Effect of Culture Acclimation on the Kinetics of Aldicarb Insecticide Degradation under Methanogenic Conditions

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This study reports on the kinetics of aldicarb transformation under methanogenic conditions using batch reactors containing acclimated and unacclimated cultures under controlled conditions. Culture acclimation was accomplished by exposing anaerobic microorganisms maintained in a semi-batch reactor to low concentrations of aldicarb. Results of the kinetic studies showed that in an anaerobic system aldicarb is converted to aldicarb nitrile by the hydrolytic pathway. Analysis of the hydrolysis/dehydration rate constants showed that anaerobic cultures enhanced the rate of conversion of aldicarb by 4-fold for acclimated cultures and by 2-fold for unacclimated cultures compared to the rate of abiotic hydrolysis ($p < 0.05$). Only the acclimated cultures were able to further mineralize the reaction intermediate aldicarb nitrile. Michaelis−Menten and Monod kinetics adequately defined the aldicarb nitrile degradation.

Keywords: *Aldicarb; biodegradation; anaerobic; culture acclimation; kinetics*

INTRODUCTION

Aldicarb [2-methyl-2(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime], an active ingredient in the insecticide Temik, is a soil insecticide used in the agricultural sector worldwide for over 25 years for the control of insects, mites, and nematodes (Baron, 1994). Aldicarb supplied as a powder is applied (drilled) into the soil, where it is solubilized and distributed by the groundwater, is adsorbed by the roots and translocated throughout the plant, and serves as a systemic insecticide. Therefore, aldicarb's propensity to contaminate aquifers due to heavy application and pervious soil conditions is great. In fact, there have been reports of groundwater contamination by aldicarb in the central sand plains of Wisconsin (Rothschild et al., 1982) and in Suffolk County, New York (Zaki et al., 1982).

There have been reports of microbial mineralization of aldicarb under anaerobic conditions (Ou et al., 1985; Kiene and Capone, 1986). Also, Vink and van der Zee (1997) reported on the increase in aldicarb transformation with decreasing redox potential (anaerobic conditions) in soil microcolumns and anaerobic lake sediments. A prior study conducted by the authors showed that aldicarb was neither acutely nor chronically toxic to active anaerobic cultures maintained in serum bottle reactors (Khandaker and Young, 1997). This opened the possibility that anaerobic cultures may be exposed to concentrations of aldicarb under controlled engineered conditions to achieve culture acclimation toward the degradation of aldicarb. This paper reports on an investigation whose objectives were (1) to determine the potential of acclimating anaerobic microorganisms to-

ward the degradation of aldicarb, (2) to study the kinetics of aldicarb degradation by using acclimated and unacclimated cultures, and (3) to mathematically model the kinetics of aldicarb degradation.

The ability to acclimate anaerobic cultures toward the degradation of aldicarb and elucidating the kinetics of aldicarb degradation would facilitate: (1) the design and operation of anaerobic systems for the treatment of groundwater contaminated with aldicarb, and (2) the design of in situ anaerobic remediation systems for aldicarb-contaminated waterlogged soils under anaerobic conditions.

EXPERIMENTAL PROGRAM

The experimental program was divided into three steps. Step I involved the development of acclimated cultures by exposing anaerobic microorganisms to low concentrations of aldicarb, under controlled conditions. In step II the acclimated cultures were transferred to test reactors and dosed with various concentrations of aldicarb. The residual aldicarb and persistent reaction intermediate concentrations were tracked over time and fitted to kinetic equations using nonlinear curve fitting. Step III was essentially a repeat of step II, but using a culture that had never been exposed to aldicarb. The reason for carrying out the third step was to investigate the effect of culture acclimation on the kinetics of aldicarb mineralization.

MATERIALS AND METHODS

Chemicals. Aldicarb and aldicarb nitrile were supplied by Chemical Service, West Chester, PA, and Rhone-Poulenc, Research Park, NC, respectively.

