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ORIGINAL PAPER

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 ± 2°C, 500 atm pressure, pelagic red clay could fix carbon at rates ranging from 100 to 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.

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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 and 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 (Hatzikioseyian 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 calcareous foraminiferal ooze (Fig. 1a; Rao and Nath 1988; Nath and Mudholkar 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

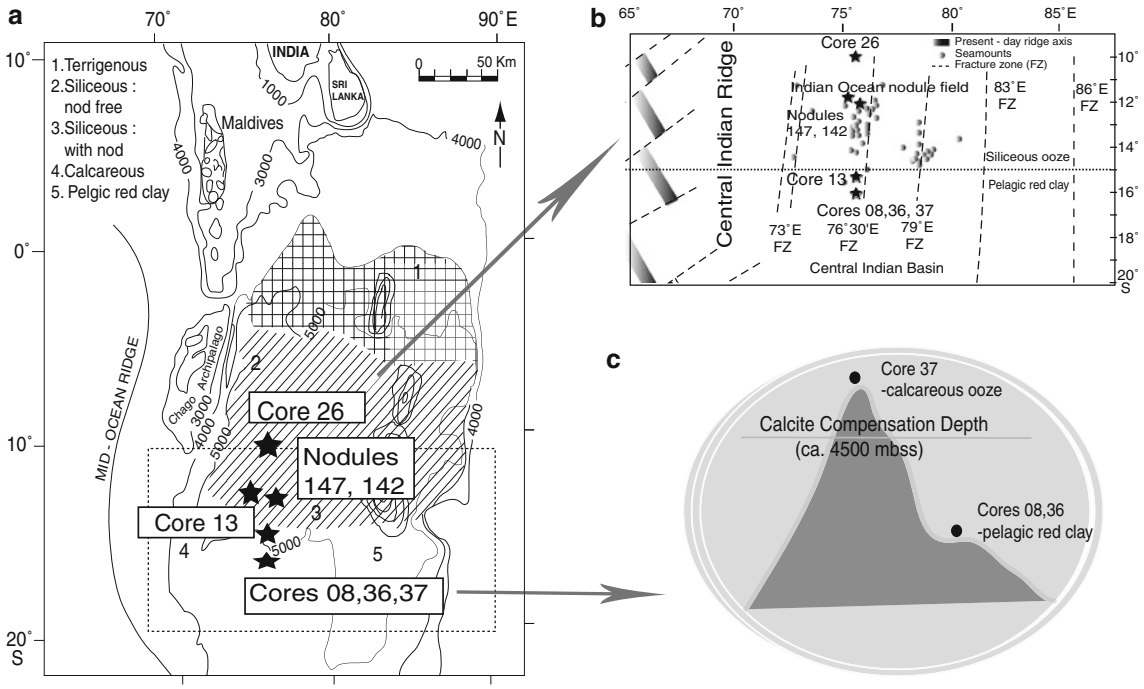

Fig. 1 Site location. **a** Central Indian Basin and its sediment types. **b** Station locations vis-à-vis geological features (adapted from Mascarenhas-Pereira et al. 2006). **c** Schematic representation of station on seamount in the pelagic clay realm

harbour most of the polymetallic nodules both at the sediment–water interface and buried under the sediment (Sudhakar 1989). Hydrothermal alterations are seen in some pelagic clays (Mascarenhas-Pereira et al. 2006; Nath et al. 2008). The calcareous 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 hydrovolcanic signatures, calcareous 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 × 50 × 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 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 to 30 cm bsf, at 2-cm interval up to 10 and 5-cm interval thereafter.

Autotrophic ^{14}C uptake by sediments and nodules

Microbial uptake of carbon was measured using $NaH^{14}CO_3$ uptake [5 μCi/ml activity, Board of Radiation and Isotope Technology (BRIT), Navi Mumbai, India] adopting methods described earlier (Tuttle and Jannasch 1977; Nelson et al. 1989). Briefly, about 1 g of sediment/nodule was suspended in 9 ml sterile seawater and incubated with 0.08 μCi ml^{-1} final concentration of $NaH^{14}CO_3$ for 24 h in the dark. Unincorporated labelled carbon was carefully washed with sterile seawater. The filtered slurry was acidified to remove unbound ^{14}C 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 h in a Liquid Scintillation counter (Model Perkin Elmer, Wallac

