Chapter 1

General Introduction
Cyanobacteria are an important entity of phytoplankton in the marine environment. These are single celled microscopic blue green algae in the ocean those utilize solar energy and fix carbon dioxide through a process of photosynthesis. This auto-trophic process of assimilation of CO₂ and production of organic matter is of immense significance as it forms the base of the food chain in oceanic environments. These cells occur in solitary form, large chains or spherical shaped colonies, some large enough to see with the naked eye. Based on their size, phytoplankton are classified as macroplankton (>200 µm in diameter), microplankton (20-200 µm), nannoplankton (2-20 µm) and picoplankton (0.2-2 µm) thus showing great size based diversity. Phytoplankton are of enormous importance in the aquatic environment. They act as a source for a variety of organisms (fish and shellfish) which in turn form food for larger animals. The phytoplankton are also known to produce most of the oxygen through photosynthesis in aquatic environment, playing an important role in maintaining equilibrium between oxygen and carbon dioxide. The marine phytoplankton are classified depending upon their pigments into 1) Cyanophyta (containing c-phycocyanin, c-phycoerythrin), 2) Rhodophyta (red algae; having r-phycoerythrin, r-phycocyanin), 3) Chromophyta (having carotenoids) which is further divided into Cryptophyceae, Dinophyceae, Prymnesiophyceae, Chrysophyceae, Dictyochophyceae, Bacillariophyceae, and Raphidophyceae and 4) Chlorophyta (having Chl b) divided into Euglenophyceae, Prasinophyceae and Chlorophyceae (green algae).
Cyanobacteria are a group of prokaryotes that possess higher plant-type oxygenic photosynthesis whereas, most algae or phytoplankton are eukaryotic. They obtain their energy through photosynthesis and are not strictly unicellular, but can be found in colonial and filamentous forms. Few cyanobacteria are also known to produce toxins. Interestingly, several forms of these organisms are capable of fixing atmospheric nitrogen either as free-living organisms or in symbiosis with many other species including protists, animals and plants (Sinha and Hader, 1997). They use the enzyme nitrogenase to reduce atmospheric nitrogen into ammonium ions ($\text{NH}_4^+$) which are made available for aquatic eukaryotic phytoplankton (Sinha, et al., 1996; Sinha, et al., 1996; Kumar, et al., 1996). Cyanobacteria are cosmopolitan in distribution and most possess a high potential to adapt to diverse environmental factors. However, UV-B radiations are known to affect cyanobacterial processes such as growth, survival, pigmentation, motility, as well as the enzymes of nitrogen metabolism and CO$_2$ fixation (Donkor and Hader, 1996).

Cyanobacteria carry out photosynthesis directly in the cytoplasm of the cell, rather than in specialized organelles (Chloroplasts). In fact, the Chloroplasts found in other plants are probably evolved from cyanobacteria. Considering that cyanobacteria possess their own DNA, it is stated that more sophisticated plant cells brought cyanobacteria into their structure through the initiation of an endosymbiotic relationship (Fogg, et al., 1973). Photosynthesis in cyanobacteria uses water as an electron donor and produce oxygen as a byproduct. The photosynthesis occurs in membranes called thylakoids, with Chl $\alpha$ being employed to absorb the radiant
energy. Unlike most photosynthetic organisms, cyanobacteria are blue-green or grayish-brown in color rather than plain green.

Based on the modern method, the cyanophyta has been divided into two families. The cyanophyceae includes all cyanobacteria, which possess phycobilisomes and phycobilin pigments whereas, prochlorophyceae include cyanobacteria which lack phycobilisome and phycobilin pigments. The cyanophyceae can be divided into five orders: Choroococcales (included genera are *Cyanothece, Aphanothee, Merismopedia, Chroococcus, Gloeocapsa, Microcystis, Chemaesiphon and Eucapsis*); Pleurocapsales genera: *Cyanocystis Chamaesiphon* and *Pleurocapsa*; Oscillatoriales genera: *Oscillatoria* also called *Trichodesmium*, *Lyngbya, Microcoleus, Phormidium, Arthrotips* and *Spirotilina*; Nostocales genera: *Nostoc, Anabaena, Climodrospermum, Aphanixomenon, Scytonema, Gloeotrichia* and *Rivularia* and Stignomatales genera: *Stigonema, Hapelosiphon* and *Fisherella*. The class Prochlorophyceae has only one order: Prochloroales (included genera are *Prochloron, Prochlorococcus* and *Prochlorothrix*).

