

Chemosynthetic activity prevails in deep-sea sediments of the Central Indian Basin

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Abstract

It is hypothesized that in the deep-sea, under psychrophilic, barophilic and oligotrophic conditions, microbial community of Central Indian Basin (CIB) sediments could be chemosynthetic. In the dark, at near ambient temperature, $4\pm 2^\circ\text{C}$, 500atm pressure, pelagic red clay could fix carbon at rates ranging from 100-500 nmol C g^{-1} dry wt day^{-1} . These clays accumulate in the deepest and the most remote areas of the ocean and contain < 30% biogenic material. These clays with volcanic signatures fixed 230–9,401 nmol C g^{-1} dry wt day^{-1} while siliceous radiolarian oozes of the basin fixed only 5–45 nmol C g^{-1} dry wt day^{-1} . These rates are comparable to those of white smoker waters and are 1–4 orders of magnitude less than those of bacterial mats and active vents recorded at other localities worldwide. The experimental ratios of carbon fixation to metal oxidation in the sediments were 0-1 order of magnitude higher than the corresponding average theoretical ratio of 0.0215 (0.0218, 0.0222, 0.0207 and 0.0211 for Fe, Mn, Co and Ni respectively) in the siliceous ooze. In case of pelagic red clay it was 0-2 orders higher than theoretical ratio. Thus, chemosynthetic activity could be more widespread, albeit at low rates, than previously considered for abyssal basins. These environments may be dependent partially or even wholly on in situ microbial primary production for their carbon requirements rather than on photosynthetically derived detritus from surface waters.

Keywords: Chemosynthesis, metal oxidation, bacteria, bioenergetics, Central Indian Basin.

Introduction

Chemosynthetic bacteria are primary producers that use chemical energy to produce biomass. Chemolithoautotrophic bacteria oxidize reduced inorganic compounds to obtain both energy and reducing power for fixing inorganic carbon. Access to both oxygenated seawater and reduced compounds is important for chemosynthetic communities. The term *chemosynthesis* is generally used to describe chemolithoautotrophic processes at hydrothermal vents and seeps (Jannasch 1989). Chemoautotrophy to some extent is also possible without dissolved oxygen by using nitrate as electron acceptor to oxidize sulphide. Thiotrophic nitrate reduction may also be an important contributing process especially in diffuse flow regimes (Childress *et al.*, 1991) with temperatures varying between 2 to 25°C (Chevaldonne *et al.*, 1991). It is being increasingly appreciated that this activity is more widespread than commonly thought, as synthesis of organic carbon by primary producers is one of the essential functions in any ecosystem. A huge expanse of the dark deep sea waters and sediments has the potential for primary production through chemosynthesis. Autotrophic bacterial processes, other than those of cyanobacteria have been shown to be significant in oxic-anoxic interfaces (e.g. Casamayor *et al.* 2001 and references therein). In the case of primary production, dark incorporation is either subtracted from carbon incorporation in light bottles or ignored (Casamayor *et al.* 2008 and references therein). The importance of dark carbon fixation has been shown for oxic–anoxic interfaces, anoxic waters in lakes (Culver and Brunskill 1969; Jørgensen *et al.* 1979; García-Cantizano *et al.* 2005 and references therein) and seas (Tuttle and Jannasch 1977; Juniper and Brinkhurst 1986; Jørgensen *et al.* 1991). It is therefore hypothesized that the perpetually dark, deep abyssal basins with scanty amounts of organic detritus would therefore be constrained to fix carbon to various extents depending upon the accessibility of reduced inorganic substrates.

Reduced compounds may become available in an environment either via the degradation of organic matter or from magmatic/geothermal sources. Ambient oxygen, nitrate and sulphate in seawater might be consumed to varying extents depending on the amount of organic matter available for early or late diagenesis (Schulz and Zabel, 2000). These processes have long been associated with the degradation of organic matter settling from surface waters. Although well known to co-occur with nitrification, metal oxidation and even oxic respiration, the process of fixation of carbon dioxide is disproportionately biased towards the iron, sulphur and methane cycles.

The present work therefore focuses on the chemosynthetic potential of the microbial community of abyssal oxic sedimentary basin harbouring polymetallic nodules in the CIB. Microbial

autotrophic carbon fixation at the expense of reduced inorganic substrates (reduced metals Ni, Co, Mn, Fe) is evaluated in deep-sea sediments. The approach is based on the bioenergetic concepts developed by McCarty (1965, 1975), according to which microbial redox reactions can be formulated by combining three half-reactions: the electron donor reaction, the electron acceptor reaction and the cell (biomass) synthesis reaction.

Theoretical ratios of carbon fixation to metal oxidation are compared with experimental values (Hatzikioseyan and Tsezos 2006). It is hypothesized that chemosynthetic activity could be more widespread than commonly thought and that deep ocean oligotrophic sediments could have retained their chemosynthetic potential. Additionally, the ratio of carbon fixation to metal oxidation could vary among different sediments, possibly depending on their geological and geochemical origins and the type of bacterial communities.

Geological setting

The CIB has five sediment types, namely terrigenous mud, siliceous radiolarian ooze with and without nodules, pelagic red clays and carbonaceous foraminiferal ooze (Fig. 1a; Rao and Nath 1988; Nath et al. 1989). The basin is bordered by the Indian Ocean Ridge system and marked by prominent fracture zones and seamounts hosting normal Mid-Ocean Ridge Basalts (Fig. 1b; Kamesh Raju and Ramprasad 1989; Mukhopadhyay et al. 2002). The oxygen- and nutrient-rich Antarctic Bottom Water Current entering the CIB from 5°S (Gupta and Jauhari 1994) maintains oxic conditions. The pH is near-neutral with sub-oxic pockets in sediments (Nath and Mudholkar, 1989). The siliceous oozes harbour most of the polymetallic nodules both at the sediment–water interface and buried under the sediment (Sudhakar, 1989). Volcanic signatures and hydrothermal alterations are seen in some pelagic clays (Mascarenhas-Pereira et al. 2006; Nath et al. 2008). The carbonaceous oozes are available on the ridge flanks and the seamount tops that rise above the calcite compensation depth ~ 4,500 m below the sea surface.