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	^{14}C incorporation[a]	Conditions	Reference
Hydrothermal vents	21°N, EPR[b]	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)
	Galapagos, Rose Garden	Water	1,500–5,000	In situ	Mandernack and Tebo (1999)
Anoxic basins	Black Sea	Water	2–833	In situ	Sorokin (1972)
	Framvaren Fjord	Water	5,800–11,200	In situ	Mandernack and Tebo (1999)
	Solar Lake, Sinai	Water	12,000–22,000	In situ	Jørgensen et al. (1979)
Oxic basins	Central Indian Basin	Siliceous ooze[c]	5–45	1 atm, dark, 5°C	Present work
		Red clay with Volcanic signature[d]	230–9,401	1 atm, dark, 5°C	
		Red clay with va[e, h]	100–500	1 atm, dark, 5°C	
		Red clay with va[e, h]	100–500	500 atm, dark, 5°C	
		Calcareous ooze[f]	1,000–15,000	1 atm, dark, 5°C	
		Manganese nodules[g]	58–448	1 atm, dark, 5°C	

[a] ^{14}C incorporation units are nmol l^{-1} day^{-1} CO_2 for water samples and nmol g^{-1} dry wt day^{-1} for sediments and bacterial mats. Rates are normalized to bacterial numbers; [b] East Pacific Rise; [c] Box-core 26; [d] Box-cores 08 and 36; [e] Box-core 13; [f] Box-core 37; [g] BN-142, SN-147; [h] Volcanic ash

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 min) 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 ± 2°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-treated (10 mM) 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 (1 ml) at zero hour were centrifuged (8,000 rpm for 10 min at 4°C) and the supernatant was acidified with 1 N HCl and stored at 4°C until analysis for assessing the metal concentration at 0 h. 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 and 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 was 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 Hatzikioseyian and Tsezos (2006). These theoretical ratios available for 1 atm, 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 and 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 μgC 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 analysed 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–sulphuric 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 and 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; 3 ml 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 Hz for 15 s. The supernatant (1 ml) was stained with 75 µl of 0.01% acridine orange (3 min, 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 calcareous 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, 2). Values vary from 100 to 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 to 1.54%, TIC varied from non-detectable in most of the deep-sea sediment to 10% in calcareous oozes. LOM varied from 0.025 to 0.14%. Bacterial densities ranged from 10^6 to 10^9 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 BC 26. 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 BC 36. 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).

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 (mbss)	TOC (%)	TIC (%)	C/N	Bacterial counts (cells g^{-1})	LOM (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
Calcareous 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

mbss metres below sea surface, α infinitely large C/N ratio due to nitrogen below detection level, *nd* not detected

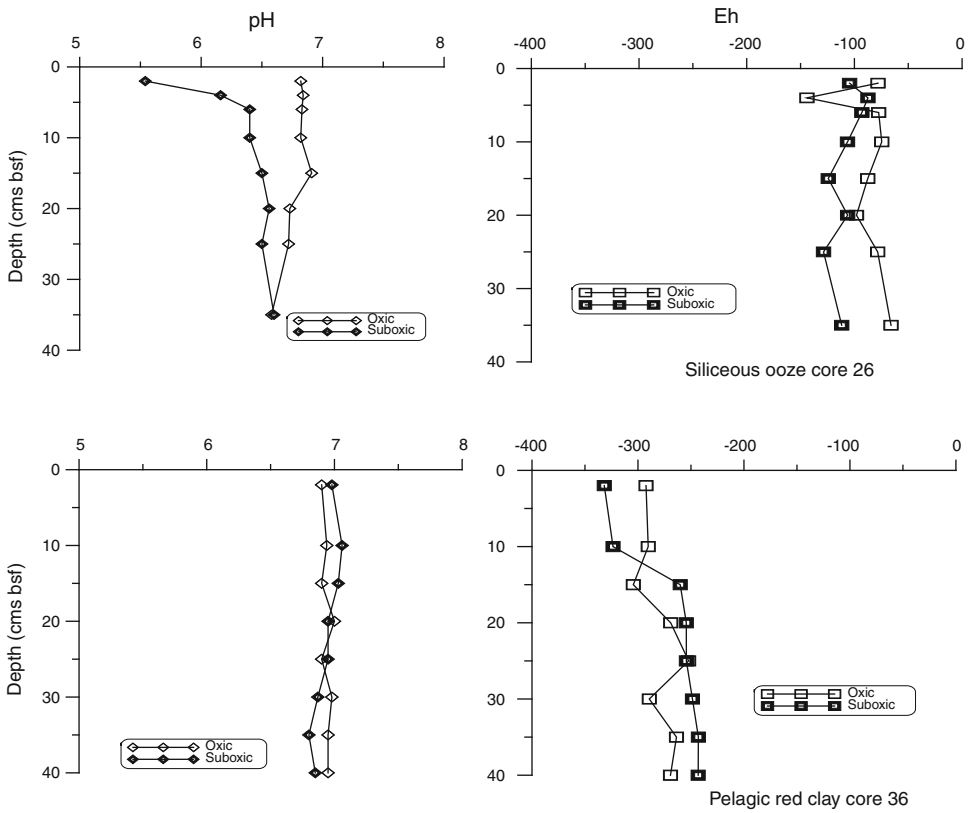

Fig. 2 pH and Eh profiles at the end of the incubation experiment

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 μM g^{-1} 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 4.59×10^{-6} g CO_2 g^{-1} dry sediment (15.82–104.22 nmol $g^{-1}day^{-1}$). In core 36 it varies from 1.97×10^{-5} to 4.14×10^{-4} g CO_2 g^{-1} dry sediment (447.92–9400.62 nmol $g^{-1}day^{-1}$).

