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Comparison of Chemical Biodegradation Rates in BOD Dilution and Natural Waters

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Laboratory studies of chemical biodegradation are generally conducted using synthetic media inoculated with sewage, natural water, soil or hydrosoil. They tend to focus, for example, on identification of intermediate metabolites and end products, determination of mineralization rates and other kinetic parameters, and evaluation of physicochemical factors affecting the rates. Results from such studies provide the bases for appraising chemical persistence in native environments. However, inspection of the literature shows the behavior pattern of a chemical in the field can vary from that predicted by the laboratory characterization (Kaplan 1979). Consequently, legitimate questions have been raised about the reliability of biodegradability screening tests, and the ability of such tests to predict the fate of compounds in "real world" (Alexander 1981).

In view of the above, the objectives of this study were: (1) to measure biodegradation rates of nine chemicals in BOD dilution water, and (2) to compare the rates with those reported in literature for the same chemicals in five natural waters. The comparison allowed an assessment of the environmental relevance of rates measured using BOD dilution water.

MATERIALS AND METHODS

Mixed microbial cultures capable of using eight phenols, an ester of 2,4-D, and glucose (all analytical grade) as sole carbon and energy sources were separately isolated by an enrichment culture technique (Vaishnav and Lopas 1985). The cells were then grown in mineral salts medium (MSM) containing 100 mg/L (solid) or 100 µL/L (liquid) chemical substrate. The biomass was centrifuged, suspended in physiological saline and incubated on a shaker at 150 rpm for 24 h. Following incubation, the stock inoculum was diluted to 7.0×10^7 cells/mL for use in biodegradation tests.

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Generation times (tg) of cultures metabolizing 3.2 or 100 mg/L phenol, p-bromophenol, p-acetylphenol and glucose were determined in 300-mL BOD bottles each containing 200 mL of BOD dilution water (APHA 1980) or MSM. Duplicate bottles were inoculated to contain approximately 10^3 cells/mL and incubated at 21 ± 3° C for 8 days. Samples were removed at regular time intervals and duplicates combined. Each pooled sample was then diluted and plated in triplicate on MSM agar containing 100 and 250 mg/L corresponding chemical substrate. Plates were incubated in sealed plastic bags for 21 days. Subsequently, CFU were counted and tg were calculated (Koch 1981).

Degradation of eight phenols and an ester of 2,4-D by 2.3 x 10^8 microorganisms/L was measured separately by the BOD technique (APHA 1980). A test chemical and 1 mL of the diluted inoculum were added to 20 mL of dilution water in a 300-mL BOD bottle. Duplicate bottles were filled to capacity with the same water, and sealed and incubated at 21 ± 3° C. An inoculum control and chemical concentrations of 1.6, 2.5 and 3.2 mg or µL/L were employed in each test. Glucose-glutamic acid controls were included for assessing the dilution water quality. Dissolved oxygen (DO) concentrations were measured initially and at regular time intervals using a YSI 54 oxygen meter 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 DO depletions were adjusted for the inoculum control and then used to calculate the mean mmol BOD per mmol chemical from duplicate tests. Mean values were transformed to the percents of chemical theoretical BOD and subsequently, unoxidized substrate concentrations remaining at various time intervals were computed for each initial concentration. Natural logarithms of the ratios of initial to residual chemical concentrations were correlated with incubation times. Correlations were calculated for the three initial concentrations and the mean slope of the linear portions of the regression lines was taken as a pseudo-first-order biodegradation rate constant (K'-DW).

Paris et al. (1984, 1983) reported for five natural waters the second-order biodegradation rate constants of nine chemicals used in this study. To relate biodegradation rates in dilution water with those in natural waters, second-order rate constants were multiplied by 2.3 x 10^8 cells/L and transformed to pseudo-first-order rate constants (K'-NW). The K'-DW and K'-NW data were then correlated to determine the environmental significance of biodegradation rates obtained using BOD dilution water. In addition, K'-NW data for each compound were compared to determine the effect of the type of natural water on biodegradation rates.

