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NOTE

Comparison of the carbon monoxide oxidation and ^{15}N tracer methods for estimating *in situ* chemolithotrophic ammonium oxidation

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ABSTRACT: The use of ^{14}CO oxidation to estimate *in situ* chemolithotrophic ammonium oxidation was compared to $^{15}NH_4^+$ tracer methodology. Samples collected from a variety of natural and artificial marine systems with a wide range of ammonium oxidation rates were analyzed using both techniques. Results for most samples compared well with a 0.971 linear correlation coefficient (r^2) between the 2 methods. Samples collected from the photic zone in oligotrophic areas and samples containing high CO concentrations (> 50 nM) did not correlate well. Experiments using 6 different ammonium oxidizers gave a value of $1.44 \pm 0.56 \times 10^{-2}$ for the factor relating the ratio of CO to ammonium oxidized, used in calculating NH_4^+ oxidation rates. Additional experimentation demonstrated that pH between 7.5 and 9.0 does not effect the calculations.

Simple and accurate methods for the measurement of *in situ* ammonium oxidation, the step in the nitrogen cycle in which NH_4^+ is oxidized to NO_2^-, have been elusive. Ammonium oxidation rates in unpolluted waters are usually low and the ammonium oxidizing bacteria are slow growing and affected by a number of environmental parameters (Painter 1970). Techniques currently used to estimate ammonium oxidation suffer from a number of limitations (for reviews see Jones et al. 1984, Hall 1986 and Ward 1986).

Many of the difficulties with previous techniques could potentially be avoided by using the ^{14}CO oxidation technique for estimating chemolithotrophic ammonium oxidation (Jones et al. 1984). This technique relies on the fact that the enzyme system responsible for the oxidation of ammonium to nitrite also oxidizes CO to CO_2 (Jones & Morita 1983b). This method has been applied in several environments with some success (Butler et al. 1987, Dodds & Jones 1987, Butler et al. 1988). However, the utility of this method has been limited by 2 factors. First it relies upon the empirical calculation of rates based on *in situ* ammonium concentrations and a laboratory-derived factor, the ratio of CO oxidation to ammonium oxidation by pure cultures of ammonium oxidizers. Second, no direct comparison between ^{15}N tracer methodologies and ^{14}CO oxidation has been made.

This note addresses both the calculation of the factor involving the ratio of CO and ammonium oxidation and the comparison of established ^{15}N tracer and ^{14}CO oxidation methodologies for the estimation of chemolithotrophic ammonium oxidation.

Materials and methods. Samples for comparison of methods were collected from a variety of marine and freshwater habitats (Table 1). Samples were examined using both the ^{14}CO oxidation technique described by Jones et al. (1984) and the ^{15}N tracer technique described by Olson (1981). Subsamples of 25 and 2000 ml were used respectively. ^{15}N determinations were made using a VG Isogas PRISM isotope ratio mass spectrometer after micro-Dumas combustion (Wada et al. 1977). Cells of ammonium oxidizers for pure culture work and pH experiments were grown in chemostats (Jones & Hood 1980) and analyzed for activity as described by Jones & Morita (1983a, b). Ammonium was analyzed by the phenol-hypochlorite colorometric method (Strickland & Parsons 1972). Nitrite was analyzed using the method described by Bendschneider & Robinson (1952).

Results and discussion. Calculation of estimated *in situ* ammonium oxidation rates by the ^{14}CO oxidation technique are based on the *in situ* concentrations of ammonium, the N-Serve (nitrapyrin) inhibited CO oxidation rates (Jones et al. 1984) and a laboratory-derived factor relating the ratio of CO oxidation (at 2.25 nM) to ammonium oxidation (at 1.0 µM) using pure cultures of ammonium oxidizers. The equation used in the calculations is:

$$O = (C/F) \times N$$

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Table 1. Values used to calculate the ratio of CO oxidized to NH_4^+ oxidized by pure cultures (F). Assay concentration for CO oxidation was 2.23 nM and for NH_4^+ oxidation 1.0 µM

Organism	Source	CO oxidation (nM h^{-1})	NH_4^+ oxidation (nM h^{-1})	F ($\times 10^{-2}$)
Nitrosomonus europaea	Soil; ATCC 25978	0.61	24.9	2.45
Nitrosomonus cryotolerans	Marine; Alaska, USA	5.78	385.9	1.50
Nitrosococcus oceanus	Marine; ATCC 19707	1.99	127.6	1.56
Nitrosomonas sp.	Estuarine; Florida, USA	0.37	40.6	1.91
Nitrosomonas sp.	Soil; Oregon, USA	0.27	27.8	0.97
Nitrosomonas sp.	Seawater; Oregon, USA	0.17	13.8	1.23
			Average ± SD	1.44 ± 0.56

where O = estimated *in situ* ammonium oxidation rate (nM h^{-1}); C = N-Serve inhibited portion of the ^{14}CO oxidation rate (nM h^{-1}); F = factor derived from the ratio of CO oxidized (nM h^{-1}) to NH_4^+ oxidized (nM h^{-1}) from pure cultures (Table 1) and; N = *in situ* ammonium concentration in µM.