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 is shown by reduced Fe both in oxic and in sub-oxic tubes of core 26. This is denoted by lowest Fe concentration in the aqueous phase. The highest metal dissolution/reduction/mobilization is shown by Mn in the sub-oxic tubes of core 26 at the deeper layers of the sediment column (Fig. 5a, b). In case of core 36 the highest metal oxidation is shown by reduced Mn under both oxic and sub-oxic conditions (Fig. 5c, 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 ± 0.1457 g C fixed g^{-1} in the pelagic red clay with volcanic signatures at 4 ± 2°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 ± 0.7776 g C fixed g^{-1} in the pelagic red clay 36 (Fig. 6). Uncertainty in down-core

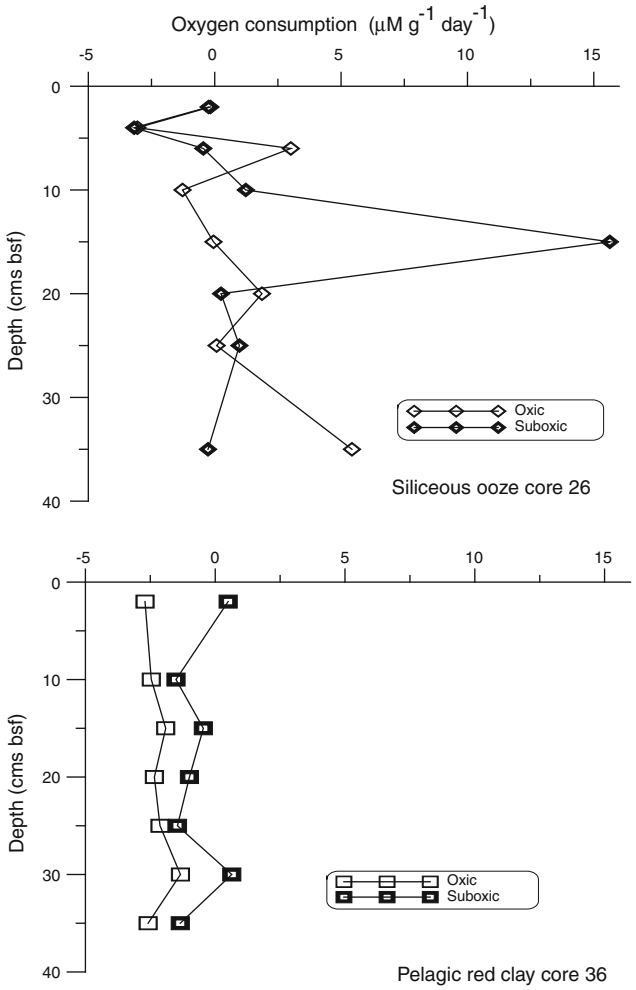

Fig. 3 Bacterial oxygen consumption rates in cores 26 and 36

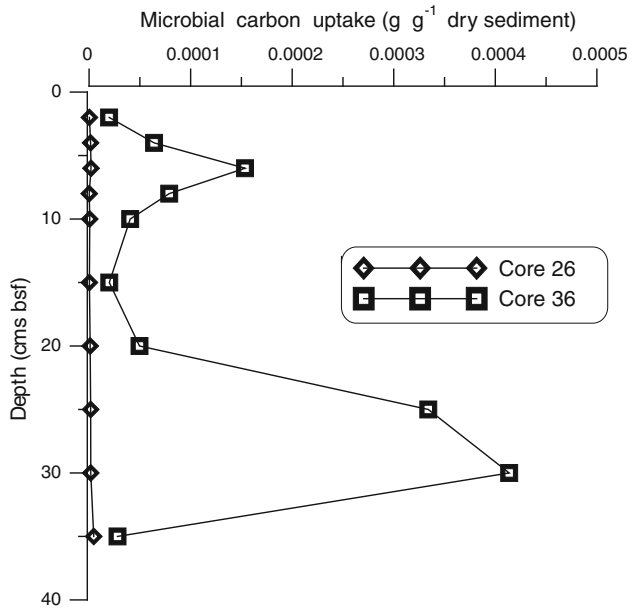

Fig. 4 Carbon fixation by sediments in cores 26 and 36

variation was ±0.1044 in core 26 and ±1.5241 in core 36 under sub-oxic condition.

