Relationship between substrate concentration and oxidation of ammonium and methane in a stratified water column

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Abstract—Distributions and oxidation rates of methane and ammonium were investigated during two cruises in Saanich Inlet, British Columbia in late summer. Distributions of inorganic nutrients were related to oxygen distribution, exhibiting large gradients associated with the oxic–anoxic interface. The depth distributions of oxidation rates were also defined by the oxic–anoxic interface: ammonium oxidation occurred at variable rates (up to 121 nM day$^{-1}$) between the photic zone and the oxic–anoxic interface. Methane oxidation occurred throughout the oxic layer and increased near the interface. The possibility of interactions such as inhibition and competition between the two substrates, methane and ammonium, were investigated in kinetic experiments. Ammonium oxidation rate was independent of both ammonium and methane concentrations. Methane oxidation rates were linearly related to methane concentration, both in manipulation experiments, and in relation to ambient methane concentrations. There was no evidence of interaction between methane and ammonium as alternative substrates for methanotrophic and ammonium oxidizing populations, which were both present in the environment. In September, we observed a bolus-type mixing event, which introduced oxygenated deep water into the inlet beneath a wedge of anoxic, methane-rich water. This kind of event is probably important in determining the rate of methane loss, due to increased microbial oxidation at the boundaries of the anoxic wedge.

INTRODUCTION

Methane and ammonium are analogous compounds in some aspects of the environmental carbon and nitrogen cycles, respectively. Both are produced as end products of anaerobic metabolism and thus tend to accumulate in anoxic environments. Saanich Inlet is an intermittently anoxic fjord on Vancouver Island, British Columbia, Canada, where methane and ammonium accumulate in the stratified bottom waters on a seasonal basis. In surface waters, both compounds occur at trace levels ([CH$_4$] $\leq$ 30 nM, [NH$_4$] $\leq$ 100 nM), while below the oxic–anoxic interface, concentrations of both increase dramatically ([CH$_4$] $\geq$ 100 nM and [NH$_4$] $\geq$ 1 μM) (Ward et al., 1989b). The ratio [CH$_4$]/[NH$_4$] also varies with depth, being greater in the anoxic waters. This site was chosen for investigations of the possible interactions between methane and ammonium oxidation by naturally occurring bacterial assemblages in oxic, anoxic and interface environments.

Both ammonium and methane are used as chemolithotrophic substrates by select groups

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of aerobic bacteria, that possess unique abilities for autotrophic growth at the expense of these molecules. Ammonia, rather than ammonium ion, is actually the substrate for "ammonia"-oxidizers (Suzuki et al., 1974; Ward, 1987a), but we will refer to ammonium, because it is the ion actually measured in our nutrient assays. At the pH of seawater, $[\text{NH}_4^+]$ is estimated as $[\text{NH}_4^+] / 25$ (Stumm and Morgan, 1981). Both groups possess characteristic intracytoplasmic membrane systems which are assumed to be the sites of enzymes involved in lithotrophic metabolism. The intermediates and reaction sequences by which methane and ammonium are oxidized exhibit certain similarities:

$$\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NOH} \rightarrow \text{HNO}_2$$

$$\text{CH}_4 \rightarrow \text{CH}_3\text{OH} \rightarrow \text{HCOH} \rightarrow \text{HCOOH} \rightarrow \text{CO}_2.$$  

Laboratory experiments demonstrate that methanotrophs and ammonium-oxidizers are both capable of oxidizing both substrates to some degree (Ferenci et al., 1975; O'Neill and Wilkinson, 1977; Dalton, 1977; Hyman and Wood, 1983; Jones and Morita, 1983; Ward, 1987a). The kinetic behavior of cultured bacteria suggests that interactions between the two substrates, such as competition and inhibition, are possible at naturally occurring concentrations (Ward, 1990). Experiments were designed to identify possible interactions between methane- and ammonium-oxidizing populations and to evaluate the possible relationships between oxidation rates and substrate concentrations.

