CHAPTER 6

Biochemical evaluation of selected strains of cyanobacteria
6.1 Introduction

Cyanobacteria are distributed in the whole aquatic ecosystem serving as the primary food source for all growth stages of bivalves, and for the larvae of some crustaceans and fishes (Brown and Farmer, 1994). The biochemical composition of the algae plays an important role in their nutritional value and is crucial to the growth and development of these animals. Besides, cyanobacteria have the potential to produce a wide range of fine chemicals including polyunsaturated fatty acids, carotenoids, biloproteins, antibiotics, vitamins, polysaccharides, bioflocculants, biosurfactants, growth promoters etc. and thus can be utilised on a commercial scale.

The cyanobacterium *Spirulina* has been commercially exploited in several countries (Benemann, 1988; Venkataraman and Becker, 1985). It has been used as food for many centuries in Central America. For the past two decades *S.platensis* has been a focus of interest among researchers in various fields because of its commercial importance as a source of proteins, vitamins, essential aminoacids and fatty acids. (Ciferri and Tiboni, 1985; Vonshak and Richmond, 1988; Vonshak, 1990; Tanticharoen et al., 1994) and more recently, for its potential in therapeutic effects (Amha Belay et al., 1993). Basic studies on this species include studies on the growth kinetics by Ogawa et al. (1971) and lehana (1983); growth and growth yield by Aiba and Ogawa (1977) and Ogawa and Aiba (1978).

Although work on the screening and selection of efficient *Spirulina* strains for their nutritive value as well as production of particular end products has been done, much attention has not been paid to screening and selection of other cyanobacteria. Cyanobacteria such as *Phormidium*, *Chroococcus* and *Nostoc* have regularly been used as food in Mexico, Mongolia, China, Fiji and Thailand (Cannell, 1989). However, the commercial significance of cyanobacteria was realized only very recently and so it is in its infancy. Now-a-days, scientists are motivated to search for more potential species available
in nature for exploiting them in a variety of ways to meet our needs. It needs an extensive screening, which is expected to result in the discovery of better cyanobacterial strains of industrial interest.

The merits of an organism for commercial exploitation are maximum yield and utility of cellular constituents (Borowitzka and Borowitzka, 1988). Higher growth rate and nutrient profile of cyanobacteria make them a potentially valuable source of nutrients (Cannell, 1989). Growth of a living organism is defined as an increase in mass or size accompanied by synthesis of macromolecules, leading to the production of a newly organized structure. In unicellular cyanobacteria, the increase in number of cells is a measure of growth. In filamentous species, growth may include differentiation to produce cells for particular function. Any cell of such a filament is potentially capable of binary fission, and hence, growth in length of the chain is mainly the sum of growth of the individual cells. Therefore, it is very complicated to measure the growth of cyanobacteria in terms of increase in cell numbers by microscopic observation.

Microbiologists use a variety of techniques to quantify microbial growth other than determining direct cell count. These include the measurement of macromolecules in the cells (Healy and Henzdel, 1976), the cell quota of specific elements (Rhee and Gotham, 1980), or the kinetic parameters for nutrient uptake (Zevenboom et al. 1982). Photosynthetic rate was higher in cultures grown at faster rates. Although relative incorporation into protein was constant, the absolute rate of protein incorporation increased at higher growth rates because the photosynthetic rate had increased. Chlorophyll-a is another component of biomass, which can be estimated as a measure of growth (Kobayasi, 1961).

The various phases of growth in a microbial culture constitute a typical growth curve. The growth cycle in the system passes through four phases such as lag, log, stationary and death phase. Lag phase is the initial period in
which there appears to be no growth. Although cells are not dividing during the lag phase they are metabolically active, repairing cellular damage and synthesizing enzymes. The lag phase is followed by a period of rapid balanced growth, the logarithmic or the exponential growth phase commonly called the log phase. Next is stationary phase, during which no new growth is apparent and finally there is decline in the viable population until all microbial cells die, i.e. the decline or death phase. Very often, one or more of these phases may not be recognizable. The presence and duration of various phases of growth are indicative of the eco-physiological response of the organism to the physicochemical parameters to which the culture is exposed.

Venkataraman and Mahadevaswamy (1992) pointed out that good culture management with suitable strain is one of the basic needs to get promising yields with quality material on commercial scale. Therefore, cultivation techniques are to be improved with the main objective of obtaining higher algal biomass that exhibits specific qualities (Lobban and Harrison, 1994). Mass cultivation of cyanobacteria is essentially a complex process involving a large number of variables and for the successful growth of cyanobacteria, the environment must be conditioned to meet as many of the essential requirements of that organism as possible. The limitations imposed in the cultivation process can be due to the physical (photon, nutrients, temperature and pH), physiological (organism-environmental-interrelationship) and economic constraints (Radha Prasanna et al., 1998). The environmental factors may be either physiological such as salinity and pH or chemical which provide all the raw materials used for structural and protoplasmic synthesis of cyanobacterial cells (Becker, 1994). Physical and chemical factors such as temperature, salinity and light (Lobban and Harrison, 1994), aeration (Chen and Johns, 1991) or nutrient concentration (Bjornsater and Wheeler, 1990) influence the biochemical composition, physiological status and ultrastructure of the cyanobacteria. Culture medium has been found to play a significant role in the growth kinetics of algae, since it has to stimulate the natural conditions as closely as possible. Cyanobacteria are known to exhibit a wide adaptability...
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to pH and salinity, but for mass cultivation of cyanobacteria, it is essential to determine their optimal conditions.

The measurement of photosynthetic pigments and other products can be used to monitor their physiological state (Li et al., 1980; Smith and Morris, 1980; Morris et al., 1981; Konopka, 1982), but by itself, it will not provide conclusive information on the nutritional status of the population (Konopka, 1983). Evaluation of their physiological as well as biochemical characteristics lead to the selection of more prospective strains.

The aim of the present investigation was to screen and select the most promising cyanobacteria. They were investigated both physiologically and biochemically. Determination of the most favourable pH, salinity and nutritional requirements for low cost production of selected cyanobacterial strains has been done. Preliminary screening was done based on their biochemical components such as total sugars, proteins and lipids, maintaining optimal growth conditions. Further characterisation of the selected species was carried out by studying the growth kinetics, pigment composition and productivity.

