

Relationship of sediment-biochemistry, bacterial morphology and activity to geotechnical properties in Central Indian Basin

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Abstract

Distribution of biochemical properties, morphological and functional characteristics of bacteria in extreme sedimentary environment of Central Indian Basin (CIB) was examined in relation to geotechnical properties and sediment texture. Interrelationships between these parameters in siliceous northern sediments were compared to southern pelagic clay to understand relationships and to appreciate mining implications. Clay impacted lipids negatively, and silt positively in the south. Geotechnical properties had opposite influence on the distribution of coccoidal and rod forms of bacteria. Rods were affected negatively by water content/porosity and positively by wet bulk density in the north suggesting their preference for deeper niches. Shear strength affected coccoidal distribution negatively in the south. Competition for labile substrates by coccoidal forms in the surface layers could also have restricted the rods to the deeper regions. Principal component analysis (PCA) further strengthened our inference that the distribution of rod forms increased with depth. Enzymatic properties of bacterial isolates also showed that the rods were able to degrade substrates like gelatin and DNA. These observations suggest that they are capable of degrading relatively more recalcitrant compounds in the deeper layers. The minor components like the rod morphotypes and sand content could wield a large influence on the variability of other parameters.

Key words: Deep-sea bacteria, biochemical distribution, geotechnical properties, sediment texture, Central Indian Basin.

Introduction

The deep sub-seafloor biosphere supports a diverse population of prokaryotes belonging to Bacteria and Archaea. The seafloor environment is a dynamic geo- biosphere that provides a diverse range of living conditions (Fry et al., 2008 and references therein). The deep-sea floor at Central Indian Basin (CIB) is an extreme environment where average temperature can be as low as 2°C, and pressure as high as 500bars. Besides, the total organic carbon (TOC) is <0.1% and lability as low as 100µg g⁻¹ (Das et al. 2011). These sediments have been explored for nature, abundance and distribution of nodules (Sudhakar, 1989; Sharma and Nath, 2000, Banerjee and Mukhopadhyay, 1991; Ghosh and Mukhopadhyay, 1999; Jauhari and Pattan, 1999). Early diagenetic processes like nitrification (Nath and Mudholkar, 1989), and sediment erosional characteristics (Banakar et al., 1991) have also been examined. The other studies include hydrothermal activity and alterations (Iyer et al., 1997, 2007; Nath et al., 2008) and tectonic features (Kamesh Raju and Ramprasad, 1989; Dymont, 1993), distribution and origin of seamounts (Das et al., 2005). Studies have also shown that CIB is dominated by siliceous sediments in the north and pelagic red clay in the south (Nath et al., 1989; Borole, 1993; Mudholkar et al., 1993). With clay and silt dominating sediment textures, sediment grain size and associated bacterial properties were suggested as indicators of biogeochemical processes that could be useful for mineral exploration and harnessing (Khadge, 2002; Valsangkar, 2004; Sharma, 2005). The microbial and biochemical studies in CIB have predominantly discussed the role and response of bacterial community to artificially simulated disturbance (Nair et al., 2000; Raghukumar et al., 2001a, b) and long-term response to the same (Raghukumar et al., 2006). Specific observations of deep-sea cultures and their role in ammonia oxidation under simulated deep-sea conditions suggest their participation in nitrification (Ram et al., 2001). In addition, Environmental Impact Assessment (EIA) studies following simulated mining in CIB have delved into the potential impact of mining on marine ecosystem (Sharma et al., 2003). Experiments conducted to assess the potential impacts of deep-sea mining in the Pacific and the Indian Oceans have indicated the immediate changes and restoration patterns of environmental conditions in the marine ecosystem (Foell et al., 1990; Trueblood, 1993; Fukushima, 1995; Tkatchenko et al., 1996; Sharma, 2005). Earlier studies on the effect of artificial disturbance on microbiology of deep-sea sediments were restricted to culturable fraction and suggested the revival of dormant bacteria and enhancement of bacterial culturability (Loka Bharathi and Nair, 2005). The present study focuses on the distribution of two main morphological forms, cocci and rod morphotypes, in the total direct counts of bacteria *vis-a-vis* the textural and geotechnical properties. Geometrical constraints, mechanical interactions and consolidation of deep sediment layers may restrict bacterial movement and activity (Fredrickson et al., 1997; Rebata-Landa and Santamarina, 2006), limit nutrient transport (Wellsbury et al., 2002) as well as diminish space availability (Zhang et al., 1998). The effects of all

these properties on the sediment biochemistry and bacterial abundance have been examined here. Thus, the present study evaluates the interrelations of microbial and biochemical properties with geotechnical and textural characteristics of sediment in Central Indian Basin for the first time and indicate some of the plausible effects that deep-sea mining could have on them. It also discusses certain functional aspects related to cell morphology and the implications of natural or artificial disruption in deep-sea sediment on geomicrobial processes.

Materials and methods

Study area

Under Environmental Variability Data Collection (EVDC) covering programs I, II, III and IV belonging to March 2003, March–April 2005, December 2006–January 2007 and September–October 2009 respectively, this study is mainly restricted to EVDC I. However some comparison has been drawn with the subsequent programs. Nine sediment cores were collected from CIB during the cruise on board *Akademik Aleksandr Sidorenko* (AAS-61) during EVDC I (Fig. 1; Table 1).

Sampling and sample sectioning on board

Sediment samples were retrieved from a depth of 5000 meters using USNEL- type box corer of 50 x 50 x 50cm dimensions. After siphoning the overlying water, sub-samples were collected in clean plexi-glass cylinders of 6.3 cm inner diameter. The 0-2, 2-4, 4-6, 6-8 and 8-10cm sections were extruded into sterile plastic bags. These sediment samples were homogenized before sub-sampling them for different parameters.

