OCEANOLOGICA ACTA 1981 - VOL. 4 - N° 4

An experimental study of the regulation of nitrification in marine sediments

Nitrification
Marine sediment
Organic matter
Ammonia
Oxygen

Nitrification
Sédiment marin
Matière organique
Ammoniac
Oxygène

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Received 12/2/81, in revised form 4/5/81, accepted 5/6/81.

ABSTRACT

The regulatory influence of organic matter, ammonia and oxygen saturation on nitrification in the marine environment was studied by means of laboratory incubations of seawater and sediments, chemical changes in the incubation medium being used to monitor the kinetics of the nitrification process. The results indicate the existence of both auto- and heterotrophic nitrification, the predominance of either type being determined by the concentration of organic matter in the culture media. When such matter is present in only very low concentrations or minute amounts, autotrophic nitrification predominates. Higher levels of organic matter lead to heterotrophic nitrification, this process increasing with the concentration of organic matter present. The influence of ammonia also varies according to the concentration in which it is present. At concentrations of ammonium sulphate equal to or less than 1 mg/l ammonia (7.6 μM) inhibits nitrification; at between 8 and 100 mg/l (60.5 and 756.8 μM) ammonia is toxic and nitrification is virtually non-existent. Saturated oxygen concentration favours nitrification, while decreasing oxygen levels lead to the remplacement of nitrification by the converse process of nitrate respiration.
The results of the study indicate that certain factors exercise a similar influence on the process of nitrification in the marine and terrestrial environments alike. Given the difference between experimental and natural conditions, however, it is difficult to determine the direct applicability of these results to the marine environment.

Oceanol. Acta, 1981, **4**, 4, 457-463.

RÉSUMÉ

Étude expérimentale sur la régulation de la nitrification dans des sédiments marins

Nous avons étudié quelques facteurs de régulation de la nitrification en milieu marin : taux de matière organique, d'ammoniaque et d'aération. Pour cela, on a réalisé des incubations d'eau et de sédiments marins dans des fioles expérimentales où l'on a suivi chimiquement la cinétique de la nitrification.
Deux types de nitrification existent et selon la concentration de matière organique, l'une ou l'autre de ces nitrifications domine : quand la quantité de matière organique est faible ou nulle, c'est une nitrification autotrophe, lente mais très intense, qui domine. Dès que le taux de matière organique est élevé, la nitrification est de type hétérotrophe; elle est rapide et peu productive mais augmente en fonction du taux de matière organique.
Cette étude a montré également que l'action de l'ammoniaque varie en fonction de sa concentration dans le milieu de culture. Pour des quantités de sulfate d'ammonium inférieures ou égales à 1 mg/l, l'ammoniaque a un rôle inhibiteur sur la nitrification;

quand ces concentrations sont entre 8 et 100 mg/l, la nitrification est maximale; et au-delà de 1 000 mg de $(NH_4)_2 SO_4$/l, l'ammoniaque est toxique et la nitrification est presque nulle.
On a aussi mis en évidence la nécessité d'une bonne aération du milieu de culture. En effet, dès que le taux d'aération diminue, la nitrification est remplacée par le métabolisme inverse qui est la respiration des nitrates. D'après cette étude, la régulation de la nitrification en milieu marin semble se faire de la même façon que dans un environnement terrestre.
Étant donnée la différence entre les conditions expérimentales et l'environnement marin, il est difficile de transposer ces résultats directement dans le milieu naturel.

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INTRODUCTION

Few studies exist concerning the regulation of nitrification in the marine environment. According to Vaccaro (1962) and Carlucci *et al.*, (1970) organic matter in the environment, degraded by heterotrophic marine bacteria, might constitute a source of the ammonia necessary for the initiation of nitrification. Rajendran and Venugopalan (1977) have shown that both organic matter and ammonia play an important role in the initiation of nitrification. Oxygen was also recognized as an essential factor in marine nitrifying activity (Zobell, 1935; Carlucci, McNally, 1969), while Kawai *et al.* (1965) have shown, in their studies on experimental marine ecosystems, that the intensity of nitrification might be a function of oxygenation in the environment.