Ratios of CO oxidation to NH_4^+ oxidation (F) ranged from 0.91×10^{-2} for an estuarine isolate from Louisiana, USA to 2.45×10^{-2} for *Nitrosomanas europaea* (ATCC 25978). The average ratio was 1.44×10^{-2} with a standard deviation of $\pm 0.56 \times 10^{-2}$ (Table 1). This ratio is an average of 6 diverse groups of ammonium oxidizers and should represent a more accurate value than that reported earlier by Jones et al. (1984) which relied on only 2 organisms, both marine isolates.

Several factors affect both CO oxidation and NH_4^+ oxidation, principal among these is pH. Ammonia ionizes to NH_4^+ with an equilibrium that is highly pH-dependent while CO does not undergo such a transformation. Research has demonstrated the pH affects NH_4^+ oxidation (Painter 1970) to a much greater extent than CO oxidation (Jones & Morita 1983b). This makes it important to understand the effects that pH may have on the ratio of CO to NH_4^+ oxidation (F). Several experiments using *Nitrosococcus oceanus* to examine the effects of pH on CO and NH_4^+ oxidation are summarized in Table 2. CO oxidation rates remained relatively constant between pH values of 6.5 and 9.0 while ammonium oxidation rates were constant between pH values of 7.5 and 9.0. Thus, the useable pH range for the factor (F) reported in Table 1 is 7.5 to 9.0. At pH values less than 7.5 upward adjustments of F are necessary to estimate ammonium oxidation rates (Table 2).

An additional parameter that affects the calculation is the measurement of the *in situ* ammonium concentration (N). Although it is possible to detect NH_4^+ at concentrations below 0.1 µM, accuracy and reproducibility are difficult at these levels. Given the nature of N in the above calculation it is important that *in situ* NH_4^+ concentrations be determined as accurately as possible. At low NH_4^+ oxidation rates the largest source of error is represented by this term. Recently Jones (1991) has reported on a fluorescent method for the determination of nM levels of NH_4^+ which should decrease the errors associated with the measurement of N if utilized in conjunction with this method.

A direct comparison between the ^{14}CO oxidation method for estimating *in situ* ammonium oxidation rates and the ^{15}N tracer method is made in Table 3 and Fig. 1. Ten different water samples ranging from a recirculating aquarium to the nitrite maximum in the southeastern Sargasso Sea (Atlantic Ocean) were compared (Table 3). Ammonium oxidation rates determined using ^{15}N ranged from 65.26 nM h^{-1} in the recirculating aquarium to 0.02 nM h^{-1} for the nitrite maximum in the Sargasso Sea. For the most part values calculated using the ^{14}CO method gave nearly identical results. Samples divided themselves into 2 groups: (1) a group where ^{15}N and ^{14}CO calculated values were highly correlated (ratio ^{15}N:^{14}CO ranged from 0.87 to 1.27; Table 3) and (2) a group where there was no observed correlation (ratio ^{15}N:$^{14}C < 0.015$). For these comparisons it was assumed that the ^{15}N method was

Table 2. Effect of pH on CO and NH_4^+ oxidation rates and the ratio of CO oxidized to NH_4^+ oxidized (F) for *Nitrosococcus oceanus*. Assay concentration for CO oxidation was 2.23 nM and for NH_4^+ oxidation 1.0 µM

pH	CO oxidation (nM h^{-1})	NH_4^+ oxidation (nM h^{-1})	F ($\times 10^{-2}$)
5.5	1.49	12	12.4
6.0	1.58	32	4.94
6.5	1.84	58	3.17
7.0	2.04	83	2.45
7.5	1.99	119	1.67
8.0	2.00	121	1.65
8.5	1.98	121	1.64
9.0	1.88	109	1.72

Table 3. Comparison of ammonium oxidation rates estimated using ^{14}CO and ^{15}N methodologies

Sample collection	*In situ* NH_4^+ (μM)	Calculated ammonium oxidation rate (nM h^{-1})		Ratio $^{15}N/^{14}CO$
		^{15}N method	^{14}CO method	
Flow-through aquaculture system (lobster)	1.23	3.16	2.66	1.19
Flow-through aquaculture system (prawns)	1.89	2.39	1.88	1.27
Recirculating aquaculture system (lobster)	2.51	65.26	63.00	1.04
Orinoco River plume (28 ‰; Venezuela)	1.68	17.03	19.40	0.88
Caribbean Sea (Nitrite maximum; 100 m)	0.27	0.48	0.41	1.17
Sargasso Sea (Nitrite maximum; 120 m)	0.05[a]	0.02	0.02	0.87
Lake Erie (surface: USA)[b]	0.91	0.07	39.01	0.001
Biscayne Bay (surface; Miami, Florida, USA)[b]	0.95	0.02	37.03	0.001
OE Lake (surface; Miami, Florida, USA)[c]	5.16	0.43	30.91	0.014
Nitrosococcus oceanus[d]	0.40	44.16	39.76	1.11

