Nitrate reductase activity in the subsurface waters of the Peru Current

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ABSTRACT

In March 1976 and 1977, nitrate reductase, nitrate, nitrite, and oxygen were measured between depths of 30 and 250m in the subsurface waters overlying the Peruvian continental shelf and shelf edge at 15S latitude. Oxygen concentrations of less than 1 ml/l began between 20 and 30m in both years. The oxygen deficient waters extended to the bottom on the shelf and to 440m at the shelf edge. Nitrate depletion was observed often throughout the water column in 1976, but it was observed only below 100m in March 1977. Sulfide was occasionally detected below 100m in March 1976, but was not detected at any location in 1977. Nitrate reductase activity was observed at depths ranging between 30 and 250m. The activity between 30 and 50m was likely of phytoplankton origin, while the activity from deeper waters was probably of bacterial origin. The distribution of NR activity could not be predicted from the nutrient, oxygen or chlorophyll distribution. In the core of the secondary nitrite maximum between 200 and 250m, nitrate reductase activity ranged from 1.1 to 1.4 ng-at N h^{-1} l^{-1} in March 1977. A year earlier, at the same location and depth, sulfide was observed. Because of the depth at which the activity occurred, its association with the secondary nitrite maximum, and the previous occurrence of both nitrate depletion and sulfate reduction, we concluded that this nitrate reductase activity represented bacterial respiration.

1. Introduction

Nitrate reduction to nitrite occurs in the oceanic euphotic zone as a result of phytoplankton nitrate assimilatory activity and it occurs in the oxygen minimum zone as a result of bacterial nitrate respiration (Richards, 1965 and Spencer, 1975). The reduction of nitrate beyond nitrite in the euphotic zone is rate-limited by the availability of a photosynthetically produced reducing agent, ferredoxin, and photosynthetically provided carbon skeletons for amino acid synthesis (Morris, 1974). In the oxygen minimum zone, the reduction of nitrate beyond nitrite is inhibited by oxygen and rate-limited by nitrate availability (Payne, 1976), but to a greater extent by the availability of denitrifying bacteria (Zobell, 1946; Delwiche and Bryan, 1976; Payne, 1973a and 1976). Because assimilatory nitrite reduction is

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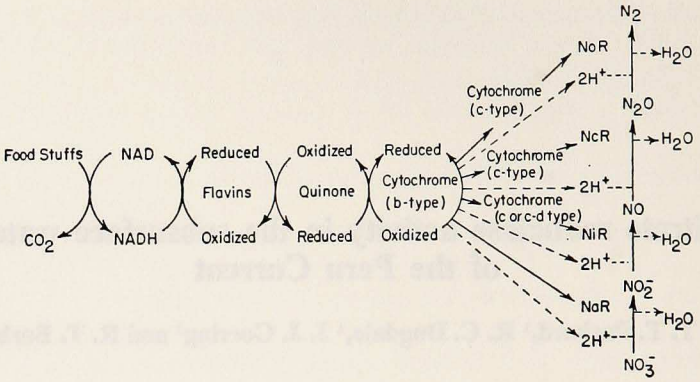

Figure 1. Reaction scheme for denitrification in bacteria according to Payne (1973b and 1976), Cox, Payne and Dervartanian (1971), Cox and Payne (1973) and Rowe *et al.* (1977). The proton flow (. . . .) is hypothetical. Abbreviations: NAD and NADH, nicotinamide adenine dinucleotide (oxidized and reduced forms); NaR, nitrate reductase; NiR, nitrite reductase; NcR, nitric oxide reductase; NoR, nitrous oxide reductase.

coupled to photosynthesis, the entire process of nitrate assimilation including nitrate reduction is limited to the euphotic zone unless the phytoplankton are cycled frequently from the darkened subsurface waters to the illuminated surface waters allowing photosynthetic maintenance of sufficient levels of ferredoxin and carbon skeletons. When the requirements for photosynthesis are not met, the phytoplankton will excrete nitrite and net growth ceases. This event is the likely origin of the primary nitrite maximum that is widely observed in the ocean (Vaccaro and Ryther, 1960), and as Blasco (1971) and Kiefer, Olson, and Holm-Hansen (1976) suggest, is an index of the bottom of the euphotic zone.

Below the euphotic zone and below the primary nitrite maximum, a secondary nitrite maximum is frequently observed if the *in situ* oxygen concentration is lower than 0.2 ml/l (Fiadeiro and Strickland, 1968). This deep nitrite maximum, so commonly observed in the eastern Pacific Ocean off the coasts of Mexico and Peru (Brandhorst, 1959; Codispoti, 1973a and b; and Wooster, Chow and Barrett, 1965), is the result of nitrate respiration, *i.e.* the metabolic process employed by bacteria when oxygen is no longer present in sufficient quantity to serve as an electron acceptor for the respiratory electron transport system. Nitrate respiration is the first step of denitrification (Fig. 1) in which NO_3^- is reduced to NO_2^- by the enzyme nitrate reductase. The NO_2^- is ultimately reduced to N_2 by the action of nitrite reductase, nitric oxide reductase and nitrous oxide reductase. The sequence is:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

