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Microbial Aldicarb Transformation in Aquifer, Lake, and Salt Marsh Sediments

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The microbial transformation of [*N-methyl*-^{14}C]aldicarb, a carbamate pesticide, occurred in aquifer, lake, and salt marsh sediments. Microbial degradation of aldicarb took place within 21 days in aquifer sediments from sites previously exposed to aldicarb (Jamesport, Long Island, N.Y.) but did not occur in sediments which were not previously exposed (Connetquot State Park, Long Island, N.Y.). At the Jamesport sites, higher aldicarb transformation rates occurred in deep, anoxic sediments than in shallow, oxic sediments. There was a significant negative relationship ($P < 0.05$) between transformation rates and ambient dissolved O_2 levels. Aldicarb hydrolysis rates in Jamesport sediments were 10- to 1,000-fold lower than rates previously reported for soils. In addition, aldicarb degradation rates were not significantly correlated with measurements of bacterial activity and density previously determined in the same sediments. Substantially higher aldicarb degradation rates were found in anoxic lake and salt marsh than in aquifer sediments. Furthermore, we investigated the anaerobic microbial processes involved in aldicarb transformation by adding organic substrates (acetate, glucose), an alternative electron acceptor (nitrate), and microbial inhibitors (molybdate, 2-bromoethanesulfonic acid) to anoxic aquifer, lake, and salt marsh sediments. The results suggest that a methanogenic consortium was important in aldicarb transformation or in the use of aldicarb-derived products such as methylamine. In addition, microbial aldicarb transformation proceeded via different pathways under oxic and anoxic conditions. In the presence of O_2, aldicarb transformation was mainly via an oxidation pathway, while in the absence of O_2, degradation took place through a hydrolytic pathway (including the formation of methylamine precursors). Under anoxic conditions, therefore, aldicarb can be transformed by microbial consortia to yield products which can be of direct benefit to natural populations of methanogens present in sediments.

The carbamate insecticides (which include aldicarb) account for over 25% of the world's annual pesticide production; these compounds are widely used because of their high specificity to target organisms and their low bioaccumulation in lipids and body tissues when compared with the chlorinated hydrocarbon pesticides (e.g., DDT and dieldrin) (12). Aldicarb is relatively soluble in water (octanol-water distribution coefficient [K_{ow}], 0.85 to 1.57) (3), and excess aldicarb may percolate through the soil and into the underlying aquifer. Once there, aldicarb can migrate readily along hydrogeologic flow paths, not only contaminating groundwater reserves but also potentially affecting lakes and coastal regions.

Aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, commercially known as Temik] is a persistent and widespread contaminant in the Long Island, N.Y., aquifer; aldicarb and its residues have also been detected in groundwaters of Wisconsin, California, and Florida (20, 21). Because of public health concerns, studies on aldicarb transformation have focused mostly on estimating the kinetics of transformation of aldicarb to its equally toxic residues, aldicarb sulfoxide and aldicarb sulfone. Although it is well established that the presence of bacteria accelerates aldicarb transformation (23, 26), few studies have investigated microbial utilization of aldicarb by naturally occurring bacteria, especially in the absence of oxygen. Our objectives were to estimate rates of aldicarb transformation in aquifer sediments with a history of aldicarb contamination and to determine whether these results could be related to a previous study (15) in which measurements of bacterial activity and density were taken in sediments from the same sites. Furthermore, we investigated the anaerobic microbial processes involved in aldicarb utilization and whether the addition of other organic substrates (acetate, glucose), an electron acceptor (nitrate), and microbial inhibitors (molybdate, 2-bromoethanesulfonic acid [BES]) can promote aldicarb transformation by aquifer sediment microorganisms. Other studies have reported that the addition of alternative electron acceptors (e.g., nitrate and sulfate), nutrients (e.g., phosphorus and mineral salts), or organic substrates (e.g., acetate, formate, propionate, and butyrate) stimulated the degradation of contaminants such as phenols and herbicides by native aquifer bacteria (9, 29). We also investigated the potential for aldicarb utilization by microbial communities in lake and salt marsh sediments and whether aldicarb transformation could be promoted in these sediments.

In addition, identifying aldicarb breakdown products may provide further information on the environmental importance of bacterial processes in aldicarb transformation and degradation. Bacteria have been implicated in two primary routes of aldicarb transformation: one pathway is the oxidation of aldicarb to aldicarb sulfoxide and subsequently to aldicarb sulfone; the other is the hydrolysis of aldicarb and its oxidized residues to their corresponding oximes (23, 24, 28, 30). Because hydrolysis or transformation of aldicarb at the *N*-methyl carbamoyl group produces residues that are not as toxic as the parent

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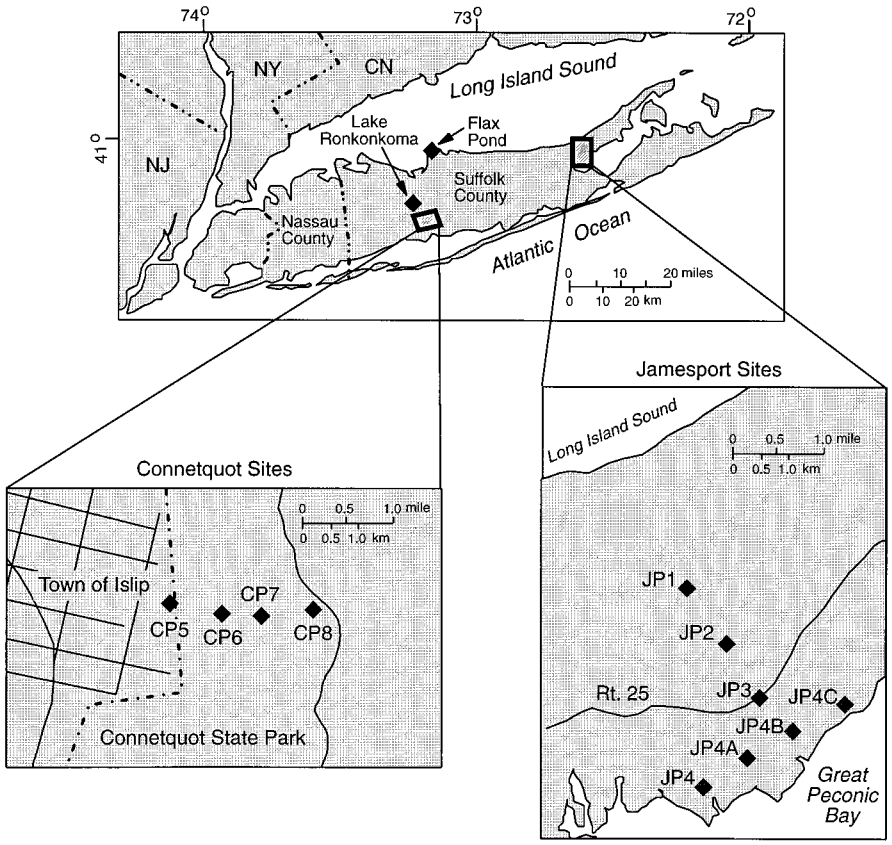

FIG. 1. Map of site locations on Long Island, N.Y.

compound, differences in aldicarb transformation pathways become important, especially when considering its fate in the environment. In this study, we investigated aldicarb transformation pathways mediated by natural sediment bacteria by monitoring the loss of aldicarb and the appearance of transformation products in sediments incubated under oxic and anoxic conditions.

