DEVELOPMENT OF PREDICTIVE STRUCTURE-BIODEGRADATION RELATIONSHIP MODELS WITH THE USE OF RESPIROMETRICALLY GENERATED BIOKINETIC DATA

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ABSTRACT

Biodegradation is an important mechanism determining the fate of chemicals in the aquatic environment. In this paper, experimental data, determined from electrolytic respirometry, for 27 compounds were analyzed using first order and Monod kinetics. Additional data from the literature were also used in our analysis. A method based on group contribution to predict first-order and Monod kinetic rate constants was developed and validated. The group contribution approach gave reasonable results for a variety of compounds. More kinetic data are required to extend the group contribution approach.

KEYWORDS

Biodegradation, Kinetics, Electrolytic Respirometry, Group Contribution.

INTRODUCTION

Information regarding the extent and the rate of biodegradation of organic chemicals is very important in evaluating the relative persistence of the chemical in the environment. This, in turn, can be used to regulate its manufacture and use. Due to the large number of these chemicals, gathering information on them is labor intensive, time consuming and expensive. Thus, there is a need to develop correlations and predictive techniques to assess biodegradability (Strier, 1980). Lack of an adequate database on biodegradation kinetics has prevented the development of such techniques.

A considerable amount of information concerning biodegradation kinetics is available in the published literature. Early literature shows widely differing kinetic rates in different studies. The evaluation and prediction of the extent and the rate of bio-oxidation is affected by methodological and experimental factors (Sayler, 1984). Many theoretical and experimental studies have been conducted on the kinetics of biodegradation and a comprehensive review is given by Gaudy and Kincannon (1977). Regardless of the different assumptions involved, the measurement of biodegradation rates is generally associated with microbial cell growth, and so most of the models for biodegradation are the same as those used to model growth and substrate removal.

The Monod model in combination with the linear law for substrate removal provides an adequate description of microbial growth behavior. It states that the cell growth rate is first order with respect to biomass concentration ($X$) and mixed order with respect to substrate concentration ($S$).

$$\frac{dX}{dt} = \frac{(S\mu_m X)}{(K_s + S)} \quad (1)$$

Also cell growth is linearly related to substrate removal rate

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\[ \frac{dX}{dt} = -Y \frac{dS}{dt} \]  

(2)

The kinetic parameters of interest are maximum specific growth rate \( \mu_m \), half saturation constant \( K \), (it is the concentration of substrate when \( \mu = 0.5\mu_m \)) and the yield coefficient \( Y \). Several authors, especially Schroeder (1977) and Grady and Lim (1980), have analyzed in depth the factors to be considered in biological kinetics and the pitfalls with indiscriminate use of the Monod equation.

Different techniques to evaluate biodegradation kinetics are reviewed in detail by Howard, et al. (1981) and discussed by Grady (1985). The chosen technique should not be labor intensive and time consuming. Also, the kinetic parameters obtained should be intrinsic: that is they should be dependent on the structure of the compound and degrading microbial community and not on the configuration of the experimental set up.

Gaudy and Gaudy (1971) have shown that the course of substrate degradation can be determined by following oxygen consumption of the degrading microbial community. Thus, intrinsic kinetic parameters can be obtained from oxygen consumption data from the single batch experiment. Grady, et al. (1989) have shown that the biokinetic parameters obtained from oxygen consumption data are similar to those obtained from traditional methods.

Jenkins (1960) has given a comprehensive review of the use of respirometers and Montgomery and coworkers (1967, 1971) have summarized its design and application in the measurement of BOD. Since then they have been widely used in effluent treatment studies (Nichols, 1981). Studies by Larson and Perry (1981), Young and Clark (1965), Young, Clark and Garner (1965), Birdie (1969), Tool (1967), Tebbutt and Berkun (1976), Young and Baumann (1976), Tabak, Lewis and Oshima (1984), Halbertschlager, Kohler and Szwerinski (1984) and Dosanjh and Wase (1987) have shown that the electrolytic respirometer eliminates most of the technical difficulties associated with other methods for determining BOD. King and Dutka (1980) have reviewed in detail the respirometric techniques and their application to determine biodegradability and toxicity of organic compounds.

Structure-activity relationships have been widely used in pharmacology and medicinal chemistry. In the field of biodegradation, interest in structure-activity relationships between the biodegradability of the chemical and its structure is not very recent (Ludzack and Ettinger, 1960). There are several studies which have attempted to correlate some physical, chemical or structural property of a chemical with its biodegradability. The literature reveals both qualitative and quantitative structure-biodegradability correlations.