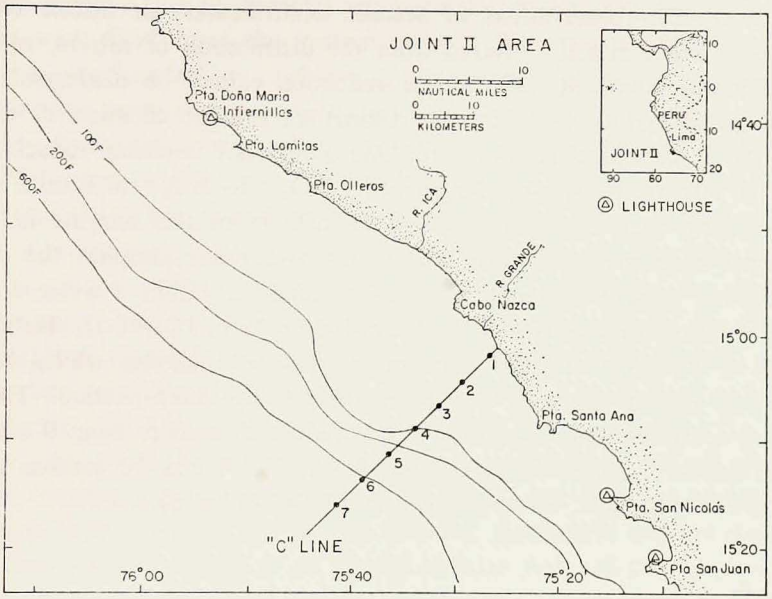

Figure 2. The location of the C-line stations on the continental shelf and shelf edge of southern Peru. This line was the area of focus for the CUEA expedition, JOINT-II, from March 1976 to June 1977. The R.V. *Anton Bruun* cruise 15 and the R.V. *T.G. Thompson Pisco* cruise also worked the same region.

This process would normally be inactive in waters closer than 75m to the sea surface unless the mixing by winds or currents were unusually weak, allowing oxygen depletion in the near-surface waters. In the Peru Current at 15S latitude, the primary nitrite maximum normally occurs between 20 and 50m and the secondary nitrite maximum between 75 and 350m (Wooster, Chow and Barrett, 1965; Fiadeiro and Strickland, 1968; and Dugdale, 1972). The depth limits of the secondary nitrite maximum are delineated by the extent of the O_2 minimum zone (Fiadeiro and Strickland, 1968; Dugdale, 1972) and because the oxygen gradients surrounding the secondary nitrite maximum are weak, these depth-limits must vary. Thus nitrate reduction and/or denitrification could expand into the shelf waters and to within 40m of the sea surface if the circulation became weaker and/or the organic load in the water became greater.

In March 1976 an expansion of the denitrification zone was observed (Dugdale *et al.*, 1977) in the region under study by the Coastal Upwelling Ecosystems Analysis (CUEA) program. Stations were made along a line (C-line) normal to the coast at 15°5′S and extending across the shelf and slope waters (Fig. 2). Along this line from location C-3 to C-5, NO_3^- depleted waters, indicating denitrification, extended from near bottom to as close as 40m to the surface. However,

as with most other observations of oceanic denitrification or nitrate reduction, the evidence was indirectly inferred from the distribution of nitrate, nitrite and oxygen. By this approach, relic nitrate reduction cannot be distinguished from active nitrate reduction. To make this distinction, a labile chemical or biological index of the process must be measured. One such index is nitrate reductase (E.C. 1.6.6.1), an enzyme responsible for reducing NO_3^- to NO_2^- in nitrate reducing and denitrifying bacteria. By measuring the activity of this enzyme in cell-free homogenates of particulate matter filtered from seawater samples, the detection of the site and relative magnitude of nitrate reduction within a water column is possible. On two recent cruises of the CUEA expedition, JOINT-II, this approach was used to investigate nitrate reduction in seawater samples which, from the nutrient and oxygen analyses, had been affected by denitrification. This communication describes observations of nitrate reductase activity from 9 stations of leg 1b of the R.V. *Alpha Helix* cruise (March, 1976) and 10 stations of leg 1 (March, 1977) of the R.V. *Wecoma* cruise (WELOC 77, leg 3).

2. Methods

Seawater samples for determination of nitrate reductase activity, chlorophyll, and some of the nutrient samples were collected using 30 1 PVC Niskin bottles attached to a rosette sampler. Samples for salinity and oxygen, and some nutrient samples were collected with 5 1 PVC Niskin bottles in a standard hydrographic cast. Soluble reactive nitrate, nitrite, ammonium, phosphate and silicic acid were measured within 2h in unfiltered samples by the methods of Murphy and Riley (1962) for phosphate, Wood *et al.* (1967) and Armstrong *et al.* (1967) for nitrate and silicic acid, Bendschneider and Robinson (1952) for nitrite, and Slawyk and MacIsaac (1972) for ammonium. All analyses were performed by the automated procedure of Friederich and Whitledge (1972). Chlorophyll α was measured by both the UNESCO (1966) and fluorescence (Lorenzen, 1966) methods. Oxygen was measured with a polarographic O_2 electrode (Radiometer-Copenhagen) on the R.V. *Alpha Helix* cruise and by the Winkler Technique (Carpenter, 1965) on the R.V. *Wecoma* cruise. Electrode readings from the R.V. *Alpha Helix* cruise were standardized against saturated surface seawater and nitrate depleted seawater from a depth just above the $S^=$ zone. Dissimilatory nitrate reductase (D-NR) activity was measured by a modification of the assay used by Fewson and Nicholas (1960 and 1961) in their study of the denitrifiers, *Micrococcus denitrificans* and *Pseudomonas aeruginosa*. This method is based on the colorimetric detection of NO_2^- that is produced in NO_3^- enriched cell-free extracts of bacteria (Hewitt and Nicholas, 1964, and Payne, 1973a) and is nearly the same as the assay for assimilatory nitrate reductase in phytoplankton (Eppley, Coatsworth, and Solórzano, 1969). Four liter subsamples were drawn from the Niskin bottles in

