

Title: Enumeration of bacteria from *Trichodesmium* spp. bloom of the Eastern Arabian Sea: elucidation of their possible role in biogeochemistry

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Abstract

The carbon-flux via algal bloom events involves bacteria as an important mediator. The present study, carried out during the spring inter-monsoon month of April 2008 onboard CRV *Sagar Manjusha-06* in the Eastern Arabian Sea, addresses the bloom specific flow of Carbon to bacteria via chromophoric dissolved organic matter (CDOM). 11 stations monitored, were located in the coastal, shelf and open-ocean areas off Ratnagiri (16°59'N, 73°17'E), Goa (15°30'N, 73°48'E) and Bhatkal (13°58'N, 74°33'E) coasts respectively. Visible bloom of “saw-dust” color in the Ratnagiri shelf were microscopically examined and the presence of cyanobacteria *Trichodesmium erythraeum* and *Trichodesmium thiebautii* with cell concentrations as high as 3.05×10^6 trichomes L⁻¹ was recorded. Total bacterial counts (TBC) varied between 94.09×10^8 cells L⁻¹ in the bloom to 1.34×10^8 cells L⁻¹ in the non-bloom area. Chromophoric Dissolved Organic Matter (CDOM) concentrations averaged 2.27 ± 3.02 m⁻¹ in the bloom to 0.28 ± 0.07 m⁻¹ in the non-bloom waters respectively. CDOM composition varied from a higher molecular size with lower aromaticity in the bloom to lower molecular size and increased aromaticity in the non-bloom areas respectively. Strong positive relationship of TBC with Chlorophyll *a* ($R^2=0.65$, $p<0.01$) and CDOM concentrations ($R^2=0.8373$, $p=0.01$) in the bloom area indicated hydrolysis and/or uptake of CDOM by bacteria. Absorbtion by mycosporine like amino acid palythene ($\lambda_{\max} = 360$ nm) was recorded in the filtrate of bloom. Morphotypes of *Trichodesmium* associated bacteria revealed a higher frequency of Gram positive rods. The role of bacteria in relation to changing CDOM nature and as a factor in affecting oxygen content of the water column is finally discussed in context of the Arabian Sea.

Keywords: Arabian Sea, *Trichodesmium* bloom, Bacteria, Chromophoric dissolved organic matter

Introduction

The cyanobacteria *Trichodesmium spp* is of global significance owing to their efficiency in fixing atmospheric nitrogen (Capone et al., 1997). *Trichodesmium* blooms appear seasonally in the tropical Eastern and Southern Arabian Sea basin during oligotrophic conditions of late March, extending till May (Devassy, 1987; Matondkar et al., 2006). Decomposition of the bloom biomass leads to nutrient regeneration, indicating the strong involvement of water column microbes (Devassy et al., 1979). Such bacterial interactions with bloom organic matter (dissolved and particulate), may trigger complex biogeochemical feed-backs (Arrigo, 2005; Azam et al., 1994) and are particularly not well understood.

The importance of bacteria in the Arabian Sea “microbial food-web” was underscored during the last decade (Ducklow et al., 2001), but their distribution during these characteristic algal blooms remains unaddressed. Although bacterial colonization and association with *Trichodesmium* blooms have been previously recognized in other oceans (Sellner, 1992; Sheridan et al., 2002; Renaud et al., 2005), data from the Arabian Sea is not available. The lack of bacteriological studies, thus led us to enumerate bloom associated bacteria and address its possible role in relation to chromophoric dissolved organic matter (CDOM).

CDOM, the dynamic pool of photo-reactive dissolved organics in the marine environment, has gained significance in recent times (Mopper and Kieber, 2002; Nelson and Siegel, 2002). CDOM spectral characteristics can reveal distinct differences in the CDOM nature, and the nature can further indicate a different CDOM source, as algal (Romera-Castillo et al., 2010; Steinberg et al., 2004), microbial (Balch et al., 2002; Brophy and Carlson, 1989; Nelson et al., 2004; Ogawa et al., 2001) or photo-chemical (Miller and Moran, 1997). This makes it useful as a potential biogeochemical marker (Bracchini et al., 2010; Helms et al., 2008; Carder et al., 1989).

The biogeochemically responsive Arabian Sea basin (Naqvi et al., 2000; Goes et al., 2005), thus provided, an important environment to explore the distribution of bacteria and CDOM and understand their relationship from the bloom and non-bloom areas of *Trichodesmium* respectively.

Materials and Methods

Sampling of water and bloom Water samples were collected during the spring inter-monsoon season (April 1st-7th, 2008) in the Eastern Arabian Sea during cruise CRV *Sagar Manjusha* 06. Sampling

stations were located along three transects off Bhatkal (BS), Goa (GS) and Ratnagiri (RS) coasts respectively (Fig. 1). Clean techniques were employed to collect water samples from various depths using 10L Niskin sampling bottles, mounted onto a Sea Bird Electronics[®] CTD rosette.

“Saw-dust” patches of bloom were also collected separately from surface waters, pre-screened through a 20 µm nytex mesh, followed by: (a) microscopic examination of bloom patches and (b) filtration on sterile 0.22µm nuclepore filters with a gentle vacuum to isolate bloom associated bacteria. The bacteria free < 0.22 µm bloom filtrates were stored at 4°C to study utilization of dissolved carbohydrates.

Evaluation of bloom biota Total phytoplankton and *Trichodesmium spp* concentrations were determined from microscopic analysis of water samples preserved with lugol’s iodine as previously described (Matondkar et al., 2006). Phytoplankton Chloropyll *a* were derived from HPLC analysis as exactly outlined by Parab et al. (2006).

Bacteria in bloom For enumeration of bacteria, water samples were fixed with 2% final concentration (v/v) of glutaraldehyde and stored at 4 °C during the cruise. Aliquots of 2-5 ml water samples were stained with 0.02% Acridine orange (Hobbie et al., 1977) and filtered through black polycarbonate filters (0.22µm pore size, nuclepore). Filters were mounted on glass slides and observed under an eyepiece with 100µm x 100µm counting grid and 100X objective in an Olympus BH2 eplifluorescent microscope fitted with blue filter sets. Images obtained were further processed for estimating total counts, frequency of dividing cells (Hagstrom et al., 1979) and bacterial morphotype distributions.

Trichodesmium filaments collected on 0.22 µm filters were further suspended in sterile 3.5% NaCl solution and triplicate dilutions were plated separately on 2216E Zobell’s full strength and Zobell’s 1/10 strength media. Morphological and biochemical studies of the isolates were carried out following standard protocols (Gerhardt et al., 1981). Gram staining was performed by both traditional and the KOH method (Buck, 1982). The enzyme activities of isolates were studied by measuring zones of hydrolysis on starch, casein and tributyrin agar (Podogorska and Mudryk, 2003).

Growth of bacteria on bloom filtrate Utilization of dissolved carbohydrates from the <0.22 µm fraction of bloom by bacterial isolates was studied by the Anthrone-sulfuric acid method (Herbert, 1971). Fixed

volumes of bacterial suspension were inoculated in 10 ml of the bacteria free *Trichodesmium* filtrate in duplicates and incubated upto 48 hours to measure the decrease in the total carbohydrate concentration.

