CHAPTER 5

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5.1 Introduction

Phytoplankton photosynthesize organic matter by taking up dissolved inorganic carbon (DIC) and nutrients in the euphotic zone. A part of this organic matter sinks from the euphotic zone to subsurface waters where it is mineralized releasing DIC and nutrients back to seawater. Thus, the biological activity leads to sequestration of carbon from the surface to subsurface waters. This process is known as the “biological pump”.

The organic matter produced by phytoplankton contains the principal elements carbon (C), nitrogen (N) and phosphorus (P) in approximately fixed ratios of 106:16:1, by atoms. A.C. Redfield was the first to point it out and recognize the importance of the near-constancy of C:N:P ratios (appropriately called the Redfield ratios) in ocean biogeochemistry (Anderson and Sarmiento, 1994). These ratios have since been used widely by oceanographers for the conversion of the concentration/flux of one nutrient to that of another. For example, an evaluation of new production in the euphotic zone can be made based on the fluxes of fixed nitrogen from outside.

Organic matter in the ocean is generally decomposed by bacteria using O₂ as the oxidant, and this process causes O₂ concentration to fall below the saturation in aphotic layers of the ocean. The degree to which such O₂
depletion takes place depends upon the balance between the rates of its consumption and supply through circulation. In most parts of the ocean, physical processes facilitate enough O$_2$ replenishment to make up for its consumption. However, there are a few areas where this balance is not achieved and the O$_2$ concentrations fall to near-zero levels. When this happens, the bacteria switch over to NO$_3^-$, the next preferred oxidant, resulting in its reductive removal from seawater.

After NO$_3^-$ has also been removed completely, sulphate, one of the major constituents of seawater, begins to act as an electron acceptor thereby producing H$_2$S. Sulphides are highly toxic and are hazardous to marine organisms (Richards, 1965).

Each of the above pathways of respiration (oxic, suboxic and anoxic metabolisms which respectively make use of O$_2$, NO$_3^-$ and sulphate as the terminal electron acceptors) has its own stoichiometry i.e. the ratios in which the oxidant is consumed and metabolic products are regenerated (Richards, 1965). However, departures from these expected ratios have been known to occur and such departures provide important insights into biogeochemical cycling in a particular environment. One of the principal objectives of the present study is to investigate the ratios of concomitant changes in order to gain such insights for the region under investigation that experiences diverse redox conditions. Limited data on natural nitrogen isotope abundance in NO$_3^-$ will also be examined and discussed.
5.2 Pathways of Oxidation of Organic Matter

For expressing the composition of organic matter in the oceans the oxidation states of carbon, nitrogen and phosphorus are assumed to be as in carbohydrates, ammonia, and orthophosphoric acid, respectively, and the organic matter is empirically formulated as \((CH_2O)_{106} (NH_3)_{16} H_3PO_4\); degradation of this hypothetical organic matter through different pathways can then be expressed as follows (Richards, 1965):

### 5.2.1 Aerobic Respiration

\[
(CH_2O)_{106} (NH_3)_{16} H_3PO_4 = 106 CH_2O + 16 NH_3 + H_3PO_4
\]

\[
106 CH_2O + 106 O_2 = 106 CO_2 + 106 H_2O
\]

\[
16 NH_3 + 32 O_2 = 16 HNO_3 + 16H_2O
\]

Addition of above equations yields,

\[
(CH_2O)_{106} (NH_3)_{16} H_3PO_4 + 138 O_2 = 106 CO_2 + 16 HNO_3 + H_3PO_4 + 122 H_2O
\]  \(\text{---- (i)}\)

This reaction implies that for the complete oxidation of organic matter 276 atoms of oxygen are consumed that leads to the regeneration of 106 atoms of carbon as CO\(_2\), 16 atoms of nitrogen as NO\(_3^-\) and 1 atom of phosphorus as H\(_3PO_4\).

