AN ABSTRACT OF THE THESIS OF

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Chemolithotrophic nitrifying bacteria were examined with respect to their abilities to oxidize methane and carbon monoxide in the presence and absence of ammonium. All of the ammonium oxidizers tested, including Nitrosomonas europaea and Nitrosococcus oceanus, were able to oxidize both CH_4 and CO to CO_2 in both the presence and absence of ammonium. All of the ammonium oxidizers were capable of incorporating varying amounts of CH_4-C into cellular components, both in the presence and absence of ammonium. None of the ammonium oxidizers were able to incorporate any CO-C in the absence of ammonium. None of the nitrite oxidizers examined were capable of either CH_4 or CO oxidation.

The effects of CH_4 or CO concentration, temperature, pH, nitrogen source, and the interactions between ammonium and CH_4 and CO were examined with respect to CH_4 and CO oxidation by several of the ammonium oxidizing bacteria. The ammonium oxidizers tested were capable of oxidizing trace concentrations of CH_4 (<1.38 nM) and CO (<0.5 nM). All of the organisms were

able to oxidize significant amounts of CH_4 and CO over wide ranges of temperature and pH.

Evidence is presented that suggests that the chemolithotrophic ammonium oxidizers may play an important role in the biogeochemical cycles of CH_4 and CO.

METHANE AND CARBON MONOXIDE OXIDATION BY NITRIFYING BACTERIA

by

Ronald Dean Jones

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Redacted for Privacy

Professor of Microbiology and Oceanography in charge of major

Redacted for Privacy

Chairman of Department of Microbiology

Redacted for Privacy

Dean of Graduate School

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CHAPTER I

INTRODUCTION

INTRODUCTION

The transformations of nitrogen and its recycling are extremely important to the productivity of all natural ecosystems. Nitrification is the phase in the nitrogen cycle where ammonium is oxidized to nitrite and then nitrate. This is a microbial process mediated by two separate groups of bacteria, the ammonium oxidizers and the nitrite oxidizers. Both of the groups are composed of chemolithotrophic organisms that obtain energy from the oxidation of reduced nitrogen compounds (NH_4 and NO_2) and fix CO_2 for the production of cellular material. The nitrite oxidizers have been shown to be able to grow heterotrophically but the ammonium oxidizers are incapable of heterotrophic growth.

In recent years increasing attention has been paid to the atmospheric trace gasses, methane and carbon monoxide, due to their suggested role in the global warming trend. A great deal of interest has also been generated because of the role these gasses play in both the cycling of carbon and energy in both marine and freshwater environments. Information dealing with the production and fate of these gasses is therefore of great interest to several groups of researchers. From the viewpoint of a microbiologist, the utilization of both CH_4 (Sansone and Martens 1978; Rudd and Taylor 1980) and CO (Ingersoll et al. 1974; Liebe et al. 1976; Bartholomew and Alexander 1979; Spratt and Hubbard 1984; Conrad and Seiler 1982) in the environment have been shown to be microbial processes. It has always been assumed that the

only group to play a role in methane oxidation is the methane oxidizers (Rudd and Taylor 1980; Harrits and Hanson 1980). With the oxidation of CO the organisms involved are not clearly recognized. There are two views concerning the organisms responsible for the oxidation of CO. Bartholomew and Alexander (1979) suggest that the oxidation of CO is due to non-specific microorganisms which utilize CO by cooxidation. This view is supported by their findings that little carbon from CO is incorporated into cellular material. Conrad and Seiler (1980, 1982) believe that the utilization of CO is due to specific organisms, which gain a distinct advantage from the oxidation of CO. Evidence for this comes from the extremely low Km values and from the use of CO at trace levels, reported in their studies. Their results indicating that the organisms involved are able to out compete others which may be able to oxidize CO at higher concentrations.

The methane oxidizing bacteria and the ammonium oxidizing bacteria have been shown to be quite similar in both structure and function (Ferenci et al. 1975; Whittenbury et al. 1970; Wilkinson 1975). The oxidation of ammonium in the ammonium oxidizers and the oxidation of methane in the methane oxidizers proceed along similar pathways and the structural similarities between NH_4 and CH_4 are obvious. Both of the pathways start with a cytrochrome based mono-oxygenase which requires an unknown reducing equivalent and molecular oxygen. Both pathways start with the hydroxylation of the substrates. Many of the methane oxidizing bacteria will slowly oxidize ammonium to nitrite (O'Neill and Wilkinson 1977; Whittenbury et al. 1970) and all examined thus far will oxidize

carbon monoxide to CO_2 (Ferenci et al. 1975; Colby et al. 1977; Dalton 1977). Despite the similarities between the ammonium and methane oxidizers and the enzymes involved, prior research using oxygen uptake measurements has indicated the Nitrosomonas europaea is unable to oxidize CH_4 or CO (Suzuki et al. 1976; Drozd 1976). With the use of $^{14}CH_4$ and ^{14}CO, however, we found that the chemolithotrophic ammonium oxidizers including Nitrosomonas europaea and Nitrosococcus oceanus are able to oxidize significant amounts of CH_4 and CO to CO_2. There are several possible explanations as to why prior researchers had not demonstrated CH_4 or CO oxidation by ammonium oxidizers. The use of oxygen uptake measurements, used by others, are much less sensitive than the use of ^{14}C-labeled compounds for the detection of substrate utilization. Suzuki et al. (1976) found that the rate of NADH oxidation by a membrane fraction was stimulated when CH_4 or CO was added. Nevertheless, they reported that no CH_4 or CO oxidation occurred, since there was no CH_4 or CO stimulated O_2 consumption. Why they did not observe any O_2 consumption is unknown since the results presented in this thesis indicate that they should have. Drozd (1976) probably used too low of a CH_4 concentration and too high of a NH_2OH concentration to detect activity by O_2 uptake. Work in our laboratory has shown that the rate of CH_4 and CO oxidation depends upon the physiological state of the cells. This can be dramatically affected by the methods used for culture storage and cell growth. Cells in our studies were grown in ammonium limited chemostats with a generation time of 24 h, and cells were used within 1 h of collection. Varying the generation time in the chemostats or

storage of the cultures for extended times always affected oxidation rates. In addition to these reasons, simply autoclaving the butyl rubber serum stoppers causes them to release an unknown toxic substances which inhibits NH_4, CH_4, and CO oxidation by as much as 90%. These factors may explain why prior workers could not demonstrate what we found to be a series of very active oxidations.

The studies reported in this thesis are the first of their kind to demonstrate that the classical ammonium oxidizers oxidize CH_4 and CO and furthermore, may play a significant role in the cycle of CH_4 and CO in the oceans, lakes, soils and atmosphere. The ability of ammonium oxidizers, a group once though to be limited to the oxidation of NH_4, to oxidize CH_4 and CO suggests that these organisms may use these compounds as alternative energy sources. When NH_4 is limiting or absent, CH_4 and CO could provide a source of energy of maintenance for some ammonium oxidizers and thus may be involved in a survival mechanism. In addition the investigations reported herein point to a strong interrelation between the carbon (at the level of CH_4 and CO) and the nitrogen (at the level of NH_4) cycles in the environment and an increased role for the ammonium oxidizers. In the marine environment where there are dramatic diurnal cycles in CO concentration the ability of the ammonium oxidizers to oxidize CO should help to explain these variations. This work will hopefully help answer questions dealing with the fate of CH_4 and CO in the environment, while expanding our view of both the physiology and ecology of nitrifiers.

The publications bound together to form Chapters II, III, and IV of ths thesis were all co-authored with Dr. Richard Morita

and have all been published or accepted for publication. Chapter II, Methane Oxidation by Nitrosococcus oceanus and Nitrosomonas europaea, was published in Vol. 45 p. 401-410 (1983) of Applied and Environmental Microbiology. Chapter III, Carbon Monoxide Oxidation by Chemolithotrophic Ammonium Oxidizers, was published in Vol. 29 p. 1545-1551 (1983) of the Canadian Journal of Microbiology and Chapter IV, Effects of Various Parameters on Carbon Monoxide Oxidation by Ammonium Oxidizers, was just accepted for publication in the Canadian Journal of Microbiology.

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CHAPTER II

METHANE OXIDATION BY *NITROSOCOCCUS OCEANUS* AND *NITROSOMONAS EUROPAEA*

ABSTRACT

Chemolithotrophic ammonium oxidizing and nitrite oxidizing bacteria including Nitrosomonas europaea, Nitrosococcus oceanus, Nitrobacter sp., Nitrospina gracilis, and Nitrococcus mobilis were examined as to their ability to oxidize methane in the absence of ammonium or nitrite. All ammonium oxidizers tested had the ability to oxidize significant amounts of methane to CO_2 and incorporate varying amounts into cellular components. None of the nitrite oxidizing bacteria were capable of methane oxidation.

The methane oxidizing capabilities of N. oceanus and N. europaea were examined with respect to ammonium and methane concentrations, nitrogen source and pH. The addition of ammonium stimulated both CO_2 production and cellular incorporation of methane-carbon by both organisms. Less than 0.1 mM CH_4 in solution inhibited the oxidation of ammonium by N. oceanus by 87%. Methane concentrations up to 1.0 mM had no inhibitory effects on ammonium oxidation by N. europaea. In the absence of NH_4-N N. oceanus achieved a maximum methane oxidation rate of 2.20×10^{-2} µmol $CH_4 \times h^{-1} \times$ mg dry wt cells^{-1} which remained constant as methane concentration was increased. In the presence of NH_4-N (10 ppm) its maximum rate was 26.4×10^{-2} µmol $CH_4 \times h^{-1} \times$ mg dry wt cells^{-1} at a methane concentration of 1.19×10^{-2} mM. Increasing the methane concentration above this level decreased CO_2 production, while cellular incorporation of methane-carbon continued to increase. N. europaea showed a linear response throughout the test range with an activity

of 196.0 x 10^{-2} μmol CH_4 x h^{-1} x mg dry wt $cells^{-1}$ at a methane concentration of 1.38 x 10^{-1} mM. Both nitrite and nitrate stimulated the oxidation of methane. The pH range was similar to that for ammonium oxidation, but the points of maximum activity were at lower values for the oxidation of methane.

INTRODUCTION

Ammonium oxidation by chemolithotrophic ammonium oxidizers and methane oxidation by methane oxidizing bacteria have been shown to be quite similar (Ferenci et al. 1975; Whittenbury et al. 1970; Wilkinson 1975). Many methane oxidizing bacteria will oxidize ammonium to nitrite (O'Neill and Wilkinson 1977; Whittenbury et al. 1970). This ability is linked to the lack of specificity of the methane oxygenase system (Ferenci et al. 1975; O'Neill and Wilkinson 1977). The oxidations of ammonium and methane both start with the hydroxylation of the substrates (Wilkinson 1975). Despite these similarities prior research using oxygen uptake measurements has indicated that N. europaea is unable to oxidize methane (Drozd 1976; Suzuki et al. 1976). However, employing ^{14}CO-methane we found that ammonium oxidizers including N. europaea and N. oceanus are able to oxidize significant amounts of methane to $^{14}CO_2$ and incorporate some of the carbon from methane into cellular components.

Ammonium oxidizers are considered to be metabolically limited to a very few substrates (Clark and Schmidt 1967; Williams et al. 1968) and their ability to oxidize methane poses interesting questions as to the interactions between the oxidation of ammonium and methane.

This paper addresses the ability of ammonium oxidizers to incorporate methane into cellular components as well as respire the methane to carbon dioxide in the presence and absence of ammonium and other nitrogen sources.

MATERIALS AND METHODS

Cultures and inoculum. Cultures of N. oceanus, Nitrosomonas marinas sp. C-15, Nitrococcus mobilis, Nitrospina gracilis, Nitrobacter sp. Nb297, provided by S. W. Watson, N. europaea, provided by E. L. Schmidt, and several of our isolates representing marine, estaurine, freshwater and soil nitrifiers, were employed in this study. The ammonium oxidizers were grown and assayed for purity using the medium and continuous flow methods described by Jones and Hood (1980). Nitrite oxidizers were cultured in an identical manner using the medium described by Watson and Waterbury (1971) with seawater being replaced by Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) in distilled water to give a salinity of $32^{o}/oo$. A standard inoculum was prepared by filtering cells onto a Millipore membrane filter (HA, 0.45 μm). The cells were washed twice with sterile NH_4 of NO_2-free medium, pH 7.8, and resuspended to an OD of 0.2 at 550 nm (Bausch and Lomb Spectronic 20). One ml portions of these suspensions were used as the inoculum to test for methane oxidation by the various nitrifiers.

The medium used for preparing these suspensions and for methane oxidation determinations had the following composition: $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.02 g; K_2HPO_4, 0.114 g; Fe-EDTA (77 mg $FeSO_4 \cdot H_2O$ + 103 mg NaEDTA in 50 ml distilled water), 1.0 ml; $Na_2MoO_4 \cdot 2H_2O$, 1.0μg; $CoCl_2 \cdot 6H_2O$, 2.0μg; $ZnSO_4 \cdot 7H_2O$, 100μg; K_2CO_3, 0.1 g; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma Chem. Co.) 2.4 g; distilled H_2O, 1.0 liter. The pH of the medium was adjusted to 7.8 using 5N NaOH. The salinity

of the medium was adjusted with Instant Ocean Synthetic Sea Salts to 10^{o}/oo for Nitrosomonas sp. 1S10, 18^{o}/00 for Nitrosomonas marinus sp. C-15, and 32^{o}/oo for the remaining marine isolates.

Suspensions of N. oceanus and N. europaea used for the evaluation of the interactions of methane and ammonium were subjected to cell counts using acridine orange fluorescent microscopy (Hobbie et al. 1977) and dry weight determinations. For dry weights 5.0 ml of culture was filtered onto a predried preweighed Millipore membrane filter (HA, 0.45 μm) and dried at 80^{o}C until a constant value was obtained.

The mean density of N. oceanus suspensions prepared in the described manner was 1.1×10^{8} cells x ml^{-1} with an average dry weight of 36 μg x ml^{-1}. The values for N. europaea suspensions were 3.2×10^{8} cells x ml^{-1} and 26 μg x ml^{-1}. One ml portions of these suspensions were used as the standard inoculum.

Methane oxidation determinations. The methods used for $^{14}CH_4$ preparation and methane oxidation rate determinations were similar to those described by Griffiths et al. (1982). One ml of standard inoculum was inoculated into 60 ml serum bottles containing 25 ml of NH_4-free medium buffered with 0.2 M HEPES, pH 7.8. The bottles were then sealed with serum stoppers and 1.0 ml (unless otherwise noted) of $^{14}CH_4$ (1.0 μCi per ml, Amersham, specific activity 59 mCi per mmole) in nitrogen was added to the headspace. Final concentration of $^{14}CH_4$ in solution ranged from 13.8 nM for the N. europaea (freshwater) cultures to 11.9 nM for the N. oceanus

(marine) cultures (Yamamoto et al. 1976). All bottles were prepared in triplicate and all experimental sets were duplicated. Controls without cells and with acid killed cells were run with each experiment. The bottles were incubated for 48 h at 25°C at 100 rpm on a rotary shaker. After incubation $^{14}CO_2$ production and ^{14}C incorporated into cellular material was measured and methane oxidation rates were determined from these data. Confidence intervals were calculated for each data point. The variation among replicates did not exceed the 95% confidence interval for the population mean.