Biomass yield on Fe, Mn, Ni and Co

In the organically richer core 26, the biomass yield was entirely contributed by Fe oxidation. Yield varied from 10 to 100 μgC g^{-1} metal oxidized under oxic condition. Under sub-oxic condition yield varied from 10 to 1,000 μgC g^{-1} metal oxidized. In core 36 the biomass yield was contributed by Fe and Mn both in oxic and in 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 thioxidans)*, 3. metal oxidizers (e.g. *Thiobacillus ferroxidans*) and 4. methane oxidizers (e.g. *Methylomonas*). Facultative autotrophs like *Pseudomonas and Alcaligenes* may also exhibit chemoautotrophy or mixotrophy (Karl 1995; LokaBharathi 1989; LokaBharathi 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. 2000).

The chemosynthetic CO_2 fixed/metal oxidised ratio in siliceous ooze under both oxic and sub-oxic experimental conditions (Fig. 6) is 0–2 orders of magnitude higher than

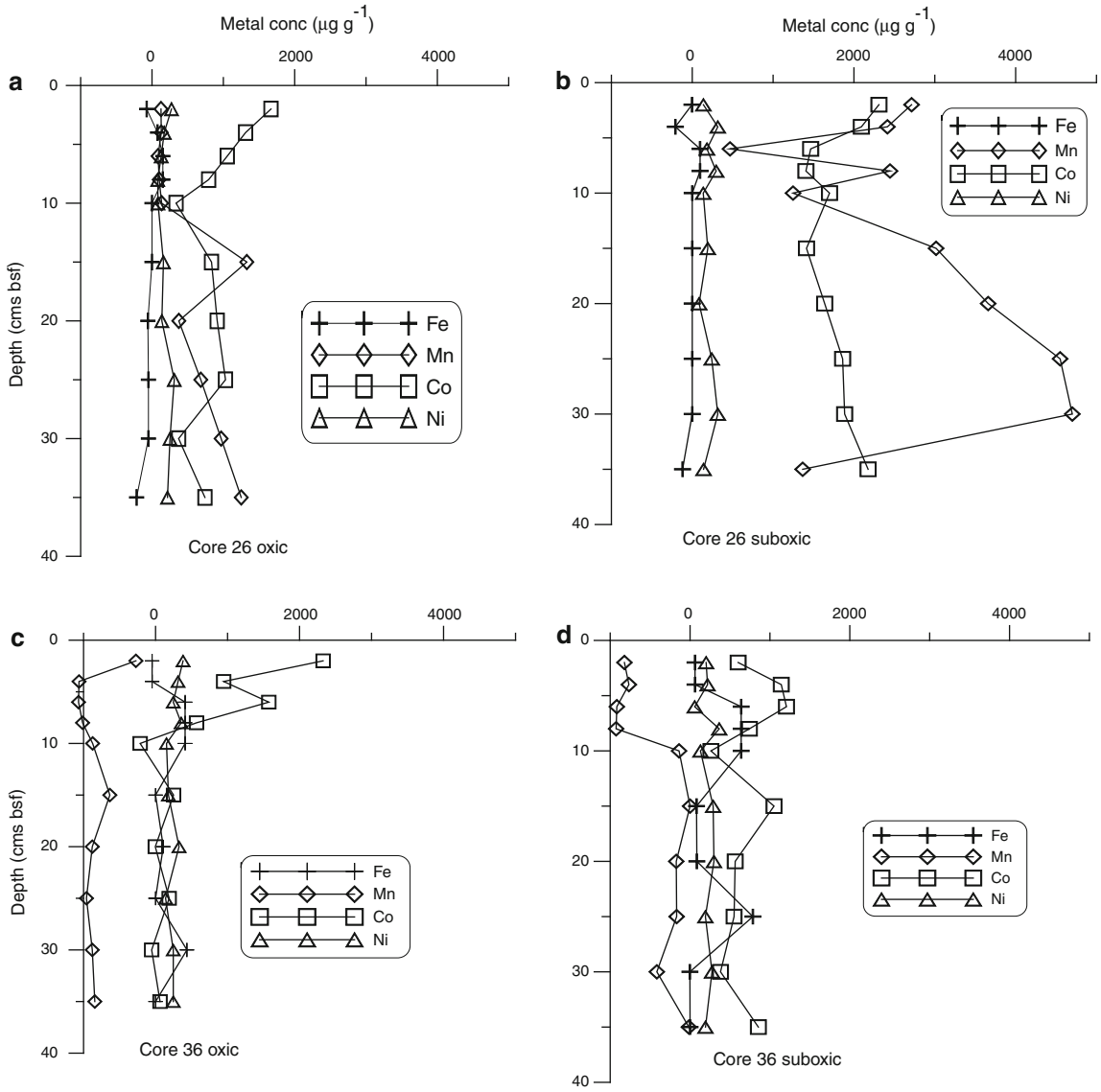

Fig. 5 Metal concentration in aqueous phase of the slurry set ups. Higher metal concentrations indicate dissolution or reduction while lower concentration indicates oxidation or precipitation

