Chapter IV

Discussion
Cyanobacteria are photosynthetic prokaryotes which require both macro and micro nutrients for their growth and development. So nitrogen is one of the important bio metal which is assimilated by some of the cyanobacteria are unable to fix nitrogen therefore they depend on the nitrogen source in the medium for their survival. Due to changes in the environment some of the cyanobacteria are forced to grow under nitrogen depletion conditions (Allen and Smith, 1969; Allen et al., 1990). This nitrogen depletion leads to the loss of chlorophyllic chlorosis in cyanobacteria. Several workers have made an attempt the responses of cyanobacteria under chlorosis conditions (Collier and Grossman, 1992; Warner et al., 1986; Grossman, 1994). The studies related to the organization and functions of thylakoid membranes under nitrogen starvation in cyanobacteria are scanty. Therefore in this investigation an attempt has been made to study the effect of nitrogen deprivation on the electron transport, energy transfer and thylakoid organization in the cyanobacteria *Synechococcus* 6301. This organism has been extensively used for both biochemical and biophysical studies by earlier workers (Newmann and Sherman, 1978). *Synechococcus* being a unicellular organism can be easily manipulated in genetic studies.

For the study log phase *Synechococcus* cells were taken and suspended in the medium containing different concentrations of nitrate. The cells which were grown in 10 µM nitrate containing medium exhibited no changes in the growth parameters. Nitrogen stress can be induced when the cells were incubated in the growth medium
which contain nitrate concentrations from 10 to 40 µM. Hence the cells were suspended in the growth medium which contains 10, 20, 40 µM of nitrate to induce the nitrogen stress and photosynthetic electron transport measurements were made using oxygen electrode in terms of either O₂ evolution or consumption. Since electron transport donors and acceptors are unable to enter inside the cell of *Synechococcus* 6301 spheroplasts have been prepared by lysozyme digestion and then they were used for electron transport measurements studies control spheroplasts, exhibited whole chain electron transport activity equal to that of 235 µmoles of O₂ consumed mg Chl⁻¹ h⁻¹. Transfer of cells to nitrogen depletion medium (10-40 µM showed time dependent inhibition in whole chain electron transport activity (Table 6 and 7). The inhibition in whole chain electron transport could be due to 2 reasons a. Alteration at the level of PS II and b. Changes at the level of PS I or both. To confirm above preposition both PS II and PS I catalyzed electron transport activities have been studied individually by the following procedure Trebst (1975). The incubation for 24 hrs period caused inhibition in PS II activity by 53% with 10 µM of nitrate concentration in growth medium (Table 8). The inhibition in PS II mediated electron transport could be due to three reasons 1) Alteration at water oxidation complex 2)changes at LHC II 3)Modification of D1 protein of PS II our results are in agreement with the earlier observations with Allen (1990) who showed loss of PS II activity due to shift cells to nitrogen depletion medium.
The further analysis of PS I catalyzed electron transport assays in spheroplasts of *Synechococcus* 6301 showed that 34% loss in the activity of PS I (Table 9). This loss of PS I activity could be due to the changes in carotenoids of PS I as reported earlier by Biswal (1994). To examine the possibility of study the inhibition of PS II at different light intensities in both control and nitrogen depleted cells. The inhibition was more at light saturating conditions (420 wm⁻²) then the light limiting conditions could be due to the changes of accessory pigments present in the LHC II of PS II in the above cyanobacterium *Synechococcus* 6301 (Table 10).

Phycobiliproteins are major components in the LHC complex of PS II in cyanobacterium. In PBsomes the energy flow will be from PC to Chl a through APC to APCB. Hence a study has been made by depleting the nitrogen concentration to 20µM a spectral study. The decreases in the absorption of PC clearly demonstrate that there are changes between apoproteins and chromophore interaction. The shift in the peak position supports the nitrogen stress induced structural alterations in phycobiliproteins (Fig 16). Duke *et al.* (1989) reported that nitrogen stress induces the degradation of PC hexamer and linear polypeptides, during the nitrogen stress in cyanobacteria. This was further supported by the observations of Grossman *et al* (1993). Regarding the involvement of serine type protease in protein degradation during nitrogen starvation. As nitrogen deprivation affected the PC absorption, fluorescence emission spectra of PC has been measured at room temperature. In treated fluorescence emission peak is shifted from 647 to 652 nm with 40% decrease
of in the fluorescence intensity (Fig 17). Murthy (1991) has already reported the alteration of energy transfer from PC to Chl a during mercury stress in *spirulina platensis*. Other workers also reported the degradation of linear polypeptides and alterations of energy transfer in other cyanobacteria under nitrogen stress (Boussibu and Richmond, 1980; Sauer *et al.*, 1999). From the above study it is clear that nitrogen depletion affects the spectral properties and induces alterations in energy transfer.

To identify the main target in PBsomes they were isolated from control and nitrogen stress samples by using sucrose density gradient centrifugation. In density gradient centrifugation of treated sample a separate light blue band has been noticed above 1molar region in treated sample (Fig 18). This clearly indicates that nitrogen starvation disrupt the assembly of phycobiliproteins and affect the energy transfer. Spectral measurements of isolated PBsomes clearly demonstrated that the energy transfer from PC to APC B is getting affected under *in vitro* conditions (Fig 19 and 20). Prolonged incubation under nitroge stress induces serine type of protease which is capable of degrading the phycobiliproteins as reported by others (Lockau *et al.*, 1981; Maldener *et al.*, 1991). The amino acids released by the protein degradation provide substances to meet the needs for the synthesis of new proteins for its survival under nitrogen stress (Allen and Smith, 1969).

Thylakoid membranes contain non bilayer lipids in association with proteins to perform the photosynthesis. Galactolipids, sulfolipids are essential for prefer package pigment proteins in thylakoid membrane to perform light reactions of photosynthesis.
Hence an attempt has been made to study effect of nitrogen depletion on lipid peroxidation of thylakoid membranes. This lipid peroxidation was measured in terms of MDA formation. The depletion of nitrogen in *Synechococcus* cells caused the increase in lipid peroxidation (Table 12). This enhancement of lipid peroxidation has increase relationship with PS II photochemistry (Table 13). These alterations of energy transfer in pigment proteins changes of lipid peroxidation of thylakoid membrane are responsible for altered PS II photochemistry in *Synechococcus* cells. After study in the lipid changes a study has carried out to know the alterations in proteins in thylakoid membrane. SDS-PAGE analysis has clearly demonstrated the alterations of some polypeptides of total cell proteins. The disappearance of β subunit of PC (22 kDa) and linker polypeptides under nitrogen stress. The possible reason for the disappearance of 22 kDa could be protease mediated degradation of PC β-subunit as earlier reported by several workers (Lockau et al 1981; Maldener et al 1991). These spectral alterations in energy transfer and specific degradation of PC β-subunit is responsible for altered PS II photochemistry under nitrogen depletion in the cyanobacterium *Synechococcus* 6301.