In our earlier long-term investigation (Mével, Chamroux, 1980), the nitrification process in recirculating experimental tanks appeared to be related to the introduction of organic matter and ammonia, and was favoured by oxygenation in the environment. These observations were verified by short-term experiments designed to test separately the influence of these three factors on nitrification in the sandy sediments and water from the tanks. The results are given in this paper.

MATERIAL AND METHODS

Experimental tanks

Compartmentalized closed aquaria similar to those described by Boucher and Chamroux (1976) were used in the experiments. One compartment contained a false bottom and a layer of sediment 20 cm deep which constituted the sand filter, through which the water, which circulated continuously in the system, percolated. Tanks 1 and 2 were similar but differed from tank 3 in sand particle size and filtration rate. The sands used (median 500 µm in tanks 1 and 2; first median 160 µm and secund median 1 600 µm in tank 3) were much finer than the sand-gravel (1 to 5 mm) generally recommended by others.

Pre-existing bacterial populations in the water and sand were fed daily with an addition of casein extract (casamino acid Difco) amounting to 1.5 g/day/tank, i. e. 110 g $N/m^2/yr$. This method of enriching the tanks was also used by Boucher and Chamroux (1976) but the development of bacterial populations in the sand filter in similar experiments has more generally been stimulated by the introduction of bacteria into the system (Lesel, de Leffemberg, 1977), or by the addition of a portion of top sand from a conditioned into a new system (Carmignani, Bennett, 1977). After a "run-in" of five weeks, prawns (*Penaeus japonicus*), (provided by Dr. A. Laubier, Centre Océanologique de Bretagne) were introduced into tanks 2 and 3; each group comprised ten adults and thirty young, whose respective weights averaged 14.5 g and 1.01 g. Organic matter subsequently added to the tanks consisted of a daily feed to the prawns of granules 76-1-1-0 (Aquacop, 1972) at a rate of 425 g organic-N/yr/m^2.

Experimental incubations

The nitrifying microflora was grown on a basic mineral medium (Carey, 1938) composed as follows: artificial sea water (Lyman, Fleming, 1940) – 1 000 ml; ferrous chloride, one drop; calcium nitrate, 50 µg; molybdic acid, 50 µg; boric acid, 50 µg; calcium sulfate, 50 µg; copper sulfate, 50 µg; zinc sulfate, 50 µg; manganese sulfate, 50 µg; magnesium sulfate, 50 µg; cobalt chloride, 50 µg; $(NH_4)_2 SO_4$ – 100 mg; K_2HPO_4 – 100 mg; $CaCO_3$ – 3 000 mg. The pH of the medium was adjusted to 7.5 with a 5 % solution of Na_2CO_3. In the course of several experiments, this medium was supplemented with either sodium succinate or ammonium sulfate in various quantities (details are provided below).

Incubation took place in long-necked, 2 l glass flasks with cotton-wool stoppers. The high S/V ratio, permitting a broad area of contact between the medium and the air contained in the flasks, is very favourable to aerobic cultures.

The flask and the medium were autoclaved together; after cooling, the medium was inoculated with either 50 ml of water or approximately 50 g of the sandy sediment (dry weight) taken in sterile glass vessels from the experimental tanks. The oxygen saturation (5.35 cm^3/dm^3) necessary for the growth of nitrifying microorganisms was achieved by continuous shaking of the flasks on a reciprocal shaker (120 strokes/min. over 8 cm). The cultures were incubated at 17°C in a temperature-controlled room and subjected to the natural photoperiod.

The kinetics and intensity of nitrification were followed through a daily process of aseptic sampling for ammonia and oxidized nitrogen products in the experimental medium. Incubation was terminated when ammonia had disappeared completely from the culture medium.

