Summary
OM in marine environments is derived from several sources including biogenic and terrestrial. Microalgae and bacteria are generally the major sources of OM in oceanic environment. Lipid is one of the major constituent of OM and is comparatively source specific. They are relatively stable compounds for degradation processes in marine environment. Certain lipid compounds such as phospholipid fatty acids (PLFAs) are the major components of microbial cell membranes and are associated with living cells. Hence they are utilized in the measurement of live biomass and community structure of the microorganisms in the natural environment. However, it is observed that PLFAs can be affected by changing environmental conditions such as incubation periods, nutrients temperature and environmental pollutants. Fatty acids on the other hand are derived from the living organisms as well as dead OM in natural environment. Their source specificity with respect to individual compounds and their lability makes FAs suitable for tracing sources and diagenetic changes in organic material in water columns and sediments. A number of studies are available on the lipids from the temperate marine environments; however such studies are less in tropical environment. Moreover, studies on PLFAs concentrations and composition in tropical marine bacteria, diatoms and in the sediments are rarely done in the Indian waters. Alkenone distribution in marine sediment core has been used to assess paleoenvironment. If these compounds have to be used for paleoenvironmental studies, then alkenones biodegradation in water and sediment should not strongly affect temperature signal that was established during alkenone biosynthesis by the alga. Despite the widespread use of alkenones in paleothermometry, comparatively, a few studies have investigated the effect of bacterial degradation on the calculation of SST.
In this thesis, studies on the phospholipid fatty acids (PLFAs) of the marine bacteria and diatoms were carried out. Various environmental factors such as incubation period, nutrients nitrogen and phosphorus, temperature and environmental toxicant such as tributyl tin (TBT) affecting the PLFAs were studied. Based on the concentrations and composition of the PLFAs the biomass and community structure of microorganisms in the marine environment was determined. The fate of the organic matter (OM) in the marine environment was evaluated using the total fatty acids and the individual fatty acids in the deep sea sediments, where the maximum alteration of OM is possible. Further the most studied microalgal lipid biomarker (alkenones) stability is evaluated by carrying out microbial degradation of alkenones in order to study their implications for paleotemperature reconstruction.

Total PLFA concentrations changed with respect to the growth condition for the *Bacillus licheniformis*, *B. subtilis* and *Aeromonas hydrophila*. Increase in Nitrogen and phosphorus concentrations resulted in increased production of total PLFAs. Hence, it can be deduced that the PLFA production is dependent upon the nutrient availability in these bacteria. Temperature also played an important role in the total PLFA production, wherein the 30°C grown *B. subtilis* and *A. hydrophila* showed optimum production of total PLFAs. However, *B. licheniformis* showed higher concentrations of total PLFAs at higher temperature (38°C).

Both the *Bacillus* spp. showed the dominance of iso and anteiso branched PLFAs with minor contribution of saturated PLFAs (Sat), whereas *Aeromonas* showed the predominance of monounsaturated (MUFA) and saturated PLFAs. Different nutrient concentrations did not change the relative % of saturated, branched and unsaturated PLFAs in the *B. licheniformis*, *B. subtilis* and *Aeromonas hydrophila*, although their
total individual concentrations varied. However, temperature influenced the relative % of the saturated and branched PLFAs. In general Iso, saturated and Br17 were higher at low temperature in *B. licheniformis*. In *B. subtilis* iso and Br17 increased and anteiso and Br15 decreased at higher temperature. However saturated PLFAs decreased in both the *Bacillus* cultures, suggesting their minor role in cell membrane fluidity at higher temperature in these cultures. This shows that both the *Bacillus* cultures behave differently to maintain membrane fluidity. Moreover, saturated PLFAs (increased) played very important role in maintaining membrane fluidity at higher temperature, while unsaturated PLFAs (increased) were important at lower temperature in the *A. hydrophila*.

