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Method for estimating *in situ* chemolithotrophic ammonium oxidation using carbon monoxide oxidation*

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ABSTRACT: A sensitive new method for estimating chemolithotrophic ammonium oxidation (nitrification) has been developed. Carbon monoxide oxidation, using ^{14}CO, is measured with and without the addition of 100 mg l^{-1} of N-Serve, a specific nitrification inhibitor. The N-Serve sensitive CO oxidation can be related to the rate of ammonium oxidation by chemolithotrophic ammonium oxidizers. Methane oxidation, using $^{14}CH_4$, is also measured to determine the potential for ammonium and CO oxidation by methane oxidizer populations. This method was used to examine pure cultures of ammonium and methane oxidizers, as well as organisms in soil, 2 freshwater lakes, and estuarine waters. Ammonium oxidizers were found to be responsible for 28 to 97 % of the CO oxidation in the environmental samples examined. Ammonium and methane oxidizers were found to have widely different CH_4 oxidation to CO oxidation ratios, allowing distinction between environmental types where ammonium oxidizers achieve dominance.

INTRODUCTION

Several methods are currently in use or have been proposed for the estimation of nitrification in the environment. These include mass balance approaches using unlabeled substrates (Schwert and White, 1974; Billen, 1975; Webb and Wiebe, 1975); blockage of nitrite oxidation by chlorate and measurement of nitrite accumulation (Belser and Mays, 1980); estimation of nitrification potentials by adding high concentrations of NH_4^+ and determining nitrite and nitrate production; dark incorportion of ^{14}C-bicarbonate in the presence and absence of N-Serve (Billen, 1976); population estimates, using either most probable number (MPN) or fluorescent antibody (FA) and estimating activities from these values (Belser, 1979); and ^{15}N tracer techniques (Dugdale and Goering, 1967; Wada et al., 1977; Ohmori et al., 1981). All of the techniques mentioned thus far suffer from major drawbacks which have kept them from being used extensively. The major limitations include lack of sensitivity, disturbance of the populations by the addition of excessive substrate and the effects of lengthy incubation times. The mass balance approach is only applicable in situations where a differential exists between NH_4^+ and NO_2^- oxidation rates or where a specific water mass can be traced with time, as in a river. In addition it is very insensitive and is subject to a great deal of sampling error. These factors make it inadequate for nitrification rate determinations in most systems. The chlorate block method has been shown to have severe limitations in its applicability (Hynes and Knowles, 1983) in that the nitrite oxidizers are able to reduce chlorate to chlorite, which is an effective inhibitor of ammonium oxidation. The ^{14}C-bicarbonate method proposed by Billen (1976) has also been demonstrated to have several limitations (Hall, 1982). Although the method is fairly sensitive and only requires 3 to 6 h incubation times it fails to consider the lack of coupling between ammonium oxidation and CO_2 incorporation over a range of environmental conditions. It also fails to take into consideration the methane oxidizers which are also inhibited by N-Serve and incorporate CO_2 autotrophically (Topp and Knowles, 1982). The use of nitrification potentials provides limited information about natural systems and is unusable to examine most aquatic systems. Population estimates while providing some information about the composition of the system, provide little information that can be coupled

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to nitrification rates (Ward et al., 1982). In addition they severely underestimate the population size (Belser and Mays, 1982) which in turn gives excessively low nitrification rates (Wilson, 1965; Carlucci and Strickland, 1968). The ^{15}N tracer technique also results in experimental problems. The addition of substrate results in an unavoidable perturbation of the system at a point where the kinetics are most sensitive (limiting substrate levels). Large sample size (ca. 4 l) and lengthy incubation times are necessary to obtain measurable results. In addition the sensitivity of the method is not great, lower quantities of nitrite formation can be detected using colorometric methods (Bendschneider and Robinson, 1952). Despite these disadvantages the use of ^{15}N is at the present time the only method that allows the direct determination of *in situ* nitrification rates, that does not rely on other factors (CO_2 uptake, chlorate inhibition).

It has been demonstrated recently that ammonium oxidizers have the ability to oxidize both methane (Hyman and Wood, 1983; Jones and Morita, 1983a) and carbon monoxide (Jones and Morita, 1983b). In addition it has also been reported that N-Serve [2-chloro-6-(trichloromethyl)pyridine] (NS), a known inhibitor of chemolithotrophic ammonium oxidation (Goring, 1962), will inhibit these oxidations (Jones and Morita, ms submitted).

This paper will address the use of CO and CH_4 oxidation by the chemolithotrophic ammonium oxidizers as an index of their activity. It will also examine the applicability of this method to soil, lake, and an estuarine environments.

MATERIALS AND METHODS

Cultures and inoculum. Cultures of the methane oxidizers, *Methylocystis parvus* (OBBP), *Methylosinus trichosporium* (OB3B) and *Methylomonas albus* (BG8) (provided by C. Murrell) and a marine isolate, designated SA, from the surface waters of Sannich Inlet, Vancouver Island, British Columbia, isolated in our laboratory, were used in this study. Methane oxidizers were grown in 60 ml serum bottles containing 25 ml of the ammonium free medium used for the ammonium oxidizers (Jones and Morita, 1983a) supplemented with 1.0 g^{-1} of KNO_3 as a nitrogen source. The pH was adjusted to 6.8 for the freshwater isolates and 7.8 for the marine isolate. The serum bottles were inoculated and then capped with butyl rubber serum stoppers and aluminum crimp seals (Bellco) and pressurized to 10 psi with methane gas (Airco, Grade 4 ultra pure). Bottles were incubated at 25 °C on a rotary shaker. Samples were taken during log phase of growth (36 to 72 h depending upon the organism) and again during cell senescence (3 wk). These samples were subjected to cell counts, using acridine orange fluorescent microscopy (Hobbie et al., 1977). Portions (1.0 ml) of these cultures were used to examine the rates of CO and CH_4 oxidation.