**METHODS**

Experiments were performed on two cruises to Saanich Inlet in August and September 1986. All samples were collected at one central up-inlet station [48°33.1'N, 123°32.7'W; Sta. B of Emerson et al. (1979)]. Samples were obtained in 5- or 30-liter Niskin bottles, equipped with teflon-coated springs and silicone O-rings. Bottles from low oxygen waters were plumbed for a slight overpressure of nitrogen gas during sampling.

**Methane concentrations**

Dissolved methane concentration was measured on 1-liter samples by a gas extraction technique using flame ionization gas chromatography [Herr and Barger (1978), as modified by M. I. Scranton, personal communication]. Repeated assays on subsamples from the same 30-liter Niskin yielded a coefficient of variation of 2.5%. We did not determine the detection limit, but concentrations less than 1 nM could be reproducibly assayed.

**Methane oxidation rates**

A thick-walled teflon tube was used to fill several 160-ml serum bottles (without introducing bubbles), each overflowed approximately 2 volumes, from Niskin samplers. Bottles were sealed without bubbles using solid black rubber stoppers and aluminum crimp seals. Radiolabeled methane (200 μl of gas, mostly hydrogen, containing 10.4 μl CH₄; 55 μCi μmol⁻¹; biogenically produced by the method of Daniels and Zeikus, 1983) was injected through the stopper using a second needle to accept overflow as the gas displaced liquid. Tracer additions formed a small bubble in the bottles, which were
vigorously mixed following tracer addition. Dissolved methane concentrations in the bottles were computed from the equation of Rudd and Hamilton (1975). Concentrations in the kinetic experiments varied (see below); concentration in standard time course experiments was about 0.3 μM. At each time point (approximately 1.4, 8 and 18 or 24 h for the time course experiments; 12 or 24 h for the kinetic experiments) incubations were processed for labeled particulates (by filtration through 47 mm diameter, 0.3 μm pore-sized Millipore filters) and labeled CO₂ (by acidification and capture on phenethylamine soaked filters, with a recovery efficiency of 80%). Efficiency of the ¹⁴CO₂ recovery was previously reported as 50% (using a shorter recovery period; Ward et al., 1987) but the standardized and improved method used in the present study has resulted in increased recovery with good reproducibility. Samples were shielded from light during all manipulations, and were incubated in running seawater incubators in the dark. Incubation temperature was 20°C in August and 13°C in September. Reported rates are not corrected for in situ temperature. Filters were assayed by liquid scintillation counting within one week. Oxidation rates were calculated from the linear regression of the time course data (α ≤ 0.05) or by assuming linearity in the single end-point kinetic experiments. Measured oxidation rates were found to be linearly dependent upon substrate concentration (see below), so results from standard time course experiments were corrected for altered substrate concentration due to label addition based on this linear relationship.

In addition to the standard time course experiments, we also performed single end-point experiments to investigate substrate dependence of the rate reaction. Twenty-two bottles were filled from a single Niskin sampler and sealed as above. Tracer additions were varied by adding different volumes of ¹⁴CH₄ and ¹²CH₄, resulting in incubation methane concentrations from 100 nM to 1 μM (computed as above). Incubation was terminated after 12 h for all treatments, and radioactivity in particulate and CO₂ fractions was analysed as above. In some experiments, the amount of ¹⁴CH₄ added was constant while ammonium concentration was varied (final concentrations 0.2–100 μM). All treatments in the methane oxidation kinetic experiments were performed in replicate.

**Ammonium oxidation**

Samples for ¹⁵N tracer experiments were collected in 30-liter Niskin samplers, which were pressurized with nitrogen gas during sampling as described above. Incubation experiments were carried out in 4-liter Pyrex bottles covered with light-tight black plastic. In order to preserve as closely as possible the in situ oxygen concentration, incubation bottles were filled and overflowed, directly from the Niskin sampler. The standard tracer addition was designed to increase ambient substrate levels by 0.2 μM [(¹⁵NH₄)₂SO₄, 99 atom-%]. Carrier solution, Na¹⁴NO₂, resulting in an increase over ambient levels of 0.25 μM, was added prior to incubation. After addition of label and carrier solutions (total volume ≤ 1.0 ml, cooled below sample temperature so that additions sank and were not displaced by the capping procedure), the bottles were sealed with plastic stoppers and electrical tape and mixed vigorously before being placed in the darkened, running seawater incubator.