Sample processing

Total bacterial abundance (TC)

TC was estimated after fixing an aliquot of sediment slurry of 10^{-1} dilution with pre-filtered formaldehyde (2% final concentration) and staining with acridine orange for 5 minutes. The stained preparation was filtered through black polycarbonate isopore membrane filters (0.22 μ m, Millipore) and cells were counted using an epifluorescence microscope (Nikon 80i, Nikon, Tokyo, Japan) (Hobbie et al. 1977). Total counts (TC) were expressed as cells g^{-1} dry wt sediment. Rods and cocci were counted separately.

Bacterial size and shape

The size of the bacterial cells was measured using Nikon image analyzer pro plus v6. Another 1ml aliquot of the fixed sample was filtered over 0.22 μ m pore size black polycarbonate filters (Millipore, USA), air dried aseptically and sputter-coated with gold using SPI Module. The scanning electron

micrographs (SEM, model JSM-5800LV-SEM, JEOL) of the cells on these filters were then examined for the size and morphology.

Hydrolytic enzyme expression of bacterial isolates

Culturable heterotrophic population was assessed as colony forming units (CFU) on Zobell Marine Agar (ZMA) plates. A 100 μ l inoculum at 10^{-1} dilution was spread plated and incubated on board at 4°C, 1atm for 15-20days. The number of CFUs were normalized for sediment weights and expressed as CFU g⁻¹ dry wt sediment. About 20-25% Zobell Marine Broth 2216, HiMedia, Mumbai, amended with 1.5% agar was known to yield maximum CFU in CIB sediments (Raghukumar et al., 2001a, b; Nair et al., 2000; Loka Bharathi and Nair, 2005). Representative colonies were isolated, and identified up to generic levels using phenotypic traits (Oliver, 1982; Holt et al., 2000). Bacterial forms enumerated microscopically were grouped as rods or cocci, with coccobacilliary forms being grouped under the former. Bacterial isolates were checked for enzyme expression for degradation of polymers and solubilization of phosphates. Tests were carried out on agar media amended with 1% concentration of starch, gelatin, tween 80 (polyoxyethylene sorbitan mono-oleate), DNA and p-nitrophenyl phosphate as substrates. The plates were inoculated with test isolates and the presence of extracellular hydrolytic enzymatic activity was detected after 4 days incubation at 4°C, 1 atm (Raghukumar et al., 2001a, b; Loka Bharathi and Nair, 2005).

Labile organic matter

Labile organic matter comprising of proteins, carbohydrates and lipids was analyzed according to Danovaro et al., (1993). Proteins were estimated using Folin-phenol reagent (Lowry et al., 1951) with bovine serum albumin as the standard. Carbohydrates were estimated using phenol-sulphuric acid method (Kochert, 1978) with glucose as the standard. Lipids were extracted by direct elution with chloroform and methanol (5:10). (Bligh and Dyer, 1959) followed by oxidation with 0.15% acid dichromate and subsequent spectrophotometry using stearic acid as the standard (Parsons et al., 1984).

Sediment texture and geotechnical properties

The percentage of sand, silt and clay components were determined by the standard pipette analyses (Folk, 1968). Water content (dry basis) and specific gravity were measured by weight/volume (ASTM, 1995). Salt correction was made to water content for 35‰ salinity (Noorany, 1985). Porosity and wet bulk density were estimated assuming complete saturation of sediments (Bennett and Lambert, 1971). Shear strength of the sediments was measured using laboratory vane shear tester (AIMIL, India).

Statistical analyses

Pearson's correlation coefficient and Principal Component Analyses (PCA) were used to compare siliceous sediments in the north (10° to 12.5°S and 73°30' to 76°30'E) and pelagic clay in the south (12.5° to 16°S and 73°30' to 76°30'E) of CIB. Pearson's rank correlation test was performed to evaluate the relationship between biological and geological components using Statistica 6 and Excel program of MS Office 2003. Of the nine cores, five were grouped as northern region (n= 5cores x 5sections =25) and the remaining 4 as southern (n = 4cores x 5 sections = 20). PCA on the fourth root-transformed data was carried out using PRIMER v6 package (Plymouth Routines in Multivariate Ecological Research) to determine which variable contributes most to the distribution in an ordination diagram (Clarke and Warwick, 2001).

Results

Microbiology and biochemistry

Total bacterial abundance

The bacterial abundance in terms of total counts (TC) in the northern region sediments ranged between 10^7 and 10^8 cells g^{-1} dry wt. sediment. In the southern region TC varied over 2 orders of magnitude between 10^6 and 10^8 cells g^{-1} dry wt. sediment (Table 2).

Bacterial size and shape

The coccoid type of morphology dominated TC at nearly 89% in the north and the distribution varied with the increase in sediment depth. However, rod types, which comprised of only 11% increased with depth (Fig 2a; Table 2). The average size of cocci varied from 0.493 to 1.325 μ m in diameter while the length x breadth dimensions of rods ranged from 1.30 x 0.59 μ m to 2.52 x 1.12 μ m. In the south too, the coccoidal forms dominated TC by nearly 91% and decreased with depth. The rods that formed 9% in this area increased with depth (Fig 2b; Table 2). The average size of bacteria in this region was smaller. The size of the coccoidal forms varied from 0.393 to 0.706 μ m in diameter, while the length x breadth dimensions of rods ranged from 0.98 x 0.52 μ m to 2.13 x 0.82 μ m. SEM images of some typical morphotypes of CIB bacteria are shown in Fig 4.

Culturable abundance

Culturable abundance on ZMA in the form of CFU ranged from non-detectable levels (ndl) to 1.45×10^4 CFU g^{-1} dry wt sediment in the north and from ndl to 1.9×10^4 CFU g^{-1} dry wt sediment in the south.

