

# Anoxia over the western continental shelf of India: Bacterial indications of intrinsic nitrification feeding denitrification

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	ACCEPTED MANUSCRIPT
1 2 3 4	Anoxia over the western continental shelf of India: Bacterial indications of intrinsic nitrification feeding denitrification.
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Coastal anoxia - Intrinsic nitrification feeds denitrification

### Abstract:

50 51 Studies on the Arabian Sea coastal anoxia have been of immense interest, but despite its ecological 52 significance there is sparse understanding of the microbes involved. Hence, observations were carried out off Goa ( $15^{\circ}30^{1}$ N,  $72^{\circ}40^{1}$ E to $15^{\circ}30^{1}$ N,  $72^{\circ}59^{1}$ E) to understand the processes that mediate 53 the changes in various inorganic nitrogen species in the water column during anoxia. Water column 54 55 chemistry showed a clear distinct oxic environment in the month of April and anoxic condition in 56 October. Our study based on microbial signatures indicated that oxygen deficit appeared as a well-57 defined nucleus almost 40 km away from the coast during the oxic period (April) and spreads there 58 after to the entire water column synchronizing with the water chemistry. Striking results of net 59 changes in inorganic nitrogen species in nitrification blocked and unblocked experimental systems show that denitrification is the predominant process in the water column consuming available nitrate 60 61 (~ 0.5  $\mu$ M) to near zero levels within ~72 hours of incubation. These observations have been supported by concomitant increase in nitrite concentration ( $\sim 4 \mu M$ ). Similar studies on 62 63 denitrification-blocked incubations, demonstrate the potential of nitrification to feed denitrification. 64 Nitrification could contribute almost 4.5  $\mu$ M to the total nitrate pool. It was found that the relation between ammonium and total dissolved inorganic nitrogen (DIN) pool (r = 0.98, p < 0.001, n = 122) 65 was significant compared to the latter with nitrite and nitrate. The occurrence of high ammonium 66 under low phosphate conditions corroborates our observations that ammonium does not to appear to 67 68 be locked under low oxygen regimes. It is suggested that ammonium actively produced by detrital 69 breakdown (ammonification) is efficiently consumed through nitrification process. The three processes in concert viz. ammonification, nitrification and denitrification appear to operate in more 70 71 temporal and spatial proximity than hitherto appreciated in these systems and this gives additional 72 cues on the absence of measurable nitrate at surface waters, which was earlier attributed only to 73 efficient algal uptake. Hence we hypothesize that the alarming nitrous oxide input into the 74 atmosphere could be due to high productivity driven tighter nitrification-denitrification coupling, 75 rather than denitrification driven by extraneous nitrate.

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77 Key words: Denitrifiers, Nitrifiers, Coastal anoxia, Arabian Sea

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#### 82 INTRODUCTION

Progressive and cyclic developing of oxygen deficient waters over the continental shelf of the 83 84 western India has received much attention (Naqvi et al. 2000). West India Coastal Current (WICC) 85 flows equatorward along the west coast of India during June-September in response to alongshore 86 wind stress and consequent upwelling can be seen along the coast (Shetye et al. 1990). The 87 biological activity over the continental shelf is enhanced during the summer due to intense coastal upwelling and Ekman pumping (Brock et al. 1991). The upwelled water over the shelf covers an area 88 of 180,000 km<sup>2</sup> (Naqvi et al. 2000) and is extremely oxygen depleted (Banse 1968). Naqvi et al. 89 90 (2000) reported that this coastal water body could be one of the largest low-oxygen zones, which begins its annual development in June, reaching peak intensity by September-October and 91 92 dissipating by December. Further, they measured the highest accumulations of hydrogen sulphide 93 (H<sub>2</sub>S) and nitrous oxide (N<sub>2</sub>O) in open coastal waters and attributed to the addition of anthropogenic nitrate and its subsequent denitrification, which is favored by hypoxic conditions. It is now well 94 95 understood that coastal anoxia is more widespread geographically with periodic cyclicity. Hence, the 96 cues that trigger such processes could be linked to large scale oceanic processes than hitherto 97 understood. A 5-10 m thick cap of warm, relatively less saline waters over the upwelled water 98 clearly indicate the magnitude of terrestrial runoff accompanied by precipitation (Banse 1959). 99 However, despite its origin from land runoff with sufficient nutrient load, low nitrate values 100 observed in this warm-water cap is attributed to efficient algal uptake (Naqvi et. al 2000). If this 101 were to happen, then the denitrification process would be constrained by the availability of nitrate. 102 The observed high  $N_2O$  accumulation (Naqvi et al. 2000) is indicative of availability of nitrate 103 despite algal uptake. Consequently, there could be a more significant pathway dynamically linked to 104 denitrification contributing significantly to  $NO_3$  and  $N_2O$  flux. The low  $O_2$  conditions are also known 105 to enhance the yield of  $N_2O$  during nitrification, and so the possibility of contribution coming 106 through this pathway cannot be excluded (Goreau et al. 1980). Shailaja (2001) has shown that both 107 denitrification and nitrification occur in several closely spaced layers in the Arabian Sea and the

