JOURNAL OF BACTERIOLOGY, Sept. 1990, p. 5368–5373
0021-9193/90/095368-06$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 172, No. 9

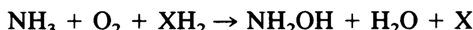
Oxidation of Monohalogenated Ethanes and *n*-Chlorinated Alkanes by Whole Cells of *Nitrosomonas europaea*

MADELINE E. RASCHE,† ROSALYND E. HICKS, MICHAEL R. HYMAN,† AND DANIEL J. ARP†*
Department of Biochemistry, University of California, Riverside, California 92521

Received 21 December 1989/Accepted 18 June 1990

We have investigated the substrate specificity of ammonia monooxygenase in whole cells of the nitrifying bacterium *Nitrosomonas europaea* for a number of aliphatic halogenated hydrocarbons. To determine the effect of the halogen substituent and carbon chain length on substrate reactivity, we measured the rates of oxidation of the monohalogenated ethanes (fluoroethane, chloroethane, bromoethane, and iodoethane) and *n*-chlorinated C_1 to C_4 alkanes by whole cells of *N. europaea*. For monohalogenated ethanes, acetaldehyde was the major organic product and little or none of any of the alternate predicted products (2-halogenated alcohols) were detected. The maximum rate of haloethane oxidation increased with decreasing halogen molecular weight from iodoethane to chloroethane (19 to 221 nmol/min per mg of protein). In addition, the amount of substrate required for the highest rate of haloethane oxidation increased with decreasing halogen molecular weight. For the *n*-chlorinated alkanes, the rate of dechlorination, as measured by the appearance of the corresponding aldehyde product, was greatest for chloroethane and decreased dramatically for chloropropane and chlorobutane (118, 4, and 8 nmol of aldehyde formed per min per mg of protein, respectively). The concentration profiles for halocarbon oxidation by ammonia monooxygenase showed apparent substrate inhibition when ammonia was used as the reductant source. When hydrazine was used as the electron donor, no substrate inhibition was observed, suggesting that the inhibition resulted from reductant limitation.

Nitrosomonas europaea is an obligate, chemolithotrophic, nitrifying bacterium which derives all of its energy for growth from the oxidation of ammonia to nitrite (23). The oxidation of ammonia in *N. europaea* is initiated by the enzyme ammonia monooxygenase (AMO) through a reductant-dependent process, as shown in the following equation (23):



$$NH_3 + O_2 + XH_2 \rightarrow NH_2OH + H_2O + X$$

In vivo, the reductant for AMO-catalyzed reactions is provided by the oxidation of hydroxylamine to nitrite by hydroxylamine oxidoreductase, as shown by the following equation (23):

$$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

In addition to oxidizing ammonia, whole cells of *N. europaea* are capable of cooxidizing a broad range of hydrocarbon substrates, including alkanes and alkenes (4, 8, 10, 12, 21), methanol (20), benzene (9), phenol (9), CO (14), and halogenated hydrocarbons (halocarbons) (1, 11, 19). These oxidations are mediated by AMO. One group of alternative AMO substrates which are of considerable interest are the aliphatic halocarbons. Many of these compounds are industrial chemicals recognized by the U.S. Environmental Protection Agency as priority pollutants (22). At present, there is concern about the presence of these chemicals in domestic water supplies since some of these compounds are potential human carcinogens (2, 16). The ability of *N. europaea* to degrade halocarbons (1, 11, 19) clearly points to their possible use in bioremediation schemes, in which the activity of these widely distributed bacteria can be stimulated by the addition of the cooxidation substrates, ammonia and O_2.

However, if *N. europaea* and related autotrophic nitrifiers are to fulfill this role in biodegradation, it is important that the products of halocarbon oxidation as well as the factors which limit halocarbon oxidations be established. This includes determination of the range of substrates oxidized, the relative rates of their cooxidation, and the nature of the oxidation products. Two factors which are likely to limit the range of halocarbon oxidations are the identity of the halogen substituent and the length of the hydrocarbon chain. In the present study, we have compared the initial rates of substrate oxidation for a series of monohalogenated ethanes and *n*-chlorinated alkanes by measuring the rates of product formation. This quantitative approach has enabled us to directly compare the effects of systematic changes in substrate composition on the ability of *N. europaea* to degrade aliphatic halocarbons.

MATERIALS AND METHODS

Growth and preparation of cells. *N. europaea* ATCC 19718 was grown in 1-liter batch cultures, harvested by centrifugation, and suspended in buffer (2 mM $MgCl_2$, 50 mM sodium phosphate [pH 7.7]) at approximately 0.2 g (wet weight) per ml (9). Cells could be stored on ice for up to 24 h with little or no loss of activity, but were typically used within a few hours of harvesting. The data presented in each figure or table were derived from a single bacterial preparation. Therefore, the data are directly comparable. However, some differences in the rates of alternate substrate oxidation were observed between individual bacterial preparations.

