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Correlation Study of Toxicity of Substituted Phenols to River Bacteria and Their Biodegradability in River Water[1]

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Objective To study the correlation of toxicity with biodegradability (BODT) in order to promote QSBR development and understand the degradation mechanism. **Methods** Toxicity of substituted phenols to river bacteria was determined by the turbidities that were measured using a spectrophotometer (UV-190) at 530 nm against a blank control. The biodegradability of substituted phenols was expressed as BODT and the DO concentrations were determined by the iodometric titration method. **Results** The BODT and toxicity(log $1/IC_{50}$) of 12 substituted phenols to bacteria from the Songhua River were determined respectively. The correlation of biodegradability with toxicity was developed: BODT=8.21 (±2.22) p*K*a -32.44 (±8.28) log $1/IC_{50}$ +89.04 (±38.20), n=12, R^2=0.791, R^2(adj)=0.745, SE=9.134, F=17.066, P=0.001. **Conclusion** The BODT of substituted phenols was influenced by their toxicity and the ionization constant p*K*a. The stronger the toxicity, the less readily the compound was degraded by river bacteria.

Key words: Biodegradability; Toxicity; Correlation; Substituted phenols

INTRODUCTION

It is important to evaluate the biodegradation of organic chemicals in understanding the transformation, fate and risk of chemicals in the environment. However, gathering this information is labor intensive, time consuming, and expensive due to the large number of these chemicals. Relevant studies have shown that the biodegradability of organic compounds could be estimated effectively and rapidly using the quantitative structure-biodegradability relationship (QSBR) models[1-3]. At present, most QSBRs have been developed using the structural or property parameters and are used to predict biodegradability, neglecting the influence of toxicity to biodegradation. In fact, the biodegradability of chemicals could be affected by the toxicity of parent substances and intermediate products. Therefore, it is useful to develop the correlation of toxicity with biodegradability in order to promote QSBR development and understand the degradation mechanism.

In this study, we determined the biodegradability and toxicity of typical phenols using bacteria from the Songhua River as an inoculum, developed QSBR models and analyzed biodegradation mechanism.

MATERIALS AND METHODS

Substituted phenols were of analytical grade. Water samples were gathered from Jilin section in the Songhua River. Dissolved oxygen (DO) was 8.3 $mg \cdot L^{-1}$, pH 6.5-6.9. The bacterial counts were about 2.4×10^3 $organism \cdot mL^{-1}$ determined by standard plate count techniques[4].

The culture was maintained in liquid medium containing 3 g beef extract, 10 g peptone, 5 g NaCl, 1 L distilled water. The pH of the culture medium was adjusted to 7.2-7.4, 40 mL of the culture medium was added in 250 mL conical flasks, and then the culture was sterilized for 20 min at 121℃.

Each compound was dissolved in 95% ethanol, and 5 concentrations were tested with the same logarithmic difference. One mL of test chemical solution and 1 mL of river water were added to 40 mL of culture medium in 250 mL conical flasks, 1 mL of 95% ethanol solution was added to 40 mL of

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culture medium as the blank control, and 1 mL of river water and 1 mL of 95% ethanol solution were added to 40 mL of culture medium as the seed control. There were three replications for each concentration and each control, respectively. All the samples were incubated in a incubator for 24 h at 20℃±2℃.

The turbidities were measured using a spectrophotometer (UV-190) at 530 nm against a blank control[5]. The absorbance values of the toxicant-amended mixtures were calculated as a percentage of the control according to the following formula:

$$\text{Absorbance rate}=\frac{\text{Absorbance of test bottle}}{\text{Absorbance of seed control}}\times 100\%$$

The concentration values causing 50% reduction of growth (IC_{50}, $M\cdot L^{-1}$) were obtained from the plot of the logarithm of the toxicant concentration against the absorbance rates (Table 1).