The preparation of *Nitrosomonas europaea, Nitrosomonas* sp. 4W30 and *Nitrosococcus oceanus* cultures used in this study was identical to that described previously (Jones and Morita, 1983b).

Field samples and sampling. Soil samples were collected from an uncultivated meadow and a garden site within the meadow near Silverton, Oregon. The soils have been under the present state of cultivation for at least the last 7 yr. The garden soil has received regular additions of fertilizer and lime during this time. Soil pH values were 5.08 for the uncultivated soil and 5.10 for the garden soil. The top 5 cm of soil was collected, air dried and returned to the laboratory. Soil was then sieved (1.0 mm), soil mositure was determined and adjusted to 23 % using distilled water. Portions of these soils were used for the analyses.

The lakes examined were 2 lakes in the blast zone of Mt. St. Helens, Washington. They were North Coldwater Lake and Spirit Lake. A more complete description of these lakes can be found in Baross et al. (1982). Water samples were collected with a 5 l Niskin bottle at the surface, top, middle, and bottom of the thermocline and at 40 m. Samples for CO, CO-NS, and CH_4 oxidation were worked up in the field within 1 h of collection. North Coldwater Lake was sampled on 18 July 1983 and Spirit Lake was sampled on 19 July 1983.

Yaquina Bay, a small estuary on the Pacific Ocean near Newport, Oregon, was also examined during this study. The estuary is ocean dominated during the summer and fall months (July–October) and river dominated during the winter and spring (November–June). On 8 November 1983 a transect was made up the estuary beginning 3.9 km from the mouth at low tide. Surface samples were collected in 5 l Niskin bottle and samples for CO, CO-NS, and CH_4 oxidation were transferred to sterile 500 ml polypropylene bottles, capped and transported to the laboratory in a styrofoam box for analysis. At the time of the sampling the estuary was river dominated (high river flow), as the winter rains had begun. In addition to this transect, single surface water samples were collected from the OSU Marine Science Center dock (approx. 3 km from the mouth of the estuary) at low tide on the 18 and 19 of October, 1983. These samples were collected in 5 l bottles and returned immediately to the laboratory for analysis.

Carbon monoxide oxidation determinations. The methods used for ^{14}CO preparation and CO oxidation rate determinations were essentially the same as those

previously described (Jones and Morita, 1983b). For rate determinations involving pure cultures of methane or ammonium oxidizers, 1.0 ml of the previously described inoculum was used. For soil samples 1.0 g wet wt was used. The prepared inocula or soils were added to 60 ml serum bottles containing 25 ml of NH_4^+-free medium adjusted to the proper salinity and pH (Jones and Morita, 1983a). When natural water samples were used, 25 ml of the sample was placed directly into the serum bottles. The bottles were then purged for 3 min with room air by bubbling it through the liquid using a small aquarium pump, flow was approximately 100 ml min^{-1}. This served to remove dissolved CO and CH_4 in the medium. Since the ambient concentrations of CO and CH_4 in the atmosphere are extremely low compared to that added for the oxidation assays, it was assumed there would be a negligable dilution effect due to ^{12}CO and $^{12}CH_4$ present in the air. The bottles were then sealed with serum stoppers, and 0.5 ml of ^{14}CO diluted in nitrogen was (0.5 μCi ml^{-1}; specific activity, 56 mCi $mmole^{-1}$; Amersham Corp.) (1 mCi = 37 mBq) injected into the headspace. All bottles were prepared in triplicate. Acid controls were run with each experiment. Bottles were then incubated for 3 h (pure cultures and soils), or 12 h (water samples) on a rotary shaker at 100 rpm. Pure cultures of ammonium and methane oxidizers were incubated at 25 °C, soils and Yaquina Bay waters were incubated at 15 °C (water temperatures ranged from 10.9 to 15.1 °C at time of collection). Samples from North Coldwater and Spirit Lakes were incubated at 10 °C. Bottles used to determine the NS differential received either 25 μl of 100 μg $μl^{-1}$ N-Serve (Chem Services, Westchester, PA) in DMSO or 25 μl of DMSO (controls). These bottles were then stoppered as before, but they were preincubated for 1 h on a rotary shaker at 100 rpm at the appropriate temperature before addition of the ^{14}CO and incubated as usual. After incubation, the reaction was terminated by the addition of 1.0 ml of 5.0 N NaOH with a syringe through the stopper. The serum bottles were then shaken for 1 h at room temperature to permit the labeled CO_2 to be absorbed into solution. The serum stoppers were then removed and the bottles were shaken an additional 30 min in an exhaust hood to remove the remaining labeled CO. Labeled CO_2 was then released, trapped and assayed using the methods previously described by Griffiths et al. (1982).

Methane oxidation determinations. The procedures used to determine methane oxidation rates were identical to those used for CO with the following exceptions; once the bottles were sealed with serum stoppers, 1.0 ml of $^{14}CH_4$ diluted in nitrogen, instead of ^{14}CO, was injected into the headspace (1.0 μCi ml^{-1}; specific activity, 59 mCi $mmole^{-1}$; Amersham Corp.). Ammonium oxidizers cultures were incubated for 48 h rather than 3 h as with CO, methane oxidizers and field samples were incubated as before.

Chemical analysis. Samples collected at North Coldwater Lake, Spirit Lake and Yaquina Bay were part of ongoing research projects. Analyses of NH_4^+, NO_2^-, and NO_3^- for North Coldwater and Spirit Lakes were provided by C. Dahm and NH_4^+ and salinity measurements for Yaquina Bay were provided by J. Garber and J. Butler.

Ancillary determinations. Water samples collected on October 18 and 19, 1983 at Yaquina Bay were subjected to a series of additional experiments to elucidate factors related to this proposed method.