6.2 Materials and Methods

6.2.1 Optimisation of Culture conditions

6.2.1.1 Salinity and pH

The effect of salinity and pH on growth of cyanobacteria was studied to find out the optimum salinity and pH for maximum growth. Three filamentous species identified as Phormidium tenue, Phormidium angustissimum, Oscillatoria salina and one unicellular species, Gloeocapsa livida, were chosen for the study. Experiments were carried out in Allen and Nelson medium. Effect of salinity was examined at 0, 10, 20, 30 and 40 ppt. and that of pH was done at pH 6.7, 8 and 9.
6.2.1.2 Medium

The influence of various culture media such as Allen and Nelson medium, BG11 medium, Sea Water Enrichment medium and Nitrogen free medium on growth rate of the above four species of cyanobacteria was studied.

The compositions of these media are given below:

6.2.1.2a Allen and Nelson
Composition of Allen and Nelson medium is given in section 2.2.2

6.2.1.2b BG11 (Stanier et al., 1971)

Table 6.1 Composition of BG 11 medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity /L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution - A</td>
<td></td>
</tr>
<tr>
<td>NaN0</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>20 mg</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>40 mg</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>95 mg</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>36 mg</td>
</tr>
<tr>
<td>Citric acid</td>
<td>6 mg</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>6 mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>1 mg</td>
</tr>
<tr>
<td>Solution - B</td>
<td></td>
</tr>
<tr>
<td>Boric acid</td>
<td>2.86 g</td>
</tr>
<tr>
<td>MnCl₂·4 H₂O</td>
<td>1.81 g</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>222 mg</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>390 mg</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>79 mg</td>
</tr>
<tr>
<td>Calcium nitrate</td>
<td>49.4 mg</td>
</tr>
</tbody>
</table>

Solution A and B were prepared separately and autoclaved. 1 ml of solution B was added to 1000 ml of solution 'A' prepared in seawater.
6.2.1.2c Sea Water Enrichment Medium (SWEM) (Subramanian et al., 1999)

Table 6.2 Composition of Seawater enrichment medium

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution – A</td>
<td></td>
</tr>
<tr>
<td>NaNO₃</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Na₂HPO₄·2H₂O</td>
<td>100mg</td>
</tr>
<tr>
<td>Sea Water</td>
<td>100ml</td>
</tr>
<tr>
<td>Solution - B</td>
<td></td>
</tr>
<tr>
<td>KNO₃</td>
<td>2.02g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.35g</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>9.7mg</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>0.75mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>100mg</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>0.1mg</td>
</tr>
<tr>
<td>Sea water</td>
<td>75ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25ml</td>
</tr>
</tbody>
</table>

Solution A and solution B were prepared separately and autoclaved. 2 ml solution A and 1 ml solution B were added to 1000 ml sterilised seawater.

6.2.1.2d Nitrogen free medium

The source of combined nitrogen (KNO₃) in the Allen and Nelson medium was omitted and replaced by corresponding chlorides to prepare the nitrogen free medium.

The observation on growth using chlorophyll-a as the biomass component was made up to 30 days at time intervals of 3 days as per the method of Strickland and Parsons (1972). Experiments were carried out in triplicate.

10 ml of the culture filtrate was filtered through GF/C filter paper under moderate vacuum, the filter paper was then transferred into a clean stoppered test tube and 10 ml of 90% acetone was added. The test tube was refrigerated.
for about 24 hrs, in order to facilitate the complete extraction of chlorophyll. The chlorophyll-acetone solution was centrifuged for about 10 minutes at 5000 rpm and the absorbance of the clear solution was measured at 665, 645 and 630 nm wavelengths using U - 2001 spectrophotometer taking 90% acetone solution as the blank. The absorbance of the sample was also obtained at 750nm, which was subtracted from the values at 665, 645 and 630 nm, thus minimizing the error in chlorophyll measure. Chlorophyll-a was estimated in µg/ml using the equation given below.

\[
Ca = 11.85 \times E_{656} - 1.54 \times E_{645} - 0.08 \times E_{630} \quad \text{Eqn -1}
\]

where E is the absorbance of chlorophyll samples at the respective wavelength.

\[
\text{Chlorophyll -a (µg/ml)} = \frac{(Ca \times v)}{(V \times 1)}
\]

where:

v = volume of acetone (ml)
V = volume of water sample filtered (L)
1 = path length of the cuvette (cm)

6.2.2 Biochemical composition, Growth kinetics, Pigment composition and productivity

Biochemical characterization of various cyanobacteria species were done under optimal conditions.

6.2.2.1 Biochemical composition

The biochemical composition (total sugars, proteins and lipids) of forty pure cultures of cyanobacteria were determined. Out of these, 12 were Oscillatoria sp., 10 Phormidium sp., 5 Lyngbya sp., 4 Gloeocapsa sp., 2 Synechococcus sp., and one species each from the genera of Synechocystis, Gloeothecce, Chroococcus, Microcystis, Chlorogloea, Tolypothrix, and Pseudanabaena.
Total sugar content was determined by Anthrone method (Roe, 1955) after hydrolysing dried samples in 2.5 N HCl.

Protein was analysed by Lowry's method (Lowry et al., 1951). Extraction was done using 1N NaOH.

Lipid content was extracted in chloroform methanol mixture and assay was done using sulphophosphovanillin method (Frings and Dunn, 1970).

6.2.2.2 Growth kinetics

In order to study growth characteristics, twenty strains of cyanobacteria were selected based on their biochemical composition and yield, of which ten were Oscillatoria sp., five Phormidium sp., three Lyngbya sp. and two Gloeocapsa sp. Growth as chlorophyll content was measured as per the methodology given in the section 6.2.1 and was expressed in terms of µg chlorophyll/ml.

6.2.2.3 Pigment composition

The composition of pigments such as chlorophyll-a, carotenoids, and phycobiliproteins of 20 strains of cyanobacteria were analysed. Concentration of phycobiliproteins such as C-phycocyanin, allophycocyanin and C-phycoerythrin were determined separately. Cells were harvested in the exponential and early stationary growth phases and these parameters were analysed.

6.2.2.3a Chlorophyll-a

Chlorophyll-a was measured as per the methodology given in the section 6.2.1 and was expressed as mg chlorophyll/100mg wet weight of the sample.

