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Determination of chemical biodegradation by direct and indirect methods

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SUMMARY

Degradation of 10 organic chemicals by pre-acclimated microorganisms in BOD dilution water was determined directly by UV spectrophotometry and indirectly by a modified BOD method. Residual chemical concentrations were periodically measured and pseudo-first-order biodegradation rate constants (k_1) were calculated. The k_1 spectrophotometry values ranged from 0.006/h to 0.077/h and k_1-BOD values from 0.002/h to 0.043/h for 1-methylnaphthalene and indole, respectively. The ratios of k spectrophotometry to k_1-BOD were between 1.5 for salicylic acid and 3.0 for 1-methylnaphthalene with a mean of 2.7. A significant ($\alpha = 0.001$) linear correlation ($r^2 = 0.854$, $F = 46.630$) existed between the two sets of rate constants. Results from this study suggest that the modified BOD method may be used to estimate chemical biodegradation rates in synthetic media.

INTRODUCTION

Determination of biodegradation of chemicals can be valuable in appraising behavior and effects of xenobiotic compounds in an aquatic environment. For example, if a chemical is readily biodegradable, then it may not bioaccumulate in the environment and reach toxic levels, unless it is toxic at low concentrations or if environmental exposure is high. In contrast, if a chemical is recalcitrant to biodegradation, then given enough exposure time, it can accumulate to toxic levels [10]. In fact, in Japan, the number-one criterion for examination and regulation of chemicals is whether a chemical is biodegradable [5].

In the U.S., the inventory of industrial chemicals is large and expanding as 200 to 1000 new chemicals are added each year [9]. The EPA also receives annually hundreds of chemicals for pre-manufacturing notice review. It would be impossible and in some instances unnecessary to test all these chemicals for their potential ecological effects. However, it would be useful to screen them for their biodegradation, mutagenic effects and toxicities to sensitive organisms. This will allow testing resources to focus on in-depth evaluations of only the potentially persistent, mutagenic or toxic chemicals.

There are several direct and indirect methods for the determination of chemical biodegradation [12]. Direct methods, such as chemical measurements by UV spectrophotometry, chromatography, organic carbon analysis or the use of radiochemicals, have lower detection limits, and are more sensitive, accurate and expensive than indirect methods. However, the use of direct methods may not be necessary and cost-effective, especially with a large number of chemicals. The indirect modified BOD method [2] appears to be reliable and accurate, and may be used to obtain estimates of chemical biodegradation, if it compares well with some of the direct methods.

In this paper, for selected chemicals, we present comparisons of pseudo-first-order biodegradation rate constants (k_1) that were determined by UV spectrophotometry (Spec) and a modified BOD method. Biodegradation of all selected chemicals was measured using BOD dilution water, preacclimated microorganisms and other uniform test conditions to obtain possibly an accurate method comparison.

MATERIALS AND METHODS

Preparation of inocula

Mixed microbial cultures capable of using 10 organic chemicals (analytical grade) as sole sources of carbon and energy were separately isolated from wastewater samples by an enrichment culture technique [14]. Subsequently, the isolates were subcultured in minimal medium [11] until 10–12 serial passages were made.

The medium contained 100 mg/l (solid) or 100 μl/l (liquid) of the respective chemical substrate. The cells from

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the final subculture were centrifuged, suspended in physiological saline and incubated on a shaker at 150 rpm for 24 h. Following incubation, the preparation was diluted to yield 1.5×10^7 cells/l in the biodegradation test. The biomass concentration in undiluted preparation was determined by spread-plate technique, using nutrient agar. Triplicate plates were incubated at 21 ± 3 °C for 48 h and then CFU were enumerated.

Determination of biodegradation

Biodegradation of test chemicals was measured directly by UV spectrophotometry and indirectly by a modified BOD technique [2]. For both methods, using micro-pipets, a test chemical and 1 ml of the diluted inoculum were added to 20 ml of BOD dilution water [1] in a 300-ml BOD bottle. Likewise, several sets of triplicate bottles were prepared and 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 un-inoculated controls containing 3.2 mg or μl/l chemical were included to assess the dilution water quality, and any chemical or oxygen loss due to abiotic processes, respectively.

For each control and chemical concentration, dissolved oxygen (DO) concentrations in one set of triplicate BOD bottles were determined initially and at regular time intervals. Each time approx. 85% of the DO determinations were made using a YSI 54 oxygen meter with a self-stirring probe, and 15% using the azide modification of the iodometric method [1]. Two or more bottles were randomly selected for analysis by the latter method. Following DO measurements, samples of control and test BOD dilution waters were also collected. The sample pH was then adjusted using 10% HCl or 10% NaOH, and absorbance measured using a spectrophotometer (Perkin-Elmer Model 200) set at a predetermined wavelength (Table 1).