Analysis of Chromophoric Dissolved Organic matter (CDOM) CDOM in water samples, with and without bloom, were obtained by gentle filtration through a 0.22µm pore size nuclepore polycarbonate filter using clean techniques (Helms et al., 2008). CDOM samples from the bloom and non-bloom areas were refrigerated in acid-washed amber colored bottles till spectrophotometric analysis in laboratory. CDOM absorption was measured in a Perkin Elmer lambda 40 spectrophotometer using a quartz cuvette of 10 cm path-length. Characteristic spectras were obtained for the 250-800 nm regions. Any temperature drifts or scattering effects in the spectra were corrected following Helms et al. (2008) and CDOM absorption coefficients ($A_c m^{-1}$) were calculated from absorbance as below:

$$A_{c\lambda} = 2.303A/l$$

where, $A_{c\lambda}$ is the absorption coefficient (m^{-1}) corresponding to absorbance A at wavelength λ and l is the optical path length (m).

The exponential spectral slopes were then obtained by performing non-linear iterative least-squares fit of the CDOM spectrum to the equation:

$$A_{c\lambda} = A_{c\lambda_0} e^{S(\lambda_0 - \lambda)}$$

where, $A_{c\lambda}$ is the absorption coefficient (m^{-1}) at wavelength λ (nm), $A_{c\lambda_0}$ is the absorption coefficient (m^{-1}) at a reference wavelength λ_0 and S is the exponential slope (nm^{-1}). The CDOM concentrations were expressed in terms of A_{c325} (Nelson and Siegel, 2002). The $A_{c250:365}$ ratios and the spectral slope ratios ($S_{ratio} = S_{275-295} : S_{350-400}$) as indicators of CDOM molecular size were computed following Helms et al. (2008). The $A_{c465:665}$ ratios were further calculated as an inverse indicator of CDOM aromaticity (Summers et al., 1987; Chin et al., 1994).

Dissolved Oxygen and Inorganic nutrients Dissolved oxygen content of water samples, drawn directly from Niskin sprout, were estimated onboard using a modified Winkler's titration using Metrohm Dosimat Titrator (Grasshoff et al., 1999). Deep freezed water samples from the cruise were analyzed for inorganic nutrients (nitrate, nitrite, phosphate and silicate) by spectrophotometric methods (Knap et al., 1996).

Statistical analysis Correlation and linear regression analysis for evaluating relationship of bacteria with Chla, CDOM and dissolved oxygen concentrations were performed using the statistical add-in package of *Microsoft excel 2003*. For analysis of CDOM spectral parameters, the non-linear regression tools of *Origin6* were used.

Results

Ratnagiri shelf (RS) harbored visible “saw-dust” patches of an algal bloom at stations RS-7, RS-5, RS-4 and RS-3 (Fig. 1). Microscopic observations revealed the presence of non-heterocystous trichomes, arranged in characteristic “puffs and tufts” arrangement, conforming to the description of cyanobacteria *Trichodesmium spp.* At these stations, *Trichodesmium spp.* comprised upto 99.9% of the phytoplankton population. Both *Trichodesmium erythraeum* and *Trichodesmium thiebautii* were observed in surface waters at all stations in the Ratnagiri transect except at RS-3, where *Trichodesmium erythraeum* was only present. *Trichodesmium spp.* concentrations varied from as high as 3.05×10^6 trichomes L^{-1} at station RS-3 and decreased to 3.24×10^2 trichomes L^{-1} at 50m depth of RS-5. Surface phytoplankton biomass, in terms of Chla, decreased from 107.49 mg m^{-3} at RS-3 to 0.56 mg m^{-3} at RS-7, as the bloom gradually weakened on moving towards the open-ocean area (Table 1).

Although, presence of *Trichodesmium* was detected in other stations off Bhatkal (BS-1, BS-9, BS-13), Goa (GS2, GS6, GS12) and in the open-ocean area off Ratnagiri at RS-13, the filament concentrations were much lower and hence will be collectively referred to as the reference or non-bloom area. Phytoplankton concentration in the non-bloom area was a maximum of 31.6×10^2 cells L^{-1} in the coastal waters off Goa (GS-2) to 14.4×10^2 cells L^{-1} off Bhatkal (BS-1). The observed bloom in the Ratnagiri slope/shelf was located ~ 560 km from the non-bloom transect off Goa and ~ 1560 km from that off Bhatkal respectively (Table 1).

Bacteria in Bloom Clustering of bacterial cells could be seen in micro-zones of *Trichodesmium* filament fragments, observed mostly as living (stained) and few damaged/dead (unstained) trichomes under epi-fluorescent microscope (Fig. 2a). Total surface bacterial counts (TBC) in the bloom area varied from a maximum of 94.09×10^8 cells L^{-1} in the bloom patch at RS-3 to a minimum of 6.94×10^8 cells L^{-1} at station RS-7 (Table 1). The Frequency of dividing cells (FDC) as an index of active growth rate of bacteria, was 8.33 ± 1.83 % (n=8) in the bloom area which decreased to 3.23 ± 1.48 % (n=29) in the non-bloom areas. Bacterial morphotypes, varied in the *Trichodesmium* bloom area with predominance of

48.5 % of rods followed by 31.54 % of Coccobacilli, 2.99 % of curved rods and 17.11 % of Cocci. Considering a conservative estimate of 10fgC per cell, bacterial cell counts corresponded to an average higher surface bacterial biomass of 42.59 $\mu\text{g C L}^{-1}$ (n=8) in the intense bloom patches which decreased substantially to 6.49 $\mu\text{g C L}^{-1}$ (n=29) in the non-bloom area. The TBC of the non-bloom areas off Goa and Bhatkal were highest in the coastal stations of 12×10^8 cells L^{-1} at GS-2(25m) to 14.3×10^8 cells L^{-1} at BS-1(10m). TBC decreased substantially in open-ocean oligotrophic areas from 1.34×10^8 cells L^{-1} at GS-12(50m) to 3.14×10^8 cells L^{-1} at BS13 (75m). The mean bacterial abundance in the dense bloom patches was as high as $54.59 \pm 21.61 \times 10^8$ cells L^{-1} while it decreased substantially to $6.48 \pm 3.30 \times 10^8$ cells L^{-1} in the non-bloom areas off Bhatkal and Goa respectively (Fig. 2b).

The relationship between TBC and Chla in the *Trichodesmium* bloom area was significant as $R^2 = 0.6599$, n=8, p=0.01; whereas, in the non-bloom areas the relationship remained as insignificant as $R^2=0.1751$, n=29, p=0.02 (Fig. 3c,f). The strong relationship indicates bloom specific flow of carbon and energy to lower trophic levels ie. bacteria.