the same manner that O_2 samples are normally drawn. The bottles for the subsamples were filled from the bottom using a 30 cm glass tube; 8 liters were allowed to overflow before a subsample was capped. These precautions minimized contamination from atmospheric oxygen. The samples were usually filtered immediately, but when a delay was unavoidable, the samples were stored in the dark at 0-4°C for less than 2 hours. Four to 8 liters were siphoned from the subsample and filtered through a glass fiber filter (Gelman, Type A) in a Millipore filtration tulip at a pressure differential of less than 1/3 atm. The effective pore size (99.9% retention) of these filters is 1 μm, but it becomes rapidly smaller upon loading. Bacteria would not have been retained until loading had reduced the effective pore size. This was accomplished before the second liter had been filtered. After filtration the filter was immediately ground at 0-4°C for 2 min. in 3 ml of 0.2 M phosphate buffer (pH 7.9) containing 9 mg polyvinyl pyrrolidone and 0.5 mg dithiothreotol. One ml of the crude homogenate was reacted with 0.8 ml of a substrate mixture containing 240 μM NADH, 8mM KNO_3 and 110 μM $MgSO_4$ in 0.2 M phosphate buffer (pH 7.9) for 20 min. at 15°C. Incubation was not done anaerobically, because Van T'Riet *et al.* (1968) showed that cell-free extracts of *Aerobacter aerogenes* retain 70% of their dissimilatory nitrate reductase activity after 1 h exposure to air at 30°C. From the data of Van T'Riet *et al.* (1968) we calculated that 1/2 h exposure to air reduced the activity in our assays by somewhat less than 20%. The reaction was quenched by adding 0.2 ml 1 M Zn acetate and 5 ml ethanol. The cloudy mixture was clarified by centrifugation. The supernatent fluid was decanted and mixed with 1 ml of 58 mM sulfanilamide. Two minutes later 1 ml of 3.9 mM N-(1-naphthyl)-ethylenediamine hydrochloride was added and mixed into solution. Controls were run through an identical procedure except that in the controls, NADH was omitted from the substrate mixture. Between 10 minutes and 1 h after the N-(1-naphthyl)-ethylenediamine hydrochloride was added, the solutions from the assays were diluted to 10 ml with ethanol and were scanned against the controls from 750 to 450 nm with a Beckman Acta IIC spectrophotometer. The diazo product of the reaction of NO_2^- with sulfanilamide and N-(1-naphthyl)-ethylenediamine hydrochloride has an absorbtion peak at 543 nm. By scanning the assay against the control, only the NO_2^- produced by NADH-stimulated nitrate reduction was detected (Fig. 3). A separate assay based on the material filtered from 1 ml of seawater was used as the "zero-level" enzyme control (lower panel, Fig. 3). The activities in units of nanogram-at h^{-1} l^{-1} were calculated from the expression: $\text{D-NR} = 3 \times F \times H \times A/(C \times V)$ where F is the calibration factor relating NO_2^- concentration to absorbance at 543 nm (435 ng-at per absorbance unit in this study), H is the homogenate volume in ml, C is the cell length (10 cm in this study), A is the absorbance corrected by the "zero-level" enzyme control, V is the volume of seawater filtered (4-8 l) and 3 converts the activity units from min to h.

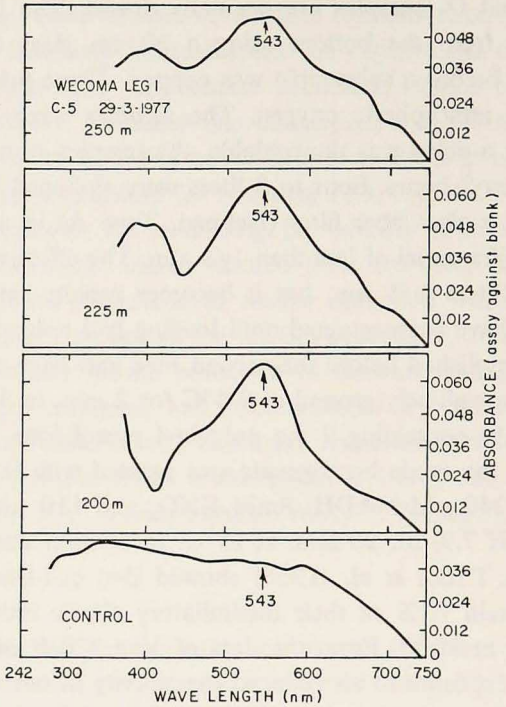

Figure 3. Difference spectra for 3 deep-nitrate reductase measurements at station 46 of the R.V. *Wecoma* cruise. The absorption maxima at 543 nm indicates the level of D-NR activity. Each assay is internally corrected for pigments and endogenous NO_2^-. The control for zero-level enzyme activity is shown in the lower panel.

3. Results and Discussion

a. Sea surface conditions. In March 1976 we observed low winds (0-10 knots), calm and stable surface waters, and NO_3^- depleted subsurface waters along the *C*-line (Dugdale et al., 1977). Dense populations of medusae and extensive blooms of the naked dinoflagellate, *Gymnodinium splendens,* an organism noted by its preference for stable waters (Margalef, in press), were found in the surface waters. The blooms extended from 5S to 15S and were so rich that chlorophyll *a* concentrations as high as 280 μg/1 were recorded during midday mapping surveys. The chlorophyll *a* at the sea surface during the morning productivity stations ranged from 1.0 to 75.6 μg/1. The mean for all stations was 18.4 μg/1.