6.2.2.3b Carotenoids

Carotenoids present in the samples were extracted using 90% acetone. After complete extraction, samples were centrifuged for about 10 minutes at 5000 rpm, and the absorbance of the clear solution was measured
at 480 and 510 nm wavelengths using U - 2001 spectrophotometer, taking 90% acetone solution as the blank (Strickland and Parsons, 1972). The absorbance of the sample was also obtained at 750nm, which was subtracted from the values at 480 and 510 nm nm, thus minimizing the error. The amount of carotenoids was determined using the equation:

\[
\text{Total carotenoids (\mu g/ml)} = 7.6 \times (E_{480} - 1.49 \times E_{510})
\]

6.2.2.3c Phycobiliproteins

Known volumes of cyanobacterial suspensions were centrifuged and the pellets were suspended in 3 ml of phosphate buffer. The contents were repeatedly frozen and thawed and centrifuged in order to facilitate complete extraction. The supernatants were pooled and the absorbance was measured at 565, 620 and 650 nm against phosphate buffer blank (Seigelman and Kycia, 1978). Calculations were done using the following equations,

\[
\text{C-Phycocyanin (PC) mg/ml} = \frac{A_{620} - (0.7 \times A_{650})}{7.38}
\]

\[
\text{Allophycocyanon (APC)} = \frac{A_{650} - (0.208 \times A_{620})}{5.09}
\]

\[
\text{C-Phycoerythrin (PE)} = \frac{A_{562} - 2.41 \times (PC) - 0.849 \times (APC)}{9.62}
\]

6.2.2.4 Productivity

Productivity of cyanobacterial cultures was estimated by light and dark bottle oxygen technique (Gaarder and Gran, 1927). The difference in oxygen concentration between the light (LB) and dark bottles (DB) was converted into its carbon equivalents using a PQ (photosynthetic quotient) of 1.25 for obtaining gross production values.

Calculation

\[
\text{Gross production (mg C/l/hr)} = \frac{\text{Oxygen content of LB} - \text{DB}}{\text{PQ} \times T}
\]

where 'T' is the time of incubation
6.2.3 Data analysis

One-way ANOVA was done by Duncan's multiple comparison of the means using SPSS 10.0 for window in order to analyze significant difference, if any, between the various culture conditions chosen.

6.3 Results

6.3.1 Optimisation of Culture conditions

6.3.1.1 Salinity tolerance

Salinity tolerance of different species of cyanobacteria viz. *Phormidium tenue*, *P. angustissimum*, *Oscillatoria salina*, *Gloeocapsa livida* was studied by growing the cultures in Allen and Nelson medium having different salinity levels (0-40ppt) and measuring growth as chlorophyll-a content upto 30 days at three day time interval.

*Phormidium tenue*

Chlorophyll content of *P.tenue* grown at zero, 20 and 30 ppt salinity began to decline markedly after 24th day of the experiment [Fig. 6.1 and Table 6.3 (Appendix 3)]. At 10 ppt, decline of growth was observed beyond 27th day. However, at 40 ppt, cells kept growing until the end of the experiment. When the species was grown at salinity 0 ppt the lag phase was very short or absent whereas, at 10, 30 and 40 ppt lag phase extended for 12 days and for 20 ppt it was upto 6 days. The specific growth rate or 'k' value obtained at various time intervals obviously showed the same trend. Negative values for 'k' indicated that final values were less than the initial values and therefore, a clear decline in cell biomass. The highest specific growth rate was obtained at 0 ppt, on the 18th day of the experiment. The maximum chlorophyll content obtained was 2.66 µg/ml at 0 ppt. However, growth was found to occur at all levels of salinity indicating that the species can tolerate wide fluctuations in salinity. ANOVA result substantiated the same finding that there was no significant difference (p< 0.05) between growth at various salinities.
**Phormidium angustissimum**

*Phormidium angustissimum* exhibited growth at all salinities initially [Fig. 6.2 and Table 6.4 (Appendix 3)]. The onset of death phase was on 21st day at zero ppt, 24th day for both 20 and 30ppt. At salinity 10 and 40ppt, cells kept growing until the end of the experiment.

Log phase started from 3, 12, 0, 9 and 9th day at 0, 10, 20, 30, 40 ppt salinity respectively. Very short lag phase upto 3 days could be observed at zero ppt and it was absent at 20ppt. The highest growth rate was obtained at
20ppt (0.066 day\(^{-1}\)). The maximum chlorophyll content obtained was 2.25 \(\mu g/ml\) at 20 ppt. It was found that at 20 ppt, yield as well as growth rate increased to double fold when compared to other salinities. One-way ANOVA showed that 20ppt gave significantly higher (p≤0.05) growth than all other salinities except zero ppt. Very low growth was observed in 30 and 40ppt.

**Oscillatoria salina**

From Fig. 6.3 and Table 6.5 (Appendix 3) it is clear that for *O. salina* at zero ppt, the lag phase was upto 3 days followed by log phase upto 24\(^{th}\) day and proceeded to death phase. The maximum chlorophyll concentration obtained at the end of the log phase was 1.84\(\mu g/ml\). At 10 and 20 ppt lag phase was upto 6\(^{th}\) day whereas, at 30 and 40 ppt there was no growth. At 10ppt, the species exhibited a log phase from 6\(^{th}\) day to 24\(^{th}\) day and reached the maximum chlorophyll concentration of 2.41\(\mu g/ml\) whereas, the log phase was upto 27\(^{th}\) day at 20ppt.

**Fig. 6.3. Salinity tolerance of Oscillatoria salina**

The maximum specific growth rates obtained at 0, 10 and 20 ppt were 0.052, 0.061 and 0.038day\(^{-1}\) respectively. The species could not survive at 30 and 40ppt. Maximum yield was obtained at 10 ppt. Duncan analysis revealed that the species could show significant (p< 0.05) growth at the salinities 0 to 20 ppt.
Gloeocapsa livida

Almost similar pattern of salinity tolerance was exhibited by G. livida [Fig. 6.4 and Table 6.6 (Appendix 3)]. At zero ppt, the onset of log phase was on 3\textsuperscript{rd} day and it sustained upto 24\textsuperscript{th} day and at 10 ppt, it was from 6\textsuperscript{th} day to 24\textsuperscript{th} day and at 20 ppt from 6\textsuperscript{th} to 27\textsuperscript{th} day. There was no growth at 30 and 40 ppt. Maximum chlorophyll content obtained was 2.46 μg/ml at 0 ppt whereas, it was 2.06 at 10 ppt, and 3.82 at 20 ppt. The maximum specific growth rate was obtained at 20 ppt (0.1 day\textsuperscript{-1}) followed by zero (0.084 day\textsuperscript{-1}) and then by 10 ppt (0.06 day\textsuperscript{-1}). From these results, it was inferred that Gloeocapsa livida is tolerant to salinity range of zero to 20 ppt. The optimum salinity was found to be 20 ppt at which the species showed maximum growth rate and maximum yield. One-way ANOVA revealed that there was no significant difference (p< 0.05) between growth at salinities 0-20 ppt and species could show good growth throughout the experiment period.

