Journal of Hazardous Materials 177 (2010) 414–419

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxicity evaluation and prediction of toxic chemicals on activated sludge system

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ARTICLE INFO

Article history:
Received 1 September 2009
Received in revised form
26 November 2009
Accepted 26 November 2009
Available online 16 December 2009

Keywords:
Toxicity
Activated sludge
QSARs
Respiration rate
Molecular descriptors

ABSTRACT

The gaps of data for evaluating toxicity of new or overloaded organic chemicals on activated sludge system resulted in the requirements for methodology of toxicity estimation. In this study, 24 aromatic chemicals typically existed in the industrial wastewater were selected and classified into three groups of benzenes, phenols and anilines. Their toxicity on activated sludge was then investigated. Two indexes of $IC_{50\text{-}M}$ and $IC_{50\text{-}S}$ were determined respectively from the respiration rates of activated sludge with different toxicant concentration at mid-term (24 h) and short-term (30 min) time intervals. Experimental results showed that the group of benzenes was the most toxic, followed by the groups of phenols and anilines. The values of $IC_{50\text{-}M}$ of the tested chemicals were higher than those of $IC_{50\text{-}S}$. In addition, quantitative structure–activity relationships (QSARs) models developed from $IC_{50\text{-}M}$ were more stable and accurate than those of $IC_{50\text{-}S}$. The multiple linear models based on molecular descriptors and K_{ow} presented better reliability than single linear models based on K_{ow}. Among these molecular descriptors, E_{lumo} was the most important impact factor for evaluation of mid-term toxicity.

1. Introduction

Currently aromatic contaminants such as benzenes, phenols and anilines entered into the drainage system with large amount, due to the development of petrochemical and energy-producing industries. When treated by activated sludge process in typical industrial wastewater treatment plant (WWTP), these kinds of contaminants are toxic and hardly degraded by activated sludge. It has been reported that the presence of aromatic compounds can reduce the affinity of the bacterial cells for carbon source, change the structure of cell envelope, and cause a drop of dissolved oxygen limitation [1]. It will further lead to sludge bulking, reduction in solids separation efficiency, low removal of organics and operation upsets of treatment plant. To minimize those impacts, it is crucial to evaluate the toxic impact of the new or overloaded chemicals discharged into WWTP on activated sludge system and then control them accordingly. However, the characteristic of industrial wastewater is difficult to be predicted due to the wide range of industrial chemicals contained and the complexity of their mixture.

Different approaches have been explored to evaluate toxicity of wastewater on activated sludge. Chemical characterization such as biochemical oxygen demand (BOD) and gas chromatography–mass spectrometry (GC/MS) analyses were executed to define the toxicity of influent; however, they were time consuming and lagging. Currently, ecotoxicological tests were developed to assay the toxicity of chemicals, e.g., Microtox® test on different bioassays, such as Vibrio fischeri etc. [2], bioluminescence assays [3], and respirometry of activated sludge [2]. To evaluate potential toxicity of chemical on activated sludge, the most preferred biological index should be activated sludge itself. Thus, the respirometry of activated sludge was more suitable assay to determine and evaluate the toxicity of certain chemical. Ubay et al. utilized respirometric analysis to estimate the biodegradability of both synthetic domestic sewage and industrial wastewaters [4]. Different time span of respirometric analyses, varied from several minutes to 24 h, were used by different researchers, however such difference may result in various toxic effects on activated sludge. Therefore the toxic expressions at different time span are greatly needed to be classified and compared.

In addition, experimental determination of the toxicity of each chemical case by case is not practical and feasible for real time operation and management of WWTP. Predictive models could play significant role in supplying the missing data of toxicity and real time control of overloaded chemicals. Quantitative structure–activity relationships (QSARs) have been developed over the last decade to establish the correlation between certain type of biochemical activity and physico-chemical properties or molecular chemical structure and then to predict the toxicity of untested chemicals [5]. In QSARs models, the octanol–water partition coefficient (K_{ow}) and molecular structural parameters derived from semi-empirical molecular orbital (MO) calculations are commonly used as descriptors [6–8]. However, most previous studies have

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doi:10.1016/j.jhazmat.2009.11.152

established the QSARs models to predict the toxicity of chemicals on alga [5], *Daphnia magna* [9], cilitate inoculated in pure culture [6], and fish [10]. Few studies have been reported on the application of QSARs models in toxicity evaluation of chemicals on activated sludge in wastewater treatment process. To control and minimize chemicals hazardous impacts, it is necessary to establish predictive QSARs models to evaluate the toxicity of the new or overloaded chemicals discharged into industrial WWTP.