In March 1977 the surface water conditions differed from the conditions in 1976. Instead of *G. spendens* blooms, the surface waters were colored by blooms of the photosynthetic ciliate, *Mesodinium rubrum.* They occurred along fronts over the shelf and slope. Ryther (1967) and Barber *et al.* (1969) have previously described these blooms along fronts in the Peruvian upwelling system and

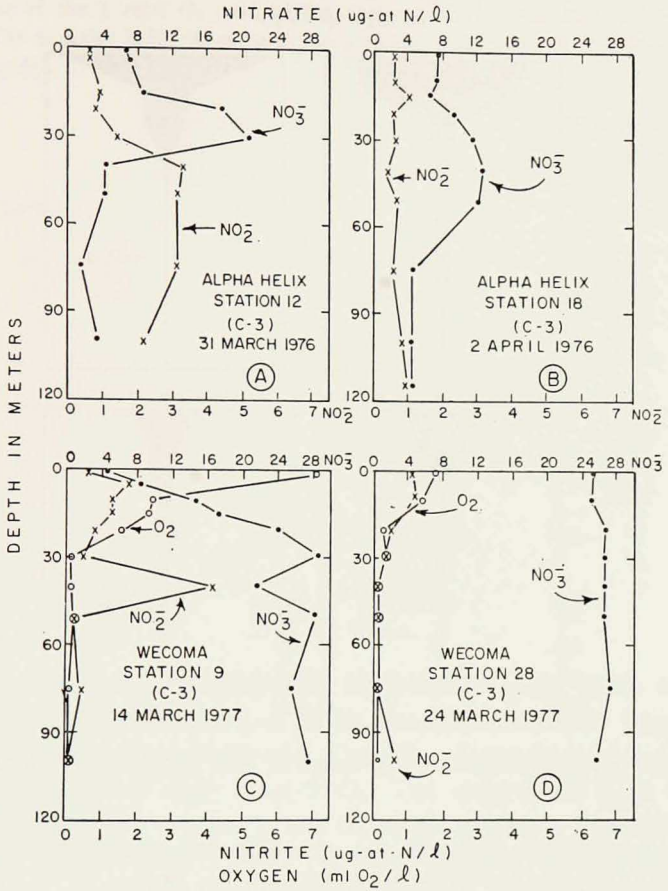

Figure 4. Nitrate and nitrite profiles for location C-3 on 2 days in 1976 (R.V. *Alpha Helix* cruise) and on 2 days in 1977 (R.V. *Wecoma* cruise). The difference in each pair represents the short term variability of the chemical properties at C-3.

Packard *et al.* (in press) predicted the location of *M. rubrum* within the frontal zones. Although dramatic in appearance, these blooms did not affect the standing phytoplankton crop as much as did *G. splendens* the previous year. Surface chlorophyll *a* at all productivity stations in March 1977 ranged from 1.3 to 19.2 μg/1; the mean was 5.1 μg/1, less than 1/3 of the value from the previous year. The upwelling in both years as indicated by temperature, was not markedly different. Sea surface temperatures at C-3 for 13 days (28 March to 8 April, 1976) averaged 16.65°C and for a slightly earlier period in 1977, they averaged 17.17°C. Surface temperature usually indicates upwelling intensity in that area (Dugdale, 1972).

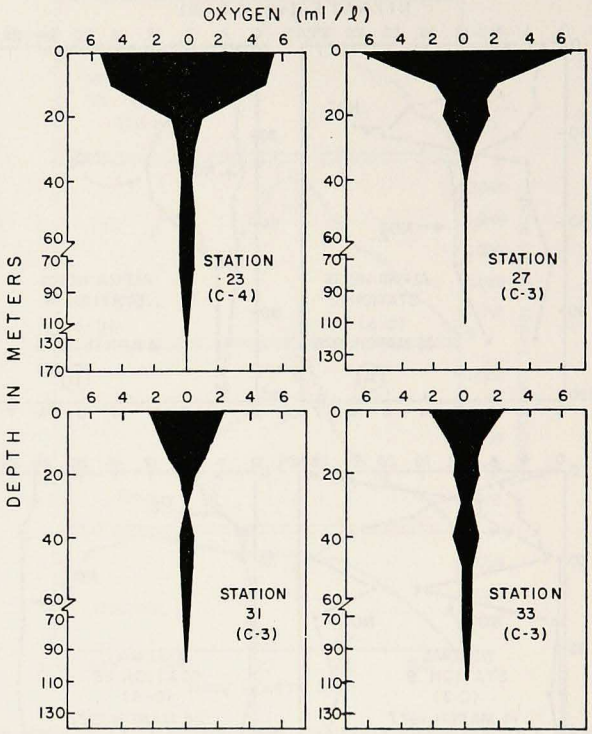

Figure 5. Oxygen profiles through the shelf waters off Peru in 1976 (R.V. *Alpha Helix* cruise).

b. Subsurface conditions. Although the upwelling was similar in the 2 years, the nitrate concentrations below 20m were different. In March 1977, the subsurface waters were twice as rich in nitrate as they were in March-April, 1976. The profiles of NO_3^- in March 1976 had a maximum just below the euphotic zone (30m) and a minimum between 40 and 300m, depending on the proximity to the coast. Nitrite profiles had subsurface maxima between 30 and 100m on the shelf (locations C-1 to C-4 in Fig. 2) and between 100 and 300m on the slope. Dugdale *et al.* (1977) discuss the conditions at the slope location C-5 where the NO_3^- in the water column had become exhausted and the bacterial electron transport system had started to use $SO_4^=$ as the electron acceptor with the result that $S^=$ began to accumulate in the water column. Less extreme cases of NO_3^- depletion at location C-3 are shown in Figure 4A and B. The profiles from stations 12 and 18 show low levels of NO_3^- between 40 and 100m. The NO_3^- levels at these stations and depths ranged from 0 to 12 μg-at/1. In other years (1969, 1970 and 1977), the NO_3^- at these depths ranged from 24 to 28 μg/1 (Dugdale, 1972 and Fig. 4C and D). Furthermore, in 1976, the nitrate minimum zone began at depths between 40 and 50m while in other years it began below 100m. In these other years, the seawater between 20 and 50m

Table 1. Depth of the 1 ml/l O_2 level at location C-3 during the R.V. *Alpha Helix* cruise (March, 1976) and the R.V. *Wecoma* cruise (March, 1977). Below this depth the oxygen decreased rapidly.

