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METABOLISM OF CO AND CH_4 BY NITRIFIERS AND THE DETERMINATION OF THE NITRIFICATION RATE

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ABSTRACT - The nitrifying bacteria were found to survive 24 weeks in the absence of ammonium without decreasing their number or cell size. Because H_2, CO, and CH_4 are present in the marine environment, these substrates were investigated as a possible source of the energy of maintenance for the nitrifying bacteria. ^{14}CO and $^{14}CH_4$ were found to be oxidized by the nitrifiers. N-serve was found to inhibit the oxidation of CO. Using the nitrifiers' ability to oxide CO, a method for the determination of the nitrification rate was developed. The ability of nitrifiers to oxidize CO may play a significant role in the cycling of CO_2 in the marine environment. Whether CO and CH_4 oxidation play a role in the survival of nitrifiers in the absence of ammonium is currently being tested.

Key words: survival, CO oxidation, CH_4 oxidation, nitrifiers, CO_2 cycling.

RÉSUMÉ - Les bactéries nitrifiantes peuvent survivre 24 semaines en absence d'ammonium sans diminution de leur nombre ou de la taille des cellules. H_2, CO et CH_4 étant présents dans l'environnement marin, ces substrats sont envisagés comme une source éventuelle d'énergie de maintenance pour les bactéries nitrifiantes. On a montré que ^{14}CO et $^{14}CH_4$ sont oxydés par les nitrifiants et que N-Serve inhibe l'oxydation de CO. En utilisant la capacité des nitrifiants à oxyder CO, une méthode pour déterminer le taux de nitrification est développée. Dans l'environnement marin, la capacité des nitrifiants à oxyder CO peut jouer un rôle important dans le cycle du CO_2. D'autre part, le rôle que joue l'oxydation de CO et CH_4 dans la survie des nitrifiants en absence d'ammonium a été fréquemment testé.

Mots-clés: survie, oxydation de CO, oxydation de CH_4, nitrifiants, cycle de CO_2.

During our studies on starvation survival, the chemolithotrophic ammonium oxidizing bacteria were selected to determine if they could survive long periods of time in the absence of ammonium as the energy source. Since ammonia cannot be detected chemically in some water masses, the ability of the nitrifiers to survive this condition was viewed to be important, especially when one considers the cycling of nitrogen in the aquatic environment. Thus far, we have been able to demonstrate that, without the addition of ammonium to the starvation menstruum, the nitrifiers remained viable until the termination of the experiment (24 weeks). During this period, the nitrifiers did not decrease in numbers or size. Therefore, the question of the possibility of an alternative energy source(s) for cellular maintenance was addressed.

In assessing the possible energy sources available in the oligotrophic waters of the oceans, it became clear that the gases (CH_4, CO, and H_2) should not be overlooked as potential sources of maintenance energy, but not necessarily as a source of energy for growth and reproduction. Some of these gases are supersaturated in the ocean. The distribution and

concentrations of these gases are well documented not only in the marine environment, but also in soil and the atmosphere. The average concentration of CH_4 in the marine environment is 49.5 nl/l (Swinnerton and Lamontagne, 1974). On the other hand, carbon monoxide is also in nl/l quantities and it is formed in the aquatic environment by photochemical action on organic matter (Wilson, Swinnerton, and Lamontagne, 1980). Approximately 10 to 20 trillion g of CO/year is added to the atmosphere with the ocean as the main biogenic source of this gas (Seiler, 1978) ; whereas 122 to 237 trillion g of CH_4/year are biogenically produced (Seiler, 1984). The aquatic environment is a source of these gases to the atmosphere whereas the soil in a sink (Conrad, 1984). The quantities of both these gases should be sufficient for maintenance energy, especially when one considers the residence time of water masses.

Methane oxidizers were found to be capable of oxidizing ammonia (Hutton and ZoBell, 1949; O'Neill and Wilkinson, 1977; and Hyman and Wood, 1983) as well oxidizing carbon monoxide (Ferenci, 1974, Hubley, Mitton, and Wilkson, 1974; and Hutton and ZoBell, 1949), but Suzuki, Kwok, and Dular (1976) and Drozd (1946) could not demonstrate the oxidation of methane or carbon monoxyde by the nitrifying bacteria employing the oxygen uptake (manometric method). Yet there is a similary in NH_4 and CH_4 structure and both start with a cytochrome based mono-oxygenase which requires an unknown reducing equivalent and molecular oxygen. Both the oxidation of CH_4 and NH_4 start with the hydroxylation of the substrates (Ferenci, Strom, and Quayle, 1975 ; Swinnerton and Lamontagne, 1974 ; and Wilkinson, 1975).