Analytical Methods. Measurement of insecticide residual and reaction intermediates were carried out by liquid chro-

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matography using a Supelco LC-8 reverse phase column, the mobile phase being water and methanol at a ratio of 1:1. The mobile phase flow rate was 1.5 mL/min, and the UV detector was set at 245 nm for aldicarb and aldicarb nitrile detection. The liquid chromatograph used was a Waters high-pressure liquid chromatograph (Khandaker, 1995). Analysis of volatile organic acids was conducted by gas chromatography, using a Varian 3300 gas chromatograph, equipped with a flame ionization detector and a 1.8 mm by 2 m gas column, packed with carbopack B-DA on 80/120 mesh 4% carbowax 20 M, and operated isothermally at 175 °C. Nitrogen was used as the carrier gas at a flow rate of 40 mL/min (Kim, 1991). The gas production was measured by the syringe method (Johnson and Young, 1983). The biomass concentration was measured by the glass fiber filter method (Standard Methods, 1980).

Master Culture Reactors (Step I). The cultures used in batch kinetic tests in step II (batch kinetic tests with acclimated cultures) were transferred from a master culture reactor containing active anaerobic cultures of constant composition. The acclimated master culture reactor was fed ethanol at a chemical oxygen demand (COD) loading of 1.0 g COD/L·d along with *1.6 mg/L·d of aldicarb.* Steady-state conditions were confirmed by monitoring daily gas production and weekly analysis of pH, fatty acids concentrations, and biomass concentrations. To ensure culture acclimation, the master culture reactor was operated in a semi-batch mode at a 20-day solid and hydraulic retention time for three hydraulic retention times (60 days) (Khandaker, 1995).

Anaerobic seed cultures used in step III (batch kinetic tests with unacclimated cultures) were transferred from an ethanol enriched master culture reactor. The ethanol master culture reactor was maintained at a solids retention time of 20 days and an organic loading of 1.0 g COD/L·d. This master culture reactor received ethanol as the sole substrate and was operated under steady-state conditions for at least three hydraulic and solids retention times prior to the initiation of any tests.

Before any culture was transferred from the master culture reactors, it was ensured that the total gas production was within the quality control limit of 500 mL/g COD ethanol loading ± 12% (2 std dev) (Young and Tabak, 1993).

Batch Kinetic Tests (Step II and Step III). The batch kinetic tests were conducted by transferring 200 mL of seed culture from the acclimated aldicarb master culture reactor for step II and the ethanol master culture reactor for step III to each of a number of 250 mL serum bottles under anaerobic conditions. Prior to the start of the tests, the cultures were stabilized for a period of 3−4 days, and only the reactors falling within the quality control limit of daily gas production of 500 mL/g COD ethanol added ± 12% (2 std dev) were used for further experimental work. A one-time slug dose of the insecticide was applied to the test units. Monitoring of gas production or sampling of the aqueous content of the test reactors was done at preset time intervals. Typically, 10 mL samples were removed from each test reactor for analysis.

Statistics. Results were expressed as mean ± SEM. Differences between means of the reaction rate constants were evaluated using one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Kinetic Models. The hydrolysis reaction of aldicarb was modeled using first-order kinetics defined by

$$Y(t)_a = A_o e^{-kt} \tag{1}$$

where $Y(t)_a$ = concentration of aldicarb at any time (*t*), mg/L; *t* = time, days; A_o = initial concentration of aldicarb in the reactor, mg/L; and *k* = first-order rate constant for aldicarb hydrolysis, day^{-1}.

Equation 2 predicts the aldicarb nitrile accumulation in the serum bottle reactors as determined by the stoichiometric conversion of aldicarb depletion, using the first-order conversion kinetic parameters derived from eq 1