MATERIALS AND METHODS

Sediment samples. Aquifer sediments were obtained from aldicarb-contaminated sites in Jamesport, N.Y., which had a history of high but variable aldicarb contamination, and from sites in Connetquot State Park (Fig. 1), which had no history of aldicarb exposure. The exact location and sampling depths were based on preliminary profiles of pH, temperature, dissolved O_2, and aldicarb residues of pumped groundwater from monitoring wells maintained by the Suffolk County Department of Health Services. Sampling sites were approximately 1 km apart (Fig. 1). Sediment cores were taken with a hollow stem auger drilling rig equipped with split barrel samplers (outside diameter, 8.9 cm). Dissolved O_2, pH, and temperature of the cores were measured in the field with a dissolved-oxygen meter (Orion model 800) and a pH meter (Corning model 105) equipped with pH and temperature probes.

Sediment cores were taken from two strata within the same borehole, generally 4.5 m below the water table (except for the core at site JP1S, which was taken at 9 m below the water table) and designated S (shallow) and 10 m below the water table (except for the core at JP1D, which was taken at 18 m below the water table) and designated D (deep); cores from the shallow depth were always taken before cores from the deeper depth. The sediment samplers were handled to maintain oxygenated (uncapped) or unoxygenated (sealed) conditions and placed on ice for transport to the Marine Sciences Research Center, Stony Brook. Sediments from Jamesport and Connetquot Park were a coarse sand.

The samplers were placed in a sterile hood and opened within 24 h after sampling. Both ends of the core were cut away, and only the center portion of the core (parallel to the barrel) was carefully scooped and reserved for use in the experiments. Several experiments were performed to determine the effects of O_2 on transformation rates of samples obtained from the two strata. In general, however, sediment cores from the upper stratum were incubated under oxic conditions while sediment cores taken from the lower stratum were incubated under anoxic conditions. At several deep sites, sediments were not fully anoxic in situ (Table 1); however, dissolved-oxygen levels were lower in deep than in shallow sediments. The deep sediments were nonetheless incubated under anoxic conditions to determine the potential for anaerobic degradation. The deep sediments to be incubated under anoxic conditions were initially placed into N_2-gassed 500-ml glass Erlenmeyer flasks, the headspace was flushed with N_2, and the flasks were capped with butyl rubber stoppers. These sediments were then set up for the experiments and dispensed into incubation vials in an anaerobic glove bag under O_2-free conditions. All glass and plasticware were acid washed and autoclaved prior to use in the experiments.

Sediments from Lake Ronkonkoma, N.Y., were collected with a benthic grab sampler from approximately 6 m below the water surface. These sediments were stored in 4-liter polypropylene jars for 1 week at 12°C prior to use in the experiments. The top 5 to 10 mm of sediment in the jar was used for oxic incubations, while sediment from 5 to 10 cm deep was used for anoxic incubations. Lake Ronkonkoma sediments were a fine sand. Salt marsh sediments from Flax Pond, N.Y., were collected with short (50-cm) lengths of Plexiglas tubing (outer diameter, 3.5 cm). The top 2 to 3 mm of the core was scraped for use in the oxic experiments, while sediment from 5 to 10 cm deep was used in the anoxic experiments. Prior to use, all sediment was sieved through a 2-mm screen to remove large debris. Sediment from this site was an organic-rich mud.

Aldicarb transformation. Aquifer sediment slurries (0.5 ml of wet sediment per 5.0 ml of 0.2-μm-filtered distilled water) were amended with custom-synthesized [*N-methyl*-^{14}C]aldicarb (Amersham Corp., Arlington Heights, Ill.; 47.3 mCi mol^{-1} or 1.8×10^3 Bq mol^{-1}, 97% radiochemical purity) to determine aldicarb transformation. In most cases, a final concentration of 500 μg liter^{-1} (or 4 μg g [dry weight]$^{-1}$) of aldicarb (solubility in water, 6 g liter^{-1}) was used. The experimental vials were incubated in a shaking water bath at 12°C to simulate field temperatures at Jamesport (15). The experiments were usually run for 21 days, and triplicate vials were removed for processing at each time point (ca. every 3 days). For anoxic experiments, the headspace of the vials was flushed with N_2. At each time point, 0.4 ml of 2 N H_2SO_4 was injected into each of the vials. In vials incubated under oxic conditions, the headspace was flushed with air and the evacuated gas was bubbled into 10 ml of Oxosol fluor (National Diagnostics, Somerville, N.J.) to trap any $^{14}CO_2$ that had evolved. In vials incubated under anoxic conditions $^{14}CO_2$ was trapped as above and the headspace also monitored

TABLE 1. Rates of aldicarb transformation in aquifer sediments

Site[a]	Depth (m)	O_2 concn (ppm)	Concn (μg/liter of:)[b]		Aldicarb transformation rate (ng/g [dry wt]/day)[c]		Lag[c] (days)	Initial $t_{1/2}$ (days)	Maximum $t_{1/2}$ (days)
			ASO	ASO_2	Initial	Maximum			
JP1S	22.5	6.6	17	24	0.3 ± 0.3	10.0 ± 2.0	13.0 ± 4.0	5,800	190
JP2S	28.5	6.0	30	30	0.8 ± BD	3.5 ± 0.4	4.5 ± 1.2	2,500	570
JP3S	14.5	6.6	BD	BD	0.4 ± 0.3	4.3 ± 0.5	10.0 ± 2.0	4,800	460
JP4S	13.5	5.5	23	11	0.6 ± 0.6	2.7 ± 0.5	4.3 ± 3.1	3,500	730
JP4AS	10.5	1.8	BD	BD	0.1 ± 0.1	3.7 ± 1.2	5.5 ± 1.6	26,000	540
JP4BS	9.5	9.8	BD	BD	0.8 ± 0.4	4.1 ± 0.6	6.7 ± 0.8	2,500	490
JP4CS	22.5	3.5	6	6	0.2 ± 0.2	2.0 ± 0.3	11.0 ± 3.0	10,000	980
Mean			10.9	10.1	0.5	4.3	7.9	7,871	566
JP1D	31.5	4.1	8	8	1.4 ± 0.5	12.0 ± 2.0	7.1 ± 0.9	1,400	170
JP2D	34	5.1	BD	BD	2.2 ± 0.4	12.0 ± 2.0	8.1 ± 1.5	930	170
JP3D	20	0.5	BD	BD	2.4 ± 0.5	28.0 ± 4.0	8.5 ± 0.7	851	70
JP4D	19	0.7	BD	BD	1.7 ± 0.2	16.0 ± 2.0	9.9 ± 0.8	1,200	130
JP4AD	16	0.5	BD	BD	2.3 ± 0.5	22.0 ± 7.0	8.4 ± 1.3	860	92
JP4BD	15	1.2	2	3	3.4 ± 0.7	23.0 ± 6.0	6.7 ± 0.8	580	86
JP4CD	28	3.2	BD	BD	0.5 ± 0.3	9.4 ± 2.3	5.2 ± 0.1	3,900	210
Mean			1.4	1.6	2.0	17.5	7.7	1,389	133
Overall mean			6.1	5.9	1.2	10.9	7.8	4,630	349