Once the ability of ammonium oxidizers to oxidize methane was determined further detailed examinations of N. oceanus and N. europaea were made with respect to ammonium and methane concentrations, nitrogen source and pH.

To test the effects of ammonium, a series of bottles containing 0, 1, 10, 50 and 100 ppm NH_4-N as $(NH_4)_2SO_4$ was prepared and incubated as previously described.

Bottles containing various nitrogen sources were prepared to examine their effects on methane oxidation. The following additions were made: 10 ppm NH_4-N, 10 and 40 ppm NO_2-N, 10 and 40 ppm NO_3-N and 10 ppm Yeast Extract (Difco). Controls which contained no additional nitrogen source was also run.

The effects of methane concentration on methane oxidation were examined. A series of bottles containing 1.0001, 0.001, 0.01, 0.1, 0.5, 1.0, 5.0, and 10% methane headspaces was prepared. The concentrations of 0.0001 and 0.001% CH_4 were prepared by

injecting 0.1 and 1.0 ml of the $^{14}CH_4$-N_2 mixture. The higher methane concentrations were prepared by injecting 1.0 ml $^{14}CH_4$-N_2 and 3.4, 38, 190, 1900 and 3800 µl of unlabeled methane into the head space to make up the balance. The addition of unlabeled methane resulted in a serial dilution of specific activity. It was assumed that there would be no significant isotopic fractionation by the microorganisms, and therefore only the dilution effects were taken into account when calculating activity. Methane concentrations ranged from 1.19×10^{-6} mM to 1.19×10^{-1} mM for N. oceanus and 1.38×10^{-6} mM to 1.38×10^{-1} mM for N. europaea. Methane series were prepared both with and without 10 ppm NH_4-N.

A pH series was prepared by adjusting the medium to various pH's between 4.0 and 11.0 using either 1.0 N HCl or 1.0 N NaOH. Series were prepared both with and without the addition of 10 ppm NH_4-N. Bottles containing ammonium were assayed for nitrite production, using the spectrophotometric method of Bendschneider and Robinson (1952), to determine ammonium oxidation.

The effects of dissolved carbonate concentration on cellular incorporation of C from $^{14}CH_4$ was determined by preparing a series of bottles that contained 0, 10, 50, 200 and 500 ppm of dissolved carbonate as K_2CO_3. The dissolved carbonates were first removed from the medium by acidification to pH 2.0 with HCl and then boiling for 10 min. The solution was then allowed to return to room temperature, and adjusted to pH 7.0 with 1.0 N NaOH. Carbonate additions were made and the final pH was adjusted to 7.8 with 1.0 N NaOH or HCl. The bottles were then sealed immediately and the $^{14}CH_4$, 10 ppm NH_4-N and cells were added.

Carbonate uptake. Bottles were prepared as usual and then $Na_2H^{14}CO_3$ was added (Amersham, specific activity 4.5 μci/mmole) to give a final concentration of 0.01 μCi per ml. The bottles were sealed and 0. 0.1, 1.0 or 10% CH_4 was added to the headspace. One set of bottles was given an addition of 1.0 ppm NH_4-N. The bottles were then incubated as usual and analyzed for ^{14}C incorporation.

Methane effects on ammonium oxidation. One ml of standard inoculum was inoculated into 60 ml serum bottles which were then filled with 1.0 ppm NH_4-N, 0.02 M HEPES buffered, pH 7.8, medium and capped in such a manner as to exclude any air bubbles. A one ml headspace was then created by injecting 1.0 ml of various CH_4-N_2 (Airco, Grade 4, Ultra Pure Gas) mixtures, and removal of 1.0 ml of medium. The methane concentrations in the injected gases were 0, 1, 10, 50 and 100% giving a concentration range of 0.0 to 1.38 mM dissolved methane for N. europaea and 0.0 to 1.119 mM for N. oceanus (Yamamoto et al. 1976). These bottles were then incubated for 12 h at 25°C at 100 rpm on a rotary shaker. The bottles were then assayed for nitrite. All bottles were prepared in triplicate and each experimental set was duplicated.

RESULTS

The rates of methane oxidation by each organism are shown in Table 1. All of the ammonium oxidizers examined were able to oxidize methane to CO_2 and incorporate it into cellular components, in the absence of ammonium. The nitrite oxidizers examined were unable to oxidize methane. The rate of methane oxidation under these conditions varies significantly between species with N. oceanus having the highest rate and Nitrosomonas sp. 2S0 the lowest. The ratio of methane carbon incorporated into cellular material to that of CO_2 produced from methane also varied significantly. With the exception of Nitrosomonas sp. 3S30, an Oregon coast isolate, all of the marine isolates had a lower incorporation rate than the freshwater, soil and estuarine isolates.

The presence of ammonium stimulated the production of $^{14}CO_2$ and ^{14}C-cellular-C by both N. oceanus and N. europaea (Fig. 1). N. europaea was stimulated to a greater extent, but both organisms showed the same trends. Ten ppm NH_4-N caused the greatest increases in methane oxidation with further increases in ammonium concentration causing a decrease in activity.

In addition to being stimulated by ammonium, nitrite, nitrate and yeast extract also stimulated methane oxidation (Table 2). Increasing the concentrations of NO_2^- and NO_3^- from 10 to 40 ppm did not cause any further increase in methane oxidation.

The response of N. oceanus to methane concentration both with and without 10 ppm NH_4-N is shown in Fig. 2. In the absence of

Table 2-1. Methane oxidation by nitrifiers in the absence of ammonium.

Organism	Source	Methane oxidaton rate (DPM)[a]		
		$^{14}CO_2$ Produced	^{14}C-cells	Ratio ^{14}C-cellular $^{14}CO_2$
Nitrosococcus oceanus	Marine North Atlantic	15051	293	.019
Nitrosomonas europaea	Soil -	596	60	.101
Nitrosomonas marinus sp. C-15	Marine South Pacific	233	13	.055
Nitrosomonas sp. 1S10	Estaurine Florida Coast	924	194	.210
Nitrosomonas sp. 2S0	Freshwater Louisiana March	179	19	.106
Nitrosomonas sp. 6S30	Marine Alaskan Coast	1285	27	.021
Nitrosomonas sp. 9W0	Freshwater Oregon Marsh	782	85	.109
Nitrosomonas sp. 3S30	Marine Oregon Coast	2590	370	.143
Nitrosomonas sp. 11W30	Marine Oregon Coast	2983	51	.017
Nitrobacter sp. Nb297	Marine -	3	0	
Nitrospina gracilis	Marine South Atlantic	0	1	
Nitrococcus mobilis	Marine South Pacific	1	0	

[a]Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1}.

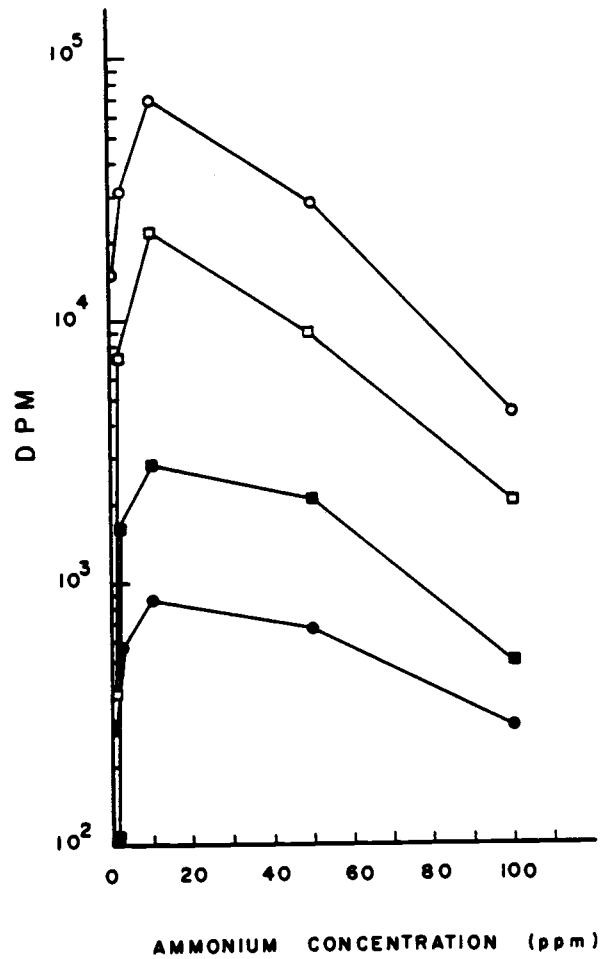

Fig. 2-1. Ammonium effects on methane oxidation. Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1}. Symbols: (○) N. oceanus, $^{14}CO_2$ produced; (●) N. oceanus, ^{14}C-cellular material; (□) N. europaea, $^{14}CO_2$ produced; (■) N. europaea ^{14}C-cellular material.

Table 2-2. Effects of ammonium, nitrite, nitrate and yeast extract on methane oxidation by N. oceanus and N. europaea.

		Methane oxidation rate (DPM)[a]						
		Additions						
Organism	Fraction	None	NH_4-N	NO_2-N		NO_3-N		Yeast Extract
			10 ppm	10 ppm	40 ppm	10 ppm	40 ppm	10 ppm
N. oceanus	$^{14}CO_2$	15558	76020	39416	38973	34776	32337	61395
	^{14}C-cells	240	575	43	ND[b]	379	ND	931
N. europaea	$^{14}CO_2$	674	26783	1618	1792	1159	1170	3513
	^{14}C-cells	31	3383	320	ND	286	ND	543

[a]Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1}.

[b]ND, Not determined.

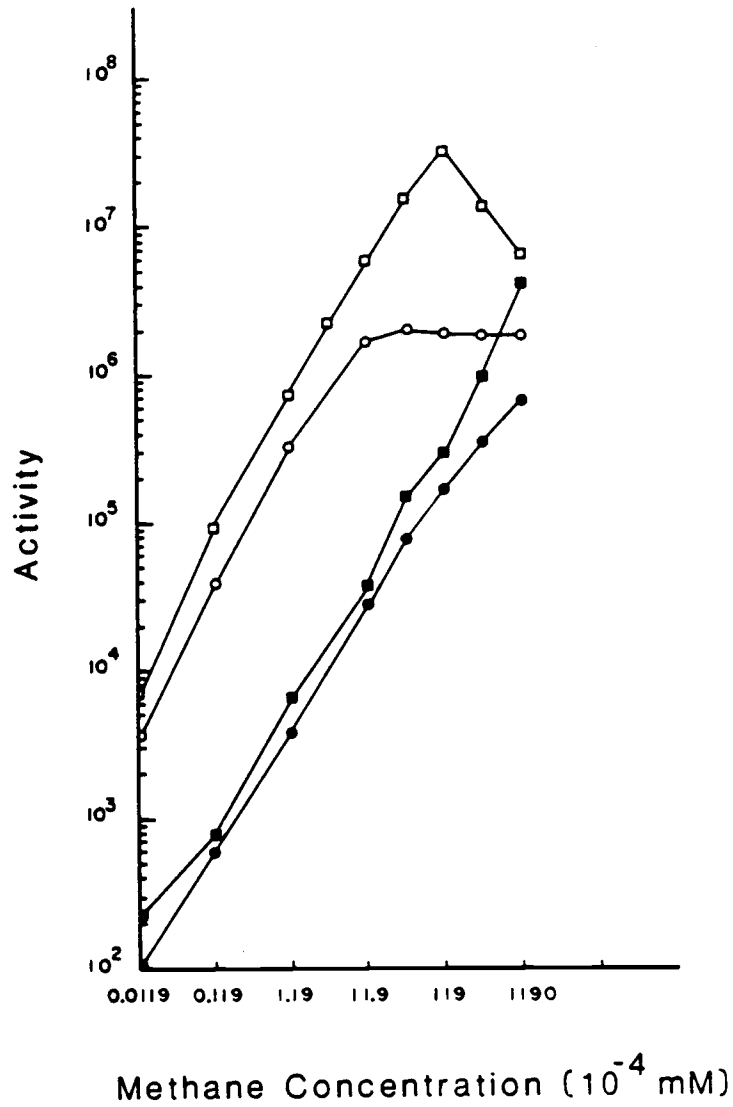

Fig. 2-2. Effects of methane concentration on the rate of methane oxidation by N. oceanus. Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1} multiplied by the dilution factor. Symbols: (○) $^{14}CO_2$ produced, 0.0 ppm NH_4-N; (●) ^{14}C-cellular material, 0.0 ppm NH_4-N; (□) $^{14}CO_2$ produced, 10 ppm NH_4-N; (■) ^{14}C-cellular material, 10 ppm NH_4-N.

ammonium, both $^{14}CO_2$ production and ^{14}C-cellular incorporation from $^{14}CH_4$ show a linear response to increasing methane concentrations up to 1.19×10^{-3} mM CH_4. At this point ^{14}C-cellular incorporation continues to increase while $^{14}CO_2$ production remains essentially constant. In the presence of 10 ppm ammonium, ^{14}C-cellular incorporation showed a similar linear trend while $^{14}CO_2$ production showed linearity to 1.19×10^{-2} mM and then decreased with further increases in methane concentration. The response of N. europaea to methane concentration are shown in Fig. 3. In both the presence and absence of ammonium the response of N. europaea to methane concentration is linear. In the absence of ammonium at methane concentrations above 1.38×10^{-2} mM the cellular incorporation of $^{14}CH_4$-C exceeds the amount of $^{14}CO_2$ produced.

The ratio of methane incorporated into cellular material to that released as CO_2 are shown in Table 3. In the absence of ammonium the values for N. oceanus remain essentially constant until a methane concentration of 0.595×10^{-2} mM, at this point the ratio increases until 48.8% of the methane oxidized is incorporated into cellular material is 1.19×10^{-1} mM CH_4. In the presence of 10 ppm NH_4-N the ratio remains constant until a methane concentration of 0.595×10^{-1} nM at which it increases an order of magnitude and then increases further until 75.7% of the methane oxidized is incorporated into cellular carbon at 1.19×10^{-1} mM CH_4. N. europaea also incorporates more ^{14}C as the methane concentration increases. In the absence of ammonium in the presence of methane

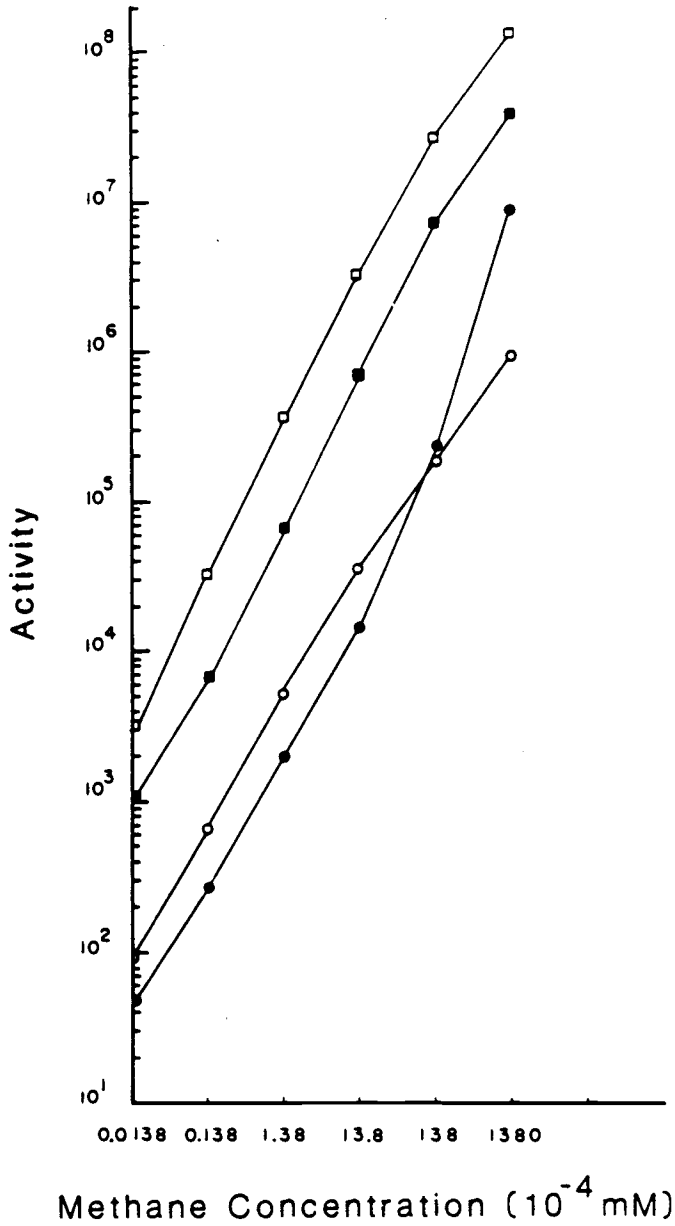

Fig. 2-3. Effects of methane concentration on the rate of methane oxidation by N. europaea. Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1} multiplied by the dilution factor. Symbols: (o) $^{14}CO_2$ produced, 0.0 ppm NH_4-N; (•) ^{14}C-cellular material, 0.0 ppm NH_4-N; (□) $^{14}CO_2$ produced, 10 ppm NH_4-N; (■) ^{14}C-cellular material, 10 ppm NH_4-N.