**Trichodesmium (Oscillatoria)**

*Trichodesmium* is a genus of filamentous cyanobacteria belong to division-Cyanobacteria and order-Oscillatoriales. They are found in nutrient poor tropical and sub-tropical ocean waters. *Trichodesmium* fixes atmospheric nitrogen into a form usable to other living things. They are most important of the marine varieties, and are being extensively studied for their role in nutrient cycling in the ocean. Unlike other nitrogen fixing bacteria, *Trichodesmium* does not have heterocyst. Instead,
Atmospheric nitrogen is processed in special, protective cells called diazocytes (Carpenter and Price, 1976). They were first described by Ehrenberg in 1823 in the Red Sea. *Trichodesmium* a name given from Greek word “Trichoma” means hair and “desmus” means bonded i.e. bonded hair. *Trichodesmium* is known to form extensive surface blooms, discolouring vast regions of the ocean. It is thought that the Red Sea was so named because of the frequent *Trichodesmium* blooms that occur giving the waters a reddish coloration. They grow in clean, optically transparent marine environment with warm temperature (>25°C), where only nitrogen fixers can grow due to paucity of the nitrogenous nutrient like NO₃, NO₂ and ammonia.

*Trichodesmium* forms large colonies which are 1-8 mm long. Colonies of *Trichodesmium* are made up of about 50 to 200 trichomes / filaments, each made up of about 100 cells (Fig. 1A and B). Thus, a colony of *Trichodesmium* containing about 5,000 to 1,60,000 cells. The colonies can be yellowish-brown to deep red in colour due to their primary light harvesting pigment, phycoerythrin. They are buoyant and able to regulate their position in the water column due to large gas-filled vacuoles or vesicles in each individual cell. *Trichodesmium* blooms can be 10-1000's of kms wide. There are six identified species of *Trichodesmium* in the world ocean viz. *T. aureum, T. contortum, T. erythraeum, T. havanum, T. hilderbrandtii, T. pelagicum, T. tenue* and *T. thiebautii*. However, in the Arabian Sea only two species were reported namely *T. erythraeum* and *T. thiebautii.*
Trichodesmium erythraeum Ehrenberg ex Gomont

*Trichodesmium erythraeum* trichomes (filaments) are straight, forming flaglike or scale-like bundles in the plankton, cells are 7-11μm in diameter and 5.4-11μm
long. The apical cell truncate — colonial or hemispherical, trichomes slightly constricted at the nodes, tapering slightly at both the ends (Fig. 1C).

*Trichodesmium thiebautii* ex Gomont

*Trichodesmium thiebautii* trichomes (filaments) are curved to spherical and forming colonies held together at the center with the ends free, 1 — 6 mm long. Cells are 7 — 16 μm in diameter, isodiametric or longer than wide, 6 — 26 μm long, the tip cell with thickened wall, trichomes briefly attenuated at the tips, without constrictions at the cross walls (Fig. 1D).

*Trichodesmium* growth and its role in elemental cycling is of great importance. In this chapter, an attempt has been made to provide information on *Trichodesmium* in the world ocean with specific reference to the Arabian Sea. It also emphasizes the importance of the present work. This is subsequently followed by the published work on similar area of research, thus highlighting the lacunae in this area. Thereafter, at the end of the chapter, the scope and objectives of the present study are mentioned.

1.1. Distribution of *Trichodesmium* in the marine environment

*Trichodesmium* have been reported throughout the tropical and sub-tropical oceanic environments (Capone, et al., 1997). This cyanobacterium has been studied in the Atlantic (Goering, et al., 1966; Carpenter and Roenhebrg, 1995; Carpenter, et al., 2004), Pacific (Letelier, et al., 1996; Karl, et al., 1997), Caribbean (Carpenter and
Price, 1977), China (Saino, 1977; Chen, et al., 2003), Sargasso (Orcutt, et al., 2001) and Arabian Sea (Qasim, et al., 1970; Devassy, et al., 1978; Capone, et al., 1998). Although, it has been reported in these areas, its distribution can be extremely irregular with large surface aggregations occurring only during optimal conditions.

*Trichodesmium* are known to occur abundantly in near surface waters. It has permanent gas vacuoles (Walsby, 1978) those make this cyanobacterium positively buoyant. *Trichodesmium* can regulate its buoyancy such that they maintain themselves at sub-surface depths (Kromkamp and Konopka, 1986; Rijn and Shilo, 1985). Often, when dense surface aggregations are encountered, the majority of the biomass lies just below a thin lens of water. In the Caribbean Sea and sub-tropical Atlantic, Carpenter and Price (1977) and Carpenter and McCarthy (1975) reported sub-surface maxima of *Trichodesmium* between 10 and 40 m depending upon season and the same has been shown to be true for the southern East China Sea (Chang, et al., 2000). More recently, on various cruises in the tropical N Atlantic, the *Trichodesmium* maxima was encountered at 12 m depth in May / June 1994 and October, 1996 and deep as 40 m in April, 1996 (Carpenter, et al., 2004). About 65% of total *Trichodesmium* biomass was found in the upper 20 m and as much as 94% of the biomass was in the upper 50 m (Carpenter, et al., 2004). It is clear that majority of the biomass is found in the upper euphotic zone however, trichomes have been found at 175 m in the Western Sargasso Sea (Carpenter and Mccarthy, 1975) and as deep as 200 m at station ALOHA in the North Pacific sub-tropical gyre (Letelier, et al., 1996). These depths are well below the nutricline and their occurrence varies depending upon time of day and level of cloud cover. There is no evidence for
chromatic adaptation of *Trichodesmium* with depth (McCarthy and Carpenter, 1979). Therefore, this phytoplankton does not adapt its light-harvesting pigments and cannot permanently make a living at depths at which it is unable to gather enough light to photosynthesize. Though, the majority of *Trichodesmium* biomass is found above 50 m, trichomes and colonies are known to occur throughout the water column. Therefore, various theories have been developed pertaining to the vertical migration of this species. A 15 m sub-surface maxima might indicate the avoidance of photo-inhibition of both photosynthesis and N₂-fixation and may support the theory that *Trichodesmium* spp. are able to descend in the water column to acquire phosphate. It is hypothesized that carbohydrate storage at the surface temporarily counteracts the positive buoyancy of trichomes and colonies, which then allows this non-motile cyanobacterium to migrate downward to the nutricline layer to acquire phosphate (Karl, et al., 1992; Villareal and Carpenter, 2003).