Hydrolytic enzyme expression of bacterial isolates

Rod shaped bacterial isolates were able to utilize the relatively more recalcitrant DNA by producing extracellular hydrolytic DNase enzyme compared to the coccoid types that showed no such activity. However, coccoid forms scored more at the utilization of the relatively labile type of substrates such as starch due to amylase production (Table 3).

Labile organic matter (LOM)

The average carbohydrate, lipid and protein concentration in the sediment was 0.16 ± 0.05 , 0.15 ± 0.03 and 0.69 ± 0.42 mg g⁻¹ dry wt respectively and decreased with depth (Fig 2c; Table 2). The contribution of these biochemical components to average sedimentary labile organic matter (LOM) was in the order proteins (69%) > carbohydrates (16%) > lipids (15%) in the north. In the south, average carbohydrate, lipid and protein concentration in sediments was 0.1 ± 0.04 , 0.15 ± 0.04 and 0.2 ± 0.13 mg g⁻¹ dry wt. respectively and decreased with depth (Fig 2d; Table 2). The contribution of the biochemical components to average sedimentary labile organic matter in the south was in the order proteins (44%) > lipids (34%) > carbohydrates (22%).

Sediment texture and geotechnical properties

Generally, the sediment grain size in the northern sector was dominated by clay ($63 \pm 8.7\%$) followed by silt ($35 \pm 8.02\%$) and sand ($2 \pm 1.97\%$). While sand and clay decreased, silt increased with depth (Fig 2e; Table 2). Like the northern sector, the average grain size in south was dominated by clay ($63 \pm 8.2\%$) followed by silt ($36 \pm 8.0\%$) and sand ($0.9 \pm 1.05\%$). Unlike the northern sector, sand and clay increased and silt decreased with depth in the south (Fig 2f; Table 2).

The geotechnical properties of the sediment in the northern sector, comprising of water content (dry basis) was $577 \pm 95\%$, porosity $93 \pm 1.3\%$, wet bulk density 1.13 ± 0.01 g cm⁻³, shear strength 2.85 ± 0.72 kPa and specific gravity was 2.33 ± 0.12 (Table 2). In the southern sector, the average water content was $560 \pm 117.2\%$, porosity was $92.3 \pm 2.4\%$, wet bulk density was 1.13 ± 0.03 g cm⁻³, shear strength was 2.4 ± 0.74 kPa and specific gravity was 2.27 ± 0.07 . Water content decreased with depth in both the sectors (Figs. 2g and 2h; Table 2).

Statistical analyses

A correlation matrix was calculated using the environmental variables and significant relationships are listed (Table 4). The Pearson's correlation coefficient between various

biological and geological parameters showed interesting results. The distribution of carbohydrates was governed by the porosity, water content, specific gravity and wet bulk density. The degree of influence

of these parameters ranged from -25 to 44% in the north and -33 to 34% in the south. However, these geotechnical parameters influenced the protein concentration only in the south (-28 to 27%). Thus, opposite influence of porosity and wet bulk density on protein content was evident in the south while these properties had no effect on lipid concentration in this region. Sand had no influence on the components of LOM while silt (35%) and clay (35%) had opposite influence on lipid concentration in the southern region. Rods avoided the less dense surface sediments preferring the denser sub-surface layers (6-8cm, 8-10cm below sea floor). The negative effect of shear strength on coccoidal forms especially in the south suggests these forms dominate the surface layers.

PCA ordination of environmental variables is presented in Fig 3. Using covariance based PCA, four components were extracted which suggested that they were responsible for 97.2% and 96.5% of total variance in the north and south respectively. The first principal component (PC1) explaining about 84.2% of the variation in the north and 73.7% in the south is mainly associated with the rod morphotypes. The coefficient in linear combinations making up the PC1, is as high as -0.99 in the north and -0.986 in the south. The second high coefficient is that of sand at 0.828 in PC3 in the north and -0.838 in the south. The PC2 comprised mainly of water content -0.695 in the north and -0.632 in the south.

Discussion

The bacterial distribution and activity along the depth of the sediment could be due to the influence of preferential paths in transport through the sediment profile (Abu-Ashour et al., 1994), limited input of fresh organic carbon (Suess et al., 1980; Danovaro et al., 1999; Dixon and Turley, 2002) and/or use of recalcitrant old buried organic matter by deep bacteria (Zhang et al., 1998; Parkes et al., 2000; Wellsbury et al., 2002). However, bacteria adapt to changes in their immediate surrounding extreme environments by undergoing morphological differentiations due to differences in the organic nutrients (Young, 2006) and their concentrations. Some microbes respond by increasing the size of individual cells when nutrient availability is more, yet others do not change their size. Under extremities of starvation or nutrient depletion the cells shrink in size and yet others form long filaments. Besides, it is the quantity and quality of LOM and not total organic matter under these extreme conditions that is responsible for density of organisms (Danovaro et al., 1993).

Bacterial Parameters

Bacterial distribution, morphology and size

Generally, the total bacterial cell number decreased with increasing sediment depth in the CIB area. This decrease with depth could be attributed to the decrease in LOM, which in turn, is dependent on the

enzymatic breakdown. Poremba (1995) showed that the hydrolytic activity of enzymes declined vertically in the upper 10cm of sediment cores. The variability in distribution was more in the southern sector than the northern (Table 2). Bacterial distribution is generally considered to be dependent upon the amount of utilizable organic matter in the sediments, which in turn is substantially controlled by the sedimentation and degradation rates in the water column (Deming and Yager, 1992; Danovaro et al., 1993).

In the present study, coccoidal forms dominated up to 90% of the total counts in both the sampling sectors. The rod forms comprised of nearly 11% of the population amounting to almost an order less than the coccoidal forms. Irrespective of the morphotypes, the bacterial cell size was relatively small in LOM-depleted southern region. According to Young (2006), the size and shape of bacteria are highly controlled by the environmental conditions in which it prevails, i.e. when the total organic matter is more, the size of bacteria would be large, however if there is less of organic carbon then the cell size begins to decrease leading to the formation of ultramicrobial forms.