Fig. 6.4. Salinity tolerance of Gloeocapsa livida

6.3.1.2 pH

The effect of pH on growth of Phormidium tenue, P. angustissimum, Oscillatoria salina, and Gloeocapsa livida were studied for 30 days by inoculating the cultures in Allen and Nelson medium having varying pH (6-9)
**Phormidium tenue**

Fig. 6.5 and Table 6.7 (Appendix 3) explain pH tolerance of *Phormidium tenue*. It was clear that at pH 6 the lag phase was up to 6 days followed by log phase up to 24th day and then proceeded to death phase. The maximum chlorophyll concentration obtained was 3.25 μg/ml on 24th day. Lag phase at pH 7, 8 and 9 were also up to 6 days but log phase extended till the end of the experiment. The highest concentration of chlorophyll at these pH were almost same, i.e., 4.88, 4.86 and 5.01 μg/ml respectively. Maximum specific growth rate obtained at pH 6 and 7 was 0.081 day⁻¹ while at pH 8 and 9, it was 0.119 and 0.124 day⁻¹ respectively. ANOVA result showed that the growth at various pH did not show any significant difference (p< 0.05).

**Phormidium angustissimum**

*Phormidium angustissimum* exhibited growth at all pH [Fig. 6.6 and Table 6.8 (Appendix 3)]. At pH 6, the onset of death phase was on 24th day whereas, at pH 7, 8 and 9, cells kept growing until the end of the experiment. There was no lag phase observed at pH 6 whereas, at all other pH lag phase extended up to 6th day. The maximum chlorophyll content obtained was 2.46, 1.61, 2.46 and 1.76 μg/ml at pH 6, 7, 8 and 9 respectively. The maximum
specific growth rate at pH 6, 7, 8, and 9 were 0.072, 0.032, 0.058 and 0.035 day\(^{-1}\) respectively. Highest growth rate was obtained at pH 6. From this experiment it was inferred that even though the species showed broad pH tolerance, pH 6 was the optimum one. pH 8 was also giving the same growth, but slower. No significant difference (p < 0.05) could be observed between the growth at various pH conditions when Duncan analysis was done.

![Fig 6.6 pH tolerance of Phormidium angustissimum](image)

**Oscillatoria salina**

From Fig. 6.7 and Table 6.9 (Appendix 3), it is clear that chlorophyll content of Oscillatoria salina grown at pH 6 began to decline markedly after 27\(^{th}\) day and that at pH 7, it declined after 24\(^{th}\) day of the experiment. At pH 8 and 9, cells kept growing until the end of the experiment. The onset of log phase was on the 6\(^{th}\) day at pH 6 and 8 and for cultures grown at pH 7 and 9, it was on the 3\(^{rd}\) day. The maximum specific growth rate of the species at pH 6 was 0.051 per day, at pH 7, it was 0.079, at pH 8, 0.03, and at pH 9, the value was 0.044 per day. Therefore, the highest specific growth rate was obtained at pH 7. The maximum chlorophyll contents obtained were 2.25, 2.75, 1.77 and 2.2 \(\mu g/ml\) at pH 6, 7, 8 and 9 respectively. From these results, it was concluded that the optimum pH for O. salina was pH 7. However, the
species showed broad pH tolerance. ANOVA revealed the same fact that no significant difference (p< 0.05) in growth could be seen at various pH levels.

**Fig 6.7 pH tolerance of Oscillatoria salina**

![Graph showing pH tolerance of Oscillatoria salina](image)

**Gloeocapsa livida**

Fig. 6.8 and Table 6.10 (Appendix 3)] reveal that at pH 6, 7 and 8 *Gloeocapsa livida* cells were growing constantly until the end of the experiment. At pH 9, a slight decline in growth was observed on the last day. Lag phase was absent at pH 6, whereas, it was upto 3 days at pH 7 as well as pH 8 and upto 6 days at pH 9. The maximum chlorophyll content obtained was 2.61 µg/ml at pH 6, whereas, it was 1.8, 1.22 and 1.15 at pH 7, 8 and 9 respectively. Maximum specific growth rate was 0.071 day⁻¹ at pH 6, 0.044 day⁻¹ at pH 7, 0.025 day⁻¹ at pH 8 and 0.026 day⁻¹ at pH 9. Therefore, for *Gloeocapsa livida*, the optimum pH was found to be 6 at which the species showed maximum growth rate. Maximum yield was also obtained at the same pH. However, single factor ANOVA revealed that there was no significant difference (p<0.05) in growth between various pH levels.
6.3.1.3 Medium

The ability of cyanobacteria to grow in different media, Allen and Nelson medium, BG11 medium, Sea Water Enrichment Medium (SWEM) and Nitrogen-free medium were tested.

*Phormidium tenue*

Fig. 6.9 and Table 6.11 (Appendix 3) reveal that in Allen and Nelson (AN) medium, *Phormidium tenue* showed a log phase between 6th to 24th days and in BG11, it was between 6 - 27 days. The log phase extended upto the end of the experiment from 9th day in SWEM whereas, in Nitrogen-free medium, the species showed no growth. In Allen and Nelson medium, maximum specific growth rate (0.074 day⁻¹) and highest chlorophyll content (2.96 μg/ml) were obtained on 24th day. In BG11, these factors were 0.018 day⁻¹ and 1.548 μg/ml and in SWEM 0.015 day⁻¹ and 1.418 μg/ml respectively. But in NF medium, k value was negative indicating that the species could not grow in nitrogen free medium, as it was unable to fix nitrogen. From these results, it was found that AN medium was the best medium for the growth of this species. One-way ANOVA also revealed the same.
Fig 6.9 Growth characteristics of *Phormidium tenue* in different media

![Growth characteristics diagram](image)