For above purposes, a structurally highly heterogeneous data set of 24 compounds was selected based on the components of practical industrial wastewater in this study. The respiration rates of activated sludge with the presence of each organic chemical at various concentrations were investigated and the effect of time span of respirometric assay on toxicity expression was compared and discussed. Furthermore, QSARs models correlating the toxicity of chemicals with their physico-chemical properties and molecular orbit parameters were established, which could predict the toxicity of aromatic contaminants on activated sludge.

2. Materials and methods

2.1. Chemicals and feed water

24 structurally highly heterogeneous chemical compounds were divided into three groups of benzenes, phenols and anilines based on their chemical structures. Benzenes contain benzene, toluene, ethylbenzene, xylene, styrene, chlorobenzene and nitrobenzene; phenols involve phenol, o-cresol, m-cresol, p-cresol, p-aminophenol, m-dihydroxybenzene, 2,4-dichlorophenol; anilines comprise aniline, o-toluidine, m-toluidine, p-toluidine, o-nitroaniline, m-nitroaniline, p-nitroaniline, o-phenylenediame, m-phenylenediame, p-phenylenediame, 2,4-diamninotoluene, 2,4-dinitroaniline. The chemicals were analytical purity and were purchased from Sinopharm Chemical Reagent Co. Ltd.

Wastewater was taken from the homogenization tank of WWTP in Shanghai Chemical Industrial Park (SCIP) and used as feed water. Activated sludge obtained from the bio-reaction tank of the same facility was used as seed sludge.

2.2. Respiration inhibition

The respiration inhibition protocol was based on OECD L133/118 [11] and ISO 8192 [12]. Tests were conducted in 1000 mL respirometric bottles. 300 mL wastewater from WWTP, 400 mL seed sludge and 300 mL toxic organic chemical with certain concentration were added in the bottle. Sludge concentration in each bottle was controlled at same value of 4000 mg mixed liquor suspended solids per liter. In order to evaluate the toxic index of each chemical, respiration tests of six different toxicant dosages and one blank without toxicant were conducted at the same time. The activated sludge oxygen consumption within 24 h was measured by electrolytic respirometer (Bioscience BI-2000, USA), and the oxygen consumption within 30 min was measured by the dissolved oxygen meter (YSI 5100 environmental, USA).

The respiration inhibition rate of tested chemical at each concentration could be calculated as:

$$\text{Inhibition ration}(\%) = \frac{r_{\text{blank}} - r_{\text{sample}}}{r_{\text{blank}}} \times 100\% \quad (1)$$

where r_{blank} and r_{sample} are the respiration rate of the blank and the tested chemical at each concentration, respectively. The relationship between each toxicant concentration and their corresponding percentage inhibition could be plotted. From the above relationship, the chemical concentration at which the percentage inhibition is 50% (IC_{50}) could be derived. Two sets of IC_{50} obtained from BI-2000 of 24 h and YSI 5100 of 30 min, were proposed to represent the mid-term toxic effects ($IC_{50\text{-M}}$) and short-term toxic effects ($IC_{50\text{-S}}$) on activated sludge system, respectively in this study.

2.3. Descriptors

The $\log K_{\text{ow}}$ data of the tested toxicants were obtained from database of National Institutes of Health of United States National Library of Medicine (http://chem.sis.nlm.nih.gov/chemidplus/). Theoretical molecular descriptors were calculated by using ChemOffice2006 (CambridgeSoft) which firstly converts chemical information into 3D structures, followed by the calculation of the quantum chemical parameters using ab initio MO theory at AM1 set. The calculated molecular descriptors included the energy of the lowest unoccupied molecular orbital (E_{lumo}), dipole moment (μ) and core–core repulsion (CCR).