Substrate oxidation by AMO. For substrate oxidation assays we used stoppered serum vials (9.4 ml) containing 900 μl of buffer (described above) with 10 mM NH_4Cl and various concentrations of halocarbon substrates. Chloromethane (Liquid Carbonic, Chicago, Ill.), chloroethane (Eastman Kodak Co., Rochester, N.Y.), and fluoroethane (Columbia Organic Chemical Co., Camden, S.C.) were delivered as gases. The molar quantity added was estimated

* Corresponding author.
† Present address: Laboratory for Nitrogen Fixation Research, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

by assuming that the compounds behave as ideal gases. The remaining substrates (Aldrich Chemical Co., Inc., Milwaukee, Wis.) were added from saturated solutions in buffer at approximately 0°C for bromoethane and at room temperature (approximately 25°C) for other substrates. The amount of each substrate added from a saturated solution was estimated from solubility tables (7).

Reactions were initiated by the addition of resuspended cells (100 μl), and vials were incubated in a shaking water bath at 30°C and 160 cycles/min. Reactions were terminated after 10 min by the addition of thiourea (a noncompetitive AMO-specific inhibitor) (6) to 50 μM. Liquid samples (5 μl) were analyzed by using a gas chromatograph (model GC-8A; Shimadzu, Kyoto, Japan) equipped with a Porapak Q column (1/8 by 16 in. [0.3 by 40.6 cm]; Waters Associates, Inc., Framingham, Mass.) and a flame ionization detector interfaced to an integrator (model C-R3A; Shimadzu). For time course experiments with fluoroethane and chloroethane, a 5-μl sample of the reaction mixture was removed 5, 10, and 15 min after initiating the assays. For other substrates, a 100-μl portion of the reaction mixture was removed at each time point and added to 2 μl of 5 mM thiourea in stoppered 1-ml glass vials to terminate the reaction. Acetaldehyde, propionaldehyde, butyraldehyde, and some alcohol products were identified by coelution with known standards. Measurement of the partitioning of the products between the gas and liquid phases showed that acetaldehyde and the alcohol products remained primarily in the liquid phase. Only 3% of the acetaldehyde and none of the halogenated alcohols were detected in the gas phase.

Formaldehyde produced upon oxidation of chloromethane was measured by a coupled assay with formaldehyde dehydrogenase from *Pseudomonas putida* (Sigma Chemical Co., St. Louis, Mo.), using a modified procedure of Ogushi et al. (17). After termination of the AMO reaction with thiourea, cells were removed by microcentrifugation (15,000 × *g* for 2 min), and 200 μl of supernatant was combined with 900 μl of 60 mM sodium carbonate (pH 8.9) and 100 μl of 12 mM NAD^+. Formaldehyde dehydrogenase (0.04 U in 10 μl of 10 mM sodium phosphate buffer [pH 6.8]) was added to the mixture, and the change in A_{340} due to the formaldehyde dehydrogenase-catalyzed production of NADH (ε = 6.22 mM^{-1} cm^{-1}) in the presence of formaldehyde was measured. A standard curve for quantifying formaldehyde production in the presence of *N. europaea* cells was generated by adding different amounts of 37% formaldehyde solution to 1 ml of reaction mixture containing cells and processing the samples as described above.

Toxicity test for chloroethane and acetaldehyde. To determine whether high concentrations of either chloroethane or its major oxidation product, acetaldehyde, were toxic to *N. europaea*, we incubated cells in reaction vials in the absence of NH_4Cl and in the presence of either chloroethane (22 μmol) or acetaldehyde (1 mM). After 10 min, the cells were sedimented, washed three times in buffer (2 mM $MgCl_2$, 50 mM sodium phosphate [pH 7.7]), and resuspended to the original volume. A 50-μl portion of cell suspension was added to buffer (1.6 ml) in the chamber of an O_2 electrode. The O_2 uptake rates coupled to ammonia oxidation were measured in the presence of 10 mM NH_4Cl. Rates of O_2 uptake were compared for cells incubated for 10 min with no addition or in the presence of 22 μmol of chloroethane or 1 mM acetaldehyde.

Protein determinations. The protein content was determined by using the biuret assay (5) after solubilizing the cells in 3 M NaOH for 60 min at 60°C. Bovine serum albumin was used as the standard.

