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Microbiology of Nutrient Regeneration: Part II—Distribution of Nitrifying Bacteria in Vellar Estuary

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Nitrifying capacity or the biological potential in nitrification of both sediments and overlying water were tested. Ammonia oxidizing bacteria and nitrite oxidizing bacteria were estimated both in water and sediments in 4 zones of Vellar estuary. Both marine and limnetic forms of bacteria were counted along with other environmental parameters. Generally, nitrification process was more in sediments than in the water column. Distribution of marine and limnetic forms of nitrifying bacteria was chiefly controlled by salinity. In the monsoon months, nitrifying bacteria of limnetic or terrestrial origin dominated the marine form. In other months, marine nitrifying bacteria were more in marine and tidal zones of the estuary with a decrease towards upstream. Salinity and substrate concentration (ammonia and nitrite) were found to be the chief controlling factors of these bacteria.

BIOGEOCHEMICAL turnover in the aquatic environment is mainly due to the metabolism of microbial population and this is performed through aerobic and anaerobic decomposition by which microbial cells are supplied with energy. Experimental studies conducted to elucidate the relative importance of bacteria in phosphorus cycle and the role of ciliates in the regeneration of phosphate have been reported[1]. The present paper deals with the chemosynthetic autotrophic nitrifying bacteria and their ecological role in the nitrogen cycle of sea. Bacteria and other microorganisms play as significant roles in the oceans as they do in terrestrial environments, but only meagre informations are available about their occurrence and activities. These studies can help a thorough understanding of various chemical and biological processes taking place in the marine environment.

For all biological growth processes nitrogen is required in some form or other for the synthesis of cellular proteins and nucleic acids. A range of nitrogen compounds are utilized by various microbes under different conditions and thus the whole of nitrogen cycle is rendered a complex one. In some microbial transformation, oxidation state of nitrogen is changed. Nitrogenous compound is utilized as a source of oxygen and in some microbial metabolic process, nitrogen is oxidized and energy is provided for synthetic reactions through inorganic nitrogen metabolism.

During the study of the ecology of nitrifying bacteria in Vellar estuary, nitrifying capacity or biological potential in nitrification of both sediments and overlying water have been tested. Studies on the distribution and abundance of both marine and limnetic forms of nitrifying bacteria and their ecological characteristics have been carried out in premonsoon and monsoon periods and reported in this paper.

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Materials and Methods

Mud and water samples were aseptically collected from various stations situated in 4 zones of Vellar estuary, viz. marine, tidal, gradient and freshwater zones. The details about the zonation of Vellar estuary were given by Rajendran[2]. Marine zone extends from the mouth of Vellar estuary to 0·8 km up in the estuary. Mean salinity difference between surface and bottom was almost nil. The tidal zone approximately extends up to 3-4 km, with mean salinity difference of 13·01‰. Gradient zone starts from 4-5 km and the mean difference in salinity was 8·70‰. Freshwater zone is situated beyond the gradient zone, where the surface to bottom difference in salinity disappears.

Mud and water samples were brought to the laboratory in sterile flasks and they were added to the enriched media for culturing ammonia oxidizing bacteria and nitrite oxidizing bacteria. The media for both ammonia oxidizers and nitrite oxidizers were essentially those of Carlucci and Strickland[3]. Aged sea water and distilled water filtered through Millipore HA membrane filters were used to prepare the media to enumerate marine and limnetic forms respectively. To each 9 ml of the medium 1 ml of the water sample or 1 g of wet mud sample were inoculated and serial dilutions made. For each dilution, additionally 2 more replicates were carried out and all the cultures were incubated at room temperature. Controls were in triplicate. After 10 days, ammonia and nitrite oxidation phases of nitrification were traced by measuring the nitrite concentration according to the method of Bendschneider and Robinson[4]. Bacterial number was

calculated by the most probable number method, using McCready's tables given by Rodina[5]. Decrease in ammonia concentration was considered as evidence of I phase of nitrification (ammonia oxidation) only when nitrite was produced at the same time. In the same way decrease in nitrite concentration was considered as evidence of II phase of nitrification (nitrite oxidation) only when nitrate was produced simultaneously. Total heterotrophic bacteria and ammonifying bacteria were also enumerated in water and sediments, using ZoBell's 2216 E nutrient agar medium[6] and the medium given by Krumbein[7] respectively.

Temperature and salinity of the water samples were measured on board with the NIO (England) pattern T-S bridge developed by Cox and Moorey[8]. Oxygen was estimated by the Winkler method. Nitrate was estimated by the method of Morris and Riley[9] using ammonium chloride as buffer. The procedure of Richards and Kletsch[10] was used to estimate ammonia. Dissolved organic nitrogen was determined by the method of Strickland and Parsons[11].