### 5.2.2 Denitrification

The uncertainty in expressing this process arises from the unknown fate of ammonia released from the organic matter. That is, while in the aerobic degradation it is known to get oxidized to NO\(_3^-\) (nitrification), it is not still clear
whether it is oxidized by NO$_3^-$ in denitrification. Without such oxidation the reaction can be expressed as:

$$(CH_2O)_{106} (NH_3)_{16} H_3PO_4 + 84.8HNO_3 =$$

$$106 CO_2 + 42.4 N_2 + 16 NH_3 + H_3PO_4 + 148.4 H_2O \quad ---- (ii)$$

and if the oxidation of NH$_3$ proceeds as follows:

$$5NH_3 + 3HNO_3 = 4N_2 + 9H_2O$$

then the overall reaction becomes:

$$(CH_2O)_{106} (NH_3)_{16} H_3PO_4 + 94.4 HNO_3 =$$

$$106 CO_2 + 55.2 N_2 + H_3PO_4 + 177.2 H_2O \quad ---- (iii)$$

Reaction (iii) predicts that in the degradation of organic matter by NO$_3^-$, for each 94.4 atoms of nitrogen used as NO$_3^-$, 106 atoms of carbon are regenerated as CO$_2$ and 1 atom of phosphorus is released as H$_3$PO$_4$.

5.2.3 Sulphate Reduction

There is lesser uncertainty in expressing the degradation of organic matter with sulphate as an electron acceptor because NH$_3$ regenerated from organic matter cannot be oxidized by sulphate. The reaction can be expressed as follows:

$$(CH_2O)_{106} (NH_3)_{16} H_3PO_4 + 53SO_4^{2-} =$$

$$106 CO_2 + 53 S^{2-} + 16 NH_3 + H_3PO_4 + 106 H_2O \quad ----(iv)$$
It indicates that for 53 atoms of sulphur used as sulphate are converted to sulphide, 106 atoms of carbon are regenerated as CO₂, 16 atoms of nitrogen are regenerated as NH₃ and 1 atom of phosphorus is regenerated as H₃PO₄.

Organic matter degradation also occurs using other electron acceptors the most important of which are Mn(IV), Fe (III), I(V) (iodate) and CO₂. For the first three oxidants the free energy change is comparable to that of denitrification, so they are expected to proceed at the same time as denitrification (Farrenkopf et al., 1997). In the case of CO₂ the free energy change is least favourable (it occurs after sulphate reduction). In any case, the concentrations of these species are much smaller than that NO₃⁻, and so their contribution to respiration in water column can be assumed to be insignificant.

5.3 Significance of the Study

The Arabian Sea is one of very few areas of the world oceans where all the three major respiratory pathways are found to occur in the open coastal waters. It therefore provides an excellent opportunity for testing the predicted stoichiometric relationships associated with the organic matter decomposition and how these might be modified by local processes. The present study provides the first ever detailed data from one single environment outside the land locked seas where both oxic and suboxic/anoxic metabolisms occur in close spatial and temporal proximity to each other. These data are expected to improve our understanding of biogeochemical transformations in the O₂-depleted oceanic environments.
5.4 Methodology

The large amount of data presented here originated from various cruises of ORV Sagar Kanya, FORV Sagar Sampada, AA Sidorenko, and CRV Sagar Sukti during the period 1997-2002 (Table 2.1). Data from 294 stations covering the western continental shelf of India (depth ≤ 200 m) have been utilized. These data includes dissolved O$_2$, dissolved inorganic nitrogen (DIN = NO$_3^-$ + NO$_2^-$ + NH$_4^+$), dissolved inorganic phosphorus (DIP = H$_2$PO$_4^-$ + HPO$_4^{2-}$ + PO$_4^{3-}$, often expressed simply as PO$_4^{3-}$, the analytically-determined "phosphate" concentration) and hydrogen sulphide (H$_2$S). Inter-relationships between various parameters were examined through least squares regression analyses for selected pairs of variables viz. DIN versus DIP, DIP versus H$_2$S; and NH$_4^+$ versus H$_2$S.