Table 2-3. Effects of methane concentration on cellular incorporation of $^{14}CH_4$-C by N. oceanus and N. europaea.

		Ratio ^{14}C-cellular material to $^{14}CO_2$						
		CH_4 concentration (mM)						
		1.19×10^{-5}[a]	1.19×10^{4}[a]	1.19×10^{-3}[a]	5.95×10^{-3}[a]	1.19×10^{-2}[a]	5.95×10^{-2}[a]	1.19×10^{-1}[a]
Organism	NH_4-N	1.38×10^{-5}[b]	1.38×10^{-4}[b]	1.38×10^{-3}[b]	6.90×10^{-3}[b]	1.38×10^{-2}[b]	6.90×10^{-2}[b]	1.38×10^{-1}[b]
N. oceanus	0 ppm	0.016	0.011	0.017	0.041	0.096	0.387	0.488
	10 ppm	0.008	0.009	0.006	0.011	0.010	0.149	0.757
N. europaea	0 ppm	0.389	0.426	0.394	ND[c]	1.133	ND	1.733
	10 ppm	0.207	0.214	0.223	ND	0.277	ND	0.316

a Methane concentrations for N. oceanus.

b Methane concentrations for N. europaea.

c ND, Not determined.

concentrations above 1.38×10^{-2} mM more ^{14}C is incorporated than released as $^{14}CO_2$. With ammonium the incorporation of methane carbon ranges from 20.7% at 1.38×10^{-5} mM to 31.6% at 1.38×10^{-1} mM.

N. oceanus in the absence of ammonium oxidizes 2.20×10^{-1} μmol $CH_4 \times h^{-1} \times$ mg dry wt cells^{-1} at 1.0% methane headspace (1.19×10^{-2} mM CH_4 dissolved) and 26.4×10^{-2} μmol in the presence of 10 ppm ammonium at the same methane concentration. N. europaea with a 10% methane headspace (1.38×10^{-1} mM CH_4 dissolved) oxidizes 11.1 μmol $CH_4 \times h^{-1} \times$ mg dry wt cells^{-1} in the absence of ammonium and 196.0×10^{-2} μmol in the presence of ammonium.

The response of methane oxidation by both organisms to pH are quite similar. N. oceanus had an optimum range for ammonium oxidation of pH 8.0 while methane oxidation occurred optimally at pH 7.0 (Fig. 4). N. europaea had an optimum for ammonium oxidation of pH 9.5 while methane oxidation occurred optimally at pH 7.5 (Fig. 5). Both organisms exhibited a broad range of high methane oxidation activity in the presence or absence of ammonium.

Increasing the carbonate concentration in solution caused a corresponding decrease in the amount of $^{14}CH_4$-C incorporated into cellular material in cultures containing ammonium (Table 4).

The uptake of $^{14}CO_3$ by either N. oceanus or N. europaea was not increased by the addition of methane at any concentration, in the absence of ammonium.

The effects of methane concentration on ammonium oxidation are quite different for the two organisms. The ability of N. oceanus

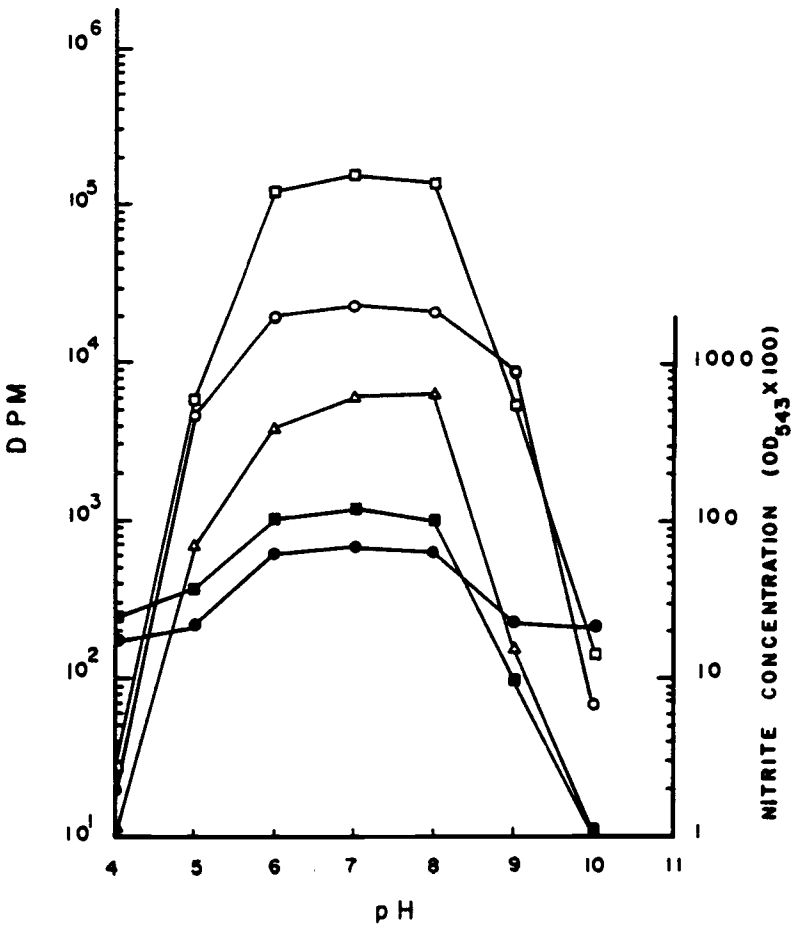

Fig. 2-4. Effects of pH on methane oxidation by N. oceanus. Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x 24 h^{-1}. Symbols: (○) $^{14}CO_2$ produced, 0.0 ppm NH_4-N; (●) ^{14}C-incorporated, 0.0 ppm NH_4-N; (□) $^{14}CO_2$ produced, 10 ppm NH_4-N; (■) ^{14}C-incorporated, 10 ppm NH_4-N; (△) nitrite produced.

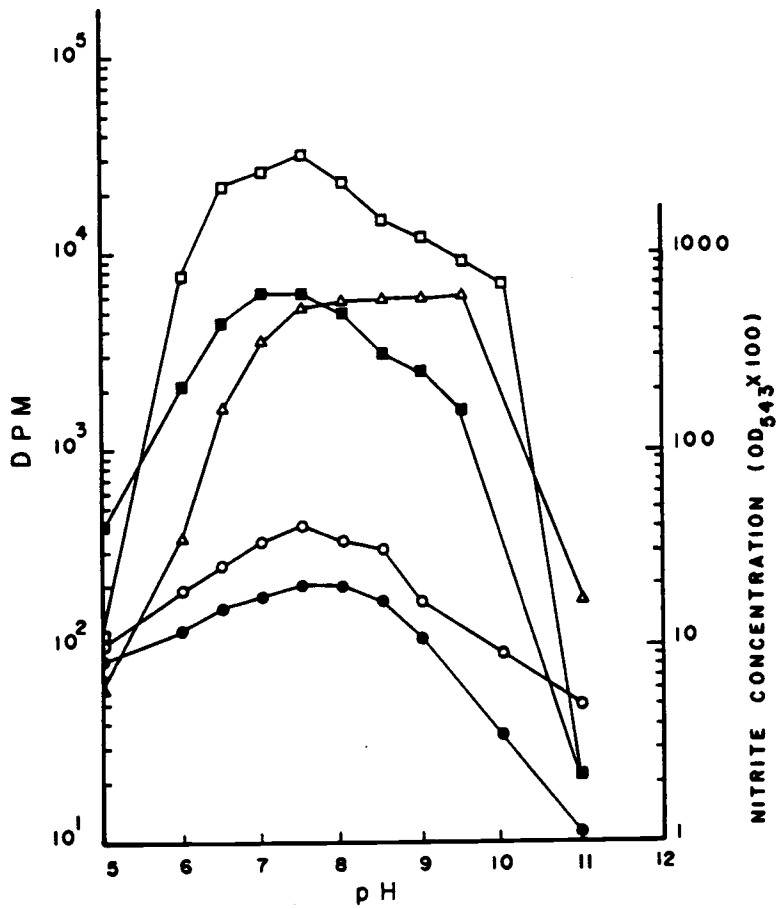

Fig. 2-5. Effects of pH on methane oxidation by N. europaea. Activity is expressed as DPM ^{14}C x ml standard inoculum^{-1} x h^{-1}. Symbols: (○) $^{14}CO_2$ produced, 0.0 ppm NH_4-N; (●) ^{14}C-incorporated, 0.0 ppm NH_4-N; (□) $^{14}CO_2$ produced, 10 ppm NH_4-N; (■) ^{14}C-incorporated, 10 ppm NH_4-N; (△) nitrite produced.

Table 2-4. Effects of carbonate concentration on cellular incorporation of $^{14}CH_4$-C in the presence of 10 ppm NH_4-N.

	Ratio ^{14}C-cellular material to $^{14}CO_2$				
	Carbonate concentration				
Organism	0 ppm	10 ppm	50 ppm	200 ppm	500 ppm
N. oceanus	0.018	0.015	0.012	0.007	0.006
N. europaea	0.276	0.239	0.200	0.193	0.185

to oxidize ammonium was inhibited by methane concentrations of less than 0.115 mM. Ammonium oxidation by N. europaea was not affected by any of the methane concentrations (Fig. 6).

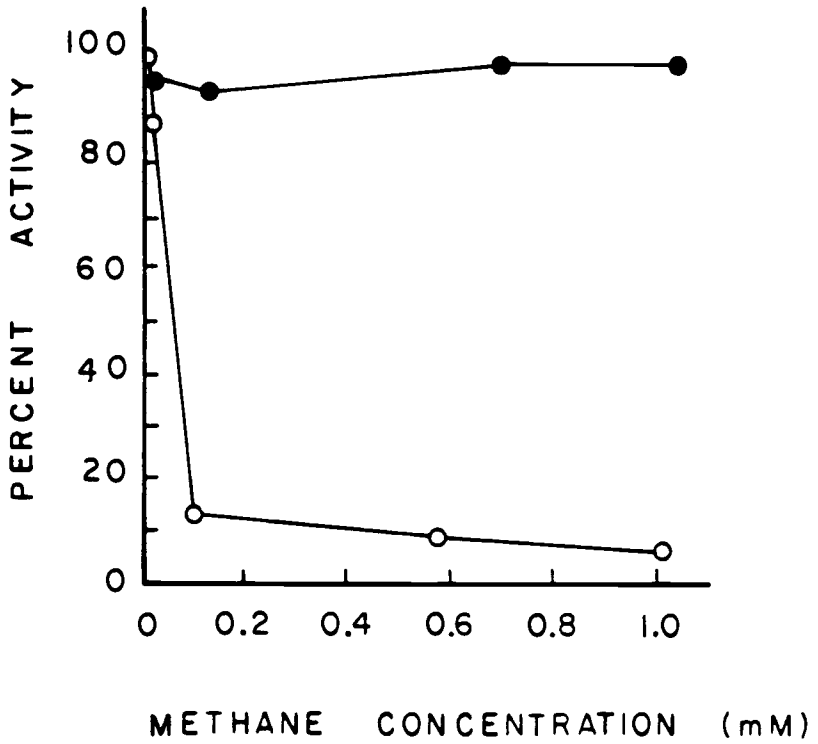

Fig. 2-6. Methane effects on ammonium oxidation by N. oceanus and N. europaea. Activity is expressed as percent of nitrite formed in 12 h at 0.0 ppm CH_4 levels. Symbols: (o) N. oceanus; (•) N. europaea.

DISCUSSION

The similarities between ammonium oxidizers and methane oxidizers have been noted by several other workers (Ferenci et al. 1975; Whittenbury et al. 1970; Wilkinson 1975). Methane oxidizers have also been shown to oxidize ammonium to nitrite (O'Neill and Wilkinson 1977; Whittenbury et al. 1970), although the rate of this process is extremely slow. Work by Drozd (1976) and Suzuki et al. (1976) using the relatively insensitive method of oxygen uptake were unable to detect any oxidation of methane by N. europaea and concluded that no methane oxidation took place. Using a more sensitive method involving the use of $^{14}CH_4$ the results of this research show that ammonium oxidizers including N. europaea can oxidize methane. Whether this is due to the lack of specificity of the ammonium oxygenase system, which seems likely, or whether separate enzymes responsible for methane oxidation exist is now known. Prior work by Suzuki et al. (1976) has indicated that oxidation of ammonium by N. europaea was inhibited by methane. We were unable to duplicate this with our strain of N. europaea. N. oceanus did show an inhibitory response similar to that reported by Suzuki et al. (1976). This indicates a marked difference between the two organisms. The stimulation of methane oxidation by low concentrations of ammonium would seem to indicate that the interactions between ammonium and methane are more than simple competitive inhibition, in fact no inhibitory response was observed for N. europaea. Ammonium concentrations above 10 ppm NH_4-N decreased the methane oxidation activity for both organisms indicating a sort of one sided 'competitive inhibi-

tion'. These relationships may be in part due to the relative insolubility of methane. Part of the increased activity caused by the addition of low levels of ammonium may be due to an activiation of the enzyme system, the other could be due to the presence of a nitrogen source. Further evidence of this is the stimulation of methane oxidation by nitrite and nitrate. These data indicate an ammonium stimulated co-oxidation of methane and ammonium. While this co-oxidation is most active in the presence of ammonium the oxidation of methane is not dependent upon the presence of ammonium.