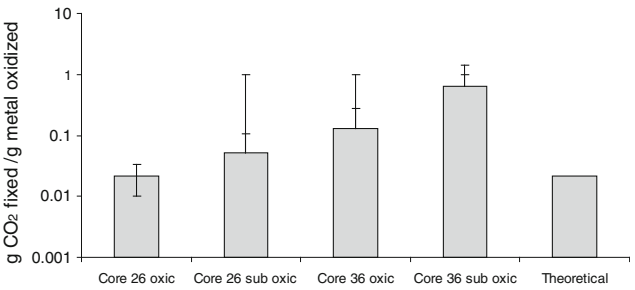

Fig. 6 Experimental ratios of carbon fixed to metal oxidized compared to theoretical value

the corresponding theoretical stoichiometric ratios calculated at 40°C at pH 1.5 by Hatzikioseyian 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 CO_2 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 (15–25 cm 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

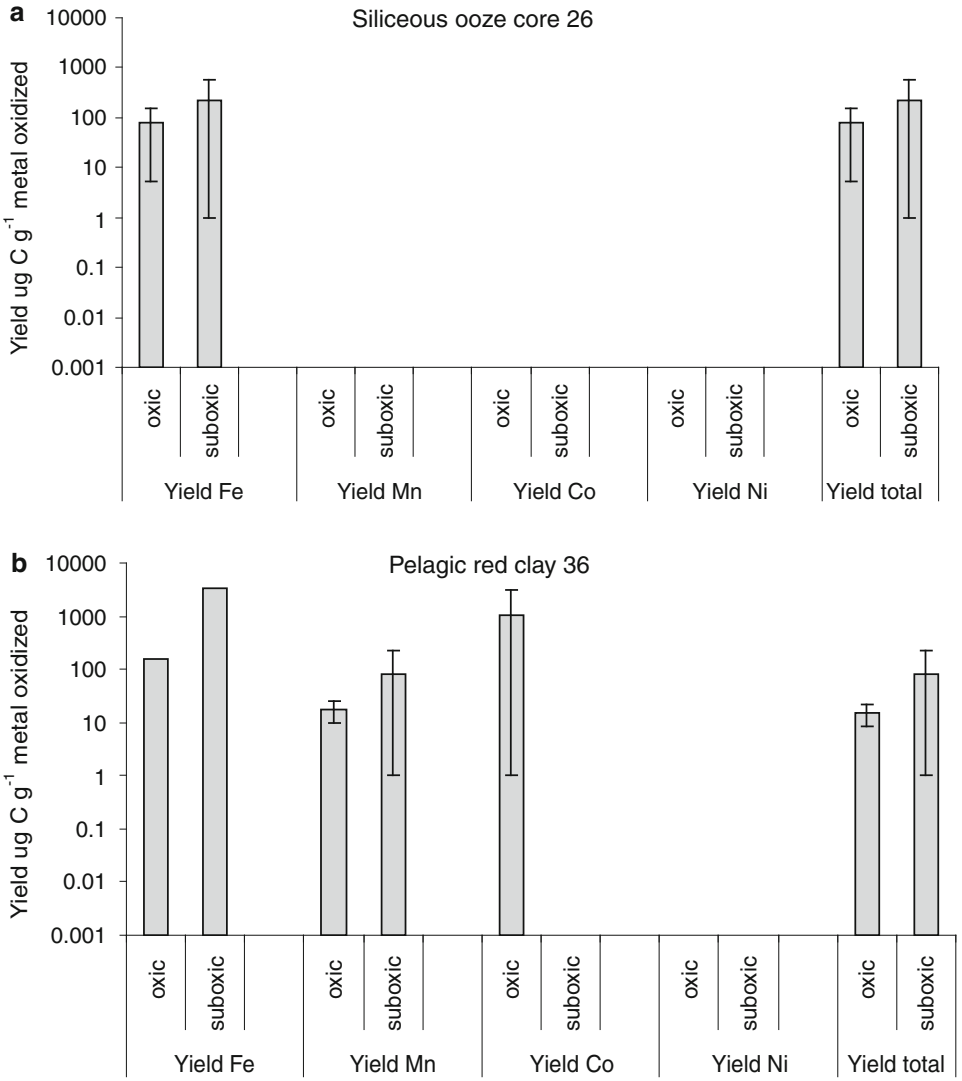

Fig. 7 Average potential yield in terms of carbon fixed by different metals. Calculations assume that single metal fixes the whole amount of carbon fixed. **a** Core 26; **b** Core 36