In *Skeletonema* sp. total PLFAs were high at d 15, while in *Amphora* sp. at d 20 of the incubation period. Higher nitrogen and phosphorus concentrations supported higher PLFA concentrations. *Skeletonema* sp. showed higher PLFAs at low temperature which decreased at higher temperature, while in *Amphora* sp. the opposite was seen. Both the microalgal cultures showed the predominance of C14:0, C16:0, C16:1 and C20:5 PLFAs. Among the PUFAs, C20:5n3 was the most abundant in both the diatoms. Although the concentrations of individual PLFAs changed with the cultivation period, the PLFA composition remained typical of diatoms during all the incubation periods. Decrease in Sat PLFAs, was associated with the increased production of PUFA in *Skeletonema* sp. while increase of Sat PLFAs, resulted in decreased production of PUFA in *Amphora* sp. This suggests that synthesis of PUFA was influenced by the Sat PLFAs in both the microalgae. Nevertheless, the cultures seem to have different requirements of PUFA during various cultivation periods. It was suggested that the increase in saturated fatty acids
in *Amphora* sp. was due to the inhibition of PUFA synthesis during aging. However, the higher relative % of PUFA during various cultivation periods in both the microalgae was also observed. PUFA were the major PLFAs under all the nutrient conditions used. Lower temperature did not induce the higher production of MUFA and PUFA, while Sat PLFAs were influenced at higher temperature.

Increasing concentrations of tributyltins (TBT) influenced growth of the cells and PLFA concentrations and composition of all the 4 cultures of *Bacillus*. Growth of all the bacterial cultures was delayed and retarded in the presence of TBT. The TBT is a membrane active substance, which alters the concentrations of PLFAs in the membrane lipids. Changes in total PLFAs in response to TBT concentrations suggest the ability of the bacteria to modulate PLFA concentrations. PLFAs are useful in maintaining the fluidity of the cell membrane. *B. subtilis* and *Bacillus* sp. synthesized higher amounts of anteiso, iso and saturated PLFAs at 6.05 µg l⁻¹ TBT. However, at higher concentrations of TBT (12.1 µg l⁻¹) production of PLFAs by the bacterial cultures was reduced, suggesting higher concentrations are toxic to the cells of the *Bacillus* cultures. The ability of the bacterial cultures to grow with various concentrations of TBT by modulating the PLFA concentration suggests adaptation of these bacterial cultures to the TBT stress.

$\delta^{13}C_{oc}$ signature and two end-members mixing model suggests that the OM was derived from mixed marine and terrestrial sources with relative abundance of latter at most of the stations in the Visakhapatnam harbour. PLFA concentrations varied spatially indicating differences in the living biomass in the harbour sediments. Furthermore, there was no correspondence between OC concentrations or sources and the concentrations or the composition of the total PLFAs. This suggest that the
community of microorganisms deduced by PLFAs was not influenced by concentration and sources of OC. Abundance of saturated PLFAs were observed throughout the stations in the Visakhapatnam harbour. However, they are ubiquitous in organisms and hence are not useful in assessing community structure. Hence a major fraction of PLFAs in these sediments cannot be ascribed to any particular microbial group. The Visakhapatnam harbour also showed the abundance of some specific PLFAs of various organisms such as monounsaturated fatty acids (MUFAs), indicated the abundance of Gram negative bacteria, cyanobacteria and microalgae. The Branched PLFAs (iso and anteiso) suggest the presence of Gram positive bacteria, Gram negative anaerobes and sulfate reducing bacteria. Similarly, PUFAs indicate the presence of eukaryotes. Moreover, the presence of trans-monounsaturated PLFAs in the harbour sediments imply that PLFA community was under stress due to contamination of the sampling sites by sewage and industrial waste, sulfur and petroleum products. Principal component analysis (PCA) based on concentrations of PLFAs was performed in order to segregate the stations which clustered the stations of the Visakhapatnam harbour into three groups. Stations #2, #7 and #13 formed one group and showed the dominance of iso and anteiso C15:0 PLFAs (branched) which are specific for bacteria, suggesting higher contribution of bacterial biomass in these stations. Second cluster was formed with stations #12, #15 and #16 wherein PUFA such as C20:3n6, C18:3n3, C20:4n6, C20:5n3 and C22:6n3 were abundant indicating the importance of marine algae at these stations. Moreover, the third cluster was formed with the stations which had lower concentrations of the PLFAs, suggesting lower microbial biomass at these stations.
Moreover, the PLFAs in the Visakhapatnam harbour sediments suggest the dominance of bacteria along with some contribution from eukaryotes.