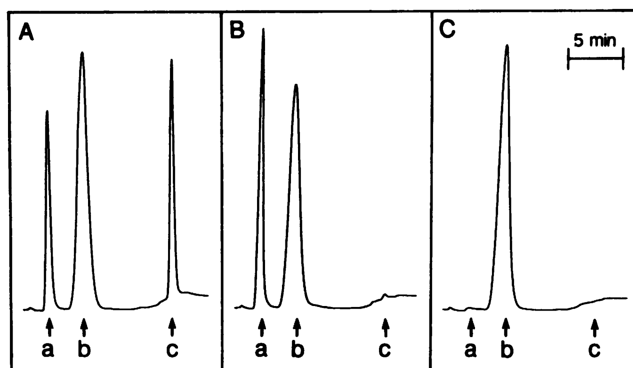


FIG. 1. Chromatograms showing the oxidation of chloroethane to acetaldehyde and chloroethanol by *N. europaea*. (A) Separation of acetaldehyde (a), chloroethane (b), and chloroethanol (c) by gas chromatography. The reference solution consisted of 1 ml of buffer with acetaldehyde (1.2 mM), chloroethanol (0.7 mM), and chloroethane (9 μmol added to the vial). Liquid samples (5 μl) were injected onto a Porapak Q column (O.3 by 40.6 cm), and chromatograms were started immediately after sample injection. The column temperature was initially set at 60°C and, after 5.5 min, raised to 150°C at a rate of 32°C/min. (B) Chloroethane oxidation to acetaldehyde and chloroethanol in the presence of whole cells of *N. europaea*. Cells were incubated for 10 min with 9 μmol of chloroethane and 10 mM NH_4Cl, as described in Materials and Methods. In this experiment, cells (1.6 mg of protein) produced 1.7 μmol of acetaldehyde and 0.030 μmol of chloroethanol. (C) Pretreatment of cells with thiourea. Cells were treated with 500 μM thiourea for 10 min and were then incubated with 9 μmol of chloroethane and 10 mM NH_4Cl. No acetaldehyde or chloroethanol was detected (chloroethanol detection limit, 85 pmol).

RESULTS

Organic products of monohalogenated ethane oxidation. By analogy to the mechanism proposed for bromoethane oxidation by AMO in *N. europaea* (11), two organic products, acetaldehyde and the 2-haloethanol, may result from the oxidation of other monohalogenated ethanes. We observed that both acetaldehyde and 2-chloroethanol were produced from the oxidation of chloroethane by whole cells of *N. europaea* (Fig. 1). However, acetaldehyde was clearly the major product of chloroethane oxidation (1,870 ± 290 nmol), accounting for more than 98% of the total product, whereas 2-chloroethanol (28 ± 6 nmol) represented only 1 to 2% of the product. The identity of the peak coeluting with 2-chloroethanol was further characterized by coelution with 2-chloroethanol on a Porapak T column (1/8 by 24 in. [0.3 by 61 cm]; Waters Associates). The amount of substrate lost (1,777 ± 520 nmol) was similar to the total amount of products detected (1,898 nmol of product), suggesting that no additional products were formed in significant quantities. Neither acetaldehyde nor 2-chloroethanol was detected when active cells were pretreated with the AMO inhibitor thiourea (Fig. 1C), supporting a role for AMO in chloroethane oxidation by *N. europaea*.

Fluoroethane, bromoethane, and iodoethane were also tested as substrates for AMO. In each case, acetaldehyde was produced, but none of the corresponding halogenated alcohols were produced in detectable quantities (data not shown). The detection limits for fluoroethanol, chloroethanol, bromoethanol, and iodoethanol were 230, 85, 150, and 85 pmol, respectively.

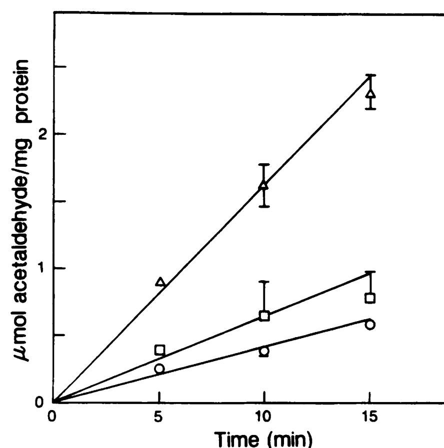


FIG. 2. Time course of chloroethane oxidation to acetaldehyde by *N. europaea*. Assays were conducted as described in the text, in the presence of 2 (□), 9 (△), or 22 (○) μmol of chloroethane. Each point represents the average of triplicate determinations ± standard deviation.