[a] S, shallow; D, deep.
[b] ASO, aldicarb sulfoxide; ASO_2, aldicarb sulfone; BD, below detection (detection limit was 1 μg liter^{-1}) for aldicarb, aldicarb sulfoxide, and aldicarb sulfone. No aldicarb was detected in any sample. Aldicarb and daughter products were analyzed by Suffolk County Department of Health Services.
[c] Values are mean ± 1 SE (n = 3).

for $^{14}CH_4$. This was achieved by extending a gas line from the $^{14}CO_2$ trap to a cuprous oxide column heated to 500°C, and the oxidation products were trapped in another two vials in series containing 10 ml of Oxosol. The Oxosol aliquots were then assessed for radioactivity by liquid scintillation counting. Recovery of the radiolabel as $^{14}CO_2$ and $^{14}CH_4$ in Oxosol was 91.8% ± 10.2% (n = 6) and 83.2% ± 4.7% (n = 6), respectively. The experimental vials were then opened, the slurry was filtered onto 0.2-μm-pore-size filters (Millipore, Bedford, Mass.), and the residue was washed several times with filtered distilled water before the filter was placed into scintillation vials. Protosol (New England Nuclear, Boston, Mass.) was added to the vials to digest organic matter. ScintiVerse II scintillation cocktail (Fisher Scientific Co., Fair Lawn, N.J.) and filtered distilled water were then added to make a homogeneous gel. Radioactivity in these samples was determined with a Packard TriCarb 300C Scintillation Counter.

To examine whether microbial aldicarb transformation under anoxic conditions was linked to the presence of sulfate-reducing and/or methanogenic bacteria, organic substrates and inhibitors were added to sediment slurries and the amount of aldicarb degraded was monitored in these cultures. Combinations of glucose or acetate (final concentration, 5 mM), nitrate (final concentration, 1 mM), and Na_2MoO_4 or BES (final concentration, 10 mM) were used. Aldicarb transformation was also determined in slurries to which no organic substrate, nitrate, or inhibitors were added. All solutions were made with autoclaved filtered distilled water and reagent grade chemicals (Fisher Scientific Co.). For experiments with lake sediments, slurries were made and processed as above for aquifer sediments; for experiments with salt marsh sediments, slurries were made with 0.5 ml of wet sediment per 5.0 ml of 0.2 μm-pore-size-filtered seawater. With salt marsh sediments, amendments were made with small volumes of solution (<500 μl added to 5.0 ml of seawater) to minimize salinity dilution (27 ppt). The experimental vials were incubated in a shaking water bath at 25°C, and the experiments were run for 14 days; at each time point, triplicate vials were removed for processing as described for aquifer sediments. The headspace of the anoxic vials was flushed with N_2.

In all experiments, formalin-killed controls (final formalin concentration, 4%) were run and treated in the same manner as the experimental vials. In the controls, less than 0.1 and 0.2% of the total radiolabel added were recovered in the Oxosol and ScintiVerse fractions, respectively, at all time points. Increases in radioactive counts in the experimental vials over time were assumed to be due to biological processes. Because aldicarb does not adsorb readily to sediment particles (8, 32), radioactivity in the ScintiVerse fraction (minus the abiotic controls) was assumed to be associated with cells.

The appearance of the radiolabel from [*N-methyl*-^{14}C]aldicarb in the cellular and respired (CO_2 and CH_4) fractions was determined after correcting for abiotic controls; total aldicarb degradation was based on the sum of the cellular and respired fractions. In most cases, total aldicarb degradation over time showed two phases: relatively slow degradation from 0 until approximately 11 to 14 days and rapid degradation from 11 to 14 until 17 to 21 days. Aldicarb degradation rates were determined by linear regression with data from the first 11 days to estimate "initial" rates and data between 14 and 21 days (or between 14 and 17 days if degradation leveled off at 21 days) to estimate "potential" rates. Initial rates provide an estimate of in situ degradation rates, while potential rates provide an estimate of the maximum capacity for contaminant removal.

Estimates of half-lives for aldicarb were based on the assumption that aldicarb degradation followed zero-order kinetics (i.e., that degradation was linear with time). The half-lives ($t_{1/2}$) were calculated from the equation $t_{1/2} = 0.5C_0/K$, where C_0 is the initial concentration (4 μg g^{-1}) and K is the weight-normalized zero-order rate constant (i.e., the aldicarb degradation rate). The minimum and maximum half-lives were estimated from potential and initial aldicarb degradation rates, respectively.

Aldicarb transformation pathways. The disappearance of aldicarb and the formation of daughter products in salt marsh sediments was followed over 14 days. Sediment slurries were prepared and incubated as described above for salt marsh sediments but were amended with unlabeled aldicarb (>99% pure; Union Carbide Agricultural Products Co., Research Triangle Park, N.C.) to a final concentration of 500 μg liter^{-1} (or approximately 4 μg g [dry weight]$^{-1}$).

To estimate the disappearance of aldicarb and the formation of daughter products, the liquid phase was separated from the sediment phase in the slurries and the liquid was analyzed by high-performance liquid chromatography (HPLC). At each time point, samples were harvested and centrifuged at 5,900 × g for 10 min. The pellet was discarded, and the supernatant was decanted into fresh centrifuge tubes and centrifuged again (5,900 × g for 10 min). The supernatant was decanted and passed through a 0.2-μm-pore-size Millipore filter. Prior to starting the experiment, we determined that by using this separation method, aldicarb recovery in the liquid phase was 88.7% ± 5.4% (n = 6). Subsamples (50 to 100 μl) of the filtered supernatant were injected into a high-performance liquid chromatograph (Waters Associates, Milton, Mass.) equipped with an Adsorbosphere C-18 column (mesh size, 5 μm; length, 15 cm) (Applied Science, State College, Pa.). Aldicarb and its residues were separated by using a linear gradient elution program and an acetonitrile-water solvent phase running from 5% acetonitrile–95% water to 35% acetonitrile–65% water. The flow rate was 1.2 ml min^{-1}. This protocol was adapted from a procedure developed by Miles and Delfino (19), which required two isocratic chromatographic runs to obtain aldicarb and its eight daughter products; by using gradient elution, we were able to determine aldicarb and its residues in a single chromatographic run. Aldicarb and its transformation products were detected on an absorbance detector (Waters model 440) at 214 nm. Aldicarb and its daughter products were identified from retention times, which, in turn, were determined from standards prepared with chemicals supplied by Union Carbide (Table 2). Both internal and external standards of methomyl were used. Triplicate analyses were run for each sample.

