Results and Discussion
3.1 Growth study of *S. platensis*

Culture of *S. platensis* in conical flask has its limitation in providing complete information related to growth, development and production of value added chemicals viz. vitamins, amino acids, fatty acids, proteins and polysaccharides both in quantity and quality and disposing off carbon dioxide one of the major causes of global warming (Capone *et al*., 1997; Ferreira *et al*., 2004). Extensive research has been conducted on production of *S. platensis* (U.N. World Health Organization, 1993; Sassano *et al*., 2004; Costa *et al*., 2003, 2004) living at salt lakes in the tropical regions (Busson, 1971). The Chad Lake has ingested *S. platensis* as a protein source from time immemorial (Richmond, 1992; Dillon and Phan, 1993; Campanella *et al*., 2002; Mazo *et al*., 2004).

The protein of *S. platensis* is composed of lot of amino acids essential to human being (Richmond, 1992; Campanella *et al*., 2002; Mazo *et al*., 2004; Singh *et al*., 2005), and richly contains minerals (Johnson and Shubert, 1986; Cohen *et al*., 1997; Blinkova *et al*., 2001; Pyufoulhouk *et al*., 2001; Gireesh *et al*., 2004) and nutrient substances except for vitamin C. *S. platensis* is
drawing public attention as a nutrition-supplementary food for human health (Mazo et al., 2004). Therefore, *S. platensis* is expected as one means for solving the food problem of the Earth (Ciferri, 1983; Belay et al., 1994, 1996, 1997; Mazo et al., 2004; Singh et al., 2005). WHO (1992) has described *S. platensis* as one of the greatest super food on the Earth and NASA considers it an excellent compact food for space travel, as small amount can provide a wide range of nutrients (Nishi et al., 1987; Oguchi et al., 1989; Khan et al., 2005).

*S. platensis* has been usually cultured at various places in world under natural growth condition but the use of a culture pool, which has a relatively shallow depth for exposing the culture containing cyanobacteria to sunlight. *S. platensis* grows better in a liquid environment or culture medium of high pH and alkalinity (Busson, 1971; Grant et al., 1990). It forms massive population in tropical and sub-tropical water bodies, characterized by high level of carbonate and bi-carbonate and high pH. The fame of *S. platensis* is a result of its economic importance, which is due to its nutritional and biomedical values (Khan et al., 2005). The mass culture becomes attractive as a source of food feed and fine
chemicals (Richmond, 1992; Belay et al., 1994, 1996, 1997; Singh et al., 2005; Chamorro et al., 2002).

Physico-chemical profiles of *S. platensis* is describing the relationship between growth and environmental factors especially irradiance flux, density and temperature (Vonshak and Tomaselli, 2000), which are important in the evolution of microalgae and cyanobacteria for biomass production, as well as their general characterization. High alkalinity is mandatory for the growth of *S. platensis* and bicarbonate is used to maintain high pH (Belkin and Boussiba, 1981; Grant et al., 1990; Huang et al., 2002). Source of nutrition also affect the growth rate of cyanobacteria (Faintuch et al., 1991)

In the present study we investigated comparative growth rate of *S. platensis* on Zarrouks Basal (ZB) and Zarrouks Modified (ZM). The growth of *S. platensis* in flask culture was monitored and expressed in terms of dry weight, chl a content, specific growth rate, turbidity, protein content and protein profile when grown on Zarrouks Basal (ZB) and Zarrouks Modified (ZM) media.
Specific growth rate of \textit{S. platensis} was 0.066 (\(\mu\text{h}^{-1}\)) on Zarrouk's basal medium and 0.052 (\(\mu\text{h}^{-1}\)) on Zarrouk's modified medium (Table 3.1). The data shows that specific growth rate of \textit{S. platensis} is higher on ZM medium. The absorption spectrum of \textit{S. platensis} on ZM culture medium was noticed to be more prominent as compared to ZB medium (Fig. 3.1 a, b). The absorption maxima of blue range (645, 665 nm) were having more optical density as compared to ZB medium. This shows that photo harvesting pigment was qualitatively increased on ZM culture medium.

The chl a content of \textit{S. platensis} was 24.805 \(\mu\text{gml}^{-1}\) on ZM medium and 19.387 \(\mu\text{gml}^{-1}\) on ZB medium (Fig. 3.2). The protein content of \textit{S. platensis} on ZB medium was 610 \(\mu\text{gml}^{-1}\) and 733 \(\mu\text{gml}^{-1}\) on ZM medium (Fig. 3.3). It is evident from results that protein content of \textit{S. platensis} is higher on ZM medium. Results on turbidity measurements revealed that the turbidity of \textit{S. platensis} is higher on Zarrouk's modified medium as compared to Zarrouk's basal medium (Fig. 3.5).
Table 3.1: Effect of micro ingredient of Zarrouk’s medium on growth: in terms of specific growth rate (μh⁻¹), dry weight (g l⁻¹) chlorophyll content (μg ml⁻¹) and protein content (μg ml⁻¹) of S. platensis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Growth parameters</th>
<th>Growth of <em>S. platensis</em> on Basal Zarrouk medium</th>
<th>Growth of <em>S. platensis</em> on Modified Zarrouk medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific growth rate</td>
<td>0.052</td>
<td>0.066</td>
</tr>
<tr>
<td>2</td>
<td>Dry weight</td>
<td>1.15</td>
<td>1.25</td>
</tr>
<tr>
<td>3</td>
<td>Chlorophyll content</td>
<td>19.387</td>
<td>24.805</td>
</tr>
<tr>
<td>4</td>
<td>Protein content</td>
<td>610</td>
<td>733</td>
</tr>
</tbody>
</table>
Fig. 3.1 Absorption spectra of chlorophyll a of *Spirulina platensis* grown on Zarrouks Basal (a) and Zarrouks Modified medium (b)
Fig. 3.2 Growth (in terms of chl a) of *Spirulina platensis* grown on Zarrouk's Basal and Zarrouk's Modified medium
Fig. 3.3 Growth (in terms of protein) of *Spirulina platensis* grown on Zarrouk's Basal and Zarrouk's Modified medium
Fig. 3.4  Protein standard curve with BSA
Fig. 3.5 Turbidity of *Spirulina platensis* on Zarrouk’s Basal and Zarrouk’s Modified medium
S. platensis was grown at different pH (8, 9, 10 and 11) in flask culture and monitored and expressed in term of dry weight (Fig.3.6a, b). The maximum bulk density about 1.25 g l⁻¹ was noticed when the pH of culture medium was maintained at 9.0 with medium volume 250 ml in a 500ml flask. The maximum bulk density was attained on 10th day of the culture with the increase of culture volume the maximum to one litre bulk density of S. platensis culture was shifted from 10th day to 12th day and remain to steady state for 24 hrs (Table 3.2). About 3.37 g l⁻¹ of S. platensis biomass was measured at this phase (Fig. 3.6b). The increase in the production of S. platensis could have been due to the availability of more space, oxygen, and light to the culture flask.