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Ammonia-oxidizing nitrifiers and sulphide-oxidizing denitrifiers (Example: Thiobacillus 113 114 denitrificans like organisms; TDLO) are physiologically diverse bacterial groups, which respond in 115 tandem to environmental chemistry. Recording the spatial and temporal distribution of these groups 116 and their synchronization with the water column chemistry gives us a better understanding of the dominant microbial processes and their potential contribution to gaseous inputs to the atmosphere. 117 118 Hence we hypothesize, that ammonification fed nitrification under hypoxic conditions could be more 119 important than previously thought of in these systems. The alarming nitrous oxide input into the 120 atmosphere could be due to a tighter nitrification-denitrification coupling, rather than denitrification 121 driven by extraneous nitrate alone.

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#### 123 MATERIALS AND METHODS

#### Study area and sampling: 124

A cross shelf transect off Goa (15°30'N, 72°40'E to15°30'N, 72°59'E) was selected to study the 125 126 spatial and temporal variation of nitrifiers and denitrifiers with respect to the water chemistry for two 127 contrasting periods viz. April (oxic) and October (anoxic) during the cruises SaSu-40 (April, 2003), 128 49 (October, 2003) and 103A (October, 2005) onboard C.R.V. Sagar-Sukti. Though the cruises 129 conducted in April and October 2003 are the best representative of the contrasting conditions, 130 analysis of additional data from other cruises almost corresponding to the period were undertaken [SaSu-26 (September 2002), 29 (December, 2002)]. A similar transect off Mangalore (13°00'N, 131 132 74°38'E to13°00'N, 74°03'E) during cruises SaSu 26, 30 (December, 2002), and 49 have also been

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- 133 included in this analysis. Such extended analyses were undertaken to get a better perspective of the
- accumulation/oxidation of dissolved ammonium and inorganic phosphate in the water column.
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Portable CTD (conductivity, temperature, depth) was used to obtain temperature and salinity profiles at sampling locations. Seawater was collected using Niskin water samplers attached to the winch wire and triggered with a messenger. Seawater sub-samples for microbiological analyses were collected into sterile containers immediately after retrieval of the bottle. Sub-samples for nitrate and phosphate measurements were frozen for onshore analysis, whereas ammonium and nitrite were measured onboard.

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#### 143 Water column chemistry:

Dissolved oxygen (DO) and nutrients (ammonium, nitrite, nitrate and phosphate) in the seawater
samples were measured by colorimetric and titrimetric methods respectively (Grasshoff 1983).

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#### 147 Abundance of nitrifiers and sulphide oxidizing denitrifiers:

148 Nitrifiers and Sulphide-oxidizing denitrifiers (*Thiobacillus denitrificans like organisms*, TDLO) 149 were enumerated as Colony Forming Units (CFU) on different selective media. Nitrifiers were 150 enumerated by spread plating onto solidified Winogradsky's medium (Rodina 1972). After 151 inoculation, the plates were incubated for 30-45 days at  $15\pm2^{\circ}$ C. The TDLO were enumerated by an 152 agar shake method on modified Leiske's medium (Loka Bharathi 1989; Loka Bharathi et al. 1988). 153 The culture tubes were incubated for ~30 days at  $28\pm2^{\circ}$ C.