Approximately 2 $mg\cdot L^{-1}$ of test chemical was added to 250 mL biochemical oxygen demand (BOD) bottles. The bottles were then filled to capacity with the water sample, sealed and incubated at 20℃±1℃. There were two replications for each chemical and each control (inoculum only), respectively. The DO concentrations were measured with the iodometric titration method[6]. The test result was expressed as BODT. If a compound could be expressed as CcHhClclNnOo, BODT could be calculated by the following formula[7]:

ThOD=16[2c+1/2(h-cl-3n)-o]/molecular weight

BODT=(BOD_5/ThOD)×100%

ThOD is the theory oxygen demand. BODT values of phenols are presented in Table 1.

The hydrophobicity parameter log *P* and the ionization constant p*K*a were obtained from Micro QSAR software (ver. 2.0, Hunter System, Washington, DC, USA). log *P* is the logarithm of n-octanol/water partition coefficient, and can reflect the hydrophobicity of a molecule. The negative logarithm of the acid ionization constant (p*K*a) reflects the proton releasing ability of a compound. The lower the p*K*a value, the stronger the proton releasing ability, and the stronger the electrophilic ability; whilst the higher the p*K*a value, the weaker the proton releasing ability, and the stronger the nucleophilic ability. The molecular connectivity indexes ${}^1X$, ${}^2X$, ${}^1X^V$ and ${}^2X^V$ were calculated by MMP software, as they are steric parameters, and can reflect the size of a molecule and the influence of substitutive group position. E_{LUMO} (eV) was calculated by the quantum chemical method MOPAC (ver. 6.0, http://ftp.osc.edu) program. E_{LUMO} is the energy of the lowest unoccupied molecular orbital and describes how susceptible the molecule is to interactions with a nucleophile and is directly related to electron affinity. The parameter values of the studied chemicals are listed in Table 1.

The equations were built by the stepwise linear regression method in the statistic package SPSS10.0.

RESULTS

The experimental results in Table 1 showed that the toxicity of phenols to river bacteria and their biodegradability were related to group variety, group sum and substitutive position. The most toxic compound was 2,4-dichlorophenol (log $1/IC_{50}$ is 4.18), while the least toxic compound was phenol (log $1/IC_{50}$ is 2.90). The order of biodegradability of phenols was: phenol > methylphenols > chlorophenols ≈ nitrophenols. The most readily biodegradable compound was phenol (BODT was up to 83%), whilst the least biodegradable chemical was 2,4-dichlorophenol containing the most groups (BODT was 15%). The parameters log *P*, p*K*a, ${}^1X$, ${}^2X$, ${}^1X^V$, ${}^2X^V$ and E_{LUMO} were selected as the descriptors to establish QSBRs. Using bivariate correlation analysis of the activity and parameters, a linear correlation matrix was obtained (Table 2).

A correlation was obtained between toxicity and biodegradability (R=0.689). Moreover, the toxicity of phenols to river bacteria was related chiefly to log *P* and the steric parameter ${}^1X^V$ and ${}^2X^V$. However, not only log *P* and the molecular connectivity index but also p*K*a and E_{LUMO} were found to be relevant to biodegradability.

The following QSBR model was obtained by stepwise regression analysis on the biodegradation data (BODT) for the 12 substituted phenols against the 7 structural parameters described above.

BODT=7.74 (±1.63) p*K*a -47.80 (±7.88) ${}^2X^V$ +67.53 (±22.74) (1)

n=12, R^2=0.889, R^2(adj)=0.864, SE=6.659, F=36.073, *P*=0.000.

Where *n* is the number of compounds. R^2 is the square of the correlation coefficient, R^2(adj) is the adjusted R^2, SE is the standard error, F is the mean square ratio and *P* is the probability.

Since there is an obvious correlation between log $1/IC_{50}$ and ${}^2X^V$ (R=0.902), the following QSBR model was derived using log $1/IC_{50}$ instead of ${}^2X^V$ as the descriptor.

