Bull. Environ. Contam. Toxicol. (2002) 69:111–116

DOI: 10.1007/s00128-002-0016-7

Bulletin of Environmental Contamination and Toxicology

Quantitative Structure–Biodegradability Relationships of Substituted Benzenes and Their Biodegradability in River Water

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Received: 17 May 2001/Accepted: 4 March 2001

Most substituted benzenes constitute a class of important environmental pollutants that are present in the Songhua River of Jilin Province, China. The presence of many of these chemicals in the natural waters is a serious public health problem. Biodegradation is an important mechanism for removing them from the ecosystem.

Biochemical oxygen demand (BOD) is the relatively simple method of determination and the information obtained is very useful for most cases. So the parameter is used to determine the ultimate degradability, which is demanded in most of the standard tests (Pagga 1997). However, gathering this information is labor intensive, time consuming, and expensive due to the large number of these chemicals. Therefore, it is necessary to develop correlation and predictive techniques in order to estimate biodegradability. The quantitative structure-biodegradability relationships (QSBRs) have been used to predict the fate of organic chemicals (Dearden and Cronin 1996b).

MATERIALS AND METHODS

Organic and inorganic chemicals were of analytical and reagent grade, respectively. Water samples were gathered from Jilin section in the Songhua River. Temperature of the water sample: 15-20℃; pH: 6.8-7.0; Dissolved oxygen (DO): about 8.0 mg/l. The bacteria counts were determined by standard plate count techniques (Wang 1988), and are about 800-3000/ml.

Biodegradation of substituted benzenes was determined using the standard iodometric titration method (Du 1994). The initial test concentration of chemicals was planned at 2mg/l on the basis of their theoretical oxygen demand (ThOD) and residual DO of at least 1mg/l at the final day (Vaishnav 1987).

The test chemical was added to 150ml of water sample in 250ml BOD bottles. The

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bottles were then filled to capacity with the water sample, sealed, and incubated for 5 d at 20±1℃.There were two replicates for each chemical and each control (only inoculum), respectively. The DO concentrations were determined by the iodometric titration method (Du 1994). The test result was expressed as BOD% by comparing the measured BOD_5 with ThOD, which is calculated from the molecular formula of the test compound (Table 1).

M_W, $H_f$and E_{HOMO} of 47 substituted benzenes were calculated by the quantum chemical method MOPAC6.0—AM1 on energy-minimized structures (Dearden 1996a). This method can automatically optimize the bond length, the bond angle and the twist angle, and yield a lot of structural information. The parameter values of studied compounds are listed in Table 1.

M_W is the molecular weight, and can reflect the size of a molecule. Generally, the smaller the molecule, the smaller the steric resistance in microorganisms, and the more easily the molecule can penetrate the cell through its cell membrane and arrive at the active site of an enzyme (Boethling 1986). The heat of formation (H_f) of a compound is a measure of its stability, and hence would be expected to reflect the ability of the compound to biodegrade (Dearden and Cronin 1996b). E_{HOMO} is the energy of the highest occupied molecular orbital, and is related to ionization potential. The higher the E_{HOMO} value, the stronger the electron donating ability (Liu 1991).

RESULTS AND DISCUSSION

The experimental results in Table 1 show that the most readily biodegradable compound is benzoic acid (BOD% is up to 86.3%), while the most non-readily biodegradable is 2,4,6-trichlorophenol (BOB% is -17%). Whilst the range of molecular weights is from 93.13 for aniline to 197.45 for 2,4,6-trichlorophenol; the range of $H_f$is from 100.50 for 3-nitroaniline to -656.09 for isophthalic acid; and the range of E_{HOMO} is from –8.27 for 4-aminophenol to –10.97 for 3-nitrobenzoic acid.

Damborsky and Schultz (1997) developed and compared QSBR models for biodegradability of groups of *m*-anilines and *p*-phenols. Anilines were degraded by an unknown bacterial strain isolated from the Onconee River, and phenols by *Pseudomonas putida* U. Biodegradability was expressed as the second-order kinetics rate constant (K_b). The QSBR equations were obtained as follows:

$\log K_b$=-11.237r_w+0.0092M_w+0.374pK_a-14.194, n=7(anilines), R^2=0.953 (1);

$\log K_b$=-13.743r_w+0.0351V_w+0.195pK_a-13.462, n=8(phenols), R^2=0.986 (2);

$\log K_b$=-11.233r_w +0.315pK_a-12.738, n=15(anilines and phenols), R^2=0.986 (3).

Table 1. Experimental and predicted BOD % of 47 substituted benzenes.