Materials and methods

Sampling and processing

Samples of siliceous ooze, pelagic red clay with volcanic signatures, carbonaceous ooze and polymetallic nodules were collected and processed during the 4th, 17th and 26th expeditions on-board the RV *Akademik Boris Petrov* (ABP-04, 17, 26 during 2005, 2006 and 2007; Fig 1) in the CIB. Samplings were done by means of USNEL-type box cores of dimensions 50 x 50 x 50 cm. Nodules along with their associated sediments were collected using van Veen grab. Station locations

and details of experiments conducted with each set of samples are presented in table 1. The in situ temperature of CIB was generally reported to be around 2°C (Warren et al., 1982). Hence, all the experiments were conducted at near ambient temperature of 4±2°C. Preliminary analyses were conducted with one of the cores (13) at 4±2°C, 1 atm and 4±2°C, 500 atm. As there was no difference in the results obtained with both the sets, experiments were conducted at 4±2°C, 1 atm. Besides in the deep-sea, the change in bacterial activity is measurably affected by change in temperature rather than pressure (Jannasch, 1989). Metal oxidation and carbon fixation experiments on sediments of cores 26 and 36 were carried out on aliquots from 0-30 cm bsf, at 2 cms interval up to 10 cms and 5 cms interval thereafter.

Autotrophic ¹⁴C uptake by sediments and nodules

Microbial uptake of carbon was measured using NaH¹⁴CO₃ uptake [5μCi/ml activity, Board of Radiation and Isotope Technology (BRIT), Navi Mumbai, India] adopting methods described earlier (Nelson et al., 1989; Tuttle & Jannasch, 1977). Briefly, about 1g of sediment/nodule was suspended in 9 ml sterile seawater and incubated with 0.08 μCi ml⁻¹ final concentration of NaH¹⁴CO₃ for 24h in the dark. Unincorporated labelled carbon was carefully washed with sterile seawater. The filtered slurry was acidified to remove unbound ¹⁴C and trace inorganic carbon. The filter with the trapped sediment was further dried at 35°C and then suspended in vial containing scintillation cocktail. The samples were counted after 12-24 hrs in a Liquid Scintillation counter (Model Perkin Elmer, Wallac 1409 DSA). Suitable controls for unlabelled and heat killed sediments, wash water and labelled carbon were included. The incorporation of carbon was read as disintegrations per minute (integrated for 5 minutes) and was expressed as nmol C g⁻¹ day⁻¹. Siliceous ooze of core 26 and pelagic red clay of core 36 were examined for carbon fixation rates under oxic and sub-oxic conditions at 4±2°C, 1 atm, pH 7. Hyperbaric incubations were done in pressurized vessels at 4±2°C, 500 atm (Tsurumi-Seiki, Japan; Kato et al., 1995).

Oxygen consumption

Oxygen in the aqueous phase of the experimental tubes was measured according to Pai *et al.*, 1993. The difference in oxygen concentration from the final day of incubation to the first day was calculated as oxygen consumption and expressed in μM g⁻¹ day⁻¹.

Metal analysis and oxidation rates in sediments

Siliceous ooze and pelagic red clays were examined for metal oxidation rates under oxic and sub-oxic conditions using a 100 μM metal spike, at $4\pm 2^\circ\text{C}$, 1 atm. About 1.5 ± 0.5 g of sediment was inoculated into 15 ml screw-capped tubes containing 100 μM concentrations of metal chlorides or sulphates prepared in sterile seawater. The incubation was carried out under oxic and sub-oxic conditions. The oxic incubation was assured by directly inoculating wet sediment in half-filled tubes and sub-oxic in completely filled tubes. Azide (10 mM) treated sediment controls were prepared as above to correct for metal adsorption. Sterile controls without any inocula to account for abiotic precipitation were also included. Samples (1ml) at zero hour were centrifuged (8000 rpm for 10 min at 4°C) and the supernatant was acidified with 1N HCl and stored at 4°C until analysis for assessing the metal concentration at 0h. Further analysis was carried out at the shore laboratory.

After 45 day-incubation, the above centrifugation step was repeated. An aliquot of 1 ml supernatant from each tube was used for estimating the residual metal concentration by spectrophotometric method using Multiskan Thermo Spectrum. The Mn concentration in the sample was determined with 1-(2-pyridylazo)-2-naphthol method at 560 nm (Chin et al. 1992). The determination of Ni with dimethylglyoxime at 460 nm and Co with nitroso-R-salt at 500 nm was done according to the scheme of Chester and Hughes (1968). Fe was performed by sampled direct current (Aldrich & van der Berg, 1998) using a Metrohm (Switzerland) voltammeter. Mean and standard deviations were calculated for microbially and non-microbially promoted metal immobilization and were corrected for chemical precipitation in different experimental setups. The sediment slurry from each tube was rinsed and poured onto a pre-weighed filter positioned in a filtration setup at the end of analysis. The filter with sediment were dried at 105°C and reweighed until constant. The filter weight was subtracted from the sediment weight to derive the actual dry weight of sediment. The residual metal concentration in the experimental tubes for the whole slurry was determined by spectrophotometric method and the concentrations were corrected for corresponding controls. The values were later normalized per gram of sediment to derive the actual metal content in the sediment (cf. Flemming and Delafontaine, 2000, for explanations on content and concentration).

Ratio of carbon fixation to metal oxidation

Cell synthesis half-reactions and bioenergetic concepts developed by McCarty were applied to calculate theoretical stoichiometric ratios of carbon fixation to metal oxidation by Hatzikioseyan and Tsezos, 2006. These theoretical ratios available for 1atm, 40°C were compared to

the present experimental values. In this study, calculations were done on the basis of net microbial oxidation of four metals Fe, Mn, Ni, Co contributing to microbial carbon fixation. Only the metals showing oxidation are considered for the calculation of ratio of carbon fixation to metal oxidation.

Biomass yield on Fe, Mn, Co. and Ni

Yield was calculated as increase in cell biomass per gram of metal oxidized and expressed as $\mu\text{gC g}^{-1}$. The total counts of bacteria of two consecutive samplings were used for the yield calculations.