Studies on the gas vacuoles found within *Trichodesmium* have revealed that the vacuoles are extremely strong and do not burst under the pressure encountered at the depth of the nutricline (Walsby, 1978). As the stored carbohydrates are respired at 5 m depth, the trichomes and colonies again become positively buoyant and return to the surface nutrient deplete waters. Empirical evidence and models show that a vertical migration of *Trichodesmium* to at least 70 m is possible (Villareal and Carpenter, 2003) though, trichomes have been found much deeper in the water column (Letelier, et al., 1996). It is also reported herein that while *Trichodesmium* is at the nutricline to acquire phosphate, cells may take up nitrate as well, which may also support growth upon return to the surface (Villareal and Carpenter, 2003). It is
generally accepted that *Trichodesmium* carbon production and N2-fixation are negatively affected by decreasing light intensity (Carpenter and Price, 1976). Carpenter, et al. (2004) reported a decrease in *Trichodesmium* volumetric C-fixation rate at compensation point in the sub-tropical N Atlantic. As a part of *Trichodesmium* N2-fixation model, Hood, et al. (2002) reported photo-inhibition of N2-fixation in 7 out of 17 incubated in 100 % Io in the sub-tropical and tropical Atlantic (Hood, et al., 2002). In the Central N Pacific, Mague, et al. (1977) reported some evidence of photo-inhibition of *Trichodesmium* photosynthesis at the highest light intensity tested and they also report that *Trichodesmium* N2-fixation attenuates with decreasing light intensity in incubator experiments with natural population.

There have been numerous reports of large blooms of *Trichodesmium* spp. along the Indian and African coasts during the winter and the inter-monsoon period (Devassy, et al., 1978; 1987; Carpenter and Capone, 1992). *Trichodesmium* colonies represent a large fraction of plant biomass in tropical, oligotropical waters and contribute substantially to primary production (Capone, et al., 1997). The detailed work of various authors indicate that, patches of the *Trichodesmium* colonies become abundant in surface waters in tropical and sub-tropical marine ecosystem during periods of calm sea conditions (Steven and Glombitza, 1972; Carpenter and Price, 1976; Bryceson and Flay, 1981; Karl, et al., 1992). It is also reported that *Trichodesmium* colonies supported epiphytic hetero-trophic bacteria (Carpenter and Price, 1976; Bryceson and Fay, 1981), and hydrozoans (Geiselman, 1977) thus implying that they play an important role in sustaining associated plant and animal biomass. Although, *Trichodesmium* is known as red tide organism having detrimental
effect on biota, Calef and Grice (1966) noticed that the survival rate of larval stages of copepod *Macrostella gracilis* increased when *Trichodesmium* blooms were present and suggested that these copepods grazed on *Trichodesmium* spp.

The colonial cyanobacterium, *Trichodesmium* spp., is responsible for most of the N₂-fixation in the open oceans (Capone, et al., 1998). Recent reports and analysis indicate that N₂-fixation is a globally significant source of new N (Carpenter and Romans, 1991; Michaels, et al., 1996) such that N₂-fixation is responsible for all of the biologically mediated net annual carbon export to the deep ocean. Eppley and Petereson (1979) stated that external sources of nitrogen (N) such as N₂-fixation were required to effect a net transport of atmospheric CO₂ from the upper ocean to the deep sea because N₂ from depth is transported upwards with dissolved inorganic carbon (DIC) in approximate Redfield proportions. The highest abundance of *Trichodesmium* colonies was counted in surface layers between surface and 50 m depth (Carpenter and Price, 1977).

