

Microbial iron reduction and methane oxidation in subsurface sediments of the Arabian Sea

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Abstract

Arabian Sea is one of the most productive regions of the world's ocean with seasonal upwelling followed by a characteristic oxygen minimum zone. It receives a continuous input of windborne iron-rich dust which possibly stimulates phytoplankton productivity. A sediment core from this area, which is on the western continental margin of India in the Kerala-Konkan basin was studied from the surface to 63 meters below seafloor, to establish the co-occurrence of microbial iron reducing activity and methane oxidation. Total bacterial abundance (TC), viable aerobic counts accounting for aerobic and facultative anaerobes (TVCa), viable anaerobic counts (TVCan), methane, sulfate, ferrous [Fe(II)], hydroxylamine extractable iron and HCl extractable iron concentrations were measured. Average TC was $52.6 \pm 29.8 \times 10^5$ cells g^{-1} sediment while TVCa and TVCan were an order less. Methane and sulphate concentrations were 1.3 ppm and 23.2 mM, respectively. Average Fe(II) concentration, hydroxylamine and HCl extractable Fe was 0.32 ± 0.26 , 0.86 ± 1.44 and 1.90 ± 2.47 mmol g^{-1} sediment, respectively. Interestingly, Fe(II) significantly correlated with TVCan ($r=0.66$, $p \leq 0.001$).

In the experimental tubes, the build-up of Fe (II) was accompanied by the disappearance of methane. Average methane concentration and Fe (II) concentration in the experimental sediment slurries was 0.56 ± 0.76 ppb and 0.50 ± 0.27 mmol g^{-1} sediment respectively. The microcosm experiment showed that Fe (II) determined was significantly higher (ANOVA, $F=6.74$, $p \leq 0.014$) after 5 days of incubation, thus implying microbial iron reducing activity. A strong negative linear correlation ($p \leq 0.001$) of methane concentration with Fe (II) suggests that increasing activity of microbial iron reduction caused suppression in methane production. This is also indicative of the co-occurrence of iron reduction and methane oxidation in these sediments.

Key words

Bacteria; iron; methane; Kerala-Konkan basin; Environmental; Abiotic

1. Introduction

The deep sub-surface sediments deposited millions of years ago comprises of a vast sedimentary ecosystem of microbial population consisting of diverse genomic and physiological repertoire (D'Hondt et al., 2004). Prokaryotes have been estimated to comprise one-tenth to one-third of the world's living biomass (Whitman et al., 1998). Estimates of the microbial abundance obtained from cores extending >1000 meters below the marine subsurface (mbsf) through dedicated deep-sea drilling expeditions, have revealed $>10^5$ microbial cells/cm³ (Teske 2013). Even ultraoligotrophic regions such as the South Pacific Gyre and the North Pacific Gyre have cell abundance of $\sim 10^3$ microbial cells/cm³ (D'Hondt, et al., 2009, Kallmeyer et al., 2012). Evidence is found for living microbial cells in the form of intact prokaryotic cells with an appreciable percentage of dividing cells in 1626 mbsf sediments on the Newfoundland Margin that are 111 My old and at 60° to 100°C (Roussel et al., 2008). Numerous studies have provided glimpses of the existence of genetically and metabolically diverse microbial communities consisting of archaea and bacteria playing an important role in global biogeochemical processes and attempts have been made to probe the link between indigenous microbial communities and biogeochemical processes, sediment geochemistry, and past geological events in sub-seafloor environments (Inagaki et al., 2003, 2006; Parkes et al., 2005; Biddle et al., 2006).

Across the world's ocean, the conditions for deep biosphere vary extremely. This is due to geological properties such as age and the type of sediment, sedimentation rate, organic matter availability, temperature, hydrostatic pressure, etc. (Whitman et al., 1998). Hence, bacterial communities are shaped by a range of microhabitat properties including pore size, availability of carbon and mineral substrates (Inagaki et al., 2002, 2003; Strong et al., 2004; Carson et al., 2009). However, in the deep sea subsurface, there is a severe limitation of nutrients and energy in the form of microbially degradable organic carbon and electron acceptor/donor, respectively (Jorgensen and Boetius, 2007). Hence, organisms may rely on under explored metabolic pathways to obtain the necessary energy and nutrients such as microbially-mediated process. Nearly 90% of the oceanic methane production is recycled in anaerobic marine sediments through methane oxidation (Reeburgh et al., 1993). In marine environments, microbially-mediated AOM could possibly act as a sink for the large amounts of methane produced in marine sediments by reducing the release of methane by 5-20% of the global methane flux (Reeburgh, 1996, 2007; Valentine et al., 2000) thus acting as gatekeepers of these sea-floor reservoirs (Joye, 2012). As an electron donor, microbially mediated methane oxidation occurs with various electron acceptors (Orcutt et al., 2011). Sulfate reduction mediated AOM has been suggested in a variety of marine environments as sulfate is the dominant electron acceptor for methane oxidation (Iversen and Jorgensen, 1985; Boetius et al., 2000; Treude et al., 2003; Knittel and Boetius, 2009).

However, recent preliminary evidence through laboratory experiments have demonstrated that microbially-mediated AOM may also be coupled to the reduction of highly reactive manganese/iron phases (Beal et al., 2009; Segarra et al., 2013). The availability of ferric iron [Fe (III)] can be a major factor controlling the amount of methane produced (Bond and Lovley, 2002; Riedinger et al., 2014).