Most of the correlations that have appeared in the literature are single parameter relations, applicable to a particular class of compounds. This demonstrates that correlations are possible, but also that single parameter correlations are limited in their applicability. The correlations by Dearden and Nicholson (1986) and Babeu and Vaishnav (1987) are applicable to different classes of compounds but they correlate gross measures like 5-day BOD. Microorganisms take 4 to 5 days to acclimatize to most of the biodegradable toxic compounds and then they degrade the compounds. Accordingly, measures like 5-day BOD would be misleading in such cases. In addition, this gross measure gives only some idea about the extent of biodegradation. It is important to know the extent as well as rate of biodegradation of the toxic compounds if their concentration in environment is to be regulated. Hence, it is necessary to find a correlation which covers different classes of compounds and correlate the rate of biodegradation with some physical, chemical or structural properties of the compound.

EXPERIMENTAL MEASUREMENT OF BIODEGRADATION KINETICS

Respirometric experiments were conducted for 20 benzenes, 8 phthalates, 6 polyaromatic hydrocarbons, 5 nitrosamines, 8 phenols, 4 ketones, hexachlorobutadiene, p-chloro-m-cresol, benzoic acid, p-nitroaniline and benzyl alcohol to collect oxygen consumption data. Aniline was used as a reference compound. The experimental conditions were as follows: compound concentration 100 mg/l, biomass concentration 30 mg/l, and temperature 25°C. Activated sludge from Little Miami wastewater treatment plant (Cincinnati), which predominantly receives domestic waste, was used as inoculum. The biomass was not acclimated to any of the test compounds. The oxygen uptake data were collected over a period of 10 - 50 days.
DEVELOPMENT OF STRUCTURE-BIODEGRADATION RELATIONSHIPS

In this study group contribution approach is selected because of its potential to predict rate constants for different classes of compounds. However, it should be emphasized that this approach requires a large amount of data for any meaningful analysis. This study was started with 56 organic chemicals with this requirement in mind, but more than half of them did not degrade under experimental conditions used. The data obtained are not sufficient for both calculation of contributions of the different groups and validation of the approach. The literature does not have the Monod constants for enough number of compounds, otherwise literature data in conjunction with the data obtained in this study could have been used to calculate group contributions and then validate approach. Hence, it was decided to use the first order biodegradation rate constants, which are available in the literature in large number, to calculate group contributions and then validate the approach using the first-order rate constants obtained from the experimental data in this study.

However, the group contributions were also calculated using the Monod constants obtained in this study and the study at the Clemson University (Grady, et al., 1989a, 1989b).

The kinetic constant values for compounds are organized into two sets — training set and testing set. This division of the compound list is based on the criterion that the chemical groups or fragments comprising the testing set are present in the training set. The main motivation for constructing the training and testing sets is to develop structure-biodegradability relationships using the training set and then test the relationship using the testing set compounds. The training and the testing sets for first order rate constants and the Monod constants are not same because of lack of availability of data. So, the group contribution results for these two should not be compared.

FIRST ORDER BIODEGRADATION RATE CONSTANTS

Biodegradation data are obtained from the literature for a series of chemicals, tested using a consistent protocol. The purpose of this requirement is to ensure, as far as possible, that the chemical structure and not the test conditions contribute towards the difference in biodegradation rate constant. Also, the number of chemicals required to calculate group contributions is comparatively large because of the nature of the analysis. Because of these restrictions, the only study considered adequate for analysis was that of Urano and Kato (1986a,b). They obtained the oxygen consumption data of different compounds using an electrolytic respirometer. The experimental conditions were as follow: temperature 25°C, pH of solution 7, sludge concentration 30 mg/L and compound concentration 100 mg/L. Urano and Kato (1986a) have obtained first-order kinetic rate constant by regressing the increasing part of the oxygen uptake curve with the following exponential relationship.

\[ \frac{dBOD}{dt} = k BOD \]  

The group contribution approach is used to estimate the first-order biodegradation kinetic constant rather than the rate of biodegradation because rate is dependent on concentration, while rate constant is not dependent on concentration but on the compound and degrading microbial community. Hence, the rate of biodegradation estimated using the first-order kinetic model will be lower at lower concentrations. However, like the Monod equation it will not identify the threshold concentration and will estimate very low rate at concentrations lower than the threshold concentration. Furthermore, the linear kinetic model used for estimating the biodegradation rate is itself an approximation of the Monod equation. However, this approximation of Monod equation by a linear kinetic model is justified since the group contribution approach provides only an estimate of the kinetic constant.