Time course experiments at one CO and one CH_4 concentration, were conducted both in the presence and absence of 10 mg l^{-1} NH_4^+-N. For CO, 3 series of bottles were prepared (60 ml bottles with 25 ml water), one containing 0.5 ml of the ^{14}CO-N_2 mixture, another containing 0.5 ml ^{14}CO-N_2 and 100 mg l^{-1} NS in DMSO, and one with 0.5 ml ^{14}CO-N_2 and 10 mg l^{-1} NH_4^+-N [as $(NH_4)_2SO_4$]. At time intervals of 1, 3, 6, 12, 24, and 48 and 72 h, 3 bottles from each series were assayed for ^{14}CO oxidized to $^{14}CO_2$. Two series of bottles were prepared for CH_4 oxidation, one with 1.0 ml of the $^{14}CH_4$-N_2 mixture and another with 1.0 ml $^{14}CH_4$ and 10 mg l^{-1} NH_4^+-N. At time intervals of 6, 12, 24, 48, 72, 96 and 102 h, 3 bottles from each series were assayed for $^{14}CH_4$ oxidized to $^{14}CO_2$.

The effects of CO concentration on CO oxidation both in the presence and absence of 100 mg l^{-1} NS were examined. Bottles were first injected with 0.5 ml of the ^{14}CO-N_2 mixture and then additional quantities of unlabeled CO to give a series of bottles containing between 2.62 and 108.1 μl l^{-1} of CO in the headspace. The bottles were incubated 3 h and assayed as before.

The effect of sample storage on CO and CO-NS oxidation was examined. Once the water sample was collected it was allowed to set in the sample container for 0, 3, 6, or 12 h before being assayed for CO and CO-NS oxidation in the usual manner.

Calculations. The equilibrium concentrations of CO and CH_4 in solution were determined by multiplying the mixing ratio of CO or CH_4 in the headspace by the appropriate Bunsen solubility coefficient. The Bunsen coefficients depend upon the temperature and salinity of the sample while the mixing ratio is independent of temperature and pressure. Bunsen coefficients were derived from Schmidt (1979) for CO and Yamamoto et al. (1976) for CH_4. Differences in both salinity and temperature of incubation were sufficiently large to affect the solubility of CO and CH_4 significantly. These differences were taken into account when calculating oxidation rates.

Methane and CO oxidation rates given for the pure

culture experiments were normalized to nmoles l^{-1} h^{-1} at a cell density of 10^6 ml^{-1}, using the epifluorescent counts of the inoculum and assuming a linear relationship between cell number and activity. Dissolved CO concentrations were 2.00 nM for the freshwater isolates and 2.23 nM for the marine isolates. CH_4 concentrations were 13.80 nM and 11.93 nM for the freshwater and marine isolates, respectively.

For comparative purposes the CO and CH_4 oxidation rates calculated for field samples were normalized to rates at a CO concentration of 2.23 nM and a CH_4 concentration of 11.93 nM. These values were chosen since they allow comparison between this work and previous studies (Jones and Morita, 1983a, 1983b). It was assumed that a linear relation exists between CO and CH_4 concentration at these low concentrations and indeed this has been demonstrated (Conrad and Seiler, 1982; Jones and Morita, 1983a, b).

The ratio of CH_4 oxidation to CO oxidation was calculated by dividing the CH_4 oxidation rate at 11.93 nM by the CO oxidation rate at 2.23 nM. When this ratio was calculated for natural samples only the NS inhibited CO oxidation was considered.

RESULTS

The CO and CH_4 oxidation rates and CH_4 to CO oxidation ratios for the pure cultures of ammonium and methane oxidizers are given in Table 1. The values given for the ammonium oxidizers represent the mean values calculated from rates determined for this paper and previous work in our laboratory. The range of these values are given for the CH_4 to CO oxidation ratios. All of the methane oxidizers examined have a much greater ability to oxidize methane than the ammonium oxidizers, even the senescent cells. The most significant result of this experiment is the difference between the CH_4 to CO oxidation ratios for the methane and ammonium oxidizers. While the log phase cells of the methane oxidizers have ratios ranging from 1.11 to 1.87, the ammonium oxidizers ratios are at least 25 times lower, 0.0007 to 0.0428. Even the ratios of senescent cells of the methane oxidizers are at least 9 times higher than those for log cells of the ammonium oxidizers.

The CO, CO-NS, and methane oxidation rates of the soil samples along with representative values from Yaquina Bay, Spirit and North Coldwater Lakes are given in Table 2. The percent of the CO oxidation varied significantly, with the uncultivated soil having the lowest value, 28.2 %, and the surface waters of North Coldwater Lake having the highest percentage, 97.3 %. While the CO oxidation rates in the absence of NS for both soils are similar, the values in the presence of NS vary significantly. With the cultivated soil 74.0 % of the CO oxidation is NS inhibited, while only 28.2 % is with the uncultivated soil.

The results of the depth profile of North Coldwater Lake are given in Fig. 1 and Table 3. Ammonium concentration increases from its surface value of 2.0 $\mu g\ l^{-1}$ and peaked at 7.0 $\mu g\ l^{-1}$ and then decreased to 2.0 μg

Table 1. Ratios of methane oxidation to carbon monoxide oxidation by methane and ammonium oxidizing bacteria