Results described in Fig 3.6 (a, b) suggest that S. platensis was grown on different pH 8, 9, 10 and 11 but the maximum yield of S. platensis is obtain on 8<9>10>11. Thus 9 pH is optimum for the growth of S. platensis. Earlier results also demonstrated that optimum pH for maximal growth of S. platensis was 9 to 9.5 ranges (Belkin and Boussiba, 1971). S. platensis is considered to be an alkalophilic organism by nature (Grant et al., 1990).
Fig. 3.6 Dry weight of *Spirulina platensis* on different pH in 500 ml flask (a) and in 1 litre flask (b)
Table 3.2: Physical and chemical composition of S. platensis

<table>
<thead>
<tr>
<th></th>
<th>Laboratory Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flask volume</td>
<td>500ml</td>
</tr>
<tr>
<td>Medium volume</td>
<td>250</td>
</tr>
<tr>
<td>Media used</td>
<td>Modified Zarrouk,s</td>
</tr>
<tr>
<td>Appearance</td>
<td>Fine powder</td>
</tr>
<tr>
<td>Colour</td>
<td>Green</td>
</tr>
<tr>
<td>Odour and Taste</td>
<td>Mild and green vegetable</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>1.25 g/l (on 10\textsuperscript{th} day)</td>
</tr>
</tbody>
</table>

**Chemical composition**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3-5%</td>
<td>3-5%</td>
</tr>
<tr>
<td>Ash</td>
<td>4%</td>
<td>5%</td>
</tr>
<tr>
<td>Proteins</td>
<td>60%</td>
<td>62%</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>1.7 g/kg</td>
<td>1.7 g/kg</td>
</tr>
<tr>
<td>PC+APC+PF</td>
<td>2.06g/Kg</td>
<td>2.15g/Kg</td>
</tr>
</tbody>
</table>
It has been observed that the culture conditions viz medium (Ciferri 1983; Venkatraman and Mahadevaswamy, 1992), temperature (Torzillo and Vonshak, 1994; Deshnium., 2000), light intensity (Vonshak and Guy, 1992; Vonshak, 1997) and pH (Grant et al., 1990; Belkin and Boussiba, 1971; Rafiquil et al., 2003, 2005) etc. play vital role in biosynthesis of chemical constituents of the S. platensis. According to different efforts of optimization of optimal temperature for laboratory cultivation of S. platensis which ranges from 30-38°C (Vonshak and Tomaselli, 2000) and the pH ranges between 9 - 9.5 (Rafiquil et al., 2003, 2005) to obtain maximum yield of S. platensis in laboratory.

Changes in the pattern of gene expression have been described by characterizing the protein profile of S. platensis under various growth conditions. Three groups can be classified in response to various environmental stresses:

i) Protein whose expression remains relatively unaffected by variation in environmental factors

ii) Proteins whose synthesis is switched off or inhibited specifically when environmental conditions are changed
Protein whose synthesis is increased or induced specifically under unfavorable environmental conditions

Proteins belonging to IIIrd category are called as stress proteins, which are of special interest because they perform important functions during adaptation to changed environment.

Chemical composition of cyanobacteria is known to be modified by composition of the culture medium (Mostert and Grobbelaar, 1981). Hence protein profile of *S. platensis* grown in Zarrouk's basal medium and Zarrouk's modified was compared using SDS-PAGE (Fig. 3.7). It has been found from results that *S. platensis* is capable to grow on ZM and ZB media and expressed and synthesized proteins. These proteins, however, differed in their molecular weights and sequence in which they are synthesized during change in the chemical composition of culture medium. There is only one protein that is expressed on Zarrouk's modified medium as compared to Zarrouk's basal medium. Protein, which was expressed only in Zarrouk's modified medium, is 21 KD, and the expressions of other proteins are similar on both
Fig. 3.7 Cellular protein profile of *Spirulina platensis*; protein markers (Lane 1); *Spirulina platensis* grown on ZB medium (Lane 2) and *Spirulina platensis* grown on ZM medium (Lane 3)
culture medium. The synthesis of the new proteins in response to

culture medium plays an important role in the maintenance of vital
cellular functions in cyanobacteria.

Zarrouk’s modified medium having some trace element for the
growth of cyanobacteria as compare to Zarrouk’s basal medium is
found to be better for the growth of S. *platensis*.

### 3.2 The effect of UV-B radiation on the morphology of S. *platensis*

*S. platensis* is a filamentous, photosynthetic, spiral shaped;
multicellular cyanobacterium (Cifferi, 1983). The filament of *S.
*platensis* consists of cylindrical cells arranged in unbranched
helicoidal trichomes. The helical structure of *S. platensis* varies
with the species and even within the same species. *S. platensis*
non-heterocystous filaments, composed of vegetative cells that
undergo binary fission in a single plane, shows easily visible
transverse cross-walls. The trichome, enveloped by a thin sheath
and no akinetes formation is occurred in any phase of life cycle.
Structurally, the cyanobacteria have similarities with plastids (chlorophyll-containing bodies) of the algae and all higher plants. They also possess a mucilaginous sheath of cellulose fibrils varying in thickness from one genus to another. *S. maxima* and *S. platensis* are the most important species in this genus and among these exist taxonomic differences in filaments, vacuoles and external cover or capsule regularity of each filament (Tomaselli, 1997).

The pitch distances of trichome of *S. platensis* vary with the climatic condition like temperature and radiation (Jeeji Bai and Seshadri, 1980; Jeejibai, 1985; Lewin, 1980; Kebede, 1997). Under specific climatic condition the helical nature of *S. platensis* vary with availability at different profiles of the pond. The *S. platensis* available at the top are having fewer spirals as compared to intermediate and bottom layer of the pond. Therefore, the spiral nature of *S. platensis* is influenced by light.

Microscopical examinations of *S. platensis* were carried out by bright field, fluorescent and scanning electron microscopy
demonstrating significant change in the trichome of UV-B treated as compared to UV-B untreated counterpart.