Culturable bacteria, their morphology and generic succession in response to artificial and natural disturbances

Cultured bacteria vary consistently from one sub-sea floor environment to another and have been shown to contribute significantly to important biogeochemical processes (D'Hondt et al., 2004). Such variability has been thoroughly recorded over space and time in CIB over a period of about 10 years.

A earlier study of simulated nodule mining and monitoring conducted in an area north of the EVDC site in CIB (Sharma, 2005) showed that the dominant retrievable forms belonged to class γ -Proteobacteria consisting of genera *Acinetobacter* and *Moraxella* sp. (coccobacillary, rods or diplococcal). After the simulated mining disturbance of the sediment, the generic diversity increased and was represented by Firmicutes comprising of *Bacilli* (rods) and *Staphylococcus* (coccoid) sp., Proteobacteria including γ -Proteobacteria, *Enterobacter* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Actinobacteria* sp., *Corynebacterium* sp., (rods) and *Micrococcus* sp. (coccoid), (Loka Bharathi and Nair, 2005).

Some more interesting features were observed while generating a natural baseline data of environmental variability (EVDC) since 2003 at the CIB on a much larger area than the simulated mining site. The data of the present study under EVDC I (2003) showed prevalence of *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Enterobacter*, *Flavobacter*, *Clostridium* and *Alcaligenes* among the rods, and *Staphylococcus*, *Streptococcus*, *Micrococcus* and *Diplococcus* among the cocci (Naik, M.Sc. Dissertation, 2006).

During the subsequent EVDC II (2005) a near-complete depletion in culturable coccoid forms was observed. The siliceous oozes in the northern sector comprised of rods belonging to *Aeromonas* and *Pseudoalteromonas*, flagellated rods belonging to *Alteromonas*, *Pseudomonas*, and *Xanthomonas* of class γ -proteobacteria and *Alcaligenes* of class β -proteobacteria. Motile and non-motile rods belonging to *Flavobacterium* of phylum *Bacteroidetes* was also present in two northern siliceous cores. The pelagic red clays in the south were relatively less diverse. They consisted of *Alteromonas*, *Pseudomonas*, *Xanthomonas* of class γ -proteobacteria and *Alcaligenes* of class β -Proteobacteria. *Flavobacterium* and *Cytophaga* (gliding rods) of phylum *Bacteroidetes* were also occasionally present (Das, PhD thesis, 2011).

Thus with time, the representation of morphotypes kept changing. Once dominant coccoid forms kept diminishing in abundance to be over-taken by the rod forms.

Activity

Microbial extracellular hydrolytic enzyme activity, psychrophily and piezophily

Culturable representative bacterial isolates checked for enzyme expression in the present study indicated that the rod shaped morphotypes were capable of degrading gelatin and DNA (Table 3) unlike the coccoidal forms that preferred starch and p-nitrophenyl phosphate. The comparatively higher phosphate concentrations as seen from the pore water nutrients studies (Nath et al., 2005) at the surface sediments could be partly due to higher living biomass in the form of ATP. The microbial activity is also higher in the surface layers (0-2cm, below sea floor) of the sediments. The coccoidal forms usually dominating the surface sediments produce the extracellular phosphatases and bring about phosphate solubilization. Thus the higher degradation of phosphates by bacteria than its consumption may lead to an increase in the concentration of this anion in the water column. This property suggested that the rods, although less in number, were capable of degrading relatively more recalcitrant compounds in the deep-sea sediments thus substantiating their abundance in the deeper layers where the denser and recalcitrant substrates tend to accumulate. Similarly, studies at the deep-sea hypersaline anoxic basins of the Eastern Mediterranean (DHABs) revealed habitat-specific behavior of esterase enzymes (Ferrer et al., 2005).

All the analyses relating to culturability have been carried out at 1atm and 4°C. It would be pertinent to mention here that, the question of decompression with bacteria is still debatable although decompression affects larger organisms. It is now being increasingly realized that the extent of damage could be appreciably less for bacteria. Previous studies have revealed that most piezophilic bacteria isolated from the deep-sea are psychrophilic (Yayanos et al., 1979, 1981; Jannasch and Wirsen, 1984;

Yayanos, 1986) and that changes in membrane fatty acid composition of bacteria with increasing pressure are analogous to changes in response to falling temperatures (DeLong and Yayanos 1985, 1986; Wirsen et al., 1987). Hence all our studies have been carried out at low temperature of ca 4°C.

The trends in the results obtained could reflect how the isolates function in the deep-sea. Yano et al., (1995) have shown that obligately barophilic bacteria could be isolated from the intestinal contents of deep-sea fish captured in the abyssal zone, without any special treatments to retain *in situ* pressure and temperature where most isolated strains later proved barophilic. While psychrophiles and piezophiles share similar genetic lineages (DeLong et al., 1997), barophiles can be grown at lower pressure provided temperature is lowered. These bacteria are capable of changing their respiratory system in response to pressure conditions (Kato and Qureshi, 1999) and many of the dominant genotypes are culturable (Rehnstam et al., 1993).

Biochemical Parametres

Protein, Carbohydrates, Lipids and Labile organic matter

Proteins, carbohydrates and lipids combine to form labile organic matter. In the north of CIB, the sedimentary organic matter is dominated by proteins that are nearly 69% of the LOM followed by carbohydrates (16%) and lipids (15%). The high protein to carbohydrate ratio also indicates that the sediment is labile. Dominance of protein in LOM is indicative of large amounts of living matter unlike the predominance of carbohydrates, which is a characteristic of detrital or oligotrophic environments (Danovaro et al., 1993; Pusceddu et al., 1999). The sedimentary organic matter in the south of CIB is also dominated by proteins (on an average 44%), followed by lipids (34%) and carbohydrates (22%). Apparently bacteria in CIB sediments preferentially utilize carbohydrates. This fraction of LOM ought also to be considered as a potentially available organic fraction for benthic organisms (Danovaro et al., 1993). This corroborates with the observation that more number of coccoidal fractions encountered in the study area could produce amylase and degrade carbohydrate more readily than proteins. In the south too, the predominance of protein is noticeable albeit at lesser percentages as compared to the north. The enzymatic profile in the study area shows that the amyolytic coccoidal forms degrades the carbohydrates much faster (Table 3) as compared to the proteins in the surface layers of the sediments, or the replenishment of this fraction of LOM is much slower than its utilization. Thus the protein concentration at the surface layers could be higher due to preferences of surface dwelling bacteria for carbohydrates.