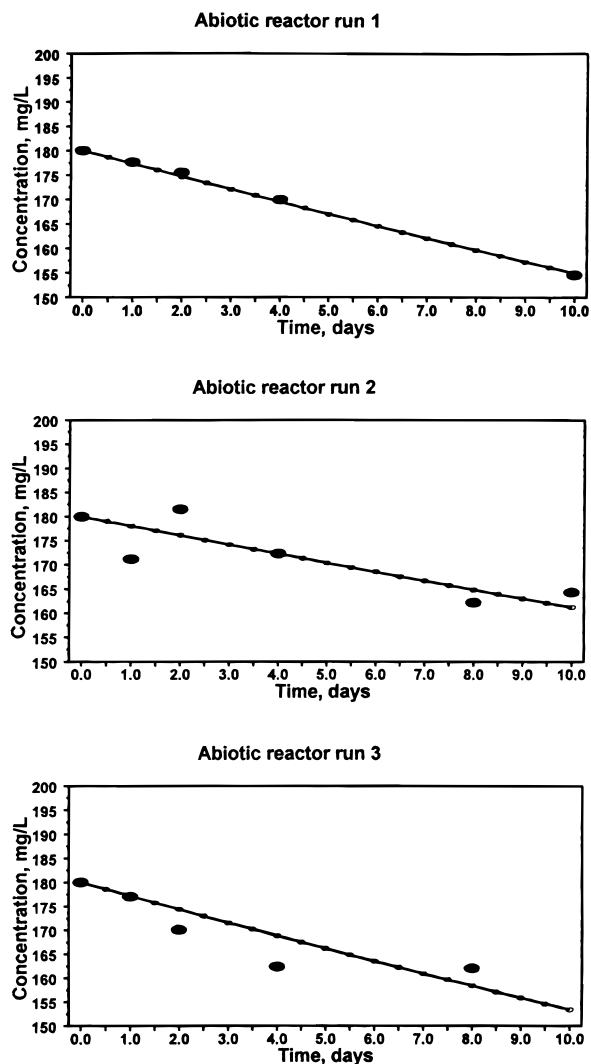$$Y(t)_n = (A_o - A_o e^{-kt}) MW_n / MW_a \tag{2}$$

Figure 1. Kinetic test results with the abiotic reactors showing the hydrolysis of aldicarb. Oversized symbols represent observed data, and small symbols with lines represent modeled data.

where $Y(t)_n$ = concentration of aldicarb nitrile at any time (*t*), mg/L; *t* = time, days; A_o = initial concentration of aldicarb in the reactor, mg/L; *k* = first-order rate constant of aldicarb hydrolysis, day^{-1}; and MW_n / MW_a = ratio of molecular weight of aldicarb nitrile to aldicarb with a 1:1 stoichiometric relationship between aldicarb depletion and aldicarb nitrile formation.

Microbial mineralization of the rate-limiting substrate (aldicarb nitrile) was modeled by eqs 3 (Michaelis−Menten, 1913) and 4 (Monod, 1949)

$$\frac{dS}{dt} = -\frac{k_1 SM}{K_s + S} = -\frac{U_m}{Y} \frac{SM}{K_s + S} \tag{3}$$

$$\frac{dM}{dt} = \frac{Yk_1 SM}{K_s + S} - K_d M \tag{4}$$

where:

dS/dt = rate of substrate utilization, mg/day;
dM/dt = net growth rate of microorganisms, mg/day;
S = concentration of growth-limiting substrate, mg/L;
k_1 = maximum specific substrate utilization rate, mg of substrate/(mg of biomass·day);
K_s = half-saturation coefficient, mg/L;
Y = growth yield coefficient, mg of solids/mg of substrate;
K_d = decay coefficient, day^{-1}; and
U_m = maximum specific growth rate, day^{-1}.

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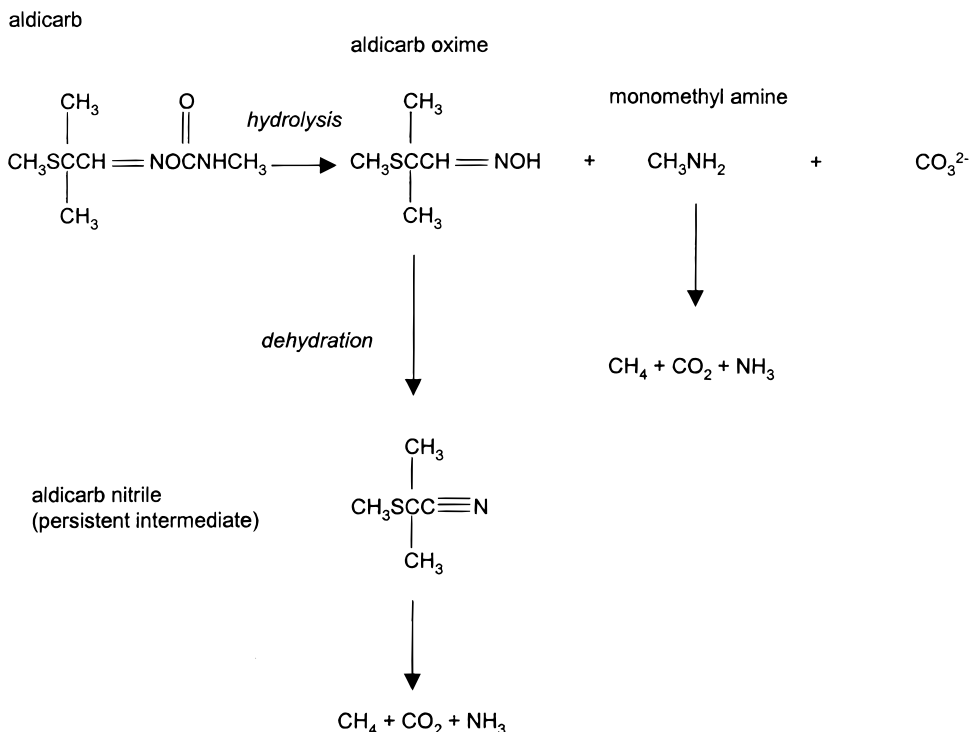*J. Agric. Food Chem.,* Vol. 48, No. 4, 2000 **1413**