$\delta^{13}C_{oc}$ values and two end-members mixing model implies that OM in sediments of the Mandovi and the Zuari estuary was derived from mixed sources consisting of phytoplankton and terrestrial C3 plants. The $\delta^{13}C_{oc}$ values indicated that stations near river end and sea end were dominated with mixed sources of marine and terrestrial origin, while the middle estuary was dominated with terrestrial OM in both the estuaries. The concentrations of PLFAs varied spatially and showed good correlation with organic carbon (OC) suggesting that the microbial community was important component of OC. However abundance of PLFA compounds in the sediments indicates that the microbial community was not dependent on the sources of OC and terrestrial and marine carbon sources were equally important in sustaining microbial biomass in both the estuaries. Saturated PLFAs are ubiquitous in nature which indicates that major proportion of PLFAs cannot be attributed to any particular microbial group in these estuaries also. Bacterial biomarkers (C15:0, C17:0, iso, anteiso, 10Me16, 10Me18 and cyclopropyl-PLFAs) associated with the gram positive bacteria, anaerobic bacteria and with sulfate-reducing bacteria were abundant in both the estuaries. MUFA (C16:1 and C18:1) abundance show the influence of gram negative bacteria, cyanobacteria, phytoplankton and other microeukaryotes in the sediments of the Mandovi and Zuari estuary. PUFA (C20:3n3, C20:5n3, C22:6n3) shows the abundance of diatoms, dinoflagellates, other algae and cyanobacteria in the sediments of these estuaries. Presence of trans- and cyclo fatty acids in Mandovi and Zuari estuary indicates that the microbial communities were under stress probably due to presence of petroleum products,
heavy metal ions, sewage, anthropogenic wastes and anoxic conditions. PCA formed cluster 1 with all the Mandovi estuary stations and three Zuari estuary stations (stations #1Z, #5Z and #8Z), while the cluster 2 was formed with the remaining Zuari estuary stations (stations #2Z, #3Z, #4Z, #5Z and #7Z). These Zuari stations #2Z, #3Z, #4Z, #5Z and #7Z contained high concentrations of PUFA such as C20:5n3 and C22:6n3 and based on this they appear to be separated from other stations of Zuari and Mandovi estuary. Moreover, none of the Mandovi estuary and the three stations of Zuari estuary (stations #1Z, #5Z and #8Z) contained C20:5n3 and C22:6n3 PLFAs. Moreover, station #4Z and station #7Z were placed relatively far from other Zuari estuary stations in cluster 2 which also contained overall high concentrations of PLFAs.

The fate of the organic matter (OM) was assessed using fatty acids (FAs). Lipid molecules such as FAs are most useful in studying the fate of OM since they are specific to various organisms and degrade at different rates. For this purpose one sediment core was collected from the Northern Indian Ocean (CC2/GCL1) and the other from the Equatorial Indian Ocean (EIO) (CC1/GC3) and analyzed for FAs using GCMS. Organic carbon (OC), lipid-phosphate and total fatty acids (TFA) were relatively higher in the CC2/GCL1 core as compared to CC1/GC3 core inferring substantially higher OM and microbial biomass in the former core. In both cores, TFA concentration decreased with the increase in downcore depth, suggesting their degradation. Monounsaturated fatty acids (MUFA) abundance was observed in both the sedimentary environments indicating contribution of microbial communities consisting of gram negative bacteria. Low abundance of branched and cyclopropyl FAs suggests the poor abundance of gram positive bacteria, anaerobic and sulfate