A bright field microscopy shows significant change in the filament of *S. platensis* during UV stress. A morphological difference in the filaments was observed when *S. platensis* was irradiated with UV-B radiation (Fig. 3.8 a, b); showing changes in terms of granulation, pigmentation and both apical and terminal end of filaments. Fluorescent microscope results (Fig. 3.9 a, b) revealed that fluorescent emission of pigment in UV-B untreated *S. platensis* was higher as compared to UV-B treated cells due to presence of high level of fluorescent pigments. The surface scanning electron micrograph (Fig. 3.10a) of UV-B untreated *S. platensis* shows the smooth morphological structure with the appearance of ridges due to coverage of sheath. However, UV-B treated *S. platensis* shows distorted and straight morphological structure (Fig. 3.10b).

It is confirmed by various microscopical examinations (Fig. 3.8 a, b; 3.9a, b and 3.10a,b) that UV-B radiation affect the
Fig 3.8 Bright field photomicrograph of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
Fig. 3.9 Fluorescent photomicrograph of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
Fig. 3.10 Scanning electron photomicrograph of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
morphology of *S. platensis*. It is evident from previous studies that environmental factor (Van Eyelenburg, 1979) such as light, temperature, and salinity, can affect the helical structure (Jeejibai and Seshadri, 1980; Jeejibai, 1985; Lewin, 1980; Kebede, 1997); for example, the filaments change from straight to the typical helical shape when they are shifted to growth with a high intensity of visible light (Fox, 1996).

Recent studies suggested that UV-B treatment not only damaged photosynthetic light-harvesting complex of cyanobacteria and but also affect the ultrastructure (Hongyan *et al.*, 2005; Holzinger and Lutz, 2006). Stress induced morphological changes are reported in microorganism *Pseudomonas aeruginosa* (Cefali *et al.*, 2002), cyanobacteria (Rajgopal *et al.*, 2000) and algae (Holzinger and Lutz, 2006).

### 3.3 Effect of UV-B Radiation on the growth and protein profile of *S. platensis*

*S. platensis* is rich in ingredients that have nutrition and biomedical values (Mazo *et al.*, 2004; Khan *et al.*, 2005). *S. platensis* is known to have components having
immunomodulating (Baojiang et al., 1994; Quershi et al., 1995; qureshi and Ali, 1996; Nemoto-Kawamura et al., 2004), antiviral (Patterson, 1993; Hayashi et al., 1994; Hernandez-corona et al., 2002; Shih et al., 2003), anticancer (Lisheng et al., 1991; Mittal et al., 1999; Liu et al., 2000; Dasgupta et al., 2001; Guan and Guo, 2002; Subhashini et al., 2004), antioxidant (Jorjani and Amirani, 1978; Gorban et al., 2000; Premkumar et al., 2000; DasGupta et al., 2001; Wang et al., 2001; Upasani and Balaraman, 2003; Chen and Zhou, 2003; Patel et al., 2006), anti allergic (Qishen et al., 1989), radioprotective (Moreno, 1997) and hypocholesterolemic (Nayaka et al., 1988; Devi et al., 1983) properties.

Cyanobacteria depend on solar radiation as the primary source of the energy in their natural environment. UV-B radiation has been a ubiquitous problem for life and particularly for the photosynthetic organisms including cyanobacteria (Capone et al., 1997; Sinha et al., 2001; Ferreira et al., 2004). The potential threat to these cyanobacterial communities is the continuous solar ultraviolet-B (UV-B 280-315) radiation reaching the Earth’s surface due to depletion of the stratospheric ozone layer (Blumthaler et al.,
1990; Crutzen, 1992; Kerr et al., 1993; Arrigo, 1994; Neale et al., 1998; Lubin et al., 1995; Sinha et al., 2003).

Ultraviolet radiation is injurious to a wide variety of biological systems. Biological effect of UV-B radiation includes DNA damage in most organism (Harm, 1980; Karentz et al., 1991, 1991b; Gour et al., 1997), killing of bacteria (Kumar et al., 2004), pigment bleaching and photoinhibition of photosynthesis in cyanobacteria (Cullen et al., 1992; Bhandari and Sharma, 2006), inhibition of motility (Donkor and Hader, 1995), inhibition of nitrogenase activity (Sinha et al., 1996; Tyagi et al, 2003), inhibition of heterocyst formation in some cyanobacteria (Sinha et al., 1996) and morphological changes (Wu et al., 2005; Hongyan et al., 2005; Holzinger and Lutz, 2006). Cyanobacteria show wide variation in tolerance to UV-B and posses a variety of defense strategies, such as avoidance of brightly lit habitats (Xiong et al., 1997; Rajgopal et al., 2005), production of UV-absorbing compounds, such as micosporine-like amino acid and scytonemin (Garcia-Pichel et al., 1993; Sinha et al., 2002; Sinha et al., 2003; Rezanka et al., 2004), and active repair or de novo synthesis of DNA (Sass et al., 1997; Sinha and Hader, 2002). Photodynamic reactions are
potential mechanisms by which ultraviolet radiation induces damage to living cells (Ito, 1983). The high energy of short wavelength photons absorbed by chromophore molecules can lead to the formation of singlet oxygen or free radicals that are known to destroy membranes and other cellular components (Benson et al., 1992; Alschcer et al., 1997; Mackerness et al., 1999; Vega and Pizarro, 2000).

In the present study we have investigated the impact of UV-B radiation on growth (in term of specific growth rate) and changes in biological compounds like chlorophyll, protein, carbohydrate content, and physiological parameters like nitrate uptake, nitrite uptake, nitrate and nitrite reductases in S. platensis.