Figure 2. Proposed microbial transformation of aldicarb under methane-generating conditions.

Table 1. Kinetic Coefficients for Aldicarb Conversion by Hydrolysis/Dehydration to Aldicarb Nitrile

	first-order hydrolysis/dehydration rate day^{-1} (CV %)		
test run	acclimated	unacclimated	abiotic
1	0.039 (21.9)	0.024 (23.52)	0.015 (1.79)
2	0.043 (24.9)	0.019 (38.90)	0.011 (16.14)
3	0.064 (9.5)	0.029 (54.55)	0.016 (16.84)
4	0.094 (13.8)	0.051 (32.10)	
mean ± SEM	0.060 ± 0.012	0.031 ± 0.007	0.014 ± 0.002

RESULTS AND DISCUSSION

Preliminary Tests. Based on reports of nonmicrobial hydrolysis of aldicarb (Hansen and Spiegel, 1983; Lemley and Zhong, 1984; Given and Dierberg, 1985), an experimental program was carried out to investigate the chemical hydrolysis potential of aldicarb. The experimental program was designed to reflect the prevailing chemical environments present in the batch anaerobic reactors used in step II and step III of the study. The test was initiated by adding 100 mL of sterilized distilled water augmented with minerals and bicarbonate buffer to three sterilized 250 mL serum bottle reactors. The reactors were maintained at a pH of 7.2 and a temperature of 35 °C. The liquid content and the headspace of the reactors were flushed with nitrogen gas to ensure low oxygen potential. The reactors were then dosed with aldicarb and sealed with a rubber septum. At preset time intervals, 5 mL samples were withdrawn and analyzed for aldicarb concentration. The resulting variation of aldicarb concentrations with time was modeled using eq 1 and is presented in Figure 1. The results showed that first-order kinetics adequately defined the chemical hydrolysis of aldicarb, with the mean hydrolysis rate constant of 0.014 ± 0.002 day^{-1} at a pH of 7.2 and a temperature of 35 °C (Table 1).

Prior studies indicated that, under oxygen-limiting conditions, aldicarb is converted by anaerobic microorganisms via the hydrolytic pathway to aldicarb oxime and monomethylamine. The aldicarb oxime is further dehydrated to aldicarb nitrile (Lemley and Zhong, 1984; Miles and Delfino, 1985). The monomethylamine is rapidly mineralized to methane and carbon dioxide, whereas the aldicarb nitrile is more persistent (Kiene and Capone, 1986). To verify the prior findings that, under strictly methanogenic conditions, the persistent reaction intermediate is aldicarb nitrile, 100 mL of anaerobic culture was transferred from the aldicarb master culture reactor to a 250 mL serum bottle reactor under anaerobic conditions. The serum bottle reactor was dosed with 400 mg of aldicarb and incubated for 7 days. At day seven, a 10 mL sample was withdrawn from the reactor and analyzed by chemical ionization spectrometric analysis for aldicarb, aldicarb oxime, and aldicarb nitrile, run by direct insertion probe, using a Kratos MS-25 mass spectrometer. The ionization gas used was isobutane at the source temperature of 125 °C. The analysis revealed the significant presence of aldicarb nitrile (molecular weight of 115, MH$^+$ = 116, M$^+$ = 115) and a weak signal of aldicarb oxime (molecular weight of 145, MH$^+$ = 146, M$^+$ = 145) and aldicarb (molecular weight of 190, MH$^+$ = 191, M$^+$ = 190), substantiating the prior findings that aldicarb nitrile is the persistent reaction intermediate in the microbial mediated mineralization of aldicarb under strictly anaerobic conditions (Figure 2).