Chemical analysis

Parnas' and Wagner's method was used for the quantitative estimation of nitrogen species (Bremner, 1965). This technique is based on the distillation of ammonia in an alkaline medium and its recovery in an acid medium. The quantity of ammonia present is subsequently estimated by the colorimetric method, using the Nessler reagent.

The ammoniacal nitrogen (NH_3 and NH_4^+) present in the sample was distilled first from the alkaline medium. The oxidized forms of nitrogen (NO_2 and NO_3) were then reduced by adding Devardas' alloy to the samples and the nitrite and nitrate thus converted to ammonia were measured as before. The quantity of organic nitrogen was measured by the micro-Kjeldahl' method: after mineralization, part of the sample was distilled to measure the ammoniacal nitrogen resulting from the destruction of the organic nitrogen, as described by Chamroux and Boucher (1979).

The quantity of nitrite in the water samples was measured directly from a second sample, using a colorimetric reaction with the Griess reagent (Robinson, Thompson, 1948). The NO_3 concentration in the water sample was then obtained as the difference between the nitric nitrogen measured by distillation method and nitrite measured colorimetrically.

This method, used to determine nitrogen content in soils, was the only one which permitted measurement of nitrogen components in the sand. To obtain comparable results, it was also applied to the water sample.

The intensity of nitrification was calculated in terms of the amount of oxidized nitrogen produced during the period of incubation, and expressed as production of oxidized nitrogen/day/litre of the medium, with both water and sand samples.

This parameter was selected in preference to oxygen consumption since it takes account of both autotrophic and heterotrophic nitrification. Values obtained in the incubation with sand were extrapolated to 50 g of sandy sediment.

RESULTS AND DISCUSSION

Role of organic nitrogenous matter

Nitrification in the presence of organic matter

Inoculation of the basic mineral medium with eutrophic sediments naturally resulted in an enrichment of the medium with organic matter. This condition was then exploited to demonstrate the influence of organic matter on the nitrification process. For this, the "transfer method" (Laurent, 1972) was used, the organic matter introduced into the medium by inoculation being exhausted by successive transfers. After inoculation, nitrification in the medium was monitored by determining the increase in the concentrations of nitrite and nitrate; when an increase in the quantities of nitrate and nitrite, indicative of nitrification occurred, 10 ml of the medium were withdrawn and re-inoculated into a fresh medium. This transfer reduced the content of organic matter in the culture medium by a factor of 100. With the progression of nitrification, successive transfers will eventually exhaust all organic matter, thus permitting identification of the influence of organic nitrogenous matter on the intensity of the nitrification process.

In two of the three incubations carried out with sediments over a period of 12 days, organic matter was completely exhausted by a single transfer; and in the third, by two transfers (Table 1). The rates of production of oxidized nitrogen given in Table 1 reveal the influence of organic nitrogenous matter on the rates of nitrification in the experimental cultures and confirm that heterotrophic nitrification actually occurs.

Since the concentrations of ammonia remain at nearly the same levels, a reduction in the activity of nitrifying microorganisms brought into the cultures with the sediments is obviously related to a sharp decrease in the quantity of organic matter in the culture medium.

Table 1

Influence of the concentration of total nitrogenous organic matter (POM) on the intensity of nitrification. Ammonia and organic nitrogen are expressed in µg at N/l and production of oxidized nitrogen in µg at NO_2+NO_3-N/l/day.

Influence de la quantité de matière organique azotée totale sur l'intensité de la nitrification. Ammoniaque et azote organique sont exprimés en µatg N/l et la production d'azote oxydé en µatg $N-NO_2+NO_3$/l/jour.