Lipids are the next dominating fractions suggesting the relatively more recalcitrant nature of the sediment. In highly oligotrophic environments, the utilization of labile compounds results in a highly

refractory composition of sedimentary organic material (Pusceddu et al., 1999). The presence of highly refractory compounds might significantly slow the conversion of organic matter into bacterial biomass with consequences for benthic microbial loop functioning (Fabiano and Danovaro, 1998). However, sediment bioturbation (Rhodes and Boyer, 1982) is a key parameter which influences a broad range of phenomena such as distribution of microbial substrates and translocation of particle bound compounds within the seabed.

Inter-relationship between bacterial morphology and geotechnical properties

The geotechnical properties had opposite influence on the distribution of coccoidal and rod shaped forms. In the LOM-rich north, the major morphotype i.e., coccoid do not show significant down-core variation. The distribution of the minor morphotype or rods increased with respect to increase of recalcitrance of LOM, decrease in water content and increase in wet bulk density. In the LOM-depleted southern sector negative interrelationship between cocci and shear strength is evident. With porosity and wet bulk density not varying significantly down-core, the decreasing water content and increasing shear strength govern the morphotype distribution (Fig. 2; Table 4). PCA analysis based on correlation further confirmed that the distribution of the rod shaped bacterial forms increased with depth. The covariance based PCA analysis clearly isolates the rod shape forms in a single dominant component in PC 1 suggesting that the distribution of rods is relatively more independent of the other sediment textural parameters (Fig 3).

Interrelationship between labile organic matter and sediment geotechnical properties

Carbohydrate distribution and sediment porosity correlated positively by 44 and 32% in the northern and southern region of CIB, respectively. Consequently, the interrelationship between wet bulk density and carbohydrate varied from -25% in the north to -33% in the south possibly suggesting that carbohydrate associates more with water and less with sediment. While clay distribution and lipids were negatively interrelated in the south due to negative charges on both, silt and lipids related positively due to optimal size, which in turn could facilitate adsorption. The geotechnical properties of the sediments influence the biochemical constituents like proteins and carbohydrates. The variation in the lipid content is brought about by the silt and clay fractions of the sediment. Thus changes artificial or natural would evoke changes in a number of parameters.

Some of these processes could have implications when harnessing the deep-sea sediments for resources. These have to be examined against the backdrop of natural disturbances. Natural processes that can disturb deep-sea sediments are bioturbation, sedimentation and physical mixing with horizontally advected material (Turnewitsch et al., 2000) and re-suspension by near bottom currents

(Lampitt, 1985). Artificial disturbances have been conducted to simulate deep-sea mining activities. Such experiments have been conducted in the Pacific and Indian oceans to evaluate the effect of natural and artificial disturbance in the deep sea (Thiel and Forschungsverbund, 2001; Sharma et al., 2001). The immediate impact observed on sediment characteristics has been highlighted by a number of workers (Sharma et al., 2001; Valsangkar, 2001; Ingole et al., 2001). Long-term changes have shown mixed effects of sediment disturbance (Raghukumar et al., 2006). Hence, it is possible that these parameters with the changes in their relationships could be used as indicators of deep-sea sediment disturbance, brought about either naturally or artificially by anthropogenic intervention like deep-sea mineral mining.

The present study also highlights variability in microbial parameters in space and time in response to both natural and artificial disturbance. The facets of changes caused by episodic natural turbulences can often surpass the regular natural and low intensity artificial disturbances by orders of magnitude. Such possibilities have been highlighted by the generic changes of culturable bacterial fractions in CIB in a time-course of nearly 10 years. A regime change has been noticed from coccoid-dominated (before 2003) to variable (during 2003, present study samples) to predominantly flagellated forms (after 2003). Thus with time, the representation of morphotypes kept changing. Once dominant coccoid forms kept diminishing in abundance to be over-taken by the rod forms. Consequently, there could be changes in the biogeochemistry.

Conclusion

Thus the biogeochemical analyses of CIB sediments suggests that the minor components like the rod morphotypes amongst bacteria and sand component in sediment texture of Central Indian Basin, wield a large influence on the variability of other parameters. This study thus adds another important dimension to earlier findings in this area that deeper sediments are richer in DNA (Fernandes et al., 2005) and that artificial disturbance could improve culturability and functions of deep-sea sediment bacteria (Loka Bharathi and Nair, 2005). If deep-sea sediments were to be disturbed, deeper dwelling rods could surface. This would alter the microbiological properties of the sediment by improving the lability of organic matter and consequently change some of the biogeochemical processes in the deep sea ecosystem.

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LEGEND TO TABLES

Table 1. Water depths at the sampling stations in the Central Indian Basin (CIB).

Table 2. Average values of the measured parameters from nine cores down to 10cm depth in the CIB (TC= total bacterial counts; SD= standard deviation; LOM = labile organic matter)

Table 3. Hydrolytic enzyme expression in bacterial isolates from CIB core samples with coccoidal and bacillary/coccobacillary morphology

Table 4. Correlation of biochemical and bacterial parameters with the geotechnical properties and sediment characteristics.

