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Use of Nitrifier Activity Measurements To Estimate the Efficiency of Viable Nitrifier Counts in Soils and Sediments

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A procedure for estimating the efficiency of the most-probable number (MPN) technique for counting ammonium-oxidizing bacteria was tested on sediments and soils collected from Delaware Inlet, Nelson, New Zealand. The procedure involved estimating the nitrifier populations required to produce observed activities and comparing these estimates with the MPN-countable populations. MPN counts ranged between 0.15×10^3 to 3.0×10^3 cells g^{-1} in sediments and between 4.4×10^3 to 19×10^3 cells g^{-1} in soils. These counts were only 0.1 to 5.0% of the estimated populations that would be required to produce the observed activity. Similar efficiency calculations were made for data already in the literature, and these calculations gave much higher percentages. Thus, we concluded that for the soils and sediments we studied, the MPN counting technique greatly underestimated the populations present and that the efficiency calculation could be used as a counting efficiency index.

The most probable number (MPN) viable counting technique for nitrifying bacteria is one of the most commonly used methods in ecological studies of nitrification. However, it is possible that under some conditions the MPN method greatly underestimates indigenous nitrifier populations (2). When MPN counts are used in a study, it is desirable to have an idea of their relative counting efficiency. It has been proposed that estimates of counting efficiency could be made by making nitrifier activity measurements in addition to MPN counts (2). This procedure requires that a theoretical estimate be made of the population necessary to produce the observed activity. The MPN count as a percentage of this theoretical population can be used as an index of counting efficiency.

In this report a simple method for measuring maximum activities of indigenous ammonium oxidizer populations (nitrifying potentials) was applied to a number of soils and intertidal sediments (3). A shaken soil or sediment slurry was incubated, and the second step of nitrification, nitrite oxidation, was inhibited by chlorate (10 mM). Under these conditions the rate of ammonium oxidation was equal to the rate of nitrite accumulation. Nitrapyrin, a specific inhibitor of nitrification, was added during the incubation to demonstrate that nitrite was produced from autotrophic ammonium oxidation.

When making the estimates of the ammonium oxidizer populations associated with a given amount of activity, we assumed that the rate of nitrite production, r [i.e.; $r = \mathrm{d}(NO_2^-)/\mathrm{dt}$], was related to the density of cells, X, by the parameter k:

$$X = r/k \quad (1)$$

Although the proportionality parameter, k, obeys Michaelis-Menten kinetics [i.e.; $k = k_0(NH_4^+)/(Km + NH_4^+)$], equation (1) is modified by setting k equal to its maximum value ($k = k_0$). Since k is always $\leq k_0$, the resulting population estimate will be less than or equal to the estimate of equation 1. This is a minimum estimate and is denoted X_{min} (i.e., $X_{min} = r/k_0$). A pure culture value for k_0 was used. Thus, X_{min} represents the theoretical minimum number of pure culture cells required to produce the amount of activity observed in the sample. A k_0 of 0.023 pmol of NH_4^+ oxidized per cell per h was used since this appears to be the maximum value of k_0 for the three major genera of ammonium oxidizers (5).

MPN counts were made for ammonium oxidizers by using one marine and two types of freshwater media for intertidal sediment, whereas only freshwater media were used for soil counts. The marine medium, which was made with aged, filtered seawater, contained ammonium (final concentration, 2.0 mM) and chelated iron (1.0 ml/liter of a solution containing 0.246 g of $FeSO_4 \cdot H_2O$ and 0.331 g of $EDTA \cdot Na_2$ in 100 ml of H_2O). The two freshwater media were those described by Alexander and Clark (1) and Soriano and Walker (13). Five milliliters of media were added per MPN tube and the tubes were autoclaved for 15 min at 121°C. Counts

TABLE 1. Description of sediments at Delaware Inlet

Site	Classification and description	Infiltration rate (cm h^{-1})	Particle size distribution (%)		
			<4 μm	4–60 μm	>60 μm
A1	Sand: relatively undifferentiated profile	6.3	<0.5	1.8	98.3
A5	Sand: cockles associated with top 4 to 5 cm overlying a sandy FeS layer	11.5	<0.5	<0.5	99.6
A6	Silty sand: 0.5-cm oxidized layer overlying a reduced FeS layer (0.5–7 cm); consolidated layer below 7 cm; surface grazed by snails (*Amphibola*)	<0.02	3.6	30.4	66.0
A7	Sand: relatively undifferentiated profile, characterized by occasional *Euglena-Oscillatoria* blooms	3.4	1.2	3.3	95.4
B7	Silty sand: fresh water stream sediment in tidal basin; 1–2 cm oxidized surface layer overlying reduced FeS layer	<0.02	2.2	27.9	69.9

were made either at the beginning of the incubation or during the slurry incubations on flasks which had no chlorate added. MPN tubes were incubated for at least 3 months at 25°C with periodic inspection for positive tubes (4).

Studies were carried out with five intertidal sediments types and two soils collected at Delaware Inlet, Nelson, New Zealand. These sediments, along with particle size analysis and infiltration rates, are described in Table 1. The sites were selected from sediment types studied by Mountfort et al. (10). Particle size analysis was done by the pipette method (8), and infiltration rates were measured by the flooding method (6). Sediments were classified by texture (9).

Two soil types were collected adjacent to the inlet and are described as Wakapuaka sandy loam and Ronga silty loam. Both soils were in permanent clover grass pastures (grazed by either sheep [Wakapuaka] or cattle [Ronga]).