	Culture	First transfer	Second transfer
Sediment 1 :			
Organic nitrogen	1996.00	0.00	—
Ammonia nitrogen	1053.00	1504.00	—
Production of oxidized nitrogen	20.40	3.57	—
Sediment 2 :			
Organic nitrogen	2323.00	173.00	0.00
Ammonia nitrogen	1040.00	1611.00	1450.00
Production of oxidized nitrogen	222.50	14.80	6.00
Sediment 3 :			
Organic nitrogen	2110.00	0.00	—
Ammonia nitrogen	1053.00	1324.00	—
Production of oxidized nitrogen	149.20	28.80	—

Influence of carbon source concentrations

Among the several substrates used as carbon sources, amino acids are best assimilated by bacteria. These cannot, however, be used in nitrification studies, since amino acid metabolism provokes the release of ammonia into the culture medium in quantities which would effectively mask the oxidation of ammonia. For this reason, sodium succinate, which is also widely utilized by a large spectrum of marine bacteria, was preferred as a carbon source.

In these experiments, the basic mineral medium was variously supplemented with sodium succinate in

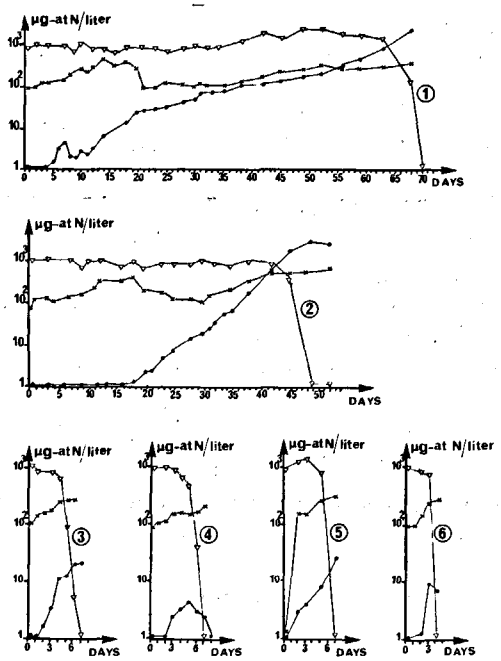

Figure 1

Intensity of nitrification as a function of different concentrations of sodium succinate (1, 0 *mg;* 2, 100 *mg;* 3, 500 *mg;* 4, 1 000 *mg;* 5, 2 000 *mg;* 6, 5 000 *mg*). ▽———▽; NH_4-N; ●———●, NO_2-N; ×———×, NO_3-N.

Intensité de la nitrification en fonction des concentrations de succinate de sodium (1, 0 mg; 2, 100 mg; 3, 500 mg; 4, 1 000 mg; 5, 2 000 mg; 6, 5 000 mg). ▽———▽, $N-NH_4$; ●———●, $N-NO_2$; ×———×, $N-NO_3$.

concentrations of 100, 500, 1 000, 2 000 and 2 500 mg/l. The media were then inoculated with 10 ml of water drawn from the experimental tank. Parallel incubation using the basic mineral medium itself served as a control.

The evolution of nitrification in the control and the five experimental cultures (Fig. 1) clearly reveals two types of distinctly different nitrifying activities.

The first of these is observed in cultures containing little or no organic matter (0-100 mg of sodium succinate per litre of the medium).

In the succinate-free culture, the disappearance of ammonia and the oxidation of nitrogen were complete after 70 days. Where sodium succinate was present, these processes required 49 days. Although the nitrification process began slowly, the production of nitrate and nitrite was significantly higher (67.71 and 56.77 µg at $NO_3^+ + NO_2 - N$/l/day respectively) in the cultures containing no succinate and 100 mg sodium succinate/l. These characteristics indicate an autotrophic nitrification.

On the other hand, in cultures containing higher concentrations of sodium succinate (500-5 000 mg/l), the nitrification process was markedly different. In all the cultures, ammonia was exhausted rapidly (4-7 days), its decrease matched by the increase in nitrate and nitrite. Nitrification was significantly low (17.31, 26.86, 41.14 and 49.35 µg at $NO_2 + NO_3 - N$/l/day respectively in cultures with 500, 1 000, 2 000 and 2 500 mg sodium succinate/l. This rapid nitrification, stimulated sharply by the addition of organic matter appears to be due to the activity of heterotrophic microorganisms.