Because of the dearth of NH_4 in most marine environments, the presence of the above mentioned gases in the marine environment, the similarity of structure between NH_4 and CH_4, and the mono-oxygenase required for metabolism of NH_4 and CH_4, we decided to investigate the possibility of the oxidation of methane by the nitrifiers employing radioactive $^{14}CH_4$ instead of the less sensitive manometric technique. It was found that all nitrifiers (Tab. 1) tested including *Nitrosococcus oceanus* and *Nitrosomonas europaea,* possessed the ability to oxidize CH_4. Most of the CH_4 was respired as $^{14}CO_4$; however, some of the CH_4 was incorporated into the cell (Jones and Morita, 1983a). The nitrite

Organism	Source	Methane oxidation rate (dpm)*		Ratio ^{14}C-cellular Material/$^{14}CO_2$
		$^{14}CO_2$ produced	^{14}C-cells	
Nitrosococcus oceanus	Marine, North Atlantic	15,051	293	0,019
Nitrosomonas europaea	Soil	596	60	0,101
Nitrosomonas marinus sp. strain C-15	Marine, South Pacific	233	13	0,055
Nitrosomonas sp. strain 1S10	Estuarine, Florida coast	924	194	0,210
Nitrosomonas sp. strain 2S0	Feshwater, Louisiana marsh	179	19	0,106
Nitrosomonsa sp. strain 6S30	Marine, Alaskan coast	2,285	27	0,021
Nitrosomonas sp. strain 9W0	Freshwater, Oregon marsh	782	85	0,109
Nitrosomonas sp. strain 3S30	Marine, Oregon coast	2,590	370	0,143
Nitrosomonas sp. strain 11W30	Marine, Oregon coast	2,983	51	0,017
Nitrobacter, sp. strain, Nb297	Marine	3	0	
Nitrospina gracilis	Marine, South Atlantic	0	1	
Nitrococcus mobilis	Marine, South Pacific	1	0	

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

* Activity is expressed as dpm of ^{14}C ml of standard inoculum^{-1} 24 h^{-1}.

oxidizers did not oxidize CH_4. Methane oxidation by *Nitrosococcus oceanus* occurred at 0.0119×10^{-4} mM, the lowest concentration employed in the study (Fig. 1). The presence of NH_4 stimulated the oxidation of $^{14}CH_4$ to $^{14}CO_2$ and cellular-^{14}C (Tab. 2). Increasing the carbonate concentration decreased the amount of $^{14}CH_4$-C incorporated into the cells

in cultures containing NH4 (Tab. 3) indicating that the organisms have the ability as chemolithotrophs to incorporate CO_2. The ability of nitrifiers to oxidize CH_4 was also confirmed by Hyman and Wood (1983).

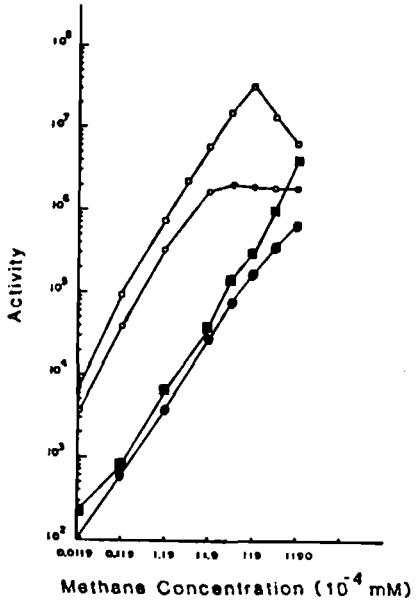

Figure 1 : Effects of methane concentration on rate of methane oxidation by *Nitrosococcus oceanus*. Activity is expressed as dpm of ^{14}C ml of standard inoculum^{-1} 24 h^{-1} multiplied by the dilution factor. Symbols : (○) $^{14}CO_2$ produced, 0,0 ppm of NH_4-N : (●) ^{14}C-cellular material, 0.0 ppm of NH_4-N : (□) $^{14}CO_2$ produced, 10 ppmp of NH_4-N : (■) ^{14}C-cellular material, 10 ppm of NH_4-N

