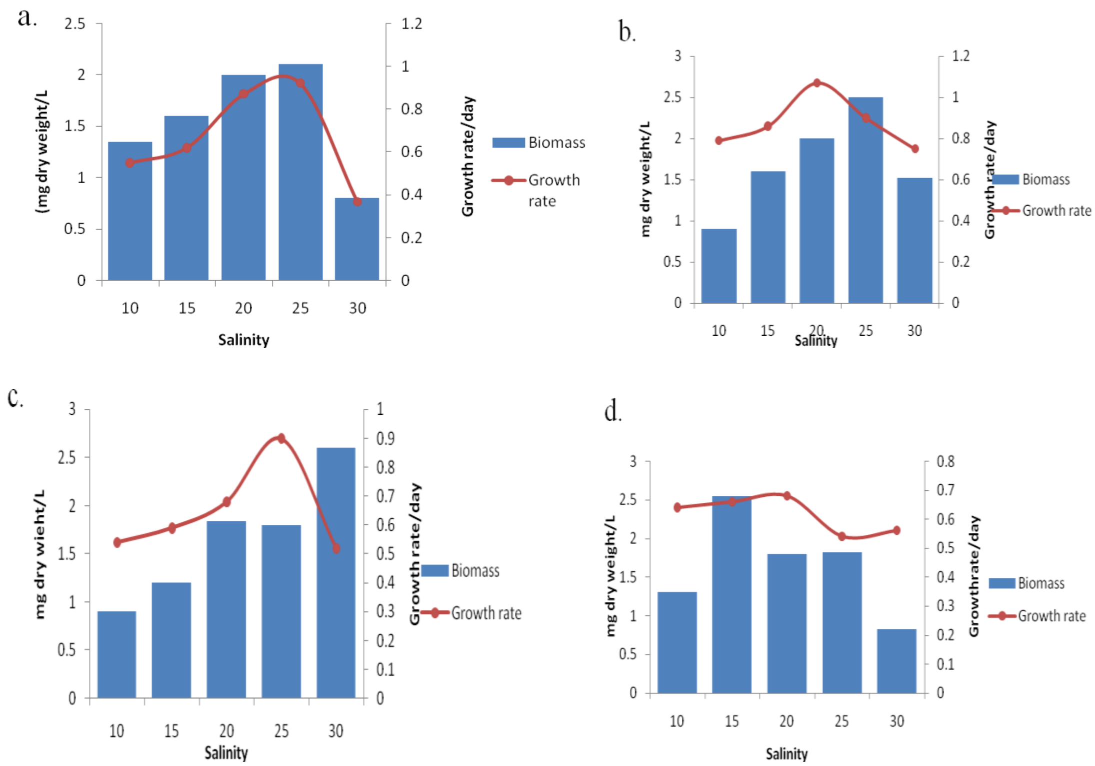

Figure 2(a-d) : Comparison of specific growth rate (μ) with the biomass production in terms of chl-a (mg dry weight/L) at varying salinity conditions for the cyanobacteria a. *Oscillatoria tenuis,* b. *Plectonema boryanum,* c. *Lyngbya aestuarii*, d. *Oscillatoria splendida*

The patterns of growth curve of all the species at varying pH is shown in the Figure 3. All the species showed maximum biomass production between the pH 6.5 and 7.5. *Oscillatoria tenuis* and *Plectonema boryanum* showed optimum growth at 7.5, whereas *Lyngbya aestuarii* and *O. splendida* produced maximum biomass at pH 7. The growth rates of all the species ranged between 0.37 and 1.02 per day. It showed no much variation with changing pH except for *Plectonema boryanum* which showed highest growth rate at 7.5 and lowest at pH 6 (Fig. 4 b). Both growth rate and biomass production showed an insignificant correlation with pH in the laboratory condition. The pH of the medium plays an important role in culturing as it determines the solubility of CO_2 and minerals in the medium which in turn directly or indirectly influence the metabolism of a species (Markl, 1977). Several works have been reported about pH tolerance of cyanobacteria (Venkataraman, 1972, Radhaprasanna, *et al.* 1998). The organisms have been reported to grow well in the pH range 6.5-10 (Roger and Reynaud, 1979, Nagle, *et al.* 2010). However, reports are available regarding cyanobacteria growing at pH as low as 3.5 (Aiyer, 1965).