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reducing bacteria. Moreover, in both the cores dominance of saturated C16:0 and C18:0 was observed. Bacteria and eukaryote group appear to be the most abundant throughout the core sections in CC1/GC3. While these organisms were also dominant in upper core (0-14 cm) sections of the CC2/GCL1 core, whereas at greater depths (38-40cm) FAs of terrestrial OM were more abundant. Zooplankton marker FAs (C20:1, C22:1 and C24:1) were prevalent in middle of the core (14-26 cm) and towards the deeper sections (34-40 cm) of the CC2/GCL1 core. In the CC1/GC3 core, these zooplankton markers were less abundant.

Studies on *E. huxleyi* culture have demonstrated that alkenone biomarkers are attractive geochemical tools for palaeoceanographic studies. The proportion of di- to triunsaturated C37 alkenones in cultured cells increases with increasing water temperature. On the basis of this finding and of the ubiquity of C37-C40 alkenones in recent and ancient marine sediments, the ratio \([C_{37:2}] / ([C_{37:2}] + [C_{37:3}])\), commonly referred to as \(U_{37}^{E}\), has become a reference standard for assessment of SST in palaeoceanographic studies. For alkenones to be useful as measures of SST in the geological record, it is essential that any effects of alkenone degradation in the water column and in sediments do not affect the temperature signal that was established during their biosynthesis by the alga, or if there is a change its extent can be reasonably estimated. Despite the widespread use of alkenones for paleothermometry (paleotemperature assessment), comparatively few studies have investigated the effects of bacterial degradation of alkenones. Although various other studies have inferred biodegradation of alkenones in different environments, very few have yet examined the details of the biodegradative processes.
Four bacterial communities isolated from *E. huxleyi* strain TWP1 cultures before (TAB- total aerobic bacteria) and after different antibiotic treatments (ATB1, ATB2 and ATB3, antibiotic treated bacteria) were used in the present work to study the biodegradation of alkenones under laboratory-controlled conditions. Incubation of sterilized *E. huxleyi* (as a substrate for alkenones) for 20 days with these bacterial communities resulted in effectively none to extensive degradation of alkenones. Only minor (5.1 to 18.9 %) degradation of alkenones was observed with the ATB1 community, while incubation with the ATB2 community resulted in major (21.1 to 50.7 %) degradation. But, in both cases the degradation of di- and triunsaturated alkenones appeared to be non-selective. In contrast, incubation of *E. huxleyi* strain TWP1 cells with the ATB3 and TAB communities resulted in major (21.2 to 77.7 % and 23.3 to 49.4 %) and selective degradation of alkenones. Because of degradation of alkenones, the observed increases in $U_{3K}^{K}$ are equivalent to a +2°C and +3.3°C change in the inferred temperature when interpreted using a standard calibration equation. The differences are sufficiently large to cause concern about data interpretations of palaeotemperature reconstructed from the alkenone unsaturation index, because some reconstructed temperature differences between the last glacial and interglacial period are only 1–3 °C in magnitude. These results clearly show that the various antibiotic treatments of *E. huxleyi* strain TWP1 cells significantly changed the composition of the bacterial communities associated with the cells. The differences in the bacterial communities were confirmed by different DGGE bands for the four communities. The very distinct results obtained with the four communities strongly suggest the existence of at least two functional classes of
aerobic bacteria capable of degrading alkenones, i.e. those able to degrade them either non-selectively or selectively. It is likely that these differences in degradative outcomes are strongly dependent on the particular metabolic pathways used by the two groups of bacteria. The epoxyketones resulting from bacterial oxidation of alkenone double bonds could be useful indicators of aerobic bacterial alteration of the alkenone unsaturation ratio in situ. The detection of epoxy ketones in some cultures (ATB3) indicates that metabolic pathways involving attack on the terminal groups of the molecule are essentially non-selective, while those acting on alkenone double bonds are selective. The production of alkenols during incubation with ATB3 demonstrated for the first time that bacterial reduction of alkenones can be a potential source of these compounds in the environment. Small amounts of monounsaturated alkenones were also observed in the degradation experiment using this community also raises the possibility of a bacterial reduction of alkenone double bonds.