Four bottles were incubated for each standard rate experiment. Incubation was terminated (one bottle each at approximately 2, 5, 10 and 24 h) by filtration through precombusted GF/F filters. Filters were dried and stored over desiccant. A portion of the filtrate was frozen and nitrogenous nutrient concentrations were determined in this
...sample by standard methods a few weeks after the cruise (see below). The remaining filtrate (about 4 liters) was extracted at sea by procedures described previously (Olson, 1981a; Ward et al., 1982). Nitrogen isotopic ratios of the extracted nitrite and of particulate material on the filters were determined on a JASCO emission spectrometer after combustion using a modified micro-Dumas procedure (Barsdate and Dugdale, 1965).

Initial atom-% of the substrate pool was calculated from the measured ambient ammonium concentration and standard tracer addition. The equations of Dugdale and Goering (1967) were used to calculate the amount of nitrite produced. Rates of ammonium oxidation were calculated from linear regressions of the time course data. Rates reported here are derived from experiments with at least three points defining the regression ($a \leq 0.05$). The coefficient of variation of isotope ratio determination on replicate subsamples of nitrite extracts averaged 6.7% in the data set from time course experiments. The limit of detection under these conditions is about 0.1 nM day$^{-1}$.

In addition to the standard time course experiments, we also performed single end-point experiments to investigate substrate dependence of the rate reaction. Fifteen bottles were filled from repeated casts to the same depth (oxygen and methane concentration were measured on each sample to ascertain that the same depth was being sampled). After tracer addition [(15NH$_4$)$_2$SO$_4$, 50 atom-%; up to final concentration of 62 $\mu$M in August, and 150 $\mu$M in September, total concentration achieved by combination of labeled and unlabeled substrate in equal amounts], methane (unlabeled) was added to some treatments. Gaseous additions were made by injection through a black serum stopper sealed into a clear silicone stopper, which was used to seal the bottles (without bubbles) for kinetic experiments, instead of plastic caps (see above). Incubation was terminated after 12 h for all treatments, and nitrite extracted as above. Average coefficient of variation for atom-% measurements in the kinetic experiments was 9.5%.

Water chemistry

Nitrate, nitrite (Strickland and Parsons, 1972) and ammonium (Koroleff, 1983) concentrations were measured in frozen subsamples after the cruise by standard methods. Oxygen concentration near the oxic-anoxic interface was measured using the colorimetric method of Broenkow and Cline (1969) on samples collected by syringe immediately after the bottles came on deck. Sulfide was assayed by the methylene blue method as described by Cline (1969). Temperature was measured using reversing thermometers on the Niskin samplers and thermometric depth corrections were computed.

RESULTS

Results describing the depth distribution of methane oxidation in August 1986 have been published (Ward et al., 1989b). The reader is referred to that paper for hydrographic, methane concentration and oxidation rate data from August 1986, which will not be repeated here.

Hydrographic characteristics

In September 1986, upon arrival at station, 20 samples evenly distributed over the water column were collected immediately so that the oxic-anoxic interface and other possible features of interest could be identified in aid of planning incubation experiments. The
dissolved methane profile (obtained within a few hours of arrival on station; Fig. 1) implied that a mixing event might have occurred in the interval since the August cruise to the same site. Distributions of oxygen (real time microcolorimetric method) and nutrients (obtained from analysis of frozen nutrient samples after the cruise) (Fig. 2) confirmed that a mixing event was indeed occurring during the September cruise. Methane concentration did not exhibit a subsurface maximum in September, as has usually been found in the inlet (Lilley et al., 1982). We attribute this to the weather; in August, we had detected such a feature (WARD et al., 1989b) at 30 m. At that time, the upper water column was temperature stratified, with a very warm (20°C) layer on top. By September, the surface water was much cooler and stormy weather prevailed.