LEGEND TO FIGURES

Fig. 1. Map of Central Indian Basin showing the sampling locations

Fig. 2. Downcore profiles of bacterial (rods and cocci) [a,b], biochemical [c,d], sediment grain size [e,f] and geotechnical [g,h] parameters for Northern and Southern sectors in CIB at 0-10cm sediment layer below sea floor. Horizontal bars represent intersample variability within each sector.

Fig. 3. Principal component analysis performed on bacterial, biochemical, geotechnical and sediment grain size parameters in northern and southern sector of CIB.

Cumulative percentages explained by axes I and I+II respectively:

a. correlation in northern sector: I – 34.7%; I+II – 57.2%.

b. correlation in southern sector: I –36.9%; I+II – 56.2%.

c. covariance in northern sector: I – 83.8%; I+II – 89.6%.

d. covariance in southern sector: I – 73.2%; I+II – 88.4%.

Fig. 4. Scanning electron micrograph of the bacterial morphology (cocci, coccobacilli and rod shape) from the sediment samples of CIB.

Table 1. Water depths at the sampling stations in the Central Indian Basin (CIB).

Sampling sector	Station no.	Depth (m)
North (Siliceous ooze)	BC 1	5355
	BC 26	5290
	BC 25	5300
	BC 3	5280
	BC 22	4900
<hr/>		
South (Pelagic red clay)	BC 10	4900
	BC 9	5350
	BC 8	5010
	BC 7	5070

Table 2. Average values of the measured parameters from nine cores down to 10cm depth in the CIB (TC= total bacterial counts; SD= standard deviation; LOM = labile organic matter).

Variables	Northern CIB (n=25)	Southern CIB (n=20)
TC (cells g ⁻¹) ± SD	2.34 × 10 ⁸ ± 1.56	2.02× 10 ⁸ ± 2.21
Cocci (cells g ⁻¹) ± SD	2.08× 10 ⁸ ± 1.45	1.84× 10 ⁸ ± 2.16
Rods (cells g ⁻¹) ± SD	0.27× 10 ⁸ ± 0.39	0.18× 10 ⁸ ± 0.27
LOM (mg g ⁻¹) ± SD	1.00±0.45	0.45±0.17
Carbohydrates (mg g ⁻¹) ± SD	0.16±0.05	0.10±0.04
Proteins (mg g ⁻¹) ± SD	0.69±0.42	0.20±0.13
Lipids (mg g ⁻¹) ± SD	0.15±0.03	0.15±0.04
Water content (%) ± SD	576.80±95.37	560.20±117.17
Porosity (%) ± SD	92.78±1.28	92.28±2.38
Wet bulk density (g cm ⁻³) ± SD	1.13±0.01	1.13±0.03
Shear Strength (kPa) ± SD	2.85±0.72	2.40±0.74
Specific gravity ± SD	2.33±0.12	2.27±0.07
Sand (%) ± SD	2.04±1.97	0.91±1.05
Silt (%) ± SD	35.08±8.02	36.21±8.00
Clay (%) ± SD	62.89±8.71	62.88±8.15

Table 3. Hydrolytic enzyme expression in bacterial isolates from CIB core samples with coccoidal and bacillary/coccobacillary morphology

Enzyme	Substrate utilizers	Percentage positive isolates	
		<i>Rods/coccobacilli morphology (n=91)</i>	<i>Coccoidal morphology (n=34)</i>
Gelatinase	Gelatin hydrolyzers	13%	9%
Lipase	Tween-80 hydrolyzers	18%	24%
DNase	DNA hydrolyzers	12%	0%
Amylase	Starch hydrolyzers	12%	35%
Phosphatase	p-nitro phenyl phosphate hydrolyzers	4%	12%

Table 4. Correlation of biochemical and bacterial parameters with the geotechnical properties and sediment characteristics.

Sediment properties	Biochemical and bacterial properties	Correlation coefficient	
		<i>Northern CIB (n=25)</i>	<i>Southern CIB (n=20)</i>
Water content %	CHO (mg g ⁻¹)	31% (r=0.553)	34% (r = 0.581)
	Protein (mg g ⁻¹)	-	23% (r = 0.478)
	Rods (x10 ⁸ cells g ⁻¹)	-30% (r= -0.541)	-
Porosity %	CHO (mg g ⁻¹)	44% (r=0.666)	32% (r = 0.595)
	Protein (mg g ⁻¹)	-	27% (r = 0.520)
	Rods (x10 ⁸ cells g ⁻¹)	-47% (r= -0.687)	-
Wet bulk density g/cm ³	CHO (mg g ⁻¹)	-25% (r= -0.496)	-33% (r = -0.574)
	Protein (mg g ⁻¹)	-	-28% (r = - 0.525)
	Rods (x10 ⁸ cells g ⁻¹)	35% (r=0.587)	-
Sheer strength	Cocci (x10 ⁸ cells g ⁻¹)	-	-27% (r = -0.523)
Specific gravity	CHO (mg g ⁻¹)	31% (r=0.554)	-
Silt	Lipids (mg g ⁻¹)	-	35% (r = 0.592)
Clay	Lipids (mg g ⁻¹)	-	-35% (r = - 0.593)