Total Organic Carbon (TOC) and C/N ratio

Total carbon and nitrogen was measured by NCS 2500 Elemental Analyser (Patience et al., 1990) using L-Cistina (Therma Quest Italia SpA) as standard. Total carbon was counter-checked with UIC CM 5014 coulometer and found similar in range. Total inorganic carbon was analyzed by UIC CM 5014 coulometer using CaCO_3 (Merck, Germany) as standard. The accuracy of measurements was verified by analysis of a standard reference material (USGS-MAG-1). Total organic carbon was determined by subtracting total inorganic carbon from total carbon. The C/N was calculated as the ratio between total organic carbon and total nitrogen.

Labile organic matter (LOM)

Total proteins in sediments were estimated by Lowry's Folin Ciocalteu method using bovine serum albumin as standard (Lowry et al., 1951). Total carbohydrates in sediments were estimated by phenol-sulfuric acid method using glucose as standard (Kochert, 1978). Total lipids in sediments were estimated by Bligh and Dyer method using stearic acid as standard (Bligh & Dyer, 1959). The sum of total proteins, carbohydrates and lipids was expressed as LOM (Danovaro et al., 1993).

Total counts of bacteria

Total bacterial cells were counted according to Hobbie et al., 1977. About 1 g of sediment was diluted with 9 ml of sterile seawater; 3ml of this slurry was fixed with buffered formalin at an end concentration of 2% and stored at 5°C until analysis. At the on-shore laboratory the aliquot was sonicated at 15 hertz for 15 seconds. The supernatant (1ml) was stained with 75 μl of 0.01% acridine orange (3mins, in dark) and filtered on 0.22 μm black polycarbonate filter paper (Millipore, USA). This procedure minimized masking by sedimentary particles. About 10-15 microscopic fields were counted to include a total of 300-600 cells per sample using Nikon 80i epifluorescence microscope. The counts were normalized per gram dry sediment.

Results

Carbon fixation

In the study area, the highest carbon uptake rates were recorded for southern carbonaceous oozes, and the lowest for siliceous oozes in the north of CIB (Table 1). Polymetallic nodules show carbon uptake rates intermediate between those of siliceous oozes and pelagic red clays with volcanic alterations. Carbon fixation in sediments below the Calcite Compensation Depth, (siliceous ooze and pelagic red clay) generally increases with the decrease in TOC and LOM (Tables 1 and 2). Values vary from 100-500 nmol g⁻¹ dry wt day⁻¹ without showing any statistically significant difference between the results at 4±2°C, 1 atm and 4±2°C, 500 atm. The difference could not be discerned in the time frame used for the experiment (Table 1).

C/N ratios, TOC, TIC, LOM and bacterial counts

In the CIB sediments, elemental C/N ratios varied from 0.7 to infinitely large values due to very low levels of total nitrogen. TOC varied from <0.05-1.54%, TIC varied from non detectable in most of the deep-sea sediment to 10% in carbonaceous oozes. LOM varied from 0.025-0.14%. Bacterial densities ranged from 10⁶-10⁹ cells.g⁻¹ dry sediment (Table 2).

pH and Eh

The initial pH was 7.8± 0.2 in the experimental tubes. At the end of incubation the pH varied from 6.58 to 6.91 in the oxic tubes and 5.54 to 6.6 in the sub-oxic tubes of BC 26. The pH varied from 6.97 to 7 in oxic tubes and from 6.8 to 7.06 in the sub-oxic tubes of core BC 36 (Fig 2).

The Eh is a measure of oxidizing and reducing conditions. At the initial stage BC 26 is slightly more reducing than BC 36. However, at the end of the incubation the situation reverses.

The initial Eh in the experimental tubes ranged from +75.81 to +79.96 mV in the incubations for BC26. At the end incubation, the Eh ranged from -66 to -144 in the oxic tubes and from -87.4 to -128.7 in the sub-oxic tubes of BC 26.

The initial Eh in the experimental tubes ranged from +77.32 to +80.2 mV for BC36. In the oxic tubes of BC 36 the Eh varied from -252.2 to -304.4 while in sub-oxic tubes the Eh varied from -243 to 331.8 (Fig 2).

Oxygen consumption

Oxygen consumption in the oxic tubes of BC 26 varied from non-detectable to 5.43 μM g⁻¹ day⁻¹. In the sub-oxic tubes of BC 26, the consumption varied from non-detectable to 15.63 μM g⁻¹ day⁻¹. A mid depth maximum was noted in the sub-oxic condition in core BC 26. In BC 36 oxic

tubes, the oxygen consumption was below detection level. In BC 36 sub-oxic tubes the consumption varied from non-detectable to $0.64 \mu\text{M g}^{-1} \text{ day}^{-1}$ (Fig 3).

Carbon fixation

The carbon fixation in the southern pelagic clay with volcanic signatures is 1-3 orders higher than the northern siliceous ooze (Table 1; Fig 4). Carbon fixation in core 26 varies from 6.96×10^{-7} to $4.59 \times 10^{-6} \text{ g CO}_2 \text{ g}^{-1} \text{ dry sediment}$ (15.82 - $104.22 \text{ nmol g}^{-1} \text{ day}^{-1}$). In core 36 it varies from 1.97×10^{-5} to $4.14 \times 10^{-4} \text{ g CO}_2 \text{ g}^{-1} \text{ dry sediment}$ (447.92 - $9400.62 \text{ nmol g}^{-1} \text{ day}^{-1}$).

Ratio of carbon fixation to metal oxidation

The change in level of metal in the aqueous phase has been measured to infer either oxidation or reduction. The fall in level of metal could be due to removal of the metal from aqueous phase for precipitation/ oxidation/ immobilization in the biotic tubes. Likewise, the rise in level of the metal in aqueous phase could be due to release of metal for dissolution/ reduction / mobilization in these tubes.

The highest metal oxidation/ precipitation/ immobilization are shown by reduced Fe in both oxic and sub-oxic tubes of core 26. This is denoted by lowest Fe concentration in the aqueous phase. The highest metal dissolution/ reduction / mobilization are shown by Mn in the sub-oxic tubes of core 26 at the deeper layers of the sediment column (Fig 5a and b). In case of core 36 the highest metal oxidation is shown by reduced Mn under both oxic and sub-oxic conditions (Fig 5c and d). Oxidation to some extent is also shown by Co in core 36 under oxic condition (Fig 5c). Ni shows only dissolution and no oxidation in both the cores under both oxic and sub-oxic conditions. Dissolution of metals is prominent in the TOC-richer core 26, while oxidation is greater in the TOC-poor core 36.