The pH range for methane oxidation was quite wide as for ammonium oxidation. The lower optimum pH for methane oxidation is interesting since methane does not have a similar ammonia-ammonium shift with pH, and its corresponding effect on activity as reported by Anthonisen et al. (1976).

The oxidation of methane by N. europaea follows the rules for first order kinetics. N. oceanus also follows this approach until a methane concentration of 1.19×10^{-2} mM in the presence of 10 ppm ammonium. The continued increase in cellular incorporation and decrease in $^{14}CO_2$ production indicates a shift in the metabolic pathway. This is further demonstrated by the continued increase of the ^{14}C cellular incorporation to $^{14}CO_2$ ratios for both organisms. This shift depends upon the organism, the presence or absence of ammonium, and could represent a point at which the methane concentration in solution becomes high enough for the organism to stop using methane solely for energy to maintain itself

and start building structural components. That the presence of 10 ppm NH_4-N shifts this point to a higher value in N. oceanus is indicative of this. Another factor in this shift is that at low methane concentrations ammonium oxidation appears to be responsible for at least 50% of the $^{14}CH_4$-C incorporation (Table 4), and that the uptake of $^{14}CO_3$ was not stimulated by the addition of methane in the absence of ammonium.

The rate of methane oxidation by ammonium oxidizers ranged from 2.2 x 10^{-2} μmol CH_4 x h^{-1} x mg dry wt $cells^{-1}$ for N. oceanus in the absence of ammonium to 196.0 x 10^{-2} μmol CH_4 x h^{-1} x mg dry wt $cells^{-1}$ for N. europaea in the presence of 10 ppm NH_4-N. These rates indicate that methane may play a substantial role in the metabolism of these bacteria under certain conditions. The rates of methane oxidation are from 3.0 to 120% of the methane oxidation rate reported for the methane oxidizer Methylosinus trichosporium, the organism with the highest methane oxidation rate and 10.1 to 389% of the rates for Methylomonas agile the organism with the lowest methane oxidation rate as reported by Whittenbury et al. (1970). These values demonstrate that the rate of methane oxidation by ammonium oxidizers is significant and may actually exceed that of the classical methane oxidizers under some conditions.

From an energetics viewpoint the oxidation of one mole of methane provides more energy than the oxidation of one mole of ammonium. It is probable that the co-oxidation of methane by ammonium oxidizers is a significant source of energy and cellular

material under certain conditions, especially when the optimum pH for ammonium oxidation by N. europaea is 9.5. It is also possible that methane could serve as a source of energy when ammonium is limiting. It appears that the oxidation of methane is most likely not limited just to these organisms but seems likely to be a characteristic of all of the classical chemolithotrophic ammonium oxidizers. The ability of ammonium oxidizers to be able to switch from the oxidation of ammonium to methane and vice versa could be a survival mechanism when one or the other is not available as the energy source. Since methane is nearly always present in low concentrations, the survival advantage of being able to use either substrate is obvious. In situations where both ammonium and methane are present in non-growth sustaining concentrations the combination of the two and co-oxidation by ammonium oxidizers may be able to supply the necessary carbon and energy requirements.

Attempts to grow the ammonium oxidizers on methane as the sole source of carbon and energy have failed even in the presence of 1.0 ppm NH_4-N as evidenced by cell counts. These results are indicative of a difference between the ammonium and methane oxidizing bacteria.

Griffiths et al. (1982) report that in their study of naturally occurring methane oxidation that the proportion of CH_4 incorporated was low indicating that methane was used as an energy source. This is suggestive of a role by ammonium oxidizers. They also report that there is a distinct difference between their results for a marine system and those reported for freshwater systems.

Our data show that there is a distinct difference between marine and freshwater, estaurine, and soil ammonium oxidizers, in the amount of CH_4 incorporated. With the exception of Nitrosomonas sp. 3S30, marine isolates incorporate significantly less $^{14}CH_4$-C than the other isolates. Some of this discrepancy does appear to be due to the relative carbonate concentration in freshwater and marine systems.

In aerobic sediments and water columns overlying methanogenic sediments, ammonium oxidizers could account for a significant portion of the methane oxidation. This type of environment is usually high in ammonium and other forms of inorganic nitrogen such that methane oxidation by ammonium oxidizers would be at an optimum. Harrits and Hanson (1980) in their work with Lake Mendota (Wisconsin) show that nitrite is confined to the part of the water column where rapid methane oxidation occurs. They also state that this indicates that since methanotrophs are known to oxidize ammonium (O'Neill and Wilkinson 1977; Whittenbury et al. 1970) methanotrophs may contribute significantly to the nitrogen cycle in Lake Mendota. This study indicates that classical ammonium oxidizers may be responsible for a portion of the methane oxidation and that the presence of high levels of nitrite helps to confirm this. The oxidation of methane by Nitrosomonas europaea has been confirmed by Wood and Hyman (paper presented at the Society for General Microbiology Annu. Mtg., 8 Sept. 1982). The observations made in this study indicate that much more work needs to be done to characterize the physiology and role that ammonium oxidizers may play in the cycling of methane.

ACKNOWLEDGEMENT

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CHAPTER III

CARBON MONOXIDE OXIDATION BY CHEMOLITHOTROPHIC AMMONIUM OXIDIZERS

ABSTRACT

Chemolithotrophic nitrifying bacteria were examined with respect to their ability to oxidize carbon monoxide in the presence and absence of ammonium. All of the ammonium oxidizers tested, including Nitrosomonas europaea and Nitrosococcus oceanus, were abot to oxidize CO to CO_2 in the presence and absence of ammonium. None of the organisms tested incorporated any of the carbon from CO into cellular components in the absence of ammonium. All were able to oxidize CO at trace concentrations of 0.5 nM. None of the nitrite oxidizers examined were capable of CO oxidation. CO oxidation by Nitrosmonas europaea, Nitrosococcus oceanus and Nitrosmonas sp. 4W30, a marine ammonium oxidizer, were examined with respect to CO concentration and time course of CO oxidation, both in the presence and absence of 10 mg/L NH_4-N. The CO oxidation rates at a CO concentration of 300 nL/L in the absence of ammonium ranged from 0.048 nmoles/h/10^6 cells for N. sp. 4W30 to 0.0025 nmoles/h/10^6 cells for N. europaea.

INTRODUCTION

It has been recently demonstrated that ammonium oxidizers have the capability to oxidize methane to CO_2 (Jones and Morita 1983). This oxidation can be linked to the lack of specificity of the ammonium mono-oxgenase (Hyman and Wood 1983). It has also been demonstrated that methane oxidizers possess the ability to oxidize CO (Ferenci 1974; Hubley et al. 1974; Ferenci et al. 1975) and that this oxidation is linked to the lack of specificity of methane mono-oxygenase (Ferenci et al. 1975). Despite the similarities between the enzymes involved, prior research using oxygen uptake measurements has indicated that Nitrosomonas europaea is unable to oxidize CO (Suzuki et al. 1976; Drozd 1976). With the use of ^{14}CO, however, we found that ammonium oxidizers including Nitrosomonas europaea and Nitrosocossus oceanus are able to oxidize significant amounts of CO to CO_2 even at extremely low CO concentrations. The consumption of CO in soils, lakes and oceans have been shown to be a microbial process (Ingersoll et al. 1974; Liebe et al. 1976; Bartholomew and Alexander 1979; Conrad and Seiler 1980; Spratt and Hubbard 1981; Conrad and Seiler 1982). There are two views concerning the organisms responsible for the oxidation of CO. Bartholomew and Alexander (1979) suggest that the oxidation of CO is due to nonspecific microorganisms which utilize CO by co-oxidation. This view is supported by their findings that little carbom from CO is incorporated into cellular material. Conrad and Seiler (1981, 1982) feel that the utilization of CO is due to specific organisms, which gain a distinct advantage from the oxida-

tion of CO. Evidence for this comes from the extremely low Km values and from the use of CO at trace levels, reported in their studies.

This paper will discuss the ability of ammonium oxidizers to oxidize CO to CO_2 and present a possible role for the ammonium oxidizers in the cycling of CO in the environment.

MATERIALS AND METHODS

Cultures and inoculum

Cultures of Nitrosococcus oceanus, Nitrosococcus mobilis and Nitrobacter sp. Nb297 (provided by S. W. Watson), Nitrosomonas europaea (provided by E. L. Schmidt), and several of our isolates representing marine, estaurine freshwater and soil nitrifiers were used in this study. Ammonium oxidizers were grown in 125 mL Erlenmeyer flasks containing 50 mL of the medium described by Jones and Hood (1980). Flasks were incubated at 25°C and shaken at 100 rpm on a rotary shaker for 14 days. Five mL portions of these cultures were filtered onto sterile membrane filters (type HA, 0.45 μm nominal pore size; Millipore Corp.). The cells were washed twice with ammonium free medium, and then the filters with the cells were used in the assay for CO oxidation. Nitrite oxidizers were cultured and prepared in an identical manner, using the medium of Watson and Waterbury (1971) with seawater being replaced by Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) in distilled water to give a salinity of 32 o/oo.

Nitrosomonas europaea, Nitrosomonas sp. 4W30 and Nitrosococcus oceanus cultures used for the determination of the effects of CO concentration and time course experiments were grown using the medium and continuous flow methods described by Jones and Hood (1980). A standard inoculum was prepared by filtering 100 mL of cells from the chemostat onto a sterile 0.45 μm membrane filter (Millipore Cor.), washing twice with NH_4 free medium. These suspensions were subjected to cell counts, using acridine orange

fluorescent microscopy (Hobbie et al. 1977). Portions (1.0 mL) of these suspensions were also used to test the CO oxidation capabilities of these organisms.

Carbon monoxide oxidation determinations

The methods used for ^{14}CO preparation and CO oxidation rate determinations were similar to those described by Griffiths et al. (1982) and Jones and Morita (1983) for methane oxidation. The filters with the various nitrifiers on them were placed in 60-mL serum bottles containing 25 mL of NH_4 free medium buffered with 0.2 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.8. Bottles were then gassed with CO free air (Airco, synthetic air) for 30 s. The bottles were then sealed with serum stoppers, and 0.5 mL of ^{14}CO diluted in N_2 (0.5 μCi/mL; specific activity, 56 mCi/mmole; Amersham Corp.) (1 mCi = 37 MBq) was added to the headspace. The final concentration of CO in solution ranged from 49.78 nL/L for the marine cultures to 44.54 nL/L for the freshwater cultures (Schmidt 1979). All bottles were prepared in triplicate. Controls without cells and with acid killed cells were run with each experiment. The bottles were incubated for 18 h at 25°C at 100 rpm on a rotary shaker. 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 and trapped and ^{14}C incorporated into cellular material was measured using the methods previously described by Griffiths et al. (1982). Control values were subtracted and CO oxidation rates were determined from these data. Control values were small (70 cpm for $^{14}CO_2$ determinations and 30 cpm for cellular incorporation). Confidence intervals were calculated for each data point. The variation among replicates did not exceed the 95% confidence interval for the population mean.

Once the ability of ammonium oxidizers to oxidize CO had been determined, the effect of CO concentration and time course experiments both in the presence and absence of 10 mg/L NH_4-N were examined using Nitrosomonas europaea, Nitrosomonas sp. 4W30 and Nitrosococcus oceanus. For these experiments 1.0 mL of the inoculum described was inoculated into 60 mL serum bottles containing 25 mL of NH_4-free medium, pH 7.8 HEPES, or medium containing 10 mg/L NH_4-N as $(NH_4)_2SO_4$. Unless otherwise noted, bottles were then incubated for 3 h and assayed as previously described.

To examine the effects of CO concentration on CO oxidation, a series of bottles containing between 0.525 and 528.95 µL/L of CO in the headspace was prepared. The low concentrations were prepared by injecting 0.1, 0.3, 0.5, or 1.0 mL of the ^{14}CO-N_2 mixture into the headspace. The higher CO concentrations were achieved by first injecting the bottles with 0.5 mL of ^{14}CO-N_2 and then 0.2, 0.6, 1.0, 2.0, 4.0, 6.0, 10, or 20 µL of unlabeled CO in the headspace to make up the balance. The addition of the

unlabeled CO resulted in a serial dilution of specific activity. It was assumed that there would be no significant isotopic fractionation by the microorganisms, and therefore only the dilution effects were taken into account when calculating the rates of CO oxidation. CO concentrations dissolved in the medium ranged from 9.956 to 10050 nL/L for N. oceanus and Nitrosomonas sp. 4W30 and from 8.908 to 8992 nL/L for N. europaea. CO series were prepared both with and without 10 mg/L NH_4-N.

An additional concentration series was conducted to determine the effects of short term nutrient deprivation on CO oxidation. To achieve this, a series of bottles without ammonium was prepared as before. These bottles were then preincubated for 24 h at 25°C at 100 rpm on a rotary shaker. At the end of this time period ^{14}CO-N_2 and unlabeled CO was injected into the headspace to make up the concentration series. The bottles were then incubated and assayed as before.

Time course experiments, at one CO concentration, were conducted both in the presence and absence of ammonium over a 24 h period. A series of bottles was prepared containing 0.5 mL of the ^{14}CO-N_2 mixture with or without ammonium. At time intervals of 30 min, 60 min, 3 h, 5 h, 10 h, and 24 h, 3 bottles from each series were assayed for ^{14}CO oxidized to $^{14}CO_2$ and ^{14}C incorporated into cellular material.

RESULTS

The rates of CO oxidation are shown in Table 1. All of the ammonium oxidizers examined were able to oxidize CO to CO_2 in the absence of ammonium; however, none of these organisms incorporated carbon from CO into cellular material in the absence of ammonium. The low counts exhibited by several of the isolates did not significantly differ from the background. The rate of CO oxidation varied greatly between species under these conditions. Nitrosomonas sp. 4W30 exhibited the highest rate of CO oxidation and Nitrosomonas sp. 9W30 the lowest. Both of the nitrite oxidizers showed no oxidation of CO at this low CO concentration.

The response of the test organisms to CO concentration in the presence and absence of ammonium are shown in Figs. 1, 2 and 3. At low CO concentrations, in the absence of ammonium, the rate of CO oxidation is linear with a doubling of oxidation occurring with a doubling of concentration. At higher concentrations saturation kinetics were observed for all three bacteria. The presence of 10 mg/L NH_4-N inhibited the oxidation rate at the lower CO concentrations for all the organisms (Figs. 1B, 2B, 3B), while increasing the amount of CO required to start saturation of the system and therefore, causing an increase in the CO oxidation rate at higher concentrations with N. europaea and Nitrosomonas sp. 4W30. This effect was most pronounced with Nitrosomonas sp. R230 (Fig. 2) and to a lesser extent with N. europaea (Fig. 1). CO oxidation by N. oceanus (Fig. 3B) was inhibited by 10 mg/L NH_4-N throughout the CO concentration range examined. No satura-

Table 3-1. Carbon monoxide oxidation by nitrifiers in the absence of ammonium.

	Source	Carbon monoxide oxidation rate (OPM)[a]	
		$^{14}CO_2$ Produced	^{14}C-cells
Nitrosococcus oceanus	Marine North Atlantic	31565	0
Nitrosomonas europaea	Soil -	36888	9
Nitrosomonas sp. 4W30	Marine Alaskan Coast	110256	3
Nitrosomonas sp. 1S10	Estaurine Florida Coast	23431	10
Nitrosomonas sp. 2S0	Freshwater Louisiana Marsh	28834	0
Nitrosomonas sp. 6S30	Marine Alaskan Coast	36561	2
Nitrosomonas sp. 9W0	Freshwater Oregon Coast	17205	0
Nitrosomonas sp. 3S30	Marine Oregon Coast	21762	0
Nitrosomonas sp. 11W30	Marine Oregon Coast	17327	3
Nitrobacter sp. Nb297	Marine -	9	6
Nitrococcus mobilis	Marine South Pacific	1	0

[a]CO oxidation rate is expressed as OPM ^{14}C/5 mL cells and filters/3 h.

