Denitrification Rates in an Island Bay in the Equatorial Pacific Ocean

Abstract. *Experimental data show that ^{15}N-labeled nitrate can be converted to molecular nitrogen in sea water containing little or no oxygen. The process is presumed to be biochemical. No evidence of the reaction was found in the water of the Peru current, which is low in oxygen but high in nitrite.*

Previous studies of denitrification in the oceans (*1*) have depended on the calculation of rates from nitrogen balances or from estimates of the amount of decomposition of organic matter as indicated by changes in the contents of oxygen, sulfide, and phosphorus in the water. We have now collected direct evidence that ^{15}N-labeled nitrate, when added to sea water containing little or no oxygen, can be converted to molecular nitrogen.

Our data were collected in the bay of Isla Genovesa, one of the Galápagos Islands, located about 1200 km off the coast of Ecuador. The exchange of water with the ocean is limited by a shallow entrance, about 15 m deep and 2 km long, which probably prevents horizontal exchange of water much deeper than the thermocline (about sill depth). Inside the entrance, the depth increases sharply to a maximum of about 240 m. Hydrogen sulfide odors in water collected from 175 and 225 m suggest that the water is anoxic.

The ^{15}N method used to measure denitrification rates in this study has been described (*2*). Briefly, ^{15}N-labeled nitrate is added to sea water, each liter containing 160 μg-atom of ^{15}N incorporated into KNO_3 (95.7 atom percent ^{15}N). After incubation in the dark at *in situ* temperatures for 108 hours, the dissolved molecular nitrogen is analyzed by mass spectrometry for increase in ^{15}N. The gases extracted from the water were passed through a liquid-nitrogen trap. With this procedure the oxides of nitrogen are trapped, allowing only molecular nitrogen to be analyzed for nitrogen isotopes. The ^{15}N method eliminates the necessity of estimating the amount of nonbiogenic molecular nitrogen dissolved in the water, a step which is required when denitrification rates are estimated from quantities of nitrogen in excess of that expected from its solubility. Phosphate was measured by the method of Murphy and Riley (*3*); nitrate by the method of Grasshoff (*4*); nitrite by the Griess method outlined in Strickland and Parsons (*5*); ammonia by the hypochlorite method of Richards and Kletsch (*6*); and oxygen by the classical Winkler procedure.

The fraction of total molecular nitrogen evolved from ^{15}N-labeled nitrate during incubation was calculated by the method of Hauck, Melsted, and Yankwich (*7*). Their method considers the situation in which the nitrogen molecules originate from more than one source (original dissolved nitrogen and that released from nitrate) and in which no common intermediate, where isotopic pooling can occur, is formed. The weight of nitrogen dissolved in the experimental samples multiplied by the fraction of the total evolved gives an estimate of the weight of nitrogen produced during incubation. The weights of nitrogen evolved given in Table 1 were calculated, the saturation with nitrogen being assumed at a chlorinity of 20 per mil and a temperature of 18°C, the approximate chlorinity and temperature *in situ*. The nitrogen solubility value was taken from Rakestraw and Emmel (*8*).

The fraction of total dissolved molecular nitrogen evolved as a result of denitrification of nitrate (highly enriched with ^{15}N) in the water of Isla Genovesa bay is given in Table 1. Measurable rates were obtained at 125, 175, and 225 m; the rate was significantly higher at 175 m than those at 125 and 225 m, which were similar. The rates measured are comparable to denitrification rates in anoxic lake water (*2*). These results confirm the indirect evidence obtained by others (*9*, *10*) that reduction of nitrate to molecular nitrogen does proceed at significant rates in sea water containing little or no dissolved oxygen.

Dissolved oxygen was not measured in this bay, but it was presumed to be low or absent in the water at 175 and 225 m because odors of hydrogen sulfide were detected. Sulfides are oxidized rapidly by oxygen; therefore, concentrations of hydrogen sulfide large enough to allow detection by odor would not be expected in the presence of oxygen.

The concentration of nutrients at the various sampling depths is included in Table 1. The absence of nitrate and nitrite at 125, 175, and 225 m, or their presence in low concentrations, is clearly the result of denitrification, where nitrate and nitrite ions serve as electron acceptors during the biochemical oxidation of organic matter. Large concentrations of ammonia and phosphate below the thermocline indicate restricted circulation which results in a nutrient trap. The concentrations of phosphate are about two times greater and ammonia about five times greater in the bay than concentrations at corresponding depths in the ocean.

Because of the gas-trapping procedure used, only molecular nitrogen was analyzed for nitrogen isotopes. However, earlier work with lake water showed molecular nitrogen as the only significant end product of denitrification (*2*). In the lake study, no evidence of nitric or nitrous oxide production was found.

Presumably the rates of denitrification measured here are realistic, although large concentrations of ^{15}N-labeled nitrate were added to the experimental flasks. The results of earlier experiments with lake water indicated that denitrification rates do not

Table 1. Rates of denitrification and concentrations of phosphate, ammonia, nitrite, and nitrate. Concentrations expressed as microgram-atoms per liter, and rate in micrograms per liter per 24 hours.