The experimental ratio of carbon fixed to metal oxidised in CIB sediments varied from 0.0216 ± 0.0116 in the siliceous core 26 and $0.1292 \pm 0.1457 \text{ g C fixed g}^{-1}$ in the pelagic red clay with volcanic signatures at $4 \pm 2^\circ\text{C}$ under oxic condition. Uncertainty in down-core variation was ± 0.227 in core 26 and ± 0.2857 in core 36 under oxic condition. Under sub-oxic condition the experimental ratio of carbon fixed to metal oxidised was 0.0521 ± 0.0532 in the siliceous core 26 and $0.6392 \pm 0.7776 \text{ g C fixed g}^{-1}$ in the pelagic red clay 36 (Fig 6). Uncertainty in down-core variation was ± 0.1044 in core 26 and ± 1.5241 in core 36 under sub-oxic condition.

Biomass yield on Fe, Mn, Ni, Co

In the organically-richer core 26, the biomass yield was entirely contributed by Fe oxidation. Yield varied from 10-100 $\mu\text{gC g}^{-1}$ metal oxidized under oxic condition. Under sub-oxic condition yield varied from 10-1000 $\mu\text{gC g}^{-1}$ metal oxidized. In core 36 the biomass yield was contributed by Fe and Mn in both oxic and sub-oxic condition and by cobalt under oxic condition. Ni did not show any significant contribution both under oxic and sub-oxic conditions (Fig 7).

Discussion

Both generalists and specialists could be involved in the chemosynthetic processes. While specialists could be high in their activity and restricted to unique niches like black smokers, generalists could be more widespread with lower ability to fix CO_2 . Many bacteria possess the RuBisCO enzyme for carbon fixation using the Calvin Benson Cycle. Yet others like green sulphur bacteria use the rTCA cycle. The methanotrophs are known to use the Serine and RuMP Pathways (Karl, 1995).

Chemosynthesis may be exhibited by generalists like facultative autotrophs or mixotrophs and specialists like strictly autotrophic bacteria. Strict autotrophs could be any of the following: 1. nitrifiers (e.g., *Nitrococcus*, *Nitrosomonas*), 2. sulphide oxidizers (e.g. photosynthetic *Chromatium* and chemosynthetic *Thiobacillus thiooxidans*), 3. metal oxidizers (e.g. *Thiobacillus ferrooxidans*), and 4. methane oxidizers (e.g. *Methylomonas*). Facultative autotrophs like *Pseudomonas* and *Alcaligenes* may also exhibit chemoautotrophy or mixotrophy (Karl, 1995, Loka Bharathi 1989, Loka Bharathi et al., 1994). Methanotrophs could be mixotrophic or a combination of both autotroph and heterotroph. However in deep-sea oligotrophic systems where organic matter is lean these bacteria may be constrained towards autotrophy.

General bacteria can thus switch to chemosynthetic behaviour and not necessarily be specialists of the Hydrothermal Vents. This process can be triggered under various situations. In oligotrophic environments, chemosynthesis sustains microbial life at the expense of diverse electron donors available at redox fronts of fractures, seamounts, nodules and crusts (Bach and Edwards, 2003, Edwards et al., 2004, Wenxuan et al., 2002).

The chemosynthetic CO_2 fixed/metal oxidised ratio in siliceous ooze under both oxic and sub-oxic experimental conditions (Fig 6) are 0-2 orders of magnitude higher than the corresponding theoretical stoichiometric ratios calculated at 40°C at pH 1.5 by Hatzikioseyan and Tsezos (2006). Interestingly, the experimental ratios obtained in the present study are much higher than theoretical

values given by these authors. This difference could be attributed to better chemosynthetic ability that is promoted under deep-sea environmental conditions like higher dissolution of CO₂ at lower temperatures. Although the pH is different from that of seawater, these values are used due to paucity of theoretical values in literature.

In the north, the profile shows increased carbon fixation in the deeper layers. This is attributed to the downward diffusion of metals by bacterial reduction to sub-surface (15-25cm bsf). In the south, the carbon fixation shows two peaks one at 0-10 cm bsf and the other at the deeper 25-35 cm bsf. This could perhaps be attributed to a small amount of reduced metal released from the organic rain from the euphotic layers above and a greater availability of reduced metal from abiogenic sources below which move upward by diffusion from the deep-biosphere.

Comparison of carbon fixation rates of CIB sediments with those recorded in other chemosynthetic settings worldwide show that CIB values are 1–2 orders of magnitude smaller than for bacterial mats, anoxic fjords, waters near hydrothermal vents, and also solar salterns, and up to 3 orders of magnitude smaller than for other active vents like the Rose Garden. However, they are comparable to waters of white smokers of 21°N East Pacific Rise. Carbon fixation rates of carbonaceous oozes are comparable to those of bacterial mats and solar salterns (Table 1). The elevation of chemosynthetic potential in the CIB pelagic clays can be explained by depletion of organic matter, both TOC and LOM and possible increase in inorganic substrates due to hydrothermal alterations (Nath et al. 2008; Das et al., 2010).

The present results suggest that even at cold temperature, microbial metal oxidation and the resultant elevated microbial immobilization of metals could be responsible for the high metal contents in bulk sediments reported by Pattan and Jauhari, (2001). Such conditions could be accompanied by microbial CO₂ fixation. Metal toxicity could probably play a role in explaining some of these patterns. It is suggested that higher CO₂ fixation in the presence of Co could be partly attributed to Co toxicity. Carbon fixation at the cost of metal oxidation could be both for obligate fixation of carbon or to mitigate/modulate the toxic effect of some metals. This is achieved by oxidizing/precipitating the excess metal. Our recent study on Co immobilization supports the above mechanism to counteract metal toxicity in Mn oxidizing bacteria (Antony *et al.*, 2010). Microbes from similar environments have been shown to precipitate metal salts in different forms under the natural oligotrophic condition (Sujith et al., 2010).