Some of the free - living bacteria and those attached to filament could be retrieved separately on Zobell's full strength and Zobell's 1/10 strength media containing 3.5% NaCl. Culturable counts averaged $36 \pm 18.4 \times 10^5$ cfu ml^{-1} on Zobell's and $13.4 \pm 6.43 \times 10^5$ cfu ml^{-1} on Zobell's 1/10 strength media. A total of 6 free-living bacteria (isolated from *Trichodesmium* free filtrate of bloom) and 21 associated bacteria were obtained. 55% of both attached and free-living isolates grew anaerobically on thioglycollate media. All isolates were catalase positive and of the 17 isolates which reduced nitrate, 7 isolates could reduce nitrate beyond nitrite (Table 2). 60% of the bacterial isolates showed amyolytic activity. 25% of these isolates showed starch hydrolysis zones having a reddish-pink tinge, indicating incomplete hydrolysis to dextrans. The starch hydrolysis zones for the *Trichodesmium* associated bacteria varied between 3-0.5 cm compared to that of 2-0.8 cm for the free-living bacteria. 44% of the isolates also had the ability to produce protease on casein agar and proteolytic zones varied between 4-0.4 cm in case of the *Trichodesmium* associated bacteria while it decreased to 2-1.2 cm for the free living bacteria. 33% of the isolates hydrolyzed tributyrin with zone sizes varying between 0.1-0.3 cm. Further, 15% isolates had the ability to produce all the three extracellular enzymes - amylase, protease and lipase (Fig. 4a). Ability of the cultured isolates to utilize dissolved carbohydrates varied from a maximum of $1.84 \mu\text{gC hr}^{-1}$ to $0.18 \mu\text{gC hr}^{-1}$ (Fig. 4b).

Chromophoric dissolved organic matter (CDOM) dynamics The CDOM spectral parameters of the bloom and non-bloom areas are summarized in Table 3. CDOM concentration averaged as high as $2.2789 \pm 3.02 \text{ m}^{-1}$ in the *Trichodesmium* bloom to as low as $0.28 \pm 0.07 \text{ m}^{-1}$ in the non-bloom waters respectively. The bloom was characterized by a significantly low mean $A_{C_{250:365}}$ ratio of 5.54 ± 3.57 and S_{Ratio} of 2.05 ± 1.4 . In comparison, the non-bloom area was characterized by $A_{C_{250:365}}$ ratio of 20.17 ± 13.75 and S_{Ratio} of 4.09 ± 2.05 . As both these parameters inversely relate to CDOM molecular size, it indicates a predominance of higher molecular size CDOM present in the bloom. The S_{Ratio} decrease in the bloom was due to an overall decrease in the mean $S_{275-295}$ slope of $0.0156 \pm 0.0041 \text{ nm}^{-1}$ in the bloom to $0.03 \pm 0.004 \text{ nm}^{-1}$ in the non bloom area, with the average $S_{350-400}$ slope (0.0099 nm^{-1}) remaining relatively constant. Interestingly, in the most intense *Trichodesmium* bloom area (RS-3 and RS-4), the $S_{275-295}$ was shallower than $S_{350-400}$ which is a characteristic feature of terrestrially dominated estuarine CDOM and uncharacteristic of marine waters (Table 3). The spectral absorbption at RS-3 also showed an absorbption peak at 360 nm, indicative of the presence of mycosporine-like amino acids (Fig. 2c). As the averaged $A_{C_{465:665}}$ ratio decreased significantly from 4.46 ± 0.9 in the *Trichodesmium* bloom to 1.68 ± 0.9 in the non-bloom area, the more aromatic nature of CDOM present in the non-bloom waters were reflected. The mean $S_{250-350 \text{ nm}}$ CDOM spectral slope varied from 0.0138 nm^{-1} ($\pm .0037$, $n=6$) in the *Trichodesmium* bloom area to 0.0255 nm^{-1} (± 0.0027 , $n=9$) in the non-bloom area respectively. The 300-700 nm spectral slopes averaged $0.0168 \pm 0.0155 \text{ nm}^{-1}$ in the bloom to 0.0385 ± 0.0556 in the non-bloom waters. Correlations between CDOM absorbption coefficients and Chla varied from $R^2=0.9831$ ($n=6$, $p<0.0001$) in the bloom to $R^2=0.9831$ ($n=9$, $p=0.51$) in the non-bloom area (Fig. 3 c,f). Similar strong relationship ($r = 0.9150$, $p<0.0001$) between $A_{C_{325}}$ and TBC indicated a significant increase in TBC as CDOM concentration increased in the bloom (Fig.3 b,e). TBC and $A_{C_{443}}$ relationship also showed strong correlations in the bloom area ($r=0.9066$, $p=0.01$, $n=6$), which was however negative and insignificant in the non-bloom area ($r= -0.2806$, $p=0.46$, $n=9$) (Fig. 3 b, e). An overall inverse relationship of TBC with the CDOM molecular size indicators - S_{Ratio} and $A_{C_{250:365}}$, was seen in both the bloom and non-bloom areas. The overall correlation between TBC and S_{Ratio} was $r=0.5354$, $p=0.03$, $n=15$. Similar relationships were obtained between TBC and $A_{C_{250:365}}$ ratio of $r =0.5362$, $p=0.04$, $n=15$ which became more insignificant as $r=0.207$, $p=0.592$, $n=9$ in the non-bloom area respectively (Fig. 3g). Further, a strong overall positive correlation of $r = 0.8417$, $p<.001$, $n=15$ were noted between $A_{C_{465:665}}$ ratios, as a relative indicator of CDOM aromaticity and TBC (Fig. 3h).

Dissolved oxygen (DO) and inorganic nutrients The distribution of dissolved oxygen with reference to the *Trichodesmium* bloom and non-bloom water types showed marked variations. The surface DO increased from open-ocean to coastal areas except off Bhatkal, where an opposite trend was observed (Fig. 5a). Surface DO ranged from 201.4 - 123.4 μM in the *Trichodesmium* bloom to 195.42 - 110.43 μM in the non-bloom areas. Off Goa, the highest recorded DO was 248.92 μM in the shelf at GS6 (25m) while it decreased to 80.16 μM (100m) at station GS12 (Fig. 6b). A significantly strong negative correlation of both TBC and Chl a with DO was seen in the areas harboring *Trichodesmium* bloom patches. While the correlation with TBC was $r = 0.81$, $n=8$, $p=0.01$, the correlation with Chl a was $r = 0.83$, $n=8$, $p=0.01$ (Fig. 5f). The relationship remained insignificant in the non-bloom areas respectively. The distribution of inorganic nutrients revealed prevailing oligotrophic conditions in the study area. The surface nitrate concentrations remained mostly depleted in both the bloom and non-bloom areas, reflecting ideal conditions for *Trichodesmium sp* to grow to bloom proportions (Fig. 5 b, c, d, e).

Discussion

Use of CDOM-bacterial dynamics as a potential biogeochemical marker can be advantageous in detecting possible water quality changes, albeit imperceptible at times, but significant for the marine environment. As recent trends of intensifying coastal anoxia (Naqvi et al., 2000), increasing productivity (Goes et al., 2005) and unusual algal blooms (Gomes et al., 2008) are raising concern about the response of the Arabian Sea to climatic and eutrophication effects, such monitoring of CDOM-bacterial relationships may provide valuable understanding of her gradual biogeochemical changes. A study of CDOM – bacterial interactions from the *Trichodesmium* bloom was therefore necessary, as they tend to be seasonally more predictable in the Arabian Sea.