In order to determine whether associated bacteria play a role in the selective degradation of alkenones, two starter culture *E. huxleyi* TWP1 were grown at 20°C and 15°C, and these cultures were designated as DK1 and DK2, respectively. These two starter cultures were re-grown at 15°C and then transferred to dark condition. In both cases several days of continuous darkness resulted in a strong decrease (more than 80%) in alkenone concentration. Concerning the selectivity of the degradation between di- and triunsaturated compounds, strongly contrasted results were obtained. Increase in $U_{27}^{37}$ (+ 0.54) was observed in the case of the DK1 experiment, while no significant increase appeared in the case of DK2. A selective consumption
of these reserve substances by the algae in DK1 and not in DK2 being very unlikely, the very strong alteration of \( U_{37}^{Ep} \) index observed during the DK1 experiment was attributed to the simultaneous involvement of selective bacterial and auto-oxidative degradation processes. The presence of bacteria able to degrade selectively alkenones in DK1 is well supported by the detection of significant amounts of epoxyalkenones exhibiting an unsaturation ratio (ep\( U_{37}^{Ep} \)) lower than the \( U_{37}^{Ep} \) ratio of the residual alkenones. The detection of specific auto-oxidation products of alkenones during the DK1 experiment attests that free radical oxidation processes also contributed to the selective degradation of these compounds. Evolution of bacteria number during the incubation (strong increase under darkness and subsequent decrease after return to light) in DK1 was attributed to a transfer of photochemically produced singlet oxygen from senescent \( E. huxleyi \) cells to the associated bacteria limiting their growth under light/dark regime but not under continuous darkness. Prolonged incubation of two non-axenic cultures of a same strain of \( E. huxleyi \) under darkness gave very contrasting results and demonstrated that the strong decrease in alkenone concentration generally observed under such conditions resulted not only from the well known consumption of reserve substances by the algae, but also from the simultaneous involvement of autooxidative and bacterial degradation processes. Free radical oxidation (auto-oxidation) of alkenones, which is induced by homolytic cleavage of photochemically-produced hydroperoxides, affords specific allylic hydroperoxides and is strongly selective towards di- and triunsaturated alkenones. In contrast, degradation of these compounds by the bacteria associated to the algal cells (attested by the detection of
epoxyalkenones) may be selective or non-selective according to the bacterial community present. The alteration of alkenone unsaturation index during incubation of *E. huxleyi* cells under darkness thus appears to be strongly dependent on the initial physiological state of the cells (favouring or inhibiting autooxidative processes) and on the bacterial communities associated with them. This finding could explain the various degrees of selectivity previously observed during incubation of different strains of *E. huxleyi* under darkness. The results obtained in the present work confirmed the potential of aerobic bacterial degradation and auto-oxidation processes to induce significant increases in $U_{37}^{K'}$ values in *E. huxleyi* cells and thus raising concern about data interpretations of palaeo-temperature reconstructed from the alkenone unsaturation index. While the importance of the warming effect resulting from selective bacterial degradation of alkenones might be estimated on the basis of measured $(U_{37}^{K'} - e_p U_{37}^{K'})$ data (Prah et al., 2009), autooxidative alteration of $U_{37}^{K'}$ values could not be estimated. Indeed, hydroperoxides resulting from these processes are insufficiently stable to be used as tracers of alkenone free radical oxidation *in situ.*