2.4. Chemometric methods

Variable selection and model regression analyses were conducted with Microsoft Excel. After comparison between several physico-chemical and molecular descriptors, the variables of $\log K_{\text{ow}}$, E_{lumo}, μ and CCR were observed to be the most related significant variances. Several parameters, such as n, R^2 (adj.), SE, F, were used to evaluate the robustness and internal reliability of the models: "n" is the number of observations, "R^2 (adj.)" was the adjusted coefficient of determination, "SE" was the standard error of estimation; "F" was the Fisher criterion. Variance inflation factor (VIF) values were also calculated to determine the interdependence of the descriptors [13]. To check the external validation of the established model, several chemicals (about 20% of the original data) which did not participate in the model development were picked up from data set. They were used to compare the error between experiment value and model predicting value.

3. Results and discussion

3.1. Toxicity determined by respiration inhibition

Electrolytic respiration was tested for each chemical compound. The accumulated oxygen consumption was observed to decrease with the increase of toxicant concentration. Taking o-toluidine as an example, the percentage inhibition of at each concentration was calculated from Eq. (1), and then the percentage inhibition against each concentration can be plotted as shown in Fig. 1. $IC_{50\text{-M}}$ value of o-toluidine could be derived from this curve. As shown, the $IC_{50\text{-M}}$ value of o-toluidine tested within 24 h was 2.663 g/L. Similarly, the IC_{50} values of all tested chemicals within 24 h and 30 min could be obtained, ranging from 0.211 to 23.000 and 0.002 to 4.996 g/L, respectively. The $IC_{50\text{-M}}$ and $IC_{50\text{-S}}$ values representing mid-term and short-term toxicity on activated sludge were presented in Fig. 2. The results were compared between the three tested groups of chemicals. The value of $IC_{50\text{-M}}$ was observed to be higher than that of $IC_{50\text{-S}}$, which should be attributable to the adaptation of activated sludge to toxicants during the relative longer time of 24 h. It is noted the $IC_{50\text{-M}}$ and $IC_{50\text{-S}}$ of toluene was 0.474 and 0.053 g/L, respectively. Dalzell and his colleagues have reported IC_{50} of toluene was 0.1 g/L during 3 h [14]. Their value is relatively higher than $IC_{50\text{-S}}$ but lower than $IC_{50\text{-M}}$ obtained from this study, further demonstrating the reliability of this study.

3.2. Toxicity comparison of each chemical

Comparing the toxicity of the tested chemicals (Fig. 2), it was found that the benzenes were the most toxic, followed by phenols and anilines. The toxicity of each chemical is determined by their functional fragments [15]. Phenolic group's identical fragment is

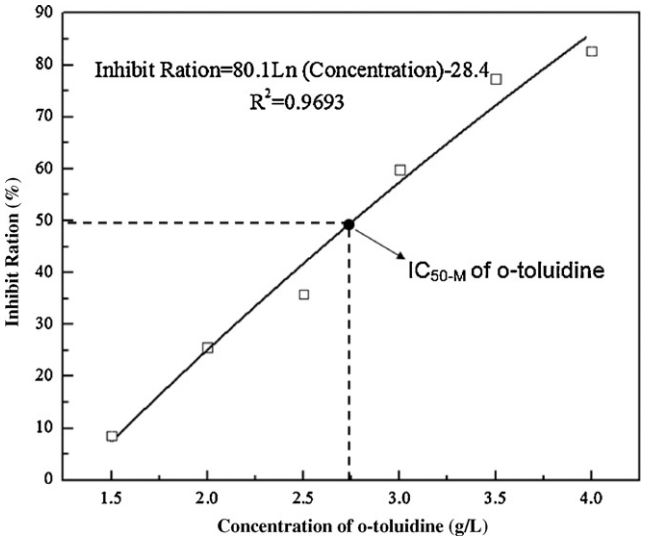

Fig. 1. The relationship of inhibit ration against concentration of o-toluidine.