TABLE 2. HPLC retention times and limits of detection for aldicarb and its residues

Compound	Retention time (min)[a]	Limit of detection (μg/liter)
Aldicarb sulfoxide oxime	4.3 ± 0.1	10
Aldicarb sulfoxide nitrile	4.8 ± 0.1	33
Aldicarb sulfone oxime	6.4 ± 0.1	15
Aldicarb sulfoxide	8.3 ± 0.2	33
Aldicarb sulfone	10.0 ± 0.2	17
Methomyl (standard)	11.1 ± 0.2	10
Aldicarb sulfone nitrile	12.9 ± 0.2	11,000
Aldicarb oxime	16.5 ± 0.2	8
Aldicarb	20.1 ± 0.2	10
Aldicarb nitrile	20.7 ± 0.2	50

[a] Retention times are mean ± 1 SE (n = 15).

In experiments examining pathways of degradation, concentrations of aldicarb and its residues were estimated by comparing areas with that of methomyl (standard) in the HPLC readout after correcting for abiotic controls. The limit of detection for each compound is shown in Table 2.

RESULTS AND DISCUSSION

Microorganisms from Jamesport sites previously exposed to aldicarb were found to incorporate and respire radiolabeled aldicarb (Table 1; Fig. 2) when compared with microorganisms from relatively pristine Connetquot Park sites (data not shown). Table 1 shows that microbial aldicarb degradation occurred in Jamesport sediments at almost all sites and depths. Furthermore, aldicarb transformation rates were higher and half-lives estimated from initial or potential degradation rates were shorter in deep than in shallow sediments. Overall, the average initial and potential aldicarb degradation rates in Jamesport sediments were 1.2 and 11 ng g (dry weight)$^{-1}$ day^{-1} (or 0.03 and 0.28% day^{-1}), respectively, and the average minimum and maximum half-lives ($t_{1/2}$) were 350 and 4,600 days, respectively, assuming zero-order kinetics (Table 1). These values are 10- to 1,000-fold lower than those reported for nonsterile soils, in which aldicarb hydrolysis was found to follow first-order kinetics with rate constants between 0.07 and 1.6 day^{-1} (or 1.2 to 40% day^{-1}) (4, 32), which correspond to a $t_{1/2}$ of 0.4 to 10 days. The aldicarb half-lives we report are also longer than those predicted (120 to 1,100 days) by laboratory studies for the Long Island saturated zone (13) and are consistent with the fact that aldicarb and its residues are still present in portions of the aquifer, even 10 years after its use was terminated (5a, 14).

In a previous study, microbial activity, estimated by [^{14}C]glucose metabolism and total [^{3}H]thymidine incorporation, and density (acridine orange direct counts) were determined in samples taken from the same sites in Jamesport as those in the present study (15). When all the results were statistically analyzed, no significant relationships between bacterial activity or biomass and aldicarb residue concentrations, aldicarb degradation rates, or lag times were found. This lack of correlation may be due to a number of factors. One possibility is that the methods used to estimate bacterial activity did not accurately assess the metabolic activity of the bacteria involved in aldicarb degradation, as a result of differences in the response of a given bacterial subpopulation to exogenous tracer compounds. Another possibility is that our estimates of bacterial numbers involved in aldicarb degradation were high, because we determined total microscopic counts, which do not distinguish between metabolically active and inactive cells.

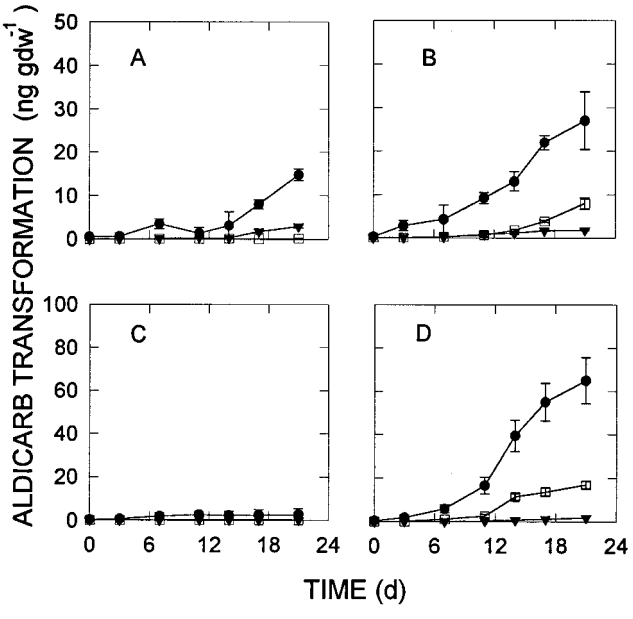

FIG. 2. Aldicarb transformation in shallow and deep aquifer sediments (site JP4) incubated in the presence (+O_2) and absence (−O_2) of oxygen. (A) Shallow, +O_2; (B) shallow, −O_2; (C) deep, +O_2; (D) deep, −O_2. ●, cellular uptake; ▼, CO_2 production; □, CH_4 production. Note the difference in the y-axis scale between the top (A and B) and bottom (C and D) graphs. Killed controls did not show aldicarb transformation. When present, bars are ±1 standard error (SE).

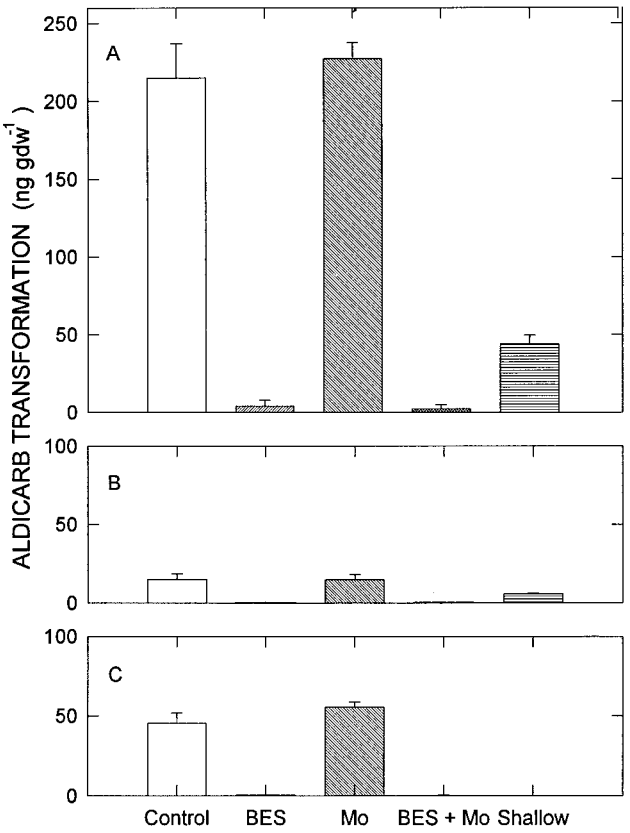

FIG. 3. Effect of BES and molybdate on the amount of aldicarb transformed in deep, anoxic aquifer sediments (site JP3D) within 21 days. (A) Cellular uptake; (B) CO_2 production; (C) CH_4 production. Killed controls did not show aldicarb transformation. When present, bars are ±1 SE.