Station	March 1976 Depth of the 1 ml/1 O_2 level (m)	Station	March 1977 Depth of the 1 ml/1 O_2 level (m)
20	28	9	23
22	37	12	30
24	29	13	30
26	27	15	27
27	15	18	35
28	40	25	21
29	39	28	14
31	22	38	18
33	28	43	18
		44	27
$\bar{x}$	29	$\bar{x}$	24
S.D.	8	S.D.	7

was oxygenated (Fiadeiro and Strickland, 1968 and Dugdale, 1972), but in March 1976, the oxygen-deficient (O_2 < 1 ml/1) seawater was found between 20 and 30m and occasionally between 10 and 20m (Fig. 5 and Table 1). At times, the surface waters contained only 2 ml/1 O_2. The oxygenated layer was slightly shallower in March 1977 (Table 1). The O_2 profile at station 9 (location C-3, Fig. 2) showed that the O_2 fell to 0.2 ml/1 or lower at depths greater than 30m (Fig. 4C). The NO_3^- and NO_2^- profiles from station 9 showed NO_3^- minima and NO_2^- maxima at 40 and 75m (Fig. 4C). These maxima and minima, along with the low O_2 levels, provide evidence of dissimilatory NO_3^- reduction. A similar decrease in NO_3^- and an increase in NO_2^- between 30 and 40m at station 12 of the R.V. *Alpha Helix* cruise (Fig. 4A) provided similar evidence for dissimilatory NO_3^- reduction during the 1976 study. The NO_3^- and NO_2^- profiles from R.V. *Alpha Helix* stations 20, 23, 24, 27, 28, and 31 bore close resemblance to the profile from station 12. However, on the strength of the observations from station 12 alone, and before we had occupied the other stations, we predicted bacterial NO_3^- reduction was present below 30m and had begun to analyze homogenates of the particulate matter from these depths for NO_3^- reducing activity.

c. Nitrate reductase activity. Nitrate reductase in the oxygen-deficient seawater below the euphotic zone was measured in both years. The 1976 measurements ranged from 0 to 3.5 ng-at h^{-1} l^{-1} at depths between 50 and 125m (Table 2).

Table 2. Chemical and biological measurements in the NO_3^- impoverished subsurface waters of the Peru Current during the aguaje (Dugdale *et al.*, 1977) of March 1976. The units of D-NR are ng-at h^{-1} l^{-1}; NO_3^- and NO_2^- are in μg-at/l; O_2 is in ml/l; and chlorophyll *a* (Chl) is in μg/l. Sulfide was determined absent (−) or present (+) by smelling the seawater sample and the filtered particulate matter from it. All stations were taken from R.V. *Alpha Helix* and were located at C-3 (Fig. 2) except 24, which was at C-4.

Station	Depth	D-NR	NO_3^-	NO_2^-	O_2	$S^=$	Chl
18	100	0.03	5.48	0.57	—	—	0.91
20	100	3.53	0.37	0.36	0	—	0.77
22	100	0	0.21	1.17	0	—	1.12
23	75	0.98	1.30	0.23	0.2	—	0.52
	100	1.46	5.02	1.30	0	—	0.49
	125	1.94	0.07	0.11	0.1	—	0.76
24	75	0	0	0.98	0	—	1.28
	100	0.04	0.02	0.33	0.1	—	1.26
27	100	0	0.09	0.70	0	+	0.80
28	100	0.32	0	0.10	—	+	0.84
31	50	3.43	0.05	0.08	0.2	—	1.06
	100	0	0.79	1.46	0	—	0.89
33	75	0.74	0.02	0.07	0.1	+	0.66
	100	0.93	0.03	0.07	0	+	0.54

Table 3. Nitrate reductase activity (D-NR), NO_3^-, NO_2^-, O_2 and chlorophyll *a* (Chl) below the euphotic zone in the Peru Current. The stations are from the R.V. *Wecoma* and most are located off Punta San Juan (Fig. 2). The units of D-NR are ng-at h^{-1} l^{-1}; for NO_3^- and NO_2^-, μg-at/l; for O_2, ml/l, and for chlorophyll *a*, μg/l.

Station	Depth	D-NR	NO_3^-	NO_2^-	O_2	Chl
18(C-3)	50	2.10^{ψ}	25.76	0.27	0.23	0.25
	100	0.47*	25.61	0.66	0.12	0.11
19(C-3)	50	0^{ψ}	26.18	0.16	0.27	0.11
	100	0*	19.38	2.65	0.09	0.08
25(C-3)	40	0.40^{ψ}	21.31	0.12	0.10	0.24
28(C-3)	40	0^{ψ}	21.89	0.11	0.15	0.31
	100	0.12*	25.95	0.67	0.10	0.19
38(C-3)	100	0.07*	23.00	1.18	0.12	0.20
44(C-3)	43	1.10^{θ}	27.09	0.30	0.18	0.29
	100	0.29*	19.51	1.52	0.11	0.15
46(C-5)	43	1.10^{θ}	25.53	0.66	0.24	0.27
	100	0.29^{ψ}	27.67	0.05	0.15	0.06
	200	1.35*	9.90	10.58	0.18	0.15
	225	1.37*				0.13
	250	1.10*	22.17	5.25	0.14	0.06

θ indicates that the sample coincided with the bottom of the primary nitrite maximum.
* indicates that the sample is from the middle of the secondary nitrite maximum.
ψ indicates that the sample is from the NO_2^- minimum between the two maxima.

