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Comparison of Occurrence and Rates of Chemical Biodegradation in Natural Waters

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Anthropogenic materials can enter natural waters through domestic and industrial effluents, agricultural run-off, seepages from surface and sub-surface chemical repositories, atmospheric deposition, and accidental spills. An assessment of the biological effects of chemicals in aquatic environments requires an understanding of the fate of such xenobiotic compounds. Biodegradation may be a principal mechanism which dictates the longevity of chemical pollutants in terrestrial and aquatic environments (Bollag 1974). However, it is the environment that actually controls the biodegradation process and has a greater influence on the process than the microorganisms *per se* (Kaplan 1979).

In recent years attempts have been made to measure chemical biodegradation in natural waters and to characterize the influence of particular environmental conditions on the process (Howard and Banerjee 1984, Larson 1983, Paris et al. 1981, Rubin et al. 1982, Rubin and Alexander 1983, Wang et al. 1984). Many of these studies, however, have generally focused on homologous chemicals and mesotrophic lotic or lentic systems. Consequently, a comparison of biodegradation for the same chemicals in diverse natural waters cannot readily be made. Information derived from such comparisons can help improve the understanding of interactions between environment, substrate and microorganisms, and may facilitate the transposition of laboratory data to the native environment. Thus, the present study was conducted to measure and compare the degradation of ten organic chemicals in ground, Lester river and Superior harbor waters by autochthonous microorganisms.

MATERIALS AND METHODS

Ground water was sampled at the tap of a 42 m submersible-pump well located about 12 km north of Lake Superior. Prior to sampling, approximately 20 L of the water were voided. River water was collected by surface (upper 15 cm) sampling from the Lester River. The sources of both waters were located in southern St. Louis County, MN. Superior harbor water was sampled (upper 15 cm) at a station located about 0.5 km northeast of Barker's Island

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in the main navigation channel of Superior Bay, Superior, WI. Each water was filtered by gravity flow through 1-cm-thick cotton layer. Aeration was started within 1 h of sampling and continued for about 24 h, at which time the water was used in biodegradation tests. All water samples were procured between May-July, 1985, during periods of similar meteorological conditions.

Microbiological and physicochemical analyses of the waters were made using unfiltered and filtered samples, respectively. The latter were done in triplicate by standard methods (APHA 1980) and included pH, conductivity, nephelometric turbidity, total, dissolved and suspended solids, alkalinity, hardness, nitrite/nitrate, ammonia, and orthophosphate.

Biodegradation of ten organic chemicals (analytical grade) in each water was separately measured by the BOD technique (APHA 1980). A test chemical was added into 20 mL of water contained in a 300-mL BOD bottle. The bottles were filled to capacity with the same water, and sealed and incubated in duplicate at 21 ± 3^{o}C. Chemical concentrations of 0.0, 0.8, 1.6 and 3.2 mg/L (solids) or µL/L (liquids) were employed. Controls to assess the water quality were included. Dissolved oxygen (DO) concentrations at 0, 5, 10, 14 or 15, and 20 days of incubation were determined using a YSI 54 oxygen meter equipped with a self-stirring probe. The DO concentrations in randomly selected bottles were measured by the azide modification of the iodometric method (APHA 1980).

Test BOD values showing unadjusted DO depletions of at least 2 mg/L and residual DO of at least 1 mg/L were acceptable. These values were adjusted for the water blank and then used for calculating the mean BODs from duplicate tests. The latter were transformed to mmol BOD/mmol chemical for each applicable concentration and subsequently used for computing the percents of theoretical (Th) BOD. The mean BOD values obtained at two different concentrations were also compared to determine the order (n) of chemical biodegradation process (Barrow 1979). It was found that all chemicals followed first-order kinetics, and thus, chemical half-lives were estimated from the first-order biodegradation rate constants.

Biodegradation of four chemicals, chosen from the above ten, was selectively assessed in ground, river and harbor waters supplemented with nutrients and/or acclimated microbial seeds. The latter were of wastewater origin and isolated by a conventional enrichment culture technique (Vaishnav and Lopas 1985). Chemical biodegradation was monitored as described earlier, except the natural water received phosphate buffer solution (1 mL/L) and/or acclimated microbial seed (about 10^5 cells/300 mL). The buffer contained 8.6 mg KH_2PO_4, 21.8 mg K_2HPO_4, 33.4 mg $Na_2HPO_4 \cdot 7H_2O$ and 1.8 mg NH_4Cl in each mL of distilled water (APHA 1980). Initial and 5-(with added nutrients and/or acclimated microbes) or 14-day (with added nutrients) DO concentrations were determined and acceptable test BODs were adjusted for the control values. Biodegradation rate constants and half-lives were derived for the test chemicals. These values were compared to gauge the effects of both individual and a combination of supplements on chemical biodegradation in natural waters.