Organism	Culture conditions	CH_4 oxidation rate (nmoles l^{-1} h^{-1})*	CO oxidation rate (nmoles l^{-1} h^{-1})*	Ratio CH_4 oxidation to CO oxidation
Methylocystis parvus (OBBP)	Log phase cells	6.60	3.53	1.87
	Senescent cells	0.526	0.910	0.578
Methylosinus trichosporium (OB3B)	Log phase cells	19.73	16.46	1.20
	Senescent cells	1.127	2.804	0.402
Methylomonas albus (BG8)	Log phase cells	5.25	3.59	1.46
	Senescent cells	0.178	0.468	0.380
Marine isolate (SA)	Log phase cells	6.05	5.44	1.11
	Senescent cells	1.033	1.883	0.549
Nitrosomonas europaea	w/o NH_4^+-N	0.0003	0.422	0.0007 ± .0003
	10 mg l^{-1} NH_4^+-N	0.0134	0.422	0.0319 ± .018
Nitrosomonas sp. (4W30)	w/o NH_4^+-N	0.0098	6.423	0.0015 ± .0005
	10 mg l^{-1} NH_4^+-N	0.0504	6.423	0.0078 ± .004
Nitrosococcus oceanus	w/o NH_4^+-N	0.0601	2.456	0.0245 ± .019
	10 mg l^{-1} NH_4^+-N	0.1052	2.456	0.0428 ± .022

* Oxidation rates expressed as nmoles l^{-1} h^{-1} $^{14}CH_4$, or ^{14}CO oxidized to $^{14}CO_2$ at a cell density normalized to 10^6 ml^{-1}

Table 2. Carbon monoxide, carbon monoxidè oxidation in the presence of 100 mg l^{-1} N-Serve, and methane oxidation by soil, lake and estuarine samples

Sample	CO oxidation rate (nmoles h^{-1})*	rate with 100 mg l^{-1} N-Serve (nmoles h^{-1})*	% CO oxidation inhibited by 100 mg l^{-1} N-Serve	CH_4 oxidation rate (nmoles h^{-1})*
Soil, uncultivated, meadow Silverton, OR	0.305	0.219	28.2	0.0096
Soil, cultivated, garden Silverton, OR	0.277	0.072	74.0	0.0003
Water, North Coldwater Lake, surface, Mt. St. Helens, WA	0.075	0.002	97.3	0.0001
Water, Spirit Lake, surface, Mt. St. Helens, WA	0.297	0.102	65.7	0.0377
Water, Yaquina Bay, surface, salinity 33‰, Newport, OR	0.136	0.018	88.5	0.0006
Water, Yaquina Bay, surface, salinity 2.2‰, Newport, OR	1.144	0.357	68.8	0.0144

* Rates expressed as nmoles g dry weight^{-1} h^{-1} for soils, and nmoles l^{-1} h^{-1} for water samples

l^{-1} and remained constant to 40 m. Nitrite remained constant at 3.0 µg l^{-1} throughout the depth profile, while nitrate decreased steadily through the first 20 m and then held relatively constant through 40 m. CO oxidation inhibited by NS and CH_4 oxidation peaked at 10 m with values of 0.143 nmoles l^{-1} h^{-1} and 0.008 nmoles l^{-1} h^{-1} respectively (Fig. 1B). CO oxidation inhibited by NS dropped to 0.65 nmoles l^{-1} h^{-1} at 15 m and then remained essentially constant throughout the profile. The ratios of CH_4 oxidation (at 11.93 nM) to CO

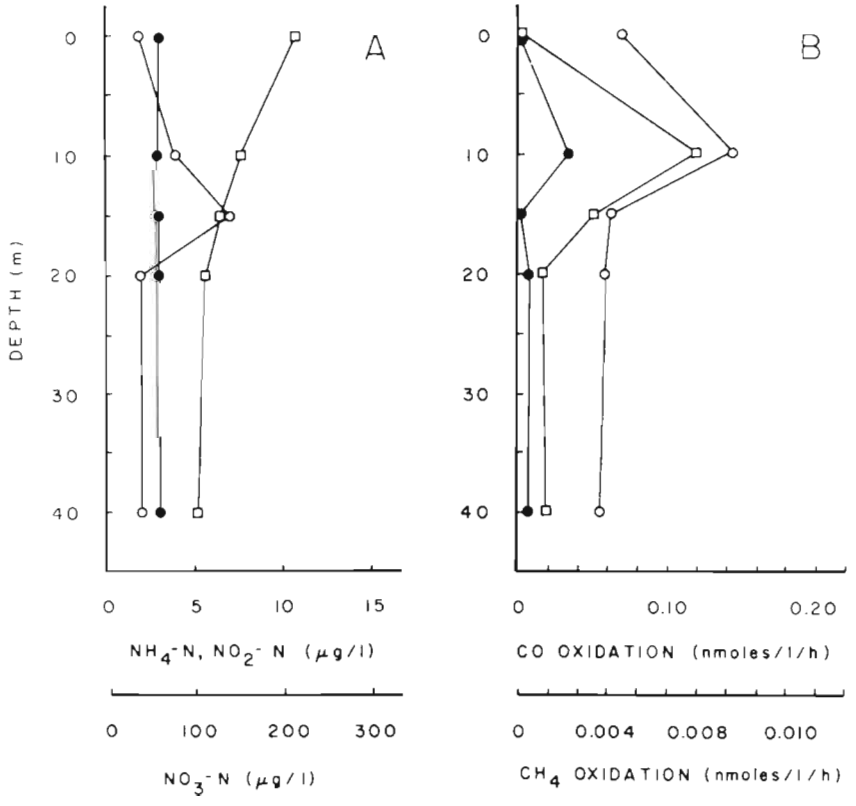

Fig. 1. North Coldwater Lake profile, 18 July 1983. (A) Inorganic nitrogen distribution; ○ NH_4^+-N; ● NO_2^--N; □ NO_3^--N. (B) CO and CH_4 oxidation; ○ N-Serve inhibited CO oxidation; ● CO oxidation not inhibited by N-Serve; □ CH_4 oxidation

Table 3. Ratios of methane oxidation to carbon monoxide oxidation for North Coldwater and Spirit Lakes

Sample site	Depth (m)	Ratio CH_4 oxidation to CO oxidation
North Coldwater Lake	0	0.001
18 July, 1983	10	0.056
	15	0.052
	20	0.019
	40	0.022
Spirit Lake	0	0.262
19 July, 1983	10	0.508
	15	0.477
	25	1.292
	40	0.934

oxidation (at 2.23 nM) range from 0.001 at the surface to 0.056 at 10 m (Table 3).