There thus appears to be a threshold concentration of organic matter at between 100 and 500 mg sodium succinate/l which demarcates autotrophic and heterotrophic nitrification. Below these levels, the former process predominates; above these levels, the latter.

Autotrophic and heterotrophic nitrification

During this series of experiments, designed to demonstrate the concurrent existence of both auto- and heterotrophic nitrification in the experimental tanks, autotrophic nitrification was suppressed by the addition of 2-chloro-6-[trichloromethyl]-pyridine or N-Serve (Goring, 1962 *a* and *b*); introduction of these substances which are highly toxic to autotrophic organisms oxidizing ammonia to nitrite, permits the evaluation of heterotrophic oxidation of nitrogen (Shattuck, Alexander, 1963). The selective inhibition of autotrophic nitrification by N-Serve has often been used as a mean of differentiating between autotrophic and heterotrophic nitrification (Wallace, Nicholas, 1969; Vanderborght, Billen, 1975; Ben Bohlool *et al.*, 1977).

Four culture media were prepared. Two of these contained only the basic mineral medium; one of them received an addition of N-Serve in a concentration of 0.5 mg/l. The other two contained the basic mineral medium enriched with sodium succinate (5 g/l); one of these was treated with N-Serve in the same concentration as above. After incubation with water drawn from the experimental tank, nitrification in all four media was monitored, and the results are presented in Figure 2.

In the mineral medium without N-Serve, ammonia disappeared slowly (16 days) and the production of

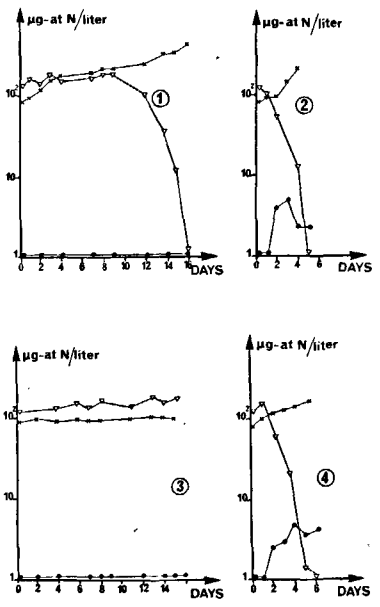

Figure 2

Effect of N-Serve on bacterial mixed populations in mineral and organic media (1, *mineral medium;* 2, *organic medium;* 3, *mineral medium* + *N-Serve;* 4, *organic medium* + *N-Serve*). ▽———▽, NH_4-N; ●———●, NO_2-N; ×———×, NO_3-N.

Effet du N-Serve sur des cultures mixtes en milieu minéral et organique (1, milieu minéral; 2, milieu organique; 3, milieu minéral + N-Serve; 4, milieu organique + N-Serve). ▽———▽, $N-NH_4$; ●———●, $N-NO_2$; ×———×, $N-NO_3$.

oxidized nitrogen amounted to 18.81 µg at NO_3+NO_2-N/l/day, indicating that the nitrification observed was due to the activity of autotrophic microflora. In the mineral medium to which N-Serve was added, ammonia disappeared over the same period (16 days) and the production of oxidized nitrogen was very weak (0.87 µg at NO_3+NO_2-N/l/day). The autotrophic nitrifiers in this medium were evidently inhibited by N-Serve, and the very weak production of oxidized nitrogen, about 4.62% of the total activity observed in the mineral medium alone, could have been due to a process of heterotrophic nitrification.