All data in this study were analyzed using Minitab^R statistical software on a DEC-350 microcomputer (Minitab, Inc. 1982). A minimum precision for triplicate CFU data was 70% and 83% for duplicate BOD data. Differences were less than 10% between the DO concentrations determined iodometrically and by the probe

method. Biodegradation measurements for the two halogenated phenols were repeated with 76% or greater accuracy. Glucose-glutamic acid control exerted at least 200 mg/L 5-day BOD in each test.

RESULTS AND DISCUSSION

Chemical mineralization rate in growth-linked biodegradation process may be dependent on both substrate concentration and the growth rate of an active microorganism (Subba-Rao et al. 1982). Consequently, phenol, p-bromophenol and p-acetylphenol were selected for measuring microbial growth because their biodegradation rates (Paris et al. 1983) implied rapid, slow and moderate turnover times, respectively. Glucose was included to help identify factors which may limit the microbial growth under test conditions. Initial concentration of each culture was considered to be optimum for detecting increases in cell density and for enduring the latent phase of growth.

Measurable growth of the cultures did not occur in the absence of corresponding chemical in BOD dilution water or MSM. Plate counts of phenol, p-acetylphenol and glucose metabolizing cultures were similar on MSM agar containing 100 and 250 mg/L chemical substrate. However, the higher concentration of p-bromophenol inhibited the culture growth. Thus, all tg except for phenol were calculated from the CFU data obtained with 100 mg/L chemical. The tg of phenol degrading culture was calculated from plate counts at 250 mg/L phenol.

Table 1. Generation times of phenol, p-bromophenol, p-acetylphenol and glucose metabolizing cultures.

Test system (200 mL growth medium/300-mL BOD bottle)	Generation time (h)
BOD dilution water + 3.2 mg/L phenol	68.6
BOD dilution water + 3.2 mg/L p-bromophenol	95.9
BOD dilution water + 3.2 mg/L p-acetylphenol	89.4
BOD dilution water + 3.2 mg/L glucose	25.3
BOD dilution water + 100 mg/L glucose	23.7
Mineral salts medium + 3.2 mg/L glucose	12.1
Mineral salts medium + 100 mg/L glucose	4.5

A comparison of tg of the glucose metabolizing culture (Table 1) indicated about a 30-fold decrease in substrate concentration had less effect on culture growth rate in BOD dilution water than in MSM. It also indicated the tg in MSM were shorter than in dilution water. The MSM contained greater amounts of inorganic nutrients than dilution water. The largest differences were 212 and 810 mg/L for nitrogen and phosphorus, respectively. Insufficient concentrations of these nutrients were implied to impede

Table 2. Pseudo-first-order biodegradation rate constants of various chemicals in BOD dilution and natural waters.

Chemical	Rate constant per hour[a]						
	Type of water						
	BOD dilution	Williams Pond	Overlook Pond	Park Pond	Hickory Hills	Oconee River	Mean for five natural waters
Phenol	0.020	0.069	0.110	0.055	0.046	0.097	0.075
p-Methylphenol	0.028	0.025	0.106	0.055	0.060	0.069	0.063
p-Methoxyphenol	0.015	0.003	0.003	0.008	0.004	0.007	0.005
p-Chlorophenol	0.008	0.019	0.011	0.016	0.016	0.021	0.017
p-Bromophenol	0.012	0.022	0.022	0.018	0.023	0.019	0.021
p-Acetylphenol	0.012	0.041	0.046	0.094	0.048	0.025	0.051
p-Cyanophenol	0.012	0.001	0.001	0.001	0.001	0.002	0.001
p-Nitrophenol	0.013	0.011	0.004	0.009	0.008	0.012	0.009
Methyl(2,4-dichlorophenoxy)acetate	0.026	0.136	0.168	0.122	0.120	0.127	0.135

[a] For natural waters calculated based on second-order rate constants reported by Paris et al. (1984, 1983).

chemical biodegradation in natural waters (Floodgate 1979) and the above data appeared to follow the same trend.