Batch Kinetic Tests Using Acclimated and Unacclimated Cultures. The results of the batch kinetic tests with acclimated and unacclimated culture reactors are shown in Figures 3 and 4, respectively. The results indicated that aldicarb was converted by the hydrolysis/dehydration reaction to aldicarb nitrile in both the acclimated and unacclimated culture reactors. First-order reaction kinetics (eq 1) adequately defined the conversion reaction (Table 1). The mean first-order hydrolysis/dehydration rate constants for the acclimated (0.060 ± 0.012 day^{-1}) and unacclimated (0.031 ± 0.007 day^{-1}) culture reactors were greater than the first-order
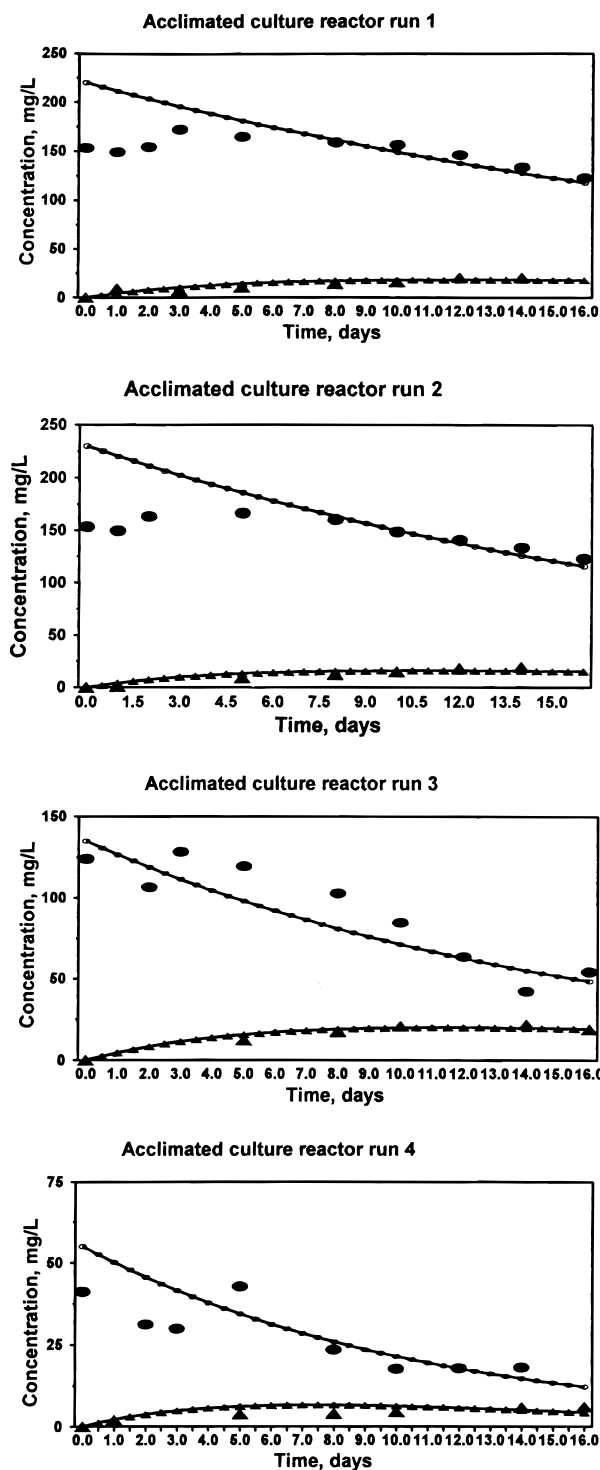
Figure 3. Kinetic test results with the acclimated culture reactors showing the conversion of aldicarb and the accumulation and sequential degradation of aldicarb nitrile. Oversized symbols represent observed data, and small symbols with lines represent modeled data.
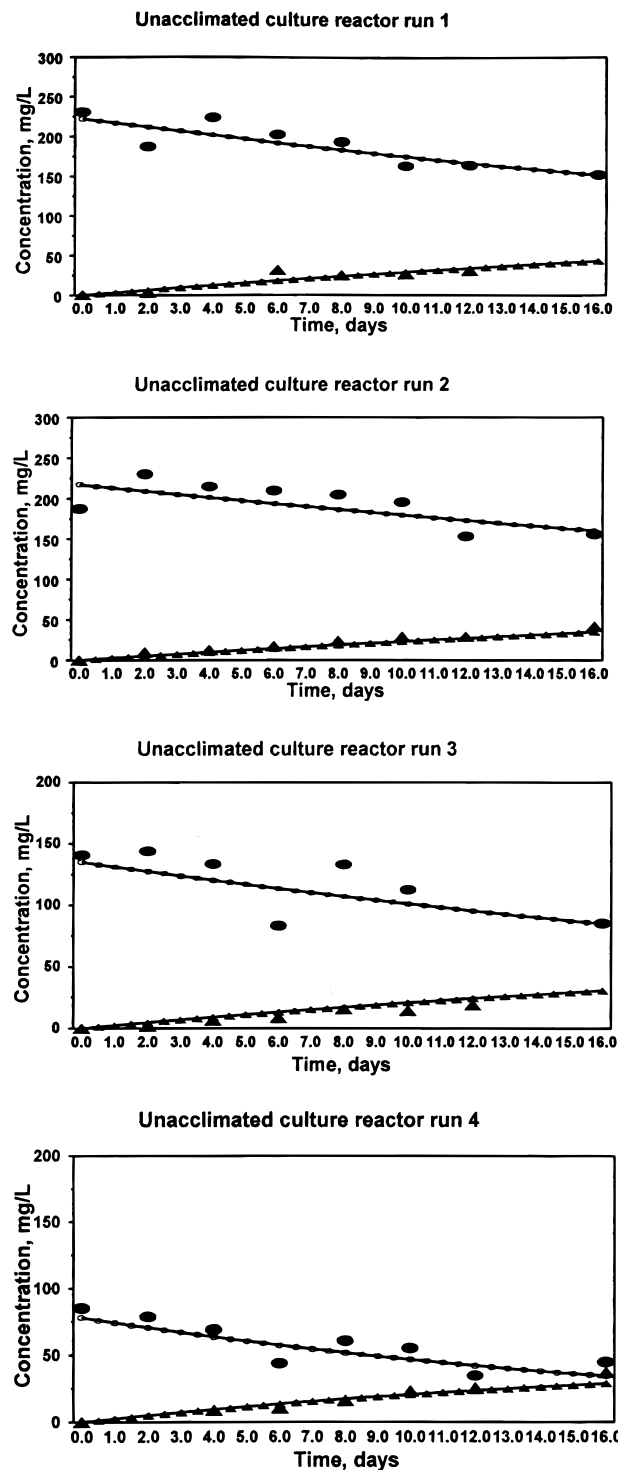
Figure 4. Kinetic test results with the unacclimated culture reactors showing the conversion of aldicarb and the accumulation of aldicarb nitrile. Oversized symbols represent observed data, and small symbols with lines represent modeled data.