*Phormidium angustissimum*

Growth characteristics of *Phormidium angustissimum* in various media are shown in Fig. 6.10 and Table 6.12 (Appendix 3). In AN medium, growth curve was in such a way that lag phase was completely absent and log phase started directly from zero day and extended up to 24th day which was followed by a death phase. $K_{max}$ was 0.049 day$^{-1}$ and chlorophyll content reached the peak value of 1.656 $\mu$g/ml. In BG11 medium, lag phase was found up to 6th day followed by log phase up to the end of the experiment. Maximum specific growth rate of 0.014 day$^{-1}$ and chlorophyll content of 0.909 $\mu$g/ml were obtained on the 30th day of the experiment. In SWEM, lag phase was up to the 3rd day followed by log phase up to 27th day followed by the death phase. $K_{max}$ of 0.024 day$^{-1}$ and chlorophyll content of 1.117 $\mu$g/ml were obtained in this medium. In nitrogen-free medium, no growth was obtained. From these results, it was inferred that AN medium was the optimum one for the growth of *P. angustissimum* and it was also found that the species was unable to fix nitrogen as it could not grow in NF medium. Duncan analysis demonstrated that AN medium showed significantly higher (p≤ 0.05) growth than BG 11. The species could give comparatively good growth in SWEM also.
Fig 6.10 Growth characteristics of *Phormidium angustissimum* in different media

![Graph showing growth characteristics of Phormidium angustissimum in different media.](image)

**Oscillatoria salina**

Fig. 6.11 and Table 6.13 (Appendix 3) reveal that in AN medium, *Oscillatoria salina* showed exponential phase from 6th day to 27th day and then an immediate death phase whereas in BG 11 medium, cells were growing constantly until the end of the experiment and in SWEM the species attained maximum growth without much delay and the stationary phase was prolonged afterwards. In this case also, in NF medium, no growth was observed. The maximum chlorophyll content obtained was 1.473 µg/ml in AN medium, whereas, it was 2.327 and 1.48 in BG11 and SWEM respectively. Maximum specific growth rate obtained in these media were 0.028, 0.054 and 0.035 day⁻¹ respectively. Therefore, for *Oscillatoria salina* the best medium for growth was found to be BG11 at which the species showed maximum growth rate and yield. However, the species could show considerable growth in both AN medium and SWEM and it was also found that the species did not have the capacity to fix nitrogen as it could not grow in NF medium. One-way ANOVA revealed that the organism showed significantly good ($p \leq 0.05$) growth in SWEM and least growth in NF medium.
Ecological and biochemical studies on cyanobacteria of Cochin estuary and their application as source of antioxidants

![Fig 6.11 Growth characteristics of *Oscillatoria salina* in different media](image)

**Gloeocapsa livida**

*Gloeocapsa livida* also exhibited significant growth in AN medium [Fig. 6.12 and Table 6.14 (Appendix 3)]. The onset of log phase was on 6th day and was extended up to the end of the experiment. $K_{\text{max}}$ was 0.121 day$^{-1}$ and chlorophyll content reached the peak value of 4.438 $\mu$g/ml. In BG11 medium, SWEM, and NF medium $k$ value was found always negative indicating that there was no growth in these media. From this, it was concluded that AN medium was the only suitable medium for the favourable growth of this unicellular species. Single-factor ANOVA substantiated the same finding.

![Fig 6.12 Growth characteristics of *Gloeocapsa livida* in different media](image)
6.3.2 Biochemical composition, Growth kinetics, Pigment composition and Productivity

In order to select the most promising strains, biochemical studies of the isolated cyanobacterial species were carried out in the laboratory keeping them in Allen and Nelson medium at the optimum salinity (20 ppt) and pH (7). Cultures were grown at 28°C with an illumination of 2000 lux for 12 hours duration and were harvested in the exponential /early stationary growth phase (24th day of incubation).

6.3.2.1 Biochemical composition

The biochemical composition of forty strains of cyanobacteria has been determined. The results are expressed as percentage of dry weight and are given in Figs 6.13 to 6.15 and Table 6.15.

6.3.2.1a Total sugars

Sugar content of various species was found to be in the range of 1.1 – 13% (Fig 6.13) and Oscillatoria pseudogeminata (C81) yielded the highest amount of sugars (13.02%). O. accuta (C49), O. jasorvensis (C23), O. wiliei (C30), O. fremyi (C32), O. foreauii (C72), O. limnetica (C77), Phormidium dimorphum (C9), P. corium (C13), Lyngbya semiplena (C34), Gloeocapsa gelatinosa (C28), G. compacta (C52), G. quaternata (C94), and Tolypothrix tenuis (C113) were also composed of comparatively higher amount of sugars (more than 8%).

6.3.2.1b Proteins

Protein content was found to be in the range of 1.84 – 39% (Fig 6.14); maximum was encountered in Phormidium dimorphum (C9 - 38.88%). Other species with high protein content (more than 20%) were Oscillatoria salina (C21), O. foreauii (C72), O. pseudogeminata (C81), O. laete-virens minimum (C85), O. accuminata (C112), Phormidium abronema (C11), P. corium (C13), Lyngbya martensiana (C1), L. semiplena (C34) and Gloeothecce rupestris (C19).
Table 6.15 - Biochemical composition of forty different species of cyanobacteria