Organism	Fraction	Methane oxidation rate (dpm)* with given addition						
		None	NH_4-N (10 ppm)	NO_2-N		NO_3-N		Yeast extract 10 ppm
				10 ppm	40 ppm	10 ppm	40 ppm	
Nitrosococcus oceanus	$^{14}CO_2$	15,558	76,020	39,416	38,973	34,776	32,337	61,395
	^{14}C-cells	240	575	43	ND	379	ND	931
Nitrosomonas europaea	$^{14}CO_2$	674	26,783	1,618	1,792	1,159	1,170	3,513
	^{14}C-cells	31	3,383	320	ND	286	ND	543

Table 2 : Effects of ammonium, nitrite, nitrate and yeast extract on methane oxidation by *Nitrosococcus oceanus* and *Nitrosomonas europaea*.

* Activity is expressed as dpm of ^{14}C ml of standard inoculum^{-1} 24 h^{-1}
ND. Not determined.

Organism	Ratio ^{14}C-cellular material/$^{14}CO_2$ at given carbonate concn (ppm)				
	0	10	50	200	500
Nitrosococcus oceanus	0.018	0.015	0.012	0.007	0.006
Nitrosomonas europaea	0.276	0.239	0.200	0.193	0.185

Table 3 : Effects of carbonate concentration on cellular incorporation of $^{14}CH_4$-C in the presence of 10 ppm of NH_4-N

Using ^{14}CO, the nitrifying bacteria were found to be capable of oxidizing CO at extremely low CO concentrations (Jones and Morita, 1983a). All the nitrifiers tested had the ability to oxidize CO but extremely little or none of the carbon monoxide was incorporated into the cells (Tab. 4). The rate of CO oxidation for *Nitrosomomas* sp. 4W30 is shown in Figure 2. During short incubations (up to approximately 4 h) the presence of NH_4 did not

stimulate the oxidation of CO and attempts to grow NH_4 oxidizers on CO as the sole source of carbon and energy failed.

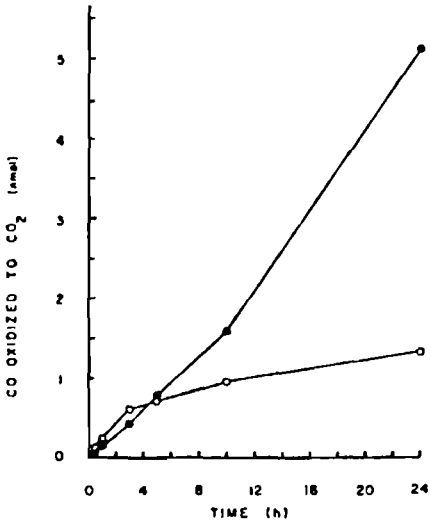

Figure 2 : Time course of CO oxidation by *Nitrosomonas* sp. 4W30. Oxidation is expressed as nanomoles per hour per 25 ml at a cell concentration of 10^6/ml.
Symbols:
○, 0.0 mg/L NH_4 N.
●, 10 mg/L NH_4 N.

Organism	Source	Carbon monoxide oxidation rate (DPM)*	
		$^{14}CO_2$ produced	^{14}C incorporated into cells
Nitrosococcus oceanus	Marine, North Atlantic	31565	0
Nitrosomonas europaea	Soil	36888	
N. sp. 4W30	Marine, Alaskan coast	110256	3
N. sp. 1S10	Estuarine, Florida coast	23431	10
N. sp 2S0	Freshwater, Louisiana marsh	28834	0
N. sp 6S30	Marine, Alaskan coast	36561	2
N. sp. 9WO	Freshwater, Oregon coast	17205	0
N. sp. 3S30	Marine, Oregon coast	21762	0
N. sp. 11W30	Marine, Oregon coast	17327	3
Nitrobacter sp. Nb 297	Marine	9	6
Nitrococcus mobilis	Marine, South Pacific	1	0

Table 4 : Carbon monoxide oxidation by nitrifiers in the absence of ammonium

* CO oxidation rate is expressed as DPM ^{14}C per 5 ml of cells and filters per 3 h.