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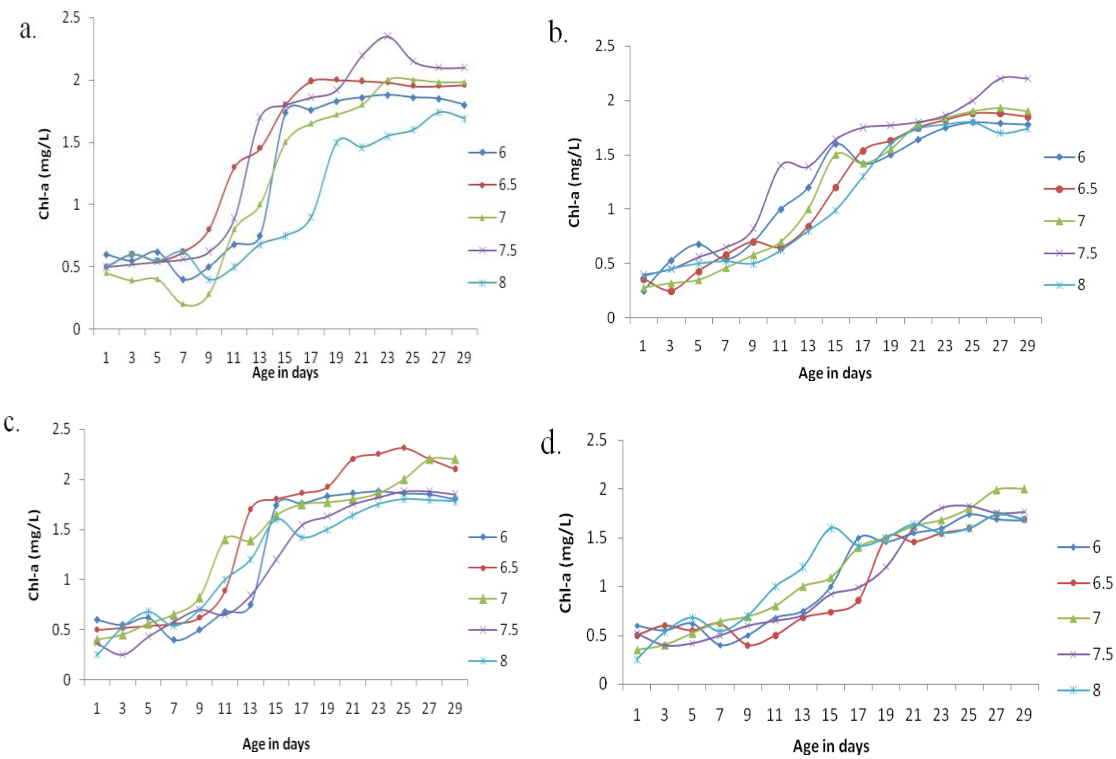

Figure 3 (a-d) : Effect of different concentration of pH on the growth curve of four cyanobacteria species : a. *Oscillatoria tenuis,* b. *Plectonema boryanum,* c. *Lyngbya aestuarii*, d. *Oscillatoria splendida*