Arabian Sea is unique as it has productivity driven seasonal pattern of dioxygen-deficient waters. It is one of the most biologically productive regions of the world's ocean having a characteristic seasonal upwelling followed by the production of an oxygen minimum zone in the water body (Wyrski, 1971; Madhuratap et al., 1996; Naidu, 1998). The seasonal upwelling in return has been reported to have prolonged and distinct effect on biological production and sedimentation (Madhuratap et al., 1996). Another important feature unique to the Arabian Sea is that it is open to the ocean only from the Southern side since it is surrounded on three sides by arid land. Due to the proximity of arid land, Arabian Sea receives continuous input of windborne iron-rich dust which possibly stimulates phytoplankton productivity (Measures and Vink, 1999; Witter et al., 2000). Further, iron has been known to drive many reactions involving cycles of carbon, nitrogen, phosphorus, sulphur, etc due to the ability of iron to oxidize and reduce easily. It is well known that high primary production in the water column results in an accumulation of organic carbon and subsequently high (anaerobic) respiration in sediments leading to accumulation of H₂S and CH₄ in the porewaters (Bruchert et al., 2006). Considering the high rates of primary production in Arabian Sea, the presence of high concentration of accumulated organic matter and its subsequent degradation is of vital interest since it may act as a potential source of CH₄ input to the overlying water column. Thus, Arabian Sea presents a unique region which is uncommon to other parts of the world. To our knowledge, there is some information on the geology of subsurface seafloor sediments of Arabian Sea (Schnetger et al., 2000; Pattan et al., 2012), whereas, information on the microbiological aspects is very scarce. In this study, we evaluated the importance of sedimentary ferric minerals in the microbially-mediated oxidation of methane in Arabian Sea marine sediments. To understand whether the process of iron reduction might regulate methane cycling and further examine the potential coupling between AOM and iron reduction, we measured methane oxidation and iron reducing activity through short-term incubation of sediment slurries.

2. Materials and Methods

2.1 Sampling site

The sampling site NGHP-01-01 (National Gas Hydrate Program-01-01) was on the western continental margin of India in the Kerala-Konkan basin of the Arabian Sea. The investigated piston core - NGHP-01-1A was cored to 290mbsf (15°18.366'N, 70° 54.192'E) and was retrieved from water depth of ~2663m during D/V JOIDES Resolution NGHP 01 expedition in May 2006. The sedimentary sequence in the Hole NGHP-01-1A was divided into four major lithologic units (Units I to IV) based on visual description, biogenic and terrigenous composition, trends in logging and physical properties data (Collett et al., 2007). Sediment samples up to a depth of 63 mbsf from lithostratigraphic unit I (LSI) were considered for the present study. LSI was composed of 80-100% clay followed by 5-20% silt and consisted of foraminifera-bearing nannofossil ooze alternating with foraminifera-rich nannofossil ooze of Quaternary age (Collett et al., 2007). Further, gas headspace analyses of these samples did not indicate detectable levels of hydrogen sulphide (H₂S, 20ppmv), methane (15ppmv) or other hydrocarbon gas. However, carbon dioxide (CO₂) concentrations expressed as equivalent concentration of gas volume to sediment volume ranged between 0.2 and 0.7 mM porewater (Collett et al., 2007).

2.2. Sample processing and analysis

2.2.1. Enumeration of bacterial abundance

Sediment cores were sectioned at various depths. Sub-samples were diluted using 0.2 µM filtered and autoclaved seawater in the ratio 1:10 (wet wt. vol⁻¹). Bacterial parameters such as total bacterial abundance (TC), viable aerobic counts (TVCa) and viable anaerobic counts (TVCan) were estimated.

Briefly, for TC, bacterial cells were stained using the method described by Deming and Colwell (1982). A 2.5% final concentration of pre-filtered formaldehyde was used to fix 5 ml aliquot of sediment slurry. One ml of the fixed sample was stained with acridine orange (AO; Sigma) at a concentration of 0.01% (wt. vol⁻¹) for 5 min and filtered onto 0.22 µm Nuclepore black polycarbonate filter papers (Millipore). The filter papers were examined by epifluorescence microscopy (Nikon 50i), and bacterial counts were enumerated according to Hobbie et al.(1977). The detection limit was 1.79 x 10⁵ cells g⁻¹ sediment and was calculated according to Kallmeyer et al. (2008).

To enumerate cells responsive to nutrient enrichment under aerobic conditions (TVCa) as well as the facultative anaerobes, yeast extract (0.01% final concentration) and an antibiotic cocktail was added to

5 ml of sediment slurry. Antibiotic cocktail comprised of nalixidic acid, piromedic acid and pipemedic acid and was used to inhibit DNA synthesis and inhibit cell division (Kogure et al. 1979; Joux and LeBaron, 1997). Slurry was incubated for a period of 7 h (prestandardized), subsequently fixed, stained and counted as described by Deming and Colwell (1982) and Hobbie et al. (1977). Similarly, to enumerate responsive cells under anaerobic conditions (TVCan), in addition to yeast extract and antibiotic cocktail, a reducing agent, Na₂S.9H₂O (0.0125% final concentration) was added to induce anaerobic conditions (Loka Bharathi et al., 1999, Kerkar and Loka Bharathi, 2011). Only elongated and enlarged cells with clearly visible invagination were counted as viable cells (Roszak and Colwell, 1987).

2.2.2. Geochemical Parameters

Sediment cores were sectioned on board at various depths, placed into sample bags and purged with nitrogen gas and frozen at -20°C.