Spirit Lake had a considerably different profile (Fig. 2). Ammonium decreased steadily from a surface high of 5 μg l^{-1} to undetectable values at 25 m. Nitrite as before was essentially constant throughout the water column at 2 μg l^{-1}. Nitrate decreased rapidly from 0 to 25 m and then increased at 40 m. CO oxidation inhibited by NS and CH_4 oxidation values were high throughout the water column (Fig. 2B). CO oxidation inhibited by NS peaked at 15 m and 40 m with values of 0.409 nmoles l^{-1} h^{-1} and 0.460 nmoles l^{-1} h^{-1} respectively. The ratios of CH_4 oxidation to CO oxidation were considerably higher than those for North Coldwater Lake and ranged from 0.262 at the surface to 1.292 at 25 m (Table 3).

The results of the Yaquina Bay transect are shown in Fig. 3. CO oxidation inhibited by NS increased steadily, peaked, decreased slightly, leveled off and then peaked again at the last station. This final peak was likely due to terrestrial input, as this station was nearly freshwater and was sampled just after a record rainfall. Ammonium nitrogen also increased peaked and then decreased progressively up river, with the peak at 8.5 to 10.5 km (Fig. 3). Methane oxidation increased steadility up river over the entire length of the transect (Fig. 3). The ratio of CH_4 oxidation to CO oxidation remained low with values ranging from 0.005 for the incoming ocean water and 0.018 for the 2.6 ‰ water at the station furthest upstream (Table 4).

The results of the time course of CO oxidation experiments are shown in Fig. 4. The oxidation of CO in the absence of added NH_4^+-N was linear through 48 h and then increased rapidly. In the presence of 10 mg l^{-1} NH_4^+-N, CO oxidation paralleled that without ammonium added for 24 h and then increased rapidly. CO oxidation in the presence of 100 mg l^{-1} NS was linear through 6 h and then decreased through 72 h.

The time course of CH_4 oxidation both in the presence and absence of added NH_4^+-N were essentially linear through the first 48 h and then decreased through 120 h (Fig. 5). Ammonium nitrogen had little effect on CH_4 oxidation.

The relation between CO concentration and CO oxidation both in the presence and absence of 100 mg l^{-1}

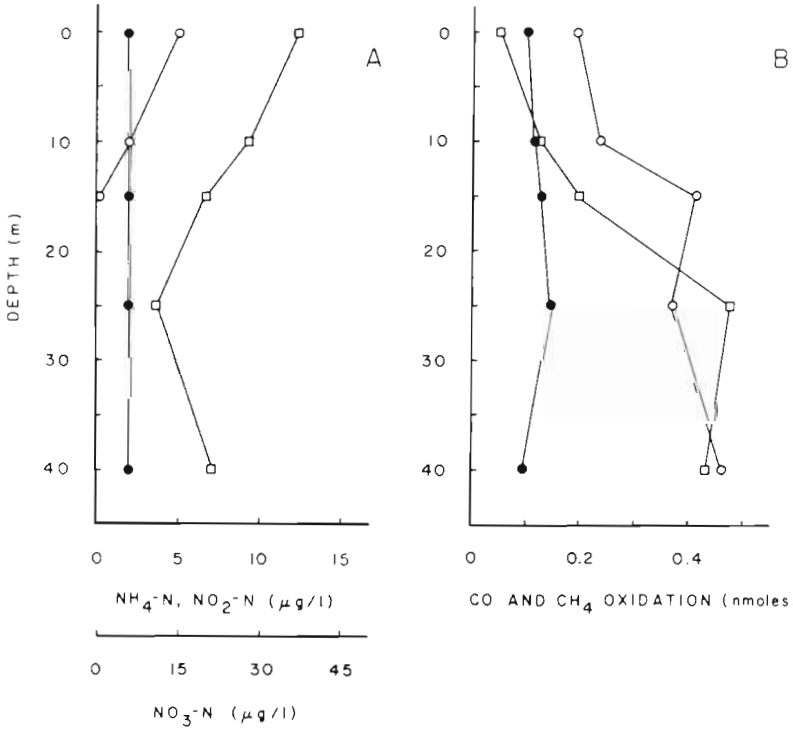

Fig. 2. Spirit Lake profile, 19 July 1983. (A) Inorganic nitrogen distribution; ○ NH_4^+-N; ● NO_2^--N. (B) CO and CH_4 oxidation; ○ N-Serve inhibited CO oxidation; ● CO oxidation not inhibited by N-Serve; □ CH_4 oxidation