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#### 155 Nitrification and denitrification studies:

Seawater for incubation experiments were collected at definite intervals (surface, 8, 13 and 18 m)
from a 25m deep station off Goa on R.V. Sagar Sukti #103A in the month of October (2005). Sub-

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158 samples in triplicates were then transferred into sterile, plugged, 250 ml, glass conical flasks, which 159 were incubated in the dark onboard under ambient temperature. The temperature in the incubation flasks varied between 26-29°C. Three sets of incubation experiments were carried out viz., -(1)160 161 Nitrification was inhibited by adding Allyl thio urea to a final concentration of 86 µM (Ginestet et 162 al. 1998), (2) Denitrification was inhibited by adding Chloramphenicol to a final concentration of 300 mg  $L^{-1}$  (Brooks et al. 1992), and (3) Sub-samples of third set were monitored without adding 163 any inhibitors (controls). Aliquots were analyzed at 24 hrs intervals up to 144 hrs (6 days) for nitrate, 164 165 nitrite and ammonium.

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#### 167 **Contours, plots and statistical analysis:**

Bacterial abundance contour map was generated using Surfer 8.0 (Golden Software, Golden, CO).
Gridding used a kriging algorithm with a linear variogram and no anisotropy, as employed in
standard topographic applications. Scatter plots and line graphs were constructed using Grapher 4.0
(Golden Software, Golden, CO, USA). Statistical analyses were done using Analysis Tool Pack in
Microsoft Excel.

173

#### 174 **RESULTS**

175 Surface temperatures roughly followed the solar heating pattern with primary and secondary heating 176 during the March-April and September-October. The salinity profile for October showed the 177 influence of riverine input as evidenced by the development of a less saline, warm water cap. 178 Variations in temperature and salinity during October (Figs.1a and 1b respectively) showed an 179 upwelling signature, possibly during the waning phase of the WICC. The WICC flows northward 180 during January-April, which results in downwelling along the coast. The temperature/salinity 181 profiles for April (Figs.-2a and 2b respectively) also showed downwelling signatures, though weak. 182 The SST was cooler during October than in April, possibly due to the upwelling and the imminent

183 winter. However, the signatures were weak during the transition periods i.e. April and October.

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#### 185 Changes in water column chemistry (Figs.1 c-e and 2 c-e):

During October, the shelf was characterized by low-oxygenated bottom waters ( $<0.5 \text{ mlL}^{-1}$ ) of  $\sim 40 \text{ m}$ 186 overlying the shelf. Nitrate values increased gradually to reach >4  $\mu$ M at around 90m depth. It was 187 188 observed that the thickness of nitrate-poor surface waters had decreased considerably. It was also 189 interesting to note that nitrite and nitrate values in the month of oxygen deficit (October) showed a similar distribution pattern as that of the oxic month (April) but with enhanced concentrations. 190 191 Patchiness in nitrite distribution seen during April was lost but replaced by a core of elevated nitrite 192 at around 50 km from the coast (Fig.1e). In April, the concentration of nitrate and nitrite was very 193 low in the top 20 m of the oxic water and it gradually increased to  $>1.4\mu$ M at around 90m water depth. The distribution of nitrite was patchy and elevated concentrations were noticed at around 40 194 195 km from the coast (Fig.2e).

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### 197 Bacterial abundance (Table 1):

During April, the abundance of nitrifiers were in the order of  $10^{7-8}$  CFU L<sup>-1</sup> and patchy. The TDLO numbers showed that there is a core of elevated abundance almost 40 km away from the coast. By October nitrifier counts fell sharply to even reach non-detectable levels. In October, the distribution of TDLO was uniform and generally increased away from the coast. There was not much difference in the abundance of TDLO ( $10^{4-5}$  CFU L<sup>-1</sup>) in both the contrasting periods.