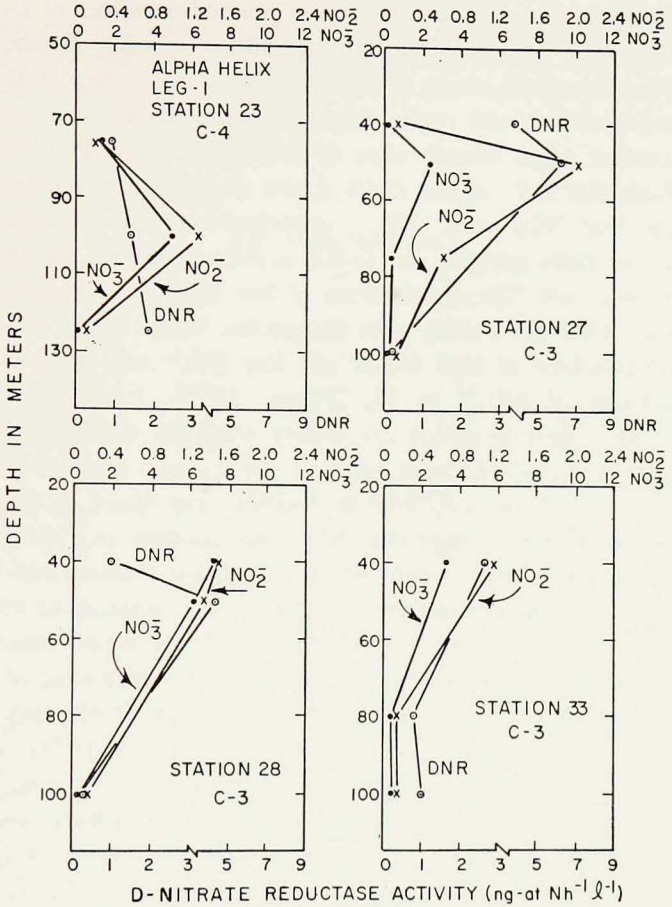

Figure 6. Deep-nitrate reductase, nitrate and nitrite profiles in the shelf waters off Peru in 1976 (R.V. *Alpha Helix* cruise).

Vertical profiles of D-NR activity, nitrite, and nitrate are shown in Figure 6; chlorophyll is given in Table 2. The 1977 measurements of D-NR activity (Table 3) ranged from 0 to 2.1 ng-at N h^{-1} 1^{-1} at depths between 40 and 100m and from 0 to 1.37 ng-at N h^{-1} 1^{-1} at depths between 100 and 250m. D-NR activity was independent of *in situ* O_2 within the low levels observed. Goering (1968) showed that denitrification can proceed at an O_2 concentration of 0.2 ml/1, but the rate was 1.7 times greater at an O_2 concentration of 0.02 ml/l. Payne (1973a and 1976) describes tolerance by denitrifiers of varying levels of O_2. These reports are not consistent with the oxygen inhibition described by Delwiche (1956), but a possible explanation is that enzyme synthesis, rather than the denitrifying reactions, is the most oxygen sensitive phase of denitrification (Payne, 1976).

Chlorophyll and nitrite were investigated as predictive indices of D-NR activity. If D-NR activity were associated with phytoplankton nitrogen metabolism, then a correlation with chlorophyll would be expected. None was found. If D-NR activity were associated with bacterial respiration, then elevated NO_2^- concentrations in NO_3^--rich seawater might identify sites of bacterial activity (Payne, 1976). Only one sample from the R.V. *Alpha Helix* cruise and five samples from the R.V. *Wecoma* cruise had NO_3^- and NO_2^- concentrations greater than 1 μg-at/l simultaneously. In these samples, the D-NR activity could not be predicted from the NO_2^- concentration. The observations of low D-NR and high NO_2^- are not easily explained, although mixing with oxygenated water could cause this condition. The observations of high D-NR and low NO_2^- are probably caused by complete reduction of NO_3^- to N_2 (Payne, 1976). Low NO_2^- and lower than normal NO_3^- concentrations are widely observed and reported as NO_3^- anomalies in the oxygen minimum zone of the eastern tropical Pacific Ocean (Codispoti, 1973a), but this condition is evidently not found in culture studies. Payne (1973a and 1976) reported that NO_2^- accumulates in stoichiometric proportion to the amount of NO_3^- reduced in denitrifying cultures and then is converted to N_2O or N_2 only after all the NO_3^- has been reduced. In explaining this phenomenon in cultures, Payne (1976) cites evidence from experiments with marine denitrifiers, *Pseudomonas perfectomarinus* and *P. aeruginosa,* of the suppression by NO_3^- of nitric oxide reduction (Payne and Riley, 1969) and the suppression by NO of nitrous oxide reduction (Payne, Riley and Cox, 1971). These studies showed that even as NO_2^- was being produced by nitrate reductase, the enzymes responsible for the reduction of NO_2^-, NO and N_2O (Fig. 1) were present, but did not function until NO reductase was derepressed by low levels of NO_3^-. Nitric oxide evidently is not released from bacterial cells (Rowe *et al.*, 1977, Barabee and Payne, 1967, and Payne, 1973a), so NO_2^- cannot be reduced sequentially to N_2O. This repression mechanism is not consistent with our observations or the observations of Thomas (1966), Goering (1968), Cline and Kaplan (1975), Codispoti (1973a) and Codispoti and Richards (1976) in the eastern tropical Pacific Ocean. This inconsistency suggests that the denitrifiers in the eastern tropical Pacific may reduce NO_3^- by a different mechanism that the one functioning in *P. perfectomarinus* and *P. aeroginosa.* Yoshinari and Knowles (1976) have recently presented evidence of different mechanisms for N_2O reduction in marine denitrifiers so differences in the NO_3^-, NO_2^- and NO reduction mechanisms are possible.