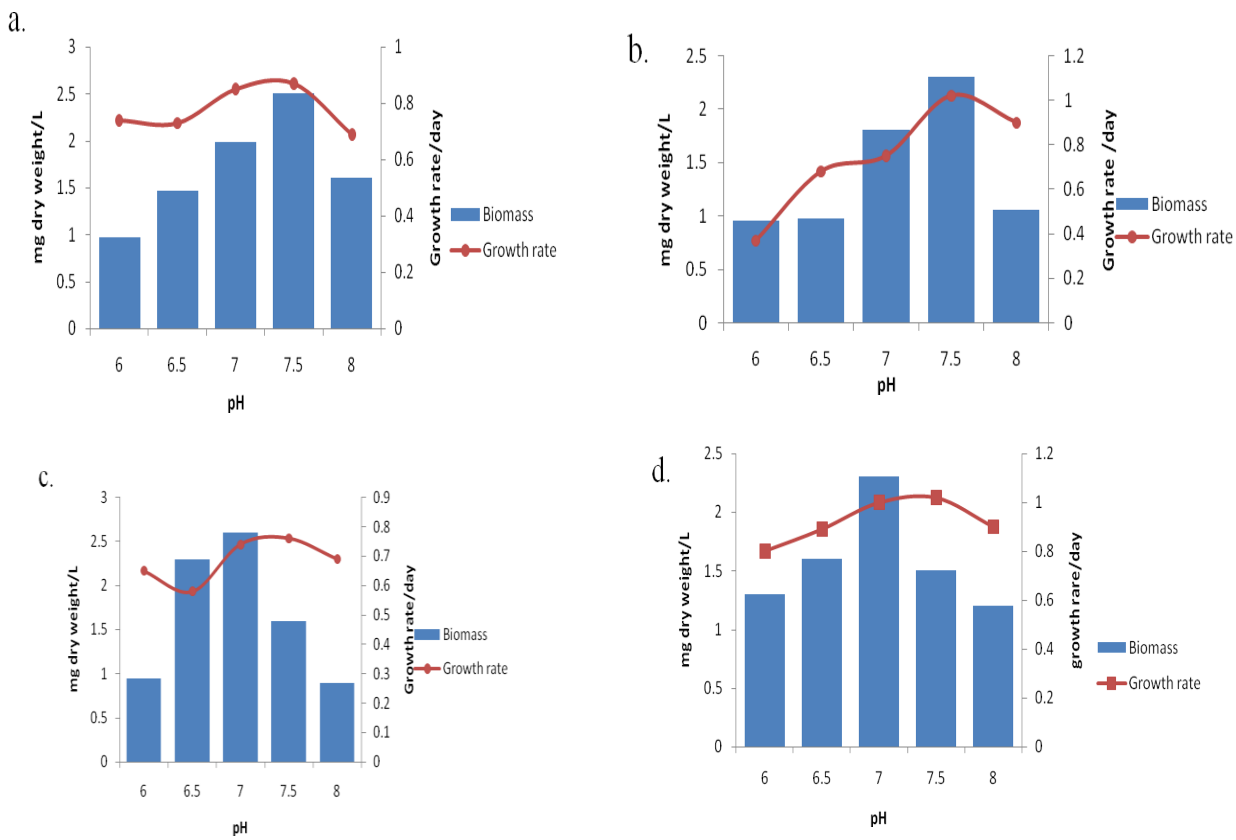

Figure 4 (a-d): Comparison of specific growth rate (μ) with the biomass production in terms of chl-a (mg dry weight/L) at varying pH conditions for the cyanobacteria a. *Oscillatoria tenuis,* b. *Plectonema boryanum,* c. *Lyngbya aestuarii*, d. *Oscillatoria splendida*

The protein, carbohydrate and lipid contents of four cyanobacteria cultured in the optimum growth conditions were studied and expressed in terms of percentage dry weight (Table 1). The present study showed that the protein was the major component in all the four species and it was maximum in *Oscillatoria tenuis* (33.7% of dry weight), followed by *O. splendida* and *Lyngbya aestuarii* which showed higher concentration of total carbohydrate (18.4% of dry weight) and total lipid was highest in *Plectonema boryanum* (17.2% of dry weight). The total lipid and fatty acid content in some freshwater cyanobacteria was studied by Sharathchandra and Rajashekhar (2011). The biochemical constituents of cyanobacteria depend on the nature of strains, physiological state of the culture and the environment (Vargas *et al.* 1998; Subhashini, *et al.* 2003; Gatenby, 2003). Protein make up a large fraction of biomass of actively growing microalgae and cyanobacteria, although they are generally undervalued compared to minor products such as omega fatty acids. Rapidly growing cells are characterized by a high protein and low carbohydrate content, and when cells have reached stationary phase, more carbon is incorporated into carbohydrate and/or lipids (Zhu, *et al.* 1997).

Table 1: Total protein, total carbohydrate and total lipid (% dry weight) of four species of cyanobacteria grown in optimum culture conditions

Species	Protein (% dry weight)	Carbohydrate (% dry weight)	Lipid (% dry weight)
Oscillatoria tenuis	33.7	6.2	9.4
Oscillatoria splendida	25.7	15.0	7.3
Plectonema boryanum	18.5	8.3	17.2
Lyngbya aestuarii	13.5	18.4	14.4

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Few species of cyanobacteria were found to be versatile by having wide range of salinity tolerance. For instance *Phormidium* species is found to tolerate a wide range (0-100‰) of salinity (Subramanian and Thajuddin, 1995). Cyanobacteria also show a wide range of tolerance to pH in the laboratory studies. Many cyanobacteria have evolved to grow at extremes of pH and have been found to tolerate low pH (Nagle, *et al.* 2010). Wide range of salinity and pH tolerance indicates that these variations in natural conditions are of some importance for the ecology of cyanobacteria. But, due to the direct dependence of eco-physiological parameters on the biomass produced and biosynthesis of biochemical constituents in cyanobacteria, there is a need to achieve a balance between these parameters during their culture in the laboratory (Ogbonna, 1996). Development of efficient culture system is necessary for algal biomass production for aquaculture practices and for industrial and pharmaceutical use.

CONCLUSION

The effect of pH and salinity on the growth and biomass production in the four species of estuarine cyanobacteria namely, *Lyngbya aestuarii, Oscillatoria splendida, O. tenuis* and *Plectonema boryanum* were studied and the study indicates that their growth and biomass production vary with varying pH and salinity concentrations. They are a good source of protein, carbohydrate and lipids and could be employed in aquaculture/ mariculture practices.

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ISSN: 2229 – 6905

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