Depth (m)	Concentrations				Nitrogen evolved during incubation	
	Phosphate-P	Ammonium-N	Nitrite-N	Nitrate-N	Fraction	Rate
25	1.56	2.4	0.06	14.3	U	
75	2.28	2.1	.03	10.5	U	
125	2.65	2.4	U†	2.8	0.00452	12
175*	3.34	5.5	U	U	.00663	18
225*	3.46	5.6	U	0.4	.00490	13

* Hydrogen sulfide present. † U, undetectable.

vary greatly with changes in nitrate concentration (*2*).

Water collected from below the thermocline at five stations in the Peru current, which has low oxygen and high nitrite concentrations (*11*), were examined for nitrate reduction to molecular nitrogen. Twenty-four experiments were conducted with water containing oxygen concentrations from 0.11 to 0.37 ml per liter and nitrite from 0.05 to 3.23 μg-atom per liter. We found no evidence of the reduction of nitrate to molecular nitrogen in these experiments. Apparently in this water nitrate reduction does not proceed at significant rates beyond nitrite, if indeed these large concentrations of nitrite are the result of biochemical reduction of nitrate, as suggested by Brandhorst (*12*).

Production of molecular nitrogen in the ocean is presumed to be biochemically mediated. Microorganisms such as *Pseudomonas*, which are capable of reducing nitrate to molecular nitrogen, have been isolated from the water and the sediments. It is unlikely that gaseous nitrogen is produced by any mechanism other than bacterial denitrification. Gas chromatographic and mass spectrometric studies of anaerobic fermentation of sewage sludge by Brezonik and Lee (*13*) showed that nitrogen gas is not produced by any mechanism other than bacterial reduction of nitrate. The nonenzymatic (Van Slyke) reaction for the conversion of ammonia, amino acids, and nitrite to molecular nitrogen occurred only with a *p*H less than 3.

This study indicates that denitrification is a significant process in oxygen-deficient sea water by which microorganisms convert nitrate and certain intermediate products (nitrite, and so on) to molecular nitrogen. Determination of the critical concentration of oxygen at which reduction to molecular nitrogen occurs requires further study. We are now of the opinion that oxygen must be absent, or present perhaps at concentrations of less than 0.10 ml per liter, in order to have significant molecular nitrogen production. Probably most of the denitrification in water masses of the type studied occurs in a narrow zone near the sulfide-oxygen interface, where large concentrations of nitrate and little or no oxygen are present (*10*).

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2 September 1966

Shattuckite and Planchéite: A Crystal Chemical Study

Abstract. *The orthorhombic crystal structures of shattuckite, $Cu_5(SiO_3)_4(OH)_2$, and planchéite, $Cu_8(Si_4O_{11})_2(OH)_4 \cdot H_2O$, have been solved. Shattuckite contains silicate chains similar to pyroxene in a complex association with copper atoms, while the closely related planchéite contains silicate chains similar to amphibole.*

As an initial phase of a program to establish the chemical and structural nature of the copper silicates, the crystal structures of shattuckite and planchéite have been solved and partially refined with two-dimensional data. Because of the scarcity of material and close association with other copper silicate minerals, the chemical composition of these two minerals has been in doubt ever since shattuckite was first reported by Schaller (*1*), and planchéite was first mentioned by LaCroix (*2*). Although similar in appearance, they can be readily distinguished by their optical properties (Table 1) and the data from x-ray powder diffraction patterns.

Previous chemical analyses of shattuckite, including those recently reported by Sun (*3*) and Newberg (*4*), have been hampered by the difficulty of obtaining a sample free of other intimately admixed minerals, such as planchéite, ajoite ($Cu_6Al_2Si_{10}O_{29} \cdot 5\frac{1}{2}H_2O$), quartz, and hematite. A recent analysis by Vlisidis (see *5*) of carefully purified samples conforms closely to the formula $Cu_5(SiO_3)_4(OH)_2$. This formula was further supported by a crystallographic study (*6*) and has now been finally established by a complete structure analysis.

The crystal unit cells of shattuckite and planchéite were determined by the Buerger precession method, and the parameters are given in Table 1. The optical properties and unit-cell parameters for shattuckite agree closely with those reported by Newberg (*4*). Because of their fibrous nature, suitable crystals for single-crystal study are difficult to isolate, and the x-ray patterns often show a uniform streaking of reflections normal to the *c*-axis. Nevertheless, complete three-dimensional intensity data have been collected for both crystals, and thus a satisfactory refinement of their detailed structures is made possible.

The structure of shattuckite was first solved by locating the Cu positions through a straightforward interpretation of the *c*-axis Patterson projection. The phases determined by the Cu atom structure factors were used to calculate a preliminary electron density map which revealed all the other atoms in the structure. Full-matrix, least-squares refinement of the atomic parameters and an overall temperature factor have been carried out separately on 131 ($h0l$) reflections and 79 ($0kl$) reflections, to a conventional reliability factor of 0.122 for the former and 0.093 for the latter. Since the interatomic distances still have rather high uncertainties at this stage, refinement is being continued with three-dimensional data.

The structure consists of $(CuO_2)_n$ layers similar to the $Mg(OH)_2$ layers in brucite, normal to the *b*-axis, centered at $b = 0$ and $\frac{1}{2}$. The oxygen atoms on both sides of the layer are provided by OH groups and the unshared oxygen atoms of $(SiO_3)_n$ chains which are parallel to the *c*-axis. These chains are nearly identical in configuration to the well-