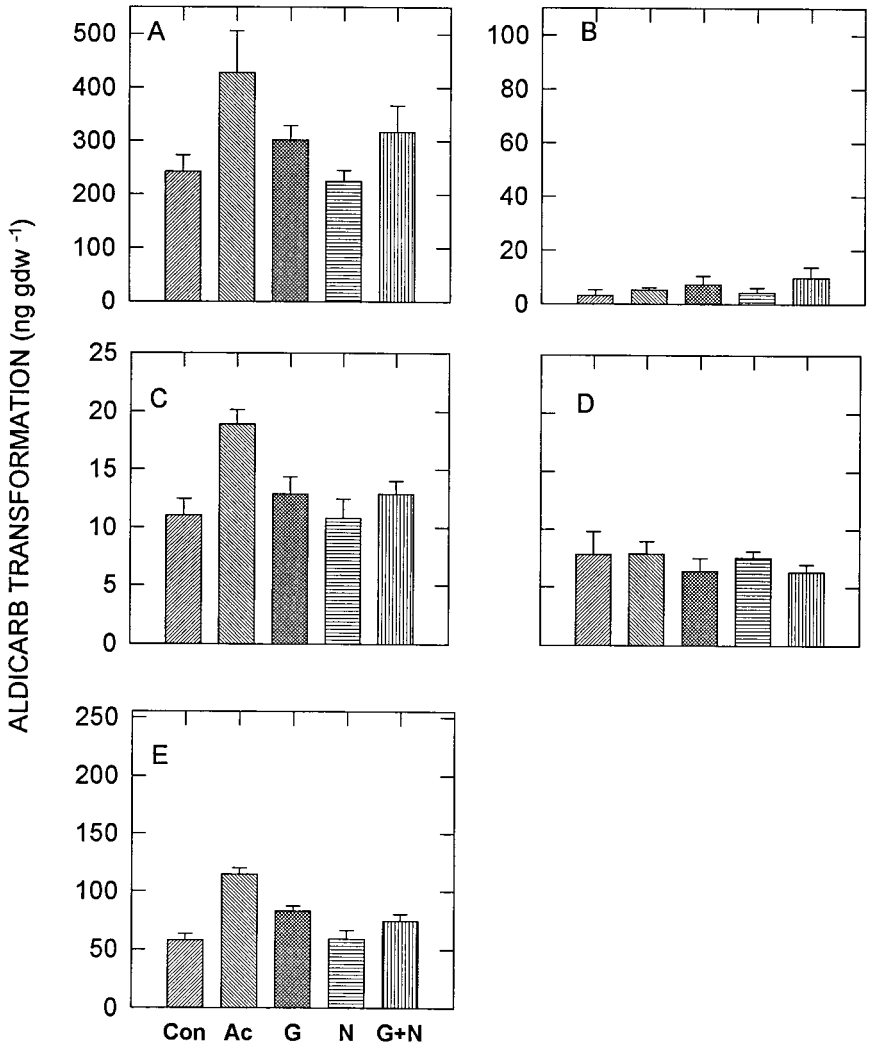

FIG. 4. Effect of substrates and BES on the amount of aldicarb transformed in anoxic aquifer sediments (site JP3) within 21 days. In the absence of BES (A, C, and E), the control sample (Con) has no amendments. In the presence of BES (B, D), the control sample has BES only. Amendments are acetate (Ac), glucose (G), nitrate (N), and glucose plus nitrate (G+N). (A and B) Cellular uptake; (C and D) CO_2 production; (E) CH_4 production. Little CH_4 was produced in BES-amended samples (data not shown). Note the difference in the *y*-axis scale between panels A and B. Results are corrected for formalin-killed controls, which were <2, 1, and 1 ng g (dry weight)$^{-1}$ for cellular uptake, CO_2 production, and CH_4 production, respectively, in all treatments. When present, bars are ±1 SE.

In addition, we attempted to relate aldicarb degradation rates with ambient temperature, pH (from reference 15), or dissolved O_2 at the Jamesport sites. There were no significant relationships between aldicarb degradation rates and temperature or pH. There was, however, a significant negative correlation between aldicarb transformation rates and ambient dissolved O_2 ($P < 0.05$ for potential rates). Experimental studies (see below) corroborate the importance of low O_2 concentration as a factor which promotes aldicarb degradation and hydrolysis. Measurements of dissolved O_2 may thus be useful in predicting in situ bacterial aldicarb degradation potential and the fate of aldicarb in portions of the Upper Glacial Aquifer.

It should be noted that aldicarb transformation often occurred in two phases: initially slow degradation over 11 to 14 days followed by faster degradation between 14 and 21 days (e.g., Fig. 2D). Lag periods before microbial utilization of contaminants have been noted in many other studies; for example, the microbial degradation of *p*-nitrophenol and *tert*-butyl alcohol in aquifer sediments took place after lag periods of 7 to 200 days (10, 29). Higher contaminant degradation after an initial lag period may be due to general growth of the overall microbial population and/or the preferential use of more labile organic substrates by bacteria before degradation of the contaminant. Other possibilities include the adaptation of the microbial population and growth enhancement of specific bacterial groups able to degrade aldicarb via mechanisms such as enzyme induction, mutation, or genetic transfer.

Aldicarb transformation under oxic and anoxic conditions. We further investigated the potential for aldicarb degradation in the presence and absence of O_2 in aquifer sediments (Fig. 2). Aldicarb utilization occurred within 14 days in shallow aquifer sediments incubated in the presence of O_2 (Fig. 2A) and even earlier in the absence of O_2 (Fig. 2B). There was little activity however, in deep sediments incubated under oxic conditions (Fig. 2C). Figure 2D shows that aldicarb utilization was greatest in deep sediments which were incubated anoxically and that significantly more aldicarb was transformed within 21 days in these cultures than in the other treatments ($P < 0.05$). Furthermore, the total (sum of cellular uptake, CO_2 production, and CH_4 production) amount of aldicarb transformed