2.2.2.1. Estimation of methane concentrations

Methane concentrations were measured using the headspace method (Gonsalves et al., 2011). The pre-weighed sample vials containing sediments were analyzed for methane concentration using a gas chromatograph (SHIMADZU 2010, Japan) in which a flame ionization detector was used, nitrogen gas was the carrier gas with a flow rate of 9.0 ml min⁻¹. The column temperature was 60°C, while both injector and detector temperatures were set at 100°C (Nakibuuka et al., 2012). All the above instrument parameters were kept constant throughout the whole study.

2.2.2.2. Estimation of sulfate concentrations

Sulfate (SO₄²⁻) concentrations were measured by turbidometry (Clesceri et al., 1998). Briefly, sediment samples were acidified (pH 1) with 4N HCl. Conditioning solution, containing NaCl, ethanol, HCl and glycerine was subsequently added. Finally, barium chloride at a final concentration of 30% was added. The amount of sulfate present in the sample was measured at 365 nm (Jasco V-650, Japan), as the concentration of sulphate is directly proportional to the precipitate formed as barium sulfate.

2.2.2.3. Estimation of sedimentary iron concentrations

Frozen sediment samples (-20°C) were used to measure different fractions of sedimentary iron concentrations consisting of ferrozine extractable ferrous iron [Fe(II)], hydroxylamine extractable ferrous iron (Hy-Fe) and 1N HCl extractable ferrous iron (HCl-Fe) (Lovley and Phillips 1986, 1987). Thawed wet sediment was extracted with ferrozine in 50mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic

acid (HEPES) buffer (pH 7) to represent adsorbed ferrous iron. Similarly, extraction with hydroxylamine represented as HY-Fe and with 1N HCl represented as HCl-Fe was carried out for 24h, before adding the buffered ferrozine solution. HY-Fe represented the Fe fractions present as reactive/bioavailable ferric iron and HCl-Fe represented the reactive solid phase Fe. Ferrozine is essentially used as an indicator as it is specific to Fe (II) and complexes with Fe(II) to form a magenta ferrozine-ferrous iron complex $[\text{Fe(II)(FZ)}_3]$ with a maximum absorbance at 562 nm (Stookey, 1970). The amount of soluble Fe(II) was then quantified by measuring the absorbance at 562 nm using a spectrophotometer (Jasco V-650, Japan). Calibration curve was obtained using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 0.1 N HCl as the standard.

2.3. Iron reduction and methane oxidation by indigenous microflora in microcosms containing Kerala-Konkan basin sediments

Briefly, sediment slurry was prepared by adding one gram of sediment to 10 ml of modified ferrozine medium in pre-weighed 15ml gas chromatography vials under inert atmosphere (Fernandes et al., 2011). The ferrozine medium contained 20 mM HEPES and 350 mM ferrozine in artificial seawater (pH 7.8 ± 0.2). Artificial seawater consisted of NaCl, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , KCl and NaHCO_3 (Atlas, 2004). Interference by sulfate reduction was inhibited by the addition of 20 mM sodium molybdate (Coates et al., 1996, Nielsen et al., 2002). The vials were incubated without shaking under dark and anaerobic conditions at $4 \pm 0.2^\circ\text{C}$. Control sets were prepared using a final concentration of 20mM sodium azide. Methane and Fe(II) concentrations were measured on 0-day and 5-day in the experimental vials. Methane concentration in the headspace was measured as previously mentioned in section 2.2.2.1. This measurement was immediately followed by the quantification of Fe(II) as mentioned before in section 2.2.2.3.

2.4. Statistical analyses

Bacterial parameters comprising of TC, TVCa and TVCan were log transformed for statistical analysis. Interrelationships between bacterial and geochemical parameters in the sediment were analyzed using Pearson's rank correlation. Bonferroni correction was incorporated to make an adjustment to the p values. This correction is very conservative and usually made when multiple comparisons are made on a single dataset so as to reduce the number of false positives (Legendre and Legendre, 1998). However, although Bonferroni correction controls false positives it must be noted that, due to the conservative nature, there is likelihood of significantly increasing the false negatives; hence in this analysis it is used with caution. Bonferroni correction, sets the significance p cut-off value at $\alpha' = \alpha/k$,

where k =number of treatments, α =uncorrected significance level, α' =corrected significance level. Here, $k=12$, $\alpha=0.05$, $\alpha'=0.004$.

Data for statistical analysis for the microcosm experiment consisted of two parameters i.e. methane and Fe(II) concentration. Initially, one-way analysis of variance (ANOVA) was performed on data obtained on 0-day and 5th day to check whether there was significant variation between initial and final dataset. Pearson rank correlation analysis was done to determine the interrelationship between methane and Fe(II) concentration in the microcosms. All statistical analysis was done using Statistica version 10.0. (StatSoft, Tulsa, Oklahoma, USA).

3. Results

3.1 Distribution of bacterial parameters

Average (avg.) cell counts in this region was $52.6 \pm 29.8 \times 10^5$ cells g^{-1} sediment (Table 1). Generally, TC significantly decreased with sediment depth (Table 2), although, peaks were recorded at the surface layer of <2 mbsf and at ~19 mbsf (Fig 1). Maximum abundance of 1.03×10^7 cells g^{-1} sediment was recorded at 0.4-1.4m. In addition, TC positively correlated with porosity ($r=0.591$, $p \leq 0.001$). TVCa and TVCan abundance were on an average 10^4 cells g^{-1} sediment and an order less than TC (Table 1). TVCa and TVCan did not show any significant interrelationship with each other ($r=-0.187$). In addition, TVCa followed a decreasing trend, although three peaks were recorded at different sediment depths (Fig 1). TVCan showed an increasing trend with sediment depth with a positive correlation which was not significant (Table 2). However, TVCan significantly correlated with Fe(II) ($r=0.66$, $p \leq 0.001$).