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Name of the species</th>
<th>Total sugars</th>
<th>Proteins</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oscillatoria cortiana</td>
<td>7.51 ± 0.22</td>
<td>10.26 ± 0.22</td>
<td>12.90 ± 0.79</td>
</tr>
<tr>
<td>2</td>
<td>Oscillatoria salina</td>
<td>9.50 ± 1.07</td>
<td>10.53 ± 0.89</td>
<td>19.25 ± 0.42</td>
</tr>
<tr>
<td>3</td>
<td>Oscillatoria jasorvensis</td>
<td>6.58 ± 0.31</td>
<td>30.02 ± 0.15</td>
<td>12.62 ± 0.67</td>
</tr>
<tr>
<td>4</td>
<td>Oscillatoria willei</td>
<td>8.04 ± 0.22</td>
<td>5.61 ± 0.05</td>
<td>15.51 ± 0.69</td>
</tr>
<tr>
<td>5</td>
<td>Oscillatoria fremyi</td>
<td>8.45 ± 0.37</td>
<td>9.58 ± 0.21</td>
<td>16.95 ± 2.07</td>
</tr>
<tr>
<td>6</td>
<td>Oscillatoria acuta</td>
<td>9.24 ± 0.80</td>
<td>6.82 ± 0.42</td>
<td>17.66 ± 2.07</td>
</tr>
<tr>
<td>7</td>
<td>Oscillatoria minnesotensis</td>
<td>2.90 ± 0.98</td>
<td>13.41 ± 0.23</td>
<td>8.16 ± 1.02</td>
</tr>
<tr>
<td>8</td>
<td>Oscillatoria foreauii</td>
<td>12.58 ± 0.73</td>
<td>21.17 ± 0.34</td>
<td>4.25 ± 0.40</td>
</tr>
<tr>
<td>9</td>
<td>Oscillatoria limnetica</td>
<td>8.52 ± 0.41</td>
<td>6.42 ± 0.11</td>
<td>13.95 ± 4.15</td>
</tr>
<tr>
<td>10</td>
<td>Oscillatoria pseudogeminata</td>
<td>13.02 ± 0.92</td>
<td>23.31 ± 0.38</td>
<td>11.10 ± 1.17</td>
</tr>
<tr>
<td>11</td>
<td>Oscillatoria laete-vires minimus</td>
<td>6.02 ± 0.49</td>
<td>31.93 ± 0.45</td>
<td>15.69 ± 2.64</td>
</tr>
<tr>
<td>12</td>
<td>Oscillatoria pseudogeminata</td>
<td>13.02 ± 0.92</td>
<td>23.31 ± 0.38</td>
<td>11.10 ± 1.17</td>
</tr>
<tr>
<td>13</td>
<td>Phormidium tenue</td>
<td>6.65 ± 0.31</td>
<td>6.92 ± 0.14</td>
<td>13.59 ± 0.33</td>
</tr>
<tr>
<td>14</td>
<td>Phormidium dimorphum</td>
<td>9.80 ± 0.68</td>
<td>38.88 ± 1.30</td>
<td>12.50 ± 1.19</td>
</tr>
<tr>
<td>15</td>
<td>Phormidium abronema</td>
<td>7.22 ± 0.34</td>
<td>23.51 ± 0.85</td>
<td>10.34 ± 2.76</td>
</tr>
<tr>
<td>16</td>
<td>Phormidium corium</td>
<td>10.01 ± 0.30</td>
<td>21.07 ± 1.10</td>
<td>12.74 ± 4.10</td>
</tr>
<tr>
<td>17</td>
<td>Phormidium mucicola</td>
<td>2.68 ± 0.03</td>
<td>5.82 ± 0.69</td>
<td>2.90 ± 0.21</td>
</tr>
<tr>
<td>18</td>
<td>Phormidium purpurescens</td>
<td>4.85 ± 0.12</td>
<td>13.60 ± 0.14</td>
<td>5.64 ± 1.21</td>
</tr>
<tr>
<td>19</td>
<td>Phormidium foveolarum</td>
<td>3.05 ± 0.10</td>
<td>6.47 ± 0.12</td>
<td>7.82 ± 1.19</td>
</tr>
<tr>
<td>20</td>
<td>Phormidium angustissima</td>
<td>5.15 ± 0.15</td>
<td>5.36 ± 0.75</td>
<td>8.35 ± 0.86</td>
</tr>
<tr>
<td>21</td>
<td>Phormidium molle</td>
<td>1.36 ± 0.19</td>
<td>4.39 ± 0.76</td>
<td>2.25 ± 0.13</td>
</tr>
<tr>
<td>22</td>
<td>Phormidium bohneri</td>
<td>1.99 ± 0.06</td>
<td>8.71 ± 0.77</td>
<td>4.26 ± 0.16</td>
</tr>
<tr>
<td>23</td>
<td>Lyngbya martensiana</td>
<td>6.83 ± 0.30</td>
<td>29.68 ± 0.70</td>
<td>4.67 ± 1.39</td>
</tr>
<tr>
<td>24</td>
<td>Lyngbya semiplena</td>
<td>9.45 ± 0.80</td>
<td>30.71 ± 0.70</td>
<td>13.45 ± 1.34</td>
</tr>
<tr>
<td>25</td>
<td>Lyngbya aeruginoe</td>
<td>4.32 ± 0.49</td>
<td>6.64 ± 1.05</td>
<td>13.71 ± 0.61</td>
</tr>
<tr>
<td>26</td>
<td>Lyngbya cryptovaginata</td>
<td>3.06 ± 0.35</td>
<td>6.82 ± 1.02</td>
<td>6.49 ± 0.29</td>
</tr>
<tr>
<td>27</td>
<td>Lyngbya putealis</td>
<td>2.96 ± 0.42</td>
<td>6.21 ± 0.90</td>
<td>3.85 ± 0.08</td>
</tr>
<tr>
<td>28</td>
<td>Gloeocapsa gelatinosa</td>
<td>10.26 ± 0.32</td>
<td>8.89 ± 0.70</td>
<td>13.17 ± 2.86</td>
</tr>
<tr>
<td>29</td>
<td>Gloeocapsa livida</td>
<td>7.08 ± 0.57</td>
<td>4.40 ± 0.85</td>
<td>12.90 ± 0.63</td>
</tr>
<tr>
<td>30</td>
<td>Gloeocapsa compacta</td>
<td>9.11 ± 0.98</td>
<td>18.65 ± 0.83</td>
<td>1.60 ± 1.40</td>
</tr>
<tr>
<td>31</td>
<td>Gloeocapsa quaternata</td>
<td>8.40 ± 0.04</td>
<td>16.74 ± 0.93</td>
<td>4.37 ± 0.37</td>
</tr>
<tr>
<td>32</td>
<td>Synechocystis aquatilis</td>
<td>5.36 ± 0.29</td>
<td>15.90 ± 0.74</td>
<td>0.59 ± 0.10</td>
</tr>
<tr>
<td>33</td>
<td>Synechococcus cedrorum</td>
<td>3.61 ± 0.00</td>
<td>8.13 ± 0.71</td>
<td>1.56 ± 0.52</td>
</tr>
<tr>
<td>34</td>
<td>Synechococcus elongatus</td>
<td>5.77 ± 1.10</td>
<td>11.86 ± 0.70</td>
<td>8.15 ± 0.12</td>
</tr>
<tr>
<td>35</td>
<td>Gloeothecae rupestris</td>
<td>4.82 ± 0.25</td>
<td>21.16 ± 1.32</td>
<td>3.10 ± 0.94</td>
</tr>
<tr>
<td>36</td>
<td>Chroococcus tenax</td>
<td>4.76 ± 0.87</td>
<td>9.12 ± 0.75</td>
<td>2.14 ± 0.17</td>
</tr>
<tr>
<td>37</td>
<td>Microcystis orissica</td>
<td>4.55 ± 0.02</td>
<td>6.47 ± 3.50</td>
<td>9.68 ± 0.34</td>
</tr>
<tr>
<td>38</td>
<td>Chlorogloea fritschii</td>
<td>3.05 ± 0.08</td>
<td>15.14 ± 0.80</td>
<td>6.54 ± 0.34</td>
</tr>
<tr>
<td>39</td>
<td>Tolypothrix tenuis</td>
<td>8.90 ± 1.35</td>
<td>18.09 ± 0.95</td>
<td>3.84 ± 0.78</td>
</tr>
<tr>
<td>40</td>
<td>Pseudanabaena schmidlei Robusta</td>
<td>1.08 ± 0.09</td>
<td>1.84 ± 0.76</td>
<td>1.90 ± 0.50</td>
</tr>
</tbody>
</table>