Samples were collected once in a fortnight and altogether 12 collections were made in both premonsoon and monsoon periods.

Results and Discussion

Ammonia oxidizing and nitrite forming bacteria — Numbers of ammonia oxidizers in both sediment and overlying water are shown in Table 1 for both premonsoon and monsoon periods. The number of marine forms of ammonia oxidizers was high in sediment and water in the premonsoon period, but their abundance showed a decreasing trend from the marine zone to the freshwater zone in the estuary. Further the number was much less in the tidal zone than in the gradient zone during this period.

The limnetic forms were maximum in the freshwater and gradient zones. Ammonia oxidation by limnetic forms of ammonia oxidizing bacteria was very meagre since their number in the marine and tidal zones was very low (Table 1). In the monsoon months (October-December) the marine species of ammonia oxidizing bacteria were not present in the water column of the gradient and freshwater zones, and only very few of them were found in the marine and tidal zones. However, the limnetic forms were found in considerable numbers throughout the estuary during this period and the maximum was observed in the gradient and freshwater zones.

There was no lag period in the oxidation of ammonia to nitrite in the sediments of the marine zone and the oxidation was very gradual. Ammonia oxidation was very quick in the sediments of the marine zone and slow in the muds of the freshwater zone.

Nitrite oxidation and nitrate forming bacteria — Nitrite oxidation started only after a short lag period of 7 days and the process was comparatively slow. The number of nitrate forming bacteria was lower than that of nitrite forming bacteria in all the zones of the estuary during both premonsoon and monsoon months. The nitrite oxidizing bacteria also were more abundant in the sediments than in the overlying water (Table 1). In the freshwater zone nitrite oxidation was not detectable in the premonsoon months in the water column, though the sediments showed only a very low number of bacteria. Likewise the limnetic nitrite oxidizers also were absent in both marine and tidal zones in the water column, but the sediments showed very low numbers of these forms (1 cell/g and 7 cells/g in the marine and tidal zones respectively).

In the monsoon months, the estuary was flooded with freshwater and hence nitrite oxidation by the marine form of nitrite oxidizers was not observed. Still the sediments showed low numbers. Limnetic forms of nitrite oxidizing bacteria were found in both sediments and water column throughout the estuary in the monsoon months.

Table 2 shows variations in environmental parameters, from which it would be obvious that salinity is the important factor regulating the distribution and abundance of marine and limnetic forms of nitrifying bacteria. Daiber and Gooch[12]

TABLE 1 — DISTRIBUTION OF AMMONIA OXIDIZING AND NITRITE OXIDIZING BACTERIA IN VARIOUS ZONES OF VELLAR ESTUARY

Zones	Ammonia oxidizing				Nitrite oxidizing			
	Marine forms		Limnetic forms		Marine forms		Limnetic forms	
	Sediment cells × 10^2/g	Water cells × 10^2/litre	Sediment cells × 10^2/g	Water cells × 10^2/litre	Sediment cells × 10^2/g	Water cells × 10^2/litre	Sediment cells × 10^2/g	Water cells × 10^2/litre
				PREMONSOON PERIOD				
Marine	575·00	1·5	0·32	nil	0·7	0·12	0·01	nil
Tidal	75·00	0·12	0·6	nil	0·5	0·11	0·07	nil
Gradient	100·00	0·16	0·65	0·45	0·2	nil	1·2	0·03
Freshwater	100·00	0·03	0·150	3·7	0·2	nil	0·16	1·2
				MONSOON PERIOD				
Marine	0·1	0·05	0·5	0·07	0·09	nil	0·2	0·05
Tidal	0·05	0·01	·3	0·5	0·1	nil	0·6	0·07
Gradient	0·03	nil	·70	·2	0·03	nil	·7	0·5
Freshwater	nil	nil	·170	·6	nil	nil	·30	0·2

observed a higher percentage of the population was represented by the autotrophic nitrifiers in Delaware marshes during the late winter and early spring when high levels of nitrate were observed. The distribution of total heterotrophic bacteria and ammonifying bacteria in water and sediments, is shown in Table 3.