No.	Compound	BOD%		M_W	$-H_f$	$-E_{HOMO}$
		Exp.	Pre.	(g · M^{-1})	(kJ · M^{-1})	(eV)
Training Set						
1	Phenol	69.8	68.6	94.1	93.9	9.1
2	Catechol	65.1	69.6	110.1	277.5	8.9
3	Resorcinol	68.6	72.6	110.1	279.5	9.1
4	3-chlorophenol	31.7	42.9	128.6	121.5	9.3
5	4-chlorophenol	44.0	40.0	128.6	122.6	9.1
6	2-Methylphenol	69.0	56.7	108.1	123.2	9.0
7	3-Methylphenol	54.0	57.2	108.1	124.9	9.0
8	4-Methylphenol	62.9	55.0	108.1	124.6	8.9
9	2-Nitrophenol	36.0	36.8	139.1	66.1	10.0
10	3-Nitrophenol	37.0	38.1	139.1	75.1	10.0
11	4-Nitrophenol	50.3	51.7	139.1	81.8	10.8
12	4-Methoxyphenol	45.9	49.5	124.1	248.3	8.6
13	2,4-Dichlorophenol	19.1	10.7	163.0	138.7	9.2
14	2,4,6-Trichlorophenol	-17.0	-16.7	197.5	160.3	9.4
15	2-Aminophenol	31.0	42.7	109.1	97.5	8.4
16	4-Aminophenol	28.0	41.1	109.1	96.0	8.3
17	2,3-Dimethylphenol	44.0	40.7	122.2	107.2	8.9
18	2,4-Dimethylphenol	9.0	44.8	122.2	150.8	8.9
19	Benzoic acid	86.3	78.4	122.1	284.3	10.1
20	2-Hydroxylbenzoic acid	64.9	73.7	138.1	470.1	9.5
21	2-Aminobenzoic acid	62.7	44.7	137.1	296.0	8.8
22	3-Aminobenzoic acid	56.2	45.5	137.1	289.1	8.9
23	4-Aminobenzoic acid	78.0	46.9	137.1	297.3	8.9
24	3-nitrobenzoic acid	42.0	46.7	167.1	261.6	11.0
25	4-nitrobenzoic acid	50.2	48.1	167.1	259.4	10.9
26	Phthalic acid	78.4	80.9	166.1	631.2	10.5
27	Isophthalic acid	60.7	84.4	166.1	656.1	10.5
28	2-Chlorobenzoic acid	55.3	43.8	156.6	288.9	9.9
29	3-Chlorobenzoic acid	45.3	46.7	156.6	310.7	9.9
30	4-Chlorobenzoic acid	65.0	47.9	156.6	312.2	10.0
31	2-Methoxybenzoic acid	55.8	58.7	152.2	422.1	9.7
32	4-Methoxybenzoic acid	71.5	58.1	152.2	446.1	9.5
33	3-Methylbenzoic acid	52.9	63.5	136.2	316.9	9.8
34	Aniline	55.0	30.5	93.1	-85.8	8.5
35	2-Methylaniline	41.0	30.1	107.2	-55.2	8.4
36	4-Methylaniline	46.0	30.2	107.2	-53.4	8.4
37	2-Nitroaniline	-5.0	7.1	138.1	-86.8	9.1
38	3-Nitroaniline	6.0	8.7	138.1	-100.5	9.3
39	2-Chloroaniline	-10.0	13.4	127.5	-54.9	8.6
40	3-Chloroaniline	19.0	15.0	127.5	-55.2	8.7
Test Set						
1	2-Chlorophenol	38.0	39.4	128.6	111.6	9.2
2	3-Aminophenol	46.0	46.5	109.1	100.8	8.6
3	4-Hydroxylbenzoic acid	63.0	76.1	138.1	472.2	9.6
4	2-nitrobenzoic acid	43.2	43.8	167.1	240.7	10.9
5	Terephthalic acid	72.0	82.8	166.1	653.8	10.4
6	3-methylaniline	50.0	29.7	107.2	-58.1	8.5
7	4-Nitroaniline	-9.0	10.1	138.1	-90.2	9.3

In which, r_w is Van der Waal's radii; V_w is Van der Waal's volume; M_w is molecular weight; and pK_a is the ionization constant. Damborsky and Schultz thought that electronic and steric or size properties should be necessary for modeling biodegradation.

Boethling (1986) developed correlation equations for biodegradability, the second order kinetics rate constant (K_b) of 12 Phthalic acids and phthalate esters using M_w: $K_b=-0.977\times 10^{-3}\ M_w + 0.533$, n=12, R=-0.954 (4). The results showed that the biodegradability of studied compounds appears to be negatively correlated with molecular size.

Dearden and Cronin (1996b) investigated the correlation of the biodegradability (final BOD) and structure parameters. Step-wise regression indicated that three parameters, namely heat of formation, six order chain molecular connectivity and the total topological index based on electrotopological state indices, were important in modeling final BOD. Discriminant analysis on the 222 compounds gave good discrimination between readily and non-readily biodegradable compounds, with an overall correct cross-validated prediction rate of 78.6%.

In addition, Lu et al (2001) also developed QSBR models on the maximum specific removal rates (Q_{TOD}) of substituted aromatic hydrocarbons with the structure parameters as follows: $Q_{TOD}=-0.614M_W-9.704E_{HOMO}-0.129H_f$, n=52, $R^2=0.860$, S.E.=14.89, F=100.21, Sig.=0.000 (5). Lu concluded that the biodegradation of studied compounds is related mainly to steric and electronic parameters.

