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Effect of microbial concentration on biodegradation rates of phenols

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SUMMARY

Biodegradation rates of 12 phenols were measured with respect to acclimated microbial biomass ranging from 2.3×10^4 to 2.3×10^8 cells/l. Rates ranged between 0.02 mg l^{-1} day^{-1} for 1.6 mg/l *p*-bromophenol exposed to 2.3×10^4 cells/l and 1.41 mg l^{-1} day^{-1} for 3.2 mg/l *p*-methylphenol exposed to 2.3×10^8 cells/l. Generally, rates for all phenols were first-order in substrate concentration and zero-order in biomass concentration. Bromophenol biodegradation was preceded by lag periods of varying lengths and to a small extent the rate was dependent on microbial biomass. Results from this study suggest chemical biodegradation generally exhibits pseudo-first- and occasionally, second-order kinetics.

INTRODUCTION

Microbial degradation of xenobiotic compounds in wastewater treatment systems, natural waters and sediments has been shown to follow first-order kinetics with respect to substrate concentration [8,12,13,22]. Attempts to normalize first-order rate constants for biomass to allow extrapolation of biodegradation rates from one environment to another have met with variable success [11]. Biodegradation of phenols in various natural waters was shown to be a second-order process, that is, rates were first-order in both substrate and microbial concentrations [19]. Second-order rate constants for corresponding phenols were also shown to be similar for all waters, indicating biodegradation rates were site-independent [19]. Limited data, however, from other studies suggested biodegradation rates were dependent on chemical concentration and not on microbial biomass [11,15,23].

Consensus among microbiologists is pseudo-first-order rate constants would allow estimation of chemical biodegradation rates. However, there is a need to establish whether rates vary proportionally with biomass concentration. This information can aid in appraising longevity of chemical pollutants in

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various environments. The present study was conducted to determine the possible effect of microbial concentration on biodegradation rates of phenols.

MATERIALS AND METHODS

Preparation of inocula

Mixed microbial cultures capable of using 12 phenols and glucose (all analytical grade) as sole carbon and energy sources were separately isolated by an enrichment culture technique [24]. The cells were then grown in mineral salts medium (MSM) [16] 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, it was serially diluted to yield five concentrations between 2.3×10^4 and 2.3×10^8 cells/l in biodegradation tests.

Measuring microbial growth

Generation times (t_g) of the 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 [3] 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 of the chemical substrate. Plates were incubated in sealed plastic bags for 21 days. Subsequently, CFU were counted and the t_g were calculated [9].

Measuring biodegradation

Degradation of 12 phenols by five concentrations of acclimated microbes was measured separately by a modified BOD technique [4]. 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 and uninoculated controls containing 3.2 mg or μl/l chemical were included to assess the dilution water quality and any oxygen consumption due to abiotic processes, respectively. Dissolved oxygen (DO) concentrations were determined 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 [3]. The test DO depletions were adjusted for the inoculum control and then used to calculate the mean mmol BOD per mmol chemical at each chemical concentration. Mean values were transformed to the percents of chemical theoretical BOD and unoxidized substrate concentrations remaining at various time intervals were calculated.

Calculation of biodegradation kinetic parameters

Biodegradation rate at each substrate and microbial concentration was determined from the linear region of the correlation between residual chemical concentration and incubation time. The rate data were fitted to the Michaelis-Menten equation to calculate a first-order biodegradation rate constant ($K_1 = V_{max}/K_m$) [6]. Rate constants for each phenol at various microbial concentrations were then fitted to a linear model

$$\log K_1 = \log K_b + n_b \log (B)$$

to derive biodegradation rate constant (K_b = intercept) and order (n_b = slope) in respect to initial biomass concentration (B). The value of n_b was used to quantify the effect of microbial concentration on biodegradation rates.

All data in this study were analyzed using Minitab^R statistical software on a DEC-350 microcomputer [14]- The minimum agreement between replicates of CFU and BOD data and their mean values was 70% and 83%, respectively. Differences were less than 10% between DO concentrations determined iodometrically and by the probe method. Biodegradation measurements for the three halogenated phenols were repeated and the new BOD values agreed within 76% with those determined

previously. The glucose-glutamic acid control exerted at least 200 mg/l 5-day BOD in each test. The final DO depletions in uninoculated chemical controls did not exceed 0.3 mg/l.