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#### 204 Nitrification and denitrification:

In October (2005), the temperature and salinity (Fig.3a) ranged from 26.3-28.6°C and 35-35.5 psu respectively throughout the water column. The levels of nitrite, nitrate and ammonium which served to define the initial conditions during the experimental incubations is illustrated in Fig.3b. The concentration of nitrite increased with depth while nitrate remained almost constant. Ammonium

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values showed two peaks viz subsurface and bottom indicating that there is considerable amount of
spatial heterogeneity in ammonium levels. The results of the incubation experiments are presented in
Figs. 4-6.

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### 213 Denitrification blocked system (Fig 4 a-e):

At the surface (Fig.4a) the levels of nitrite did not vary much over an incubation period of 144 hours 214 when denitrification was inhibited by chloramphenicol, but nitrate increased to almost 2 µM in 96 215 216 hours and plateaued at the end of incubation (144 hours). The ammonium levels increased at a slow 217 rate over the incubation period to reach final values of around 5.5 µM. At 3m (Fig.4b), the values of 218 ammonium fluctuated with a decrease followed by an increase with very little variation in nitrite but 219 with constant increase in nitrate, which reached 2.6µM in 144 hours. At 8m (Fig.4c), the variation in 220 nitrite values fairly resembled the surface and 3m incubations except for a sharp decrease in the first 221 24 hours. At 13m (Fig.4d), nitrite and nitrate values evolved as in the 8m water depth though 222 absolute values differed. The irregularities observed in the rise and fall of ammonium values were 223 more pronounced at this depth. At bottom i.e., 18 m (Fig. 4e), it could be observed that both nitrite 224 and ammonium values vary in phase at higher frequency, while nitrate values continuously increase over time, which was to all the sampled depths. 225

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#### 227 Nitrification blocked system (Fig. 5a-e):

At the entire sample depths nitrate fell below the levels of detection within 48-72 h. At the surface (Fig.5a), when nitrification was inhibited by the addition of allyl thiourea, the levels of nitrite initially increased and followed an elliptical curve to reach values below the limits of detection. The nitrate values decreased to non-detectable levels within 48 hours of incubation due to nitrate reduction/denitrification, while change in concentrations of ammonium was sigmoidal though less pronounced. The trend in the variation of all the three nitrogen species at 3m (Fig.3b) water depth

Coastal anoxia - Intrinsic nitrification feeds denitrification 234 were similar to that observed at the surface waters. The concentrations in nitrite and ammonium 235 varied inversely. Nitrite level increased at the beginning of incubation and then fell to below 236 detection. The peak of nitrite is broader at 8m depth (Fig.5c) and decreased with increasing depth. 237 Changes in ammonium at 8m resembled that of the surface; while at 13 (Fig.5d) and 18m (Fig.5e) it 238 increased rapidly after an initial stationary phase and then flattened out. At 13m the peak of nitrite 239 accumulation was observed at 48 hours while at 18m it was observed at 24 hours. It could also be observed that the fall in level of nitrite at 13m is more pronounced between 96-120 hours where as 240 241 the depletion of nitrite was more gentle at 18m.

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#### Monitoring the changes in 'N' species in the controls (Fig. 6a-e): 243

At the surface (Fig.6a) and 3m (Fig.6b), when neither of the inhibitors were added, the levels of 244 245 nitrite initially increased and then followed an elliptical curve to reach values below the limits of detection, while ammonium levels increased at a very slow rate. At all the depths nitrate values fell 246 to non-detectable levels within 72 - 120 hrs. The pattern of changes in ammonium and nitrite at 8m 247 248 (Fig.6c), 13m (Fig.6d) and 18m (Fig.6e) were very similar to that of the experiment where nitrification was inhibited by allyl thiourea (Figs 5c-e). 249

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#### DISCUSSION 251

The presence of a 5-10 m thick cap of warm, less saline water over the upwelled water clearly 252 253 indicates the magnitude of terrestrial runoff accompanied by precipitation (Banse 1959). Low nitrate 254 values observed in this warm water cap were solely attributed to efficient algal uptake (Naqvi et al. 255 2000). A more significant pathway dynamically linked to denitrification could contribute 256 significantly to NO<sub>3</sub> and N<sub>2</sub>O flux and mediate N<sub>2</sub>O production. It was observed that the relationship 257 between ammonium and total dissolved inorganic nitrogen (DIN) pool (r = 0.98, p < 0.001, n = 122; 258 data obtained from various cruises as described in legend to Fig.7) was stronger than the relationship 259 between DIN to nitrite and nitrate. It is therefore inferred that ammonification is a predominant