Specific growth rate ($\mu h^{-1}$) for UV-B untreated S. platensis was 0.065, and for UV-B treated S. platensis was 0.049 (Table 3.3). The maximum bulk densities were 1.25 g l$^{-1}$ for UV-B untreated S. platensis and 1.10g l$^{-1}$ for UV-B treated S. platensis (Fig. 3.11). The specific growth rate and biomass were found to be lower for the UV-B treated cells of S. platensis as compared to UV-B untreated counterpart.
Table 3.3: Effect of UV-B stress on growth: in terms of specific growth rate ($\mu h^{-1}$), dry weight (g l$^{-1}$), chlorophyll content (in %), protein content (in %), carbohydrate content (in %), NR activity ($\mu$mol NO$_2^-$ produced mg$^{-1}$protein min$^{-1}$) and NiR activity ($\mu$mol nitrite reduced mg$^{-1}$protein min$^{-1}$) of UV-B treated and UV-B untreated S. platensis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Growth Parameters</th>
<th>UV-B UV-B untreated S. platensis</th>
<th>UV-B treated S. platensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific growth rate</td>
<td>0.065</td>
<td>0.049</td>
</tr>
<tr>
<td>2</td>
<td>Dry weight</td>
<td>1.25</td>
<td>1.10</td>
</tr>
<tr>
<td>3</td>
<td>Chlorophyll content</td>
<td>1.82%</td>
<td>1.41%</td>
</tr>
<tr>
<td>4</td>
<td>Protein content</td>
<td>61.0%</td>
<td>51.5%</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrate content</td>
<td>21.97%</td>
<td>34%</td>
</tr>
<tr>
<td>6</td>
<td>NR activity</td>
<td>8</td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td>NiR activity</td>
<td>5</td>
<td>2.25</td>
</tr>
</tbody>
</table>
Fig. 3.11 Dry weight of *Spirulina platensis* in response to UV-B stress
The chlorophyll a content was achieved 1.82% for UV-B untreated cells of *S. platensis* and 1.41% for UV-B treated cells of *S. platensis* (Fig. 3.12). Result shows that chlorophyll a content is reduced significantly for UV-B treated *S. platensis* as compared to UV-B untreated counterpart.

Protein content achieved for UV-B untreated cells of *S. platensis* was 61.0% and for UV treated cells was 51.5% (Fig. 3.13). Carbohydrate content achieved for UV-B untreated *S. platensis* was 21.97%, and for UV-B treated cells of *S. platensis* was 34% (Fig. 3.14). Result shows that protein content was higher (9.5%) in UV-B untreated *S. platensis* as compared to UV-B treated counterpart. It is evident that the carbohydrate content increased 12% in UV-B treated *S. platensis* as compared to UV-B untreated counterpart. Thus UV-B radiations stimulate the synthesis of carbohydrate for the protection of cell from stress.

Protein content and chlorophyll content is reduced under stresses (Sinha *et al.*, 1995; Vonshak, 1997, 1996). Carbohydrate synthesis is stimulated under stress (Tomaselli *et
Fig. 3.12 Growth (in terms of chl a) of *Spirulina platensis* in response to UV-B stress
Fig. 3.13 Growth (in terms of protein) of *Spirulina platensis* in response to UV-B stress
Fig. 3.14 Effect of UV-B stress on carbohydrate content of *Spirulina platensis*
Fig. 3.15 Carbohydrate standard curve (glucose)
Thus stress cells have a lower biosynthesis capacity for protein but higher biosynthesis capacity for carbohydrate.

Cifferi (1983) and Rafiqul et al., (2005) suggested that the culture conditions, temperature, light intensity, irradiation and pH etc. are known to change the biochemical composition of the cyanobacterium *S. platensis*. Protein content of *S. platensis* is grown commercially for health food, which may range from 55 to 70% dry weight (Belay and Ota, 1993). UV-B radiation affects cyanobacterial photosynthesis and nitrogen metabolism is also studied in cyanobacteria (Newton et al., 1979; Gour et al., 1997).

NO$_3^-$ and NO$_2^-$ is probably the most abundant source of nitrogen for cyanobacterial nutrition. The first step in the metabolism of any nutrient by cyanobacteria is the entrance of that nutrient into the cell with the help of specialized uptake systems. The assimilation of NO$_3^-$ by cyanobacteria involves NO$_3^-$ uptake and reduction of intracellular NO$_3^-$ (via NO$_2^-$) to NH$_4^+$, which is incorporated into organic compound (Manzano et al., 1976). Nitrate metabolism in cyanobacteria suggested that the uptake of NO$_3^-$ and NO$_2^-$ is an ATP-dependent process, which takes place
through a membrane, bound permease system (Singh et al., 1996a). The NO$_3^-$ uptake system is mainly dependent on light as the dark incubated cyanobacterial cells showed drastic decline in its NO$_3^-$ accumulating ability as compared to its light grown counterpart (Singh et al., 1996b).

Fig. 3.16 showed nitrate uptake pattern in *S. platensis*. It is evident from results that the rate of nitrate uptake for UV-B treated cells of *S. platensis* was lower as compared to UV-B untreated *S. platensis*. Nitrate uptake pattern was characterized by a faster uptake rate for first 120 min. followed by a slower uptake at least up to 3 hrs. Fig. 3.17 showed nitrite uptake pattern in *S. platensis*. It is evident from results that the rate of nitrite uptake for UV-B treated cells of *S. platensis* was lower as compared to UV-B untreated *S. platensis* (Fig. 3.17). The rate of uptake of nitrate was found to be more as compared to nitrite under similar conditions. It is evident from the results (Fig. 3.16, 3.17) that UV-B untreated cells of *S. platensis* had high affinity for NO$_3^-$ and NO$_2^-$ as compared to the UV-B treated cells of *S. platensis*. An UV-B radiation is affected nitrate, nitrite uptake and NR activity of *Spirulina platensis*.
Fig. 3.16 Effect of UV-B stress on nitrate uptake of *Spirulina platensis*
Fig. 3.17 Effect of UV-B stress on nitrite uptake of *Spirulina platensis*
Further to determine the correlation between nitrogen-metabolizing enzymes, nitrate reductase (NR), nitrite reductase (NiR) and NO$_3^-$ and NO$_2^-$ uptake, were also studied in both UV-B treated and UV-B untreated *S. platensis*. It is evident from the data that the UV-B treatment inhibited both NR and NiR activity by more than 50% in UV-B treated cells of *S. platensis* as compared to UV-B untreated *S. platensis* (Table 3.3).

It has been reported that UV-B exposure has a deleterious effect on the photosynthetic apparatus leading to the reduction in the supply of ATP and NADPH$_2$ (Kulandaivelu and Noorudeen, 1983). As such, disruption of cell membrane and/or alteration in thylakoid integrity as a result of UV-B radiation may partly damage the photosynthetic apparatus (Vu *et al.*, 1981). Thus, there is a possibility that the inhibition in NR activity might be due to the reduced supply of reductants and energy following the UV-B treatment.