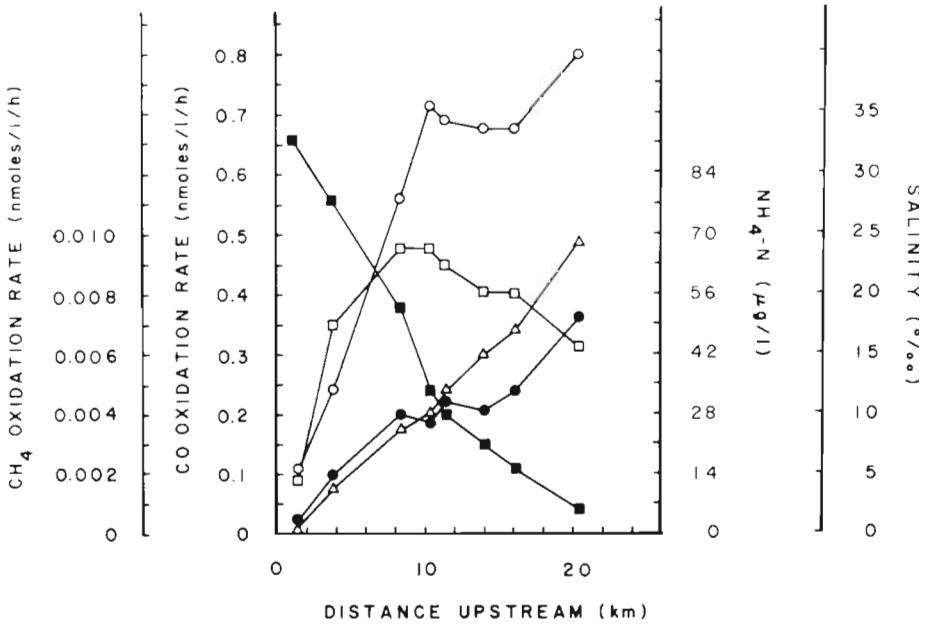

Fig. 3. Yaquina Bay transect, 8 Nov. 1983. ○ N-Serve inhibited CO oxidation; ● CO oxidation not inhibited by N-Serve; □ NH_4^+-N concentration; ■ salinity; △ CH_4 oxidation

NS demonstrated first-order kinetics, with the rates doubling with every doubling of CO concentration, at low CO concentrations (< 6.0 nM) and saturation at higher concentrations. Analysis of the data using Lineweaver-Burke plots yielded straight lines and gave Km values of 27.8 nM and a Vmax of 7.1 nmoles l^{-1} h^{-1} for CO oxidation in the absence of NS, 8.1 nM and 0.26 nmoles l^{-1} h^{-1} in the presence of 100 µg l^{-1} NS, and 42.0 nM and 5.0 nmoles l^{-1} h^{-1} for the difference (NS inhibited CO oxidation).

Sample storage up to 12 h had no effect on CO oxidation rates either in the presence or absence of 100 mg l^{-1} NS.

DISCUSSION

Several groups of organisms have been demonstrated to have the ability to oxidize CO to CO_2 aerobically. Among these are the carboxydobacteria (Zavarzin and Nozhevnikova, 1977), several actinomycetes (Bartholomew and Alexander, 1979), the methane oxidizing bacteria (Ferenci, 1974; Hubley et al., 1974; Ferenci et al., 1975), and the chemolithotrophic ammonium oxidizers (Jones and Morita, 1983b). It is likely that all of these organisms and others yet to be discovered play a role in the oxidation of CO in the

Table 4. Ratios of methane oxidation to carbon monoxide oxidation for Yaquina Bay

Date	Distance from mouth (km)	Ratio CH_4 oxidation to CO oxidation
18 Oct 1983	3.0	0.031
19 Oct 1983	3.0	0.020
8 Nov 1983	1.5	0.005
8 Nov 1983	3.9	0.009
8 Nov 1983	8.5	0.008
8 Nov 1983	10.5	0.007
8 Nov 1983	11.4	0.009
8 Nov 1983	14.2	0.012
8 Nov 1983	16.2	0.014
8 Nov 1983	20.3	0.018

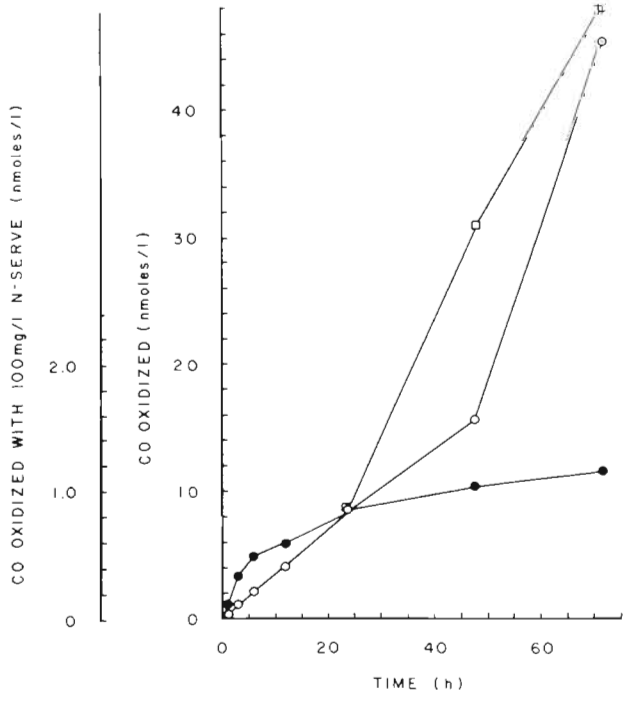

Fig. 4. Time course of CO oxidation by a Yaquina Bay water sample. ○ no additions; ● 100 mg l^{-1} N-Serve; □ 10 mg l^{-1} NH_4^+-N

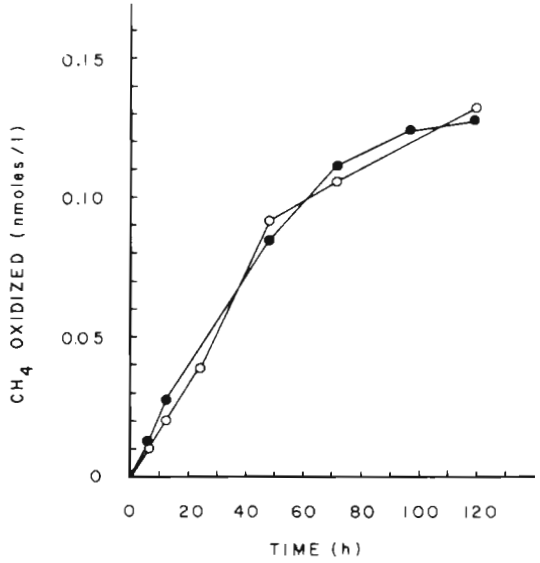

Fig. 5. Time course of CH_4 oxidation by Yaquina Bay water sample. ○ no additions; ● 10 mg l^{-1} NH_4^+-N

environment. The results of this study and previous studies would seem to indicate that the ammonium oxidizers could play an important role in CO oxidation in the environment (Jones and Morita, 1983b). It has been demonstrated by Conrad et al. (1981) and Conrad and Seiler (1982) that the carboxydobacteria do not play a major role in the oxidation of CO in the soils, oceans or lakes. This is evidenced by their high Km values (390 to 930 nM) (Conrd et al., 1981) and the low values of incorporation of ^{14}CO-C into cellular material found by Bartholomew and Alexander (1979) for soils. This paper presents additional support for this in that Km values for Yaquina Bay waters ranged from 8 to 42 nM, well below those for the carboxydobacteria. In addition to this no incorporation of ^{14}CO-C was detected in Yaquina Bay waters (Jones and Morita, unpubl.).