Results of interrelationship between bloom, CDOM and bacteria showed a strong dependence of: (a) CDOM concentration on Chl a ($R^2=0.9831$, $p < 0.01$) implying CDOM production almost entirely from the bloom (Fig. 3a); (b) bacteria on CDOM ($R^2 = 0.8373$, $p=0.01$) implying significant increase in bacterial counts in response to higher CDOM concentrations (Fig 3b) and (c) bacteria on Chl a ($R^2 = 0.6599$, $p=0.01$) implying a bloom-specific increase in bacterial counts (Fig. 3c). An anticipated decrease in CDOM concentration in response to active bacterial utilization is not reflected in these results. This possibly is because an active bloom continues to be a constant source of CDOM. However, the significantly higher biomass of bacteria in the bloom strongly suggests the role of CDOM in

supporting it (b). It may be further argued, such a possibility of actual decrease in CDOM concentration following active bacterial utilization would exist, but only when the rate of newly produced CDOM from an active bloom (source) decreases as the bloom declines (Zhang et al., 2009). In such cases, the correlation of Chla (aging/post bloom) with CDOM concentration will be weak, unlike in our study. Higher CDOM concentrations during these post-bloom periods will not mean a higher Chla. If bacterial consumption still predominates during these post-bloom stages, CDOM uptake may be reflected as a negative relationship with TBC; but not always, as other possible sources of CDOM, such as extracellular products of microbial hydrolysis or even viral lysis of bacteria (Balch et al., 2002; Nelson et al., 2004; Ogawa et al., 2001; Miller and Moran, 1997) may be important during these stages.

The nature of CDOM produced by *Trichodesmium* bloom at RS-3 and RS-4 was characterized by $S_{350-400} > S_{275-295}$ and an absorption peak (λ_{max} , 360 nm) of the mycosporine-like amino acid palythene (Subramaniam et al., 1999). The shallower slopes of the spectral regions $S_{275-295}$, $S_{250-350}$ and $S_{300-700}$ in the bloom (Table 3), reflected a possible increase in absorption by colloidal dissolved organics $>1\text{KD}$ (Simeon et al., 2003). In comparison, CDOM quality was a mix of low molecular - higher aromatic compounds with steeper slopes, from the larger area covering non-bloom locations in the Eastern Arabian Sea. CDOM nature supporting lower bacterial counts in these areas were possibly of a more refractile/semi-refractile nature. Such intermediate pools of CDOM in the water column can be climatologically important sinks of photo-oxidization to CO (Zuo and Jones, 1995) or export to the deep (Druffel et al., 1992), as they evade bacterial degradation.

Reported bacterial count of 94.09×10^8 cells L^{-1} from the intense *Trichodesmium* bloom patch at RS-3 (Table 1) was much higher in comparison to that reported from New Caledonia, Bay of St Marie (Renaud et al., 2005). Significantly low bacterial counts of 9.3×10^6 cells ml^{-1} were recorded in this area during blooms of *Trichodesmium spp.* In contrast, bacterial counts averaged as high as 8.2×10^{11} cells ml^{-1} per *Trichodesmium* colony in the Sargasso Sea (Sheridan et al., 2004). Such heterogeneity in bacterial distribution associated with *Trichodesmium* reflects the influence of geographic habitat and conditions. A comparison of TBC in the bloom and non-bloom areas in the present study, further revealed a ~ 19 -fold decline of TBC at non-bloom RS-13, located only ~ 120 km offshore from the intense bloom at RS-3 (Fig. 2b).

Mucopolysaccharides surrounding *Trichodesmium* colonies (Sellner, 1992) forms an important micro-niche for bacteria (Fig. 2a). Culturable bacteria isolated from *Trichodesmium* filaments were mostly gram-positive rods, varying from branched actinomycetes to short cocco-bacillary morphotypes. Although uncharacteristic of the normal marine flora, ability of gram positive bacterium to release extracellular enzymes more efficiently (Chrost, 1991), should confer a selective advantage in the organic rich phycosphere of the *Trichodesmium* bloom. While the incomplete hydrolysis of starch to dextrans indicated the difference in amylase types expressed by these bacteria, ability to grow on bloom-filtrates without any organic supplements directly corroborates their role in utilizing *Trichodesmium spp* photosynthates (Fig. 4b).

Trichodesmium blooms were observed to be associated with low oxygen waters (Fig 5a). Higher respiration rates by these bacteria perhaps create such oxygen poor zones and may actually benefit *Trichodesmium spp* to fix nitrogen. Although any hypoxic conditions developing at depths were not detected, the surface dissolved oxygen of the entire study area mostly remained lower than 200 μM and interestingly decreased on moving from the coastal to the open-ocean stations (Fig 5a). Bacterial mineralization of the decaying bloom and dissolved organic matter is likely to leave the system finally enriched in nitrate at depths (Devassy et al., 1987). In this context, the study of Naqvi et al. (2000) provides insight. As sub-oxic waters shoal into the Western Indian shelf and coasts during upwelling, vigorous denitrification occurs. Appearance of intense *Trichodesmium* blooms just prior to the monsoonal upwelling (late March-May) has therefore important implications. Production of more bio-refractile CDOM, through a combination of bacterial and photochemical processing of bloom exudates (Brophy and Carlson, 1989; Ogawa et al., 2001; Miller and Moran, 2007), could also be important in naturally mitigating such processes. However, no available datasets to quantify such effects are available and requires exhaustive post-bloom observations.

The C-N flux can split through multiple pathways, involving unknown intermediates in the marine environment (Druffel et al., 1992; Chin et al., 1998). Flow of carbon from algal blooms via CDOM \leftrightarrow bacterial interactions, is an important step in this direction. In conclusion, our study is the first report on bacterial distribution and CDOM-bacterial relationship during a *Trichodesmium spp* bloom. Characteristic CDOM properties of *Trichodesmium spp* are clearly indicated in the results. Significant variations in CDOM spectral slopes and absorption coefficient ratios over short distance scales in the bloom and non-bloom area, reiterates its suitability for use as a biogeochemical marker. Although,

bacterial utilization/hydrolysis of *Trichodesmium spp* bloom generated high-molecular low aromatics CDOM is highlighted, the occurrence of relative bio-refractile CDOM pools, detected in the non-bloom waters were also highly significant. The study leaves us with future strategies to determine the resident time of CDOM carrying specific bloom signatures and their gradual transformation to these relative bio-refractile pools in relation to bacteria in the Arabian Sea.

Acknowledgement

We gratefully acknowledge the encouragement and support of Dr SR Shetye, Director NIO for conducting this work. This is NIO contribution number.