3.2 Distribution of methane, sulfate, and iron and its association with various environmental parameters

Methane concentrations ranged between non detectable levels (ndl) to 3706 ppm and significantly increased ($p \leq 0.001$) with depth (Table 1). A peak was recorded at ~ 7-8 mbsf. SO_4^{2-} concentrations varied between 17.78 – 28.38 mM and showed an increasing trend up to a depth of 5 mbsf. The concentration then decreased steeply at a depth of 7-8 mbsf and peaked again at ~ 25 mbsf only to reduce again further. Fe(II) ranged between ndl to 0.96 (avg. 0.32 ± 0.26) mmol g^{-1} sediment and varied with depth in an irregular manner. The addition of hydroxylamine (Hy) and HCl as an extractant to the sediment resulted in an increase in reactive Fe(II) that was higher than adsorbed Fe(II) concentrations. Hy-Fe and HCl-Fe extractable Fe(II) fractions accumulated over a period of 24h and was on an average of 0.86 ± 1.44 and 1.90 ± 2.47 mmol g^{-1} sediment, respectively (Table 1). Thus addition of these

extractants resulted in Fe(II) concentration that was 27 to 57 times higher than adsorbed Fe(II). Depth wise, the Fe(II) and Hy-Fe did not show a consistent trend in distribution, while, HCl-Fe concentrations increased with depth (Fig. 2). Pearson's correlation analysis revealed that methane concentration correlated negatively with porosity ($r=-0.481$, $p\leq 0.004$) and TC ($r=-0.491$, $p\leq 0.004$) while SO_4^{2-} concentration correlated negatively with methane ($r=-0.462$, $p\leq 0.01$) and TVCa ($r=-0.432$, $p\leq 0.05$). Concentrations of HY-Fe and HCl-Fe correlated significantly ($r=0.530$, $p\leq 0.001$), Table 2. HCl-Fe fractions positively correlated with Fe (II) ($r=0.394$, $p\leq 0.05$), SO_4^{2-} ($r=0.419$, $p\leq 0.05$) and negatively with TC ($r=-0.391$, $p\leq 0.05$).

3.3 Iron reduction and methane oxidation by indigenous microflora

Over the duration of a 5-day experiment, results showed that the average methane concentration in the sediment slurries was 0.56 ± 0.76 ppb. However, in surficial sediments and at 57mbsf, the concentration of methane increased over 500 ppb (Fig. 3a) with a corresponding decrease in the Fe(II) concentration. Also a decrease in methane concentrations at ~38mbsf had a corresponding increase in Fe(II) concentrations. In the sediment slurries Fe(II) concentrations ranged from ndl to 1.08 mmol g^{-1} sediment with an average of $0.50\pm 0.27 \text{ mmol g}^{-1}$ sediment (Fig 3b). The Fe(II) concentrations at the end of 5-day was significantly higher (ANOVA, $F=6.74$, $p\leq 0.014$) than the 0-day. Iron reducing activity was negligible in the control tubes containing sodium azide (data not shown). Sodium azide is a known microbial growth inhibitor. Linear correlation analysis showed a significant negative interrelationship between Fe(II) concentration and methane production ($r=-0.56$, $p\leq 0.001$, $n=36$, Fig. 3c).

4. Discussion

4.1 General distribution of bacterial abundance down the sediment core

Arabian Sea is one of the most productive regions of the world with a characteristic monsoon induced upwelling followed by the development of an oxygen minimum zone in the water body (Wyrki, 1971; Madhuratap et al., 1996; Naidu, 1998). The monsoon induced upwelling brings about high productivity in the Arabian Sea which leads to a continuous accumulation of organic matter. Arabian Sea is unique as it is surrounded on its three sides by areas which contain deserts. The windborne dust containing iron from these areas probably contributes to the excess iron concentration in the water column which ultimately settles at the bottom sediments (Measures and Vink, 1999; Witter et al., 2000). Due to the ability of iron to oxidize and reduce, iron has been known to drive many reactions involving cycles of carbon, nitrogen, phosphorus,

sulphur etc. Bacteria are known to play a major role in these different cycles (Madsen, 2011). Depth profiles of TC in the Arabian Sea sediments showed that the bacterial abundance fluctuated between 10^{5-7} cells g^{-1} sediment and significantly decreased with depth ($r=-0.775$, $p\leq 0.001$). Comparison of Arabian Sea subseafloor cell abundance to that of other subseafloor sediments was similar. In addition, the decrease in TC with depth is consistent with the general depth distribution pattern of microbial abundance (Parkes, et al., 1994, 2000, D'Hondt et al., 2004, Roussel et al., 2008). The occurrence of high percentage of clay between 80-100% in the studied sedimentary core (Collett et al., 2007) might have a possible role in the distribution of the bacterial abundance in the Arabian Sea sediments. Numerous studies have suggested that bacterial communities in the sediment are considerably influenced by various sedimentological properties such as pore size and mineral substrates (Inagaki et al., 2002, 2003, Strong et al., 2004, Carson et al., 2009). Inagaki et al. (2002) while studying the deep-sea siltstone collected from the Japan Trench suggested that the distribution of the microbial communities apparently correlated with the porosity and permeability of the geological matrices. In the present study too, the cell numbers significantly correlated ($r=0.591$, $p\leq 0.001$) with porosity. In turn, porosity was also correlated to depth which could be co-correlated to pressure, organic carbon availability, etc (Ying et al., 2012). However, in the present study these parameters have not been measured.