The transition to anoxic waters below 120 m was signalled by the rapid increase in ammonium and methane concentrations, and the coincident decreases in nitrate and oxygen concentrations. However, these trends were reversed by 160 m, and the bottom water below about 180 m was similar in characteristics to the subsurface water just above the interface. From these observations, we infer the occurrence of a bolus mixing event (ANDERSON and DEVOL, 1973), in which cold surface water outside the inlet flows in over the sill and sinks, displacing the anoxic deep water. Over the period of 4 days, several casts to the interface region were assayed for dissolved methane. These profiles document the vertical displacement of the methane peak on the scale of 20 m (Fig. 1).

**Depth distribution of ammonium and methane oxidation**

Ammonium oxidation rates varied by about 6-fold over the oxygenated water column. Absolute rates (up to 120 nM day⁻¹ in August) were generally higher than observed in
coastal and oceanic waters previously studied. The August data (Fig. 3) cover the water column between the euphotic zone and the oxic-anoxic interface (about 140 m). In September, only three depths were sampled for standard rate measurements, and many more kinetic experiments were done (see below).

Methane oxidation rates also were measured at only three depths in September 1986 (Fig. 3). Rates were lowest at 30 m (two experiments) and highest at 112 m. The two 30-m experiments in September differed significantly from each other (see also kinetic experiment results below). The two experiments were separated by 4 days of stormy weather during a mixing event. Different populations may have been present at 30 m on different days due to displacement of the water column caused by influx of water over the outer sill. A more detailed depth distribution of methane oxidation in August 1986 can be found in Ward et al. (1989b).

Meanwhile oxidation rates are reported as total oxidation, the sum of label accumulated in particulate matter (presumably cells) plus that in CO₂. The fraction which appears in cells varied from 0.3 to 0.7 (one point outside this range). There was no systematic variation in the fraction which was incorporated into cells, either in relation to ammonium concentration (Fig. 4) or to total methane oxidation rate. The fraction incorporated into cells was correlated with [CH₄] within individual experiments (see Discussion) but...
this correlation was not evident in the pooled data (Fig. 4). The fraction was much more variable at low rates (greater variability among samples from 30 m) than at higher rates (nearly constant among samples at 112 m).

**Kinetics of methane and ammonium oxidation**

Methane oxidation rate increased in response to increased methane concentration up to 1 \( \mu \text{M} \). Rates increased on the order of 5-fold in response to an increase in methane concentration of about 10-fold. The data are in reasonable agreement with the Michaelis-Menten interpretation, i.e. double reciprocal plots are approximately linear (Fig. 5). Kinetic constants derived from these experiments are given in Table 1. Methane oxidation rates were not affected by ammonium concentration in the range from 0.2 to 10 \( \mu \text{M} \) nor at 100 \( \mu \text{M} \) NH\(_4^+\) (Fig. 6).

Two ammonium oxidation kinetic experiments were performed in August with water from just below the photic zone. No systematic variation in rate with substrate concentration was observed in two separate experiments covering overlapping substrate ranges from low (0.2–2.0 \( \mu \text{M} \)) to high (0.2–62 \( \mu \text{M} \)) ammonium levels (Fig. 7a and b, respectively). Error bars in the low ammonium range experiment include only error due to atom-% measurement. Including error from other sources would only increase the amount of overlap among samples. Similar experiments covering the ammonium concentration range up to 150 \( \mu \text{M} \) were performed in September at depths representing the bottom of the euphotic zone, mid-depth and the oxic–anoxic interface. No systematic variation in rate with substrate concentration was observed (Fig. 8). In both August and September, sets of replicate bottles from each ammonium range received varying amounts of methane. There was no discernible difference among treatments that received methane (up to 200 nM, measured in incubation bottles) and those that received no methane (Figs 7 and 8).
DISCUSSION