[a] NH_4^+ concentrations < 0.1 μM have a ± 50 % uncertainty associated with them
[b] Samples were collected from high light intensity areas
[c] Ambient CO concentrations = 293 nM
[d] Value measured by nitrite formation gave a rate of 40.56 nM h^{-1}

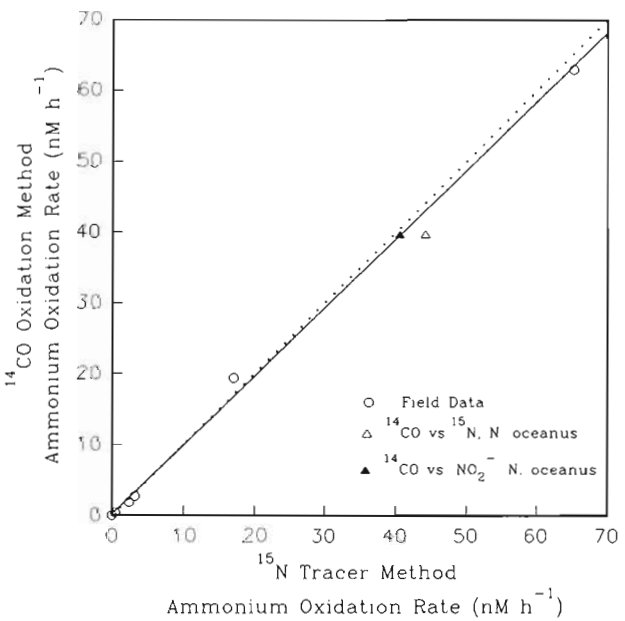

Fig. 1. Comparison of the ^{14}CO oxidation and the ^{15}N tracer methods for estimating *in situ* ammonium oxidation rates ($r^2 = 0.971$). *Nitrosococcus oceanus* data not used in calculating r^2 Dotted line represents 1:1 relationship

accurate and the ^{14}CO calculation was subject to error. This is likely to be the case since it is known that both light and ambient CO concentrations play an important role in regulating/inhibiting NH_4^+ oxidation (Vanzella et al. 1989). These factors would therefore be expected to affect the indirect ^{14}CO method and not the more direct ^{15}N method.

CO oxidation does not appear to show the same inhibitory effects of light (Vanzella et al. 1989, 1990, Guerrero & Jones unpubl.); in fact increasing CO concentrations are stimulatory (Jones & Morita 1983b). Although other explanations are possible it is likely that the second group, where the values did not compare well, can be explained by 1 of these factors. Samples collected from Lake Erie and Biscayne Bay were from high light environments and the OE Lake samples contained 293 nM of CO, a known inhibitory concentration of CO for the ammonium oxidizers (Vanzella et al. 1989). Although samples from the flow-through aquaculture systems were exposed to light, they did not demonstrate significant differences between the 2 methods. This could possibly be explained by the observation that high rates of ammonium oxidation protect the ammonium oxidizers from the effect of light (Vanzella et al. 1989, 1990).

With the exception of the samples (Group 2) reported above, 6 of the 9 environmental samples examined demonstrated ≥ 79% compatibility between the 2 methods. A graphical comparison of these 6 samples is shown in Fig. 1. The methods compared well with a linear correlation coefficient of 0.971 (r^2), thus demonstrating that for this range of samples the ^{14}CO method provides a reliable technique for estimating *in situ* ammonium oxidation rates. Linearity of the method was excellent and rates ranged from 0.02 to 63 nM h^{-1} demonstrating the utility of this method. Other samples comparing ^{15}N with ^{14}CO have shown that this relationship holds for rates well over 500 nM h^{-1} (Jones unpubl.). Experiments using pure cultures of the marine ammonium oxidizer *Nitrosococcus oceanus* demonstrated that ^{14}CO, ^{15}N and NO_2^- production methods all gave nearly identical results (Table 2, Fig. 1).

Despite the fact that this method appears to accurately estimate *in situ* ammonium oxidation rates, is simple to perform, requires short incubation times, and is highly sensitive, it is important to note that it cannot be universally applied to all sample matrices without first taking into consideration environmental characteristics (pH, light, system productivity, etc.). In addition to this, CO is a competitive inhibitor of ammonium oxidation (Suzuki et al. 1976) and thus the relationship between CO and ammonium concentrations could affect the estimates. The ^{14}CO method is an indirect method. However, if proper controls are performed and the method is calibrated using ^{15}N or some other direct measurement of ammonium oxidation, this method represents an accurate and simple way to estimate *in situ* ammonium oxidation rates in many aquatic environments.

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