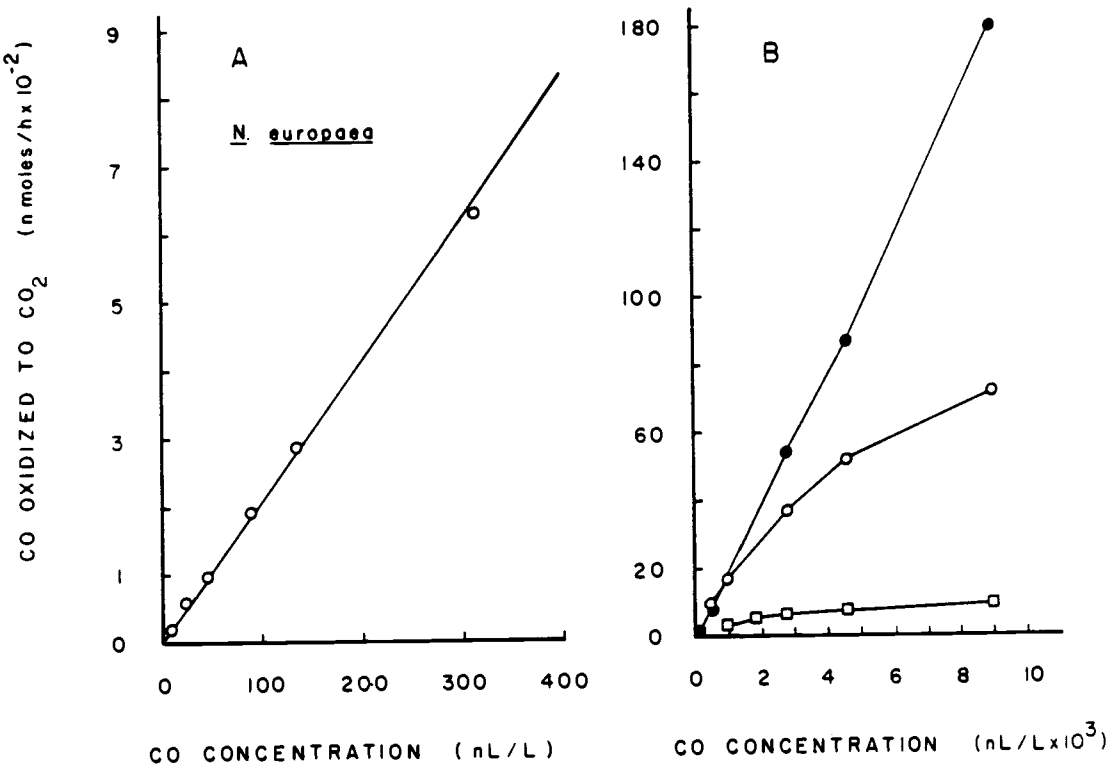

Fig. 3-1. Effects of CO concentration on CO oxidation by N. europaea. Oxidation rate is expressed as nmoles/h/25 mL at a cell concentration of 10^6/mL. (A) CO concentration range 0-313 nL/L and (B) 134-8992 nL/L. Symbols: (o) 0.0 mg/L NH_4-N; (•) 10 mg/L NH_4-N; (□) after 24 h starvation.

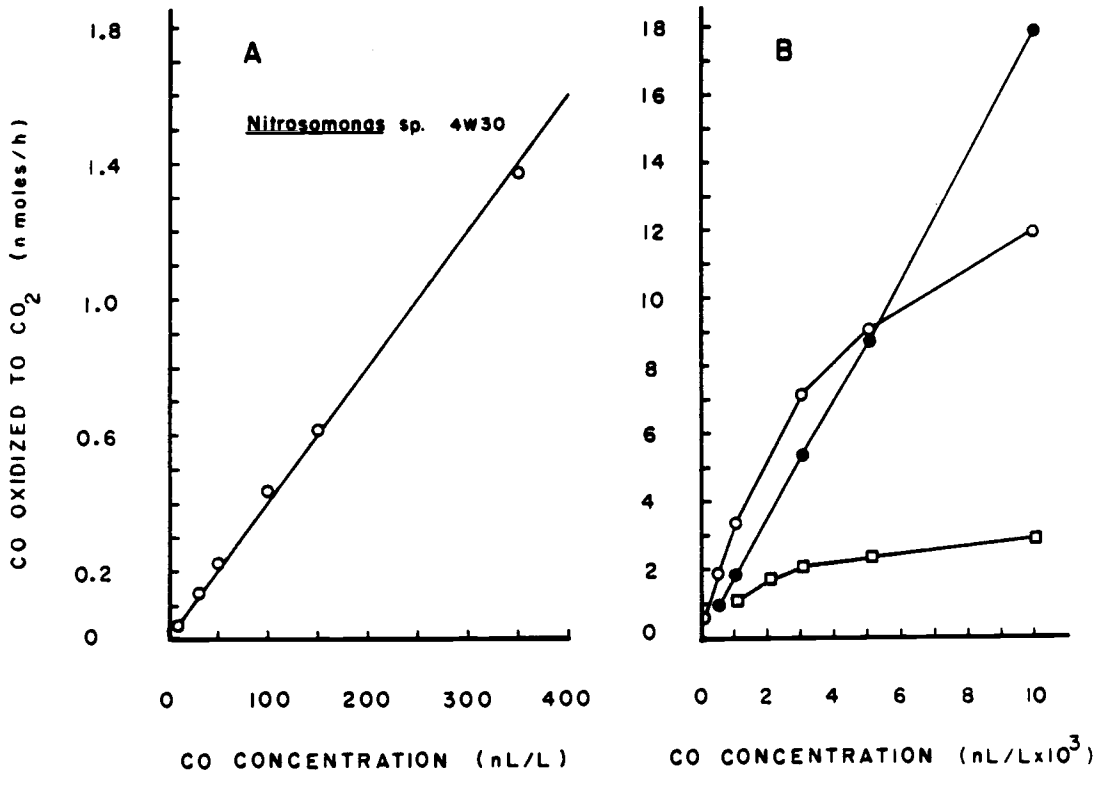

Fig. 3-2. Effects of CO concentration on CO oxidation by Nitrosomonas sp. 4W30. Oxidation rate is expressed as nmoles/h/25 mL at a cell concentration of 10^6/mL. (A) CO concentration range 0-350 nL/L and (B) 150-10050 nL/L. Symbols: (○) 0.0 mg/L NH_4-N; (●) 10 mg/L NH_4-N; (□) after 24 h starvation.

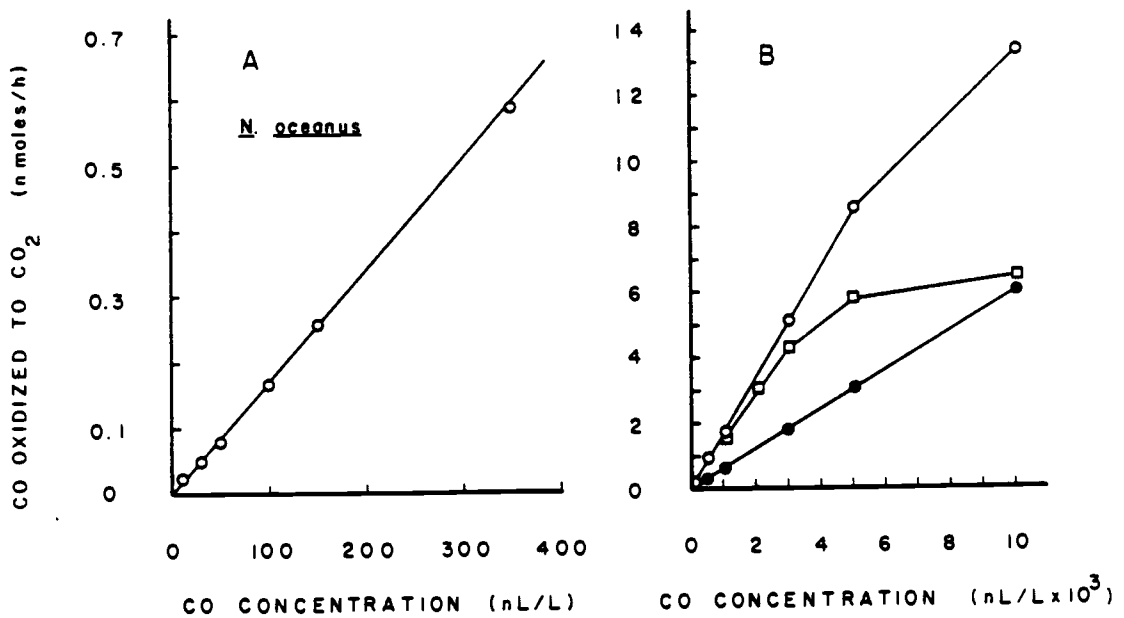

Fig. 3-3. Effects of CO concentration on CO oxidation by N. oceanus. Oxidation rate is expressed as nmoles/h/25 mL at a cell concentration of 10^6/mL. (A) CO concentration range 0-350 nL/L and (B) 150-10050 nL/L. Symbols: (o) 0.0 mg/L NH_4-N; (•) 10 mg/L NH_4-N; (□) after 24 h starvation.

tion was observed in any of the organisms with ammonium present at the CO concentrations used. Analysis of the data using Lineweaver-Burk plots yielded straight lines and gave Vmax values of 41.67 nmoles/L/h at 10^6 cells/mL and a Km of 222 nM for N. europaea; Vmax = 645 nmoles/L/h at 10^6 cells/mL, Km = 195 nM for Nitrosomonas sp. 4W30; Vmax = 1587 nmoles/L/h at 10^6 cells/mL, km = 889 nM for N. oceanus.

After being incubated 24 h in a medium devoid of an energy yielding substrate, all three test cultures showed saturation at lower CO concentrations (Figs. 1B, 2B, 3B). Lineweaver-Burk plots of these points yielded much lower Vmax and Km values. Under these conditions N. europaea had a Vmax of 4.76 nmoles/L/h at 10^6 cells/mL and a Km of 97 nM; Nitrosomonas sp. 4W30, a Vmax of 140 nmoles/L/h at 10^6 cells/mL and a Km of 105 nM; and N. oceanus, a Vmax of 408 nmoles/L/h at 10^6 cells/mL and a Km of 200 nM.

The results of the time course experiments are shown in Figs. 4, 5, and 6. N. europaea (Fig. 4) and Nitrosomonas sp. 4W30 (Fig. 5) exhibited the same trends. The rate of CO oxidation in the absence of ammonium showed linearity for the first 3 h and then decreased thereafter, such that the rate at 24 h was less than 20% of the initial rate. The presence of 10 mg/L NH_4-N had just the opposite effect. There was an initial decrease in activity when compared with the cultures without ammonium but as the length of incubation increased, the rate of CO oxidation increased (Fig. 4, 5). Nitrosococcus oceanus showed a different

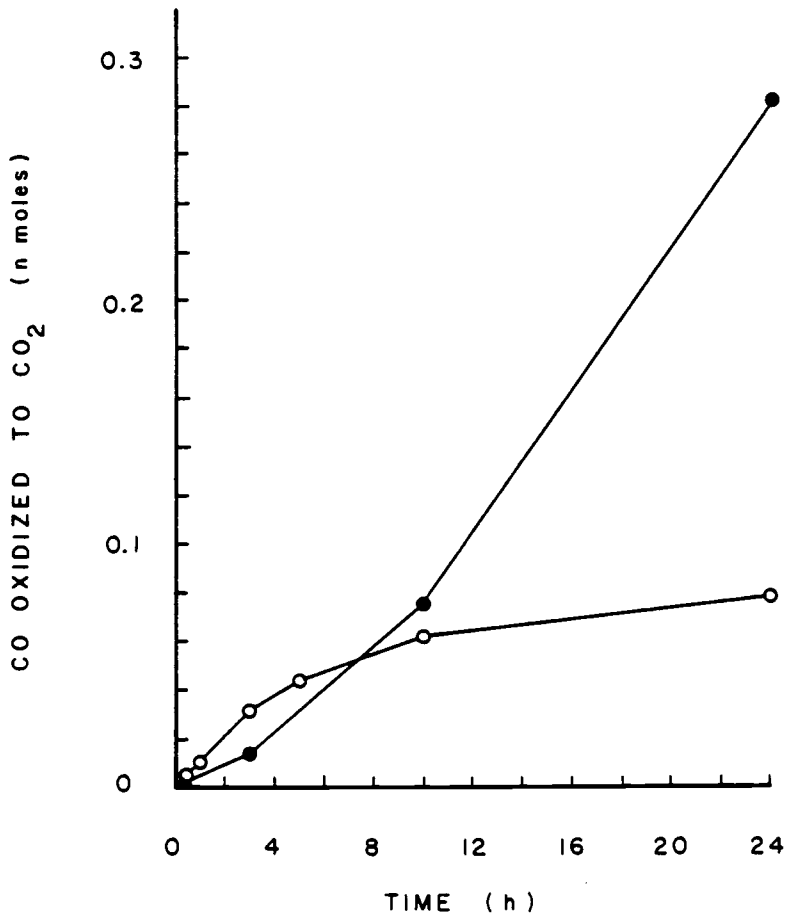

Fig. 3-4. Time course of CO oxidation by N. europaea. Oxidation is expressed as nmoles/25 mL at a cell concentration of 10^6/mL. Symbols: (o) 0.0 mg/L NH_4-N; (•) 10 mg/L NH_4-N.