5.5 Results

The DIP versus DIN plot for the pooled data shows three interesting trends (Fig 5.1a). The trend of oxic degradation can be easily discerned from the cluster of points around the expected Redfield relationship (16:1). The second trend is that of denitrification. The reason for the third trend (rapid increase in DIP at low DIN levels) is less apparent. All the three trends exhibited a great deal of scatter. In order to better resolve the effects resulting from various metabolic pathways, the data were further processed as follows:

For the first trend DIP and DIN data were selected for the samples containing NO$_2^-$ < 1μM, DIP < 2.5μM and NO$_3^-$ > 0. The purpose of this filtering procedure was to isolate the samples affected only by aerobic respiration. The reason for selecting NO$_2^-$ < 1μM is that NO$_2^-$ is an immediate
Fig. 5.1. Plots of DIN vs DIP for
(a) all samples taken for depths < 200m.
(b) samples not experiencing reducing conditions
(c) samples experiencing denitrification
(d) samples experiencing sulphate reduction
5.6 Discussion

The slope of the DIN/DIP regression line in oxic waters (13.74) is close to the Redfield value (16). It is quite likely that in spite of the efforts to filter out data affected by denitrification, the relationship might still have been influenced by this process (i.e. waters not experiencing denitrification might still show NO$_3^-$ deficits due to mixing with waters affected by denitrification; Codispoti et al., 2001).

The slope of the DIN/DIP ratio for denitrifying water (-79.1) is higher than Richards' (1965) value (-94.4) for the case when NH$_3$ is oxidized by NO$_3^-$ but close to the value (-84.8) for the case when such an oxidation does not take place. While it may be argued that, given the large scatter in the data, the observed difference could well be within the margin of error of computations, it should be pointed out that there is some evidence in the literature indicating that a substantial part of NH$_3$ released from organic matter may, in fact, be assimilated by organisms instead of getting oxidized to N$_2$ (Lipschultz et al., 1990). There are no previous estimates in the literature based on field measurements of DIN/DIP ratio for denitrification with which to compare the present results. It may be pointed out that the DIN/DIP ratio may be even higher (i.e. less negative) in case of a nitrification denitrification coupling (e.g. -81.6; Codispoti and Christensen, 1985). However, results of the present study cannot differentiate between these possibilities.

The results presented in Fig. 5.1 may be visualised in terms of spatio-temporal evolution of anoxia in a parcel of initially oxygenated water parcel as it moves up over the continental shelf during upwelling and undergoes sequential changes in redox conditions. First, the decay of organic matter in
the presence of O₂ increases the concentrations of both DIN and DIP in conformity with the Redfield stoichiometry (represented by line I in Fig. 5.1a) until the O₂ falls below the threshold for denitrification to set in (<0.02 ml l⁻¹; Morrison et al., 1999; Naqvi et al., 2003). Once this threshold is crossed (at this point DIN concentration is on an average ~24 μM), NO₃⁻ is used by the bacteria, and the concentration of DIN decreases rapidly, but that of DIP increases slowly. As discussed above, the relative changes in DIN and DIP during this transition (represented by Line II in Fig. 5.1a) conform to theoretical considerations [reaction (ii)].

When the system turns anoxic (after complete loss of NO₃⁻ that seems to take place when DIP concentration reaches on an average 2.15 μM), the relative changes in DIN and DIP exhibit complete departure from the theoretical value. That is, as NH₃ released from organic matter cannot be oxidized by sulphate, one would expect the DIN and DIP values to fall along the hypothetical Line III in Fig. 5.1a. Such is not the case. Instead, all data points are located to the left and above this line. Moreover, while there is considerable scatter in data, there seems to be a suggestion of a nearly linear trend (Line IV in Fig. 5.1a) extending toward the origin of the graph. It is obvious that processes other than simple degradation of organic matter by sulphate should be responsible for this trend.

