

**A NEW TECHNIQUE FOR MEASURING DENITRIFICATION IN THE OCEANS:
ITS IMPORTANCE AND APPLICABILITY TO THE RED SEA**

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"This paper covers the occurrence of denitrification in the Red Sea and its hot brines. It discusses the importance of the process in calculating nitrogen budgets and the contribution of the oceans to atmospheric nitrous oxide (N_2O). The author presents a descriptive and critical discussion on the biochemistry and methods of measurement of the process. The new technique is based on selective use of acetylene to inhibit reduction of nitrous oxide to nitrogen gas. It was tested in Lake Balaton, the Koros River (Hungary), Eastern Harbor (Alexandria, Egypt), and the Suez Canal (Egypt). The results, when compared with those of other areas studied, are in the accepted range of denitrification rates in aquatic ecosystems."

Introduction

In recent years, a large literature has accumulated on bacterial denitrification in aquatic ecosystems. The great concern over this process has stimulated research in two major areas: (a) the relative importance of the process in the quantification and modeling of the nitrogen balance in the ocean, and (b) the contribution of the cycle of oceans to atmospheric nitrous oxide (N_2O , one of the products of denitrification), which catalyzes ozone (O_3) reduction and depletion in the stratosphere (Crutzen, 1970). This paper reviews the current state of knowledge regarding the significance of denitrification in modeling the nitrogen cycle in the ocean. A new technique for simple analysis of frequent sediment samples is discussed and applied to different ecosystems.

Nitrogen Budget

In all nitrogen budgets calculated for the oceans and the Red Sea (Emery et al., 1955; Eriksson, 1959; Morcos, 1970; and others), the annual inflow of combined nitrogen -- nitrite, in particular -- exceeds the annual outflow. Maintaining a steady state with respect to nitrogen in the sea requires conversion of combined nitrogen to gaseous nitrogen and its release to the atmosphere; oceanic denitrification appears to be the only mechanism that accomplishes this. However meaningful the calculation of inflow and outflow of nutrients are, they are only part of the complex nitrogen cycle.

A model for the marine nitrogen cycle, taken from the global model of Liu et al. (1977), is shown in Figure 1. It includes all the inputs and outputs to the cycle; the denitrification rate is $130 \text{ Tg-N year}^{-1}$ ($1 \text{ Tg} = 10^{12} \text{ g}$).

Investigations on the tropical Pacific Ocean indicate that, when the dissolved oxygen content of the water falls below about $5 \mu \text{ M/l}^{-1}$, denitrification is the predominant respiratory process (Cline and Richards, 1972; Goering, 1968). Hence, the bottom sediments and the poorly oxygenated deep water of the

Red Sea are probably suitable sites for denitrification, especially in the hot brines. Emery et al. (1969) stated that denitrification could account for the nearly 70 percent excess of molecular nitrogen relative to argon in the hot brines, as compared to the ratio of nitrogen to argon in ordinary ocean water. Weiss (1969) attributed the excess gaseous nitrogen of about 5 cc/kg in each brine to decomposition of organic matter in sediments. Oxidation of ammonia equivalent to this amount would consume about 7.5 cc/kg of oxygen. Assuming the solubility of oxygen in the initial seawater to be about 4.1 cc/kg, Craig (1969) concluded that half of the excess nitrogen would result from denitrification of nitrate in sediments.

Methods of Denitrification Measurement

Nitrogen gas, the product of denitrification, is naturally abundant, thus rendering precise determination of denitrification rather difficult. This may be one of the reasons why there are so few quantitative studies on denitrification in the aquatic environment. Many methods are in use, including indirect analyses, such as (a) measuring the redox potential (by knowing the E_h value, one can be fairly certain about the degree of anoxia); (b) using the presence of Fe^{+3} and Mn^{+4} as indicative of nitrate reduction (many aerobic bacteria utilize Fe^{+3} and Mn^{+4} as terminal electron acceptors); and (c) enumerating denitrifying bacteria, which is meaningless for field studies. The simplest approach for direct determination of the rate of denitrification is to measure the disappearance of nitrate after the water has become anoxic. However, this method overestimates the rate, since some assimilatory nitrate reduction may occur concurrently.