TVCa is generally used as a representative to estimate metabolically active forms under aerobic conditions (Kogure et al., 1979) and also accounts for the facultative anaerobes. TVCa decreased with depth yet their presence was recorded even at 63 mbsf. Moreover, peaks in abundance were recorded at different depth suggesting the possibility of metabolically different communities at various depths, where the presence of facultative anaerobes cannot be ruled out. Facultative anaerobes have been isolated from a depth of 900mbsf in sediments of Sea-do located towards the western part of Dokdo Island in the East Sea of Republic of Korea (Chang et al, 2013). In addition, the present study also showed that there was no significant correlation between TVCa and TVCan indicating that the overlap between these two groups was less, possibly suggesting an existence of physiologically different communities. This supports our observation on the presence of facultative anaerobes. TVCan increased with depth reflecting that these anaerobic forms thrived under increasing anaerobic conditions. Previous studies of deep subseafloor sediments have also reported the existence of microbial cells that exhibit anaerobic metabolic activity (Roussel et al., 2008, D'Hondt et al., 2009). Moreover, reducing conditions in this sediment core were recorded by Collett et al (2007). The strong correlation between abundance of TVCan and Fe(II) ($r=0.66$, $p\leq 0.001$) may be attributed to the involvement of this anaerobic community in the iron oxide reduction.

4.2 Distribution of methane, sulfate and iron, and its association with environmental parameters

Methane is a well known greenhouse gas and has mass-based warming potential up to 72 times greater than carbon dioxide (ICCP, 2007). In the Arabian Sea sediments the distribution of methane, sulfate and iron and their interrelationship clearly demonstrated the presence of a significant potential for iron reduction and methane oxidation.

In the present study, sulfate increased up to 5 mbsf and decreased concomitantly when methane started to build up at ~ 7 mbsf, indicating a sulfate–methane transition zone (Iversen and Jørgensen, 1985, Martens and Berner, 1977). Below this zone, sulfate apparently regenerated. This regeneration of sulfate concentrations, appears to be due to the downward diffusing sulfide reacting with Fe(III) species, which is present in the form of poorly-reactive iron oxides or iron bound in reactive silicates (Holmkvist et al., 2011). Furthermore, HCl extractable Fe(II) is often associated with solid phase Fe such as acid-soluble minerals like FeS (van der Zee et al., 2002). This may explain the significant interrelationship ($r=0.419$, $p\leq 0.05$) of HCl-Fe with sulfate. The background concentration of sulfate in these sediments was 23.19 ± 3.70 mM. In these sediments, H₂S was not detectable at concentrations < 20 ppmv (Collett et al., 2007). FeS, green mottling and bands with Fe(II)–rich clays have also been observed in this sediment core (Collett et al., 2007). The hydrogen sulfide produced by sulfate reduction, subsequently reacts with reactive iron to produce iron sulphide as an initial product and consequently pyrite (FeS₂) (Rickard and Luther, 1997, Schoonen, 2004).

Methane and sulfate were negatively correlated in the present study, ($r=-0.462$, $p\leq 0.001$). The average methane concentration was ~1.3 ppm in the sediments. On the other hand in these sediments, concentration of CO₂ was 0.46 ± 0.17 mM pw while methane concentrations >20 ppmv were only considered for recording for gas hydrate detection, on board (Collett et al., 2007). It is a known fact that in sulfate-linked AOM, the end product of methane oxidation is CO₂ with the reduction of sulfate to hydrogen sulfide (Joye, 2012). Taken together, it is probable that the hydrogen sulfide formed possibly combines with Fe to produce FeS and subsequently FeS₂ which may influence the initiation of metal-oxide dependent AOM.

The Fe(III)-bearing minerals increased continuously with sediment depth and is indicated by the increase in the content of reactive iron in the form of hydroxylamine extractable iron and HCl extractable fractions of Fe. In particular, addition of hydroxylamine and HCl as extractants resulted in Fe(II) concentrations that were 27-57 times higher than adsorbed Fe(II) (avg. 0.32 ± 0.26 mmol g⁻¹ sediment) indicating the availability of iron for microbial use as an energy source. Fe(II) significantly

correlated with TVCan ($r=0.667$, $p\leq 0.001$) implying that the anaerobic fraction of metabolically active microbial cells were actively involved in the iron cycling. Moreover, TVCan and HCl-Fe related to each other ($r=0.344$, $p\leq 0.05$). HCl extraction of iron is usually a proton promoted dissolution and it is possible that the anaerobic viable forms may be using the proton-promoted mechanism for the dissolution of Fe oxides. Proton-promoted dissolution is one of the mechanisms that have been suggested for dissolution of Fe oxides for microorganisms in deep terrestrial subsurface due to their inherent ability to produce acidic conditions by excreting organic acids (Hersman, 1997). It may be noted here that HCl-Fe fractions positively correlated with Hy-Fe ($r=0.530$, $p<0.004$). A possible explanation of this correlation, is that the HCl-Fe extractable fraction represent Fe(III) oxide assemblages spanning amorphous Fe(III) oxides and is possibly extracted by HCl. Further, the amorphous Fe(III) (hydr)oxide could also be extracted with hydroxylamine-HCl (Lovley and Phillips, 1987).