In the remaining two flasks, both containing the mineral medium supplemented with sodium succinate, ammonia disappeared rapidly and nitrification progressed normally, irrespective of whether or not the medium contained N-Serve. In both the succinate-enriched media, nitrification was high (27.0 µg at NO_3+NO_2-N/l/day without N-Serve and 15.0 µg at NO_3+NO_2-N/l/day with N-Serve). In the presence of N-Serve, however, the rate of production of oxidized nitrogen was much lower, suggesting some influence of N-Serve on the nitrification process in the organic medium. This further suggests that part of the nitrification occuring in the organic medium in absence of N-Serve is due to autotrophic activity. By contrast, the nitrification achieved in the organic medium treated with N-Serve was of an exclusively heterotrophic nature, and accounted for 55.6% of the total activity.

These observations reveal the simultaneous occurrence of both types of nitrification, but their relative importance varies according to the substrate available. In the mineral medium alone, autotrophic processes predominate (95.38%) whilst in the presence of organic matter, both autotrophic and heterotrophic processes are of the same range (44.4 and 55.6% respectively).

Influence of ammonia concentrations

In these experiments, which were designed to demonstrate the influence of ammonia on nitrification, the culture media were inoculated with sand sediments from the experimental tanks. Ammonia was added to the media in the form of ammonium sulfate in concentrations ranging from 0.264 to 5000 mg/l (2 to 37800 µM (Table 2)).

Table 2

Intensity of nitrification (µgat NO_2+NO_3/l/day) as a function of ammonia concentration added to the medium.

Intensité de la nitrification (µatg $N-NO_2+NO_3$/l/jour) en fonction de la concentration d'ammoniaque dans le milieu.

Culture	$(NH_4)_2SO_4$ (mg/l)	NH_4-N (µg-at N/l)	Intensity of nitrification
1	0.264	4.00	7.00
2	0.500	7.60	6.70
3	1.000	15.10	6.66
4	8.000	121.10	52.50
5	10.000	151.50	50.00
6	16.000	242.40	45.00
7	100.000	1515.15	52.37
8	1000.000	15151.50	1.00
9	5000.000	75757.60	0.45

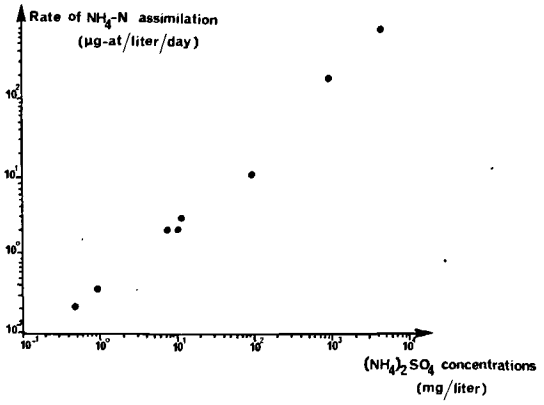

Figure 3

Rate of assimilation of ammonia nitrogen as a function of ammonium sulfate concentrations in the mixed populations.

Taux d'assimilation de l'azote ammoniacal en fonction de la concentration de sulfate d'ammonium dans le milieu de culture.

Assimilation of ammonia

Figure 3 shows the rate of disappearance of ammonia as a function of its initial concentration in the culture medium. At lower initial concentrations, ammonia was exhausted very rapidly; the time required for complete utilization increased in relation to the initial concentration. At very high concentrations (1000 and 5000 mg ammonium sulfate/l) ammonia still remained in the culture media, even after 40 days of incubation; the rate or utilization expressed as µg at NH_4-N assimilated/l/day, increased in relation to the concentration of ammonia added to the medium (Fig. 4). At the maximal concentration of ammonia-nitrogen ($7\text{-}8\times10^4$ µg at NH_4-N/l) used in this experiment, the assimilation of ammonia was not inhibited.

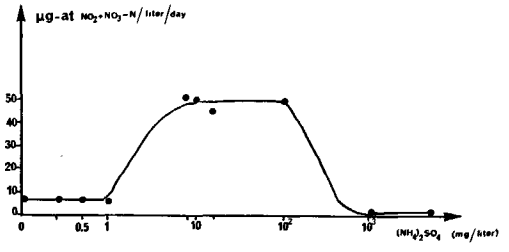

Figure 4

Intensity of nitrification as a function of ammonium sulfate concentrations.