Measurement of denitrification with ^{15}N (Brezonik and Lee, 1968; Goering, 1968; Goering and Dugdale, 1966) is complicated and requires special equipment. Moreover, because nitrogen lacks a long-lived radioisotope, a stable, less sensitive isotope than ^{15}N must be used.

The underlying assumption in the N_2/Ar technique is that argon is conservative. Hence, any increase in the ratio above the value expected from solubility/atmospheric partial pressure considerations implies an excess of N_2 , presumably from denitrification. This method is tedious and quantitation is difficult because increases in N_2 concentration are not necessarily indicative of N_2 production. According to Focht (1978), the idea of N_2/Ar is theoretical-

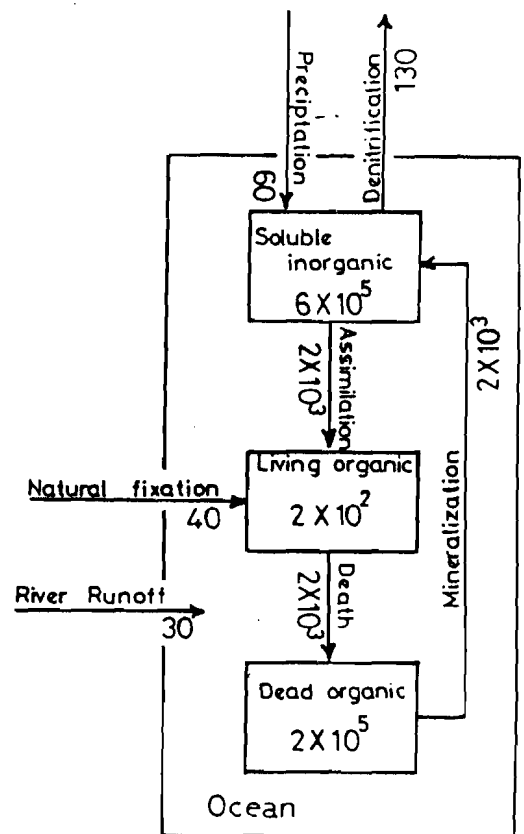


FIGURE 1. The marine nitrogen model (part of Figure 2 after Liu et al., 1977). Units: $1 Tg = 10^{12} g$; reservoir contents in $Tg-N$; transfer rates in $Tg-N yr^{-1}$.

ly sound and experimentally feasible.

A New Technique

The recent discovery of Yoshinari and Knowles (1976), that 0.1 percent acetylene blocks further reduction of nitrous oxide to nitrogen, has prompted considerable thought on how to design in-situ studies whereby one sediment core would be treated with acetylene and another left untreated. The difference in N_2O amount between the two cores would give a quantitative estimate of denitrification rate.

Procedure

For sample treatment and incubation (Figure 2, part I) the sediment core is taken in a polyvinyl tube of about 5 cm inside diameter. The core is cut into 2-cm segments, each of which is placed in another smaller tube 12 cm long and about 5 cm inside diameter. Injection of acetylene-saturated distilled water (2 ml of C_2H_2 per ml of water) into the sediment at the bottom of the tube is performed through a silicone rubber-stoppered hole. One ml C_2H_2 saturated water is injected through the sediment while the needle is slowly withdrawn in horizontal and decline levels in five directions. The injections give about 5 percent saturation of the interstitial water. The core is then stoppered, and 5 percent v/v acetylene gas is added to the air space through another hole. After a period of incubation (5 hours) $HgCl_2$ solution (0.1 M) is injected through the hole to block the reaction.

For nitrous oxide assays a proposed simple system should provide easy and accurate measurements of many samples, facilitating low-cost study of samples collected from various locations and at different times. The suggested system of N_2O gas-purging is shown in Figure 2, part II: The 2-cm sediment segment is placed in a round-bottom flask, which is then put into the system (Figure 2, a). N_2 gas carrier flowing to the flask (40 ml/min) purges N_2O from the sediment and passes through a 20 ml volume trap (Figure 2, d) attached to the system and completely covered with a liquid nitrogen-cooled jacket. Purging is facilitated by a magnetic stirring bar inside the flask. Figure 2, b and c, show the absorption traps with potassium hydroxide for removal of CO_2 , and calcium chloride for removal of water vapor from nitrous oxide gas. After 30 min purging, the liquid N_2 -jacket is removed and nitrous oxide gas is immediately withdrawn from a rubber tube from the trap with a clean syringe. The nitrous oxide gas is then injected into the gas chromatograph, where a molecular sieve 5A column 2 m long and 0.2 cm in diameter is used at 320°C in a N_2 carrier flow of 40 ml/min. An electron capture detector is suitable for N_2O