Species selected for further study are given in bold letters.
6.3.2.1c Lipids

Lipid content varied between 0.6-19.25% (Fig 6.15) and the maximum being in Oscillatoria accuta (C49), for which 19.25% of the total biomass was lipid. Oscillatoria cortiana (C7), Oscillatoria salina (C21), Oscillatoria jasorvensis (C23), Oscillatoria willei (C30), Oscillatoria fremyi (C32), Oscillatoria limnetica (C77), Oscillatoria pseudogeminata (C81), Oscillatoria laete-virens minimum (C85), Oscillatoria accuminata (C112), Phormidium tenue (C8), Phormidium dimorphum (C9), Phormidium abronema (C11), Phormidium corium (C13), Lyngbya semiplena (C34), Lyngbya aerugineo (C15), Gloeocapsa gelatinosa (C28), and Gloeocapsa livida (C35) were also composed of comparatively higher amount (more than 11%) of lipid.

Species with high composition of all the three components viz. total sugars, proteins and lipids were Oscillatoria pseudogeminata (C81), Phormidium dimorphum (C9), Phormidium corium (C13) and Lyngbya semiplena (C34).

6.3.2.2 Growth profile

Twenty species of cyanobacteria with high composition of carbohydrates, proteins and lipids were selected for the growth characterization. The cultures were kept under optimum conditions. Growth was measured in terms of chlorophyll-a content. Figs 6.16 (1-20) and Tables 6.16 (1-20) (Appendix 3) depict growth characteristics of various species of cyanobacteria. From these results, the following inferences were made:

Out of ten Oscillatoria sp. studied, O. laete-virens minimum (C85), O. jasorvensis (C23) and O. foreaui (C72) showed maximum specific growth rate and the values were 0.301, 0.244 and 0.235day⁻¹ respectively. The cultures were continuously growing upto 24th day and reached their zenith and then a diminution was observed.
Fig 6.16 Growth characteristics of selected cyanobacterial strains

1. Oscillatoria cortiana

2. Oscillatoria salina

3. Oscillatoria jasorvensis

4. Oscillatoria fremyii
(5) Oscillatoria accuta

(6) Oscillatoria willei

(7) Oscillatoria foreauii

(8) Oscillatoria limnetica
(9) *Oscillatoria pseudogeminata*

(10) *Oscillatoria leaetevirens minimum*

(11) *Phormidium tenue*

(12) *Phormidium dimorphum*
(13) Phormidium abronema

(14) Phormidium corium

(15) Phormidium angustissimum

(16) Lyngbya martensiana
(17) *Lyngbya aerugineo*

(18) *Lyngbya semiplena*

(19) *Gloeocapsa gelatinosa*

(20) *Gloeocapsa livida*
Phormidium corium (C13) was the outstanding species in the genus Phormidium as it showed high specific growth rate, 0.213 day\(^{-1}\). P. corium showed continuous growth from the 6\(^{th}\) day to the end of the experiment. Of the species selected from Lyngbya, L. martensiana (C1) showed excellent growth with a Kmax of 0.186 day\(^{-1}\). But the species reached the log phase very late, i.e., beyond 9\(^{th}\) day. In case of Gloeocapsa, G. gelatinosa (C28) was grown with the maximum specific growth rate of 0.345 day\(^{-1}\). Gloeocapsa gelatinosa and Oscillatoria laete-virens minimum (C85) showed maximum growth rate among the tested cyanobacteria.

6.3.2.3 Pigment composition

Percentage composition of pigments such as chlorophyll-a, carotenoids and phycobiliproteins of 20 species of selected cyanobacteria were analyzed. Figs 6.17 to 6.21 and Table 6.18 (Appendix 3) show the pigment composition in various species.

Chlorophyll- a

Out of 20 species studied, Oscillatoria jasorvensis (C23), O. laetevirens minimum (C85), Phormidium corium (C13) and Gloeocapsa gelatinosa (C28) were composed of more than 3% chlorophyll (Fig 6.17).

Carotenoids

Carotenoids were more in O. fremyi (C32), Phormidium corium (C13), Phormidium angustissima (C84), Lyngbya aerugineo (C15) and Gloeocapsa gelatinosa (C28) in which more than 0.6% could be obtained (Fig 6.18).

Phycobiliproteins

Phycocyanin was maximum in Lyngbya semiplena (C34) and in Phormidium corium (C13), it was 10% of the weight, whereas, Gloeocapsa gelatinosa (C28), 7% and Oscillatoria foreau (C72), 5.5% and others were with less than 2.5% phycocyanin (Fig 6.19). Lyngbya semiplena (C34), Phormidium corium (C13), Gloeocapsa gelatinosa (C28) Oscillatoria willei
Composition of pigments: chlorophyll-a, carotenoids and phycobiliproteins

Fig 6.17 Chlorophyll-a content of selected cyanobacterial species

Fig 6.18 Carotenoid content of selected cyanobacterial species
Fig 6.19 C- Phycocyanin content of selected strains

Fig 6.20 Allophycocyanin content of selected strains

Fig 6.21 C- Phycoerythrin content of selected strains
(C30) and Oscillatoria foreaui (C72) were composed of more than 0.5% allophycocyanin (Fig 6.20). Phycoerythrin was also present in high level in these strains where more than 2% of the weight was phycoerythrin (Fig 6.21).

From the analysis, it was found that the prominent pigments present in cyanobacteria are chlorophyll and phycobiliproteins whereas, carotenoids were comparatively less. The proportion of phycobiliproteins to chlorophyll was more than one in case of species like O. cortiana (C7), O. willei (C30), O. accuta (C49), O. foreaui (C72), P. tenue (C8), P. corium (C13), P. angustissima (C84), L. semiplena (C34) and G. gelatinosa (C28) as phycobiliproteins were more in these species.