N-Serve is a powerful inhibitor of chemolithotrophic nitrification (Goring, 1962) and has been used previously for estimating nitrification rates (Webb and Wiebe, 1975; Christofi et al., 1981; Hall, 1982). Jones and Morita (ms submitted) have demonstrated that NS can also be used to inhibit the oxidation of CO by ammonium oxidizers. CO oxidizers can be divided into two groups using NS inhibition, those that are inhibited and those that are not inhibited by NS. The relative proportion of these groups can vary significantly (Table 2) and is not necessarily related to the total CO oxidation rate. The best examples of this are the 2 soil types examined for this study. While both soil types have high CO oxidation rates in the absence of NS, in the uncultivated soil only 28.2 % of the CO

Table 5. Calculated ammonium oxidation rates for North Coldwater Lake and Yaquina Bay

North Coldwater Lake Depth (m)	Rate (nmoles l^{-1} h^{-1})	Yaquina Bay Distance upstream (km)	Rate (nmoles l^{-1} h^{-1})
0	0.39	1.5	4.06
10	1.55	3.9	32.20
15	1.23	8.5	101.50
20	0.32	10.5	129.36
25	0.30	11.4	117.32
		14.2	103.88
		16.2	104.16
		20.3	94.22

Table 6. North Coldwater Lake and Yaquina Bay nitrification rates compared to other waters

Locality	Rate (nmoles l^{-1} h^{-1})	Reference
Lake Vanda	0.25–5.8	Vincent et al. (1981)
Lake Taupo	1.5–1.2	Vincent and Downes (1981)
Lake Mendota	2–44	Brezonik (1968*)
Belelham Tarn	134	Christofi et al. (1981)
North Coldwater Lake	0.39–1.55	This work
Scheldt Estuary	170–490	Somville (1978)
Scheldt Estuary (potential)	50–1700	Somville (1978)
Sagami Bay	0.16–5.0	Miyazaki et al. (1973)
California coastal waters	0.67–1.67	Ward et al. (1982)
Yaquina Bay	4.06–129	This work

* Ph. D. thesis, Univ. of Wisconsin, Madison, USA

oxidation was inhibited by NS, while 74.0 % of the activity was NS inhibited with the garden soil. At the same time the uncultivated soil had MPN values of 1.7×10^5 ammonium oxidizers g dry wt^{-1} while the garden soil contained 2.4×10^6 g dry wt^{-1} (Jones and Morita, unpubl.). The Km values for Yaquina Bay also demonstrate that there are 2 separate nonrelated groups. While the NS inhibited group had a Km value of 42.0 nM and a Vmax of 5.0 nmoles l^{-1} h^{-1}, the noninhibited group had a Km of 8.1 nM and a Vmax of 0.26 nmoles l^{-1} h^{-1}. In addition, none of the Lineweaver-Burke plots were parallel or shared either Km or Vmax values. This demonstrates that the group that was not inhibited by NS does not simply represent competitive, noncompetitive or uncompetitive inhibition of the NS inhibited group. The oxidation of methane by the methane oxidizers has also been

demonstrated to be inhibited by NS (Topp and Knowles, 1982).

Studies in our laboratory have shown that CO oxidation by the methane oxidizers is also completely inhibited by 100 mg l^{-1} NS (Jones and Morita, unpubl.). Although it is possible that there are other organisms involved in the NS sensitive oxidation of CO at the present time, it seems likely that this group contains mainly the ammonium and methane oxidizers. Because of these findings it is necessary to distinguish between the ammonium and methane oxidizers. Since both methane and ammonium oxidizers oxidize methane (Jones and Morita, 1983a) and CO, the ratios of CH_4 to CO oxidation provide this distinction. While the methane oxidizers had values of between 0.380 for senescent cells of *Methylomonas albus* (B68) to 1.87 for log cells *Methylocystis parvus* (OBBP), the ammonium oxidizer values were much smaller, 0.0007 for *Nitrosomonas europaea* in the absence of NH_4-N to 0.0428 for *Nitrosococcus oceanus* in the presence of 10 mg l^{-1} NH_4^+-N (Table 1). The depth profiles from North Coldwater and Spirit Lakes demonstrate that this distinction holds for environmental samples. While North Coldwater Lake is an ammonium oxidizer dominated systems (ratios of 0.001 to 0.056) Spirit Lake is a methane oxidizer dominated system with ratios ranging from 0.262 to 1.292 (Table 3). The values for the soil samples and estuarine water samples show they are also dominated by ammonium oxidizers (Tables 2 and 4). In situations where the ratios are high (> 0.2), it becomes difficult to determine how much of the CO oxidation is due to ammonium oxidizers, and from the viewpoint of this method it will work best where the system is ammonium oxidizer dominated, unless ammonium oxidation rates for the methane oxidizers are determined. The use of these ratios will, however, allow distinction between these 2 types of systems. In a paper dealing with methane oxidation in Lake Mendota (Wisconsin), Harrits and Hanson (1980) demonstrate that ammonium oxidation closely parallels the rates of methane oxidation, and suggest that since methane oxidizers are known to oxidize ammonium (Whittenbury et al., 1970; O'Neill and Wilkinson, 1979) they may contribute to the nitrogen cycle in Lake Mendota. Jones and Morita (1983a) suggest that an alternative explanation for this may be methane oxidation by the classical ammonium oxidizers. The results of the present study provide a method of determining the answer to this question.