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Legend of Figures

Fig. 1 Cruise track of CRV *Sagar Manjusha-06* in the Eastern Arabian Sea along three transects off Bhatkal (BS), Goa (GS) and Ratnagiri (RS); (inset) “saw-dust” patches of *Trichodesmium spp* observed off Ratnagiri

Fig. 2 (a) Clustering of bacterial cells around *Trichodesmium spp* trichomes (b) Comparative profiles of TBC from bloom and non-bloom areas (c) CDOM absorption in *Trichodesmium spp* bloom

Fig. 3 Relationship of Chla, CDOM concentration (Ac_{325}) and Total bacterial counts (TBC) in the *Trichodesmium spp* bloom and non-bloom areas respectively as: (a) Ac_{325} and Chla (Bloom) (b) TBC with Ac_{325} and Ac_{443} (bloom) (c) TBC and Chla (bloom) (d) Ac_{325} and Ac_{443} with Chla (non-Bloom) (e) TBC and CDOM concentration (non-bloom) (f) TBC and Chla (non-bloom) and overall relationship of TBC with (g) CDOM molecular size indicators (S_{ratio} and $Ac_{250:365}$) and (h) aromaticity indicator $Ac_{465:665}$ in the study area

Fig. 4 *Trichodesmium spp* associated bacterial isolates: (a) producing extracellular enzymes and (b) utilizing dissolved carbohydrates from the bloom filtrate

Fig. 5 Distribution of (a) dissolved Oxygen and inorganic nutrients (b) nitrate (c) nitrite (d) phosphate and (e) silicate in the bloom and non-bloom areas; (f) Relationship of bacteria with dissolved oxygen in the bloom

Legend of Tables

Table 1 Phytoplankton biomass (Chla), *Trichodesmium spp* counts (TC) and Total Bacterial Counts (TBC) in the Eastern Arabian Sea basin during April 2008

Table 2 Morphology and biochemical characteristics of *Trichodesmium* associated and free-living bacterial isolates

Table 3 Comparative mean CDOM spectral parameters in *Trichodesmium spp* bloom and non-bloom areas and their relationship with TBC

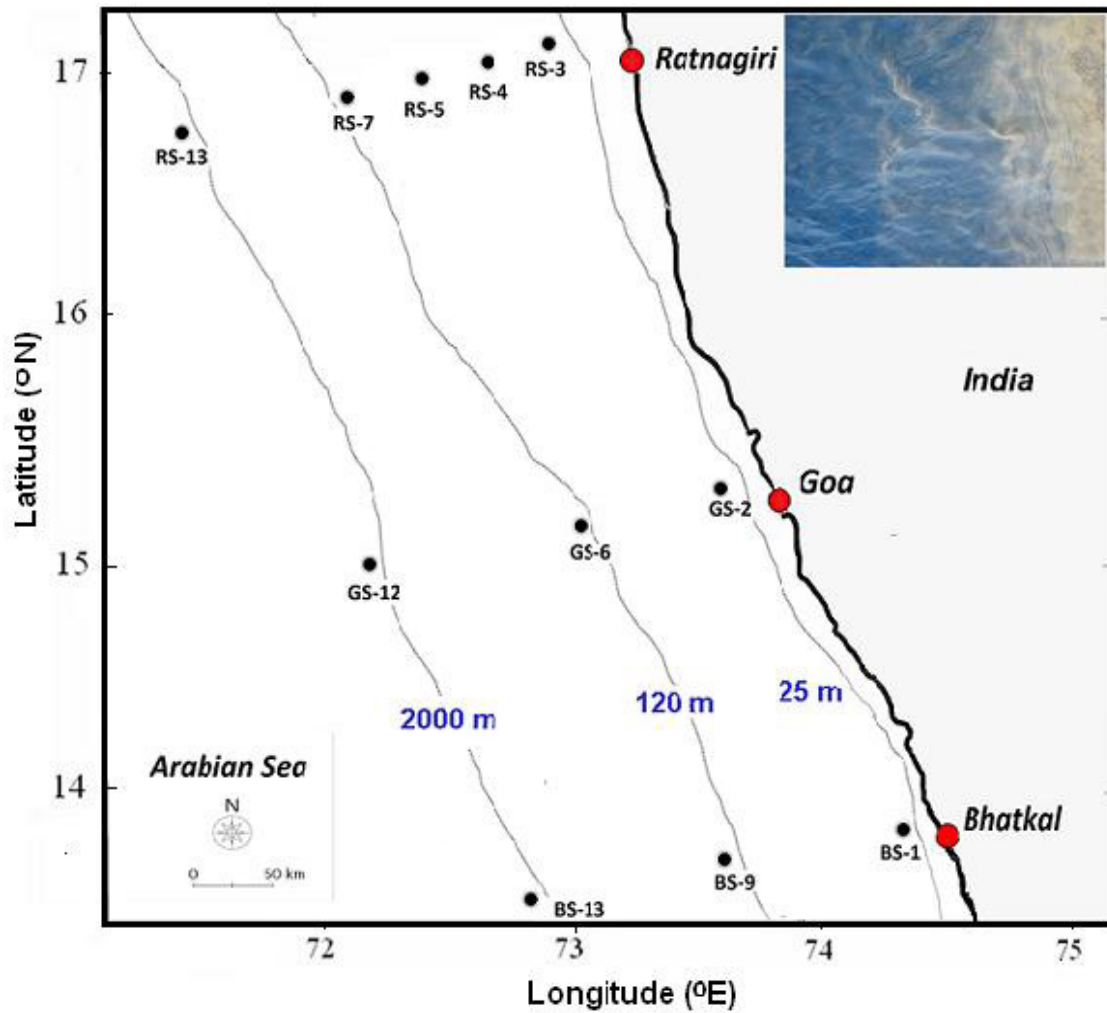
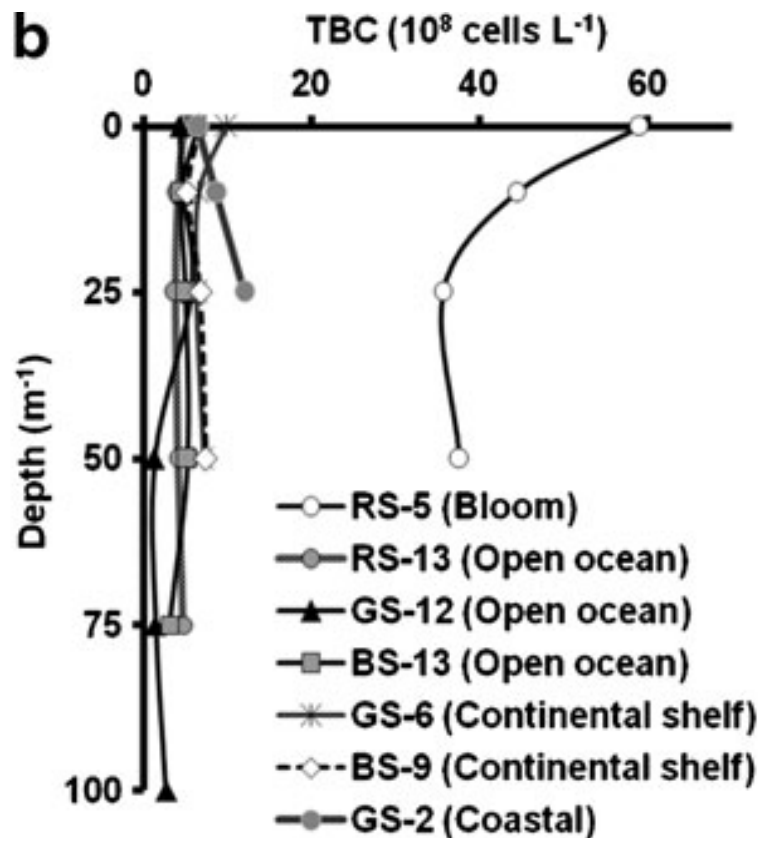
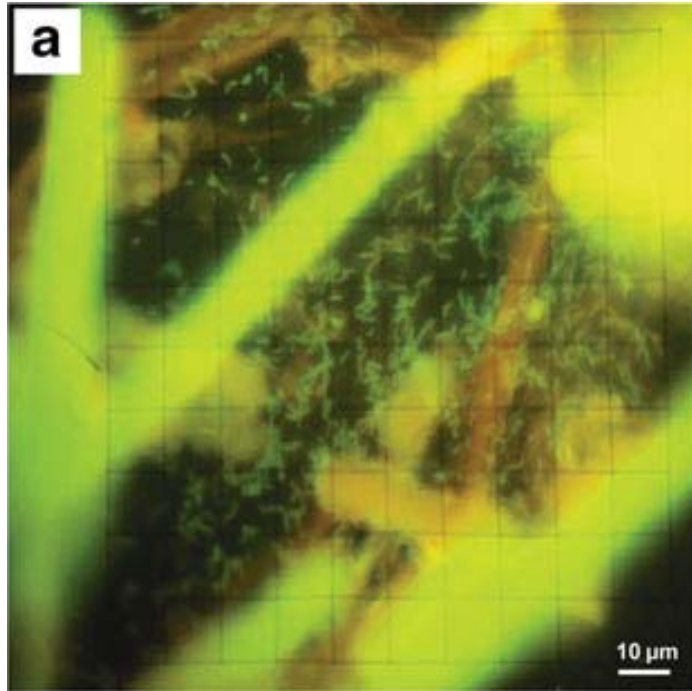