Two sampling dates one month apart demonstrated the seasonal nature of trace gas and nutrient distributions in Saanich Inlet, due to the transition from stratified summer conditions to autumn mixing conditions in the interim. The transition between oxygenated and anoxic waters was slightly shallower in September (about 120 vs 140 m). The thin warm surface layer of August had been replaced by a cooler surface layer in the process of mixing deeper with autumn weather, and the subsurface methane concentration maximum present in August was not detected in September. Methane, oxygen and nutrient concentration in the upper layer were, however, otherwise similar in August (WARD et al., 1989b) and September. The characteristics of the deep water were radically different between the two sampling dates, due to the intrusion of cold oxygenated water below the anoxic layer. Our observations are consistent with the bolus-type mixing suggested by ANDERSON and DEVL (1973), who drew their conclusions on the basis of nitrate distributions. We observed the effect of such a mixing event on methane distributions, and suggest that discrete mixing events like these have significant impact on the distribution and fate of methane originating in the anoxic sediments. We did not follow the methane

![Graph showing methane to ammonia ratio vs fraction in cells](image)

Fig. 4. Fraction of total methane oxidized (particulate plus CO2) that appeared in the particulate fraction. (a) Experiments in which [NH4+] was constant and [CH4] varied. (b) Experiments in which [CH4] was constant and [NH4+] varied. Different symbols represent samples from different depths: ○, 30 m, ambient [CH4] = 37 nM; △, 80 m, ambient [CH4] = 18.5 nM; ●, 112 m, ambient [CH4] = 306 nM; □, 30 m, ambient [CH4] = 39.0 nM.
distribution long enough to quantify its dispersion by mixing; however, it is likely that enhanced mixing would lead to enhanced oxidation as oxygenated and methane-rich waters are mixed at the edges of the distributions. ANDERSON and DEVOI, (1973) concluded that mixing at the lower boundary between the old bottom water and the bolus was minimal and that nitrate acted in a conservative manner on the time scale of the mixing event. We suspect that we observed the beginning of seasonal weakening or destruction of the anoxic boundary, eventually leading to mixing of methane with adjacent oxygenated waters. Seasonal mixing on this scale is analogous to autumn overturn in lakes. Also by analogy, this suggests that much of the methane which accumulates in the deep water over the summer stratification season is vented over a very short time in the autumn. Ventilation may mix methane up into contact with oxygenated waters where it is oxidized in situ, or, less likely, it could be directly vented to the atmosphere.

Ambient ammonium concentration was sampled only once in September, on the initial

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>V_{max} (nmol h^{-1})</th>
<th>K_s (nM)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.043</td>
<td>463</td>
<td>0.90</td>
</tr>
<tr>
<td>30</td>
<td>0.053</td>
<td>68</td>
<td>0.74</td>
</tr>
<tr>
<td>80</td>
<td>0.203</td>
<td>222</td>
<td>0.85</td>
</tr>
<tr>
<td>112</td>
<td>0.426</td>
<td>129</td>
<td>0.52</td>
</tr>
</tbody>
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vertical profile cast. Its distribution was very similar to that of methane, and clearly shows the accumulation of ammonium in the anoxic wedge. Ammonium oxidation experiments were concentrated in the oxygenated upper layer, including one time course and two kinetic experiments in the ammonium gradient at the upper edge of the anoxic wedge. The distribution of ammonium oxidation rates in August in Saanich Inlet differed from that found in oceanic sites, in that there was no regular decrease with depth below the euphotic zone. [The extent of the photic zone, estimated from the chlorophyll distribution (MAFRAI and VETTER, 1988), was taken to be about 30 m.] Lowest rates were found just above the oxic-anoxic interface, with one high point in the interface (130 m). This one point might suggest a rate maximum in a narrow interface region, but more complete coverage would be necessary to test this hypothesis. In September, the variability among replicate samples in kinetic experiments (Fig. 8) ensures that the oxidation rate in the interface region (112 m) was indistinguishable from rates measured at 30 and 80 m.