The substantial degradation of these colonies occurs in surface layers. During degradation of colonies a part of the nitrogen bound by N₂-fixation will be transferred to the pool of dissolved nitrogen and can be used by other auto-trophic and hetero-trophic organisms (Carpenter and Price, 1977). The bacteria are attached to the trichome and also to the mucopolysaccharide layer. *Trichodesmium* releases NH₄⁺ (Prufert-Bebourt, et al., 1993) and dissolved organic nitrogen (Capone, et al., 1994; Glibert and Bronk, 1994) during the process of N₂-fixation. It releases nitrogen at high rate, as high as 50 % of fixed nitrogen (Mulholland and Capone, 1999). Letelier and Karl (1996) reported that *Trichodesmium* spp. comprised, on an average, 18 % of
the Chl $a$, 4 % of the photosynthetic carbon assimilated, 10 % of the particulate nitrogen and 5 % of the particulate phosphorus. A comprehensive information on occurrence of *Trichodesmium* spp. in the world oceans has been tabulated in Table 1.

### Table 1. Reports of *Trichodesmium* around the world ocean

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Location</th>
<th>Months</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Northern Gulf of Mexico</td>
<td>August</td>
<td>Eleuterius et al., 1981</td>
</tr>
<tr>
<td>3.</td>
<td>Honduras, Nicaragua</td>
<td>Sep-Dec</td>
<td>Hulburt, 1968</td>
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<td>5.</td>
<td>West Indies, Atlantic</td>
<td>Sep-Dec</td>
<td>Carpenter and Romans, 1991</td>
</tr>
<tr>
<td>6.</td>
<td>Barbados</td>
<td>2 years</td>
<td>Sander and Steven, 1973</td>
</tr>
<tr>
<td>7.</td>
<td>Barbados</td>
<td>3 years</td>
<td>Steven and Glombitza, 1972</td>
</tr>
<tr>
<td>8.</td>
<td>Amazon, Brazil</td>
<td>Mar-May</td>
<td>Calef and Grice, 1966</td>
</tr>
<tr>
<td>10.</td>
<td>Brazil</td>
<td>October</td>
<td>Sato, 1966</td>
</tr>
<tr>
<td>11.</td>
<td>Sierra Leone</td>
<td>Jan-Apr</td>
<td>Aleem, 1980</td>
</tr>
<tr>
<td>12.</td>
<td>Georgia Bight</td>
<td>All year</td>
<td>Dunstan and Hosford, 1977</td>
</tr>
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<td>13.</td>
<td>Red Sea</td>
<td></td>
<td>Wille, 1904</td>
</tr>
<tr>
<td>15.</td>
<td>Madagascar</td>
<td>Nov-Feb</td>
<td>Sournia, 1968</td>
</tr>
<tr>
<td>16.</td>
<td>Laccadive Islands</td>
<td>April</td>
<td>Qasim, 1972</td>
</tr>
<tr>
<td>17.</td>
<td>Off Cochin</td>
<td>Feb-Apr</td>
<td>Joseph and Pillai, 1975</td>
</tr>
<tr>
<td>18.</td>
<td>West Coast of India</td>
<td>Feb-Apr</td>
<td>Devassy, et al., 1978</td>
</tr>
<tr>
<td>19.</td>
<td>Palk Straits</td>
<td></td>
<td>Chidambaram et al., 1944</td>
</tr>
<tr>
<td>20.</td>
<td>Bay of Bengal</td>
<td></td>
<td>Madhupratap, et al., 1980</td>
</tr>
<tr>
<td>21.</td>
<td>Java Sea</td>
<td></td>
<td>Delsman, 1939</td>
</tr>
<tr>
<td>22.</td>
<td>East Coast of Borneo</td>
<td></td>
<td>Mohler, 1941</td>
</tr>
<tr>
<td>23.</td>
<td>North Western Australia</td>
<td></td>
<td>Wood, 1965</td>
</tr>
<tr>
<td>24.</td>
<td>Great Barrier Reef</td>
<td>June</td>
<td>Jones et al., 1986</td>
</tr>
<tr>
<td>25.</td>
<td>Great Barrier Reef</td>
<td>Aug-Jan</td>
<td>Revelante et al., 1982</td>
</tr>
<tr>
<td>26.</td>
<td>NW African Upwelling</td>
<td>Nov</td>
<td>Vallespinos, 1985</td>
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<td></td>
<td>Region</td>
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</tbody>
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### 1.2. Ecology

High sea surface temperature and irradiance, low ambient nutrients, and calm seas (Carpenter, 1983b; Capone, et al., 1998) can promote dense aggregations of
*Trichodesmium* bloom. Under optimal conditions surface aggregations can cover thousands of square kilometers (Capone, et al., 1998). Recent modeling efforts have confirmed that *Trichodesmium* distribution is defined by high light intensity, weak vertical mixing, and low concentrations of nutrients (Hood, et al., 2004). At species level, *T. thiebautii* as the most common species encountered in the Sargasso Sea, tropical North Atlantic and Caribbean Sea (Carpenter and Price, 1977; Carpenter, et al., 2004). The distribution of *Trichodesmium thiebautii* has been inversely related to wind speed (Bryceson, et al., 1981) and directly related to nutrient limitation (Logan and Hunt, 1988). In the Pacific, single filaments of *Trichodesmium thiebautii* are common (Letelier, et al., 1996) than in the Atlantic where, colonies make up as much as 89 to 92 % of the *Trichodesmium* biomass (Carpenter, et al., 2004).