4.3 Iron reduction and methane oxidation by indigenous microflora in microcosms containing Kerala-Konkan basin sediments

Production of Fe(II) in sediment slurries with native microflora increased with time. There was significant difference (ANOVA, $F=6.74$, $p\leq 0.014$) in the concentrations of Fe(II) between the 0-day and 5th day. In the control tubes i.e. in the presence of sodium azide, the process of iron reduction was inhibited. Sodium azide has previously been shown to inhibit iron reduction (Fernandes et al. 2011). The build-up of Fe(II) in the experimental tubes was accompanied by the disappearance of methane (Fig. 3c). A strong negative linear correlation ($r=-0.56$, $p\leq 0.001$) of methane concentration with the adsorbed Fe(II) suggests that increasing rates of microbial iron reduction caused a suppression in methane production. Abundance of Fe(III)-bearing minerals have been suggested to have a potential for a coupling of AOM to Fe(III) reduction in sediments (Wankel et al., 2012). Moreover, iron oxides, manganese oxides, and/or nitrate, have been known to serve as alternate electron acceptors for AOM (Raghoebarsing et al., 2006, Beal et al., 2009, Sivan et al., 2011). Studies have demonstrated that, poorly crystalline Fe(III) oxides present in sediments were capable of inhibiting methane production (Lovley and Phillips, 1987; Roden and Wetzel, 1996; Frenzel et al., 1999; Yao and Conrad, 1999; Chidthaisong and Conrad, 2000). Moreover, when Fe(III) is present in the sediment, Fe(III)-reducing organisms are known to divert electron flow away from sulfate reduction and methane production even though there were populations of potentially active sulfate reducers and methanogens in the sediments (Lovley et al., 1987). It may be recalled here that percentage of clay in these sediments is around 80-100% and the average adsorbed Fe(II) concentration and reactive Fe like hydroxylamine and HCl extractable Fe was 0.32 ± 0.26 , 0.86 ± 1.44 and 1.90 ± 2.47 mmol g⁻¹ sediment, respectively. The high percentage of clay as well as the concentration of reactive species of Fe in these sediments implies the

potential availability of Fe (III) for iron reduction. Studies on marine sediments have shown that a fraction of iron is released from structurally coordinated Fe^{3+} of clay minerals by microbial extraction (Vorhies and Gaines, 2009).

In conclusion, Arabian Sea is one of the most productive regions of the world with a characteristic monsoon induced upwelling and an oxygen minimum zone. In this region the windborne dust containing iron probably contributes to the excess iron concentration in the water column which ultimately settles at the bottom sediments. These sediments have a bacterial abundance between 10^{5-7} cells g^{-1} sediment. Anaerobic fraction of viable bacteria was actively involved in the iron cycling. In these sediments iron reduction is seen to influence methane oxidation rates. Thus, the presence of both methane oxidation and iron reduction activities in the Arabian Sea sediments, indicates the co-occurrence of their indigenous physiological microbial groups with the potential to carry out these activities.

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Figure captions

Fig. 1: Distribution pattern of bacterial parameters down the sediment core up to 63 mbsf

Fig. 2: Distribution pattern of different fraction of iron concentration down the sediment core up to 63 mbsf

Fig. 3: Methane concentration at 0-day and 5-day (a), Ferrozine-extractable Fe(II) concentration at 5-day (b) and the linear correlation (c) between the two parameters in a microcosm experiment after 5-day incubation under anaerobic conditions.