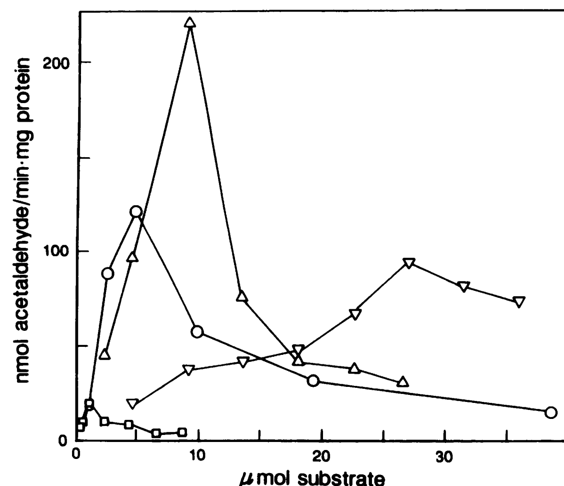


FIG. 3. Effect of substrate concentration on the rate of monohalogenated ethane oxidation by *N. europaea*. Cells were incubated for 10 min in the presence of 10 mM NH_4Cl and various amounts of iodoethane (□), bromoethane (○), chloroethane (△), or fluoroethane (▽). Data represent the average of triplicate determinations. The following values were obtained for partitioning of the substrates into the liquid phase when the amount of haloethane supporting the greatest rate of acetaldehyde production was added to the vial: iodoethane, 22%; bromoethane, 21%; chloroethane, 15%; and fluoroethane, 12%.

Determination of initial rates of substrate oxidation. To compare the reactivities of the different halocarbon substrates, it was necessary to determine the period over which the oxidation rates were constant. Over the range of chloroethane concentrations used in this experiment (2 to 22 μmol added to reaction vials), the rate of acetaldehyde production was constant for at least 10 min (Fig. 2). The rate of aldehyde production from all other substrates was also constant during the first 10 min (data not shown).

Effect of halogen substituent on the oxidation rate of monohalogenated ethanes. When *N. europaea* was incubated in the presence of various amounts of the haloethane substrates with ammonia as the reductant source, the rate of alternate substrate oxidation increased with increasing substrate concentration to an optimal substrate level and then decreased at higher concentrations (Fig. 3). The most reactive substrate for oxidation by AMO was chloroethane at its optimal concentration of 9 μmol added to the vial (Fig. 3; Table 1). The maximum observed rate of acetaldehyde production decreased with increasing halogen molecular weight for bromoethane and iodoethane. The maximum rate of fluoroethane oxidation was lower than that observed for chloroethane (Fig. 3; Table 1). Interestingly, the optimal substrate level for monohalogenated ethanes was shifted to progressively higher values with decreasing molecular weight of the halogen substituent from iodoethane to fluoroethane (Fig. 3). This trend extended even to ethane, for which the optimal level of substrate was 89 μmol, compared with 27 μmol for fluoroethane (data not shown).

Acetaldehyde production for all substrates tested was inhibited by pretreatment with the AMO inhibitors thiourea (6) and acetylene (13) (Table 1). The small amount of acetaldehyde detected when cells were incubated with bromoethane under conditions which inhibit other AMO-catalyzed oxidations may have resulted from nonbiological hydrolysis of bromoethane (11).

The oxidations of alternate substrates by AMO in the presence of ammonia are regarded as cooxidations. This is because the hydroxylamine generated by ammonia oxidation is subsequently oxidized by hydroxylamine oxidoreductase, which returns electrons to AMO to support further oxidation reactions. Alternate substrates for AMO do not generate an equivalent source of reductant, and therefore their oxidation requires the concomitant oxidation of ammonia or the presence of an exogenous source of reductant. The possibility that the halocarbon oxidations described above were catalyzed by a reductant-requiring enzyme other than AMO can be tested by inhibiting AMO with thiourea and providing an alternate source of reductant, such as hydrazine, to the cells. When cells were pretreated with thiourea and subsequently incubated with haloethane, ammonia, and hydrazine, no haloethane oxidation was observed (Table 1). This result provides further evidence that AMO is the enzyme which catalyzes the oxidation of the halocarbon substrates.

Effect of carbon chain length on the reactivity of *n*-chlorinated alkanes. Because chloroethane was the most reactive haloethane substrate for oxidation by AMO, we used *n*-chlorinated alkanes (chloromethane, chloroethane, chloropropane, and chlorobutane) to study the effect of carbon

TABLE 1. Oxidation of monohalogenated ethanes by *N. europaea*

Substrate (amt)[a]	Rate of acetaldehyde formation (nmol/min per mg of protein) with[b]:			
	10 mM NH_4Cl	22 μmol of C_2H_2	50 μM thiourea	50 μM thiourea and 600 μM hydrazine
Fluoroethane (26.8 μmol)	93 ± 22	0	0	0
Chloroethane (8.9 μmol)	221 ± 40	0	0	0
Bromoethane (4.8 μmol)	122 ± 32	1	0	1
Iodoethane (1.1 μmol)	19 ± 2	0	0	0

[a] Amount of substrate which supported the greatest rate of product formation (see Fig. 3).

[b] Data represent the average of triplicate determinations ± standard deviation where indicated. The standard deviations in this table are indicative of a single bacterial preparation.