The tg of the cultures oxidizing three phenols were long (Table 1). This implied the initial concentrations of phenol and substituted phenol degrading cultures would not change significantly over short periods of time. Consequently, the microbial biomass employed in deriving K'-DW could be used for adjusting the second-order rate constants reported by Paris et al. (1984, 1983) with natural waters.

The K'-DW ranged from 0.008/h for p-chlorophenol to 0.028/h for p-methylphenol (Table 2). Correlation coefficients (r) were significant (α=0.05) for linear relationships between natural logarithms of the ratios of initial to residual chemical concentrations against time. These plots implied biodegradation rates decreased considerably when initial chemical concentration had declined by about 55%.

Both statistically significant r values for linear correlations and relatively high levels of precision and accuracy, as measured in this study suggest a conventional BOD method (APHA 1980) can be adapted for estimating biodegradation rates of a large number of chemicals. This would be cost-effective, as it does not require the use of a radioactive chemical or quantification of substrate or product. This may be also environmentally safe, as the BOD technique conservatively estimates chemical biodegradation. Critical limitations of the method are that only a narrow range of substrate concentration can be used and chemicals with less than 1 mg/L water solubilities may not be successfully tested (Babeu and Vaishnav 1987).

The mean K'-NW ranged from 0.001/h for p-cyanophenol to 0.135/h for methyl(2,4-dichlorophenoxy)acetate, but K'-NW for individual chemicals were similar in all five waters (Table 2). This suggested the majority of site-dependent variations in biodegradation rates were due to differences in bacterial concentrations in the waters. The concentrations of bacteria in five waters ranged from 10^7 to 10^9 cells/L (Paris et al. 1983).

Correlations of K'-DW had r values between 0.59 with K' in Park Pond and 0.86 with K' in Overlook Pond water (Fig 1). The adjusted pooled r (0.69) for these correlations was significant (α=0.05). The rate constant for p-acetylphenol in Park Pond water was an outlier in the above relationship. A new correlation without this rate constant value had r statistic that increased from 0.59 to 0.81.

The K'-NW of all chemicals, except p-methoxy-, p-cyano- and p-nitrophenol were about four times greater than the corresponding K'-DW. Larson (1983) observed biodegradation rates of surfactants were also greater in natural waters than in a synthetic medium containing preacclimated microbes. A possible reason for