BODT=8.21 (±2.22) p*K*a -32.44 (±8.28) log $1/IC_{50}$ +89.04 (±38.20) (2)

n=12, R^2=0.791, R^2(adj)=0.745, SE=9.134, F=17.066, *P*=0.001

DISCUSSION

Apparently, there is an obvious negative correlation between log $1/IC_{50}$ and BODT, i.e., the stronger the toxicity, the lower the BODT value, the less readily the compound is degraded by river bacteria. Among the compounds studied in this paper,

TABLE 1

Structural Parameters, Toxicity and Biodegradability Data of Substituted Phenols

Compounds	log P	^1X	^2X	$^1X^V$	$^2X^V$	E_{LUMO}	pKa	log $1/IC_{50}$	BODT(%)				
									Exp.	Pre1.[a]	Er1.[b]	Pre2.[c]	Er2.[b]
Phenol	1.97	3.39	2.74	2.13	1.34	0.40	9.92	2.90	83	80	3.6	77	7.2
2-Methylphenol	2.44	3.81	3.24	2.55	1.79	0.40	10.21	3.42	67	61	11.2	62	7.5
3-Methylphenol	2.44	3.79	3.38	2.55	1.84	0.38	10.05	3.36	56	57	-1.8	63	-12.5
4-Methylphenol	2.44	3.79	3.37	2.55	1.84	0.43	10.23	3.73	63	59	6.3	52	17.5
2-Chlorophenol	2.69	3.81	3.24	2.62	1.86	0.07	8.40	3.43	43	44	-2.3	47	-9.3
3-Chlorophenol	2.69	3.79	3.38	2.61	1.92	0.02	9.09	3.84	34	46	-35.3	39	-14.7
4-Chlorophenol	2.69	3.79	3.37	2.61	1.91	0.07	9.38	3.51	40	49	-22.5	52	-30.0
2-Nitrophenol	2.13	4.72	4.17	2.73	1.82	-1.01	6.80	3.31	32	33	-3.1	38	-18.7
3-Nitrophenol	2.13	4.70	4.28	2.72	1.86	-1.16	8.27	3.58	39	43	-10.3	41	-5.1
4-Nitrophenol	2.13	4.70	4.26	2.72	1.86	-1.07	7.15	3.75	43	35	18.6	26	39.5
2,3-Dimethylphenol	2.90	4.22	3.75	2.97	2.22	0.38	10.34	3.94	45	42	6.7	46	-2.2
2,4-Dichlorophenol	3.42	4.20	3.87	3.09	2.44	-0.24	7.87	4.18	15	12	20.0	18	-20.0

Notes. [a]. Pre 1 is calculated from equation (1); [b]. Relative error (Er%) is defined as the difference between the experimental and predicted values for biodegradability divided by the experimental values; [c]. Pre 2 is calculated from equation (2).

TABLE 2

Correlation Matrix for Toxicity, Biodegradability and Parameters

R	log $1/IC_{50}$	BODT	log P	pKa	^1X	^2X	$^1X^V$	$^2X^V$	E_{LUMO}
log $1/IC_{50}$	1.000								
BODT	-0.689	1.000							
log P	0.741	-0.576	1.000						
pKa	-0.150	0.660	0.097	1.000					
^1X	0.348	-0.586	-0.140	-0.746	1.000				
^2X	0.475	-0.667	-0.023	-0.717	0.983	1.000			
$^1X^V$	0.848	-0.831	0.683	-0.388	0.617	0.695	1.000		
$^2X^V$	0.902	-0.783	0.859	-0.184	0.360	0.473	0.952	1.000	
E_{LUMO}	-0.122	0.543	0.318	0.885	-0.893	-0.864	-0.347	-0.093	1.000

the toxicity value of 2,4-dichlorophenol is the highest (log $1/IC_{50}$=4.18), and its BODT value is the lowest (15%); whilst the biodegradation of phenol is the strongest (BODT=83%), as its toxicity value is only 2.90.

In general, the substrate is the best inducer for synthesizing enzymes. However, some enzymatic synthesis may be influenced by various factors. The biodegradability controlled by the toxicity of compounds may result from the toxicant either lowering enzyme activity and enzymatic system or influencing the synthesis of relevant enzymes. Accordingly, the enzymatic reaction is affected and the biodegradation is restrained[8].