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260 process contributing to DIN. The oxygen-poor waters are conducive for reduction of iron and the 261 subsequent phosphate release from ferric phosphate (Caraco et al. 1989; 1991). Enhanced dissolved 262 inorganic phosphate in the water column is used as an indicator for the degree of anoxia in the 263 region. The reduced compounds such as  $NH_3$ ,  $H_2S$ , and  $CH_4$  tend to accumulate under anaerobic 264 conditions. Hence low oxygen regimes result in the accumulation of phosphate and ammonia. 265 However, our study showed that under low oxygen regimes with high phosphate concentrations, 266 though ammonium is a significant fraction of DIN, accumulation of ammonium was low. This 267 strengthens our observation that ammonium does not appear to be locked always under low oxygen 268 regimes. Besides, the higher diffusion coefficient of ammonium as compared to other reduced 269 compounds like sulphide (Li & Sandra, 1974) facilitates its mobility to oxic shallower layers. This 270 could probably improve the spatial coupling between the substrate (ammonium) and the utilizer 271 (nitrifier), which could get decoupled due to the development of acute anoxia. Unlimited substrate availability by degradation of organic matter could also eliminate the dependence of culturable 272 273 bacterial abundance on the distribution of ammonia.

Studies generally reveal that 99% or more of the bacterial diversity remains uncultured and 274 275 unexplored. However, estimates on data compiled for culturability indicate that in seawater and 276 unpolluted estuarine water, percentage culturability can reach up to 0.1 and 3% of direct microscopic counts respectively (Fuhrman & Campbell, 1998). Table 1 presents contrasting distribution of TDLO 277 278 and nitrifiers. While nitrifiers are mostly detectable only during the oxic period (April), TDLO could be retrieved during both the seasons (April and October) as TDLO is more metabolically versatile to 279 280 respire either oxygen or nitrate. During April (2003), the culturable fraction of nitrifier population  $(10^7 \text{ CFU/ L}^{-1})$  decreased towards the open sea. It was also observed that the nitrifier counts vary 281 282 with water depth at around 40 km from the coast. A zone of high nitrite, high TDLO and low 283 nitrifiers was observed in the same area. Increase of nitrate concentration below 20m depth indicates 284 that nitrification is more pronounced in the deeper depths than in the surface. These observations 285 provide a glimpse of the distribution pattern of bacteria well before the onset of chemical signatures

Coastal anoxia - Intrinsic nitrification feeds denitrification 286 of anoxia. In the post monsoon season (October, 2003) the nitrifier population declined to non detectable levels from ~  $10^8$  CFU L<sup>-1</sup>. During this period, very low nitrifier counts in conjunction 287 288 with a more heterogeneous and regular abundance pattern of TDLO showed that the water column is 289 at the peak of anaerobic conditions. The depth of nitrate-poor surface layers has decreased 290 considerably but showed inputs of nitrate by nitrification from subsurface levels. Though the nitrifier 291 counts have dropped by almost two orders, the elevated nitrate observed could be attributed to higher 292 specific activity of nitrifying bacteria or suboxic nitrification, perhaps at the expense of iron and 293 manganese. The nitrite and nitrate values in the anoxic period showed a similar distribution pattern 294 as that of the oxic period, but the enhanced magnitude due to net nitrate production being more than 295 nitrate reduction suggests that the nucleus of microbial activity spreads during the period of anoxia to cover the entire water column, changing the chemistry. Hence it is proposed that the microbial 296 297 indicators of oxygen deficit appear as a well-defined nucleus almost 40 km from the coast even 298 during the oxic period (April).