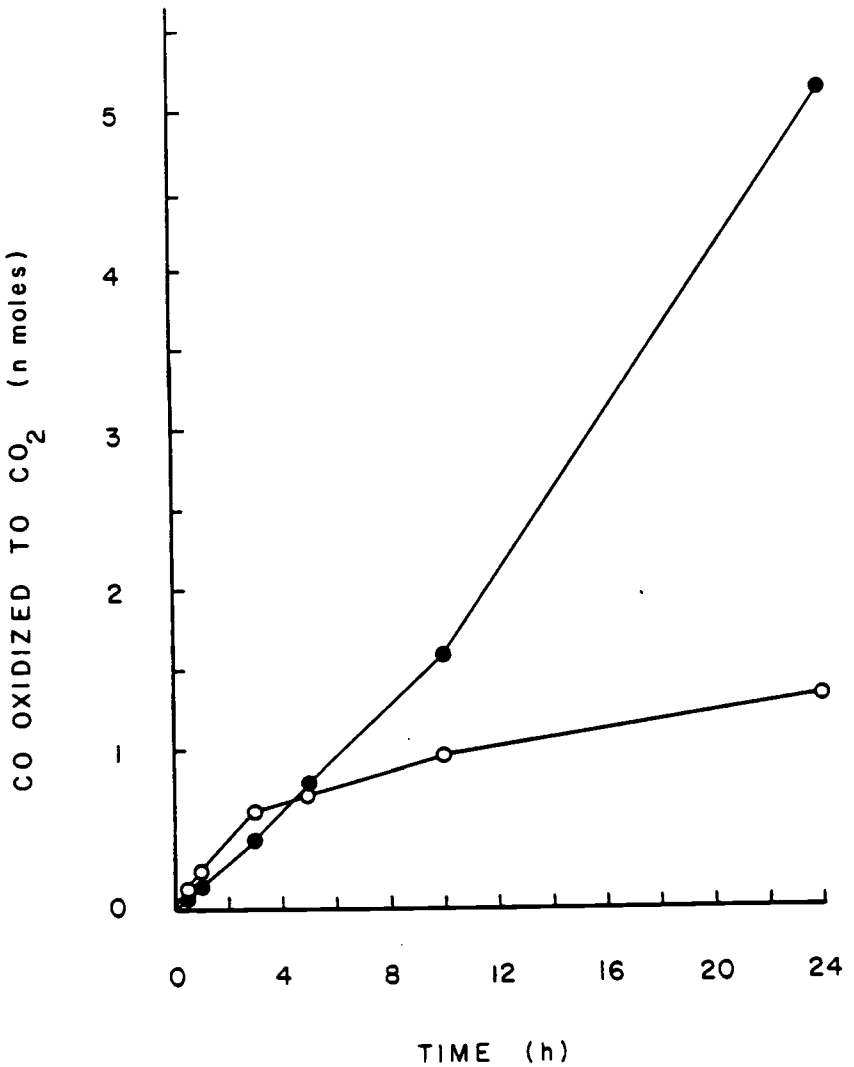

Fig. 3-5. Time course of CO oxidation by Nitrosomonas sp. 4W30. Oxidation is expressed as nmoles/25 mL at a cell concentration of 10^6/mL. Symbols: (○) 0.0 mg/L NH_4-N; (●) 10 mg/L NH_4-N.

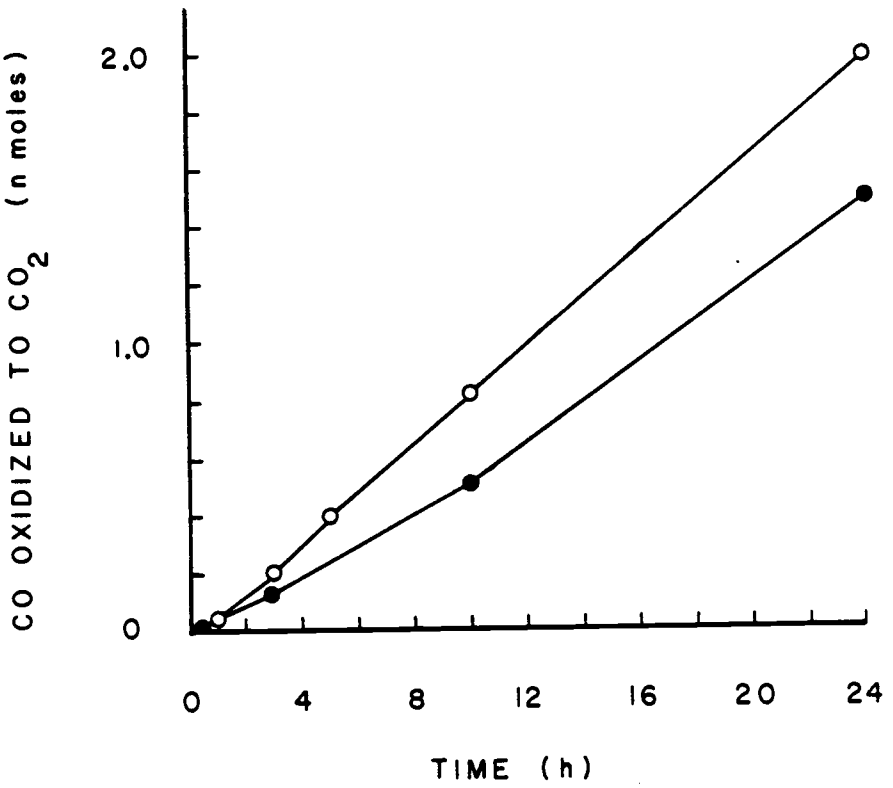

Fig. 3-6. Time course of CO oxidation by N. oceanus. Oxidation is expressed as nmoles/25 mL at a cell concentration of 10^6/mL. Symbols: (o) 0.0 mg/L NH_4-N; (•) 10 mg/L NH_4-N.

trend with the rate of CO oxidation being linear with time and ammonium having an inhibitory effect but not changing the shape of the curve only decreasing the slope of the line (Fig. 6). With the exception of N. oceanus the initial inhibition is relatively short, lasting only for approximately the first 3 h of incubation.

The amount of ^{14}C incorporated into cellular material is shown in Table 2. In the absence of ammonium, even after 24 h, no carbon from CO was detected in the cellular fraction. The cultures with ammonium present all incorporated some of the ^{14}CO into cellular components evern after only 30 min. The percentage of ^{14}C incorporated was variable with N. europaea giving a value of 7.6%. N. sp. 4W30, 1.2% and N. oceanus, 0.5%.

Table 3-2. Incorporation of ^{14}CO-C into cell material by Nitrosococcus oceanus, Nitrosomonas europaea, and Nitrosomonas sp. 4W30.

		^{14}CO-C incorporated (pmoles)[a,b]			
		Time (h)			
	NH_4-N	0.5	3.0	10	24
N. oceanus	0 mg/L	0	0	0	0
	10 mg/L	0.016	0.354	0.402	1.005
N. europaea	0 mg/L	0	0	0	0
	10 mg/L	0.193	2.317	19.550	73.500
Nitrosomonas sp. 4W30	0 mg/L	0	0	0	0
	10 mg/L	0.243	2.672	17.870	63.250

[a]Expressed as CO-C incorporated/25 mL at a density of 10^6 cells/ml.

[b]CO concentrations were 2.22 nM for N. oceanus and Nitrosomonas sp. 4W30 and 1.99 nM for N. europaea

DISCUSSION

The similarities between methane mono-oxygenase and ammonium mono-oxygenase have been pointed out by several other workers (Ferenci et al. 1975; Wilkinson 1975; O'Neill and Wilkinson 1977; Dalton 1977). The methane mono-oxygenase of methane oxidizing bacteria is capable of oxidizing a wide range of carbon compounds including CO (Hubley et al. 1974; Ferenci 1974; Colby et al. 1977). We recently demonstrated that methane is a utilizable substrate for the ammonium oxidizers, further demonstrating the similarities (Jones and Morita 1983). Prior work using oxygen uptake measurements had indicated that CO could not be oxidized by Nitrosomonas europaea (Suzuki et al. 1976; Drozd 1976). Using ^{14}CO this research demonstrates that ammonium oxidizers including Nitrosomonas europaea can oxidize significant amounts of CO. Why no CO oxidation was observed in the earlier studies is unknown. The presence of high ammonium concentrations in the experiments conducted by Suzuki et al. (1976) and the low CO concentrations used by Drozd (1976) may have masked the oxidation of CO to the relative insensitivity of oxygen uptake measurements.

The results of this research clearly demonstrate that the ammonium oxidizers are capable of significant CO oxidation even at the low mixing ratios of the atmosphere (<1 $\mu L/L$) Figs. (1A-3A). Unlike the oxidation of methane (Jones and Morita 1983), the presence of ammonium did not stimulate the initial rate of CO oxidation. The fact that ammonium eventually causes a stimulation

in N. europaea and Nitrosomonas sp. 4W30 indicates that the interactions between ammonium and CO are complex, and not explainable simply as two substrates competing for the same enzyme. Since CO oxidation by N. oceanus remained inhibited by NH_4-N after 24 h (Fig. 6) and throughout the CO concentration range (Fig. 3B), it appears that there may be a fundamental difference between this organism and the other two isolates examined. It is possible, and in fact, seems likely that this difference is at the level of ammonium mono-oxygenase.

Attempts to grow the ammonium oxidizers on CO as the sole source of carbon and energy have failed. Although the ammonium oxidizers can oxidize CO in the absence of ammonium, the oxidation can be considered a cooxidation, since they cannot grow, or even incorporate CO-carbon into cellular material in the absence of ammonium (Table 2). It has been demonstrated that CO is an inhibitor of ammonium oxidation (Hooper and Terry 1973; Suzuki et al. 1976) but, it is not known what effect the presence of CO may have on the growth yield and specific growth rate of ammonium oxidizers especially at low concentrations of both substrates. It is also possible that CO could serve as a source of energy of maintenance when ammonium is limiting but not for growth and reproduction. As with methane, the ability of ammonium oxidizers to utilize CO could serve as a survival mechanism. The distribution of CO in the environment is nearly ubiquitous. The average CO concentration in the lower atmosphere is 110 nL/L (Seiler 1974). Concentrations of CO ranging from 10 nL/L at 4000

M to >4000 nL/L at the surface have been reported for the Atlantic Ocean (Conrad et al. 1982), and aerobic lake water has been found to contain between 20 and 615 nL/L of dissolved CO (Conrad et al. 1983). At these concentrations CO may represent a constant source of energy. The combination of multiple energy yielding substrates and cooxidation by the ammonium oxidizers may be able to support growth or maintain viability of the cell where none could occur given only one substrate.

Although the consumption of CO in the soil, lakes and oceans is known to be a microbial process (Ingersoll et al. 1974; Liebe et al. 1976; Bartholomew and Alexander 1979; Conrad and Seiler 1980; Spratt and Hubbard 1981; Conrad and Seiler 1982), the organisms responsible remain largely a mystery. Conrad et al. (1981) examined the role of the carboxydobacteria in CO oxidation in the soil, and using kinetic information concluded that although the carboxydobacteria could oxidize CO at low concentrations, it was unlikely that they play a major role. Bartholomew and Alexander (1979) reported the utilization of CO by Nocardia salmonicolor and other nocardioforms but the rates of CO oxidation by these organisms appear to be relatively slow. Most of the evidence concerning the nature of the CO oxidizing organisms is derived from the extremely low Km values reported for soils, lakes and oceans (Conrad et al. 1981; Conrad and Seiler 1982; Conrad et al. 1983). They report Km values of between 4-9 nM for their own work and from 13 to 39 nM for the work of Spratt and Hubbard (1981) and Bartholomew and Alexander (1981), respectively. These low Km values led them to

conclude that organisms with a higher affinity than those already reported are needed to explain the utilization of CO by natural samples. The data presented in this paper indicates the Km values, while not as low as those found with natural samples, are low enough for the ammonium oxidizers to have a significant role in the oxidation of CO. It is interesting to note the effects that nutrient starvation and ammonium have on the Km values. After 24 h of starvation the affinity for CO increases in all of the organisms (Figs. 1B-3B). In fact the Km value for N. oceanus decreases over 4 fold. Using the techniques presented in this paper we have been unable to obtain Km values as low as those reported by Conrad and Seiler (1982). Although, our values are higher 27-50 nM for seawater, soil and stream waters (Jones and Morita, unpublished data) they are still lower than those for the ammonium oxidizers. It should be noted that the calculation of kinetic data from natural samples is far from ideal, and that a great deal of variation can be introduced due to the method used to measure rates in natural samples. Rather than placing so much importance on Km values, it is more interesting to use the rate of CO oxidation in culture and estimate relative importance from these values. Using this approach, Bartholomew and Alexander (1979) reported a CO oxidation rate for soil of 0.81 nmoles/h/5.0 g at a CO concentration of 17 µL/L in the headspace. Using the values of CO oxidation in the absence of ammonium from this ppaer, would require from 2.4×10^6 cells/g of Nitrosomonas sp. 4W30 to 6.8×10^7 cells/g for N. europaea to account for this activity. The

value of 3.0 nL/L/h at a CO concentration of 100 nL/L (dissolved) reported by Conrad and Seiler (1982) for Atlantic Ocean waters would require from 6.3 x 10^4 cells/mL of Nitrosomonas sp. 4W30 to 1.2 x 10^6 cells/mL for N. europaea. These cell concentrations are not unacceptably high values given the estimated populations of nitrifiers in the environment (Matulewich et al. 1975; Belser 1979; Belser and Mays 1982). Preliminary results in our laboratory indicate that ammonium oxidizers can be responsible for from 4 to 70% of the CO oxidation in natural samples depending upon the source (Jones and Morita unpublished data).

It is not known what advantage the ammonium oxidizers get from their cooxidation of CO. It appears likely that they play an important role in the consumption of CO in the environment. Given the elevated concentrations and nearly ubiquitous distribution of CO in oceans and lakes, CO may serve as a significant source of energy for these organisms especially in the absence of ammonium. Additional work needs to be conducted to further define the relationships of the alternative substrates of the ammonium oxidizing bacteria and their effects on the growth, survival, physiology and ecology of this unique group of organisms.

Since this manuscript was submitted a paper has been published demonstrating the oxidation of cytochrome c_{554} by CO in cell free Nitrosomonas preparations (Tsang and Suzuki 1982).

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CHAPTER IV

EFFECTS OF VARIOUS PARAMETERS ON CARBON MONOXIDE OXIDATION BY AMMONIUM OXIDIZERS

ABSTRACT

The effects of temperature, pH, nitrogen source, cell concentration and the interactions between ammonium and carbon monoxide were examined with respect to the oxidation of carbon monoxide by several chemolithotrophic ammonium oxidizing bacteria. The ammonium oxidizers Nitrosomonas europaea, Nitrosomonas sp. 4W30, a marine isolate, and Nitrosococcus oceanus were examined. All of the organisms were able to oxidize significant amounts of CO over wide ranges of temperature and pH. Ammonium at concentrations as low as 1 mg/L NH_4^+-N initially inhibited CO oxidation in all three organisms, however after 48 h, the presence of ammonium stimulated the CO oxidizing ability of N. europaea and Nitrosomonas sp. 4W30, while N. oceanus remained inhibited. None of the other nitrogen sources examined had a significant effect on CO oxidation. No carbon from CO was incorporated into cellular material in the absence of ammonium, even in the presence of alternate nitrogen sources. Cells incubated in the presence of ammonium in concentrations as low as 1.0 mg/L NH_4^+-N were able to incorporate CO-carbon into cellular material. Increasing NH_4^+-N concentrations to 50 mg/L stimulated the incorporation of CO-carbon by N. oceanus. Concentrations of 10 mg/L gave the highest incorporation levels for N. europaea and Nitrosomonas sp. 4W30 and 50 mg/L inhibited the incorporation by these two organisms. The presence of CO-inhibited the oxidation of ammonium by all of the organisms tested.

INTRODUCTION

The oxidation of carbon monoxide by ammonium oxidizers has recently been demonstrated. Tsang and Suzuki (1982) demonstrated the oxidation of cytochrome c_{554} by CO in a cell free Nitrosomonas system. Jones and Morita (1983b) have shown that a wide variety of ammonium oxidizers including N. europaea, N. oceanus and Nitrosomonas sp. 4W30 are able to oxidize CO to CO_2 and, in the presence of ammonium, incorporate some of the carbon from CO into cellular components. It appears that the oxidation of CO by nitrifiers may have a significant effect on the cycle of CO in the environment (Jones and Morita 1983b). The purpose of this paper is to further define some of the parameters which may affect CO oxidation in natural systems and to elucidate more information about the interactions between ammonium and CO.