RESULTS AND DISCUSSION

Standard plate counts on nutrient agar showed that bacterial concentrations in ground, river and harbor waters were 55, 420 and 310 cells/mL, respectively. The agar plates with ground water sample produced seven types of morphologically distinct colonies and those with the river water sample produced six types of distinct colonies. Similarly, the bacterial concentration in harbor water was distributed among five types of morphologically diverse colonies.

Nitrogen and phosphorus concentrations were relatively low in the ground water, and that of phosphorus was low in the river water (Table 1). Other physicochemical characteristics, except total solids of river and harbor waters were generally comparable (Table 1).

Table 1. Physicochemical characteristics of ground (GW), Lester River (RW) and Superior harbor (HW) waters.

Characteristic	GW	RW	HW
pH	8.23	7.76	7.56
Conductivity (μmhos/cm)	322.65	148.50	138.20
Nephelometric turbidity	0.76	1.19	10.90
		mg/L	
Total solids	170.3	93.2	131.7
Dissolved solids	156.7	80.7	109.0
Suspended solids	<1.2	<1.2	5.6
Alkalinity (as $CaCO_3$)	122.60	51.80	40.90
Hardness (as $CaCO_3$)	121.35	58.59	50.10
Nitrite/nitrate-N	<0.05	<0.05	0.15
Ammonia-N	0.07	0.59	1.03
Orthophosphate-P	<0.01	<0.01	0.02
Total sulfides			
pre-aeration	0.15	ND[a]	ND
post-aeration	<0.02	ND	ND

[a] ND = Not determined.

Ten chemicals from various chemical classes were selected for measuring biodegradation. Their water solubilities ranged from about 7 ng/L for hexadecane to about 17 g/L for isopentyl alcohol. Test concentrations used for each chemical, except hexadecane, were below the chemical water solubility.

The extent (% ThBOD) of chemical biodegradation and duration of incubation were correlated for each water (Fig 1). There were five chemicals that were biodegraded in all three waters, however, their % ThBODs varied among the water types. For example, 88% of the initial phenol concentration was oxidized in 15 days in the harbor water, but 31% was oxidized in the ground water during the same incubation period. These differences for 2-decanone and isopentyl alcohol biodegradation decreased with increased incubation, and were less than 12% for the three waters at 20 days. Variations among chemical biodegradation rates in natural waters can arise from the differences in physicochemical and/or microbial composition of waters (Floodgate 1979, Spain et al. 1980).

There were eight chemicals that were biodegraded in one or more water types. For these chemicals, linear relationships were observed between the amount of biodegradation and incubation time for up to 10-15 days (Fig 1). For each chemical, natural logarithms of the percentages of initial concentration which remained were correlated with time. This produced a linear relationship in which the slope of the line was regarded as the first-order biodegradation rate constant (Barrow 1979). Correlation coefficients of 21 linear regression equations for all three water types ranged from -0.933 to -1.000 for benzene in river and ground waters, respectively, with a mean value of -0.985 ± 0.018.

Chemical half-lives ranged from 3 days for phenol in the harbor water to 50 days for amyl acetate in the ground water (Table 2). The half-lives for hexadecane may be overestimates, since the added chemical concentrations were in excess of the theoretical water solubility (7.4 ng/L). Generally, chemical half-lives were the shortest in the harbor water, followed by those in the river and ground waters. Increased chemical biodegradation rates in the harbor water could have been due to the water fertility and/or the presence of microorganisms that were pre-exposed to test or similar chemicals. The latter was possible because trace levels of organic acids, aldehydes, ketones, amines, esters, alcohols, phenols, polychlorinated biphenyls, and polynuclear aromatic hydrocarbons have been detected in the harbor water and sediment samples (Bahnick and Markee 1985).

Benzene, 4-tertbutylbenzoic acid, hexadecane, N-methylaniline, and naphthalene were found resistant to biodegradation in one or more water types (Table 2). This recalcitrance in natural waters was also observed for the respective chemicals by Hart et al. (1975) and Traxler and Bhattacharya (1978). Available forms of nitrogen and phosphorus (Floodgate 1979), and/or the lack of emergence of an active microbial community (Spain et al. 1980) are frequently implied to impede chemical biodegradation in natural waters. Accordingly, all of these chemicals except N-methylaniline were incubated in the respective supplemented natural waters in which they had otherwise failed to undergo biodegradation (Fig 1, Table 2). The waters were amended with sources of nitrogen and phosphorus and/or acclimated microbes.