Net changes of inorganic nitrogen species in nitrification blocked and unblocked 299 300 experimental systems during the period of anoxia (October) show that denitrification is the predominant process in the water column consuming available nitrate (~  $0.5 \mu$ M) to near zero levels 301 within 48 – 96 hours of incubation. Similar experiments conducted by Naqvi et al. (2000) showed 302 303 that majority of the available nitrate (21.8µM) is converted to nitrite in the first 48 hours of 304 incubation. Initial levels of nitrite varied with depth and could range between 0.44-3.87µM. The 305 levels of nitrite encountered in the incubation experiments were comparatively higher (~7-65 times) 306 than that reported by Naqvi et al. (2000). When denitrification was blocked, the absence of a well-307 defined nitrite peak coupled to a steady increase in nitrate clearly indicates the efficiency with which 308 ammonium is oxidized to nitrate. It was found that nitrification could contribute almost 4.5  $\mu$ M to 309 the total nitrate pool, suggesting that ammonium is actively produced by detrital breakdown, but 310 efficiently consumed through the nitrification process. Moreover, the highest rate of ammonia

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Coastal anoxia - Intrinsic nitrification feeds denitrification oxidation (0-144 hours) was found to be at the surface (2.34 ng at  $NH_4^+$ -N  $l^{-1}$   $h^{-1}$ ) and the lowest 12 311 rate of 0.72 ng at  $NH_4^+$ -N  $I^{-1}$  h<sup>-1</sup> was observed at 13 m depth. But contrary to the observed oxidation 312 maxima, nitrite and nitrate production maxima of 5.85 and 2.34 ng at  $NO_3^{-1}N l^{-1} h^{-1}$  were observed at 313 314 18m depth. Since the incubation systems were isolated and denitrification was blocked it could be 315 assumed that a major fraction of nitrate was produced by the oxidation of ammonium with an 316 intermediate production of nitrite. The production of nitrate occurred at a much higher rate than 317 ammonium oxidation. This highlights the predominance of ammonification and its contribution to 318 denitrification via nitrification. In addition, the highest rate of nitrate reduction in nitrification blocked systems (0-144 hours) was found to be at 13 m depth (0.05 ng at NO<sub>3</sub><sup>-</sup>N  $l^{-1}$   $h^{-1}$ ) and the 319 lowest rate of 0.03 ng at NO<sub>3</sub><sup>-</sup>-N l<sup>-1</sup> h<sup>-1</sup> at the surface. High ammonium oxidation and low nitrate 320 321 reduction in surface waters suggest that the physiologically forced synthetic pathway as electron sink is much more dominant in these systems than hitherto accepted. Oxidation of  $NH_4^+$  to  $NO_2^-$  in the 322 323 euphotic zone has been observed in many regions (McCarthy et al. 1984; Ward et al. 1989; Dore & 324 Karl, 1996). Studies by Sambrotto (2001) have also shown that ammonium regeneration rates and 325 concentrations are higher along the coastal waters of Oman during the southwest monsoon season. The unaccounted ( $\sim 20\%$ ) portion of total ammonium sink in the study was attributed to nitrification. 326 Moreover, Mantoura et al. (1993) have attributed elevated N<sub>2</sub>O production to nitrification from the 327 Omani coastal region. Hence, the sequential processes viz. ammonification, nitrification and 328 329 denitrification operate in more spatial and temporal proximity than hitherto believed suggesting their 330 significant role in the dynamics of nitrogen species. Further, the study suggests the potential role of 331 anaerobic/suboxic nitrification that could intrinsically feed the denitrification process.

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#### 333 CONCLUSION

The microbial indicators of ensuing oxygen deficit appear as a well-defined nucleus almost 40 km from the coast even during the oxygenated period and spreads during the period of anoxia to cover the entire zone governing the water column chemistry. Nitrification operates at the base of this

Coastal anoxia - Intrinsic nitrification feeds denitrification 337 suboxic system, very efficiently feeding denitrification. The alarming nitrous oxide input into the 338 atmosphere could be relatively more due to a tighter intrinsic coupling of nitrification-denitrification, 339 rather than denitrification driven by extraneous nitrate alone.

340

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#### 353 REFERENCES

354

355 Banse K (1959). On upwelling and bottom-trawling off the southwest coast of India. J. Mar. Bio. 356 Assoc. Ind. 1:33-49.