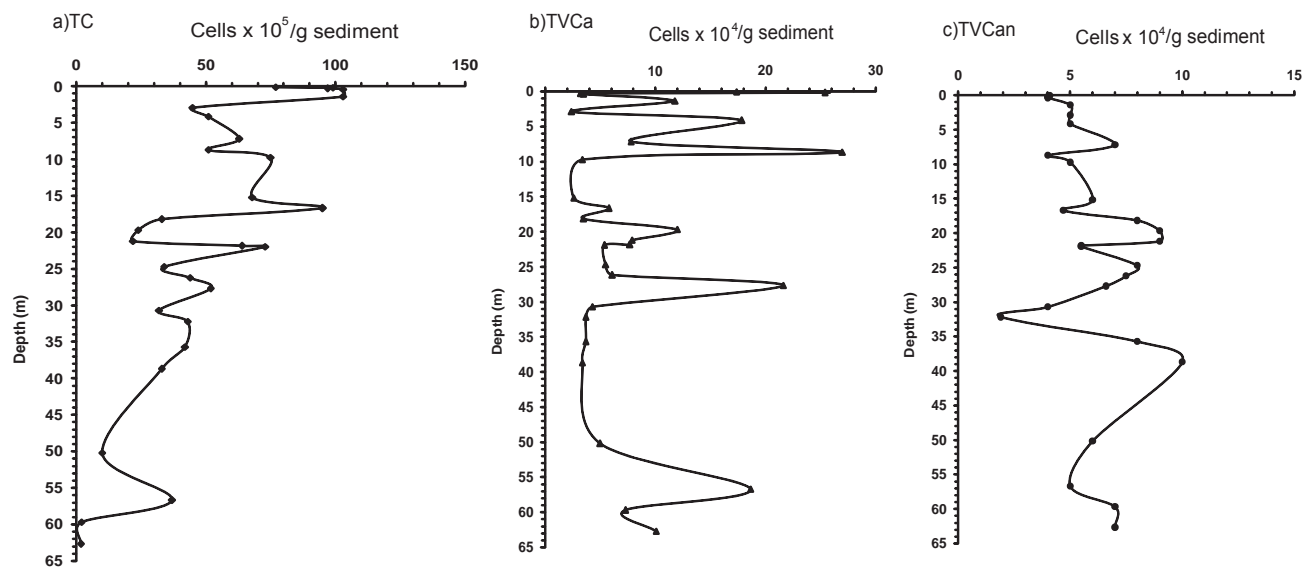


Fig.1

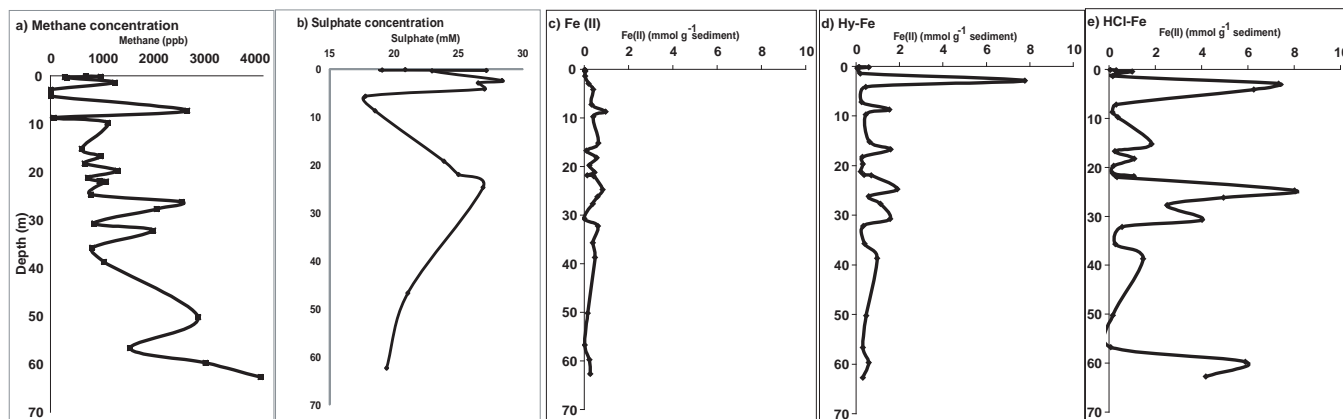
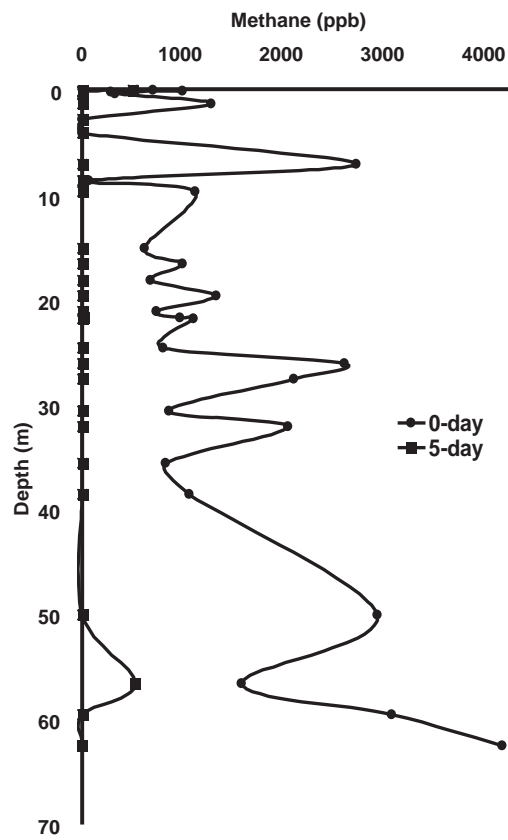
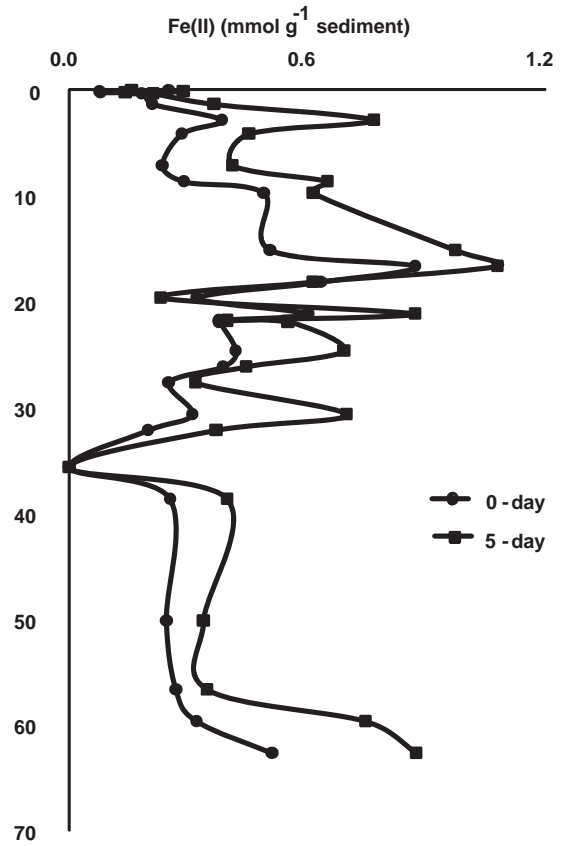


Fig.2

a) Methane concentration



b) Fe(II) concentration



c) Linear correlation between methane and Fe(II)