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 shows 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 calcareous 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 CO_2 fixation. Metal toxicity could probably play a role in explaining some of these patterns. It is suggested that higher CO_2 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 deplete 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 CO_2 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 CO_2 (Tang et al. 2009). It is probable that some bacterial communities use this mode of CO_2 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, under review).

It is suggested that chemosynthesis in oligotrophic deep-sea environments of the present study could be facilitated by higher availability of dissolved CO_2 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.

CO_2 is not limiting in the deep-sea environment, however, due to severe depletion of organic carbon, the microbes are constrained/facilitated to fix CO_2 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 to 1,000 nmol $g^{-1}day^{-1}$. Biomass yield of 10–1,000 μg C g^{-1} metal oxidized has 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; Hatzikioseyian 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., 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 and 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 CO_2 is much higher at elevated pressures. In this study, excess CO_2 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, 1 atm and 4°C, 500 atm did not show much difference.

It is therefore hypothesised that in the deep sea sediments of CIB, higher ratio of CO_2 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.

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References

Aldrich AP, van der Berg CMG (1998) Determination of Fe and its redox speciation in seawater using catalytic cathodic stripping voltametry. Electroanal 10(6):369–373

Antony R, Sujith PP, Fernandes SO, Verma P, Khedekar VD, LokaBharathi PA (2010) Cobalt immobilization by manganese oxidizing bacteria from the Indian Ridge System. Curr Microbiol. doi:10.1007/s00284-010-9784-1

Bach W, Edwards K (2003) Iron and sulphide oxidation within the basaltic ocean crust: implications for chemolithoautotrophic microbial biomass production. Geochim Cosmochim Acta 67(20):3871–3887

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Casamayor EO, Garcia-Cantizano J, Mas J, Pedros-Alio C (2001) Microbial primary production in marine oxic–anoxic interfaces: main role of dark fixation in the Ebro River salt wedge estuary. Mar Ecol Prog Ser 215:49–56

Casamayor EO, Garcia-Cantizano J, Pedros Alio C (2008) Carbon dioxide fixation in the dark by photosynthetic bacteria in sulphide rich stratified lakes with oxic–anoxic interfaces. Limnol Oceanogr 53(4):1193–1203

Chase RL, Delaney JR, Karsten JL, Johnson HP, Juniper SK, Lupton JE, Scott SD, Tunnicliffe V, Hammond SR, McDuff RE (1985) Hydrothermal vents on an axis seamount of the Jaun de Fuca ridge. Nature 313:212–214

Chester R, Hughes MJ (1968) Scheme for the spectrophotometric determination of Cu, Pb, Ni, V and Co in marine sediments: applied earth science. Trans Inst Miner Metall 77:37–41

Chevaldonne P, Desbruyeres D, Le Haitre M (1991) Time-series of temperature from three deep-sea hydrothermal vent sites. Deep Sea Res A 38:1417–1430

Childress JJ, Fisher CR, Favuzzi JA, Kochevar RE, Sanders NK, Alayse AM (1991) Sulphide-driven autotrophic balance in the bacterial symbiont-containing hydrothermal vent tubeworm *Riftia pachyptila*. Biol Bull 180:135–153

Chin CS, Johnson KS, Coale KH (1992) Spectrophotometric determination of dissolved manganese in natural waters with 1-(2-pyridylazo)-2-naphthol: application to analysis *in situ* in hydrothermal plumes. Mar Chem 37:65–82

Culver DA, Brunskill GJ (1969) Fayetteville Green Lake, New York. V. Studies of primary production and zooplankton in a meromictic marl lake. Limnol Oceanogr 14:862–873

Danovaro R, Fabiano M, Della Croce N (1993) Labile organic matter and microbial biomass in deep sea sediments (Eastern Mediterranean sea). Deep-sea Res 40:953–965

Das A, Fernandes CEG, Naik SS, Nagender Nath B, Suresh I, Mascarenhas-Pereira MBL, Gupta SM, Khadge NH, Prakash

Babu C, Borole DV, Sujith PP, Valsangkar AB, Mourya BS, Biche SU, Sharma R, LokaBharathi PA (2010) Bacterial response to contrasting sediment geochemistry in Central Indian Basin. Sedimentology. doi:10.1111/j.1365-3091.2010.01183.x