It is necessary to use a minimum of five data points per variable in univariate statistical analysis, otherwise there is a high risk of chance correlations being obtained. Accordingly it is necessary to ensure that each group, for which group contribution is calculated, occurs in at least five compounds. This precluded the use of whole data set of Urano and Kato (1986a,b). The compounds used for the calculation of group contribution parameters are: ethyl alcohol, butyl alcohol, ethylene glycol, acetic acid, propionic acid, n-butyric acid, n-valeric acid, adipic acid, methyl ethyl ketone, hexamethylenediamine, n-hexylamine, mono ethanol amine, acetamide, benzene, benzyl alcohol, toluene, acetonephone, and aminophenol. The group contribution parameters $\alpha_i$ for all the groups, which satisfied the above mentioned requirement, are given in Table 1. The contribution of other groups is not calculated because of the likelihood of obtaining their regression coefficients which will be dependent on a very small number of compounds, perhaps even a single compound in certain cases.
To validate the approach, cresols, phenol, 2,4-dimethyl phenol, acetone, 2-butane, butyl benzene, 1-phenyl hexane, aniline and benzoic acid data from this study are used. In addition, data for phenol (Paris, et al., 1982) and data for pentanol, hexanol and heptanol (Yonezawa, et al., 1979) are used for the validation of the approach. This latter set of compounds is not used in determination of group contribution parameters. Except for the source and the nature of biomass, the experimental conditions in this study are similar to that of Urano and Kato (1986a,b). The kinetics for the above mentioned compounds is determined from oxygen consumption data in the similar way as Urano and Kato (1986a) and is also predicted using the group contribution parameters given in Table 1.

The percentage errors are calculated by

\[ 100 \times \frac{\ln(k)_{\text{pre}} - \ln(k)_{\text{exp}}}{\ln(k)_{\text{pre}}} \]  

where "pre" and "exp" denote predicted and experimental biodegradation rate constants. The predicted values for the training set are within 10% except for aliphatic amines. The reported results for the testing set agree within 20% except for m-cresol and benzoic acid. The model does not differentiate between ortho-, meta- and para-cresols. This is because the contribution of OH group is calculated assuming that all the three positions are equivalent. The contribution of COOH group, used to calculate ln(k) value for benzoic acid, is calculated using aliphatic acids. This can be the reason for large error in prediction of the ln(k) value of benzoic acid. These problems could be alleviated if more data were available for each unique group.

The group contributions \( \alpha_i \) can be used to estimate the rate constants for any organic chemical that consists of the groups listed in Table 1. The values of \( \alpha \) in Table 1 suggest that the larger aliphatic molecules will have smaller degradation rates and vice versa. This dependence on molecular size is not generally true since some groups have a positive contribution to the ln(k) values as evident from Table 1.

Pitter (1976) measured biodegradation kinetic constants and obtained the values of 84.0, 40.0, 66.0, and 54.2 (mg COD/g h) for butanol, 1,4-butanediol, hexanol and 1,2-hexanediol, respectively. This shows that addition of another OH group in aliphatic chain decreases the rate. The biodegradation kinetic constants for benzene and benzoic acid are 0.28 and 0.56 (l/hour). Therefore, the addition of COOH group on the benzene ring increases the biodegradation rate. The rate constants estimated by group contribution approach show similar trends in both the cases.

**MONOD MODEL FOR BIODEGRADATION KINETICS**

The compounds used to calculate these parameters for the Monod constants are: benzene, toluene, ethyl benzene, xylenes, cumene, butyl benzenes, 1-phenyl hexane, dimethyl phthalate, diethyl phthalate, dipropyl phthalate, dibutyl phthalate, phenol, cresols, 2,4-dimethyl phenol, catechol, resorcinol, acetone, 2-butane, 4-methyl-2- pentane and benzyl alcohol from this study and dichlorobenzenes.
chlorophenol, 4-nitrophenol and 2,4-dinitrophenol from the study at Clemson University (Grady, et al., 1989a, 1989b). These compounds form the training set. The group contribution parameters, $\alpha_{i,\text{Monod}}$, of the ten groups occurring in the above compounds are given in Table 2. The contribution for chloro and nitro groups should be used only when acclimated biomass is present.