Although ammonium oxidation by the chemolithotrophic ammonium oxidizers is considered to be important in the cycle of nitrogen in the oceans, freshwaters and soils, to date there is not a simple, sensitive or rapid technique to assay their importance. The procedure developed in this paper offers several advantages over existing methods for determining ammonium oxidation. First, the sample size necessary is small, the present study uses 25 ml samples. Second, the incubation time necessary to get results is short, 3 to 12 h. Third, it is not necessary to add abnormally high NH_4^+ concentrations to obtain results. Finally, the use of ^{14}CO as an alternative substrate for the chemolithotrophic ammonium oxidizers offers much greater sensitivity than the use of nonradiolabeled substrates. In addition to this, sensitivity can be increased by either increasing the sample size or increasing the ^{14}CO concentration.

In order for this method to be most accurate several factors must be taken into consideration, (1) the blockage of CO oxidation by NS must be complete, (2) the oxidation of CO and CH_4 must be linear with respect to both time of incubation and concentration, and (3) the ammonium oxidizers rather than the methane oxidizers must be the major group responsible for the NS inhibited CO oxidation.

The blockage of CO oxidation by ammonium oxidizers has been demonstrated to be complete with 100 mg l^{-1} added NS (Jones and Morita, ms submitted). In addition, studies with soils and Yaquina Bay waters show that increasing NS concentrations beyond 100 mg l^{-1} causes no additional decrease in CO oxidation (Jones and Morita, unpubl.).

The results of this study with respect to time of incubation and CO oxidation (Fig. 4) indicate that while CO oxidation in the presence of NS loses linearity after 3 to 6 h, its deviation from linearity is not great enough to cause significant changes in the results when samples are incubated for 12 h, but it would be advisable that future studies use incubation times of 6 h or less. CO oxidation in the absence of NS was linear for 48 h and therefore presents no problem. Methane oxidation rates from this study (Fig. 5) and in that of Griffiths et al. (1982), show that methane oxidation is linear with time through 48 h. The effects of increasing CO concentrations have been shown to be linear in this study and in those of Jones and Morita (1983a) and Conrad and Seiler (1982).

It may be possible to convert the rate of CO oxidation inhibited by NS to a rate of ammonium oxidation if the *in situ* concentrations of ammonium are known. Since CO acts as an alternative substrate for the ammonium monooxygenase system (Tsang and Suzuki, 1982; Jones and Morita, 1983a) and since there is no transport mechanism necessary for CO, the oxidation of CO should act as a sensitive enzyme assay for ammonium monooxygenase activity. It is interesting to use the values of ammonium oxidation at 1.0 mg l^{-1} NH_4^+-N reported by Jones and Morita (in press) for the ammonium oxidizers, *Nitrosomonas europaea* oxidized 1.2 µmoles NH_4^+ l^{-1} h^{-1} at 10^6 cells l^{-1}; *Ni-*

trosomonas sp. 4W30 oxidized 21 μmoles NH_4^+ l^{-1} h^{-1}, and *Nitrosococcus oceanus* oxidized 5.8 μmoles NH_4^+ l^{-1} h^{-1}, and the CO oxidation rates reported in this paper (Table 1) (*N. europaea* at 2.23 nM CO gives an oxidation rate of 0.471 nmoles l^{-1} h^{-1}) to calculate a ratio of CO oxidized at 2.23 nM CO to NH_4^+-N oxidized at 1.0 mg l^{-1}. These ratios are 3.9×10^{-4} for *N. europaea*, 3.1×10^{-4} for *Nitrosomonas* sp. 4W30, 4.2×10^{-4} for *N. oceanus*, with an average value of 3.7×10^{-4}. Using this value, assuming linearity (first order kinetics) for ammonium concentrations of < 1.0 mg l^{-1} NH_4^+-N, knowing the *in situ* concentrations of NH_4^+-N, and value for NS inhibited to CO oxidation, an actual rate can be calculated. Ammonium oxidation rates calculated from these data are given in Table 5. These values are within the range of activity reported by other workers (Table 6). It would be interesting to correlate these with actual field determinations of NH_4^+ oxidation using ^{15}N tracer methodologies to determine if the NH_4^+ to CO oxidation ratios hold in natural samples. The NS inhibited CO oxidation rates (and calculated ammonium oxidation rates) follow the ammonium concentration in Yaquina Bay (Fig. 3). The NS inhibited CO oxidation gives a potential ammonium oxidation that reflects the activity at the *in situ* temperature, pH and salinity and the calculated rates of ammonium oxidation would therefore represent actual *in situ* rates.

The method for determining *in situ* ammonium oxidation developed here can be applied to all types of environmental samples, soils, freshwaters and ocean waters. The determinations are performed over short time frames (3 to 12 h) and have lower detection limits than any other method currently in use, 0.0008 nmoles l^{-1} h^{-1} ^{14}CO oxidized to CO_2 with 25 ml samples or 0.0004 nmoles l^{-1} h^{-1} with 50 ml samples. These would translate to ammonium oxidation rates of 2.2×10^{-4} nmoles NH_4^+ oxidized l^{-1} h^{-1} and 1.1×10^{-4} nmoles l^{-1} h^{-1} at NH_4^+ concentrations of 0.1 μg l^{-1}. The use of this method may allow the determination of ammonium oxidation in the environments where it was previously impossible to obtain such estimates. It should also simplify the determinations in other samples such as soils, estuarine waters, eutrophic lake and river waters, and coastal ocean waters.

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