Dattagupta S, Miles LL, Barnabei MS, Fisher CR (2006) The hydrocarbon seep tubeworm *Lamellibrachia luymesi* primarily eliminates sulfate and hydrogen ions across its roots to conserve energy and ensure sulfide supply. J Exp Biol 209:3795–3805

Dattagupta S, Telesnicki G, Luley K, Predmore B, McGinley M, Fisher CR (2007) Submersible operated peepers for collecting porewater from deep-sea sediments. Limnol Oceanogr: Methods 5:263–268

Edwards KJ, Bach W, McCollom TM (2004) Neutrophilic iron-oxidizing bacteria in the Ocean: their habitats, diversity, and roles in mineral deposition, rock alteration, and biomass production in the deep-sea. Geomicrobiol J 21:393–404

Ehrlich HL (1998) Geomicrobiology: its significance for geology. Earth-Sci Rev 45:45–60

Flemming BW, Delafontaine MT (2000) Mass physical properties of muddy intertidal sediments: some applications misapplications and non-applications. Cont Shelf Res 20:1179–1197

García-Cantizano J, Casamayor EO, Gasol JM, Guerrero R, Pedros-Alio C (2005) Partitioning of CO_2 incorporation among guilds of microorganisms in lakes with oxic-anoxic interfaces and estimation of in situ specific growth rates. Microb Ecol 50:230–241

Gupta SM, Jauhari P (1994) Radiolarian abundance and geochemistry of the surface sediments from the central Indian Basin. Curr Sci 66(9):659–663

Hatzikioseyian A, Tsezos M (2006) Modelling of microbial metabolism stoichiometry: application in bioleaching processes. Hydrometallurgy 83:29–34

Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescent microscopy. Appl Environ Microbiol 3:1225–1228

Jannasch HW (1989) Chemosynthetically sustained ecosystems in the deep sea. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer, Berlin, Heidelberg, New York, pp 147–166

Jørgensen BB, Kuenen JG, Cohen Y (1979) Microbial transformation of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol Oceanogr 24:799–822

Jørgensen BB, Fossing H, Wirsen CO, Jannasch HW (1991) Sulfide oxidation in the anoxic Black Sea chemocline. Deep-sea Res 38(suppl.2):1083–1103

Juniper SK, Brinkhurst RO (1986) Water-column dark CO_2 fixation and bacterial-mat growth in intermittently anoxic Saanich Inlet, British Columbia. Mar Ecol Prog Ser 33:41–50

Kamesh Raju KA, Ramprasad T (1989) Magnetic lineations in the Central Indian Basin for the period A24–A21: a study in relation to the Indian Ocean Triple Junction trace. Earth Planet Sci Lett 95(3/4):395–402

Karl DM (1995) Ecology of free-living, hydrothermal vent microbial communities. In: Karl DM (ed) The microbiology of deep-sea hydrothermal vents. CRC Press, Boca Raton, FL, pp 35–124

Kato C, Sato T, Horikoshi K (1995) Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. Biodivers Conserv 4:1–9

Kochert G (1978) Carbohydrate determined by the phenol-sulfuric acid method. In: Hellebust JA, Craigie JJ (eds) Handbook of physiological methods: physiological and biochemical methods. Cambridge University Press, Cambridge, pp 95–97

Levin LA, Michener RH (2002) Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: The lightness of being at Pacific methane seeps. Limnol Oceanogr 47(5):1336–1345

LokaBharathi PA (1989) The occurrence of denitrifying colourless sulphur-oxidizing bacteria in marine waters and sediments as shown by the agar shake technique. FEMS Microbiol Ecol 62:335–342

LokaBharathi PA, Ortiz-conde BA, Nair S, Chandramohan D, Colwell RR (1994) FiveS rRNA sequences and fatty acid profiles of colourless sulfur-oxidising bacteria Ocean technology: perspectives. In: Sushilkumar, Agadi VV, Das VK, Desai BN (eds) Symposium on ocean technology. National Institute of Oceanography, Goa, India, 27–29 August 1992. Publ and Inf Dir, New Delhi, India

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-Phenol reagents. J Biol Chem 193:265–275

Mandernack KW, Tebo BM (1999) In situ sulfide removal and CO_2 fixation rates at deep-sea hydrothermal vents and the oxic–anoxic interface in Framvaren Fjord, Norway. Mar Chem 66:201–213

Mascarenhas-Pereira MBL, Nath BN, Borole DV, Gupta SM (2006) Nature, source and composition of volcanic ash in sediments from a fracture zone trace of Rodriguez Triple Junction in the Central Indian Basin. Mar Geol 229(1):79–90