hydrolysis/dehydration rate constants of the abiotic reactors (0.014 ± 0.002 day^{-1}) ($p < 0.009$, acclimated vs abiotic; $p < 0.05$, unacclimated vs abiotic). This implies that anaerobic microorganisms enhance the rate of hydrolysis/dehydration of aldicarb to aldicarb nitrile and monomethylamine. Culture acclimation increased the mean first-order hydrolysis/dehydration rate constant of aldicarb hydrolysis from 0.031 ± 0.007 to 0.060 ± 0.012 day^{-1}, an increase of almost 94%.

Modeling of the observed aldicarb nitrile data for the unacclimated culture reactors using eq 2 indicated that there was a good correlation between the predicted and observed aldicarb nitrile concentrations (reactor 1, $r^2 = 0.805$; reactor 2, $r^2 = 0.852$; reactor 3, $r^2 = 0.962$; reactor 4, $r^2 = 0.681$). The increase in aldicarb nitrile concentration agreed stoichiometrically with the amount of aldicarb converted. In addition, the concentration of aldicarb nitrile in the reactors did not decrease with time for the duration of the test. In contrast, modeling

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Table 2. Kinetic Coefficient for Aldicarb Nitrile Degradation by Acclimated Culture Reactors

run	k_1 (day^{-1})	K_s (mg/L)	Y (mg/mg)	K_d (day^{-1})
1	1.2	4.0	0.13	0.024
2	1.4	0.4	0.13	0.024
3	0.9	5.0	0.13	0.024
4	0.9	2.6	0.13	0.024
mean ± SEM	1.1 ± 0.1	2.6 ± 0.9		

of the aldicarb nitrile data for the acclimated culture reactors using eqs 2−4, showed that aldicarb nitrile was further mineralized by the acclimated culture. The kinetic coefficients defining the biological degradation of aldicarb nitrile in eqs 3 and 4 are listed in Table 2. In eq 4 the biomass yield coefficient ($Y = 0.13$ mg/mg) was determined by thermodynamic principles (McCarty, 1971) based on the stoichiometric equation defining aldicarb nitrile mineralization, and is reported elsewhere (Khandaker, 1995). The initial concentration of the biomass was set at 1.3 mg/L, a value equal to 0.15% of the steady-state biomass concentration (830 mg/L) in the acclimated master culture reactor. The assumption that 0.15% of the biomass in the acclimated master culture was responsible for the mineralization of aldicarb nitrile was based on the daily feed COD loading ratio of aldicarb to ethanol in the acclimated master culture reactor. In addition, the decay coefficient for the aldicarb nitrile degrading biomass was set at 0.024 day^{-1} (Young and Tabak, 1993).

Least-squares simulation of eqs 2−4 to the observed aldicarb nitrile data yielded a mean specific substrate utilization rate (k_1) of 1.1 ± 0.1 day^{-1} and a mean half-saturation coefficient (K_s) of 2.6 ± 0.9 mg/L. There was also a good correlation between the predicted and observed aldicarb nitrile concentrations (reactor 1, $r^2 = 0.759$; reactor 2, $r^2 = 0.845$; reactor 3, $r^2 = 0.812$; reactor 4, $r^2 = 0.865$), indicating that eqs 3 (Michaelis−Menten, 1913) and 4 (Monod, 1949) adequately defined the aldicarb nitrile degradation. To validate the mineralization of aldicarb nitrile by the acclimated culture, a test was initiated with five serum bottle reactors containing anaerobic cultures transferred from the acclimated master culture reactor. Four reactors were dosed with 0.02 g of aldicarb, and one reactor was not dosed with any aldicarb and served as control. All five reactors were incubated for 16 days, and the methane gas generated by all the reactors at day 17 was measured and noted as excess methane gas above that of the base control. The results indicated that 2.86 ± 0.18 mol of methane gas was generated per mole of aldicarb dose at the end of 16 days of incubation. If aldicarb nitrile was not being mineralized, then only 1.74 mol of methane could be generated based on the complete mineralization of monomethylamine, a minor product of aldicarb hydrolysis (Kiene and Capone, 1986). The theoretical methane yield of 1.74 mol for monomethylamine mineralization was based on the stoichiometric equation for aldicarb hydrolysis/dehydration to monomethylamine and aldicarb nitrile (eq 5), and with the subsequent mineraliza-

$$C_7H_{14}N_2O_2S + 2OH^- \rightarrow$$
$$C_5H_9NS + H_2O + CH_3NH_2 + CO_3^{2-} \quad (5)$$
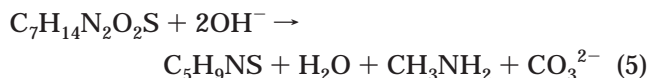
tion of monomethylamine (eq 6), and by the method of

$$CH_3NH_2 + 2.5H_2O \rightarrow 1.75CH_4 + 1.25CO_2 \quad (6)$$
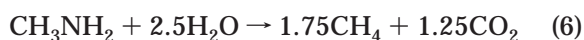
Buswell and Mueller neglecting the nitrogen fraction (1952).

The result of the methane generation study substantiates the findings of the acclimated culture batch kinetic tests, which showed that aldicarb nitrile was being mineralized.

CONCLUSIONS

In an overall perspective from this experimental program, one can conclude that under anaerobic conditions aldicarb is converted to aldicarb nitrile and that active anaerobic cultures enhance the rate of aldicarb conversion. In addition, only acclimated anaerobic cultures can further mineralize the metabolic intermediate aldicarb nitrile. Culture acclimation was accomplished by exposing anaerobic cultures to low concentrations of aldicarb for an extended period of time.