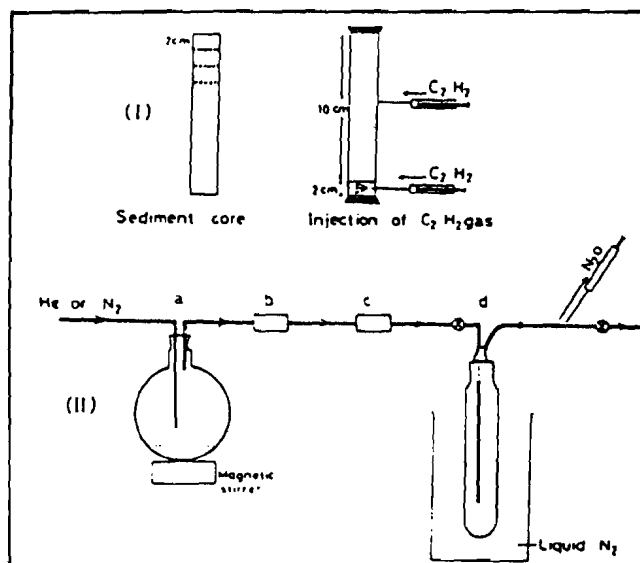


FIGURE 2. Sediment treatment and incubation (I) and nitrous oxide assay (II).

gas detection. Nitrous oxide is detected in the gas space above the sediment in the core after withdrawal with a syringe. The amount of this diffused gas must be included in the calculation of the total denitrification rate.

Application

Sediment cores were taken from Lake Balaton and the Koros River in Hungary. Two cores were also collected from the Eastern Harbor off Alexandria, and two more were taken from the Suez Canal off Kantara City and Ras El-Ish City. The denitrification rate in each environment as well as from some other ecosystems is shown in Table 1. The values represented for net loss of nitro-

TABLE 1. Rate of denitrification in sediments calculated from the original data.

Ecosystem	Denitrification rate ($\mu\text{M N m}^{-2}\text{h}^{-1}$)	Source
Lake Kul, Denmark	163	Andersen, 1977
Some Danish lakes	148	Pheiffer, 1977
Lake Balaton, Hungary		
0-2 cm depth	162	Abdel Moneim and El Samra, 1981
2-4 cm depth	181	
4-6 cm depth	74	
Koros River, Hungary		
0-2 cm depth	178	Abdel Moneim and El Samra, 1981
2-4 cm depth	124	
4-6 cm depth	63	
Eastern Harbor, Alexandria, Egypt		
Station I	14	Present study
Station II	12	Present study
Suez Canal, Egypt		
Near Kantara City (km 60)	0.8	Present study
Near Ras El-Ish City (km 45)	0.6	Present study
NO_3 -amended coastal Marine sediment	40	Sorensen, 1978
Santa Barbara basin	20	Codispoti, 1973
Darwin Bay	148	Richards and Broenkow, 1971
Soils of Arctic tundra	0.28	Barsdate and Alexander, 1975

gen fall in the accepted range of reported rates. Calculation of the magnitude of denitrification rate and the effect of ambient nitrate, nitrite, organic matter, temperature, and pH are discussed in another paper (Abdel Moneim and El Samra, 1981).

Conclusions

Denitrification is now known to occur over wide areas of tropical oceans. Even in the hot brines there are indications of denitrifying activity, but quantitative projections of oceanic nitrogen losses are still very crude.

The low cost and simplicity of nitrous oxide detection has fostered future research toward acquiring a more complete idea of nitrogen balance in the Red Sea. Applying this method, one can assay 10 samples a day. Because injected C_2H_2 provides a picture of nitrogen fixation activity, this method can be used to quantify biological nitrogen input. Thus, with one sample, investigators can measure two important dynamic processes in the nitrogen cycle.

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