In warm seeps high carbon fixation is accompanied by high biomass (Karl, 1995). Carbon fixation is also known in cold seeps from continental shelves and margins. For example, Louisiana Shelf of Gulf of Mexico (Dattagupta et al., 2006, 2007), California Bay (Levin and Michener, 2002) and Nambian shelf (Schulz and Schulz, 2005). Eastern boundary upwelling regions like west coast of India are also known for preservation of organic matter produced by photosynthetic primary production (Rao et al., 2003, Paropkari et al., 1992). Anoxic sapropels and hypersaline realms (Sorokin 1972, Jørgensen et al., 1979) are all situated on the continental shelves and margins. While sediment organic matter might reflect the surface photosynthetic primary productivity, preservation of this organic matter could promote chemosynthesis for modulating excess pools of electron donors and acceptors. Chemosynthesis can occur either under organically depleted or replete conditions. Under organically depleted conditions with high amount of electron donors like in hydrothermal vents and deep-sea volcanic environments, microbes are facilitated towards chemosynthetic mode of growth. They fix the carbon dioxide in the sea water or those emanating from vents by oxidizing electron donors like reduced metals in the plumes. Under organic rich conditions like cold seeps, chemosynthesis could be a bacterial response to counter toxicity from excess electron donors like H_2S / NH_3 .

Chemosynthesis is also observed on active vents of 21°N East Pacific Rise (Wirsen et al. 1986), Juan de Fuca (Chase et al. 1985) and Mid-Atlantic Ridge (Wirsen et al. 1993). These sites are all above the Calcite Compensation Depth (CCD) implying that high particulate inorganic carbon is available.

The chemosynthesis in warm vents is mainly driven by the rich supply of electron donors which could compensate for the poor dissolution of electron acceptor like CO_2 . In contrast, the process in cold seeps could be supported by an excess availability of both electron donors and acceptors.

Chemosynthetic carbon fixation is carried out by a number of pathways- Calvin Benson Bassham cycle, reductive acetyl-CoA, pathway, 3-hydroxypropionate (3-HP) bicycle, 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB), dicarboxylate/4-hydroxybutyrate (DC/4-HB) cycles and the reductive tricarboxylic acid (rTCA) cycle (Karl, 1995). For replenishment of the TCA or Krebs cycle, the intermediates are regenerated by anaplerotic reactions. These reactions are generally achieved through the insertion of either one carbon fragment in the form of CO_2 or two carbon fragments in the form of acetyl CoA into the appropriate metabolic pathway. There are number of microorganisms that make an appreciable fixation of CO_2 by this pathway. In the present study, though it is not clear which pathway is predominantly used, it is probable that a substantial

amount of CO₂ can be fixed through this means. *Roseobacter denitrificans* uses the anaplerotic pathways mainly via the malic enzyme to fix 10–15% of protein carbon from CO₂ (Tang et al., 2009). It is probable that some bacterial communities use this mode of CO₂ fixation when the ambient concentration of utilizable substrate is low.

In the CIB it is possible that multiple carbon fixation pathways might occur. One of the cores in the southern CIB where the community is dominated by *Erythrobacteria*, multiple carbon fixation pathways including the anaplerotic rTCA cycle could be prevalent (Das and LokaBharathi, Chemosynthesis and Aerobic Anoxygenic Phototrophic Bacteria in Central Indian Basin, under review).

It is suggested that chemosynthesis in oligotrophic deep-sea environments of the present study could be facilitated by higher availability of dissolved CO₂ due to higher pressures prevailing below CCD. Besides, there could be a moderate supply of electron donors in the form of dissolved metals. In most natural environments there could be overlaps of the above factors in varying degrees.

CO₂ is not limiting in the deep-sea environment, however, due to severe depletion of organic carbon, the microbes are constrained/facilitated to fix CO₂ at the expense of oxidation of reduced metals. The deep-sea environment can have a plethora of electron donors like ammonia and sulphide besides reduced metals, as in hydrothermal vents. However, the concentrations could be considerably lower. The CIB is a source of both oxidized and reduced metals. The oxidized metals are mostly in the form of polymetallic nodules while reduced metals occur in the pore-water of sediments.

Studies on chemosynthesis from the abyssal depths like CIB can thus provide a valuable baseline or a clear background of microbial chemosynthesis. Here, organic matter is low but dissolved inorganic carbon and metal content could be high. Diffuse hydrothermal fluid-flow and alterations predominate and persuade microbes towards chemosynthetic mode of metabolism.

The present results show that the values for carbon fixation vary from 5-1000 nmol g⁻¹day⁻¹. Biomass yield of 10-1000 μg C g⁻¹metal oxidized have been measured. Under cold oxic near neutral conditions, with high concentration of metals and very low organic matter, the yield is low with very high conversion of reduced metal to oxidised form. In contrast, warm and anoxic conditions with high sulphide concentrations and higher organic matter promote increase in biomass [Karl, 1995; Hatzikioseyan and Tsezos, 2006]. It is speculated that there are number of rates operating between these two extremes. It can therefore be suggested that chemosynthesis is widespread in this

environment with the southern locations contributing more than the north. These environments may be more dependent partially or even wholly on in situ microbial primary production for their carbon requirements, rather than on photosynthetically derived detritus from surface waters (Ehrlich 1998). Either way the processes contribute to immobilization of metals.

Microbial immobilization of metals can be either catalytic or non-catalytic; it proceeds through four different mechanisms namely biosorption, bioaccumulation, redox reaction and complex formation. Immobilization may be through cellular sequestration and accumulation, or through extra-cellular precipitation (Sujith *et al*; Immobilization of manganese, cobalt and nickel by deep-sea-sediment-microbial communities; under review).

The contribution of chemoautotrophic activity in deep-abyssal sediments is a few orders less than that of active hydrothermal vents in terms of carbon fixation per unit area per unit time. However, the vast extent of oligotrophic abyssal basins would make its contribution far from negligible. The frame-work of the present study assumes that microbial oxidation of four metals Fe, Mn, Ni, Co contributes to the microbial carbon fixation under psychrophilic and piezophilic conditions. The present experiments throw light on how differences in organic matter concentrations, temperature and relative variations in metal concentrations in geochemically contrasting sediments could induce chemosynthetic activity to varying degrees. The contribution of other major processes like nitrification, iron and sulphide oxidation could give a more holistic picture.