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LITERATURE CITED

Baron, R. L. A carbamate insecticide: a case study of aldicarb. *Environ. Health Perspect.* **1994**, *102* (Suppl. 11), 23−27.

Buswell, A. M.; Mueller, H. F. Mechanism of methane fermentation. *Ind. Eng. Chem.* **1952**, *44*, 550−552.

Ghosh, M. M. *Standard Methods for the Examination of Water and Wastewater*, 15th ed.; Greenberg, A. E., Connors, J. J., Jenkins, D., Eds.; American Public Health Association: Washington, DC, 1989.

Given, C. J.; Dierberg, F. E. Effect of pH on the rate of aldicarb hydrolysis. *Bull. Environ. Contam. Toxicol.* **1985**, *34*, 627−633.

Hansen, J. L.; Spiegel, M. H. Hydrolysis studies of aldicarb, aldicarb sulfoxide and aldicarb sulfone. *Environ. Toxicol. Chem.* **1983**, *2*, 147−153.

Johnson, L. D.; Young, J. C. Inhibition of anaerobic digestion by organic priority pollutants. *J. Water Pollut. Control Fed.* **1983**, *55*, 1441−1449.

Khandaker, N. R. Fate and effect of aldicarb, carbaryl, and carbofuran insecticides in methanogenic environments. Ph.D. Thesis, Pennsylvania State University, University Park, 1995.

Khandaker, N. R.; Young, J. C. Kinetic assessment of the effect of carbamate insecticides on anaerobic transformation of cosubstrate reactions. *J. Environ. Sci. Health* **1997**, *B32* (1), 107−125.

Kiene, R. P.; Capone, D. G. Simulation of methanogenesis by aldicarb and several other *N*-methyl carbamate pesticides. *Appl. Environ. Microbiol.* **1986**, 1247−1251.

Kim, I. S. Effect of toxic organic chemicals on the kinetics of acetogenesis in anaerobic digestion. Ph.D. Thesis, University of Arkansas, Fayetteville, 1991.

Lemley, A. T.; Zhong, W. Hydrolysis of aldicarb, aldicarb sulfoxide, and aldicarb sulfone at parts per billion levels in aqueous mediums. *J. Agric. Food Chem.* **1984**, *32*, 714−719.

McCarty, P. L. Energetics and kinetics of anaerobic treatment. *In Anaerobic Biological Treatment Processes*; Gould, R. F., Ed.; Advances in Chemistry Series 105; American Chemical Society: Washington, DC, 1971.

Michaelis, L.; Menten, M. L. Die kinetik der invertinwirkung. *Biochem. Z.* **1913**, *49*, 333.

Miles, C. J.; Delfino, J. J. Fate of aldicarb, aldicarb sulfoxide, and aldicarb sulfone in Floridine groundwater. *J. Agric. Food Chem.* **1985**, *33*, 455−460.

Monod, J. The growth of bacterial cultures. *Annu. Rev. Microbiol.* **1949**, *3*, 371−394.

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Ou, L.-T.; Sture, K.; Edvardsson, V.; Suresh, P.; Rao, C. Aerobic and anaerobic degradation of aldicarb in soils. *J. Agric. Food Chem.* **1985**, *33*, 72−78.

Rothschild, E. R.; Manser, R. J.; Anderson, M. P. Investigation of aldicarb in groundwater in selected areas of the central sand plain of Wisconsin. *Groundwater* **1982**, 20, 437−445.

Vink, J. P. M.; van der Zee, S. E. A. T. M. Effect of oxygen status on pesticide transformation and sorption in undisturbed soil and lake sediment. *Environ. Toxicol. Chem.* **1997**, *16* (4), 608−616.

Young, J. C.; Tabak, H. H. Multilevel protocol for assessing the fate and effect of toxic organic chemicals in anaerobic treatment processes. *Water Environ. Res.* **1993**, *65* (1), 34−45.

Zaki, M. H.; Moran, D.; Harris, D. Pesticides in groundwater: the aldicarb story in Suffolk County, NY. *Am. J. Public Health* **1982**, *72* (12), 1391−1395.