The ionization constant pKa reflects the proton releasing ability of a compound. The biodegradability of studied phenols appears to be positively correlated with pKa. For those phenols with similar toxicity, the higher the pKa value, the easier the compound is degraded. For example, 4-methylphenol with a higher pKa value is more readily degraded than 4-nitrophenol, suggesting that these phenols are degraded by an intracellular enzyme. For the compound with a higher pKa value, since the concentration of its molecular state is higher, it is easier to penetrate cells and arrive at the enzyme active site, leading to faster degradation.

The biodegradability of phenols is correlated with the electronic and steric parameters. Damborsky and Schultz[9] have developed a QSBR model for biodegradability of *p*-phenols. Biodegradability is expressed as the second-order kinetics rate constant (K_b): $\log K_b$=-13.743r_w+0.0351V_w+0.195pKa -13.462, n=8(phenols), R^2=0.986, where, r_w is Van der Waal's radii, and V_w is Van der Waal's volume. They found that electronic and steric or size properties are necessary for modeling the biodegradation of the studied compounds.

Moore *et al*.[10] studied the biodegradation half-life (T_{50}) of phenols and have found a correlation between T_{50} and pKa: log (T_{50})= -0.21pKa+2.0, n=20, R=0.941. They also noted that the biodegradability of the studied compounds correlates with the electron donating ability of the substitutive group.

Eq. (1) and (2) used to predict the biodegradability of the compounds studied in this report, and the predicted values and relative errors are presented in Table 1.

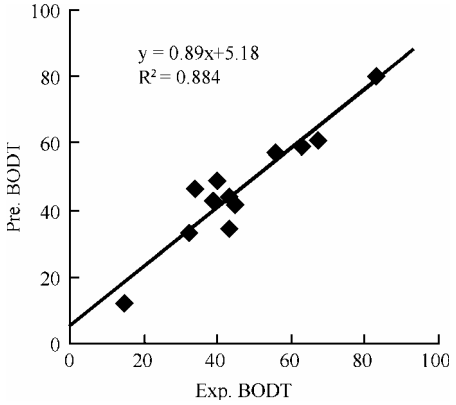

FIG. 1. Plot of predicted values by Eq.1 vs experimental BODT.

The plot of the predicted values from Eq. (1) and (2) versus the experimental BODT is shown in Figs. 1 and 2. It can be seen from Figs. 1 and 2 that the predicted values by Eq. (1) fit better than those by Eq. (2). The errors of 75% compounds are lower than 20% when predicted by either of the two equations, whilst all the errors remain within 35% for

Eq. (1) and 40% for Eq. (2).

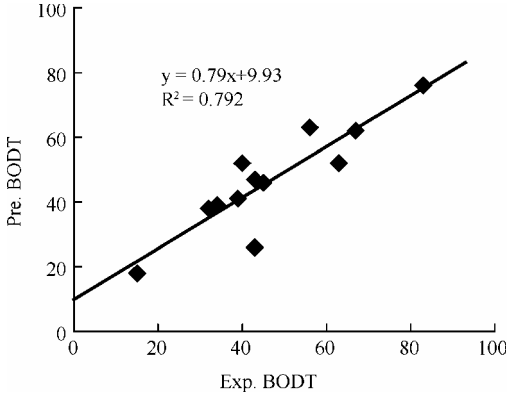

FIG. 2. Plot of predicted values by Eq.2 vs experimental BODT.

CONCLUSION

The biodegradability of substituted phenols to bacteria from the Songhua River can be predicted using p*K*a and ${}^2X^V$(R^2=0.889). There is an obvious correlation between the toxicity (log $1/IC_{50}$) of phenols to bacteria from the Songhua River and the steric parameter (${}^2X^V$) (R=0.902). A new QSBR model can be developed using log $1/IC_{50}$ and p*K*a.

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