RESULTS AND DISCUSSION

Measurable growth of the cultures did not occur in the absence of corresponding chemicals 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 t_g except for phenol were calculated from the CFU data obtained with 100 mg/l chemical. The t_g of the phenol degrading culture was calculated from plate counts at 250 mg/l phenol.

A comparison of t_g for 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 t_g 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 presumed to impede chemical biodegradation in natural waters [7] and the above data appeared to follow a similar trend.

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

The t_g of cultures oxidizing three phenols were long (Table 1). This implied the initial concentrations of phenol and substituted phenol degrading cultures in biodegradation test must have not changed significantly over short periods of time. Consequently, the values for initial concentrations were used in a linear model to measure the effect of microbial biomass on biodegradation rates.

Coefficients of determination (r^2) and *F*-statistics were significant ($\alpha = 0.05$) for linear regressions used to calculate biodegradation rates. The rates ranged between 0.02 mg l^{-1} day $^{-1}$ for 1.6 mg/l *p*-bromophenol incubated with 2.3×10^4 cells/l and 1.41 mg l^{-1} day^{-1} for 3.2 mg/l *p*-methylphenol incubated with 2.3×10^8 cells/l. The rates of all phenols, except *p*-bromophenol, increased in direct proportion to increases in chemical concentration. This implies the rates were first-order in substrate concentration. Biodegradation rates of bromophenol at the three lower concentrations of microorganisms increased by about three times more than corresponding increases in chemical concentrations. A comparison [5] of these rates showed they were approximately second-order in bromophenol concentration. The rates at other two biomass levels, however, were first-order in chemical concentration.

The t_g values for cultures oxidizing phenols (Table 1) had a negative linear correlation ($r^2 = 0.943$) with biodegradation rates of corresponding chemicals at 3.2 mg/l concentration exposed to 2.3×10^6 cells/l. These chemical and microbial concentrations were equivalent to those used for measuring culture growth. The above correlation was based on limited data and thus no inference can be made from it. However, a need to relate microbial concentration to microbial activity has been shown for predicting the extent of chemical biodegradation [11].

The r^2 and *F*-values were significant ($\alpha = 0.01$) in the Michaelis-Menten model used to derive first-order rate constants (Table 2). Generally, rate constants increased with increased microbial concentration but the increases were relatively small in

comparison to biomass increments (Table 2). Other studies have also shown the addition of several concentrations of acclimated microorganisms did not affect the first-order biodegradation rate constants of dodecyl nonylethoxylate in synthetic medium [11], 2,4-D in phosphate buffer [15] and benzoate in natural water [23]. Results from these and the present studies were in contradiction of those obtained by Paris et al. [17–20]. They observed, in natural waters, biodegradation rates of several chemicals were first-order in biomass concentration and overall the rates were second-order.

The above disagreement may be due to differences in microorganisms. In this and the other three studies [11,15,23], preacclimated microorganisms were used, while Paris et al. had used indigenous aquatic microorganisms. It is possible that the latter microbes degraded chemicals with extended lag periods, while preacclimated microorganisms degraded chemicals with minimal latencies. Lamanna et al. [10] reported the length of the lag phase generally decreases with an increased inoculum and quantitatively tends to be a linear function of the log of the number of cells in the inoculum. Accordingly, biodegradation rates in studies of Paris et al. [17–20] may have increased in proportion to increases in

Table 2

First-order biodegradation rate constants (K_1) for phenols at various microbial concentrations