The compounds from Urano and Kato’s data set (1986a,b) which could be formed using the groups given in Table 2, were selected to validate the group contribution approach for prediction of the Monod constants. Urano and Kato (1986a) have calculated the first-order rate constant by fitting an empirical exponential form of equation to the increasing part of the oxygen uptake curve. The Monod relationship, used in this study, can be reduced to linear relationship under the assumption, $K_s > > S$. Under this assumption equation 1 reduces to

$$\frac{dS}{dt} = -\mu_m S \frac{X}{(Y K_s)} = - (K X) S$$

where $K = \frac{\mu_m}{(Y K_s)}$ and $S$ and $X$ are expressed in BOD units. Additionally, if it is assumed that there is no appreciable change in biomass concentration then we get a pseudo first order constant ‘$K_X$’. Using this linear relationship and the Monod constants, calculated using group contribution parameters in Table 2, oxygen uptake values were generated for the following compounds: ethyl alcohol, butyl alcohol, iso-butyl alcohol, ethylene glycol, acetone, methyl ethyl ketone, ethyl acetate, butyl acetate, benzene, benzyl alcohol, toluene, phenol, phenyl acetate and acetophenone. These compounds constitute the testing set. Oxygen uptake values were also generated for these compounds using the first
order constant and the rate equation of Urano and Kato (1986a,b). The average absolute percentage between the experimental values (Urano and Kato, 1986a,b) and predicted values (group contribution method) were calculated by

\[
(100/n) \sum \text{ABS} \left( \frac{(\text{OU})_e - (\text{OU})_p}{(\text{OU})_p} \right)
\]

where 'e' and 'p' denote experimental and predicted values. The experimental values agree within 25% with the predicted values for most of the compounds. The error is high for most of the aliphatic compounds (alcohols and esters). These high errors may be attributed to the fact that the contribution of all the groups is determined by using aromatic compounds except for ketones and assumptions made in reducing the Monod relationship to linear form may not be valid under the experimental conditions of Urano and Kato (1986a).

Most of the substrate degradation takes place in the exponential phase (Grady and Lim, 1980 and Gaudy and Gaudy, 1980). The maximum specific rate constant, \( \mu_m \), has the major impact on the exponential part of the oxygen uptake curve. Hence, the magnitude of this constant will indicate the extent of biodegradation that can be achieved for a particular compound.

RESULTS AND DISCUSSION

The Monod rate constant \( \mu_m \) is calculated for different compounds using group contribution parameters of Table 2 and are given in Table 3. These values are used to show that the different trends observed by researchers for different groups of compounds are also predicted by the group contribution approach.

Geating (1981) has found that percentage degradation decreases with increase in molecular weight and branching and in presence of bulky side chains. The \( \mu_m \) values in Table 3 reflect similar trend with increase in molecular weight (compounds 1-4) and branching and size of side chain(s) (compounds 1,2,5-8). Babeu and Vaishnav (1987) and Boething (1986) have reported that for alcohols percentage degradation decreases with increase in carbon number. The group contribution approach predicts a similar trend for alcohols (compounds 9-14 in Table 3). Pitter (1976) and Yonezawa and Urushigawa (1979) reported that the degradation rate constant for normal alcohols is higher than alcohols with hydroxy group attached to any carbon atom other than the end carbon atoms. The values for compounds (11-14) in Table 3 show that the Monod rate constant is higher for normal alcohols.

Vaishnav et al. (1987) have shown that addition of chlorine decreases the percentage degradation with respect to parent compound. The \( \mu_m \) values of chlorinated compounds (15-16 and 17-20) in Table 3 show that a similar trend is predicted by the group contribution approach.

Paris et al. (1982, 1983) has studied the degradation of para-substituted phenols and have reported the second-order rate constants for these compounds. The rate constant with respect to phenol decreases with substitution and magnitude of decrease depends on the type of substituent. The rate constants of substituted phenols are reported in the following decreasing order: Phenol > p-methyl phenol > p-chlorophenol > p-nitrophenol. Pitter (1976) has reported the following order: Phenol > methyl phenols > dimethyl phenols > chlorophenols > nitrophenols > dichlorophenols > 2-chloro-4-nitrophenol > trichlorophenol. Pitter has reported the degradation as percentage of theoretical oxygen demand (ThOD) achieved. The values of the Monod rate constant for compounds (17-24) in Table 3 agree with the trend.