Like photosynthesis of the euphotic world, chemosynthesis of the aphotic zone could have its own implications. Surprisingly, in the present study, the difference in these rates between normal atmosphere and 500 bars are not significantly different. It is well known that the dissolution of CO₂ is much higher at elevated pressures. In this study, excess CO₂ was not supplied to the pressurized microcosm. Perhaps varying the amount of dissolved carbon dioxide in the pressurized microcosm would change the results marginally. However, it is often seen that although the amount of carbon dioxide dissolved is significantly different under hyperbaric conditions, the change in microbial rate of carbon fixation is measurably affected by change in temperature rather than pressure (Jannasch, 1989). Hence our experiments conducted at 4°C, 1atm and 4°C, 500atm did not show much difference.

It is therefore hypothesised that in the deep sea sediments of CIB higher ratio of CO₂ fixed / metal oxidized could be either due to mixotrophy or higher efficiency of this process under hyperbaric condition in the cold. Chemosynthesis is an obligate and ancient microbial process. Many microbes seem to retain this ability. They tend to express this activity either under extremely

eutrophic or oligotrophic conditions for different reasons. This process helps them to counteract either excess electron donor/acceptor or help survive under nutrient starved conditions. The present study on microbial carbon fixation and metal oxidation might find useful application in deep-sea metal mining and carbon dioxide sequestration.

Acknowledgements

The authors acknowledge the Director, NIO for encouragement and constant support. Thanks are extended to the project leaders of “Environmental studies for nodule mining in Central Indian Basin (PMN-EIA)” and PMN-Survey, and the onboard teams of the ABP-04, 17 and 26 cruises for facilities and samples. The Ministry of Earth Sciences (MoES), New Delhi, India is acknowledged for financial support, and the CNS and Particle Flux laboratories, Geological Oceanography, NIO for analytical support. AD and SPP acknowledge the CSIR, New Delhi for research grants. An abridged version of these results was presented as poster in the 4th Int Symp Chemosynthesis-based Ecosystems, 2009, Okinawa, Japan, with financial aid from Chemosynthetic Ecosystem Science (ChEss). The manuscript is NIO contribution no. XXXX.

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Legend to tables

Table 1. Comparison of carbon fixation rates of the Central Indian Basin from the present work and other chemosynthetic sites extracted from Karl 1995, and Mandernack and Tebo 1999.

Table 2. Total organic and inorganic carbon contents (TOC, TIC), elemental carbon/nitrogen ratios, bacterial counts and labile organic matter contents (LOM) of sediments in the Central Indian Basin.

Legend to figures

Fig 1 Site Location

- a. Central Indian Basin and its sediment types
- b. Station locations vis-à-vis geological features (adapted from Mascarenhas-Pereira et al. 2006).
- c. Schematic representation of station on seamount in the pelagic clay realm.

Fig 2 pH and Eh profiles at the end of the incubation experiment

Fig 3 Bacterial oxygen consumption rates in cores 26 and 36.

Fig 4 Carbon fixation by sediments in cores 26 and 36.

Fig 5 Metal concentration in aqueous phase of the slurry set ups. Higher metal concentrations indicate dissolution or reduction while lower concentration indicates oxidation or precipitation.

Fig 6 Experimental ratios of carbon fixed to metal oxidized compared to theoretical value.

Fig 7 Average potential yield in terms of carbon fixed by different metals. Calculations assume that single metal fixes the whole amount of carbon fixed.

- a. Core 26
- b. Core 36

Table 1. Comparison of carbon fixation rates of the Central Indian Basin from the present work and other chemosynthetic sites extracted from Karl 1995, and Mandernack and Tebo 1999.

Environment	Location	Type	¹⁴C incorporation^a	Conditions	Reference
Hydrothermal vents	21°N, EPR*	Water, white smoker	13	1 atm, dark, 3°C, 24 h	Wirsen et al. (1986)
	Juan de Fuca	Water	202	1 atm, dark	Chase et al. (1985)
	Guaymas Basin	Bacterial mats	12,000	1 atm, dark, 8°C	Nelson et al. (1989)
	Mid-Atlantic Ridge				
	“TAG” site	Water	54–183	in situ	Wirsen et al. (1993)
Anoxic basins	Galapagos, Rose Garden	Water	1,500–5,000	in situ	Mandernack and Tebo (1999)
	Black Sea	Water	2–833	in situ	Sorokin (1972)
	Framvaren Fjord	Water	5,800–11,200	in situ	Mandernack and Tebo (1999)
Oxic basins	Solar Lake, Sinai	Water	12,000–22,000	in situ	Jørgensen et al. (1979)
	Central Indian Basin	Siliceous ooze**	5–45	1 atm, dark, 5°C	Present work
		Red clay with volcanic signature***	230–9,401	1 atm, dark, 5°C	
		Red clay with va ⁺	100–500	1 atm, dark, 5°C	
		Red clay with va ⁺	100–500	500 atm, dark, 5°C	
		Carbonaceous ooze ⁺⁺	1,000–15,000	1 atm, dark, 5°C	
Manganese nodules ⁺⁺⁺		58–448	1 atm, dark, 5°C		

^a¹⁴C incorporation units are nmol l⁻¹ day⁻¹ CO₂ for water samples and nmol g⁻¹ dry wt day⁻¹ for sediments and bacterial mats. Rates are normalized to bacterial numbers

*East Pacific Rise **Box-core 26 *** Box-cores 08 and 36 + Box-core 13 ++ Box-core 37 +++ (BN- 142, SN-147)

⁺volcanic ash

Table 2. Total organic and inorganic carbon contents (TOC, TIC), elemental carbon/nitrogen ratios, bacterial counts and labile organic matter contents (LOM) of sediments in the Central Indian Basin.