The August ammonium oxidation rate distribution is similar to the pattern found in the Cariaco Trench (WARD and KILPATRICK, submitted), where variable rates were found throughout the oxygenated layer. Highest rates detected in the Cariaco Trench were 48 nM day\(^{-1}\). Measured ammonium oxidation rates in Saanich Inlet (up to 120 nM day\(^{-1}\) in August, 40 nM day\(^{-1}\) in September) were in the same range as rates reported for most coastal and oceanic environments by similar methods. Highest rates detected in the Southern California Bight were 40 nM day\(^{-1}\) (WARD, 1987b) and in the eastern tropical North Pacific oxygen minimum zone about 60 nM day\(^{-1}\) (WARD and ZAFIRIOU, 1988). Rates reported for the subsurface water overlying the strong oxygen minimum zone off Peru exceeded those measured here, reaching nearly 300 nM day\(^{-1}\) (WARD et al., 1989a).

The kinetic behavior of the natural ammonium oxidizing population was consistent with previous observations in marine systems (OLSON, 1981b; WARD, 1986). That is, there was no apparent relationship between oxidation rate and experimentally altered ammonium concentration. Although cultured ammonium oxidizers sometimes exhibit conventional

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**Fig. 6.** Methane oxidation rate (nM h\(^{-1}\)) as a function of experimentally increased ammonium concentration. September.
Fig. 7. Kinetic experiments in August. Ammonium oxidation rate (nM h⁻¹) as a function of experimentally increased ammonium concentration. △, Zero methane; ●, 140 nM methane; □, 200 nM methane. Two separate experiments, both at 35 m. (a) Ammonium concentration in low range (0.2–2.0 μM). (b) Ammonium concentration extending to higher range (0.2–62 μM).

Fig. 8. Kinetic experiments in September. Ammonium oxidation rate (nM h⁻¹) as a function of experimentally increased ammonium concentration. △, Zero methane; ●, 140 nM methane; □, 200 nM methane.
Michaelis–Menten kinetics (Carlucci and Strickland, 1968; Suzuki et al., 1976; Hyman and Wood, 1983), such behavior is rarely observed in natural populations (Hashimoto et al., 1983). The ranges of ammonium concentration tested in the Saanich Inlet samples are the same concentrations that elicit a normal kinetic response in cultured Nitrosococcus oceanus, a marine ammonium oxidizing bacterium (Ward, 1987a). Lack of kinetic response by natural populations is not understood, but has several possible explanations: the most obvious possibility is that the bacterial strains studied in the laboratory are not representative of members of the natural population, and that the substrate ranges that elicit responses in the laboratory are inappropriate for natural populations. This hypothesis is very difficult to test at present due to limitations of the \(^{15}\text{N}\) methodology used to measure \textit{in situ} rates. If natural populations have effective saturation constants below our measurement capabilities, ammonium may not be the limiting component. Light and oxygen concentration are known to influence ammonium oxidation rate (Horrigan et al., 1981; Olson, 1981b; Ward, 1985, 1987a, b), but these variables were constant at physiologically acceptable levels in the kinetic experiments described here. Another alternative explanation derives from the autotrophic nature of ammonium oxidizers. This behavior is consistent with the characteristics of strict chemoautotrophs (Whittenbury and Kelly, 1977). Obligate autotrophs possess constitutive Calvin cycle pathways and constitutive chemolithotrophic pathways. The natural selection argument for the evolution of obligate autotrophy put forward by Whittenbury and Kelly (1977) states that enzymes in the constitutive autotrophic pathways are not inducible and do not respond to changes in substrate concentration on short time scales.

Although single samples exposed to varying ammonium concentrations do not generally exhibit a rate response, measured rates in different samples are sometimes found to be correlated with ambient ammonium concentration (Hashimoto et al., 1979; Ward et al., 1984; Ward, 1985). This was not the case in the present data; there was no correlation between ambient ammonium concentration and measured ammonium oxidation rate at the same depth. Ammonium concentrations were uniformly low except in the surface layer, where sporadic high values were found, and in the anoxic layer where ammonium concentration was inversely related to oxygen concentration.