*Trichodesmium* colonies host a variety of organisms (Sheridan, et al., 2002) including heterotrophic bacteria (Paerl, et al., 1989) and other species of filamentous non-heterocystous cyanobacteria (Siddiqui, et al., 1992). Interestingly *T. thiebautii*, has been reported to produce a toxin (Hawser, et al., 1991) to which these associated organisms must be immune or insensitive. The toxin in approximately 50 % of *T. thiebautii* culture samples caused more than 50 % lethality in *Artemia salina* and calanoid and cyclopoid copepods but not harpacticoid copepods however, does not affect copepods (Hawser, et al., 1991).

There are few known grazers of *Trichodesmium* (O'Neil and Roman, 1992). The cyclopoid copepod, *Macrosetella gracilas* is the most well known and the distribution and life cycle of this copepod has been directly related to the distribution and abundance of *Trichodesmium* (Calef and Grice, 1966, Bottger-Schnack and
M. gracilas not only ingests Trichodesmium, up to 21 % in $^{15}$N labelled feeding assays (O'Neil, 1998) and forms 90 to 126 % of its body carbon (Roman, 1978) and also uses Trichodesmium as a place to deposit their eggs thus acting as a "nursery" for the development of naupliar stages (O'Neil and Roman, 1992). The occurrence of few known grazers in the area dominated by Trichodesmium suggests that Trichodesmium sp. is important to oligotrophic systems primarily as a supplier of New $N_2$ by way of DON excretion (Capone, et al., 1994, Glibert and Bronk, 1994, Mulholland, et al., 2000, Mulholland and Capone, 1999). In fact Glibert and Bronk (1994) report that as much as 50 % of Trichodesmium $N_2$-fixation is released as dissolved organic nitrogen and Capone et al. (1994) reported that approximately 25 % of the concurrent $N_2$-fixation in Trichodesmium is released as glutamate. Mulholland et al. (2000) reports the direct release of NH$_4^+$ by Trichodesmium in batch culture and indicated that 10 to 15 % of Trichodesmium cells along a trichome contain active nitrogenase (Lin, et al., 1998, Bergman and Carpenter, 1991) which exudes as dissolved organic nitrogen supplying $N$ within a Trichodesmium colony. Capone, et al. (1994) reported that the $K_s$ value for glutamate in Trichodesmium is high and therefore it is possible that glutamate is the common means for $N$ exchange in Trichodesmium. Isotopically light $N$ is fixed by Trichodesmium and subsequently taken up by phytoplankton cells. These cells are then ingested by zooplankton thus the diazotrophic signal is propagated up in the food chain. In a similar study, the stable isotopic signature of amino acids in zooplankton from a transect across the Atlantic Ocean has shown that Trichodesmium was a food source (McClelland, et al., 2003). Similarly, the study along the Gulf of Mexico
indicates that as much as 60% of the carbon found in the zooplankton comprising of 250 and 500 μM size fractions were generated from direct *Trichodesmium* ingestion (Holl, 2004). This may be true for other oceanic basins at times when, *Trichodesmium* is a dominant member of the phytoplankton community.

The elemental composition of the *Trichodesmium* specially C and N content has been studied and reported by various authors. Letelier, et al. (1996) reported C:N ratio for *Trichodesmium* of 6.3 in the sub-tropical N Pacific. Both, McCarthy and Carpenter (1979) and Carpenter, et al. (2004) reported a C: N ratio of 6.5 for *Trichodesmium* in the central N Atlantic and in the tropical N Atlantic respectively. Since these C: N ratios are below Redfield, it is imperative that *Trichodesmium* is not N limited, emphasizing its role as nitrogen fixer. At low growth rates and under P or N limitation, phytoplankton can exhibit large variability in their stoichiometry (Goldman, 1986).

1.3. Physiology

Physiology of *Trichodesmium* is an important aspect of study since it fixes nitrogen and evolves oxygen simultaneously. The nitrogenase activity of *Trichodesmium* increases rapidly at the start of the day, peaks at mid-day, decreases throughout the afternoon and is absent during night (Capone, et al., 1990). Further, it was also noticed that nitrogenase was completely degraded throughout the night and absent in the cells before dawn (Capone, et al., 1990).