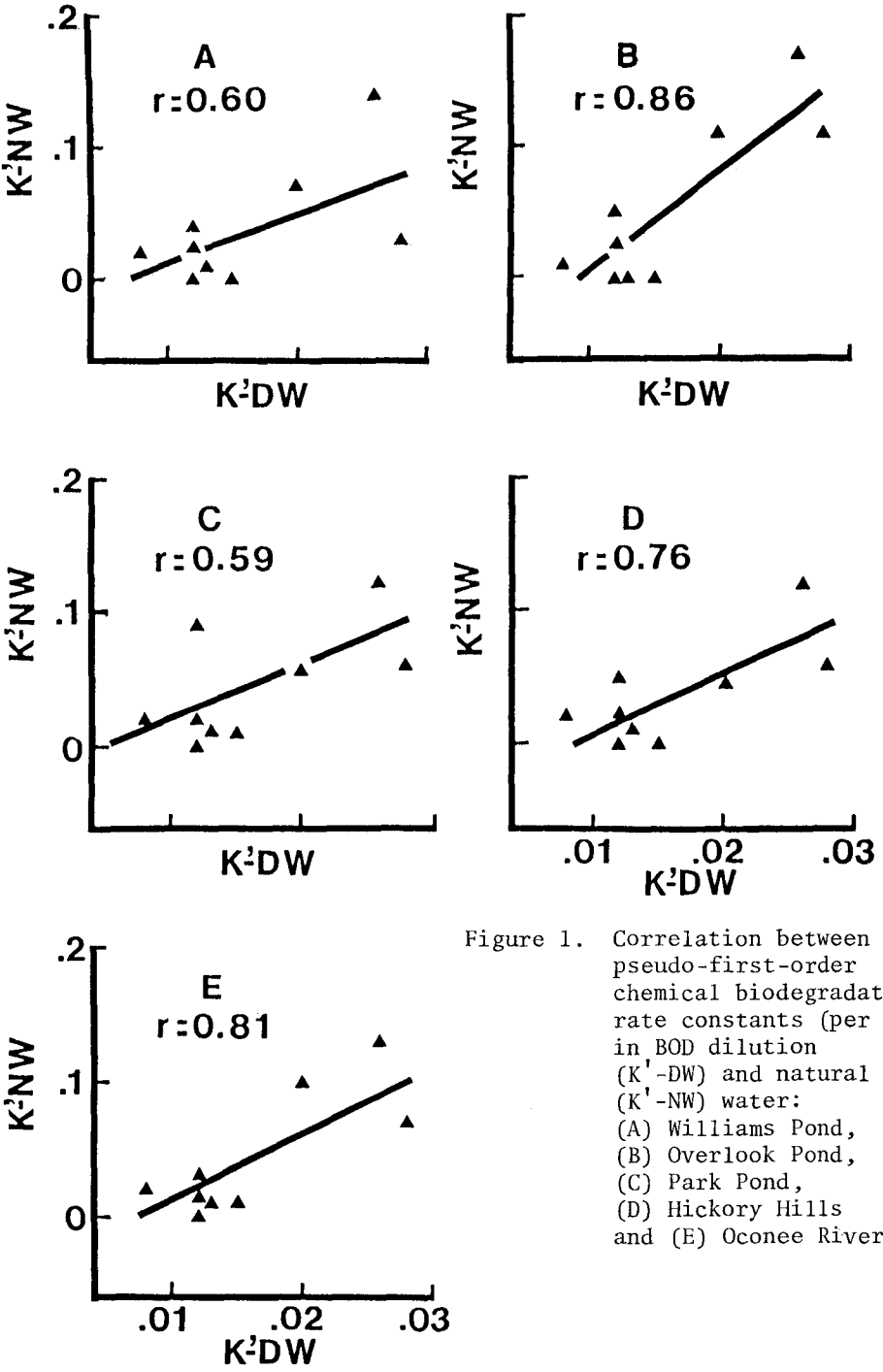

Figure 1. Correlation between pseudo-first-order chemical biodegradation rate constants (per h) in BOD dilution (K'-DW) and natural (K'-NW) water: (A) Williams Pond, (B) Overlook Pond, (C) Park Pond, (D) Hickory Hills and (E) Oconee River.

increased rates was the aquatic microbial community had prior exposure to natural substrates, which in turn enhanced the ability of microorganisms to respond to some of the test chemicals (Shimp and Pfaender 1985). In addition, it was also possible to some extent the K'-DW values were underestimated because of the conservative nature of the BOD method.

The K'-DW had a span of about 3.5 but that for the mean K'-NW was approximately two orders of magnitude. A possible reason for this difference was K'-DW and K'-NW values were based on chemical degradation by preacclimated and native aquatic microorganisms, respectively. A key distinction between the two types of microorganisms was preacclimated microbes may have possessed the needed biochemical machinery at its optimum for commencing degradation with a minimal lag period. In contrast, aquatic microbes may have evolved the necessary biochemical capabilities in response to test substrates (Stephenson et al. 1984, Torstensson et al. 1975). This may have caused extended latencies in degradation of p-methoxy-, p-cyano- and p-nitrophenol.

An alternate explanation for the above difference was the entire population of acclimated microbes may have been capable of degrading a particular test chemical. In contrast, only a fraction of the aquatic microbial population may have been able to metabolize a test chemical. Thus, lag periods in degradation of the three phenols were possibly the time required for the multiplication of initially small populations of active microorganisms, and not for evolution of enzymatic capabilities (Wiggins et al. 1987).

Results from this study suggested biodegradation rates measured using BOD dilution water were environmentally relevant, as they correlated well with rates in several of the natural waters. However, additional research to include diverse chemical structures and environments is required to determine the "real world" significance of biodegradability screening tests.

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