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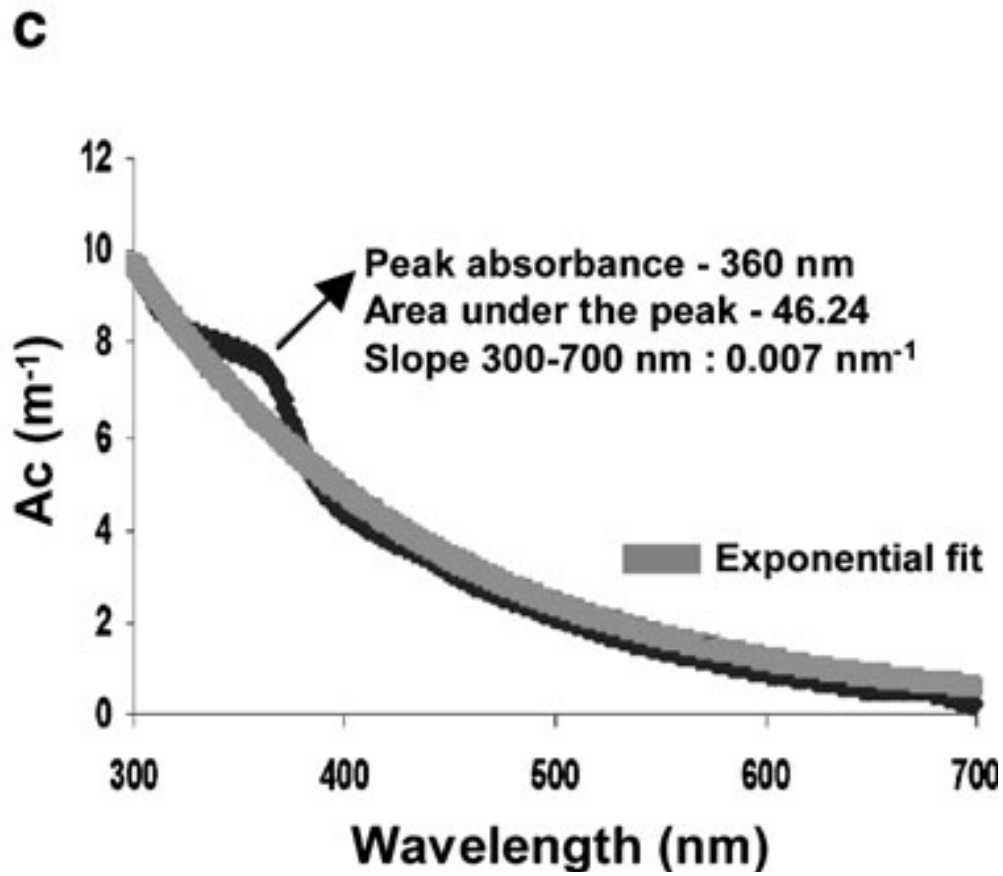


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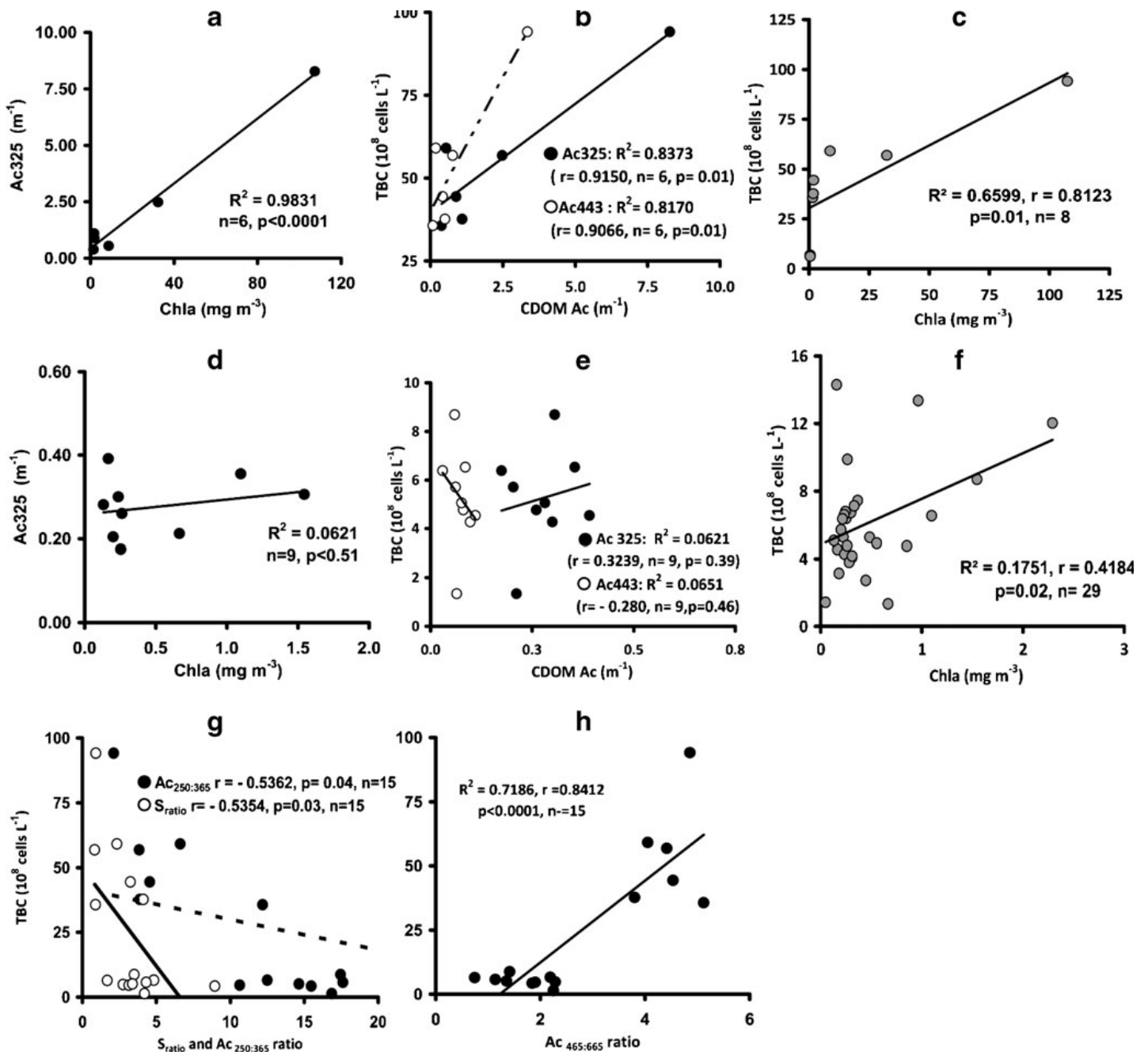