Intensité de la nitrification en fonction des concentrations de sulfate d'ammonium.

Intensity of nitrification

The rates of nitrification at various concentrations of ammonia in the medium (Table 2 and Fig. 5) indicate three levels of intensity. At concentrations ranging from 0.264 to 1.0 mg ammonium sulfate/l, nitrification was weak and amounted to between 6 and 7 µg at NO_3+NO_2-N/l/day. At concentrations ranging from 8 to 100 mg ammonium sulfate/l, nitrification was strong, and remained constant regardless of the concentration of ammonium sulfate. At very high concentrations (1000 and 5000 mg ammonium

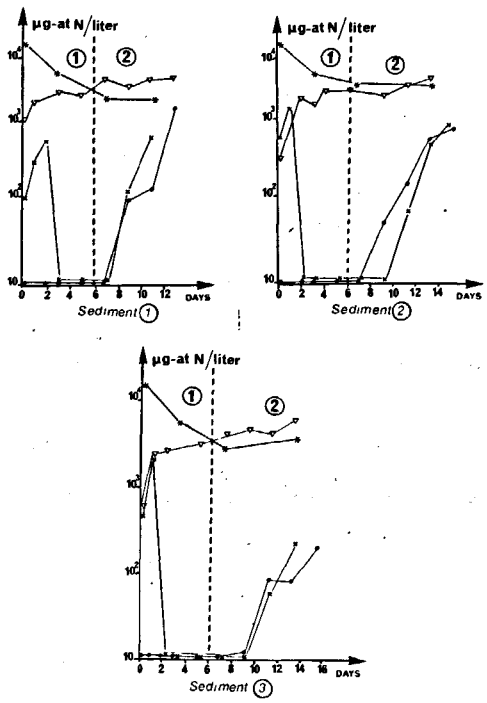

Figure 5

Influence of aeration on the nitrification activity in three marine sediments (1, *sediment* 1; 2, *sediment* 2; 3, *sediment* 3). ▽——▽, NH_4-N; ●——●, NO_2-N; ×——×, NO_3-N; ★——★, *organic-N*.

L'influence de l'aération sur l'activité nitrifiante de trois sédiments marins (1, sédiment 1; 2, sédiment 2; 3, sédiment 3). ▽——▽, $N-NH_4$; ●——●, $N-NO_2$; ×——×, $N-NO_3$; ★——★, N-organique.

sulfate/l), nitrification was very weak and almost non-existent. These observations indicate that the relation between nitrification and the quantity of ammonia available for nitrification is not linear, but that ammonia acts in a non-linear fashion (Fig. 5).

The low rates of nitrification associated with smaller concentrations of ammonia may be explained by the fact that the availability of nitrogeneous substrate for the cultures was not sufficient; nitrification in these cultures is eventually inhibited by the absence of ammonia. This is often the case in natural environments where ammonification is much lower than the potential level of nitrification (Besler, 1979). On the other hand, the high and nearly constant nitrification activity observed at concentrations of 8 to 100 mg ammonium sulfate/l shows that nitrification was at its maximum intensity. There thus appears to be a threshold concentration of ammonia (between 1 and 8 mg ammonium sulfate/l) which activates nitrification. The very weak levels of nitrification at higher concentrations of ammonia indicate an inhibitory effect of ammonia. Such an effect has been observed already in other studies (Stojanovic, Alexander, 1958; Watson, 1971; Wild *et al.*, 1971; Prakasam, Loehr, 1972; Miyazaki *et al.*, 1975; Wong-Chong, Loehr, 1975). The data in the present study show that the threshold concentration leading to the inhibition of nitrification by ammonia is situated between 100 and 1 000 mg ammonium sulfate/l. Inhibition due to ammonia (Anthonisen *et al.*, 1976; Wong-Chong, Loehr, 1978) is, however, also a function of pH. Bearing this in mind, the threshold concentration of inhibition observed in the present study corresponds to the zone of complete nitrification described by Anthonisen *et al.* (1976).