Under oxidizing conditions (Eh>~200 mV), PO₄³⁻ is known to get adsorbed onto iron hydroxide thereby forming complex iron-oxyhydroxophosphates (FeOP), which settles to seafloor; PO₄³⁻ is released back from sediments to the overlying water when FeOP complex dissolves under reducing conditions (Shaffer 1986). The complex is formed when Fe²⁺/(HPO₄²⁻-
+H₂PO₄⁻) ratio exceeds 2 (Gunnars et al, 2002). It would appear that once the overlying waters turn anoxic over the Indian shelf PO₄³⁻ is mobilized in large quantities from the sediments. The Fe²⁺ released into solution in reducing waters may be removed as its insoluble sulphide but PO₄³⁻ is expected to accumulate (Gächter and Müller, 2003). This appears to be the most probable cause of the observed departure of the DIN/DIP variations from the theoretical trend in anoxic waters.

The extension of the anoxic trend to waters with low DIN and DIP values can be explained as follows: During the later part of the SWM and early Fall Intermonsoon, the anoxic subsurface layer is directly overlain by the oxic layer without a transient suboxic layer separating the two (see, for example, Fig. 4.16). The upper layer is nutrient-depleted, while the lower one has high DIP and moderate amounts of DIN (present almost entirely as NH₄⁺). Mixing between these two end-members is expected to lead to DIP and DIN concentrations that should cluster around Line IV in Fig. 5.1a.

The relation between H₂S and DIP is linear with a slope of 7.62. This value is much lower than the theoretical value (53) given by Redfield et al. (1963) and Richards (1965). As in the case of the DIN/DIP relationship, this departure can also be ascribed to the mobilization of PO₄³⁻ from the sediments. Additionally, oxidative loss of H₂S is also expected to result in lower H₂S /DIP ratio.

Relationship between H₂S and NH₄ exhibited a slope value of 4.79, which is unexpectedly higher than the theoretical value of 3.3. However, the scatter in data is large with significant intercept on the NH₄⁺ axis (Fig. 5.3). This means that NH₄⁺ found in water at very low H₂S concentrations is
probably not produced during sulphate reduction and could have diffused out of sediments. Normally one would expect \( \text{H}_2\text{S} \) to be oxidized quickly in near surface waters, where the oxidation of \( \text{NH}_4^+ \) may be inhibited by light. Moreover, anoxic conditions have been observed to inhibit (oxygenic) photosynthesis (Fig. 4.16), and while there is a possibility that anoxygenic photosynthesis and chemosynthesis could occur in the anoxic waters, the \( \text{NH}_4^+ \) uptake rates should not be very high (much lower than the sulphide oxidation rate). The generally low concentrations of \( \text{H}_2\text{S} \) do strongly point to its loss through oxidation. Such a loss is, in fact, suggested by data from individual stations — for example, at Sta. M1a occupied on SaSuPl2, that experienced well-developed anoxic conditions, \( \text{H}_2\text{S} \) and \( \text{NH}_4^+ \) showed excellent linear relationship with a slope of 1.74 (Fig. 5.4). Thus, the higher slope value obtained from the pooled data is not easy to explain.

5.7 Implications for Biogeochemical Cycles

Fixation of molecular nitrogen is an important source of biologically useful nitrogen in oceanic surface waters that increases the productivity of phytoplankton, thus influencing the global carbon cycle (Falkowski, 1997). Most of the marine nitrogen fixation is carried out by the cyanobacterium *Trichodesmium* (Capone et al., 1997) and in some areas of the ocean as much as half of the new nitrogen could come through this pathway (Karl et al., 1997). The enzyme nitrogenase responsible for N-fixation has a high requirement for iron and this may lead to Fe limitation of N-fixation in remote parts of the sea (Falkowski, 1997; Codispoti et al., 2001). One other
Fig. 5.4. Relationship between $\text{H}_2\text{S}$ and $\text{NH}_4^+$ at the Sta SaSu M1A (see Fig. 4.16 for vertical profiles)
necessary condition for N-fixation to take place is that $\text{PO}_4^{3-}$ must be present in sufficient quantities.