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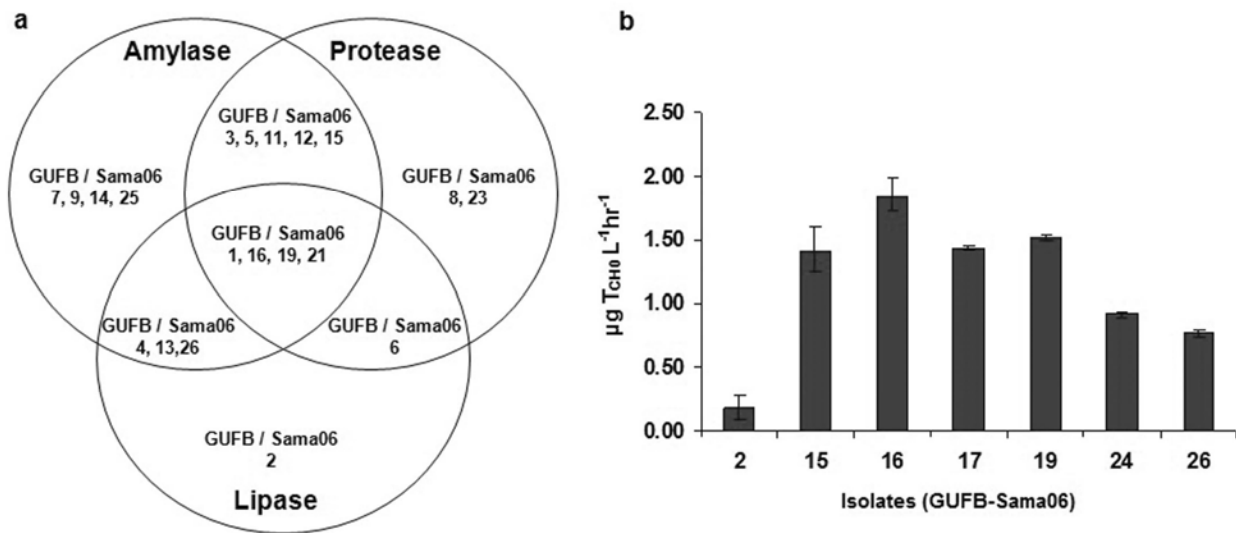


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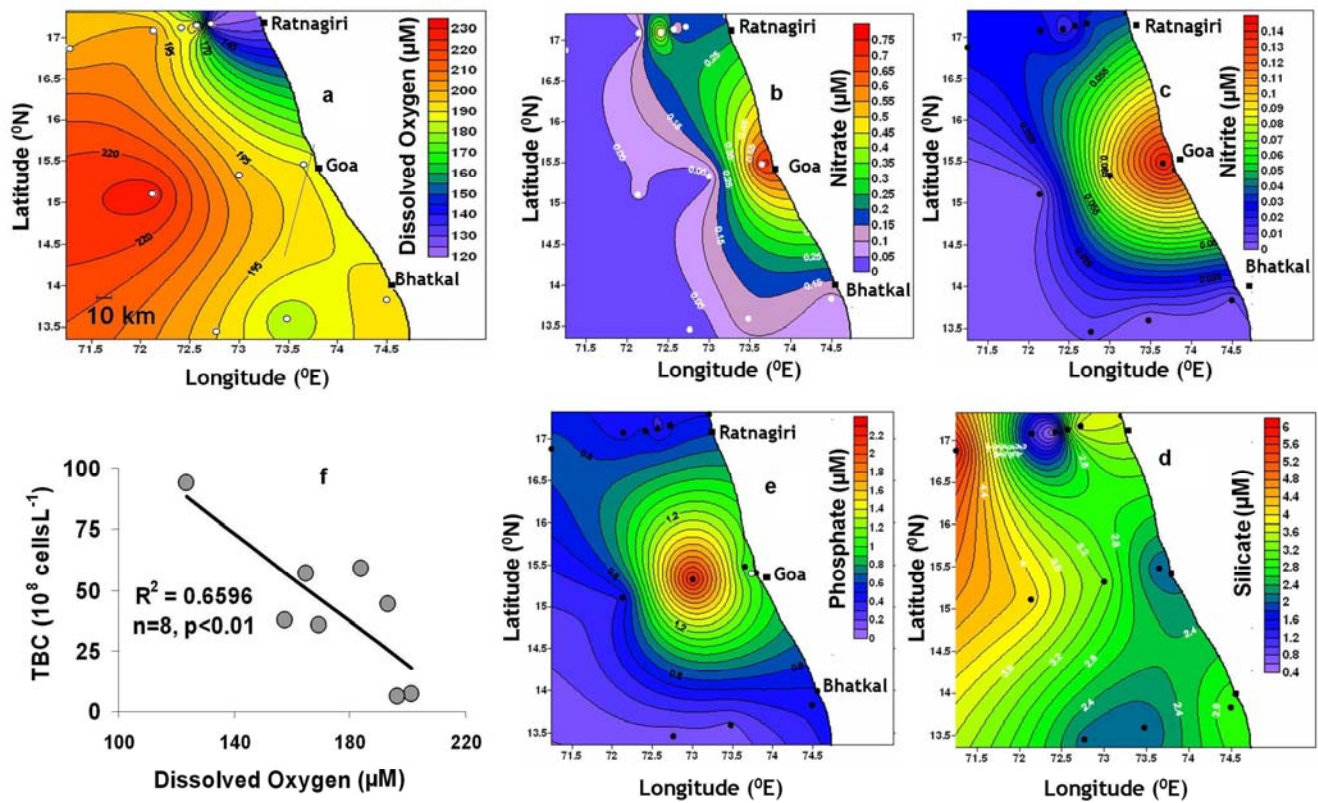