TABLE 2. Oxidation of *n*-chlorinated alkanes by *N. europaea*

Substrate (amt)	Calculated substrate depletion (nmol/10 min)[a]	Product(s) (nmol formed in 10 min)	Rate of acetaldehyde formation (nmol/min per mg of protein)[b] with:			
			10 mM NH_4Cl	22 μmol of C_2H_2	50 μM thiourea	50 μM thiourea and 600 μM hydrazine
Chloromethane (4.5 μmol)	839 ± 138	Formaldehyde (801 ± 98)	51 ± 6.1	0	0	<0.1
Chloroethane (8.9 μmol)	1,777 ± 520	Acetaldehyde (1,870 ± 290)	118 ± 18.1	0	0	0
		2-Chloroethanol (28 ± 6)	2 ± 0.4			
		Both (1,898)	120			
Chloropropane (3.2 μmol)	722 ± 90	Propionaldehyde (60 ± 5)	4 ± 0.3	0	0	<0.1
		3-Chloro-1-propanol (346 ± 46)	22 ± 2.9			
		1-Chloro-2-propanol (189 ± 26)	12 ± 1.6			
		All three (595)	38			
Chlorobutane (4.0 μmol)	733 ± 126	Butyraldehyde (120 ± 19)	8 ± 1.2	0	0	0
		4-Chloro-1-butanol[c] (510 ± 20)	32 ± 1.2			

[a] Substrate depletion was estimated from the decrease in peak area of each substrate after 10-min incubations with *N. europaea*. Data represent the average of triplicate determinations ± standard deviation.
[b] Amount of substrate which supported the greatest rate of product formation.
[c] An additional product was detected by gas chromatography in substantial quantity but was not identified.

chain length on the reactivity of aliphatic halocarbons. The highest rate of dechlorination for the *n*-chlorinated C_1 to C_4 alkanes (as measured by the appearance of the corresponding aldehyde products) was observed for chloroethane (Table 2). Chloromethane showed a lower maximum rate of aldehyde production than chloroethane, and both chloropropane and chlorobutane showed dramatically lower rates of dechlorination than chloroethane. Thiourea and acetylene inhibited aldehyde formation, and hydrazine did not restore activity to thiourea-treated cells (Table 2).

An aldehyde was the major product of chloroethane oxidation by AMO (Table 2; Fig. 1). In contrast, the major products of chloropropane oxidation were the alcohols 1-chloro-2-propanol (61% of the total products) and 3-chloro-1-propanol (29%), followed by propionaldehyde (10%) (Table 2). Similarly, with chlorobutane more 1-chloro-4-butanol was formed than butyraldehyde. Standards for the predicted 2-ol and 3-ol products of chlorobutane oxidation were not commercially available, and therefore these compounds could not be identified and quantified. However, in the presence of active cells, an unidentified peak of approximately the same area as 4-chloro-1-butanol eluted before 4-chloro-1-butanol on the Porapak Q column, indicating a lower boiling point. This feature is consistent with the possibility that the additional product peak is either 1-chloro-2-butanol or 1-chloro-3-butanol.

Decline in oxidation rate at high substrate concentrations. When ammonia was used as the reductant, the concentration profile of halocarbon oxidation exhibited apparent substrate inhibition (Fig. 3). To determine whether altering the supply of ammonia would affect the shape of the substrate concentration profile, we incubated cells with a range of chloroethane concentrations in the presence of various amounts of NH_4Cl. Under standard conditions (10 mM NH_4Cl), a maximum rate of 332 nmol/min per mg of protein was obtained with 9 μmol of chloroethane (Fig. 4). Reducing the NH_4Cl concentration to 1 mM resulted in a shift of the optimum down to 4.5 μmol of chloroethane, with an oxidation rate that was only about 19% of the maximum rate with 10 mM NH_4Cl. In the absence of ammonia the rate of oxidation was approximately 30 nmol/min per mg of protein at all substrate concentrations tested, presumably driven by endogenous reductant (8). In contrast, increasing the NH_4Cl concentration from 10 to 20 mM and 40 mM did not affect the optimal substrate concentration and increased the maximum rate by only 10% over the rate with 10 mM NH_4Cl. This result indicates that a factor other than exogenous NH_4Cl concentration (perhaps transport of ammonia to AMO) may limit the ability of the cell to derive reductant from ammonia.