The measurement of D-NR activity in the subsurface waters at C-3 was taken as evidence of dissimilatory nitrate reduction by bacteria, but the contribution to the activity of phytoplankton assimilatory enzymes should not be overlooked. Table 4 presents nitrate reductase, oxygen, nutrient and chlorophyll levels in the dysphotic zone limited by the 1% light level and 50m. The NR activity could reflect a combination of bacteria and phytoplankton metabolism at all of the sta-

Table 4. Nitrate reductase activity, nutrients, oxygen and chlorophyll *a* (Chl) at R.V. *Alpha Helix* and R.V. *Wecoma* stations. The samples were taken between 20 and 50 m where phytoplankton biomass is relatively high. All the R.V. *Alpha Helix* stations and stations 25 and 26 of the R.V. *Wecoma* were located at C-3, the others were drogue stations in the vicinity of the C-line (Fig. 2). See Table 3 for units.

Station	Depth	D-NR	NO_3^-	NO_2^-	O_2	Chl
			R.V. *Alpha Helix* cruise			
27	40	3.84	0.08	0.12	0	1.26
	50	6.22	2.46	2.04	0	1.50
28	30	2.70	9.60	0.46	—	1.90
	40	1.01	7.30	1.47	—	0.64
	50	4.43	6.16	1.36	—	0.74
31	31	2.42	5.58	0.59	0.1	1.99
33	40	2.73	3.24	1.07	0.6	1.24
			R.V. *Wecoma* Cruise			
25	26	0	21.36	0.48	0.67	0.64
36	30	1.00	24.51	0.93	0.54	0.97
38	35	4.9	27.47	0.25	0.19	0.20
39	30	0.40	21.86	1.08	1.82	0.39
42	26	0.53	5.57	1.03	4.86	0.85

tions listed in Table 4 except R.V. *Wecoma* stations 39 and 42. At these stations, the high O_2 concentrations would probably repress the synthesis of dissimilatory NR. The observed activity at these two stations most likely reflects assimilatory NR activity. The contribution of assimilatory enzymes to the subsurface NR activity measurements would have been more unlikely had the measurements been made in seawater below 200m instead of in seawater in the upper 100m. The bottom topography precluded sampling deeper than 125m at C-3, but at C-5, samples could be taken down to 500m. A reconnaissance cast was made on 17 March, 1977, (R.V. *Wecoma*, station 14) which revealed evidence of dissimilatory nitrate reduction between 100 and 300m (Fig. 7). The process was intense between 200 to 300m, so this location was sampled again at station 46 (29 March). The results are shown in Fig. 8. The PO_4^{-3} and $Si(OH)_4$ profiles, the previous year, exhibited maxima between 150 and 250m where H_2S was being produced (Dugdale *et al.*, 1977). This year, when sulfate reduction was not being employed by bacteria, these maxima did not appear (Fig. 8B and D). The NO_3^- profile shows little evidence of dissimilatory nitrate reduction down to 150m but between 150m and 200m the NO_3^- drops from 27.5 to 9.9 μg-at/1. At 250m, the NO_3^- had increased to 22.2 μg-at/1. This NO_3^- minimum was not as low, nor as extended, as it was on 17 March (R.V. *Wecoma,* station 14). The NO_2^- profile at station 46 was nearly a mirror image of the NO_3^- profile, in that it remained low until 150m, increased sharply between 150 and 200m from 2 μg-at/1 to 10.6 μg-at/1, and

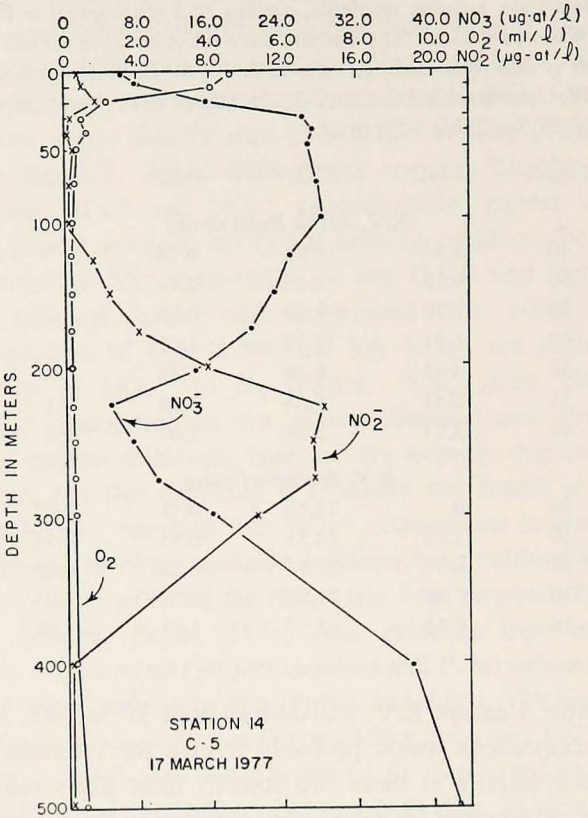

Figure 7. The secondary nitrite maximum at the shelf edge (C-5) on 17 March 1977 (R.V. *Wecoma*, station 14).

decreased to 5.3 µg-at/1 at 250m (Fig. 8). The D-NR activity was high (1.1 µg-at h^{-1} 1^{-1}) at 43m where only NO_2 (at 0.66 µg-at/1) showed any evidence of nitrate reduction. It was low (0.29 µg-at h^{1-} 1^{-1}) at 100m, but then increased to a plateau between 200 and 225m. Then it decreased from 1.37 to 1.10 µg-at h^{-1} 1^{-1} between 225 and 250m (Fig. 8). This profile confirms the evidence of nitrate reduction as given by the NO_3^- and NO_2^- profiles and provides evidence that the NO_3^- decrease in the water column resulted from a concurrent process rather than a historical one.