UV-B radiation induced changes in nitrate metabolism are reported in *Anacystis nidulans* (Sinha *et al.*, 1995; Gour *et al.*, 1997) and green algae *Chlorella vulgaris* (Rai and Rai, 1997). Singh and Singh (2000) also suggested that high light stress
induced alterations in the nitrogen assimilatory enzymes in S. *platensis*.

Thus, UV-B radiation not only affects the biomass and biological compounds like chl a content, protein content, carbohydrate content, but also affects physiological parameters like nitrate uptake, nitrite uptake, nitrate reductase and nitrite reductase activity in S. *platensis*. This study is useful to study the impact of UV-B radiation on biomass and biological compounds of S. *platensis*. It will also be useful for enhancing potential of cyanobacteria in biotechnology.

### 3.4 Isolation, purification and Characterization of thylakoid membrane from S. *platensis* under UV-B stress

Cyanobacteria are unique prokaryotes due to the presence of distinct intracellular membrane system (Stanier, and Cohen, 1977; Gantt, 1994). The fundamental membrane structure of the cyanobacterial cell is the thylakoid membrane (Nomura *et al.*, 1995; Omata and Murata, 1983; Norling *et al.*, 1997; Norling *et al.*, 1998), a peptidoglycan layer between them (Jost, 1965; Murata *et al.*, 1981; Omata and Murata, 1983; Fujita *et al.*, 1994: Allnutt,
1996; Meijer et al., 1999) and lipopolysaccharide is a major constituent of the outer membrane (Weise et al., 1970; Omata and Murata, 1983; Neisser et al., 1994; Fujita, 1996; Meijer et al., 1999; Zak et al., 2001). Cyanobacteria have the thylakoids, which is the site for both photosynthesis and respiration (Peschek et al., 1988; Gantt, 1994; Cooley and Vermaas, 2001).

Thylakoid membrane is photosynthetically active membrane found in the cyanobacteria. Rapid adaptations to number of environmental factors are accompanied by changes in the lipid and protein content of thylakoids. Thus regulation of synthesis and assembly of all these elements is required to ensure the optimal function of this membrane (Vothknecht, 2001; Vothknecht and Soll, 2005).

The envelope and thylakoid membrane of cyanobacterial cells have been separated by using lysozyme treatment followed by French Pressure and separation using sucrose density gradient with slight modification of the method as described by Omata and Murata (1983) and Fluda et al., (1999a). Membrane plays an important role during Na⁺ transport across the membrane (Apte
and Thomas, 1986; Apte and Haselkorn, 1990; Ramani and Apte, 1997; Fluda et al., 1999a).

LC-MS has revolutionized the biological science, and now biological macromolecules are mass measured with great accuracy and highly resolved spectra reveal subtle molecular heterogeneity (Whitelegge et al., 1998).

In present study, we investigated comparative analysis of the thylakoid membrane in UV-B untreated and UV-B treated S. platensis. The separation and characterization of the thylakoid membrane in S. platensis under UV-B stress has undergone tremendous changes in its thylakoid membrane morphology, pigment; chl a, absorption spectrum of chl a, fluorescent spectrum and protein profile.

The cells of S. platensis were found to be very resistant to mechanical treatment and were efficiently disintegrated only after freezing and thawing followed by lysozyme treatment at 37°C overnight. This was followed by French pressure. Unbroken cells were removed by centrifugation at 18,000 rpm for 30 min. Thylakoid membrane were harvested at 40,000xg for 90 min. on
the sucrose gradient from 30 to 90% (w/v) and were collected at interface between 39% and 50% with a light green band. Fig. 3.18 shows the light green band of thylakoid membrane of *S. platensis*.

A bright field microscopy result shows significant changes in the thylakoid membrane of UV-B treated *S. platensis* as compared to UV-B untreated counterpart (Fig. 3.19 a, b). It is evident that thylakoid membranes of *S. platensis* showed high chl *a* content and thickening on the outer side of the membrane as compared to UV-B treated counterpart. It is evident from result (Fig. 3.19 b) that under UV-B stress the membrane of *S. platensis* become partially distorted.

It is evident from the result of the absorption spectrum of thylakoid membrane of UV-B treated *S. platensis* that chl *a* content of thylakoid membrane was decreased as compared to UV-B untreated counterpart (Fig. 3.20 a, b). A dominating peak of thylakoid membrane was recorded at 660 nm that confirms the purity of membrane. Occurrence of this peak also suggests the presence of chl *a* in the thylakoid.
Fig. 3.18 Thylakoid membrane of *Spirulina platensis* showing dark green band
Fig. 3.19 Bright field photomicrograph of thylakoid membrane of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
Fig 3.20 Absorption spectra of chl a at 660nm of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
This is the first report, which deals with the separation, and characterization of pigment of thylakoid membrane of UV-B treated *S. platensis* through LC-MS. We used ODS column, utilizing methanol and 2 propanol as mobile phase by using APCI method for the analysis of pigment chl a in *S. platensis*. LCMS analysis of pigment chl a in UV-B treated *S. platensis* showed alter chl a level as compared to UV-B untreated counterpart (Fig. 3.21a, b). The level of chl a in UV-B untreated *S. platensis* was higher than that of UV-B treated counterpart (Fig. 3.21a, b). Chl a was separated and purified from other pigments through an ODS column and then subjected to PDA (photodiode array) detector. Chlorophyll is a light harvesting protein, which serve as photosynthetic antenna through absorption and funneling of excitation energy to photosystem II and I. Chl a is a porphyrin derivative having molecular weight 893.49 (Fig 3.22). Due to Na adduct of molecular ion (M+Na⁺), m/z ratio of chlorophyll altered to 915 (Fig 3.23 a, b). Chlorophyll had a specific absorbance peak at 660 nm. Our LC-MS coupled with PDA detector result demonstrated that Chl a level is decreased in UV-B treated *S. platensis* as compared to UV-B untreated counterpart.
Fig. 3.21 Part of total ion chromatogram of LC-MS analysis of pigment chl a of UV-B untreated (a) UV-B treated (b) *Spirulina platensis*
Fig. 3.22 Chemical structure of chlorophyll a having a molecular weight 893.49
Fig. 3.23 Mass spectra of chl a in UV-B untreated (a) and UV-B treated (b) Spirulina platensis
Environmental factors such as UV radiation are known to affect photosynthesis in both cyanobacteria (Vass et al., 2000). Changes in the pattern of gene expression have been described by characterizing the protein profile of *S. platensis* under various growth conditions. Protein whose synthesis is induced under stress is called stress proteins, which are of special interest because they perform important functions during adaptation to changed environment.