Sample type	Water depth	TOC	TIC	C/N	Bacterial counts	LOM
	(mbss) ^a	(%)	(%)		(cells g ⁻¹)	(mg g ⁻¹)
Siliceous ooze 26	5,325	0.25±0.09	nd–trace	8±5	10 ⁷ –10 ⁸	0.035–0.135
Red clay 08	5,210	0–0.70	nd	0.7–α	10 ⁶ –10 ⁹	0.045–0.550
Red clay 36	4,894	0.05–0.40	nd	2–31	10 ⁶ –10 ⁸	0.035–0.080
Carbonaceous ooze 37	3,992	1.02±0.52	9–10	60–1,525	10 ⁶ –10 ⁹	0.025–0.085
Polymetallic surface nodule SN-147						
(at sediment–water interface)	~5,100	0.16±0.14	nd	10–600	10 ⁸	0.040–0.140
Polymetallic buried nodule BN- 142						
(buried under sediment)	~5,100	0.09±0.00	nd	479–α	10 ⁸	0.04–0.07

^ambss, meters below sea surface; α, infinitely large C/N ratio due to nitrogen below detection level; nd, not detected

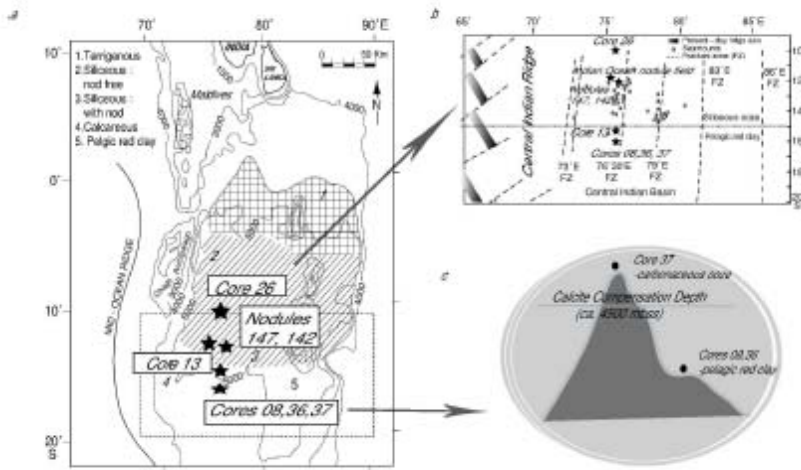


Fig. 1. Das et al. 2010.

Site Location

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247x190mm (600 x 600 DPI)

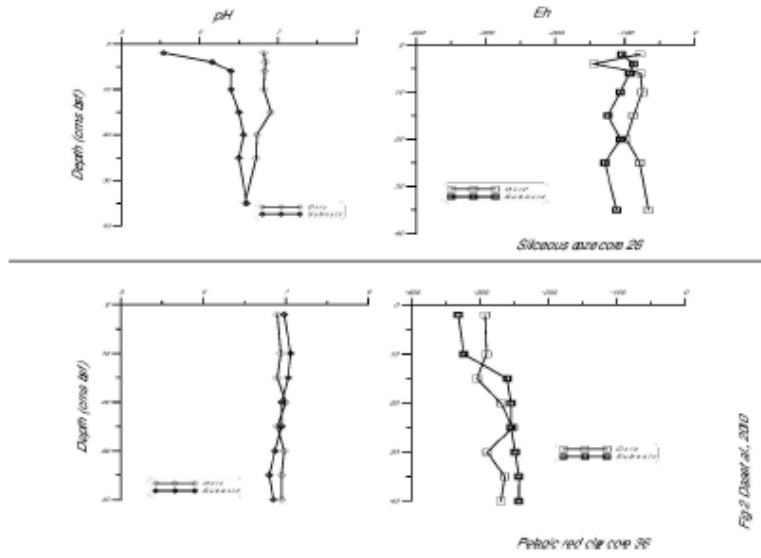


Fig. 2. Cassel et al., 2010

pH and Eh profiles at the end of the incubation experiment
279x201mm (600 x 600 DPI)

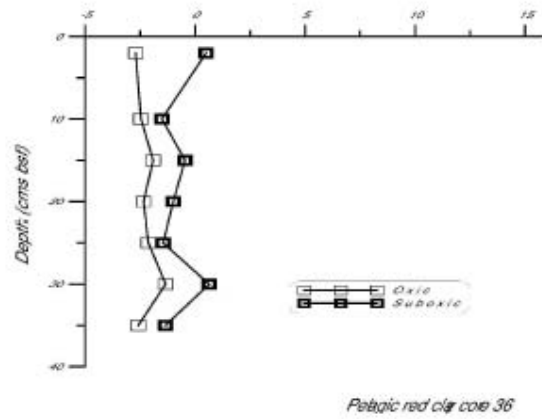
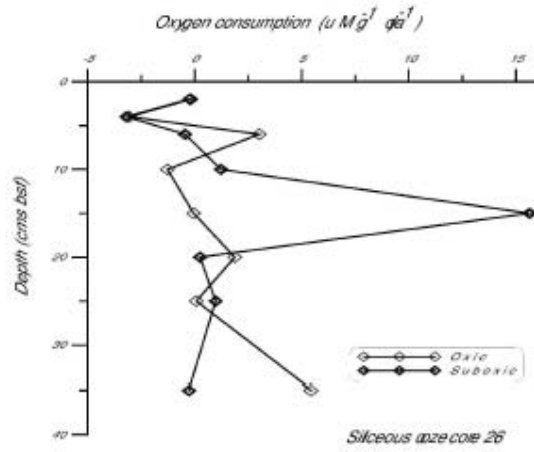


Fig. 3. Duer et al., 2010.

Bacterial oxygen consumption rates in cores 26 and 36.
215x266mm (600 x 600 DPI)