The question of whether denitrification was proceeding simultaneously with the nitrate reduction and the nitrite accumulation in the water column can be investigated by calculating the original NO_3^- level in the water column before the initiation of nitrate reduction. This can be done from the nitrate-silicic acid ratio and the *in situ* NO_3^- concentration. Using a moderate value of 1 for the nitrate-silicate ratio (Fig. 8B, 100 and 400m), the original NO_3^- at 200m at station 46 becomes

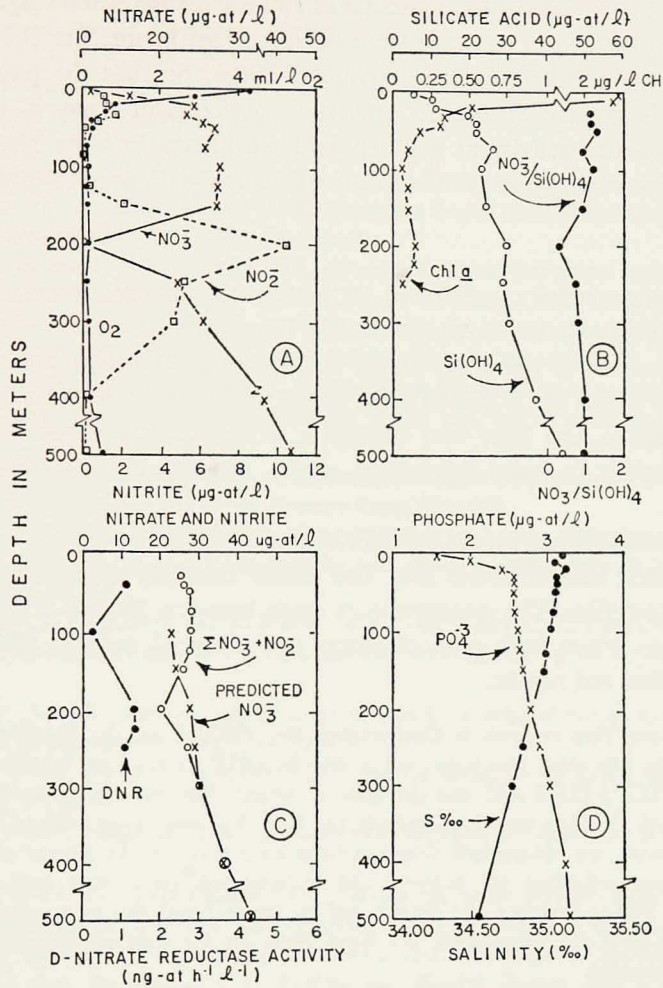

Figure 8. Deep-nitrate reductase activity, chlorophyll and associated chemical measurements in the secondary nitrite maximum at the shelf edge (C-5) on 29 March 1977 (R.V. *Wecoma*, station 46).

27.5 µg-at/1. The sum of *in situ* NO_3^- and NO_2^- was 20.5 µg-at/1, leaving a difference of 7 µg-at N/1 to have been reduced to NO, N_2O or N_2. Payne and co-workers (Rowe *et al.*, 1977, Payne, 1973a, and Barabee and Payne, 1967) have found that NO is not released from bacteria, thus the deficit in NO_3^- and NO_2^- at 200m must have resulted in the production of 7 µg-at/1 of N_2O or N_2. At station 14, even more denitrification occurred concurrently with NO_2^- accumulation.

The relative contribution of phytoplankton nitrate reductase activity at station 46 remains uncertain. The chlorophyll α profile shows a subsurface maximum of

0.15 μg/1 at 200m as compared to minima at 100 and 250m of 0.06 μg/1 (Fig. 8). However, if phytoplankton enzymes were an important factor, the D-NR activity at 43m should be greater than the activity at 200m, because the phytoplankton biomass level is greater (Table 3) and the physiological state is presumably healthier. The greater activity at depth provides evidence that the phytoplankton enzymes were not of major importance in deep water D-NR activity measurements.

4. Conclusion

Nitrate reductase activity was found in the core of the secondary nitrite maximum off the Peru coast in March 1977. The previous year, nitrate depletion and sulfide were observed at the same depths and location. The core layer, at 200 to 250m, lies well below the euphotic zone where phytoplankton enzymes might be active. Furthermore, the layer had negligible oxygen and a nitrate deficit of 7 μg-at/1, implicating bacterial respiration as the source of the nitrate reductase activity.

Nitrate reductase was measured at other depths and locations in the same region, and many of these measurements may also reflect bacterial respiration, especially the samples below 50m. The measurements made between 30 and 50m were from a transition zone where both phytoplankton and bacterial enzyme activities contribute to the observed results.

Acknowledgments: This research is Contribution No. 77020 from the Bigelow Laboratory for Ocean Sciences. The work was supported in part by ONR contract no. N00014-76-C-0271, NSF grant no. OEC-75-23718 A01 and the State of Maine. The nutrient determinations were run by R. Shepherd, W. Skea and G. Grunseich, the R.V. *Wecoma* oxygen determinations were made by D. Boisseau, the chlorophyll determinations were made by D. Blasco and J. Kogelschatz. J. MacIsaac organized the nutrient and hydrographic data from both cruises into useable form. J. Rollins executed the figures and V. Jones typed the manuscript. D. Blasco constructively criticized the manuscript. We thank them all for their conscientious assistance and cheerful cooperation.

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Received: *7 November, 1977;* Revised: *15 December, 1977.*