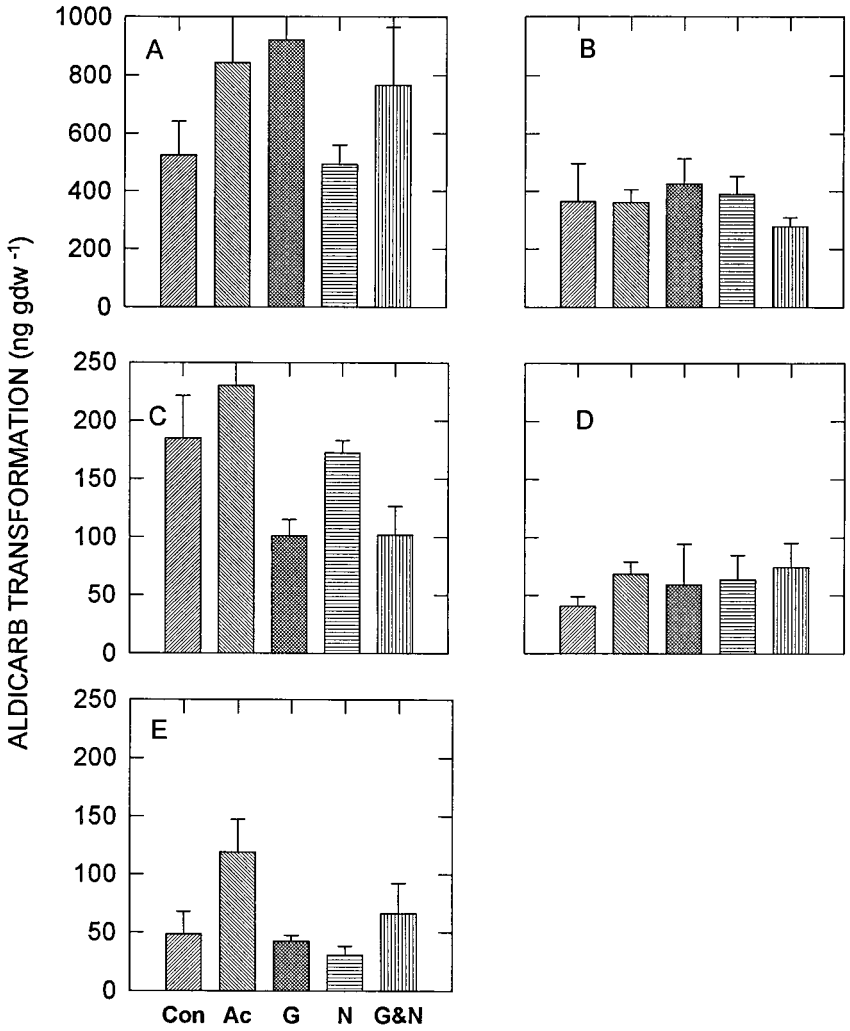

FIG. 5. Effect of substrates and BES on aldicarb transformation in anoxic lake sediments within 21 days. Conditions and labels are the same as for Fig. 4. Results are corrected for formalin-killed controls, which were <40, 1, and 1 ng g (dry weight)$^{-1}$ for cellular uptake, CO_2 production, and CH_4 production, respectively, in all treatments. When present, bars are ±1 SE.

within 21 days in deep sediments was 28-fold greater under anoxic than oxic conditions. The total amount of aldicarb metabolized within 21 days in shallow sediments was twofold greater under anoxic conditions. Methane production was noted only in those samples incubated anoxically (Fig. 2B and D).

These results indicate that anoxic conditions promoted aldicarb transformation and suggest that facultatively or obligately anaerobic microorganisms were more important than aerobic bacteria in aldicarb degradation or that different types of bacteria may be involved in zones of differing redox conditions. In another study, aldicarb disappeared faster in anoxic than oxic sandy soils, but limitations in methodology precluded the identification of the metabolized end products (23). The faster disappearance of aldicarb under anoxic conditions has been attributed to the hydrolysis of aldicarb, which occurs more readily in the absence of oxygen, although the exact mechanism is still unknown (23, 26).

Furthermore, the production of $^{14}CO_2$ and $^{14}CH_4$ in our cultures indicates transformation of aldicarb at the terminal *N*-methyl group. Production of $^{14}CO_2$ from [*N-methyl*-^{14}C]aldicarb by bacteria has been shown in a previous study with oxic soils (4), but to our knowledge, this is the first report of $^{14}CH_4$ production from [*N-methyl*-^{14}C]aldicarb. This result is consistent with a previous study in which excess CH_4 production was noted in anoxic salt marsh sediments amended with aldicarb and other carbamate pesticides (16, 17). The production of CH_4 may be due to the microbial respiration of carbamate-derived monomethylamine (NH_2CH_3), which in turn can be released by the hydrolysis of aldicarb and other carbamate pesticides at the *N*-methyl position (2, 6). Other studies have shown that methanogenic bacteria can use methylamine as a growth substrate (31).

To investigate the role of sulfate-reducing and/or methanogenic bacteria in aldicarb utilization, molybdate and BES were added to anoxic aquifer sediments. Figure 3 shows that the addition of BES alone or together with molybdate decreased the total amount of aldicarb transformed within 21 days, while the addition of molybdate alone had no effect. Relative to controls, which had no amendments, and molybdate-amended samples, samples with BES had inhibited aldicarb uptake (Fig. 3A) and respiration (Fig. 3B and C). Furthermore, there was less aldicarb uptake and respiration in BES-amended samples than in shallow sediments, which were incubated in the pres-

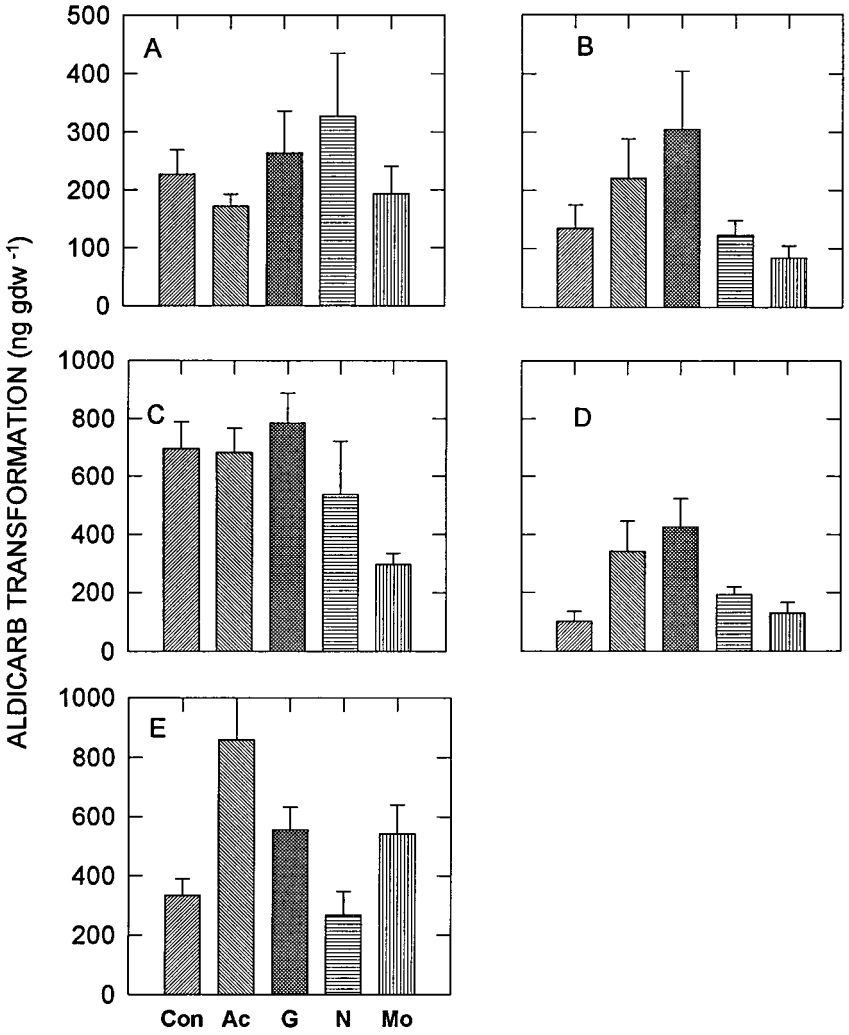

FIG. 6. Effects of substrates and inhibitors on aldicarb transformation in anoxic salt marsh sediments within 14 days. Conditions and labels are the same as for Fig. 5, except that molybdate (Mo) was also added. Results are corrected for formalin-killed controls, which were <50, 1, and 1 ng g (dry weight)$^{-1}$ for cellular uptake, CO_2 production, and CH_4 production, respectively, in all treatments. When present, bars are ±1 SE.