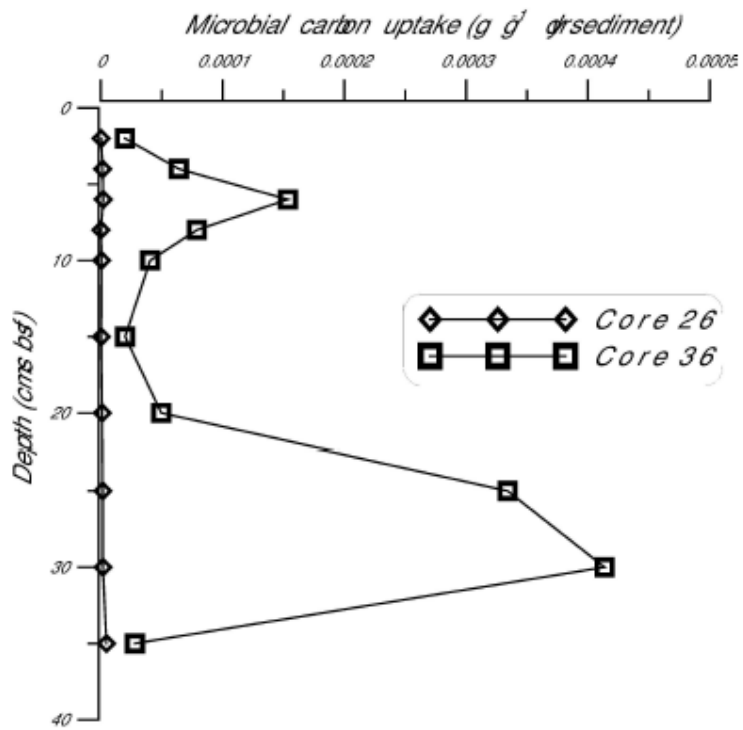


Fig 4 Daset *et al.*, 2010

Carbon fixation by sediments in cores 26 and 36.
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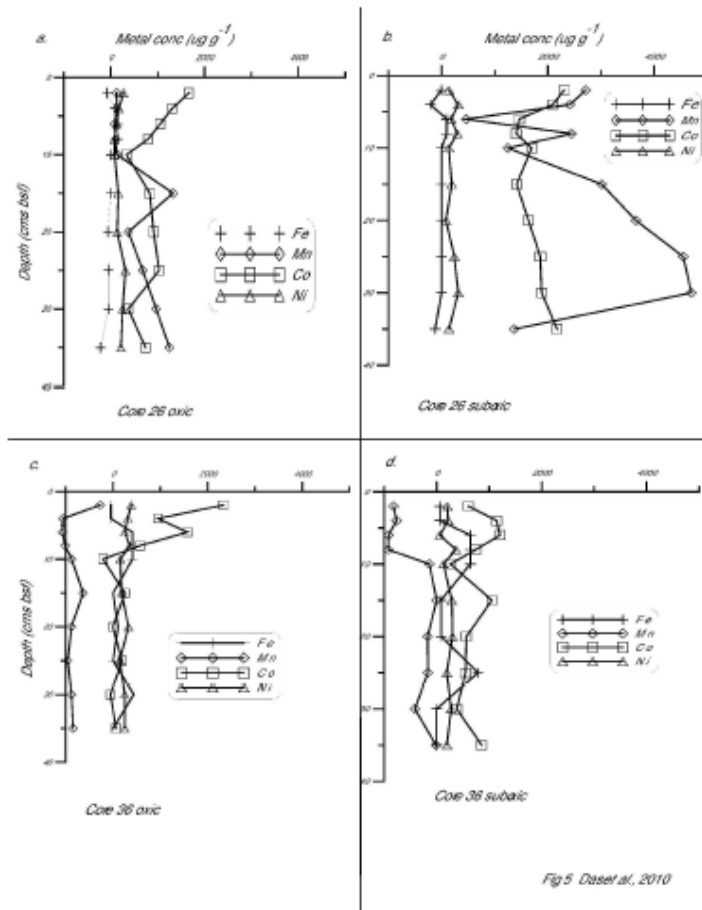


Fig 5. Daser et al., 2010

Metal concentration in aqueous phase of the slurry set ups. Higher metal concentrations indicate dissolution or reduction while lower concentration indicates oxidation or precipitation.
 215x279mm (600 x 600 DPI)

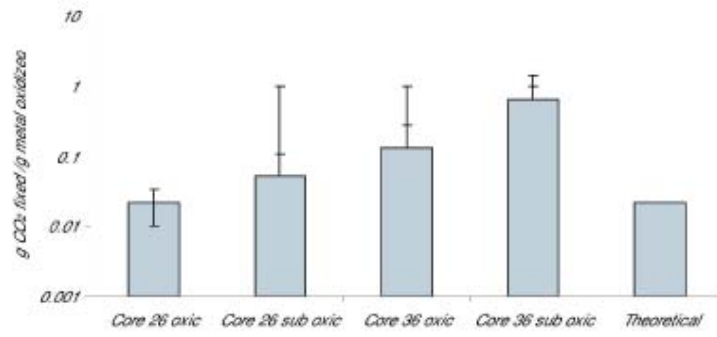


Fig 6 Dasari et al., 2010

Experimental ratios of carbon fixed to metal oxidized compared to theoretical value
218x149mm (600 x 600 DPI)

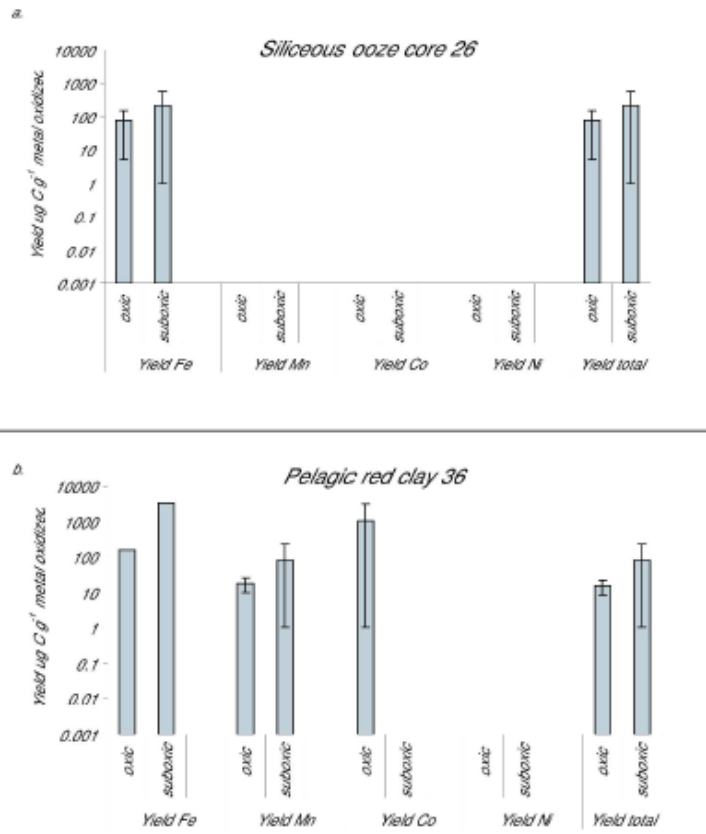


Fig 7 Das et al., 2010

Average potential yield in terms of carbon fixed by different metals. Calculations assume that single metal fixes the whole amount of carbon fixed.

a. Core 26

b. Core 36

215x264mm (600 x 600 DPI)