In *Trichodesmium*, both processes (carbon and nitrogen fixation) proceed simultaneously during the day with no obvious mechanism of separation. Initially, it
was suggested that only cells in the interior of colonies fixes N\textsubscript{2} (Fogg, 1974; Paerl, 1994). However, Ohki, et al. (1991) stated that the highest rate of N\textsubscript{2}-fixation in a cultured Trichodesmium was during exponential growth. Subramaniam, et al. (1999) determined ratio of photosystem I to photosystem II to be approximately 25, which was extremely high for a marine phytoplankton. The most recent work reported that the protection of nitrogenase was via electron flow through PSII and the subsequent oxidation of the quinone pool (Berman-Frank, et al., 2001a). It was also detected that, high respiration rates early in the photoperiod further reduce the quinone pool, which sends a negative feedback to PS electron transport, regulates PSII, and allows for high N\textsubscript{2}-fixation rates during the photoperiod when O\textsubscript{2} consumption exceeds O\textsubscript{2} production (Berman-Frank, et al., 2001a). Trichodesmium strains growing at different sites like North Pacific, North Atlantic and Great Barrier Reef were genetically different due to varying environmental conditions. Chen, et al. (1996; 1998) reported light as an important factor for nitrogen fixation by Trichodesmium. Mullholland et al. (2001b) and Fu and Bell (2003) have reported that phosphorus as the important nutrient for nitrogen fixation. However, Kustaka et al. (2003) suggested that Fe availability does influence nitrogen fixation by Trichodesmium. Since Trichodesmium in the Arabian Sea occur under wide range of environment conditions (November to May) the physiology of Trichodesmium was studied by rate of carbon synthesis and nitrogen fixation.
1.4. Primary production

*Trichodesmium* distribution is patchy and uneven at various sights in the world ocean. This distribution pattern reflects wide spectrum of primary productivity during *Trichodesmium* bloom in the marine environment. An earlier study (Beers, et al., 1968, Carpenter and Price, 1977, Taguchi, et al., 1988) suggests that average primary productivity was 5.12 mgCm\(^{-3}\)d\(^{-1}\), 22.3 mgCm\(^{-3}\)d\(^{-1}\) and 5.85 mgCm\(^{-3}\)d\(^{-1}\) in Off Bermuda, Sargasso Sea and Central Carribean Sea respectively. Similar range of primary production was recorded in tropical North Atlantic (0.12 to 37.8 mgCm\(^{-3}\)d\(^{-1}\)) during *Trichodesmium* bloom. However, a high value of primary production (60 mgCm\(^{-3}\)d\(^{-1}\)) was recorded at South of Puerto Rico (Burkholder, et al., 1967). Earlier studies conducted on variations in primary production in different parts of world suggest that there exists great variability, largely attributed to geographical location and season. In Bermuda, during Atlantic Time Series studies (BATS) the primary productivity was 302 – 394 mgCm\(^{-2}\)d\(^{-1}\) (Michaels, et al., 1994). Similar values (247 mgCm\(^{-2}\)d\(^{-1}\)) were reported from Eastern Equatorial Atlantic whereas, a value of 288 mgCm\(^{-2}\)d\(^{-1}\) was reported from NW Coast of Barbados (Steven, 1971). The high (899 – 980 mgCm\(^{-2}\)d\(^{-1}\)) column productivity was recorded in the tropical Atlantic waters (Monger, et al., 1997; Voituriez and Herbland, 1981). Interestingly, in the Arabian Sea the earlier reported values were comparatively high (34.8 – 3195.84 mgCm\(^{-3}\)d\(^{-1}\); Devassy, et al., 1978). The column primary productivity was equally variable during *Trichodesmium* bloom although major *Trichodesmium* biomass prevails in surface layers.
1.5. Nitrogen fixation

Though *Trichodesmium* has been studied for past few decades, N$_2$-fixation rate measurements still vary considerably and the source of this variation remains unclear. This is mainly due to the fact that nitrogenase has a large metallic component and limited access to molybdate (Howarth and Cole, 1985) or iron (Rueter, et al., 1992, Berman-Frank, et al., 2001b) implicating variability in N$_2$-fixation rates. As supporting evidence of iron limitation in *Trichodesmium*, a model of *Trichodesmium* distribution mimics the pattern of high dust deposition (Tyrrell, et al., 2003), which is the greatest input of Fe in the oligotrophic ocean (Duce and Tindale, 1991, Baker, et al., 2003). A model developed by Hood, et al. (2004) has highlighted that iron concentration may limit the absolute amount of *Trichodesmium* biomass but does not control the occurrence of *Trichodesmium*. Further, it has been shown that the catalytic activity of nitrogenase per mole of iron is the lowest of any iron-containing enzyme in nitrogen metabolism (Raven, 1988) suggesting the iron requirement for diazotrophs is approximately 100x higher than the requirement for non-diazotrophic phytoplankton. Kustka, et al. (2003) presented revised iron use efficiency data for *Trichodesmium* and reported that for their predicted set of requirements (*Trichodesmium* growth of 0.1 d$^{-1}$, PS I : PS II ratio from 1-4, and 48 % Mehler activity) *Trichodesmium* would require only 7-11 times more iron than a non-diazotrophic (eukaryotic) phytoplankton. The available iron in the Atlantic and Caribbean Sea could support and possibly even exceed that is necessary for the
observed rates of *Trichodesmium* N₂-fixation. In the Gulf of Mexico, iron rich dust deposition has been implicated in a 100-fold increase in the *Trichodesmium* biomass (Lenes, et al., 2001). Culture studies on the effects of iron limitation on N₂-fixation have determined that N₂-fixation is more sensitive to Fe stress than is cellular yield (Fu and Bell, 2003) and that cellular iron quota, photochemical quantum yield, PS I : PS II ratio, as well as N₂-fixation rate decline in iron-limited culture conditions (Berman-Frank, et al., 2001a). The earlier data collected on variation in Fe content indicate that it is unlikely that iron will limit the growth of the *Trichodesmium* in the Arabian Sea (Witter, et al., 2000).