Chemical	Microorganisms/l				
	2.3×10^4	2.3×10^5	2.3×10^6	2.3×10^7	2.3×10^8
	K_1/day ± SE				
Phenol	$2.9(\pm 0.01) \times 10^{-1}$	$3.7(\pm 0.01) \times 10^{-1}$	$2.8(\pm 0.17) \times 10^{-1}$	$3.3(\pm 0.05) \times 10^{-1}$	$3.3(\pm 0.06) \times 10^{-1}$
p-Methylphenol	$3.4(\pm 0.24) \times 10^{-1}$	$4.0(\pm 0.18) \times 10^{-1}$	$3.8(\pm 0.08) \times 10^{-1}$	$3.4(\pm 0.02) \times 10^{-1}$	$4.0(\pm 0.02) \times 10^{-1}$
o-Methylphenol	$1.4(\pm 0.05) \times 10^{-1}$	$1.4(\pm 0.10) \times 10^{-1}$	$1.7(\pm 0.13) \times 10^{-1}$	$2.3(\pm 0.14) \times 10^{-1}$	$2.7(\pm 0.09) \times 10^{-1}$
p-Methoxyphenol	$4.2(\pm 0.06) \times 10^{-2}$	$5.0(\pm 0.06) \times 10^{-2}$	$8.0(\pm 0.00) \times 10^{-2}$	$1.9(\pm 0.05) \times 10^{-1}$	$2.5(\pm 0.09) \times 10^{-1}$
p-Chlorophenol	NC[a]	$3.0(\pm 0.12) \times 10^{-2}$	$3.0(\pm 0.12) \times 10^{-2}$	$5.0(\pm 1.50) \times 10^{-2}$	$1.2(\pm 0.06) \times 10^{-1}$
2,4-Dichlorophenol	$7.0(\pm 0.12) \times 10^{-2}$	$9.0(\pm 0.06) \times 10^{-2}$	$6.0(\pm 0.35) \times 10^{-2}$	$1.3(\pm 0.01) \times 10^{-1}$	$2.2(\pm 0.09) \times 10^{-1}$
p-Bromophenol	$7.0(\pm 0.58) \times 10^{-3}$	$7.0(\pm 0.00) \times 10^{-3}$	$9.0(\pm 0.58) \times 10^{-3}$	$6.0(\pm 1.27) \times 10^{-2}$	$2.1(\pm 0.05) \times 10^{-1}$
p-Acetylphenol	$8.0(\pm 0.58) \times 10^{-2}$	$1.1(\pm 0.01) \times 10^{-1}$	$1.5(\pm 0.06) \times 10^{-1}$	$1.6(\pm 0.07) \times 10^{-1}$	$1.7(\pm 0.01) \times 10^{-1}$
p-Cyanophenol	$9.0(\pm 0.23) \times 10^{-2}$	$1.6(\pm 0.01) \times 10^{-1}$	$1.8(\pm 0.04) \times 10^{-1}$	$1.9(\pm 0.02) \times 10^{-1}$	$2.0(\pm 0.09) \times 10^{-1}$
p-Nitrophenol	$8.0(\pm 0.58) \times 10^{-2}$	$1.2(\pm 0.02) \times 10^{-1}$	$4.0(\pm 0.35) \times 10^{-2}$	$8.0(\pm 0.23) \times 10^{-2}$	$1.1(\pm 0.10) \times 10^{-1}$
2-Phenylphenol	$1.2(\pm 0.08) \times 10^{-1}$	$1.6(\pm 0.31) \times 10^{-1}$	$3.3(\pm 0.59) \times 10^{-1}$	$1.8(\pm 0.13) \times 10^{-1}$	$2.8(\pm 0.05) \times 10^{-1}$
2,2′-Biphenol	$1.9(\pm 0.07) \times 10^{-1}$	$2.5(\pm 0.13) \times 10^{-1}$	$2.6(\pm 0.06) \times 10^{-1}$	$4.0(\pm 0.03) \times 10^{-1}$	$4.0(\pm 0.10) \times 10^{-1}$

[a] NC = Not calculated, as difference between test and control BOD values was insufficient even after 27 days of incubation.

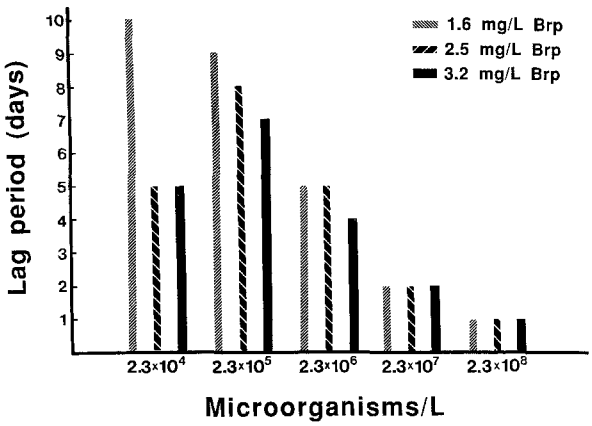

Fig. 1. Lag periods occurred during *p*-bromophenol (Brp) degradation by various concentrations of acclimated microorganisms.

biomass concentration and data could be fitted to a second-order rate equation.