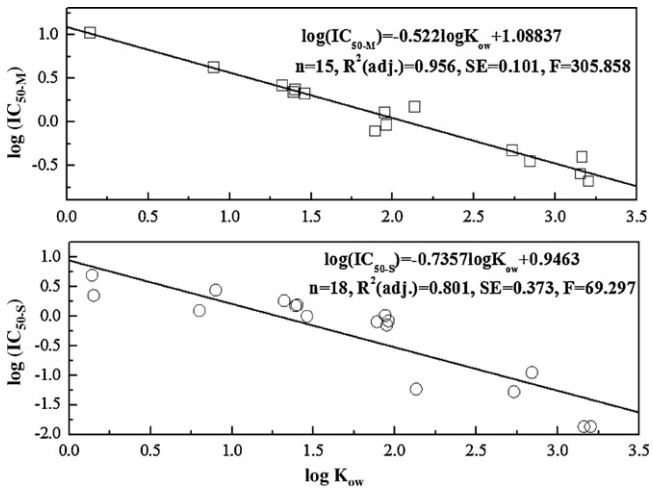

Fig. 3. Single linear model of chemical toxic concentration.

the hydroxyl moiety attached to an aromatic sp^2-hybridized carbon atom, which is strongly electrophilic function group. Amino group consists of one atom of nitrogen attached by covalent bonds to two atoms of hydrogen, leaving alone valence electron on the nitrogen which is available for bonding to another atom. Benzenes are more hydrophobic than the above two, and therefore the toxicity of benzenes increase because the hydrophobic fragments can adhere to bio-cell easily and attack cells of bacteria.

3.3. Single linear QSARs analysis of IC_{50} by K_{OW}

The single linear regression QSARs models correlating the logarithm of K_{ow} ($\log K_{ow}$) and the logarithm of $IC_{50\text{-}M}$ and $IC_{50\text{-}S}$ were developed based on around 18 of the 21 compounds (presented in Table 1). As shown in Fig. 3, the established model could fit the experimental data well, with the values of internal validation parameters, $R^2 > 0.8$, $F > F_{0.01}(1,12) = 9.33$. Comparing the developed two models, $IC_{50\text{-}M}$ was found to have better pertinency with K_{ow} than $IC_{50\text{-}S}$. It is note that the slopes of the curve obtained from $IC_{50\text{-}M}$ and $IC_{50\text{-}S}$ models are 0.522 and 0.736, respectively. Könemann et al. obtained slope values of single linear QSARs models as 0.87 when modeling the toxicities of narcotics chemicals on guppy [16]. Lee et al. obtained slope values of 1.005 when modeling the toxicity of benzoic acids to *Pseudokirchneriella subcapitata* [5]. Their slope values are relatively close to that of $IC_{50\text{-}S}$ models in this study.

The predictive capacity of the above model was validated, subsequently. Table 2 presents the predictive results on the compounds which are not used for QSARs model development. The errors within 8% were observed for m-dihydroxylene (mid-term) and o-nitroaniline (short-term). However, special and complicated reaction between certain tested contaminant and bacteria in activated sludge system may be involved, causing the scattered results, i.e. p-cresol (44.4% for mid-term). In addition, high volatility, i.e. ethylbenzene (23.8% for short-term), and limited solubility, i.e. 2,4-dinitroaniline (34.5% for short-term) also lead to outliers of the model.