Cyanobacterial sp. is capable to grow under UV-B stress but some stressed proteins are over expressed and some disappeared. It is evident from the SDS-PAGE electrogram of *S. platensis* (Fig. 3.24) that intact trichomes of *S. platensis* are exposed to UV-B radiations, which affect the protein profile of *S. platensis*. This UV-B exposure results in alterations in the pigment-protein complexes CP47 and CP43 are affected by UV-B irradiation. Furthermore, 94kD and 20KD protein band is only appeared in the sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of UV-B-exposed thylakoids membrane of *S. platensis* as compared to UV-B untreated
Fig. 3.24 UV-B induced modification of thylakoid membrane protein synthesis in *Spirulina platensis*; protein markers (Lane 1); UV-B treated (Lane 2) and UV-B untreated (Lane 3)
counterpart. This 94 kDa and 20KD protein appears not to be newly induced by UV-B exposure, but could possibly have originated from the UV-B-induced cross-linking of the thylakoid proteins.

Several studies demonstrated that thylakoid membrane proteins were affected by UV irradiation (Rajgopal et al., 1998, 2000). D1 protein of thylakoid membrane was showed as a sensitive protein to environmental stress condition: under various unfavorable conditions like drought, nutrition deficiency, heat, chemical stress, ozone fumigation as well as UV-B and visible light stresses (Giardi et al., 1997; Campbell et al., 1998; Vass et al., 2000). Similar conclusions have been reached in higher and lower plants during photoinhibitory stress (Rintamaki et al., 1994) Proteins from thylakoid membrane have studied in cyanobacterium Synechocystis sp. PCC 6803. (Wang and Chitnis, 2000; Vass et al., 2000).

The fluorescence emission spectrum of the chlorophyll of thylakoids membrane was monitored in UV-B treated and UV-B untreated S. platensis (Fig. 3.25 a, b). Fluorescence emission
Fig. 3.25 Fluorescence emission spectra of the chl a of thylakoids membrane of UV-B untreated (a) and UV-B treated (b) Spirulina platensis
spectrum of the chlorophyll was taken at room temperature. Present results clearly demonstrate that prolonged exposure of UV-B radiation alters the fluorescence emission spectral profile of the thylakoid membranes of *S. platensis* as compared to UV-B untreated counterpart (Fig. 3.25 a, b). Thus, it is evident that UV-B radiations alter the fluorescence emission spectral profile of thylakoid membrane of *S. platensis*.

Rajgopal *et al.*, (1998) suggested that prolonged exposure of UV-B irradiation affects the chl a-protein complexes of the thylakoid membranes of cyanobacteria. Thus, it is clear that chlorophyll a antennae of *S. platensis* are significantly altered by UV-B radiation. Similar conclusions have been reached in higher and lower plants during photo-inhibitory stress (Ohad *et al.*, 1984; Rontamaki *et al.*, 1994; Garnier *et al.*, 1994) and in cyanobacteria (Krause and Weis, 1991; Rajgopal *et al.*, 2000).

3.5 Effect of UV-B radiation on fatty acids profile of *S. platensis*

*S. platensis* possesses diverse biological activities and having a long history of use as food supplement (Belay, 1997;
Cifferi, 1983). It is the most commonly used species of cyanobacteria as concentrated natural source of nutrition and biomedical values, e.g. essential fatty acids (Hwang, 1989; Pascaud, 1993; Huang and Mills, 1996; Cohen et al., 1987; Mahajan and Kamat, 1995), and so on.

Fatty acids are composed of a long hydrocarbon chain and a terminal carboxylate group and a great variety of fatty acids exit in nature. In biological systems, fatty acids are mostly encountered as components of lipids. The lipids that contribute to the structure and function of biological membrane are called structural lipids. Cyanobacteria and some bacilli can introduce double bonds into fatty acids by using oxygen-dependent desaturases enzyme (Bloomfield and Bloch 1960; Shanklin and Cahoon, 1998).

Fatty acid composition is known to be affected by changes in growth rate (Liang et al., 2006) and by the concentration of substrates in the medium. The effects of changes in growth conditions on lipid accumulation and its composition have been reviewed by Rattrary et al., (1975).
Fatty acid has got a property to become less saturated at lower temperature and become more unsaturated at higher growth temperature. This is apparently due to the lower solubility of oxygen at higher temperature resulting in low concentration of unsaturated fatty acid, since low oxygen concentration causes a decrease in the desaturation of the saturated fatty acids. Oxygen is required for the conversion of stearic acid to oleic acid and to linolenic acid etc. Changes in pH, have little effect on growth and consequently on the fatty acids. Organisms accumulate more lipids in the medium containing organic source rather than inorganic source of nitrogen (Blinc and Hocevar, 1953; Witter et al., 1974). Temperature is a crucial parameter, since it may have a substantial impact on fatty acid composition itself (Wada et al., 2000; Deshnium et al., 2000) as well as on the dynamics of repair mechanisms (Roos and Vincent, 1998).

Polyunsaturated fatty acids (PUFAs) play important roles as structural components of membrane phospholipids. Polyunsaturated fatty acids have made up an essential part of the human diet (Pascaud and Brouard, 1991; Pascaud, 1993). They are nutritionally important for various reasons. PUFA such as GLA
is found in higher plants like evening primrose, black currant and borage as well as in cyanobacteria (Huang et al., 1982) and fungi. In plant oils GLA is present either in low conc. or is associated with other undesirable fatty acids, from which large scale purification could be very expensive. *S. platensis* is unique that contains substantial quantities of GLA (Huang et al., 1982; Huang and Mills, 1996; Cohen et al., 1987; Mahajan and Kamat, 1995).

In present study we investigated the effect of UV-B stress on the fatty acid profile of *S. platensis*. This is a first attempt of GC-MS analysis of fatty acids in *S. platensis* under UV-B stress.

Before GC analysis it is necessary to prepare non-reactive derivatives of fatty acids. We used bis-trimethylsilyl trifluoroacetamide (BSTFA) for the silylation of fatty acids. Trimethylsilyl (TMS) ester derivatives are used more widely than any other for the gas chromatographic analysis of hydroxy compounds. Their main value increases the volatility and reduces the polarity of the parent molecules, ensuring sharp symmetrical peaks on GC analysis (Jeong and Lachance, 2001).