The ability of nitrifieres to oxidize CO in the presence and absence of N-serve (2-chloro-6-(trichloromethyl) pyridine) was determined (Jones and Morita, 1984). It was found that 100 mg/l of N-serve would inhibit CO oxidation and this N-serve sensitive CO oxidation was related to the rate of ammonium oxidation and a detailed description of the method is given in Jones *et al.* (Jones, Morita, and Griffiths, 1984). The oxidation of CO is linear within the time range of test (3 to 6 h). Blockage of CO oxidation is complete with 100

mg/l of N-serve. Basically these calculations involve using ratios of NH_4 to CO oxidized in pure cultures and back calculation using the *in situ* NH_4 concentrations. The method should be used in a situation where the ammonium oxidizers rather than the methane oxidizers are the main group responsible for the oxidation of CO. By examining the ratio of CH_4 and CO oxidation one can determine which is major group responsible for the oxidation (CH_4 oxidizers have ratios between 0.380 to 1.87, ammonium oxidizers have a value between 0.0007 to 0.0428). Therefore, if the *in situ* NH_4 concentrations are known and the value for N-serve inhibited CO oxidation is known the rates of nitrification can be calculated, assuming that we have first order kinetics. This method has been applied not only to marine waters, but also to lakes and soil and represents a very sensitive method for rate measurements of nitrification that can reflect the activity at the *in situ* temperature, pH, and salinity.

Johnson, Davis and Sieburth (1983) suggested the CH_4 producers and CH_4 oxidizers play a significant role in CO_2 cycling. This could be through the cycling of the nonconservative gases, CH_4, CO, and H_2 and link between chemotrophy and phototrophy (Fig. 3). They also suggest that temporal TCO_2 changes appear to indicate the net direction of microbiological activity and join a body of literature showing dynamic variation in CO_2 and O_2 that exceed estimates by ^{14}C bottle assays of CO_2 fixation. We believe the contribution of the nitrifying bacteria assumes a greater role in the cycling of CO an CH_4 to CO_2 than the methane oxidizers. Carboxydobacteria do not play an important role in the oxidation of CO in the environment (Conrad and Weiler, 1982; and Conrad, Meyer, and Seiler, 1981).

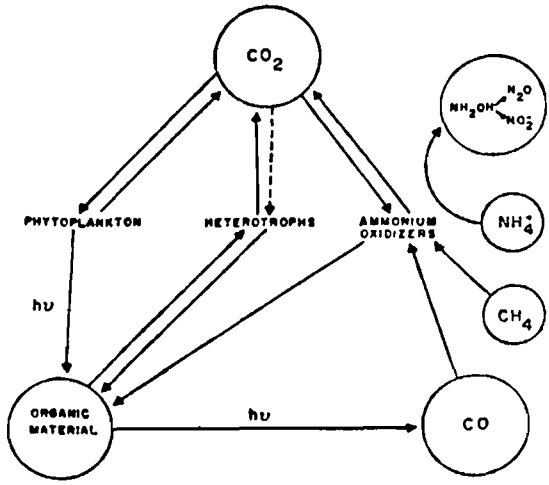

Figure 3 : Potential role of ammonium oxidizers in carbon monoxide, methane, carbon dioxide and ammonium cycling.

From all the studies dealing with microbes in the ocean, it appears that many microbes are smaller (ultramicrocells) than when cultured in the laboratory. This is also true with microbes in the soil (R. A. Olsen, personal communication). All forms of life seek energy and other nutrients for growth and metabolism but the availability of these materials is generally limited. As for microbes, the vast majority of them are not in environments where there is sufficient energy sources for growth and metabolism and there they exist in various degrees of starvation. Therefore the primary mode of bacteria in nature is a starvation mode, the «normal» states of most bacteria in nature. For some organisms, a

mechanism exists to satisfy their energy of maintenance, but not for growth and reproduction. Since all organisms survive to produce progeny, the energy of maintenance, if any is needed, must come from alternative energy sources as in the case of the nitrifiers. Some organisms may not require a maintenance energy source. We do not as yet address this «normal» state of bacteria when we study microbial ecology. For the nitrifying bacteria in the oceans we believe that the energy of maintenance for their survival lies in the ability to utilize alternative energy sources, mainly CO and CH_4. Conformation of this hypothesis is currently being tested in our laboratory.

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