MATERIALS AND METHODS

Cultures and inoculum. Growth and preparation of N. europaea, Nitrosmonas sp. 4W30 and N. oceanus cells for CO and ammonium oxidation assays were as described by Jones and Morita (1983b). A standard inoculum was prepared by filtering 100 mL of cells from the chemostat onto a 0.45 μm membrane filter, washing twice with NH_4^+ free medium, pH 7.8, and resuspending them in 25 mL of sterile NH_4^+ free medium. These suspensions were subjected to cell counts, using acridine orange fluorescent microscopy (Hobbie et al. 1977). Portions (1.0 mL) of these suspensions were also used to test the CO oxidation capabilities of these organisms. Nitrosococcus oceanus cultures were provided by S. W. Watson and Nitrosomonas europaea cultures were provided by E. L. Schmidt.

Carbon monoxide oxidation determinants. The methods used for ^{14}CO preparation and CO oxidation rate determinations were the same as those previously described (Jones and Morita 1983b). One mL of the standard inoculum (unless otherwise noted) was inoculated into a 60-mL serum bottle containing 25 mL of NH_4^+-free medium (Jones and Morita 1983a). The bottles were gassed with CO free air, sealed with serum stoppers, and 0.5 mL of ^{14}CO diluted in nitrogen (9.5 μCi/mL; specific activity, 56 mCi/mmole; Amersham Corp.) (1 mCi = 37 mBq) was injected into the headspace. CO concentrations ranged from 49.8 nL/L for N. europaea to 44.5 nL/L for Nitrosomonas sp. 4W30 and N. oceanus (Schmidt 1979). Bottles were then incubated for 3 h (unless otherwise noted) on a rotary shaker at 100 rpm and assayed for $^{14}CO_2$ production and ^{14}CO-C incorporated into cellular

material, as previously described (Griffiths et al. 1982). All bottles were prepared in triplicate and each experiment was duplicated. Confidence intervals were calculated for each data point. The variation among replicates did not exceed the 95% confidence interval for the population mean.

To test the effects of temperature a series of bottles was prepared and incubated at various temperatures between 5^{o} and $30^{o}C$.

A pH series was prepared by adjusting the medium to various pH values between 4.0 and 10.5, using either 1.0 N HCl or 1.0 N NaOH.

Bottles containing various nitrogen sources were prepared to examine the effects on CO oxidation. The following additions were made: 1 and 10 mg/L NH_4^+-N as $(NH_4)_2SO_4$, 1 and 10 mg/L NO_2^--N as KNO_2, and 1 and 10 mg/L NO_3^--N as KNO_3. Controls contained no additional nitrogen source. An additional nitrogen source series was prepared to examine the effects of a longer term incubation period on CO oxidation with the various nitrogen compounds. Bottles were inoculated with 0.1 mL of the standard inoculum, and 10 mg/L of one of the 3 nitrogen sources. They were then incubated for 48 h and assayed for CO oxidation as usual. Controls which contained no additional nitrogen source were also run.

The effects of cell concentration on CO oxidation were determined by preparing a series of bottles as usual and then inoculating them with either 0.01, 0.1, 1.0 or 2.0 mL (3.0 mL for Nitrosomonas sp. 4W30) of the standard inoculum. In addition the effect of 10 mg/L NH_4^+-N on CO oxidation at various cell concentrations was examined using Nitrosmonas sp. 4W30. CO oxidation rates were determined and rates were calculated on a per cell basis.

To test the effect of ammonium, a series of bottles containing 0, 1, 10, and 50 mg/L NH_4^+-N as $(NH_4)_2SO_4$ was prepared, incubated and assayed.

Carbon monoxide effects on ammonium oxidation. A 1-mL amount of the standard inoculum was inoculated into 60-mL serum bottles which contained 25 mL of medium containing 1.0 mg/L NH_4^+-N. The bottles were capped and 0, 0.05, 0.1, 0.3, 0.5, or 1.0 mL of unlabeled CO was injected into the headspace. CO concentrations ranged to 450 µL/L for N. europaea and 500 µL/L for Nitrosomonas sp. 4W30 and N. oceanus (Schmidt 1979). These bottles were then incubated for 6 h at 25°C at 100 rpm on a rotary shaker. The bottles were then assayed for nitrite using the spectrophotometric method of Bendschneider and Robinson (1952) to determine ammonium oxidation. All bottles were prepared in triplicate and each experimental set was duplicated.

RESULTS AND DISCUSSION

The effects of temperatures between 5° and 30°C on the rates of CO oxidation are shown in Fig. 1. All of the organisms were able to oxidize CO over the range of temperatures examined. The CO oxidation rate increased as the temperature increased for all of the test organisms. The rate of the increase in CO oxidation showed a concomitant rise with increasing temperature for N. europaea. Nitrosmonas sp. 4W30 showed the opposite trend. The effect of temperature on N. oceanus was linear with its activity doubling with each 15°C increase in temperature. These results demonstrate the ability of ammonium oxidizers to oxidize CO over the usual range of environmental temperatures. Nitrosomonas sp. 4W30 was isolated from Alaskan waters and is able to grow at temperatures below 1°C (Jones and Morita unpublished data). It is interesting to note that this species oxidizes a considerable amount of CO at 5°C.

The pH ranges for all three organisms were wide and significant amounts of CO were oxidized outside of the normal growth ranges for these organisms (Fig. 2). N. europaea had an optimum pH for CO oxidation at 8.5 and was able to oxidize CO at pH values as low as 5.0 and as high as 10 although significant CO oxidation ceased at pH 9.5. N. oceanus did not exhibit a clear optimum pH, but rather showed a level response from 5.5 to 8.5. It oxidized CO from pH 5.0 to 9.5. Nitrosomonas sp. 4W30 had an optimum pH of 8.0 and a range of 5.0 to 10.5. Although ammonium oxidizers will oxidize ammonium to nitrite over a wide range of pH values in general as the pH falls below 6.0 the activity drops very rapidly

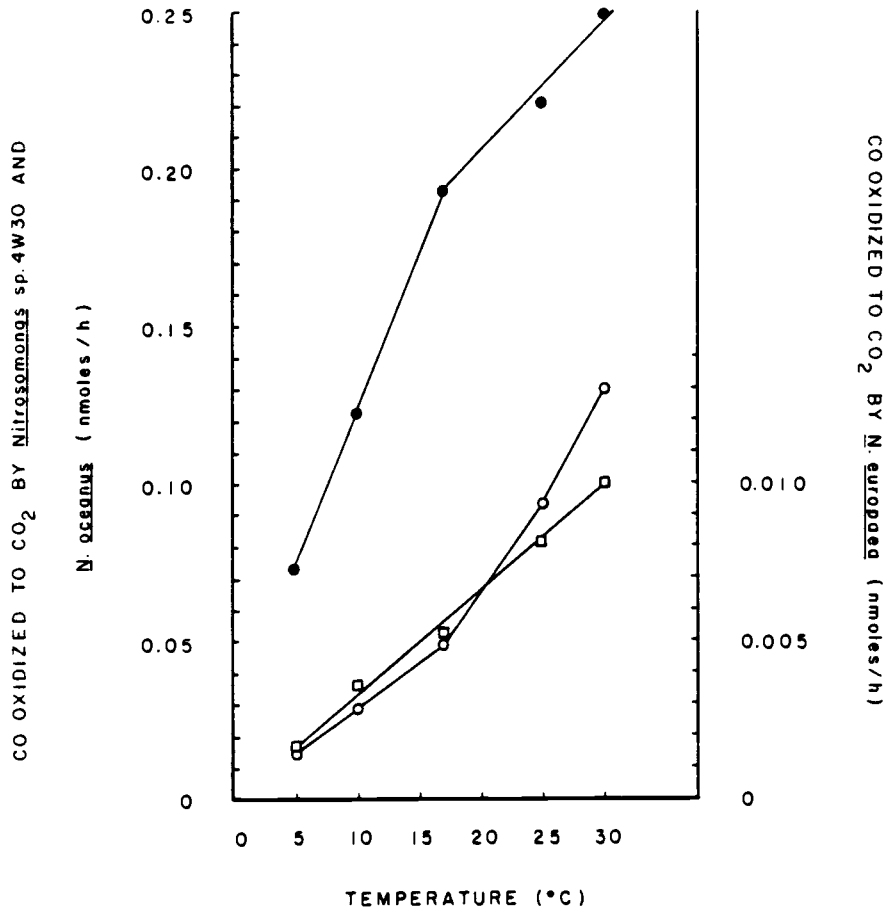

Fig. 4-1. Temperature effects on CO oxidation. Oxidation rate is expressed as nmoles/h/25 mL at a cell density of 10^6/mL. Symbols: (○) N. europaea; (●) Nitrosomonas sp. 4W30; (□) N. oceanus.

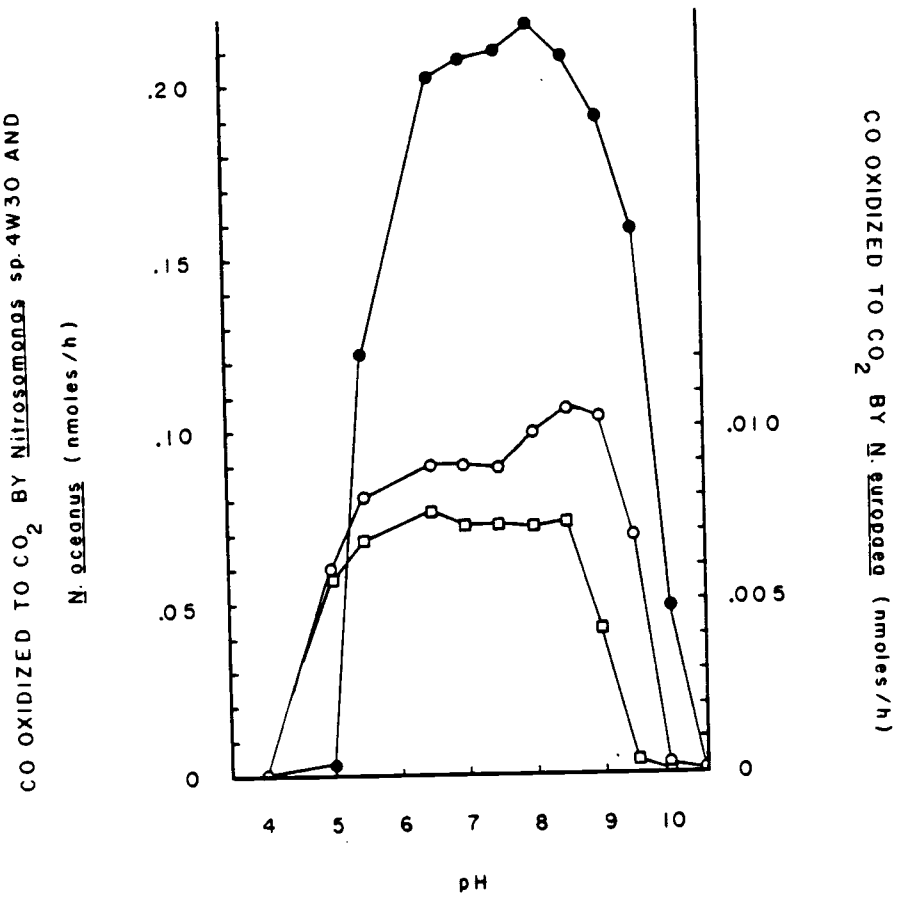

Fig. 4-2. Effects of pH on CO oxidation. Oxidation rate is expressed as nmoles/h/25 mL at a cell density of 10^6/mL. Symbols: (o) N. europaea; (•) Nitrosomonas sp. 4W30; (□) N. oceanus.

(Painter 1970). In the case of CO oxidation this was not observed with any of the organisms tested. Although activity did drop at pH 6.0 and below, with the exception of Nitrosomonas sp. 4W30, at a pH of 5.0 this drop was less than 50% of the optimum values. If ammonium oxidizers are able to obtain energy from the oxidation of CO, the ability to oxidize CO at these low pH values could be a mechanism for survival or even growth at the low pH values found in some environments.

The effects of the various sources of inorganic nitrogen on CO oxidation are shown in Table 1. Ammonium concentrations of both 1.0 and 10.0 mg/L initially inhibited the oxidation of CO in all of the test organisms. After 48 h cultures of N. europaea and Nitrosomonas sp. 4W30 showed a marked increase in the rate of CO oxidation in the presence of NH_4^+-N, while N. oceanus remained inhibited. This is in agreement with the time course of CO oxidation reported in an earlier study (Jones and Morita 1983b). The stimulation of CO oxidation by ammonium may be due to the regeneration of reduced cytochrome c_{554} by hydroxylamine oxidation as demonstrated by Tsang and Suzuki (1982). This would allow the further oxidation of CO if their proposed mechanism of ammonium and CO oxidation is correct. The lack of stimulation and, in fact, a depression of CO oxidation in the presence of ammonium with N. oceanus cannot be explained by this mechanism. Unlike the oxidation of methane where the presence of NH_4^+-N, NO_2^--N, and NO_3^--N stimulated activity (Jones and Morita 1983a), neither nitrite or nitrate had a stimulatory effect on CO oxidation. The presence of nitrite and

Table 4-1. Effects of ammonium, nitrite and nitrate on carbon monoxide oxidation by Nitrosococcus oceanus, Nitrosomonas europaea and Nitrosomonas sp. 4W30.

Organism	Incubation time (h)	Carbon monoxide oxidation rate (nmoles/h)[a] with given addition						
			NH_4^+-N		NO_2^--N		NO_3^--N	
		None	1 mg/L	10 mg/L	1 mg/L	10 mg/L	1 mg/L	10 mg/L
N. oceanus	3	0.0705	0.0637	0.0404	0.0694	0.0709	0.0688	0.0700
	48	0.0270	ND[b]	0.0237	ND	0.0285	ND	0.0279
N. europaea	3	0.0116	0.0110	0.0050	0.0104	0.0106	0.0086	0.0081
	48	0.0015	ND	0.0209	ND	0.0016	ND	0.0016
N. sp. 4W30	3	0.221	0.187	0.150	0.215	0.209	0.218	0.218
	48	0.0814	ND	0.2845	ND	0.0811	ND	0.0765

[a]Oxidation is expressed as nmoles of CO oxidized to CO_2/25 mL at a cell concentration of 10^6 mL.

[b]ND, not determined.

nitrate appear to have a slight inhibitory effect on CO oxidation by N. europaea, but this was only evident in the short (3 h) incubation experiments.

Cell density has been shown to affect the rate at which the carboxydobacteria oxidize CO (Conrad et al. 1981). The results of a similar experiment using the ammonium oxidizers are shown in Fig. 3. All of the organisms examined displayed a slightly different pattern. N. europaea cultures oxidized less CO per cell as density increased and this decrease appeared to be linear with increasing cell density. Nitrosomonas sp. 4W30 gave an initial level response, but then decreased more rapidly than N. europaea. The oxidation rate of N. oceanus remained constant throughout the cell densities examined. Conrad and Seiler (1981) found that, with the carboxydobacteria, the ability to oxidize CO decreased as cell density was increased. They attribute this to the solubility and the diffusion of CO in solution. With the ammonium oxidizers this would not appear to be the only explanation, since the addition of 10 mg/L NH_4^+-N eliminates the effect with Nitrosomonas sp. 4W30, and no effect was observed with N. oceanus. At least a portion of this effect seems to be due to residual ammonium in the medium. The changes in CO oxidation rate are relatively small, at the normally used cell densities, and do not appear to have a major impact on the results; however, future experimentation should avoid excessively high cell densities which could significantly alter the results.