In this study, biodegradation of all phenols, except *p*-bromophenol, occurred without measurable lag phases. The bromophenol biodegradation was preceded by lag periods of varying lengths, especially at the three lower biomass concentrations (Fig. 1). Generally, the extent of latency decreased with increased chemical and/or cell concentration. A possible explantation for this is that either of the increments enhanced interaction between cell and substrate and consequently, facilitated onset of biodegradation.

Biodegradation order with respect to biomass concentration can be expected to range between zero and one. The value of approximately zero or one suggests the corresponding biodegradation rate is zero- or first-order with respect to microbial concentration. Data in Table 3 indicated biodegradation of phenols did not increase considerably with increased microbial mass, as all values for biodegradation order were significantly ($\alpha = 0.05$) less than one. This implied a second-order rate constant would be useful for estimating chemical biodegradation rate only when the exponent values for both chemical and microbial concentrations approach one. Furthermore, a second-order rate constant can not be based on total bacterial biomass, since a chemical can cause adaptation of only a few specific organisms in the total microbial community [21].

In natural environments, microorganisms may be subjected to chronic or intermittent exposure to chemical pollutants. In the latter situation, extended lag periods may precede biodegradation of certain chemicals and rates of chemical mineralization can vary proportionally with microbial concentration. The rate for a particular chemical, however,

Table 3

Biodegradation order (n_b) and rate constants (K_b) for phenols in respect to microbial concentration

Chemical	$n_b \pm$ SE[a]	$K_b \pm$ SE (l microorganism^{-1} day^{-1})
Phenol	$1.0(\pm 0.89) \times 10^{-2}$	$3.0(\pm 0.36) \times 10^{-1}$
p-Methylphenol	$1.0(\pm 0.45) \times 10^{-2}$	$3.4(\pm 0.27) \times 10^{-1}$
o-Methylphenol	$8.0(\pm 0.45) \times 10^{-2}$	$6.0(\pm 0.45) \times 10^{-2}$
p-Methoxyphenol	$2.0(\pm 0.13) \times 10^{-1}$	$4.0(\pm 0.89) \times 10^{-3}$
p-Chlorophenol	$2.0(\pm 0.30) \times 10^{-1}$	$3.0(\pm 0.10) \times 10^{-3}$
2,4-Dichlorophenol	$1.2(\pm 0.22) \times 10^{-1}$	$2.0(\pm 0.89) \times 10^{-2}$
p-Bromophenol	$3.9(\pm 0.45) \times 10^{-1}$	$2.0(\pm 0.89) \times 10^{-4}$
p-Acetylphenol	$8.0(\pm 0.89) \times 10^{-2}$	$4.0(\pm 0.45) \times 10^{-2}$
p-Cyanophenol	$8.0(\pm 1.34) \times 10^{-2}$	$6.0(\pm 0.89) \times 10^{-2}$
p-Nitrophenol	$1.0(\pm 3.13) \times 10^{-2}$	$1.1(\pm 0.36) \times 10^{-1}$
2-Phenylphenol	$8.0(\pm 2.24) \times 10^{-2}$	$8.0(\pm 2.24) \times 10^{-2}$
2,2'-Biphenol	$9.0(\pm 0.89) \times 10^{-2}$	$9.0(\pm 0.89) \times 10^{-2}$

[a] All values were significantly ($\alpha = 0.05$) less than one.

can be pseudo-first-order, if previously or newly acclimated microorganisms degrade that chemical with a short period of latency. In a situation in which exposure is chronic, indigenous microbial population may acclimate to rapidly degrade some chemicals. In such cases, minimal lag phases may occur before the onset of measurable biodegradation and concentrations of active microorganisms may not be rate limiting. Factors governing microbial acclimation are not well understood at the present time but the review of the literature implies that acclimation for metabolizing a chemical can be rapid, slow or absent in a given environment [1,2].

Results from this study suggest when a threshold concentration of acclimated microorganisms is present or evolves, and other environmental conditions are favorable, chemical biodegradation will generally assume pseudo-first- and occasionally, second-order kinetics. The latter kinetics can be applicable, as inferred from the bromophenol data, to microbes requiring long lag periods before commencing biodegradation.

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