357

358 Banse K (1968). Hydrography of the Arabian Sea Shelf of India and Pakistan and effects on

359 demersal fishes. Deep-Sea Res. 15: 45–79.

360

361 Brock JC, McClain CR, Luther ME, Hay WW (1991). The phytoplankton bloom in the northwestern

Arabian Sea during the southwest monsoon of 1979. J Geophys Res. 96: 20623–20642. 362

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363

- 364 Brooks MH, Smith RL, Macalady DL (1992). Inhibition of existing denitrification enzyme activity
- 365 by chloramphenicol. Appl Environ Microbiol. 58:1746–1753.
- 366
- 367 Caraco NF, Cole JJ, Likens GE (1989). Evidence for sulphate-controlled phosphorus release from
- 368 sediments of aquatic systems. Nature. 341: 316-318.
- 369
- 370 Caraco N, Cole JJ, Likens GE (1991). A cross-system study of phosphorus release from lake
- 371 sediments. In: Comparative Analysis of Ecosystems; Patterns, Mechanisms, and Theories (eds Cole
- 372 JJ, Lovett G, Findlay S), pp. 241-258. Springer-Verlag, New York.
- 373
- 374 Dore JE, Karl DM (1996). Nitrification in the euphotic zone as a source for nitrite, nitrate and
  375 nitrous oxide at station ALOHA. Limnol Oceanogr. 41:1619-1628.
- 376
- Fuhrman JA, Campbell L (1998). Microbial diversity. Nature. 393: 410–411.
- 378
- Ginestet P, Audic JM, Urbain V, Block JC (1998). Estimation of nitrifying bacterial activities by
  measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. Appl
  Environ Microbiol. 64: 2266–2268.
- 382
- 383 Grasshoff K, Ehrhardt M, Kremling K (1983) *Methods of Seawater Analysis*, Verlag Chemie,
  384 Weinheim.
- 385
- 386 Goreau TJ, Warren AK, Steven CW, Michael BM, Frederica WV, Stanley WW (1980). Production
- 387 of NO<sup>-2</sup> and N<sub>2</sub>O by nitrifying bacteria at reduced concentrations of oxygen. Appl Environ
- 388 Microbiol. 40: 526-532.

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3	89
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- 390 Jayakumar DA, Naqvi, SWA, Narvekar PV, George MD (2001). Methane in coastal and offshore
- 391 waters of the Arabian Sea. Mar Chem. 74, 1-13.
- 392
- 393 Loka Bharathi PA, Chandramohan D, Nair S (1988). A preliminary study of anaerobic thiosulfate-
- 394 oxidising bacteria as denitrifiers in the Arabian Sea. Geomicrobiol. J. 6:195-207.
- 395
- 396 Loka Bharathi PA (1989). The occurrence of denitrifying colourless sulphur-oxidising bacteria in
- 397 marine waters and sediments as shown by the agar shake technique. FEMS Microbiol Ecol. 62: 335-
- 398 342.
- 399
- 400 Li YH, Sandra G (1974). Diffusion of ions in seawater and in deep-sea sediments. Geochim
- 401 Cosmochim Acta. 38: 703-714.
- 402
- Mantoura RFC, Law CS, Owens NJP, Burkill PH, Woodward EMS, Howland RJM, Llewellyn CA
  (1993). Nitrogen biogeochemical cycling in the northwestern Indian Ocean. Deep-Sea Res Part II.
  405 40: 651-671.
- 406
- 407 McCarthy JJ, Kaplan W, Nevins JL (1984). Sources and sinks of nitrite in the water column of the
  408 York River and the Chesapeake Bay. Limnol Oceanogr. 29: 84-98.
- 409
- 410 Naqvi SWA, Jayakumar DA, Narvekar PV, Naik H, Sarma VVSS, DeSouza W, Joseph S, George
- 411 MD (2000). Increased marine production of N<sub>2</sub>O due to intensifying anoxia on the Indian continental
- 412 shelf. Nature. 408: 346–349.
- 413