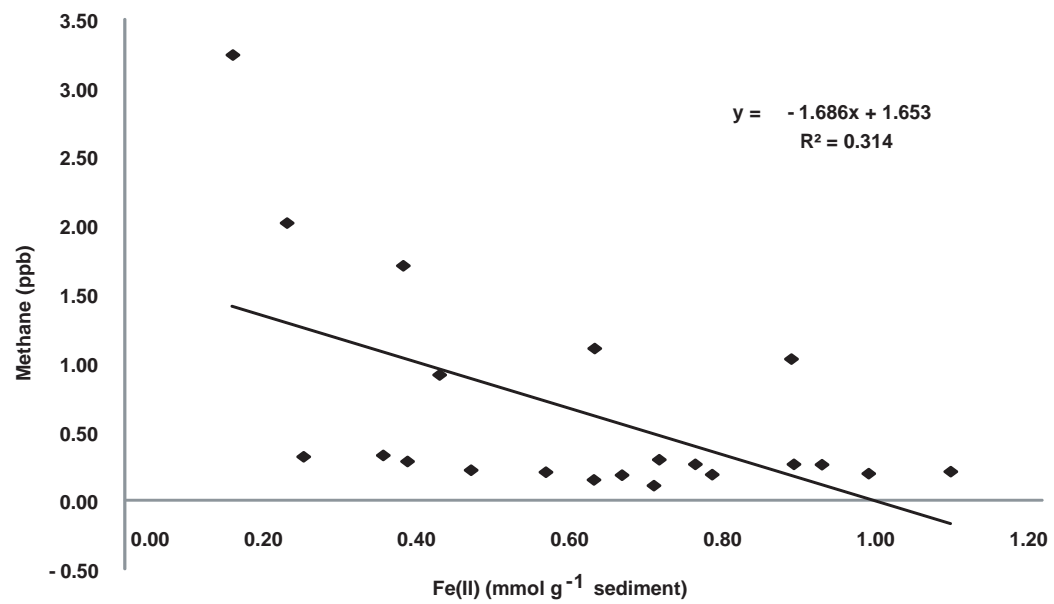


Fig.3

Table 1: Range and average values of various chemical and bacterial parameters obtained from the Konkan-Kerala basin sediment core analyzed up to 63 mbsf.

Parameters	Range	avg±sd
Sulphate (mM)	17.78 – 28.38	23.19±3.70
Methane (ppb)	ndl – 4149	1280±1022
Fe(II) (mmol g ⁻¹)	ndl – 0.96	0.32±0.26
HY-Fe (mmol g ⁻¹)	0.05 – 7.76	0.86±1.44
HCl-Fe (mmol g ⁻¹)	0.34 – 17.62	1.90±2.47
*CO ₂ (mM pw)	0.2 – 0.7	0.46±0.17
*GD (g ⁻¹ cm ³)	2.62 – 2.80	2.73±0.03
*porosity (%)	56.03 – 71.48	65.20±3.17
TC (x 10 ⁵ cells g ⁻¹)	1 – 103	52.6±29.8
TVCa (x 10 ⁴ cells g ⁻¹)	2.4 – 27	9.07±7.20
TVCan (x 10 ⁴ cells g ⁻¹)	1 – 10	5.89±1.99

avg – average, ndl – non detectable limit, pw – porewater, Fe(II) – ferrous iron, HY-Fe – hydroxylamine extractable Fe, HCl-Fe – HCl extractable Fe, GD – grain density, TC – total bacterial counts, TVCa – Viable counts under aerobic conditions, TVCan – Viable counts under anaerobic conditions.

*Data obtained from Collett et al. 2007

Suppl. Table 1: Pearson's correlation analysis using 10 parameters (n=36). Values denote correlation coefficient, r values.

Parameters	Depth	Fe(II)	HY-Fe	HCl-Fe	SO ₄ ²⁻	CH ₄	*CO ₂ pw	*GD	*Porosity	TC	TVCa	TVCan
Depth	1.000											
Fe(II)	0.051	1.000										
HY-Fe	-0.155	0.041	1.000									
HCl-Fe	0.165	0.394	0.530*	1.000								
SO ₄ ²⁻	-0.249	-0.131	0.239	0.419	1.000							
CH ₄	0.694*	-0.060	-0.280	0.069	-0.462	1.000						
+CO ₂ pw	0.131	-0.300	0.196	0.229	-0.276	0.092	1.000					
+GD	-0.471*	-0.160	0.093	-0.165	0.093	-0.302	-0.230	1.000				
+Porosity	-0.775*	0.298	0.053	0.045	0.271	-0.481*	-0.145	0.556*	1.000			
TC	-0.775*	-0.394	-0.084	-0.391	0.190	-0.491*	-0.329	0.299	0.591*	1.000		
TVCa	-0.130	-0.003	-0.132	-0.138	-0.432	-0.038	0.110	0.154	0.241	0.107	1.000	
TVCan	0.327	0.667*	-0.068	0.344	-0.082	0.204	-0.105	-0.148	-0.124	-0.509*	-0.187	1.000

Fe(II) – ferrous iron, HY-Fe – hydroxylamine extractable Fe, HCl-Fe – HCl extractable Fe, ndl – non detectable limit, pw – porewater, GD – grain density, TC – total bacterial counts, TVCa – Viable counts under aerobic conditions, TVCan – Viable counts under anaerobic conditions.

+Data obtained from Collett et al. 2007.

(Significant Bonferroni corrected p values at ≤ 0.004 are in bold and *).