6.3.2.4 Productivity

Production of organic substances by 20 different species of cyanobacteria grown in Allen and Nelson medium with salinity 20 ppt and temperature 28°C were studied. The cells were harvested on 24th day. Known weight of the samples were taken for the analysis and productivity was estimated in terms of µg C / L / hr/ gram wet weight of sample. The results are presented in Fig 6.22 and Table 6.19 (Appendix 3). Gross production was high in Lyngbya aerugineo (C15) and Oscillatoria jasorvensis (C23).

Fig 6.22 Productivity of selected species of cyanobacteria
6.4 Discussion

6.4.1 Optimisation of culture conditions

All the four species of cyanobacteria studied in the present investigation showed almost identical requirements of salinity and pH. Salinity up to 20 ppt was found to be optimum for all species. Growth was found to occur at all levels of salinity indicating that the species can tolerate wide fluctuations of salinity. This is in agreement with earlier studies reported on cyanobacteria (Kaushik and Sharma, 1997, Subramanian and Thajuddin, 1995 and Newby, 2002). However, higher salinity was found to be unsuitable for survival as it affects the protein content. Kaushik and Sharma (1997) studied the effect of salinity stress on the halotolerant forms such as Nostoc linckia, Westiellopsis prolifica and Tolypothrix ceylonica and found that there was a 3-5% reduction in total protein content when these forms were grown in 100mM NaCl. It was found that the growth of cyanobacteria in the presence of NaCl depends on the availability of nutrients and the rate and mode of carbon fixation (Atre, 1998).

Cyanobacteria showed a wide range of adaptability and flexibility in their response to pH. The pH of the medium plays an important role in culturing as it determines the solubility of CO₂ and minerals in the medium, which in turn, directly or indirectly influence the metabolism of the algae (Markl, 1977). Cyanobacteria vary, not only in their preference for different pH but also in their qualitative response which is dependent upon the composition and buffering capacity of the medium, amount of CO₂ dissolved, temperature and metabolic activity of the cell. Venkataraman (1972), Subramanian and Shanmugasundaram (1987) and Radha Prasanna et al. (1998) studied the pH tolerance of cyanobacteria. The organisms have been reported to grow well in a range of pH 7.5 - 10 (Gerolff et al., 1950; Kratz and Myers, 1955; Okuda and Yamaguchi, 1956; Prasad et al., 1978; Roger and Reynaud, 1979). However, there are reports of cyanobacteria growing at pH as low as 3.5.
(Aiyer, 1965). In the present study, the strains exhibited broad tolerance to pH.

Growth kinetics of four cyanobacterial strains in various media proved that Allen and Nelson medium was significantly superior to other media in terms of both chlorophyll and growth and was very simple to prepare. All the media showed good growth except nitrogen-free medium in which no growth was observed. Unicellular species *Gloeocapsa livida* could grow well only in Allen and Nelson medium.

### 6.4.2 Biochemical composition, Growth kinetics, Pigment composition and Productivity

Cyanobacteria constitute a large proportion of the world's organic matter. They offer the most efficient means of fixing solar energy in the form of biomass. The cyanobacterium *Spirulina* has already been commercially exploited because of its merits viz. maximum yield and utility of cellular constituents (Borowitzka and Borowitzka, 1988). Therefore, it would be appropriate if the growth characteristics and biochemical composition of the presently investigated species could be compared with those of *Spirulina*. *Spirulina* strains from CFTRI, India were found to have protein content within the range of 40 – 55% and total lipid content between 2 – 7% (Tasneem Fatma *et al.*, 1999). Phycocyanin was reported in them between 10 and 19% of the weight. In the present study, out of forty strains, *Phormidium dimorphum* (C9) contained total proteins of 39% of the dry weight, which was comparable to *Spirulina*, the reference species. Eighteen species were reported with more than 11% lipid content, which was very much greater than that of *Spirulina*. In the present study, phycocyanin was estimated as 10% of the weight in *Lyngbya semiplena* and *P. corium* (C13). Highest amount of carbohydrate (13.02%) was present in *Oscillatoria pseudogeminata* (C81) whereas, carbohydrate content of dried Spirulina (CFTRI) could be improved only up to 10% as per Venkataraman, 1989. Vaidya and Mehta, 1989 reported that the dried matter of blue green algae like *Anabaena* and *Nostoc* contained
Ecological and biochemical studies on cyanobacteria of Cochin estuary and their application as source of antioxidants

total carbohydrates varying from 14.6% to 20.2%. When nitrogen fixing Cyanobacteria were grown under diazotrophic conditions, protein, carbohydrate, and lipid comprised 37-52%, 16-38% and 8-13% of the dry weight respectively (Vargas et al., 1998). Kebede and Ahlgren (1996) found maximum chlorophyll content of 2.4% of dry weight in \textit{S.platensis} and also reported that the ratio of carotenoids to chlorophyll-a was within the range of 0.27 to 0.64 in them. In the present investigation, of the twenty strains studied, four strains contained more than 3% of chlorophyll. Seven species were reported having the ratio of phycobiliprotein to chlorophyll greater than one. In \textit{Phormidium tenue}, the ratio of carotenoids to chlorophyll was greater than one.

Since the culture conditions are known to change the biochemical composition of the algae (Ciferri, 1983), the constituents can be improved upon further by manipulating culture conditions. The overall results showed that, of the strains undertaken for the detailed biochemical evaluation, \textit{Oscillatoria pseudogeminata} (C81), \textit{Phormidium dimorphum} (C9), \textit{Phormidium corium} (C13), \textit{Gloeocapsa gelatinosa} (C28) \textit{Oscillatoria foreauii} (C72) and \textit{Lyngbya semiplena} (C34) are suitable for mass cultivation as a source of food/ feed as they showed not only good growth and productivity but also high levels of valuable biochemical constituents and pigments, very much similar to that of \textit{Spirulina}.

The techniques of genetic manipulation are likely to be applied to cyanobacterial biotechnology in the near future. Genetic manipulation may lead to increased production of valuable primary and secondary metabolites, faster growth rates, tolerance of extreme growth conditions and so on (Lewin, 1983). Considerable variation in growth and cellular constituents justify the importance of strain selection over the complex and time-consuming genetic engineering process involved in strain improvement. Cyanobacteria represent an ecologically sensible form of technology, and it is hoped that, with enduring research and development, their enormous potential would be realized by all.