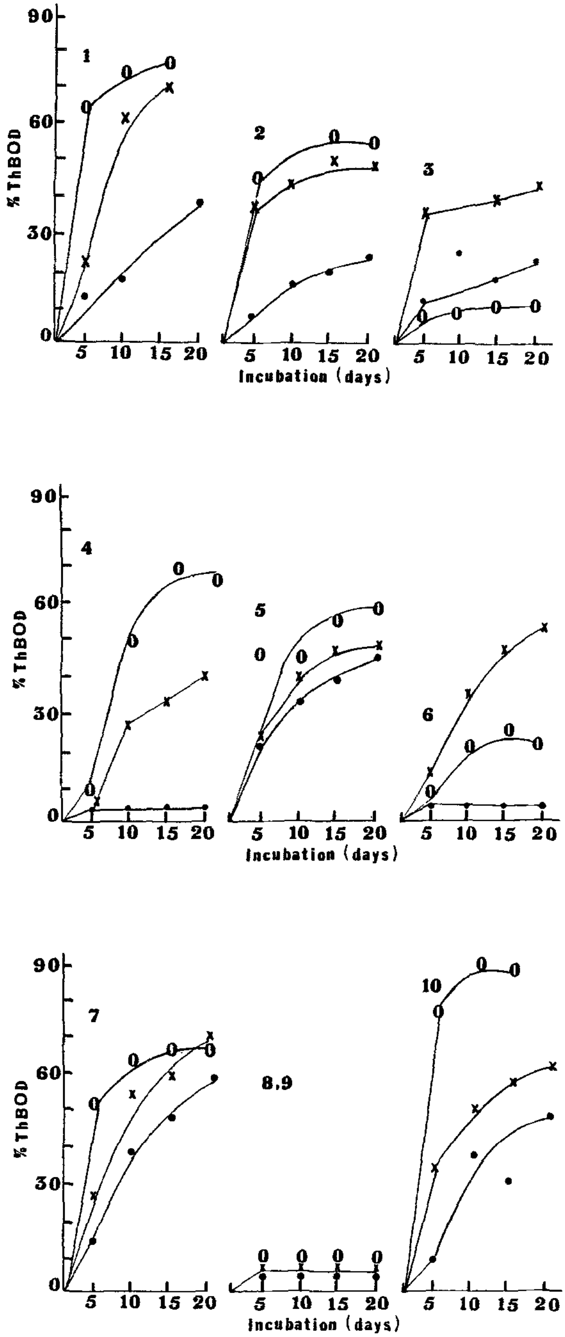

Fig 1. Biodegradation extents of (1) acetophenone, (2) amyl acetate, (3) benzene, (4) 4-tertbutylbenzoic acid, (5) 2-decanone, (6) hexadecane, (7) isopentyl alcohol, (8) N-methylaniline, (9) naphthalene and (10) phenol in ground (•), Lester River (x) and Superior harbor (o) waters.

Table 2. First-order biodegradation rate constants (per day) and half-lives (days) of selected chemicals in ground (GW), Lester River (RW) and Superior harbor (HW) waters.

Chemical	Rate constant[a] and half-life (in parenthesis) GW		RW		HW	
Acetophenone	0.022	(32)	0.083	(8)	0.155	(4)
Amyl acetate	0.014	(50)	0.054	(13)	0.069	(10)
Benzene	0.025	(28)	0.044	(16)	NC[b]	
4-tertButylbenzoic acid	NC		0.027	(26)	0.073	(9)
2-Decanone	0.032	(22)	0.045	(15)	0.061	(11)
Hexadecane	NC		0.039	(18)	0.023	(30)
Isopentyl alcohol	0.045	(15)	0.064	(11)	0.113	(6)
N-Methylaniline	NC		NC		NC	
Naphthalene	NC		NC		NC	
Phenol	0.035	(20)	0.065	(11)	0.247	(3)

[a] ± 11% mean SE for all determinations in three water types.

[b] NC = Not calculated, as difference between test and control BOD values was insufficient.

Table 3. Effects of nutrient and microbial additions on first-order biodegradation rate constants (per day) and half-lives (days) of selected chemicals in ground (GW), Lester River (RW) and Superior harbor (HW) waters.

Chemical and water type	Rate constant[a] and half-life (in parenthesis) in water supplemented with nutrients		acclimated microbes		nutrients and acclimated microbes	
Benzene						
HW	NC[b]		NC		0.082	(8)
4-tertButyl-benzoic acid						
GW	0.029	(24)	0.008	(87)	0.062	(11)
Hexadecane						
GW	0.009	(77)	0.044	(16)	0.031	(22)
Naphthalene						
GW	NC		NC		0.024	(28)
RW	NC		0.013	(53)	0.018	(39)
HW	NC		0.016	(43)	0.018	(39)

[a] ± 12% mean SD for all determinations in three water types.

[b] NC = Not calculated, as difference between test and control BOD values was insufficient.

Ground water added with nutrients could moderately sustain the biodegradation of 4-tertbutylbenzoic acid and that to a lesser extent of hexadecane (Table 3). Biodegradation of the latter, however, was enhanced when the water received acclimated microorganisms. Similarly, river and harbor waters supplemented with nutrients failed to support benzene or naphthalene biodegradation, but the latter did occur when the waters received active microbes. Generally, chemicals were found to have biodegraded more in the waters enriched with both nutrients and microbes than in those receiving either amendment alone.

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