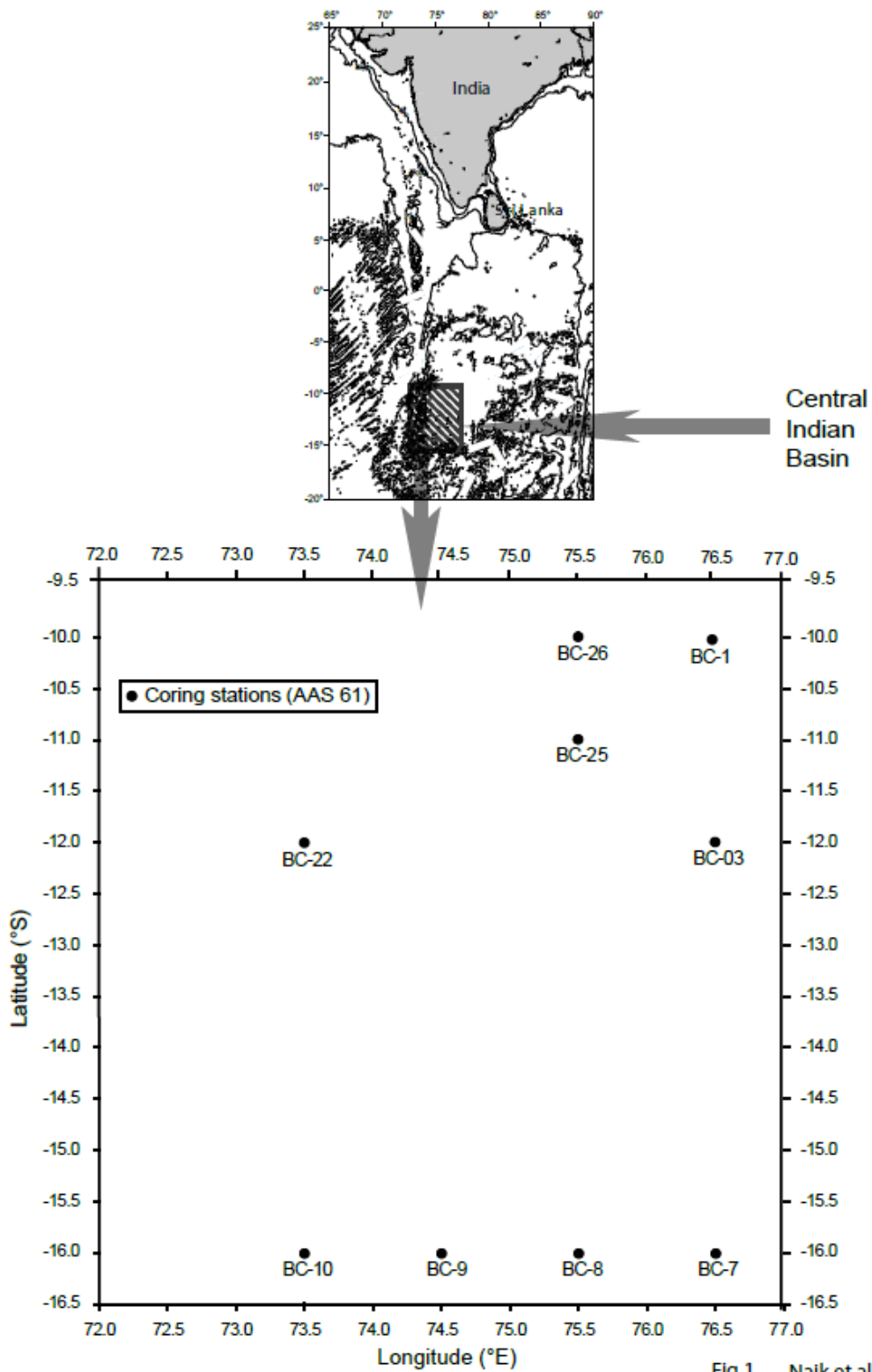


Fig 1. Naik et al 2012

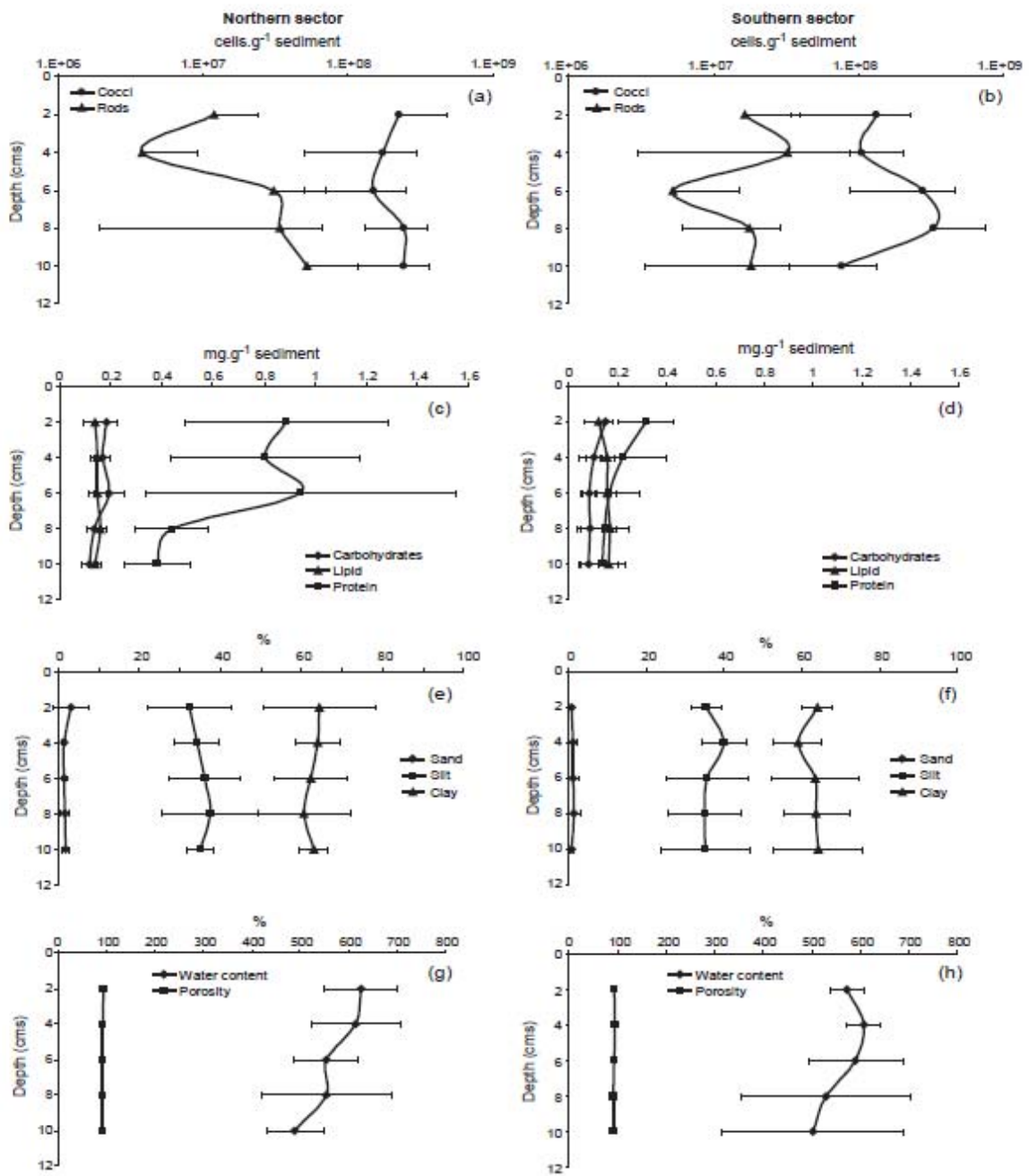


Fig 2. Naik et al 2012