The results of the present study clearly demonstrate that anoxic conditions lead to a large excess of $\text{PO}_4^{3-}$ in coastal waters, which in conjunction with adequate supply of Fe (both from land and through mobilization in anoxic waters) should prime the system for N-fixation. This implies that N-fixation could be tightly coupled with denitrification in waters over the western Indian continental shelf and also probably on a global scale. It is therefore not surprising that extensive blooms of *Trichodesmium* occur on a regular basis in the eastern Arabian Sea following the cessation of upwelling (during the NEM and SI periods). Such blooms add new nitrogen to the system, partially compensating for the loss through denitrification and contributing significantly to biogeochemical cycles (Devassy et al., 1978).

5.8 Isotopic Composition of Nitrate

Limited measurements of nitrogen isotope ratio in $\text{NO}_3^-$, expressed as $\delta^{15}\text{N}$ relative to air$^*$ ($\delta^{15}\text{N-NO}_3^-$), were made on two occasions — on cruise SS158 (in August 1997) off Mangalore and on field trip Candolim 22 (in September 2000) off Goa. On both occasions the waters sampled were affected by denitrification.

Hydrographic and chemical data at station SS 3939 along with the $\delta^{15}\text{N-NO}_3^-$ values are plotted against depth in Fig. 5.5. Temperatures and salinity profiles were typical of this region for the survey period, showing strong near-surface thermohaline stratification and isothermal and isohaline

$^*\delta^{15}\text{N} = \left[\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{sample}} / \left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{air-N}_2} - 1\right] \times 1000$
Fig. 5.5. Vertical profiles of temperature, salinity, density, inorganic nitrogen species and δ¹⁵N of NO₃⁻ at Sta 3939 (13.126°N; 74.631°E) sampled on 30/8/1997
conditions below the shallow pycnocline. Similarly, the two deepest samples also experienced near-complete O₂ depletion that is characteristic of the sampling period. NO₃⁻ profile exhibited a mid-depth maximum below which concentrations decreased due to loss through denitrification while the NO₂⁻ concentrations showed a concomitant increase with depth. NO₃⁻ deficit was calculated as the difference between the expected and observed NO₃⁻ concentrations [the former was approximately quantified by dividing the apparent oxygen utilization (AOU) with 8.65, the ratio between O₂ consumption and NO₃⁻ regeneration for aerobic respiration; Richards, 1965]. This deficit increased with depth reaching the peak value of just under 15 μM in the deepest sample. While all the above parameters exhibited expected depthwise changes, the profile of δ¹⁵N-NO₃⁻ deviated greatly from that expected. That is, given the high NO₃⁻ deficit in subsurface waters the δ¹⁵N-NO₃⁻ values should have ranged between 17 and 26‰ in subsurface waters if the isotopic fractionation factor reported by Brandes et al. (1998) for the open ocean suboxic zone (25 ‰) was also applicable to the shallow suboxic zone. The values measured were consistently lower. In fact, all the four samples taken from within or below the pycnocline yielded δ¹⁵N-NO₃⁻ values (6.65-7.41 ‰) that were quite close to the oceanic average (5.7 ‰; Altabet et al., 1995; marked by the dashed vertical line in the figure) with no depthwise variability. NO₃⁻ in the only sample taken from the surface layer was distinctly lighter (3.43).

Three possible explanations may be offered for the above anomaly: (1) The value of the isotopic fractionation factor for the deeper perennial suboxic
zone may not be valid for the seasonal suboxic zone over the shelf. In support of this view there is some experimental evidence which suggests that the factor may vary with denitrification rate (Dr. K.K. Liu, personal communication); (2) Denitrification within the sediment might account for the bulk of NO$_3^-$ deficit observed in the water column. Relative to pelagic denitrification, the process in sediments results in little isotopic fractionation (~3 \%, Codispoti et al., 2001); (3) Low $\delta^{15}$N-NO$_3^-$ could be produced as a result of complete denitrification followed by mixing with freshly upwelled water. In order to further illustrate this possibility the following hypothetical scenario may be considered: Suppose a parcel of water is subjected to complete denitrification, it will lose both $^{15}$NO$_3^-$ and $^{14}$NO$_3$. Now if it is quickly diluted with equal volumes of freshly-upwelled water having a NO$_3^-$ content and $\delta^{15}$N of, say, 24 $\mu$M and 7 \%, respectively, the resultant mixture will possess the isotopic characteristics of the upwelled water (7 \%), but it will show a NO$_3^-$ deficit of 12 $\mu$M. The relative importance of the above possible factors in lowering the $\delta^{15}$N-NO$_3^-$ cannot be evaluated with the help of available data.