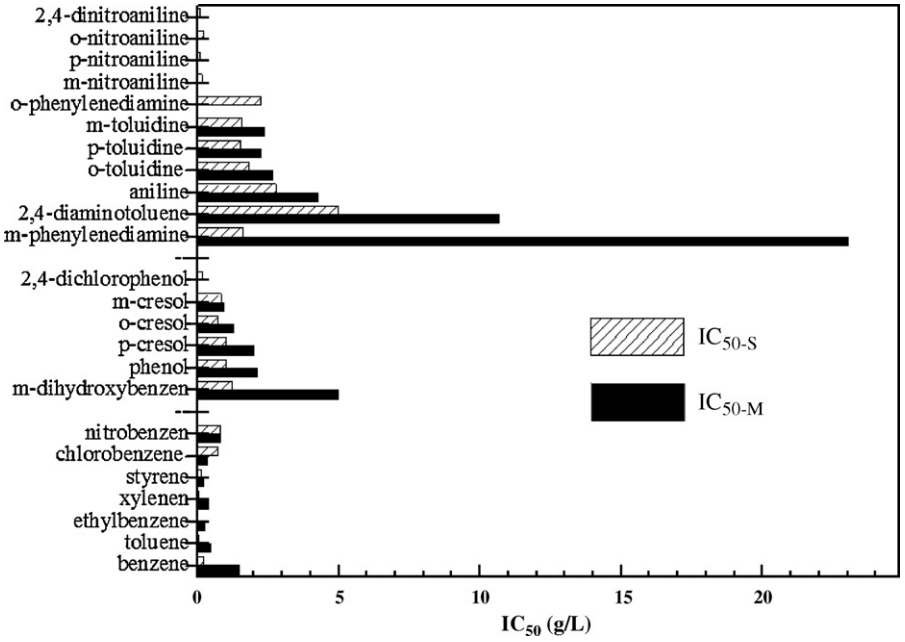

Fig. 2. The $IC_{50\text{-}M}$ and $IC_{50\text{-}S}$ of each chemicals.

Table 1
IC_{50} and $\log K_{ow}$ values of each chemical for mid and short-term.

Mid-term (24 h)				Short-term (30 min)		
No.	Chemicals	$IC_{50\text{-}M}$ (g/L)	$\log K_{ow}$	Chemicals	$IC_{50\text{-}S}$ (g/L)	$\log K_{ow}$
1	2,4-Diaminotoluene	10.686	0.14	m-Phenylenediamine	1.611	−0.33
2	Aniline	4.269	0.9	2,4-Diaminotoluene	4.996	0.14
3	o-Toluidine	2.663	1.32	o-Phenylenediamine	2.259	0.15
4	p-Toluidine	2.228	1.39	m-Dihydroxylene	1.252	0.80
5	m-Toluidine	2.372	1.40	Aniline	2.783	0.90
6	Phenol	2.133	1.46	o-Toluidine	1.836	1.32
7	Nitrobenzen	0.794	1.89	p-Toluidine	1.515	1.39
8	o-Cresol	1.291	1.95	m-Toluidine	1.558	1.40
9	m-Cresol	0.931	1.96	Phenol	1.008	1.46
10	Benzene	1.553	2.13	Nitrobenzen	0.812	1.89
11	Toluene	0.495	2.73	p-Cresol	1.04	1.94
12	Chlorobenzene	0.365	2.84	o-Cresol	0.713	1.95
13	Ethylbenzen	0.271	3.15	m-Cresol	0.839	1.96
14	Xylene	0.422	3.16	Benzene	0.235	2.13
15	Styrenen	0.215	3.20	Toluene	0.12	2.73
16				Chlorobenzene	0.743	2.84
17				Xylene	0.045	3.16
18				Styrenen	0.136	3.20

Table 2
The comparison of experimental IC_{50} and predicted IC_{50}.

	No.	Chemicals	$\log K_{ow}$	Experimental IC_{50} (g/L)	Predicted IC_{50} (g/L)	Error (%)
Mid-term (24 h)	1	p-Cresol	1.94	2.140	1.190	44.4
	2	m-Dihydroxylene	0.80	2.012	1.948	7.31
Short-term (30 min)	1	o-Nitroaniline	1.85	0.355	0.385	7.79
	2	2,4-Dinitroaniline	1.84	0.256	0.391	34.5
	3	Ethylbenzen	3.15	0.032	0.042	23.8

Based on the obtained model, K_{ow} of organic compounds should be mechanistically related to the biological activity. Hydrophobic chemicals will tend to 'escape' the aqueous phase and will concentrate in more lipophilic phases of biota and suspended particles in activated sludge system. QSARs models based on K_{ow} are capable of interpretation of chemical groups with their biological activity.