Two other factors which could have produced the apparent substrate inhibition were (i) toxicity of the halocarbon substrate or its oxidation product and (ii) reductant limitation due to binding of the alternate substrate to all of the AMO in an enzyme-substrate complex and thereby prevention of ammonia binding and oxidation. To determine whether high concentrations of either the substrate or product were toxic to *N. europaea*, we incubated cells with a supraoptimal amount of chloroethane or with acetaldehyde in the absence of NH_4Cl. After washing the cells, we measured the O_2 uptake rates in the presence of NH_4Cl. No differences in the rates of O_2 uptake were detected between control cells incubated without treatment (769 ± 41 nmol/min per mg of protein), acetaldehyde-treated cells (802 ± 22 nmol/min per mg of protein), and chloroethane-treated cells (795 ± 38 nmol/min per mg of protein). This result indicates

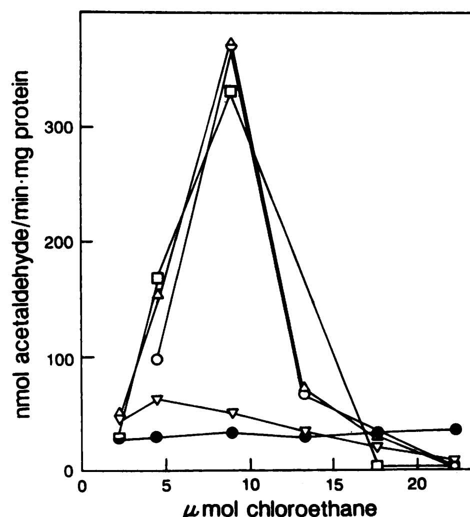


FIG. 4. Effect of ammonia concentration on the concentration profile of chloroethane oxidation. Cells were incubated with various amounts of chloroethane in the presence of 0 (●), 1 (▽), 10 (□), 20 (○), or 40 (△) mM NH_4Cl and were assayed as described in the text.

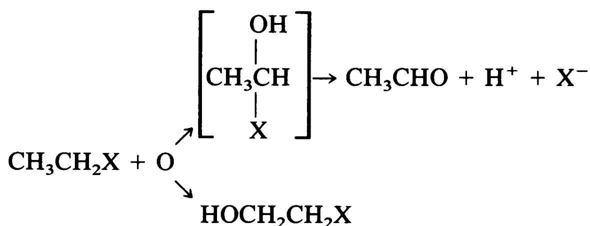
that over the time course of the assay, neither the substrate nor the product was toxic to the cells.

We tested the possibility that the decline in the halocarbon oxidation rate was due to reductant limitation by adding hydrazine to assays containing a supraoptimal amount of chloroethane (22 μmol) in either the presence or absence of NH_4Cl. The chloroethane oxidation rate in the presence of 5 mM hydrazine (122 ± 2 nmol/min per mg of protein) and 10 mM NH_4Cl plus 5 mM hydrazine (104 ± 4 nmol/min per mg of protein) was approximately sixfold higher than the rate with 10 mM NH_4Cl alone (16 ± 1 nmol/min per mg of protein). The higher rates were similar to that obtained with the optimal concentration of chloroethane in the presence of 10 mM NH_4Cl (97 ± 8 nmol/min per mg of protein). This result supports the hypothesis that at supraoptimal chloroethane concentrations the cells were reductant limited.

DISCUSSION

We investigated the ability of whole cells of *N. europaea* to oxidize a series of monohalogenated ethanes (fluoroethane, chloroethane, bromoethane, and iodoethane) and the *n*-chlorinated C_1 to C_4 alkanes. These series provided an excellent opportunity to determine the effects of the halogen substituent and carbon chain length on the rates and products of halocarbon oxidation by AMO. Identification of the oxidation products was of interest for two reasons: first, in biodegradation schemes, it is necessary to consider whether the resulting products would be more toxic or recalcitrant in the environment than the starting materials, and second, the relative distribution of the products could provide insight into the mechanism of halocarbon oxidation by AMO.

By analogy to the mechanism proposed for bromoethane (11), two organic products are possible from the oxidation of monohalogenated ethanes. Acetaldehyde would result from oxidation of the substituted carbon, while the halogenated alcohol would be produced from a C_2 attack, as illustrated in the following equation:



Because the major oxidation product for all four haloethanes was acetaldehyde (Fig. 1; Table 1), the halogenated carbon was clearly the preferred position for oxidation. Inhibition of the oxidations by thiourea and acetylene and failure of hydrazine to restore activity to AMO-inhibited cells (Table 1) suggested that AMO was the enzyme which catalyzed the halocarbon oxidations.

With the monohalogenated ethanes, the decreased maximum oxidation rate with increasing halogen molecular weight (from chloroethane to iodoethane) (Fig. 3) may reflect a decreasing ability of the enzyme to turn over the substrates containing the larger-molecular-weight halogens. The oxidation of substituted methanes (chloromethane, bromomethane, and iodomethane) by the soluble methane monooxygenase of *Methylococcus capsulatus* Bath (3) followed a similar trend of decreasing activity with increasing halogen molecular weight. However, although no oxidation of iodomethane was detected with *M. capsulatus* Bath (3), oxidation of iodoethane by *N. europaea* was observed and resulted in deiodination to form acetaldehyde (Table 1). This is the first report of the oxidation of an iodinated (as well as a fluorinated) compound by a nitrifying bacterium.