Results of GC-MS analysis of silylated fatty acids in UV-B untreated *S. platensis* shows both saturated and unsaturated fatty
acids. Major constituents of fatty acids were short chain and medium short chain fatty acids (Fig. 3.26 a, b). The solvent delay was 4 min. Therefore total ion chromatogram (TIC) is from 4 min (Fig. 3.26a). The saturated fatty acids were octanoic (C₈), nonanoic (C₉), decanoic (C₁₀), dodecanoic (C₁₂), tetradecanoic (C₁₄), hexadecanoic (C₁₆) and octadecanoic (C₁₈). Monounsaturated fatty acids were tetradecenoic (C₁₄:1), hexadecenoic (C₁₆:1) and polyunsaturated fatty acids were nonadienoic (C₉:2), decadienoic (C₁₀:2), dodecatrienoic (C₁₂:3), tetradecatrienoic (C₁₄:3), hexadecatrienoic (C₁₆:3) and octadecatrienoic (C₁₈:3).

Further in contrast to UV-B untreated, GC-MS analysis of silylated fatty acids in UV-B treated S. platensis shows short chain and medium short chain saturated, monounsaturated and polyunsaturated fatty acids (Fig. 3.26 b). The dominant saturated fatty acids were decanoic (C₁₀), tetradecanoic (C₁₄), hexadecanoic (C₁₆) and octadecanoic (C₁₈) and in unsaturated fatty acids series both monounsaturated and polyunsaturated fatty acids were obtained. Monounsaturated fatty acids were decaenoic (C₁₀:1)
Fig. 3.26 GC-MS study of trimethylsilyl derivative of fatty acids of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
dodecenoic (C_{12:1}), tetradecenoic (C_{14:1}), hexadecenoic (C_{16:1}) and octadecenoic (C_{18:1}) and polyunsaturated fatty acids were nonadienoic (C_{9:2}), decadienoic (C_{10:2}), decatrienoic (C_{10:3}), dodecatrienoic (C_{12:3}), tetradecatrienoic (C_{14:3}), hexadecadienoic (C_{16:2}), octadecadienoic (C_{18:2}) and octadecatrienoic (C_{18:3}) obtained during fatty acids separation.

MS profiles of TMS derivative of fatty acids are shown in Fig. 3.27.1 to 3.27.5. The mass spectra of TMS derivative of fatty acids octanoic, nonadienoic, decaenoic and nonanoic are shown in Fig. 3.27.1. The mass spectra of octanoic (M^+ = 216) and decaenoic (M^+ = 242) showed [M-15]^+ fragment ion at m/z value 201 and 227 respectively which is well known fragmentation of TMS ester of fatty acid (Fig. 3.27.1a, c). The mass spectra of TMS derivative of fatty acids decanoic, dodecatrienoic, dodecenoic and dodecanoic are depicted in Fig. 3.27.2. The mass spectra of TMS derivative of fatty acids tetradecanoic, tetradecatrienoic, tetradecenoic and hexadecatrienoic are shown in Fig. 3.27.3. The mass spectrum of tetradecanoic (M^+ = 300) showed [M-15]^+ fragment ion at m/z 285 which is well known fragmentation of TMS ester of fatty acid.
Fig. 3.27.1 Mass spectra of identified peaks of fatty acids of \textit{Spirulina platensis} (C\textsubscript{8} to C\textsubscript{10:1}) a) C\textsubscript{8:0} b) C\textsubscript{9:2} c) C\textsubscript{10:1} d) C\textsubscript{9:0}
Fig. 3.27.2 Mass spectra of identified peaks of fatty acids of *Spirulina platensis* (C\textsubscript{10} to C\textsubscript{12}) a) C\textsubscript{10:0} b) C\textsubscript{12:3} c)C\textsubscript{12:1} d) C\textsubscript{12:0}
Fig. 3.27.3 Mass spectra of identified peaks of fatty acids of *Spirulina platensis* (C<sub>14:3</sub> to C<sub>18:0</sub>) a)C<sub>14:0</sub> b)C<sub>14:3</sub> c)C<sub>14:1</sub> d)C<sub>16:3</sub>
(Fig. 3.27.3a). The mass spectra of TMS derivative of fatty acids hexadecadienoic, hexadecenoic, hexadecanoic and octadecatrienoic/ gamma linolenic acid are shown in Fig. 3.27.4. The mass spectra of TMS derivative of fatty acids octadecadienoic, octadecenoic, octadecanoic and decadienoic are shown in Fig. 3.27.5 and mass spectra of decatrienoic are shown in Fig. 3.27.6.

These results indicate that membrane lipid unsaturation increases the tolerance of cyanobacterium to UV radiation. It is evident from the GC-MS result of silylated fatty acid is that the percentage of SFA in UV-B untreated S. platensis is 46.7% and the percentage of MUFA and PUFA is 53.3% of total fatty acid content. The percentage of SFA in UV-B treated S. platensis is 23.6% and the percentage of MUFA and PUFA is 76.4% of total fatty acid content. Thus UV-B radiation reduced the degree of saturation in S. platensis and increased 23.1% unsaturated fatty acid content in UV-B treated S. platensis as compared to UV-B untreated counterpart. It is evident from result that in UV-B treated S. platensis gamma linolenic (C₁₈:₃) acid is an important
Fig. 3.27.4 Mass spectra of identified peaks of fatty acids of *Spirulina platensis* (C₁₆:₂ to C₁₈:₃) a) C₁₆:₂ b)C₁₆:₁ c)C₁₆:₀ d)C₁₈:₃
Fig. 3.27.5 Mass spectra of identified peaks of fatty acids of *Spirulina platensis*, a) C₁₈:2 and b) C₁₈:1  c)C₁₈:0 d)C₁₀:2
Fig. 3.27.6 Mass spectra of identified peaks of C\textsubscript{10:3} fatty acids of *Spirulina. platensis*
component of total content of PUFAs as compared to UV-B untreated counterpart.

This result indicates that in tolerance of UV-B radiation membrane lipid unsaturation play important roles in cyanobacterium \textit{S. platensis}. Previous studies showed that unsaturation of fatty acids are important in regulating membrane fluidity and physiological processes under stress (Hall \textit{et al.}, 2002, Gombos \textit{et al.}, 1997; Hessen \textit{et al.}, 1997).

3.6 Effect of UV-B radiation on Hydrocarbon profile of \textit{S. platensis}

Most cyanobacteria are a common source of a wide range of fatty acids, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacologically and industrially important products. Hydrocarbons are also used as sole carbon and energy source (Walker and Pore, 1978).