The next parameter to be examined was the effect of NH_4^+-N on CO oxidation. The results are shown in Fig. 4 and Table 2. The

Fig. 4-3. Effects of cell density on CO oxidation. Oxidation rate is expressed as fmoles/h/cell. Symbols: (○) N. europaea; (●) Nitrosomonas sp. 4W30; (□) Nitrosomonas sp. 4W30 with 10 mg/L NH_4^+-N; (■) N. oceanus.

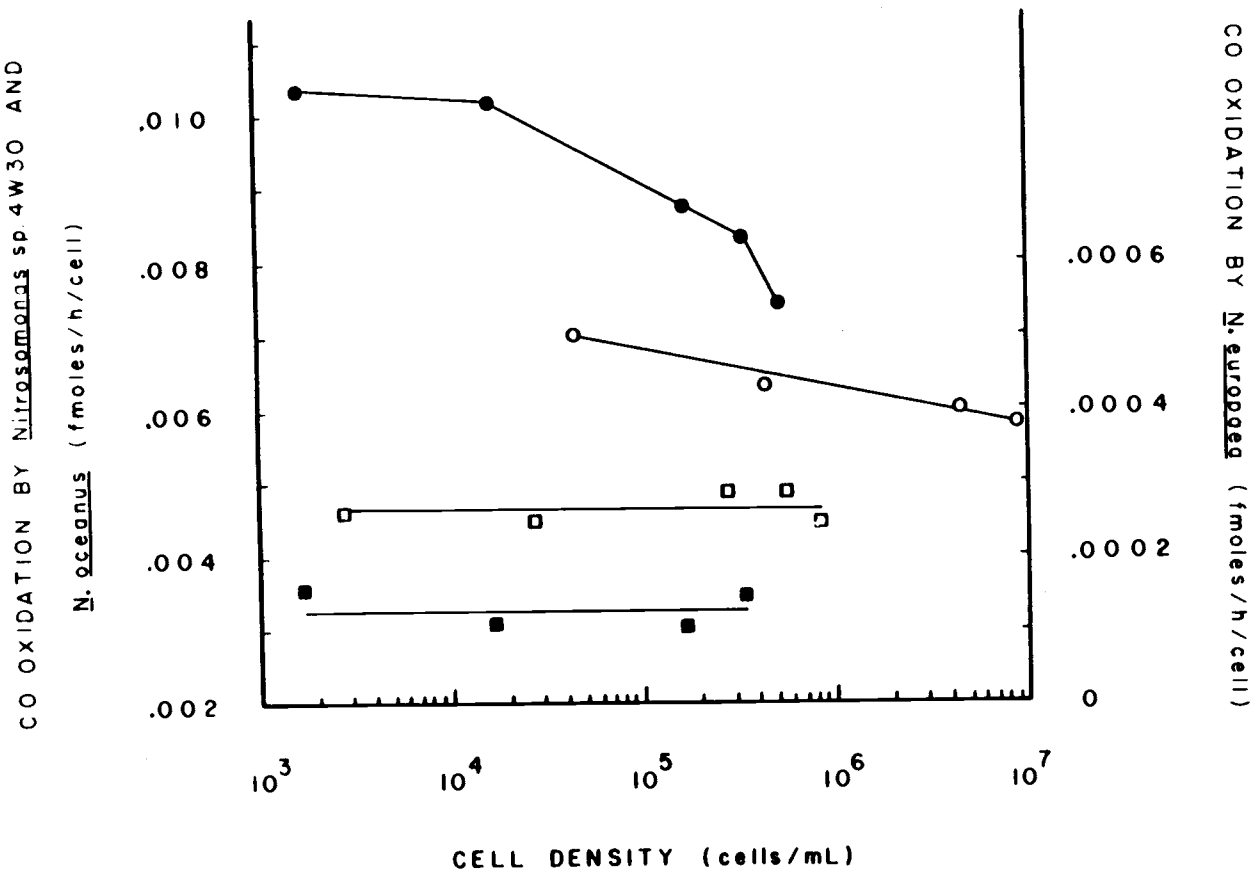
CO OXIDATION BY Nitrosomonas sp. 4W30 AND
N. oceanus (fmoles/h/cell)
.010
.008
.006
.004
.002
CO OXIDATION BY N. europaea (fmoles/h/cell)
.0006
.0004
.0002
0
10^3
10^4
10^5
10^6
10^7
CELL DENSITY (cells/mL)

Fig. 4-3.

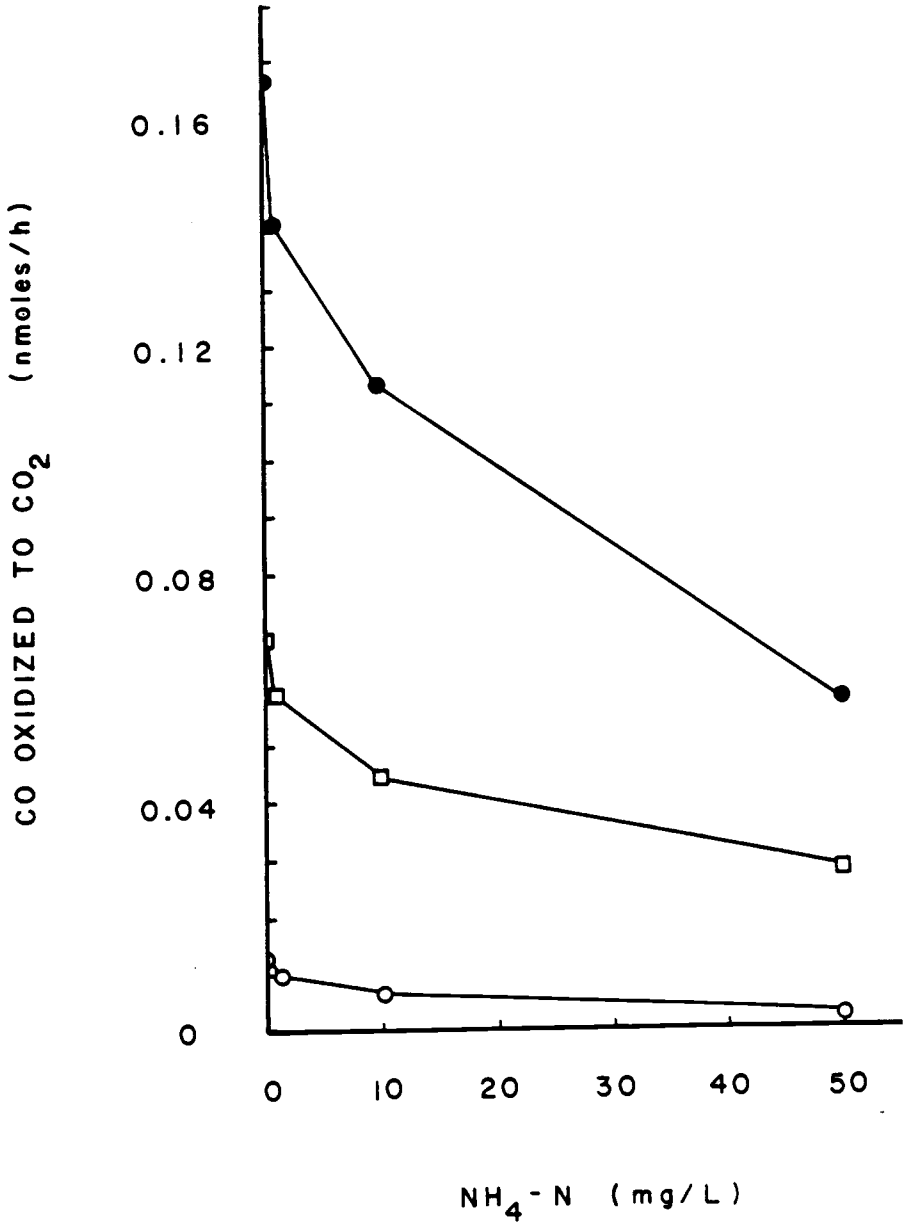

Fig. 4-4. Ammonium effects on CO oxidation. Oxidation rate is expressed as nmoles/h/25 mL at a cell density of 10^6/mL. Symbols: (o) N. europaea; (•) Nitrosomonas sp. 4W30; (□) N. oceanus.

Table 4-2. Effects of ammonium concentration on CO incorporation into cellular material by Nitrosococcus oceanus, Nitrosomonas europaea and Nitrosomonas sp. 4W30.

	Carbon monoxide incorporation rate (pmoles/h)[a]			
	NH^+-N concentration (mg/L)			
Organism	0.0	1.0	10.0	50.0
N. oceanus	0.0000	0.219	0.1025	0.1171
N. europaea	0.0000	0.0506	0.1497	0.0595
N. sp 4W30	0.0000	0.198	0.759	0.515

[a]Oxidation is expressed as pmoles of ^{14}CO-C incorporated/25 mL at a cell concentration of 10^6/mL.

production of $^{14}CO_2$ by all three organisms showed the same response to increasing NH_4^+-N concentrations. Concentrations as low as 1.0 mg/L inhibited the oxidation (3 h experiments) with a further decrease in activity as concentrations increased. The ^{14}CO-C incorporated into cellular material showed a different trend (Table 2). As pointed out earlier none of the organisms tested incorporated any ^{14}CO-C into cellular material in the absence of NH_4^+-N. However, the presence of ammonium in concentrations of 1.0 mg/L or greater allowed the cells to incorporate ^{14}C from ^{14}CO. N. europaea and Nitrosomonas sp. 4W30 gave a peak of ^{14}CO incorporation at 10.0 mg/L NH_4^+-N with a decrease as ammonium concentrations increased. N. oceanus continued to incorporate more ^{14}CO-C throughout the range of NH_4^+-N examined, again demonstrating the difference between the test organisms. Since ammonium is necessary for the incorporation of CO-C it is likely that the incorporation proceeds through CO_2 and that the oxidation of ammonium is the driving force for CO_2 reduction. This provides evidence that if the ammonium oxidizers are gaining energy from the oxidation of CO it is insufficient to support growth and may only be sufficient to provide an energy of maintenance.

The effects of CO concentration on ammonium oxidation are shown in Fig. 5. All of the organisms showed inhibition of ammonium oxidation to nitrite by CO, although the concentrations of CO necessary to inhibit NH_4^+-N oxidation were quite high (25 μL/L) compared to those found in the environment. The initial response to increasing CO concentrations was a strong inhibitory effect on

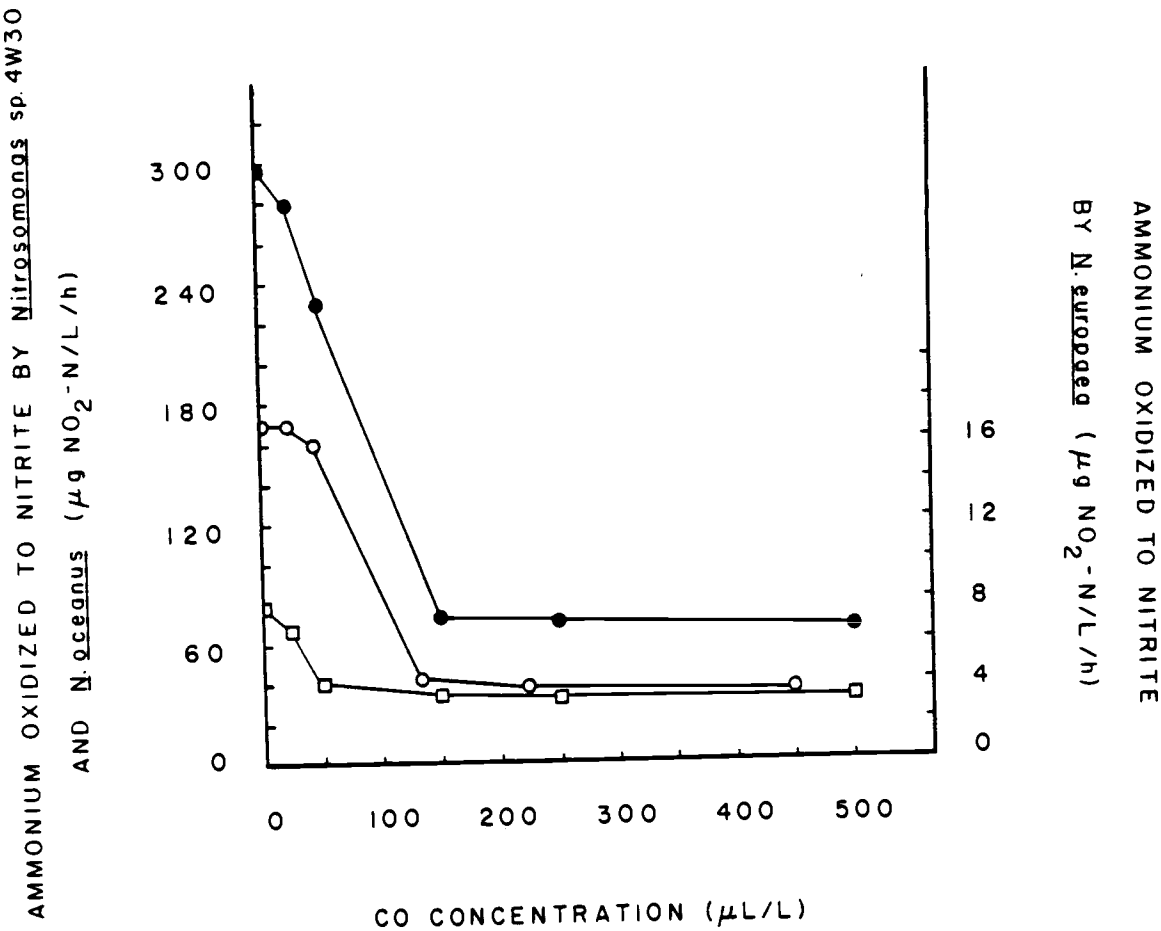

Fig. 4-5. CO concentration on ammonium oxidation. Oxidation rate is expressed as μg NO_2^--N/L/h at a cell density of 10^6/mL. Symbols: (o) N. europaea; (•) Nitrosomonas sp. 4W30; (□) N. oceanus.

all of the cultures. However, as CO concentrations were increased above 150 μL/L (50 μL/L for N. oceanus) the ammonium oxidation rate leveled off. This indicates that there is more involved than the simple competitive inhibition indicated by previous workers (Drozd 1976; Suzuki et al. 1976). This effect can be explained by the cooxidation of CO which removes it from the role of an inhibitor and makes it a co-substrate for ammonium-monoxygenase.

This paper demonstrates that ammonium oxidizers are able to oxidize CO under a variety of environmental conditions, including low temperature and low pH. It also indicates that the interrelations between ammonium and CO are complex and in need of further experimentation to understand them. The fact that CO and NH_4^+-N (and CH_4) are present in nearly every environment, although the concentrations may be extremely low, points to the ammonium oxidizers playing a role in the cycles of CO and CH_4 in nature. It is unknown what the role of CO oxidation may be but, as already reported, CO oxidation may be a mechanism by which the cells can survive periods when their primary energy and growth substrate, ammonium, is limiting (Jones and Morita 1983b).

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