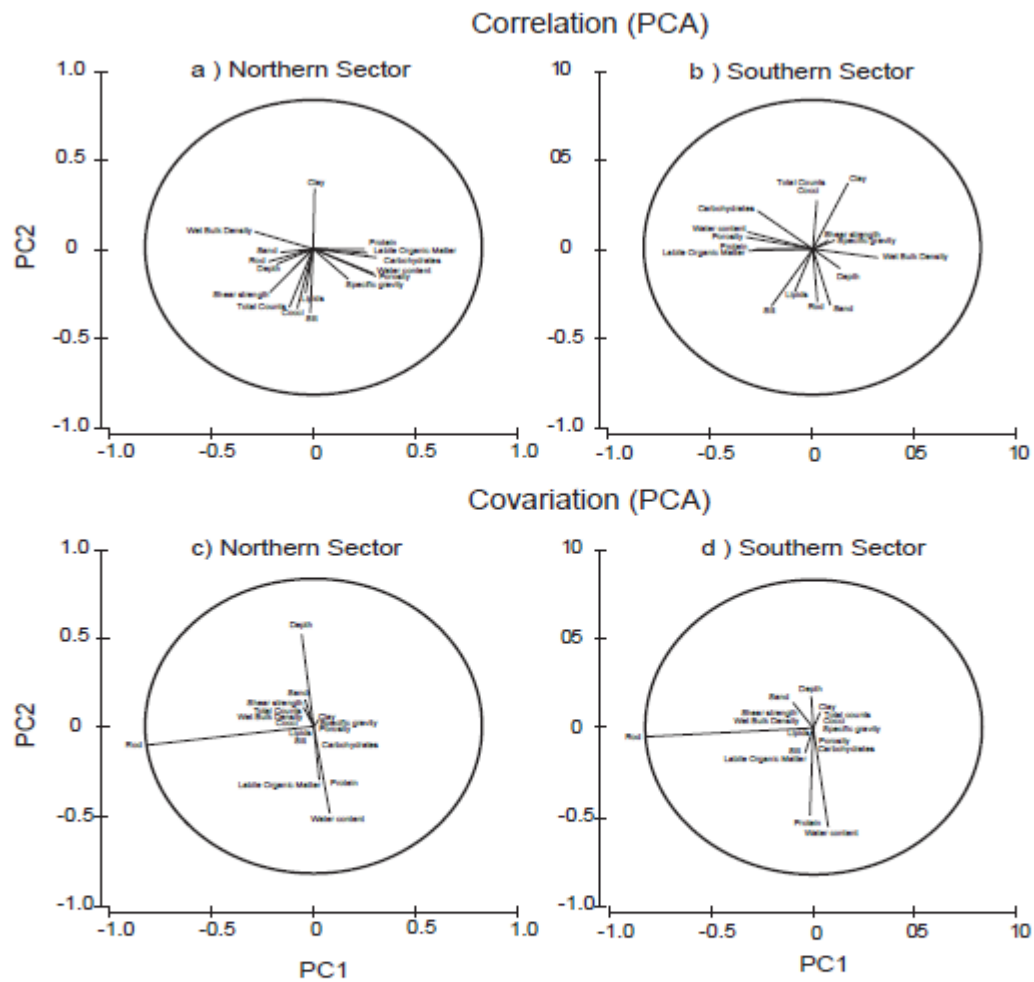


Fig 3. Naik et al., 2012

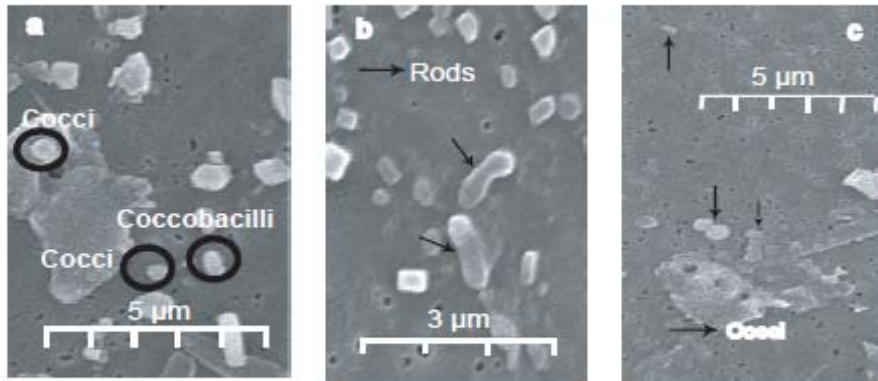


Fig. 4.