Importance of aeration

Nitrifying organisms are generally aerobic, although heterotrophic nitrifiers tolerate lower oxygen concentrations in comparison with autotrophs (Alexander, 1965). Availability of oxygen would thus appear to exert a certain influence on nitrification. This was studied on samples withdrawn from the sediments in the experimental tanks.

The experiments were carried out in triplicate and mineral medium was employed in all the three experiments. For the first 6 days, the flasks were kept in static condition; they were then transferred to a reciprocal shaker which ensured that the medium was well mixed and aerated.

The changes observed in the concentrations of ammonia, nitrate, nitrite and organic nitrogen were similar in all three experiments (Fig. 6). Concentrations of ammonia increased continuously regardless of whether or not the cultures were aerated. This increase may be attributed to ammonification.

Thus, the mineralization of organic matter was not affected by oxygen deficiency. This was not, however, the case with nitrification, which was affected by oxygen deficiency. Nitrate production, indicative of nitrification, was observed on the first day of incubation. Oxygen available in the medium at the beginning of the experiment would have been sufficient for this, but from the second day onwards, when the oxygen was presumably exhausted, nitrate decreased and disappeared from the cultures. Nitrification recommenced however when the cultures were transferred from their static phase to the shaker, where oxygen levels sufficient for nitrification were again obtained. These results indicate that although microorganisms capable of nitrification exist in these cultures, they can only initiate this process when the medium is sufficiently aerated.

CONCLUSIONS

The following conclusions may be drawn from the present study:

Concentrations of both organic matter and ammonia play a role in nitrification. Considered together with the results obtained by McLaren (1971), Ardakani *et al.* (1974) and Rajendran and Venugopalan (1977) on estuarine microbiology it may be stated that both these factors exercise a strong influence on nitrification in the marine environment. Organic matter influences nitrification in two ways: firstly, it provides an ammonia source for the nitrifiers; and secondly, depending upon its concentration, it favours either auto- or heterotrophic nitrification.

Ammonia initiates and stimulates nitrification at concentrations of 1 mg ammonium sulfate/l (7.6 µM) and above; but nitrification is inhibited when the concentration exceeds 10^3 mg/l (7 570 µM).

Oxygenation of the medium is important to the maintenance of nitrification. Even in well aerated conditions, however, nitrification can be masked by nitrate respiration, if nitrate is available in high quantities.

There appears to be a similarity between the regulation of nitrification in the experimental marine tank and in the terrestrial environment. In both environments, nitrification is essentially controlled by these three factors (Focht, Verstraete, 1977).

These results cannot be extrapolated in their entirety to the natural environment, since experimental conditions, and more specifically the shaking process which causes oxygen saturation in the sediment, are not exactly matched in nature. On the other hand, it would appear worthwhile investigating the influence of eutrophic activity in certain coastal waters on the process of heterotrophic nitrification, and studies in this connection are in progress.

Acknowledgements

This study was supported by grants from CNEXO (77/1664-78/1846-1). The authors wish to express their gratitude to Dr. A. Laubier (Centre Océanologique de Bretagne) who supplied the prawns used in the experiment and who assisted them with advice concerning the rearing and biology of Penaeids.

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Collection : Mémoires ORSTOM n° 91, 21 × 27, 558 *pages,* 225 *figures,* 39 *planches,* 15 *tableaux,* 1981, *ISBN* 2-7099-0578-7.

En vente aux éditions de l'ORSTOM, 70-74, route d'Aulnay, 93140 Bondy, au prix de : 320 FF (C.C.P. 22.272.21 Y Paris).