A second trend noted in the oxidation of the monohalogenated ethanes was a shift in the optimal substrate concentration to higher values with decreasing molecular weight of the halogen substituent (Fig. 3; Table 1). These effects of the halogen substituent on substrate oxidation by AMO can be analyzed in terms of two factors: the affinity of AMO for the halocarbon and the rate of turnover of the substrate by AMO. With low levels of substrate added, such that formation of the enzyme-substrate complex is most probably rate limiting, the rates of oxidation of iodoethane, bromoethane, and chloroethane are similar, which indicates that they have similar affinities for the enzyme. In contrast, fluoroethane gives a lower rate of oxidation at low substrate concentrations, which indicates a lower affinity. At higher halocarbon substrate concentrations, the turnover rate for each compound becomes the limiting factor. Therefore, iodoethane appears to have a high affinity for AMO, but is not turned over rapidly by the enzyme. This results in a low maximum rate and low optimal concentration. The lower optimal concentration occurs because the longer lifetime of the enzyme-iodoethane complex effectively limits the binding of ammonia, thereby limiting the reductant supply to AMO. Bromoethane and chloroethane have similar affinities to iodoethane but are turned over more rapidly, which results in higher maximal rates and optimal concentrations. On the other hand, fluoroethane has a low affinity for the enzyme, but is turned over readily once bound. Chloroethane, the best haloethane substrate for AMO, may represent the best compromise between effective binding and rapid turnover.

We studied the effect of carbon chain length on the ability of AMO to dechlorinate *n*-chlorinated C_1 to C_4 alkanes by measuring the rate of formation of the corresponding aldehydes. Of the chlorinated alkanes tested, chloroethane was the most reactive substrate, followed by chloromethane (Table 2). This is consistent with the observation for hydrocarbon oxidation reported by Hyman et al. (8), in which cells incubated with methane produced a lower level of product (68%) than did cells incubated with ethane. With increasing carbon chain length from C_2 to C_4, the rate of dechlorination decreased dramatically. In addition, the overall reactivity of chloropropane (38 nmol of product per min per mg of protein) was lower than that of chloroethane (120 nmol of product per min per mg of protein). This is in contrast with the trend noted for hydrocarbons, in which propane and butane were more reactive than ethane (8). This difference may be due to steric hindrance introduced by the halogen substituent in the larger (C_3 and C_4) chlorinated alkanes.

The relative amounts of the aldehyde and alcohol products from the oxidation of *n*-chlorinated alkanes were also determined in this study (Table 2). Formaldehyde was the only detected product of chloromethane oxidation. For chloroethane (which has two potential products), the aldehyde was the major product. The electrophilicity of the substituted carbon or a preferential binding orientation of the substrate may direct the oxidative attack by AMO to the C_1 position. In contrast, with chloropropane the C_2 position was preferred; this is consistent with the observation that 2-propanol was the major product of propane oxidation (8). The second major product was 3-chloropropanol, with only small amounts of propionaldehyde formed. In this case, steric hindrance or the more hydrophilic nature of the substituted

carbon may direct the binding of chloropropane such that either C-2 or C-3 is positioned for oxidation. The electron-withdrawing properties of the chlorine on C-1 could have an indirect effect on C-2 and make it more susceptible than C-3 to the oxidative attack. Similar results were obtained with chlorobutane (Table 2). Therefore, it seems that with longer-chain chloroalkanes, the substituted carbon is seldom positioned for oxidation.

In summary, we have identified organic oxidation products and compared the reactivities of monohalogenated ethanes and *n*-chlorinated C_1 to C_4 alkanes for oxidation by whole cells of *N. europaea*. The dehalogenating potential of AMO in *N. europaea* may have practical applications for the detoxification of contaminated soil and groundwater. Aliphatic halocarbons are a major class of environmental pollutants which include chlorinated methanes, ethanes, and ethylenes. Aerobic biodegradation of a number of these compounds has been reported for AMO in *N. europaea* (1, 19), methane monooxygenases in *M. capsulatus* Bath (3) and *Methylosinus trichosporium* OB3b (18), and haloalkane dehalogenase in *Xanthobacter autotrophicus* GJ10 (15). The current results, in combination with trends noted for hydrocarbon oxidation (8), provide a basis for predicting whether *N. europaea* is capable of initiating the biodegradation of halocarbon compounds through dehalogenation reactions. An increased understanding of the biodegradative capabilities and limitations of *N. europaea* may provide insight into the mechanism of halocarbon oxidation by AMO and may suggest procedures for enhancing the efficiency of halocarbon biodegradation in contaminated groundwater and soils.

ACKNOWLEDGMENTS

This research was supported by U.S. Department of Agriculture grant 88-37120-3956; by the Toxic Substance Program at the University of California, Riverside; and by a National Science Foundation Predoctoral Fellowship to M.E.R.