However, the other set of isotopic measurements did reveal enrichment of the heavier isotope in residual NO$_3^-$ (Table 5.1). In order to compare the magnitude of this enrichment with that observed in the offshore suboxic zone, an attempt was made to compute the fractionation factor, $\varepsilon_{\text{denit}}$ [=$10^3(1-\alpha)$], where $\alpha$ is the ratio between the rates of utilization of $^{15}$NO$_3^-$ and $^{14}$NO$_3$], using a simple advection-reaction model. This model ignores diffusion and the water mass is assumed to advect into the suboxic zone without undergoing
Table 5.1. Hydrographic, chemical and isotopic data at selected depths and locations along the Candolim transect sampled on 12/9/2000.

<table>
<thead>
<tr>
<th>Stn. No.</th>
<th>Depth (m)</th>
<th>Temperature (°C)</th>
<th>Salinity (‰)</th>
<th>( \text{O}_2 ) (µM)</th>
<th>( \text{NO}_3 ) (µM)</th>
<th>( \delta^{15} \text{N-NO}_3 ) (‰ relative to air)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15</td>
<td>22.20</td>
<td>36.03</td>
<td>4</td>
<td>2.45</td>
<td>22.5</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>22.10</td>
<td>35.70</td>
<td>4</td>
<td>9.63</td>
<td>15.4</td>
</tr>
<tr>
<td>5</td>
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<td>22.50</td>
<td>36.11</td>
<td>22</td>
<td>2.24</td>
<td>14.4</td>
</tr>
<tr>
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<td>21.60</td>
<td>35.74</td>
<td>4</td>
<td>15.37</td>
<td>6.5</td>
</tr>
</tbody>
</table>
any mixing in the vertical and horizontal directions. As the stations were located very close to each other, this assumption seems justifiable. The isotopic distribution could thus be modelled with a simple Rayleigh equation (Bender, 1990):

\[
\delta^{15}\text{N-NO}_3 = 10^3(\alpha - 1) \ln f_{\text{NO}_3} + (\delta^{15}\text{N-NO}_3)_{\text{init}}
\]

where \( f_{\text{NO}_3} \) is the ratio between the observed and expected \( \text{NO}_3^- + \text{NO}_2^- \) concentrations and \((\delta^{15}\text{N-NO}_3)_{\text{init}}\) gives the isotopic composition of the initial (unaltered) material.

The plot of \( \delta^{15}\text{N-NO}_3 \) versus \( \ln f_{\text{NO}_3} \) for the five samples (Fig. 5.6) from the Candolim transect indicates a good linear correlation \((r^2=0.91)\) with the slope of the regression \((-\varepsilon_{\text{denit}})\) line being \(-7.70\). Inclusion of data from Sta. SS3939 leads to a little change in the slope \((-7.21)\), but the correlation is deteriorated \((r^2=0.44)\). At the first glance these results appear to support the notion of lower fractionation factor in the coastal suboxic zone. However, as discussed above, the possibility of other factors being also responsible for pulling down the \( \delta^{15}\text{N} \) value of \( \text{NO}_3^- \) of coastal waters experiencing denitrification cannot be ruled out.
Fig. 5.6. The $\delta^{15}N$ - $NO_3^-$ (‰ relative to air) vs. the natural log of $f$.
(red circles indicate data at Sta. SS3939; Blue circles indicate data from Candolim transect).