However, the bioavailability of Fe is an important factor affecting the productivity of cyanobacteria, specially during photosynthesis and N₂-fixation (Wilhelm, 1995). The cyanobacteria require relatively low amounts of Fe compared to C, N and Phosphorus (P). Their biological Fe requirements are not always met because of the low inputs of Fe that are common to the open ocean and low solubility in seawater. Sanudo-Wilhelmy, et al. (2001) reported N : Fe ratios of *Trichodesmium* in the Central Atlantic Ocean and estimated that N₂-fixation may require 2.5-5.2 times more Fe than organisms relying on NH₄⁺ alone. Thus, an oceanic deficiency of bioavailable Fe has been suggested to regulate the primary production, N₂-fixation and biomass of many species of cyanobacteria (Rueter, 1988). Most of the Fe in seawater exists in the particulate form because of the low solubility of Fe (III) in oxygenated seawater. However, this insoluble portion of the Fe pool is thought to be relatively unavailable for biological uptake, as most organisms can only assimilate dissolved Fe (Bruland, et al., 1991). There are very low levels of dissolved Fe in the
open ocean, and nearly all of the soluble Fe in seawater appears to be bound in organic complexes of unknown origin and chemical composition (Rue and Luther, 1995; Wu and Luther, 1995). It is possible that the chemical nature of these organic ligand-Fe complexes either increases or reduces the bioavailability of Fe to cyanobacteria (Hutchins et al., 1999).

It has been suggested that the lack of available phosphate in the euphotic zone of oligotrophic oceans can inhibit growth and N₂-fixation (Wu, et al., 2000; Wu, et al., 2003; Sanudo-Wilhelmy, et al., 2001). Stihl et al., (2001) and Mulholland, et al. (2002) reported alkaline phosphatase activities in both phosphate depleted cultures and in natural populations and demonstrated the ability of *Trichodesmium* to take up organic phosphate in the form of glycerophosphate thus, illustrating that *Trichodesmium* has remarkable P-scavenging ability under limiting oligotrophic conditions. An enzyme-labeled fluorescence (ELF) labeling technique, which labels PhoA, the enzyme responsible for alkaline phosphatase activity, has been shown to detect cell-specific phosphorous stress (Dyhrman, et al., 2002). It is still unclear to what extent limited P availability affects the rate of N₂-fixation in natural populations. However, in culture, inorganic phosphorous up to 1.2 μM and organic phosphorous have been shown to stimulate N₂-fixation in *Trichodesmium* (Fu and Bell, 2003).

Another potential source of variability in N₂-fixation rates is the presence and preferential uptake of combined N. In addition to its ability to fix N₂, *Trichodesmium* can take up various forms of combined nitrogen (NH₄⁺, NO₃⁻, urea, amino acids, and DON) from solution (Goering, et al., 1966; Carpenter and McCarthy, 1975; Glibert and Banahan, 1988; Mulholland, et al., 1999; 2001b). Most studies of both
natural populations and cultures show that uptake of combined N is extremely low and even undetectable (Goering, et al., 1966, Mulholland, et al., 1999; 2001).

1.6. Detection and Monitoring of *Trichodesmium* blooms

Phytoplankton data collection in an open-ocean over large areas become an elaborate task hence, remote sensing technique is considered to provide a synoptic view of ocean productivity. Banse, et al. (1986) identified large blooms by late winter North of 20° N using CZCS data. Further, a protocol was developed by Subramaniam and Carpenter (1994) for the detection of the bloom of the *Trichodesmium* using CZCS imagery. Subramaniam and Carpenter (1999) studied the remote sensing reflectance model using optical absorption and backscattering due to *Trichodesmium* in the ocean. Recent study monitored *Trichodesmium* bloom and estimated nitrogen fixation using satellite derived *Trichodesmium* Chl $a$, PAR coefficients (Hood et al., 2002). Further, they reported that *Trichodesmium* population may adjust their position in the water column by resulting buoyancy to maintain optimal light condition. Recently, *Trichodesmium* blooms are detected using SeaWiFS (Subramaniam, et al., 2002) and OCM (Sarangi, et al., 2004, Desa, et al., 2005, Dwivedi, et al., 2006) imagery.