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Table 1 Phytoplankton biomass (Chla), *Trichodesmium spp* counts (TC) and Total Bacterial Counts (TBC) in the Eastern Arabian Sea basin during April 2008

Location	Chla		TC		TBC	
	Surface mgm ⁻³	Column mgm ⁻²	Surface 10 ² trichomes L ⁻¹	Column 10 ⁴ trichomes m ⁻²	Surface 10 ⁸ cells L ⁻¹	Column 10 ¹³ cells m ⁻²
<i>Off Ratnagiri</i>						
*RS-3	107.4	-	30533	-	94.09	-
*RS-4	32.34	-	447.6	-	56.82	-
*RS-5 ^c	8.8	121.63	195.2	13.6	59.04	16.1
*RS-7 ^a	0.56	7.65	4.0	1.03	6.94	1.11
^ψ RS-13 ^d	0.55	30.60	15.96	2.83	4.92	3.15
<i>Off Goa</i>						
GS-2 ^b	1.10	42.05	11.40	0.80	6.52	1.42
GS-6 ^c	0.26	12.7	2.50	0.99	9.86	3.47
^ψ GS-12 ^c	0.24	25.83	10.0	1.29	4.27	2.13
<i>Off Bhatkal</i>						
BS-1 ^a	0.96	5.6	1.0	0.13	13.35	1.38
BS-9 ^c	0.30	13.8	5.20	9.4	6.72	3.28
^ψ BS-13 ^d	0.25	20.48	18.80	2.38	6.38	3.61

Column: ^a 0-10 m and ^b 0-25 m (coastal), ^c 0-50 m (shelf), ^d 0-75 m and ^e 0-100 m (open-ocean)

* *Trichodesmium spp.* bloom area

^ψ Open-ocean stations

- Only surface bloom collected

Table 2 Morphology and biochemical characteristics of *Trichodesmium* associated and free-living bacterial isolates

Isolate Code	Pigmentation	Morphology	Gram reaction	Motility	Endospores	Anaerobic growth	Catalase	Oxidase	Nitrate Reduction
GUFB/Sama06/*									
<i>Attached</i>									
01/RS5	White	Thick Branched rods	+	+	+ ^T	+	+	-	+ ^d
02/RS5	White	Long slender rods	+	+	+ ^T	+	+	-	+
03/RS5	Colorless	Branched rods	-	-	-	-	+	-	+
04/RS5	White	Oval rods	+	-	-	+	+	-	-
05/RS5	White	Short rods	+	-	-	+	+	-	+
06/RS5	Cream	Short rods in chains	+	-	-	-	+	-	+
08/RS5	White	Very short rods	+	-	-	-	+	-	+
11/RS5	Cream	Long rods	+	-	+	+	+	-	+ ^d
12/RS5	Cream	Long rods	+	+	+ ^c	+	+	-	+
13/RS5	White	Very short oval rods	+	+	-	-	+	-	-
14/RS5	Cream	Very short oval rods	+	+	-	-	+	-	-
15/RS5	Cream	Very short oval rods	+	-	-	+	+	+	-
17/RS5	White	Curved rods	+	+	-	-	+	-	+
18/RS5	White	Short rods	+	+	-	+	+	+	+ ^d
19/RS5	White	Short rods	+	+	+ ^T	+	+	-	+ ^d
22/RS5	Cream	Short oval rods	+	-	-	-	+	+	-
23/RS5	White	Irregular rods	+	-	-	-	+	+	+
24/RS4	Orange	Short rods	-	+	-	+	+	-	+
25/RS4	Orange	Short rods	-	+	-	-	+	-	+
26/RS4	Orange	Cocci in pairs	+	-	-	+	+	-	+ ^d
27/RS4	Yellow	Cocci in pairs	+	-	-	+	+	-	+ ^d
<i>Free-living</i>									
07/RS-5	Cream	Long rods	+	+	+ ^T	+	+	-	-
09/RS-5	Orangish	Short rods	+	+	+ ^T	+	+	-	-
10/RS-5	Pale Cream	Long rods	+	-	-	-	+	-	-
16/RS-5	Cream	Long rods	+	-	+ ^T	+	+	+	+ ^d
20/RS-5	Orangish	Rods	-	+	-	-	+	-	-
21/RS-5	Beige	Rods	+	+	-	-	+	-	-

Isolate code: GUFB (Goa University Furtado Basu) /Sama06 (Cruise Sagar Manjusha 06)/*isolate code/station

Table 3 Comparative mean CDOM spectral parameters in *Trichodesmium spp* bloom and non-bloom areas and their relationship with TBC

CDOM Spectral Parameters	Bloom (n=6)	Non-Bloom (n=9)	Relationship with TBC		
			Overall	Bloom	Non-Bloom
S ₃₅₀₋₄₀₀ nm ⁻¹	0.0099 ± 0.0043	0.0099 ± 0.0056	r = - 0.8951 p < 0.0001	r = - 0.7059 p = 0.11	r = - 0.02 p = 0.95
S ₂₇₅₋₂₉₅ nm ⁻¹	0.0156 ± 0.0041	0.03 ± 0.004	r = -0.8492 p < 0.0001	r = - 0.4041 p = 0.43	r = - 0.0831 p = 0.83
S _{Ratio}	2.0539 ± 1.4091	4.09 ± 2.05	r = -0.5354 p = 0.03	r = - 0.4187 p = 0.41	r = - 0.2274 p = 0.56
S ₂₅₀₋₃₅₀ nm ⁻¹	0.0138 ± 0.0037	0.0255 ± 0.0027	r = -0.8948 p < 0.0001	r = -0.7083 p = 0.11	r = - 0.0166 p = 0.96
S ₃₀₀₋₇₀₀ nm ⁻¹	0.0168 ± 0.0155	0.0385 ± 0.0556	r = -0.2659 p = 0.34	r = - 0.4087 p = 0.42	r = -0.1475 p = 0.70
Ac _{250:365} (E ₂ :E ₃)	5.54 ± 3.57	20.17 ± 13.75	r = -0.5362 p = 0.04	r = - 0.5306 p = 0.278	r = - 0.207 p = 0.592
Ac _{465:665} (E ₄ :E ₆)	4.46 ± 0.9	1.68 ± 0.9	r = 0.8412 p < 0.0001	r = 0.2279 p = 0.66	r = -0.4986 p = 0.17
Ac ₃₂₅ m ⁻¹	2.2789 ± 3.02	0.28 ± 0.07	r = 0.7965 p < 0.001	r = 0.9150 p = 0.01	r = 0.3239 p = 0.39
Ac ₄₄₃ m ⁻¹	0.9094 ± 1.26	0.78 ± 0.02	r = 0.8034 p < 0.0001	r = 0.9066 p = 0.01	r = -0.280 p = 0.46