McCarty PL (1965) Thermodynamics of biological synthesis and growth. In: Baers J (ed) Advances in water pollution research. Proceedings of 2nd international conference on water pollution research. Pergamon Press, Oxford, pp 169–199

McCarty PL (1975) Stoichiometry of biological reactions. Prog Water Technol 7:157–172

Mukhopadhyay R, Iyer SD, Ghosh AK (2002) The Indian Ocean Nodule Field: petrotectonic evolution and ferromanganese nodules. Earth Sci Rev 60:67–130

Nath BN, Mudholkar AV (1989) Early diagenetic processes affecting nutrients in the pore waters of Central Indian Ocean cores. Mar Geol 86:57–66

Nath BN, Borole DV, Aldahan A, Patil SK, Mascarenhas-Pereira MBL, Possnert G, Ericsson T, Ramaswamy V, Gupta SM (2008) ^{210}Pb, ^{230}Th, and ^{10}Be in Central Indian Basin seamount sediments: signatures of degassing and hydrothermal alteration of recent origin. Geophys Res Lett 35:L09603. doi:10.1029/2008GL033849

Nelson DC, Wirsen CO, Jannasch HW (1989) Characterisation of large, autotrophic *Beggiatoa* species abundant at the hydrothermal vents of the Guaymus Basin. Appl Environ Microbiol 55:2909–2917

Pai SC, Gong GC, Liu KK (1993) Determination of dissolved oxygen in seawater by direct spectrophotometry of total iodine. Mar Chem 41:343–351

Paropkari AL, Prakash Babu C, Mascarenhas A (1992) A critical game of depositional parameters controlling the variability of organic carbon in Arabian Sea sediments. Mar Geol 107:213–226

Patience RL, Clayton CJ, Kearsley AT, Rowland SJ, Bishop AN, Rees AWG, Bibby KG, Hopper AC (1990) An integrated biochemical, geochemical, and sedimentological study of organic diagenesis in sediments from Leg 112. In: Suess E, von Huene R, et al (eds) Proceedings of ODP, Science Results, vol 112, College Station, TX (Ocean Drilling Program), pp 135–153. doi:10.2973/odp.proc.sr.112.191.1990

Pattan JN, Jauhari P (2001) Major, trace, and rare earth elements in the sediments of the Central Indian Ocean Basin: their source and distribution. Mar Georesour Geotechnol 19:85–106

Rao VP, Nath BN (1988) Nature, distribution and origin of clay minerals in grain size fractions of sediments from Manganese Nodule Field, Central Indian Ocean Basin. Ind J Mar Sci 17:202–207

Rao VP, Kessarkar PM, Krumbein WE, Krajewski KP, Schneiders RJ (2003) Microbial dolomite crusts from the carbonate platform of western India. Sedimentology 50:819–830

Schulz HN, Schulz HD (2005) Large sulfur bacteria and the formation of phosphorite. Science 307:416–418

Schulz HD, Zabel M (eds) (2000) Marine geochemistry. Springer, Berlin, Heidelberg, New York

Sorokin YI (1972) The bacterial population and the processes of hydrogen sulphide oxidation in the Black Sea. J Cons Int Explor Mer 34:423–454

Sudhakar M (1989) Ore grade manganese nodules from the Central Indian Basin: an evaluation. Mar Mining 8:201–214

Sujith PP, Khedekar VD, Girish AP, LokaBharathi PA (2010) Immobilization of Nickel by bacterial isolates from Indian Ridge System and chemical nature of the accumulated metal. Geomicrobiol J 27:424–434

Tang K-H, Feng X, Tang YJ, Blankenship RE (2009) Carbohydrate metabolism and carbon fixation in *Roseobacter denitrificans* OCh114. PLoS ONE 4(10):e7233. doi:10.1371/journal.pone.0007233

Tuttle JH, Jannasch HW (1977) Thiosulfate stimulation of microbial dark assimilation of carbon dioxide in shallow marine waters. Microb Ecol 4:9–25

Warren BA (1982) The deep water of the Central Indian Basin. J Mar Res 40(Suppl.):823–860

Wenxuan H, Zhijun J, Suping Y, Xiancai L, Zhilin C, Linye Z, Xuejun Z, Huaiyang Z (2000) Discovery of low-mature hydrocarbon in manganese nodules and ooze from the Central Pacific deep sea floor. Chinese Sci Bull 47(11):939–944

Wirsen CO, Tuttle JH, Jannasch HW (1986) Activities of sulfur-oxidizing bacteria at the 21°N East Pacific Rise vent site. Mar Biol 92:449–456

Wirsen CO, Jannasch HW, Molyneaux SJ (1993) Chemosynthetic microbial activity at mid-Atlantic Ridge hydrothermal vent sites. J Geophys Res 98:9693–9703