In the premonsoon period (July-September), ammonia oxidizing capacity of the water decreased gradually from the marine zone to freshwater zone and in the monsoon months the reverse was the case. This was mainly due to the terrestrial run off due to monsoon rains. Kawai *et al.*[13] dealt with the question whether the so called marine nitrifying bacteria were found in limnetic environments or not. They found that the population of nitrifying bacteria in the upper reaches of rivers was, for the most part, composed of limnetic bacteria which did not grow in sea water media and that the ratio of marine nitrifiers to limnetic ones became larger in the lower reaches of the river. Nitrifying bacteria of marine origin were more active at salt concentrations equivalent to those of the natural environment than the terrestrial strains and the terrestrial bacteria also showed greater activity in seawater after a longer lag period[14]. Finstein and Bitzky[15] found that autotrophic ammonium oxidizing cultures derived from freshwaters did not require marine salts for growth and that a portion of the test cultures from estuarine and marine littoral waters responded similarly. Yoshida[16] reported comparatively large counts of limnetic nitrifying bacteria (10^3 -10^5/g) in the upper stream and the estuarine zone of the river, but only low counts in the coastal region away from the mouth of the river (10^1-10^2 cells/g). He also found that a few almost pure cultures of these limnetic nitrifying bacteria grew well in limnetic as well as marine media. Watson[17] observed that both terrestrial and marine forms grew for a limited period in the presence or absence of sodium chloride and that the marine forms did best in a medium with 2·5% NaCl. Some of the marine forms did best in a medium with 2·5% NaCl. Some of the cultures from estuarine and littoral waters grew only in the presence of marine salts and the minimum concentration that supported development corresponded to 6% of full strength seawater[15].

The present study shows that the number of nitrifying bacteria of terrigenous origin or limnetic forms of nitrifying bacteria decreases from freshwater zone to marine zone. Marine nitrifying bacteria are comparatively more in the marine and tidal zone. On the other hand, the marine forms of nitrifying bacteria which thrive well at optimum salinity values were mostly present in marine and tidal zones. In the monsoon season when the entire estuary is flooded with terrestrial run off the limnetic forms dominate. Billen[18] also recently reported that nitrifying bacteria of terrestrial origin were present throughout the water along a longitudinal profile of the Scheldt estuary, with a regular decrease in numbers down stream. However, he has pointed out that nitrification occurred only in zone of favourable oxidation-reduction conditions, which coincided with the thermodynamic stability fields of nitrate and nitrite with respect to ammonia.

In the present study the bottom sediments always showed high concentrations of nitrifying bacteria. Kawai *et al.*[13] also made a similar observation in their study of the coastal area of Maizuru Bay. Yoshida[16] pointed out that most of the nitrification in Maizuru Bay was carried out in the mud and in the offshore region in the water rather than in the mud. Carey[19] and Watson[17] suggested that the most probable site of nitrification was within the water column rather than in the sediments. Billen[18] observed that nitrification in sediments of Scheldt estuary was very low.

The present investigation showed that microorganisms which could oxidize ammonium and nitrite were present in the water column, though

TABLE 2 — SOME ENVIRONMENTAL PARAMETERS IN VELLAR ESTUARY DURING THE STUDY PERIOD

	Premonsoon		Monsoon	
	Surface	Bottom	Surface	Bottom
Nitrite (μg at/litre)	0·01-0·746	0·01-0·535	0·088-1·870	0·061-1·940
Nitrate (μg at/litre)	0·54-15·95	0·5-11·75	1·28-19·9	1·71-16·78
Ammonia (μg at/litre)	0·01-0·891	0·01-1·705	0·02-1·968	0·175-1·565
Dissolved organic nitrogen (μg at/litre)	12·7-38·4	14·3-29·2	2·0-66·3	4·6-51·1
Oxygen (ml/litre)	3·1-4·9	3·5-4·8	4·1-6·0	3·8-6·2
Salinity (‰)	1·7-33·8	1·2-33·9	0·0-27·0	0·0-31·3
Temperature (°C)	25·0-28·0	25·0-27·0	22·0-24·0	23·-25·0

TABLE 3 — DISTRIBUTION OF SOME HETEROTROPHIC BACTERIA IN VELLAR ESTUARY

Heterotrophic form	Marine zone			Freshwater zone		
	Surface water cells/ml	Bottom water cells/ml	Sediments cells/g	Surface water cells/ml	Bottom water cells/ml	Sediments cells/g
Total heterotrophic bacteria	175×10^6	150×10^6	90×10^7	154×10^5	106×10^5	68×10^7
Ammonifying bacteria	2×10^6	5×10^6	5×10^7	98×10^5	133×10^5	84×10^7

low in numbers and that oxidation of ammonia to nitrate occurred with no accumulation of nitrite. It also indicated that nitrification was predominant in the sediments rather than in the water column. From the present study, it could also be inferred that the distribution of nitrifiers was not influenced by oxygen concentration, but by salinity and nutrient variations. Though the numbers of nitrifying bacteria of either marine or limnetic forms are greatly influenced by salinity, the potential activity of the bacteria might depend on the substrate concentration, namely ammonia and nitrite.

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