The predicted Monod rate constants decrease with increase in molecular weight of phthalates (compounds 25-30 in Table 3). Urushigawa and Yonezawa (1979), Wolfe et al. (1980) and Sugatt et al. (1984) have reported the degradation rate constants for phthalates. Sugatt et al. (1984) found that dibutyl phthalate was outlier and did not follow the trend but other two studies and experimental results of this study confirm the results predicted by the group contribution method. The molecular weight of both dioctyl phthalate and bis(2-ethyl hexyl) phthalate is 391 but bis(2-ethyl hexyl) phthalate is more branched than dioctyl phthalate. Hence, bis(2-ethyl hexyl) phthalate should be more refractory than dioctyl phthalate. The group contribution approach predicts negative Monod rate constant for both these phthalates, indicating that none of them would degrade. The predicted rate constant of bis(2-ethyl hexyl) phthalate is greater than dioctyl phthalate, which suggests that bis(2-ethyl hexyl) phthalate should be more difficult to degrade than dioctyl phthalate. This agrees with the findings of above three studies.
## Table 3. The Monod constants predicted by group contribution method

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>$\mu_{m}$/hr</th>
<th>$K_i$ mg/l</th>
<th>$Y$ mg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene</td>
<td>0.29</td>
<td>5.7</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>Methyl benzene (Toluene)</td>
<td>0.25</td>
<td>5.9</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>Dimethyl benzene (Xylene)</td>
<td>0.22</td>
<td>6.0</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>Trimethyl benzene</td>
<td>0.18</td>
<td>6.2</td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl benzene</td>
<td>0.23</td>
<td>6.4</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>Propyl benzene</td>
<td>0.20</td>
<td>6.9</td>
<td>0.37</td>
</tr>
<tr>
<td>7</td>
<td>iso-Propyl benzene</td>
<td>0.16</td>
<td>9.7</td>
<td>0.39</td>
</tr>
<tr>
<td>8</td>
<td>(2-Ethyl propyl) benzene</td>
<td>0.14</td>
<td>10.2</td>
<td>0.40</td>
</tr>
<tr>
<td>9</td>
<td>Methanol</td>
<td>0.22</td>
<td>1.7</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>Ethanol</td>
<td>0.19</td>
<td>2.2</td>
<td>0.14</td>
</tr>
<tr>
<td>11</td>
<td>Propanol</td>
<td>0.16</td>
<td>2.7</td>
<td>0.15</td>
</tr>
<tr>
<td>12</td>
<td>iso-Propanol</td>
<td>0.15</td>
<td>5.0</td>
<td>0.16</td>
</tr>
<tr>
<td>13</td>
<td>Butanol</td>
<td>0.13</td>
<td>3.2</td>
<td>0.16</td>
</tr>
<tr>
<td>14</td>
<td>2-Butanol</td>
<td>0.12</td>
<td>5.6</td>
<td>0.17</td>
</tr>
<tr>
<td>15</td>
<td>Chlorobenzene</td>
<td>0.19</td>
<td>4.6</td>
<td>0.38</td>
</tr>
<tr>
<td>16</td>
<td>Dichlorobenzene</td>
<td>0.08</td>
<td>3.6</td>
<td>0.40</td>
</tr>
<tr>
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<td>Phenol</td>
<td>0.38</td>
<td>5.7</td>
<td>0.36</td>
</tr>
<tr>
<td>18</td>
<td>Chlorophenol</td>
<td>0.28</td>
<td>4.7</td>
<td>0.38</td>
</tr>
<tr>
<td>19</td>
<td>Dichlorophenol</td>
<td>0.17</td>
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<td>0.40</td>
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<tr>
<td>20</td>
<td>Trichlorophenol</td>
<td>0.07</td>
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<td>0.34</td>
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<td>4.8</td>
<td>0.38</td>
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<td>3.8</td>
<td>0.40</td>
</tr>
<tr>
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<td>0.33</td>
<td>5.8</td>
<td>0.34</td>
</tr>
<tr>
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<td>Diethyl phthalate</td>
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<td>6.8</td>
<td>0.36</td>
</tr>
<tr>
<td>27</td>
<td>Dipropyl phthalate</td>
<td>0.22</td>
<td>7.8</td>
<td>0.38</td>
</tr>
<tr>
<td>28</td>
<td>DIBUTYL phthalate</td>
<td>0.17</td>
<td>8.9</td>
<td>0.40</td>
</tr>
<tr>
<td>29</td>
<td>Diocyl phthalate</td>
<td>-(0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Bis(2-ethyl hexyl) phthalate</td>
<td>-(0.07)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSIONS

It can be concluded that the group contribution approach to predict the Monod rate constant and hence the extent of degradation does follow the trends reported in literature for different groups of compounds.

REFERENCES


Predictive structure-biodegradation relationship models


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