\textit{S. platensis} is good source of hydrocarbons (Tulliez \textit{et al.}, 1975; Fedelio and Favini, 1980), having antimicrobial activity against wide spectrum for four Gram-positive, six Gram-negative
bacteria and *Candida albicans* ATCC 10239 and anti HIV activity (Burja et al., 2001; Ozademir et al., 2004).

The Long-chain saturated hydrocarbons represent a substantial fraction 25% of the non-saponifiable element in *S. platensis* (Bujard et al., 1970). Thus, dry *S. platensis* contains between 0.1% and 0.3% of saturated hydrocarbons. Two-thirds of these hydrocarbons consist of n-heptadecane, the remainder, in descending order, of saturated linear hydrocarbons (*C*$_{15}$, *C*$_{16}$, *C*$_{18}$) and three unidentified saturated branched-chain hydrocarbons (Tulliez et al., 1975).

In present study we investigated the n-alkanes profiles specially heptadecane and tetradecane in UV-B untreated and UV-B treated *S. platensis* through GC-MS. The cells of *S. platensis* were harvested and extracted with hexane and the unsaponifiable fraction was used for hydrocarbon analysis. The n-alkanes were separated by serially coupled capillary column to mass detector.

GC-MS analysis of hydrocarbons in UV-B untreated *S. platensis* indicated both short and long chain n-alkanes.
Fig. 3.28 a, b). The solvent delay was 6 min. Therefore, complete total ion chromatogram (TIC) is from 6 min (Fig. 3.28). The major constituents of short chain n-alkanes are nonane (C\textsubscript{9}H\textsubscript{20}), tetradecane (C\textsubscript{14}H\textsubscript{30}), hexadecane (C\textsubscript{16}H\textsubscript{34}), heptadecane (C\textsubscript{17}H\textsubscript{36}) and octadecane (C\textsubscript{18}H\textsubscript{38}) and n-cosane (C\textsubscript{20}H\textsubscript{42}), and monocosane (C\textsubscript{21}H\textsubscript{44}), docosane (C\textsubscript{22}H\textsubscript{46}), tricosenes (C\textsubscript{23}H\textsubscript{48}), tetracosane (C\textsubscript{24}H\textsubscript{50}) and hexacosane (C\textsubscript{26}H\textsubscript{54}) were the major constituents of long chain n-alkane in UV-B untreated S. platensis. The major constituents of short chain n-alkane were decane (C\textsubscript{10}H\textsubscript{22}), tetradecane (C\textsubscript{14}H\textsubscript{30}), pentadecane (C\textsubscript{15}H\textsubscript{32}), heptadecane (C\textsubscript{17}H\textsubscript{36}), octadecane (C\textsubscript{18}H\textsubscript{38}) and cosane (C\textsubscript{20}H\textsubscript{42}), and long chain n-alkanes were monocosane (C\textsubscript{21}H\textsubscript{44}), docosane (C\textsubscript{22}H\textsubscript{46}), tetracosane (C\textsubscript{24}H\textsubscript{50}), pentacosane (C\textsubscript{25}H\textsubscript{52}), hexacosane (C\textsubscript{26}H\textsubscript{54}), heptacosane (C\textsubscript{27}H\textsubscript{56}), octacosane (C\textsubscript{28}H\textsubscript{58}), triacontane (C\textsubscript{30}H\textsubscript{62}) and tetracontane (C\textsubscript{34}H\textsubscript{70}).

It is evident from the GC-MS result on hydrocarbon that the percentage of short chain n-alkanes (C\textsubscript{9}-C\textsubscript{20}) is 54.6\% and long chain alkanes (C\textsubscript{21}-C\textsubscript{34}) is 45.4\% of total hydrocarbon content in UV-B untreated S. platensis. The percentage of short chain
Fig. 3.28 GC-MS study of hydrocarbons of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
n-alkanes is 40% and long chain n-alkanes is 60% of total hydrocarbon content in UV-B treated S. platensis. Thus, the GC-MS results show that 14.6% long chain alkanes (C_{21}-C_{34}) increased of total alkanes in UV-B treated S. platensis as compared to UV-B untreated counterpart (Fig.3.28 a, b).

MS spectrum reveals that the m/z values 199; [M-1]^+ for tetracane (C_{14}H_{30}) and 241; [M-1]^+ for heptadecane (C_{17}H_{38}) are shown in Fig. 3.29a,b. The part of TIC for tetracane (C_{14}) and heptadecane (C_{17}) of UV-B untreated and UV-B treated S. platensis is depicted in Fig.3.30a, b. Result shows that the level of tetracane (C_{14}) and heptadecane (C_{17}) were increased in UV-B treated S. platensis as compared to UV-B untreated counterpart (Fig.3.30a, b).

Both hydrocarbon tetracane (C_{14}) and heptadecane (C_{17}) has potent antimicrobial activity (Ozademir et al., 2004). But further investigation is needed to understand the mode of action and also for study of antimicrobial activity of tetracane (C_{14}) and heptadecane (C_{17}).
Fig. 3.29 Part of GC-MS total ion chromatogram of hydrocarbons n-tetra decane and n-hepta decane of UV-B untreated (a) and UV-B treated (b) *Spirulina platensis*
Fig. 3.30 EI-MS of identified peak of hydrocarbon n-tetra decane (a) and n-hepta decane (b) of *Spirulina platensis*
Result shows that under UV-B stress the n-alkane content in *S. platensis* were increased as compared to UV-B untreated counterpart. The n-alkane content increased in UV-B treated *S. platensis* for protecting the cell from environmental stress. Similar results have been found in leaves of higher plants containing waxy alkanes, which are useful for protection against UV-B radiations, as well as photoinhibition (Robinson *et al.*, 1993).

Dembitsky and Srebnik, (2002) studied the dominant components of hydrocarbons in culture of filamentous cyanobacterium *Scytonema* sp. and isolated similar long chain hydrocarbons, n-alkane that ranged from C<sub>12</sub> to C<sub>25</sub> in lake water samples (Badawy *et al.*, 1999), n-alkane in lichen (Zygadio *et al.*, 1993), in photobionts a green algae *Treouxia* sp (Toress, 2003) and hydrocarbons and volatile components in cyanobacterium Nostoc sp. (Dembitsky *et al.*, 1999).

Biologically active compounds like n-alkanes isolated from *S. platensis* have an antimicrobial activity (Ozademir, 2004) and are also rich source of nutritional and therapeutic potential.