Nitrifying potentials and MPN counts for the five sediments and two soils are shown in Table 2. The two soils tested had significantly higher activities than did any of the sediments. The highest sediment activities were measured in the silty sand sediments (A6 and B7), whereas the activities of the sands were at the detection limit of the activity tests. MPN counts were also lower in the sediments than in the soils.

In the fourth column of Table 2, the MPN counts are given as percentages of the equivalent pure culture population (X_{min}) required to produce the observed nitrifying potential. If it is assumed that the activity per nitrifier, k_0, in a natural environment is similar to that in pure culture, one must conclude that the counting efficiency in this study was very low. It could be argued that the activity per cell in the natural environment was larger than in pure culture, but it is unlikely to be a factor of 20 to 5,000 higher. An increase in activity in this range would be required to give a 100% counting efficiency.

There have been several studies in which both MPN counts and nitrifying potentials have been measured, and counting efficiencies can be calculated. Calculations from data of Sarathchan-

TABLE 2. Activities, MPN counts, and counting efficiency for ammonium oxidizers for soils and intertidal sediments at Delaware Inlet[a]

Site	Activity (nmol g^{-1} h^{-1})	MPN count (Cells g^{-1} [$\times 10^3$])	Efficiency (%)
Sediments			
A1	0.54	1.7	5.1
A5	0.41	0.15	0.8
A6a	6.5	1.7	0.6
A6b	3.7	0.28–0.47	0.2–0.3
A7	0.32	0.42	2.9
B7s	7.5	1.7	0.5
B7f	5.1	3.0	1.2
Soils			
Ronga	21.1	4.4–9.6	0.5–1.1
Wakapuaka	96.4	10–19	0.2–0.5

[a] Samples A6a and A6b were sediments of similar texture, but they were separated by approximately 2 km. Sediment B7 was influenced by a freshwater stream; therefore, two nitrifying potentials were made: one with a salt water slurry (B7s) and one with freshwater slurry (B7f).

dra (11) for nine New Zealand pasture soils show counting efficiencies for ammonium oxidizers between 0.85 and 37.3%, with an average of 13.2 ± 8.0%. This is significantly higher than the 1.2 ± 1.4% resulting from our work. Data from Steele et al. (14) for 13 grassland soils show even higher efficiencies. These efficiencies range between 1.6 and 266% (mean, 53.0 ± 68.1). Similarly, values between 25.3 and 36.2 are obtained from the data of Curtis et al. (7) for ammonium oxidation in river sediments. These calculations support the theory that MPN counts were underestimating the populations in our study.

Efficiency calculations are complicated by the fact that it is not generally known which genus of ammonium oxidizer is being counted in a particular habitat. If, for example, *Nitrosospira* were the dominant genus, one would expect the efficiencies calculated above to be lower by a factor of five since *Nitrosospira* has an activity of only 0.004 pmol cell^{-1} h^{-1} in pure culture, as opposed to an activity of 0.023 pmol cell^{-1} h^{-1} for *Nitrosomonas* (5). In fact, *Nitrosospira* cells were observed in some of the MPN tubes (Ronga and Wakapuaka soils and sediment B7), but they were never the dominant genus in any of the soils and sediments we studied. *Nitrosomonas* cells were much more dominant in MPN tubes, so it is likely that *Nitrosomonas* was the dominant genus counted.

One of the reasons why the MPN technique may underestimate populations is the requirement that all genera and strains of ammonium oxidizers be able to grow in one medium. We tested three media for ammonium oxidizer counting efficiency in sediments, and the marine nitrifier medium gave the highest counts (Table 2). The ratio of counts in the marine ammonium oxidizer medium to those in the freshwater media was 15.27 ± 8.56.

There was no significant difference among the freshwater media. Therefore, it is clear that the autotrophic media used for counting greatly affects counting efficiency. Despite the improved growth conditions, the marine medium still appeared to be a rather poor medium for the marine nitrifiers. Enrichment cultures of marine nitrifiers grew very poorly in this medium, did not give MPN counts remotely near Petroff-Hauser counts (except on the one occasion when a pure culture was isolated), and often did not transfer at all. Poor growth in this medium may account for some of the low efficiencies observed in the marine environment.

It has often been assumed that low autotrophic nitrifier counts in nitrifying soils indicate the presence of heterotrophic nitrification. Despite the low nitrifier counts in this study, the ammonium-oxidizing activity in both the soils and the sediments appeared to be autotrophic in nature since nitrite accumulation was at least 95% inhibited by the presence of nitrapyrin. This inhibitor specifically blocks autotrophic ammonium oxidation (12). Thus, the low counts associated with these nitrifying soils and sediments must result from inefficient counting of the nitrifier populations.

In addition, it can be concluded that MPN counts give a poor indication of activity or potential activity in natural environments. Sarathchandra (11) concluded this when he found that there was poor correlation between counts and activities. We found a similar poor correlation (r = 0.499 for sediments). This is most obvious when the sediments are compared. One would not guess on the basis of the MPN count that on average the silty sands (A6 and B7) were 10 times more active than the sands (A1, A5, and A7). In fact, if only MPN counts had been done, this rather surprising feature would have been missed. This is surprising because sites A6 and B7 are visibly muddy with reduced FeS layers below the surface and have low infiltration rates. The silty sands would seem to be a rather poor habitat for nitrifying bacteria, especially when compared with the sands.

We conclude that nitrifying potentials are superior to MPN counts as a quantitative measurement of nitrifier biomass. We are currently using nitrifying potentials almost exclusively as a measurement of nitrifier biomass. However, there are occasions when MPN counts are a useful measurement. When MPN counts are made, we feel that it is essential to have an index of counting efficiency, such as the one proposed here. However, this requires not only the measurement of a nitrifying potential, but the establishment of the dominant genus of nitrifier present.

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