On the basis of the above work, M_W, $H_f$and E_{HOMO} are selected as the structural parameters to establish the QSBRs using the linear regression method in this paper. 47 compounds are randomly divided into two sets. Of these, 40 compounds are included in the training set and 7 compounds in the test set. The linear regression analyses were performed using the SPSS statistical package. From the stepwise regression analyses, three QSBR models are developed using the degradation data (BOD), and parameters of studied chemicals as follows:

$$BOD\%=-4.826E_{HOMO},\ n=40,\ R^2=0.771,\ S.E.=24.96,\ F=131.20,\ Sig.=0.000 \quad (6)$$

$$BOD\%=-3.213E_{HOMO}-0.077H_f \quad (7)$$

$$n=40,\ R^2=0.839,\ S.E.=21.17,\ F=99.35,\ Sig.=0.000$$

$$BOD\%=-15.833E_{HOMO}-0.109H_f-0.925M_W \quad (8)$$

$$n=40,\ R^2=0.930,\ S.E.=14.20,\ F=162.82,\ Sig.=0.000$$

Where, R^2 is the square of the correlation coefficient; S.E. is the standard error; F is the mean square ratio; Sig. is the significance level; and n is the number of compounds. It is apparent that correlation is most significant and the standard error is lowest in equation (8). Thus, this equation is used to predict the biodegradability (Table 1).

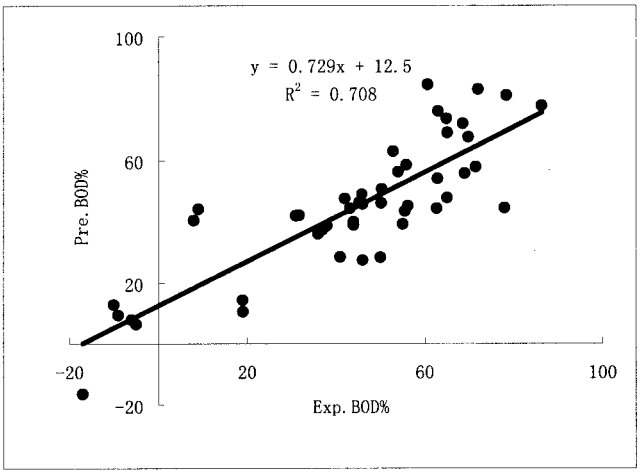

Figure 1. Plot of predicted BOD% by Eq. 8 vs experimental BOD%

The biodegradability of 47 substituted benzenes may be divided into three categories according to their BOD% values: non-readily biodegradable (BOD%≤30); biodegradable (30<BOD%<60); and readily biodegradable (BOD%≥60). If the rule is used to qualitatively estimate the predicted results, the correct prediction rates of the linear regression method (Eq. 8) is up to 72% for the training set, while more than 85% for the test set. This result shows the obtained QSBR model fits well, especially for the test set.

In order to test further the prediction capability of Eq.8, the plot of the predicted values from (8) *versus* the experimental BOD% is shown in Figure 1. It can be seen that the obtained predicted model fits well and R^2 between the predicted and the experimental values is up to 0.708.

Acknowledgment. We thank the National Natural Science Foundation of China and the Science Innovation Foundation of Hohai University for funding this research.

REFERENCES

Bhagat P (1990) An introduction to neural nets. Chem Eng Prog 86: 55-60

Boethling R S (1986) Application of molecular topology to quantitative structure-biodegradability relationships. Environ Toxicol Chem 5: 797-806

Damborsky,J, Schultz,T W (1997) Comparison of the QSAR models for toxicity and biodegradability of anilines and phenols. Chemosphere 34: 429-446

Dearden J C (1996a) Descriptors and techniques for quantitative structure- -biodegradablity

studies. QAR and QSAR in Environmental Research 5: 17-26

Dearden J C, Cronin M T D (1996b) Quantitative structure biodegradability studies: an investigation of the Miti aromatic compound data-base. In: Peijnenburg W J G M and Damborsky J (ed) Biodegradation prediction, Academic Press, New York, p 93-105

Vaishnav D D, Boethling R S, Babeu L (1987) Quantitative structure- -biodegradability relationships for alcohols, ketones and alicyclic compounds. Chemosphere 16: 695-703

Du C J, Chu D S (1994) Water quality analysis base in environmental monitoring. Science and Technology Press of Heilongjiang, Haerbin, P. R. China

Liu C Q. (1991) Quantum biology and application. Higher Education Press, Beijing, P.R. China

Lu G H, Jiang S Q, ZhaoY H (2001) Quantitative relationships study for the structure and biodegradability of substituted benzenes. Chem J Internet 3: 3-6

Pagga U (1997) Testing biodegradability with standardized methods. Chemosphere 35: 2953-2972

Wang J L (1988) Environmental microbiology. Higher Education Press, Beijing, P.R. China