The methane oxidation rates measured in September are consistent with results from a month earlier: low rates occurred in the surface waters, but increased approaching the oxic–anoxic interface (Fig. 3). A similar distribution, with a rate maximum at the oxic–anoxic interface, was found in the Cariaco Trench (Ward et al., 1987, 1989a). However, absolute methane oxidation rates in Saanich Inlet were much higher than observed in the Cariaco Trench (2 nM day\(^{-1}\) at the interface in Saanich Inlet vs 0.06 nM day\(^{-1}\) at the interface in the Cariaco Trench; Ward et al., 1987). This difference in rates between the two sites is consistent with the relative magnitude of ammonium oxidation at the sites (see above), and implies a generally more eutrophic status for Saanich Inlet. Observation of a seasonal mixing event in Saanich Inlet also suggests that the latter is a less stable system with a higher diffusive flux of methane into the oxygenated layer, and consequently, higher \textit{in situ} methane oxidation rates, at least on a seasonal basis.

It has been demonstrated that methanotrophs are capable of oxidizing ammonium to nitrite (Ferenci et al., 1975; O'Neill and Wilkinson, 1977; Dalton, 1977), and that ammonium oxidizers can oxidize methane to methanol (Hyman and Wood, 1983) or to carbon dioxide and cell material (Jones and Morita, 1983; Ward, 1987a). In some combinations of substrate concentrations, methane inhibits ammonium oxidation by
ammonium oxidizers (Suzuki et al., 1974; Hyman and Wood, 1983; Jones and Morita, 1983; Ward, 1987) and vice versa (Whittenbury et al., 1970; Ferenci et al., 1975). The rate of oxidation of the alternative substrate is generally much lower than oxidation of the primary substrate by each group and utilization of the secondary substrate for growth has not been demonstrated. Based on laboratory studies of the relative affinity of N. oceanus for CH₄ and NH₄ (Ward, 1990), it appears that marine nitrifiers might be able to utilize methane and ammonium simultaneously at concentrations at which they commonly occur in marine environments.

The interaction between methane and ammonium in pure cultures of nitrifiers has been interpreted as a form of competitive inhibition, and inhibition by methane was overcome at high ammonium concentrations (Suzuki et al., 1974; Hyman and Wood, 1983; Ward, 1987a). In the kinetic experiments performed in September, the ratio \([\text{CH}_4]/[\text{NH}_4]\) varied from <0.001 to 0.5; the upper end of this range was previously found to produce inhibition in cultured N. oceanus. Methane concentration had no discernible effect on ammonium oxidation rates in Saanich Inlet populations. The ambient concentration ratio in the water column ranged from 0.04 to >1.0. Natural populations may be acclimated to the presence of both substrates in variable ratios, and thus not subject to inhibition at the concentration ranges or ratios used in the experimental manipulations. Alternatively, as suggested above, the ammonium oxidizers studied in pure cultures are not representative of those comprising natural populations (because they are no longer adapted to very low substrate concentrations), and thus kinetic behavior of the former cannot be directly translated to understanding of the latter.

In contrast to ammonium oxidation, methane oxidation rate increased in response to experimentally increased methane concentrations (Fig. 5). This "normal" kinetic response by marine methanotrophs is typical for successful facultative autotrophs (Whittenbury and Kelly, 1977). Samples from different depths yielded different estimates of kinetic parameters, presumably due to the differences in in situ populations (Table 1). \(V_{\text{max}}\) increased with depth; the value at 112 m was 10-fold greater than at 30 m. This is in general agreement with the expected population abundance of methane oxidizing bacteria being greatest near the oxic-anoxic interface. The highest and lowest estimates for \(K_c\) were both derived from experiments in 30-m samples. Different populations may have been present at 30 m on different days due to displacement of the water column caused by the mixing event which occurred during the cruise.