ence of O_2. Methane production occurred only in the controls without amendments and in the samples with Mo alone (Fig. 3C). These results suggest that in aquifer sediments, methanogenic bacteria were more important than sulfate-reducing bacteria in aldicarb transformation. Other studies have also shown that in microbial enrichments from aquifer sediments, degradation of contaminants (e.g., haloaromatic compounds and the herbicide 2,4,5-trichlorophenoxyacetic acid) occurred in the presence of molybdate but not in the presence of BES (9, 27). This suggests that at least in some anoxic aquifer sediments, methanogens are important in the utilization of organic contaminants.

Effects of added substrates and inhibitors on aldicarb transformation. (i) Deep, anoxic aquifer sediments. We investigated whether adding organic carbon sources (acetate, glucose) and an alternate electron acceptor (nitrate) in the presence and absence of BES could promote microbial aldicarb utilization. Figure 4 shows aldicarb assimilation and respiration in the absence (Fig. 4A, C, and E) and presence (Fig. 4B and D) of BES in aquifer sediments incubated for 21 days. In samples without BES, acetate stimulated aldicarb assimilation (Fig. 4A) and respiration (Fig. 4C and E) above that of the controls, which had no amendments. Additions of glucose, nitrate, or glucose with nitrate, however, did not affect the amount of aldicarb utilized. Most of the transformed aldicarb was assimilated (Fig. 4A), with smaller fractions respired to CO_2 (Fig. 4C) and CH_4 (Fig. 4E). In samples with BES alone or with acetate, glucose, nitrate, or glucose plus nitrate, less aldicarb was utilized (Fig. 4B and D). There was little CH_4 production in BES-amended sediments (data not shown). In BES-amended cultures, aldicarb was respired primarily to CO_2 (Fig. 4D), with a substantial reduction in the amount of aldicarb assimilated (Fig. 4B).

(ii) Lake sediments. Figure 5 shows the results of an identical experiment to that conducted and described above (Fig. 4) but with lake sediments. The addition of acetate, glucose, nitrate, or glucose plus nitrate did not increase the total amount of aldicarb transformed within 21 days above that of the control ($P < 0.05$); however, there was a trend of enhanced uptake in the organically enriched samples (Fig. 5A). In addition, aldicarb transformation yielded both CO_2 and CH_4 (Fig. 5C and E). In contrast, amendments of BES alone and with acetate, glucose, nitrate, or glucose plus nitrate significantly decreased but did not eliminate the amount of aldicarb taken

TABLE 3. Comparison of bacterial aldicarb transformation in aquifer, lake, and salt marsh sediments

Site	Aldicarb transformation rate (ng/g [dry wt]/day)		Recovery (%)	Aldicarb uptake (%)	Production (%) of:	
	Initial	Maximum			CO_2	CH_4
Aquifer[a,b]	2.0	17.5	3.6	80	5	15
Lake[a]	9.6	70	19	69	24	6
Marsh[c]	45	138	31	18	55	27

[a] 21-day experiment.
[b] Deep sediments only ($n = 7$).
[c] 14-day experiment.

up or respired to CO_2 (Fig. 5B and D). In samples with BES, only trace amounts of CH_4 were produced (data not shown).

(iii) Salt marsh sediments. Figure 6 shows the results of a similar experiment to that described above and shown in Fig. 4 and 5 but with salt marsh sediments and with a second microbial inhibitor, molybdate. In the absence of BES, the addition of acetate, glucose, nitrate, or molybdate did not affect the total amount of aldicarb taken up compared with that for the controls, which had no amendments, within 14 days (Fig. 6A, C, and E). CH_4 production accounted for the largest fraction of transformation products in samples to which acetate or molybdate was added (Fig. 6E). As with aquifer and lake sediments, BES generally inhibited aldicarb transformation in salt marsh sediments (Fig. 6B and D). Glucose and acetate additions, however, slightly stimulated aldicarb uptake relative to that for the BES control (Fig. 6B). In all cases, the addition of BES decreased respiration to CO_2 (Fig. 6D) and inhibited CH_4 production (data not shown).

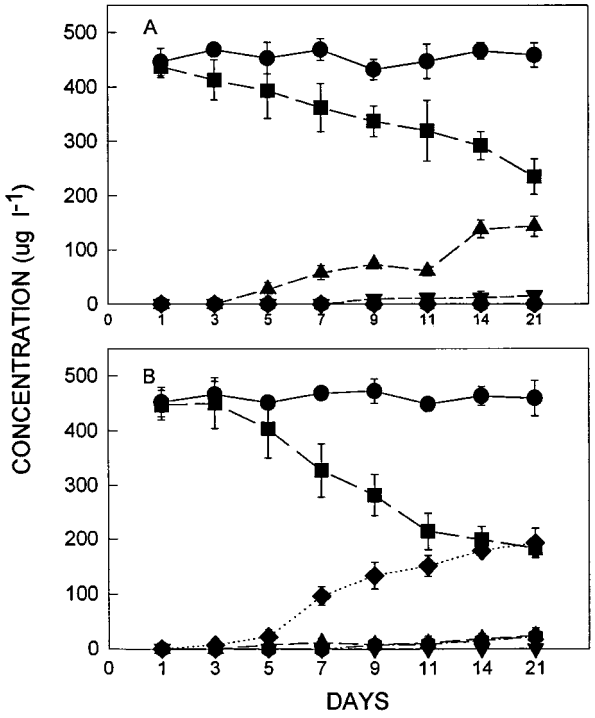

FIG. 7. Time course of aldicarb transformation in salt marsh sediments over 21 days. (A) Oxic conditions; (B) anoxic conditions. ■, aldicarb; ●, aldicarb sulfoxide oxime; ▲, aldicarb sulfoxide; ◆, aldicarb oxime; ▼, aldicarb sulfone; ●, aldicarb control.

Comparison of aldicarb transformation in aquifer, lake, and salt marsh sediments. Table 3 shows rates of aldicarb transformation and the amount of aldicarb assimilated and respired in anoxic aquifer, lake, and salt marsh sediments. Aldicarb was degraded and respired to CO_2 and CH_4 in all sediments incubated in the absence of O_2, with the fastest transformation occurring in salt marsh sediments. In addition, our results from incubations of aquifer, lake, and salt marsh material with BES indicate that a methanogenic consortium was involved in the transformation of aldicarb or products derived from aldicarb degradation.