The difficulties inherent in tracking *Trichodesmium* biomass in situ, a number of models of *Trichodesmium* distribution and their potential to supply new N to oligotrophic systems have been published of late. One of these, is a model by Fennel, et al. (2002) while working on the N$_2$-fixation dynamics of *Trichodesmium* at station 20.
ALOHA that takes into account the physical forcing that may affect N\textsubscript{2}-fixation. This model accounts for temperature, irradiance and wind speed, allows for fluctuating N:P ratios in the inorganic and organic pools, and ultimately captures the inter-annual variation in diazotrophic biomass in the sub-tropical North Pacific. Hood, et al. (2001) modeled N\textsubscript{2}-fixation by *Trichodesmium* in the North Atlantic and implications of this N\textsubscript{2}-fixation upon the decrease of dissolved inorganic carbon (DIC) as well as export flux. Their results point to significant inter-annual variation in N\textsubscript{2}-fixation rates as a result of decadal-scale climate fluctuations.

Arabian Sea is considered as one of the most important *Trichodesmium* site for global carbon and nitrogen cycle (Hood, et al., 2000). Information on *Trichodesmium* distribution and density is required to quantitatively estimate their contribution to global N\textsubscript{2}-fixation on global and regional scales. In order to estimate N\textsubscript{2}-fixation by *Trichodesmium* spp. in the Arabian Sea, it needs to define their spatial and temporal variability and one of the approach could be satellite remote sensing.

**Scope and objectives of the present work**

Capone, et al. (1997) has reviewed the work on *Trichodesmium* in the world ocean and opined that, *Trichodesmium* spp. is an ecologically most important organism that have global significance. The importance was mainly attributed to primary production (Carpenter and Price, 1977; Capone, et al., 1998; Carpenter, et al., 2004) and N\textsubscript{2}-fixation (Carpenter, 1972; Carpenter and Price, 1977; Capone et al., 1982, 1998 and 2005; Carpenter, et al., 1987). Recently, the role of *Trichodesmium* in nutrient cycle is also established (Mulholland, et al., 1999; 2001b). The *Trichodesmium* produced large amount of fixed nitrogen making it available to other
autotrophs. The role of *Trichodesmium* colony as micro-environment supporting heterotrophs and hence microbial food chain is also established (Nausch, 1996). Arabian Sea, being a unique environment supports population of *Trichodesmium erythraeum* and *Trichodesmium thiebautii* during different times, thus facilitating the diversified population of *Trichodesmium*, largely regulated by environmental factors and its ability to occur when the available nitrogen is limiting in the Arabian Sea (Capone, et al., 1998).

In the Arabian Sea, *Trichodesmium* occurrence is a regular feature from February to April, when large areas of the ocean get covered with clumps of sawdust coloured algae (Qasim, 1970; 1972). Along Goa waters, the occurrence of *Trichodesmium* spp. bloom was first reported in 1972 (Ramamurthy, et al., 1972). Thereafter, the bloom phenomenon has been observed every year from February to May indicating a well-defined periodicity and annual rhythm in its appearance (Devassy, et al., 1978). These blooms were found to enrich the environment and were responsible for succession of other organisms (Devassy, et al., 1979).

*Trichodesmium* forms dense bloom along west coast of India during March to May. However, its seasonality and prevalence in open-ocean is not known. The nitrate levels of open water get depleted after October- November and limits productivity due to non availability of the nitrate. During this period, the role of *Trichodesmium* spp. in this part of the Arabian Sea is not known quantitatively. Similarly, in coastal water, although various authors record the presence of the *Trichodesmium* for last 3 decades, the work on carbon production and nitrogen
fixation is lacking. This organism is tagged as the red tide organism and its harmful/toxic effect on environment and other flora-fauna is also not well understood.

Recently ocean colour is used as the new tool for the studying the phytoplankton in the ocean. The efforts are also necessary to evaluate application of this tool for phytoplankton with special reference to the *Trichodesmium* distribution in the Arabian Sea, since *Trichodesmium* forms dense aggregation and suitable for ocean colour detection. Equally important is its role as primary producer. Although, some information is available on primary productivity during bloom conditions (including other phytoplankton) however, exclusive studies on quantification of carbon fixed by this organism have not been initiated in the Arabian Sea. In the present study, an attempt has been made to study distribution, ecology, organic production and nitrogen fixation during *Trichodesmium* bloom. The objectives of the present study are given below:-

❖ *Trichodesmium* distribution in the coastal waters of Goa on a monthly basis for period one-year.

❖ *Trichodesmium* distribution and composition in the open waters of the Arabian Sea during November to April period (on monthly basis).

❖ Physico-chemical, biological (microscopy and chemotaxonomy) and optical aspects of the phytoplankton ecology in the Arabian Sea.

❖ Rate of primary production and nitrogen fixation during *Trichodesmium* blooms.

❖ Succession and monitoring of *Trichodesmium* using ocean colour remote sensing in the Arabian Sea.