Calculation of biodegradation kinetic parameters

For the BOD method, 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 percentages of chemical theoretical BOD and unoxidized substrate concentrations remaining at various time intervals were calculated stoichiometrically. For the spectrophotometric method, the sample absorbance was fitted to a calibration curve and residual chemical concentration was determined.

For both spectrophotometric and BOD methods, biodegradation rates at each chemical concentration were determined from the linear regions of the correlations between residual chemical concentrations and incubation times. The log of the ratios of 3.2 mg or μl/l to 1.6 mg or μl/l chemical, and corresponding rates were then taken and used to calculate the order (n) [3] of biodegradation. Also for both methods, natural log of residual chemical concentrations were regressed on incubation times to determine first-order biodegradation rate constants. The mean k_1-Spec and k_1-BOD values were then correlated, and regression statistics were evaluated.

All data were analysed using Minitab® statistical software on a Zenith 286-20 microcomputer [8]. The minimum agreements between replicates of spectrophotometric and BOD data and their mean values were 94% and 83%, respectively. Differences between DO concentrations determined iodometrically and by the probe method were less than 10%. Biodegradation measurements for the three phenols were repeated and the new rate constants for both methods agreed within 76% with those determined previously. The glucose-glutamic acid control exerted at least 200 mg/l 5-day BOD in each test. Chemical recoveries from un-inoculated controls, as determined from spectrophotometric measurements, ranged from 90% for indole to 118% for 2,4-dichlorophenol with a mean of 101%. The final DO depletions in these controls did not exceed 0.3 mg/l.

RESULTS AND DISCUSSION

We have previously shown some of the physicochemical properties of chemicals significantly influence the extent of chemical biodegradation [2]. Accordingly, chemicals (Table 1) for this study were selected to include both structural diversity and wide ranges of values for several physicochemical parameters. For example, the water solubilities of test chemicals ranged from about 7 mg/l for biphenyl to 1.6 g/l for *p*-aminobenzoic acid [7]. In general, we expected the test chemicals to range from being moderately persistent to rapidly biodegradable. This was desired to obtain a meaningful relationship between the two sets of rate constants.

Coefficients of determination (r^2) and F-statistics were significant ($\alpha = 0.05$) for linear regressions used to calculate both biodegradation rates and rate constants. The mean biodegradation order (n) for rates from spectrophotometric method was 1.05 ± 0.085 and from BOD method 0.96 ± 0.190. This shows that the rates were approximately first-order in chemical concentration. Furthermore, in this study, we also assumed a constant biomass (1.5×10^7 cells/l) with each chemical. This is because we have shown the initial concentrations of microorganisms, while degrading low levels of industrial chemicals, do not change significantly over short periods of time [17].

TABLE 1

First-order chemical biodegradation rate constants (k_1) as determined by UV spectrophotometry (Spec) and modified BOD method, and specifications for photometric measurements[a]

Chemical	k_1-Spec/h ± SE	k_1-BOD/h ± SE	Spec specifications	
			Sample pH	Wavelength (nm)
1-Methylnaphthalene	$0.6 (\pm 0.07) \times 10^{-2}$	$0.2 (\pm 0.01) \times 10^{-2}$	2	280
2,4-Dihydroxybenzaldehyde	$3.0 (\pm 0.15) \times 10^{-2}$	$0.8 (\pm 0.10) \times 10^{-2}$	6	275
p-Aminobenzoic acid	$2.3 (\pm 0.18) \times 10^{-2}$	$0.9 (\pm 0.03) \times 10^{-2}$	2	275
Thymine	$4.1 (\pm 0.20) \times 10^{-2}$	$1.1 (\pm 0.00) \times 10^{-2}$	2	265
p-Cyanophenol	$3.8 (\pm 0.12) \times 10^{-2}$	$1.2 (\pm 0.03) \times 10^{-2}$	2	243
Biphenyl	$3.7 (\pm 0.15) \times 10^{-2}$	$1.4 (\pm 0.03) \times 10^{-2}$	2	245
2,4-Dichlorophenol	$3.6 (\pm 0.78) \times 10^{-2}$	$1.6 (\pm 0.12) \times 10^{-2}$	10	245
p-Nitrophenol	$5.9 (\pm 0.06) \times 10^{-2}$	$2.6 (\pm 0.23) \times 10^{-2}$	2	316
Salicylic acid	$5.2 (\pm 0.12) \times 10^{-2}$	$3.4 (\pm 0.06) \times 10^{-2}$	2	310
Indole	$7.7 (\pm 0.18) \times 10^{-2}$	$4.3 (\pm 0.10) \times 10^{-2}$	2	265

[a] Studies were conducted using BOD dilution water containing 1.5×10^7/l pre-acclimated microorganisms; incubation was at 21 ± 3° C.