LITERATURE CITED

1. **Arciero, D., T. Vannelli, M. Logan, and A. B. Hooper.** 1989. Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. Biochem. Biophys. Res. Commun. **159**:640–643.
2. **Cohen, D. B., and G. W. Bowes.** 1984. Water quality and pesticides: a California risk assessment program. California State Water Research Control Board, Sacramento.
3. **Colby, J., D. I. Stirling, and H. Dalton.** 1977. The soluble methane monooxygenase from *Methylococcus capsulatus* (Bath). Its ability to oxygenate *n*-alkanes, *n*-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. Biochem. J. **165**:395–402.
4. **Drozd, J. W.** 1980. Respiration in the ammonia oxidizing chemotrophic bacteria, p. 87–111. *In* C. J. Knowles (ed.), Diversity of bacterial respiratory systems, vol. 2. CRC Press, Inc., Boca Raton, Fla.
5. **Gornall, A. G., C. J. Bardawill, and M. M. David.** 1949. Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. **177**:751–766.
6. **Hooper, A. B., and K. R. Terry.** 1973. Specific inhibitors of ammonia oxidation in *Nitrosomonas*. J. Bacteriol. **115**:480–485.
7. **Horvath, A. L.** 1982. Halogenated hydrocarbons: solubility-miscibility with water, p. 646–749. Marcel Dekker, Inc., New York.
8. **Hyman, M. R., I. B. Murton, and D. J. Arp.** 1988. Interaction of ammonia monooxygenase from *Nitrosomonas europaea* with alkanes, alkenes, and alkynes. Appl. Environ. Microbiol. **54**: 3187–3190.
9. **Hyman, M. R., A. W. Sansome-Smith, J. H. Shears, and P. M. Wood.** 1985. A kinetic study of benzene oxidation to phenol by whole cells of *Nitrosomonas europaea* and evidence for further oxidation of phenol to hydroquinone. Arch. Microbiol. **143**:302–306.
10. **Hyman, M. R., and P. M. Wood.** 1983. Methane oxidation by *Nitrosomonas europaea*. Biochem. J. **212**:31–37.
11. **Hyman, M. R., and P. M. Wood.** 1984. Bromocarbon oxidations by *Nitrosomonas europaea*, p. 49–52. *In* R. L. Crawford and R. S. Hanson (ed.), Microbial growth on C_1 compounds. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, D.C.
12. **Hyman, M. R., and P. M. Wood.** 1984. Ethylene oxidation by *Nitrosomonas europaea*. Arch. Microbiol. **137**:155–158.
13. **Hyman, M. R., and P. M. Wood.** 1985. Suicidal inactivation and labelling of ammonia mono-oxygenase by acetylene. Biochem. J. **227**:719–725.
14. **Jones, R. D., and R. Y. Morita.** 1983. Carbon monoxide oxidation by chemolithotrophic ammonium oxidizers. Can. J. Microbiol. **29**:1545–1551.
15. **Keuning, S., D. B. Janssen, and B. Witholt.** 1985. Purification and characterization of hydrolytic haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10. J. Bacteriol. **163**:635–639.
16. **Ochsner, J. C., T. R. Blackwood, and W. C. Micheletti.** 1979. Status assessment of toxic chemicals: trichloroethylene. EPA 600/2-79-210m. U.S. Environmental Protection Agency, Cincinnati, Ohio.
17. **Ogushi, S., M. Ando, and D. Tsuru.** 1986. Formaldehyde dehydrogenase from *Pseudomonas putida*: the role of a cysteinyl residue in the enzyme activity. Agric. Biol. Chem. **50**:2503–2507.
18. **Oldenhuis, R., R. L. J. M. Vink, D. B. Janssen, and B. Witholt.** 1989. Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. **55**:2819–2826.
19. **Vannelli, T., M. Logan, D. M. Arciero, and A. B. Hooper.** 1990. Degradation of halogenated aliphatic compounds by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. Appl. Environ. Microbiol. **56**:1169–1171.
20. **Voysey, P. A., and P. M. Wood.** 1987. Methanol and formaldehyde oxidation by an autotrophic nitrifying bacterium. J. Gen. Microbiol. **33**:283–290.
21. **Weijers, C. A. G. M., C. G. van Ginkel, and J. A. M. de Bont.** 1988. Enantiomeric composition of lower epoxyalkanes produced by methane-, alkane-, and alkene-utilizing bacteria. Enzyme Microb. Technol. **10**:214–218.
22. **Wise, H. E., and P. D. Fahrenthold.** 1981. Predicting priority pollutants from petrochemical processes. Environ. Sci. Technol. **15**:1292–1304.
23. **Wood, P. M.** 1986. Nitrification as a bacterial energy source, p. 39–62. *In* J. I. Prosser (ed.), Nitrification. Society for General Microbiology, IRL Press, Oxford.