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- Rodina AG (1972). Methods in Aquatic Microbiology. (eds Colwell Tr Rev RR, Zambruski MS),
- University Park Press, Baltimore and Butterworth Co., London.
- Sambrotto RN (2001). Nitrogen production in the northern Arabian Sea during the Spring
- Intermonsoon and Southwest Monsoon seasons. Deep-Sea Res Part II. 48: 1173-1198.
- Shailaja MS (2001). Respiratory enzyme activities in the oxygen-deficient waters of the
- Arabian Sea. Curr. Sci. 81: 684-689.
- Shetye SR, Gouveia A, Shenoi SSC, Sundar D, Michael GS, Almeida AM, Santanam K (1990).
- Hydrography and circulation off west coast of India during the southwest monsoon. J Mar Res. 48:
- 359-378.
- Ward BB, Kilpatrick KA, Renger EH, Eppley R.W (1989). Biological nitrogen cycling in the
- nitracline. Limnol Oceanogr. 34: 493-513.

- OCF.

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### 440 **Figure legends**:

- 441 Fig.1. Hydrography along the transect Off Goa transect in October a- temperature (°C); b salinity
- 442 (psu); c oxygen (mlL<sup>-1</sup>); d -nitrate ( $\mu$ M); e- nitrite ( $\mu$ M). Station # 1 was sampled at 1, 5 and
- 443 17m (bottom depth, 19m); Station # 2 was sampled at 1,5,15 and 25m (bottom depth, 35m),
- 444 Station # 3 was sampled at 1,15,30 and 60m (bottom depth 79m); Station # 4 was sampled at
- 445 1,15,30,60 and 90m (bottom depth, 100m).
- 446 Fig.2. Hydrography along the transect Off Goa transect in April. temperature (°C); b salinity (psu);
- 447 c oxygen (mlL<sup>-1</sup>); d -nitrate ( $\mu$ M); e- nitrite ( $\mu$ M). Details of sampling depths same as Fig 1.
- 448 Fig.3a-b. 3a represents T-S profiles for station #2 during the cruise SaSu 103A (October, 2005). 3b
- 449 represents the zero hour levels of nitrite, nitrate and ammonium at station #2 at the start of the
- 450 experiment.
- Figs 4a-e represents net changes in 'N' species from denitrification blocked water sample retrieved from - surface, 3,8,13 and 18m respectively. Figs 5a-e and 6a-e show, nitrification blocked and
- 453 control sample respectively from similar depths. 'x'-axis represent the hours of incubation.
- 454 ( $\Box$  Nitrite Ammonium+ Nitrate).
- Fig.7. Plot of dissolved inorganic phosphate (DIP) versus ammonium under different oxygen levels
  for the southwest monsoon cycle. Data from cruises covering the off Goa transect SaSu-26
  (September 2002), 29 (December, 2002), 49 (October 2003), 103A (December 2005) and a
  similar transect off Mangalore SaSu 26, 30 (December, 2002), and 49 during oxic and anoxic
  seasons have also been included in this analysis.
- 460















Fig.7.

	April								October							
Depth	Nitrifiers				TDLO			Nitrifiers				TDLO				
(m)	$(x10^8  \text{CFU L}^{-1})$				$(x10^5 \text{ CFU L}^{-1})$			(x10 <sup>7</sup> CFU L <sup>-1</sup> )			$(x10^5 \text{ CFU L}^{-1})$					
	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4
1	2.3	1.2	2.6	0.7	0.1	0.1	0.3	0.3	ND	ND	ND	2.2	0.8	0.5	0.6	1.4
5	4.1	2.3	3.1	0.8	0.2	0.2	0.6	0.3	ND	ND	ND	ND	0.5	0.7	0.6	1.2
15	3.1	1.0	1.2	1.3	2.2	2.2	0.1	0.08	ND	ND	ND	ND	0.07	0.5	0.3	2.4
25	-	1.0	3.7	1.6	-	0.3	0.6	0.3	ND	ND	ND	ND	-	0.5	0.3	1.5
50	-	-	1.5	0.7	-		0.2	-	ND	ND	ND	ND	-	-	0.6	1.0

### Table 1. Bacterial groups along the transect Off Goa in April and October 2003

# - station number, CFU – Colony Forming Units and ND – not detected

ACEPT