The relative importance of methanogens in aldicarb transformation is illustrated by the ratio of $^{14}CH_4$ to $^{14}CO_2$ produced from labeled aldicarb. The CH_4/CO_2 ratios were approximately 3:1, 0.3:1, and 0.5:1 for unamended aquifer, lake, and salt marsh sediments, respectively. Methanogenesis is usually considered to be responsible for most of the organic carbon oxidation in freshwater systems (7), while the availability of sulfate allows sulfate reduction to be responsible for the majority of terminal carbon metabolism in marine sediments (22). In the present study, the CH_4/CO_2 ratios in aquifer and salt marsh sediments reflect the expected overall contribution of methanogens in organic matter cycling in these environments. In lake sediments, however, a lower than expected CH_4/CO_2 ratio was noted, indicating that methanogens were relatively unimportant in the terminal metabolism of aldicarb. One possibility is that some lakes are also sites of substantial sulfate reduction (18), and this may be the case for Lake Ronkonkoma, although sulfate concentrations and bacterial sulfate reduction have not been measured (26a).

Of the organic substrates and electron acceptors added to promote aldicarb degradation, acetate had the greatest effect, with 80 to 100, 57, and 36% higher aldicarb transformation than that for the controls in aquifer, lake, and salt marsh sediments, respectively. Glucose amendments, to a lesser degree, enhanced the amount of aldicarb transformed by 10 to 30, 40, and 30% in aquifer, lake, and salt marsh sediments, respectively. Nitrate additions increased the amount of aldicarb transformed only in the presence of glucose, but not alone, in all three sediments. These results may be due to enhanced bacterial growth or microbial cometabolism of aldicarb with the addition of more utilizable organic sources. In other studies with aquifer enrichments, the addition of nutrients (e.g., phosphate), labile organics, and alternate electron acceptors has been reported to stimulate the microbial transformation of xenobiotics such as jet fuels and herbicides (1, 9, 11). This indicates that enhancement of metabolic activities of natural bacteria is possible and may be useful when considering the potential for bioremediation of contaminated aquifers.

More aldicarb transformation and higher methane production in the presence of acetate is a further indication of the involvement of methanogens in aldicarb transformation or in the use of aldicarb-derived products. Acetate can be directly used by methanogens, while more complex organic substrates such as glucose require prior metabolism by other bacteria. Furthermore, the addition of BES alone or BES with added substrates stopped or decreased the aldicarb transformation in all three sediments. The effect of BES was not as great in lake and salt marsh sediments, suggesting that a different spectrum of bacteria was involved in aldicarb transformation in these environments.

Our studies with the microbial inhibitors molybdate and BES suggest the presence of competing pathways in terminal aldicarb metabolism in anoxic aquifer and salt marsh sediments. When molybdate alone was added to aquifer sediments, the CH_4/CO_2 ratio was significantly higher than that in the

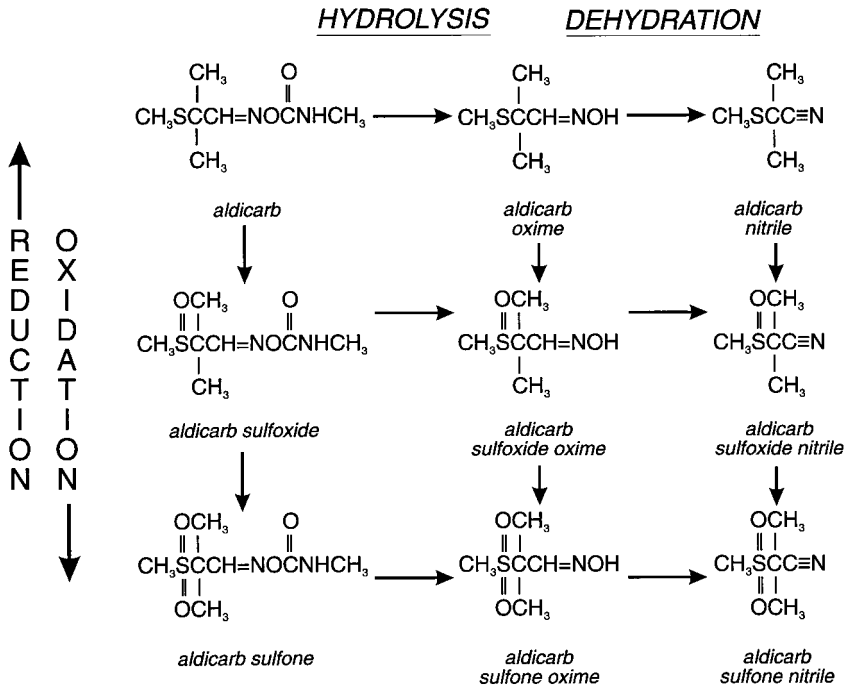

FIG. 8. Proposed aldicarb transformation pathways under oxic and anoxic conditions.

control (3.8 and 3.0, respectively; $P < 0.05$) (Fig. 3). A high CH_4/CO_2 ratio was also noted in anoxic salt marsh sediments in the presence of molybdate alone (1.8 and 0.5, respectively; $P < 0.05$) (Fig. 6). These results indicate that in the absence of sulfate-reducing bacteria, methanogens were able to produce more CH_4. Other studies have shown that in many anoxic environments, sulfate-reducing bacteria and methanogenic bacteria often compete for simple organic substrates (see reference 5 for a review). Methanogens may therefore be respiring aldicarb-derived products such as methylamine, allowing them to proliferate relative to sulfate-reducing bacteria in our assays with aquifer and salt marsh sediments.

Aldicarb transformation pathways under oxic and anoxic conditions. Aldicarb transformation mediated by microorganisms proceeded via different pathways under oxic and anoxic conditions. Figure 7 shows the loss of aldicarb and the appearance of transformation products in salt marsh sediments over 21 days. In the presence of O_2, the aldicarb concentration decreased over 21 days with a concomitant increase in the amounts of aldicarb sulfoxide and aldicarb sulfone (Fig. 7A). In anoxic sediments, in contrast, the major transformation products were aldicarb oxime, aldicarb sulfoxide, and aldicarb sulfoxide oxime (Fig. 7B). In both cases, at least 85% of the initial compound was accounted for. These results are similar to those of other studies which showed microbial oxidation of aldicarb in oxic soil microcosm and batch studies (25, 28) and microbial hydrolysis in anoxic soil column and well water incubations (28, 30). The difference in transformation pathways is notable in light of higher aldicarb transformation under anoxic conditions. In the absence of O_2, hydrolysis of aldicarb was the predominant pathway, with aldicarb oxime and aldicarb sulfoxide oxime (and presumably methylamine) forming the major residues of aldicarb transformation; in the presence of oxygen, these hydrolysis products were not formed (Fig. 8). These results suggest that in anoxic sediments, microbially mediated hydrolysis of aldicarb can yield products which can be of benefit to natural populations of methanogens present in sediments.

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