The k_1-Spec values ranged from 0.006/h to 0.077/h and k_1-BOD values from 0.002/h to 0.043/h for 1-methylnaphthalene and indole, respectively (Table 1). The ratios of k_1-spec to k_1-BOD were between 1.5 for salicylic acid and 3.0 for 1-methylnaphthalene. The mean ratio was 2.7 ± 0.75. This shows that the BOD method underestimated the rate constants. From a comparative study on various biodegradation tests, Gerike and Fischer [6] also showed that the BOD method conservatively estimates chemical degradation. Possibly some of the chemical substrate may be used by seed microorganisms to synthesize new cell materials. Consequently, residual substrate concentrations would be overestimated, as they are calculated based on the theoretical BOD of chemicals [12].

The correlation of k_1-Spec and k_1-BOD (Fig. 1) was defined by the following regression equation:

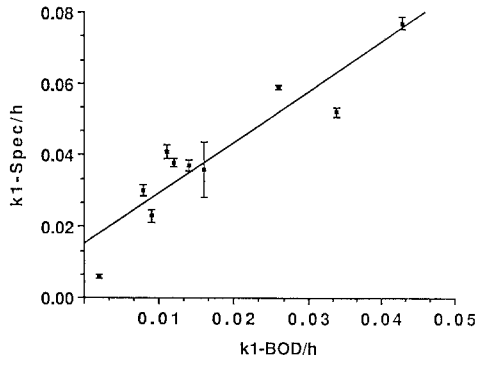

Fig. 1. Relationship between the first-order biodegradation rate constants determined by UV spectrophotometry (k_1-Spec/h) and a modified BOD method (k_1-BOD/h).

$$k_1\text{-Spec/h} = 0.015 + 1.408\ (k_1\text{-BOD/h})$$
$$n = 10,\ \overline{SD} = 0.008,\ r^2 = 0.854,\ F = 46.630$$

The r^2 and F-value were significant ($\alpha = 0.001$) for this relationship, and the average standard deviation ($\overline{SD}$) about the regression line was relatively small. We also could not find any pattern when the residuals of k_1-Spec were plotted against k_1-BOD. Thus, both regression statistics and the lack of any pattern in residual distribution suggest that a strong and unbiased correlation exists [13] between the two sets of rate constants.

The other useful features of the modified BOD method, in addition to its comparability with a direct method, are as follows:

(1) Concentrations (1 to 3 mg/l) of chemicals used for testing are below the water solubility limits [7] for most industrial chemicals. These concentrations may be less than the K_m of enzymes involved in biodegradation of many chemicals and therefore rates can be fitted to a first-order model.

(2) Chemicals, due in part to well-adapted microorganisms, are relatively rapidly biodegraded. This allows frequent DO measurements and thus, the calculation of biodegradation kinetic parameters from the BOD data that may be linear in incubation time.

(3) Only the pre-acclimated microorganisms are used. Therefore, if microbes capable of metabolizing a particular chemical can not be isolated, then that chemical would not require further testing and can be considered persistent, unless shown otherwise.

(4) It can be used to measure biodegradation of chemicals in oligotrophic natural waters by autochthonous microorganisms [15,16].

(5) It is well-suited for use with volatile compounds, as BOD bottles are hermetically sealed.

(6) Replicate data have a good agreement. For example, we determined the mean 5-day BOD for 88 chemicals, and found individual standard deviations were relatively small and the mean coefficient of variation was about 23% [2].

(7) It provides conservative and hence possibly environmentally safe estimates of chemical biodegradation.

8 It is cost-effective because test chemical analysis or use of radioactive test chemical is not required. Our work experience in Contract Laboratories suggests the cost to determine biodegradation of a chemical by the modified BOD method should be about one-third of the cost with a direct method involving the test substance analysis.

The BOD method, whether conventional [1] or modified [2], also has several limitations. For example, chemicals having less than 1 mg/l water solubilities may not be successfully tested for their biodegradation by this method [2]. The method also does not provide any information on metabolites produced from, or the amount of chemical mineralized due to, biodegradation. In addition, the BOD for liquid, low molecular mass paraffins may not be determined by this method, as they extract lipids from water-wet microbial cells and render them inactive [4].

The future studies could focus on comparing the modified BOD method with other more sophisticated direct methods, as UV spectrophotometry may not provide good estimates of residual parent compounds if UV-absorbing metabolites accumulate during biodegradation. Preferably, these studies should include a large number of diverse chemicals, and be performed using various types of synthetic media and natural waters. This will help to identify the types of chemicals and situations where the BOD method may fail to provide estimates of biodegradation. This may also help to enhance confidence in the modified BOD method, if good correlations exist between the rate constants determined by the direct and the modified BOD methods.

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