When all methane oxidation rates above the oxic-anoxic interface from August (Ward et al., 1989a) and September are combined, there is a suggestion of a relationship between measured oxidation rate and in situ methane concentration (Fig. 9). The correlation explains 82% of the variance in the combined data from August and September. Other variables, such as oxygen concentration, may also be important in determining in situ oxidation rates. Anaerobic methane oxidation rates are very low relative to \([\text{CH}_4]\) (not included in Fig. 9).

There was no effect of ammonium concentration on methane oxidation rate (Figs 7 and 8). The variability in oxidation rate between depths exceeded that between concentrations within depths, implying that ammonium concentrations between 0.2 and 100 \(\mu\)M neither limited nor inhibited in situ methane oxidation rates. If methanotrophs were capable of utilizing ammonium as an electron donor for energy generation, it might be expected that the proportions of methane utilized for carbon assimilation and oxidized to \(\text{CO}_2\) would respond to the availability of the alternative electron donor. The fraction of methane label
Fig. 9. Relationship between methane oxidation rate (nM day\(^{-1}\)) and ambient methane concentration (nM). □, Measurements made in September 1986; △, ○ and ●, measurements made on three casts in August 1986 (Ward et al., 1989b).

which appeared in cells did not vary systematically with either total methane oxidation rate or ammonium concentration. A correlation with \([\text{CH}_4]/[\text{NH}_4]\) explained part of the variation in the fraction of methane going into cell material in experiments in which ammonium concentration was constant while methane concentration varied. For individual experiments, this correlation explained 60–90% of the variation (but was not evident in the pooled data from all experiments: Fig. 4). However, this relationship is wholly due to correlation with methane concentration, rather than the ratio of the two substrates. The implication is that relatively less methane is fixed into cells as methane concentration increases. Since there is a positive relationship between methane oxidation rate and \([\text{CH}_4]\), it further implies that increased oxidation rate in response to increased substrate concentration results from disproportionately faster energy production compared to cell production.

In aerobic samples from the August profile (including the surface through the interface), there was no relationship between fraction of label in cells and \([\text{CH}_4]\). This is consistent with the lack of correlation in the pooled samples from the September experiments (see above, Fig. 4) and implies that we do not really know what controls the proportionation between particulate and CO\(_2\) production from methane oxidation. In three rate measurements from anoxic waters (\([\text{CH}_4] \geq 250 \text{nM}\)), however, the fraction of label in cells was significantly reduced (about 30% compared to a low of 50% for all other samples). These samples were inconsistent with the relationship between oxidation rate and \([\text{CH}_4]\) (Fig. 9), implying that different mechanisms may apply to the anaerobic process. Anaerobic methane oxidation was much slower in the presence of equivalent methane concentrations than was aerobic oxidation, but the kinetics of the anaerobic process were not investigated.

CONCLUSIONS

Several indirect means were used to evaluate the relationship between ammonium and methane oxidation in Saanich Inlet. Although the distribution of ammonium and methane show certain similarities, their oxidation rates are not obviously correlated. Experiments
designed to detect inhibition or enhancement of oxidation rates in the presence or absence of alternative substrates detected no effect of ammonium on methane oxidation rate nor of methane on ammonium oxidation rate. These results imply that ammonium and methane are not competitive substrates in this system (at least not over the concentration ranges tested), and that the bacterial populations which perform the two reactions are probably distinct from each other. It also implies that kinetic behavior observed in laboratory culture is not always a viable basis upon which to predict behavior of natural systems. This dichotomy is also demonstrated in the fact that ammonium oxidizers in culture exhibit normal Michaelis–Menten kinetics with respect to ammonium, and this effect is not observed in most marine systems. Either laboratory strains have evolved away from those in the environment from which they were originally derived, or our laboratory species are not representative of the organisms which dominate the environmental system studied here. Further experimentation at truly tracer substrate concentrations are necessary to exclude the possibility that natural populations do exhibit competition or inhibition by alternative substrates at natural substrate levels.

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