3.4. Multiple linear QSARs analysis of IC_{50} by K_{ow} and molecular descriptors

To further investigate the relationship of physico-chemical properties of chemicals with their toxicity to activated sludge, three main-factors of E_{lumo}, μ, CCR were selected. These descriptors were separately considered by multiple linear regression analysis based on $\log K_{ow}$ model. VIF values for the independent variables were all below 5, indicating that there was no multicollinearity. The multiple linear regression QSARs models correlating $\log K_{ow}$ and above three molecular descriptors, respectively, were developed based on around 12–13 of the 24 compounds and the remaining compounds were used for QSARs model prediction. The database for establishing the multiple linear models was listed in Table 3.

3.4.1. Multiple linear QSARs analysis of $C_{50\%}$ by K_{ow} and E_{lumo}

Descriptor E_{lumo} was introduced into the K_{ow}-dependent QSARs models. E_{lumo} was usually used to describe the electrophilicity of chemicals and measure their electron acceptance ability [17]. Wang and Li observed that, lower E_{lumo} values led to stronger electrophilicity, which played important role in chemical toxicity [18]. Multiple linear QSARs models based on IC_{50}, E_{lumo} and K_{ow} were developed for toxicity expression in this study as shown in Eqs. (2) and (3), respectively. The chemicals used to establish model were items of 1, 4–6, 8–12, and 16–19 in Table 3.

$$\log(IC_{50\text{-}M}) = -0.788E_{lumo} - 0.4263\log K_{ow} + 0.9450$$
$$n = 13, \quad R^2(\text{adj.}) = 0.9640, \quad SE = 0.0798, \quad F = 161.4757 \tag{2}$$

$$\log(IC_{50\text{-}S}) = -0.2420E_{lumo} - 0.9539\log K_{ow} + 1.4069$$
$$n = 13, \quad R^2(\text{adj.}) = 0.7867, \quad SE = 0.3722, \quad F = 23.1331 \tag{3}$$

Eq. (2) was characterized by a higher R^2 coefficient (0.964) and smaller standard error (0.0798) than Eq. (3), indicating that the mid-term toxicity is more reliable and stable than short-term toxicity. E_{lumo} is an effective model descriptor for mid-term chemicals toxicity evaluation. The negative value of slope (−0.7887) for E_{lumo} obtained in Eq. (2) indicated that the mid-term toxicity effect increased with the increase of E_{lumo}, and with the decrease in electrophilicity. Cronin and his colleagues obtained the slope value of −0.70 for E_{lumo} when studying the toxicity of the phenols to *Tetraphymena pyriformis*, whose value is similar to the result of this study [6]. However, the short-term toxicity QSARs model dependent on E_{lumo} presented a relatively low and negative coefficient of −0.2420. The differences of the above two equations might be attributable to the difference between acute toxicity and moderate toxicity to the activated sludge system.

3.4.2. Multiple linear QSARs analysis of IC_{50} by K_{ow} and dipole moment

Dipole moment is proved to be an important factor related with the toxicity of chemicals. QSARs multiple linear regression equations were established based on descriptors of K_{ow} and μ, as shown in Eqs. (4) and (5). The chemicals used for models development

Table 3
Database of toxicity concentration and molecular descriptors values for mid-term and short-term toxicity test.

No.	Toxic compounds	Toxicity Concentration		Molecular descriptors		
		$IC_{50\text{-M}}$ (g/L)	$IC_{50\text{-S}}$ (g/L)	μ (D)	E_{lumo} (eV)	CCR (eV)
1	Benzene	1.497	0.235	0.002	0.139	−157.567
2	Toluene	0.474	0.053	0.284	0.051	−163.686
3	Ethylbenzene	0.257	0.002	0.343	0.053	−148.513
4	Xylenen	0.404	0.045	0.120	−0.034	−161.316
5	Styrene	0.211	0.136	0.161	−0.353	−189.925
6	Chlorobenzene	0.357	0.743	1.948	−0.139	−295.249
7	Nitrobenzen	0.794	0.812	7.100	−1.113	−362.131
8	m-Dihydroxybenzen	5.000	1.252	1.929	0.018	−344.840
9	Phenol	2.133	1.008	1.939	0.055	−252.811
10	p-Cresol	2.000	1.040	2.071	0.037	−255.019
11	o-Cresol	1.291	0.713	1.663	−0.014	−243.215
12	m-Cresol	0.931	0.839	1.806	−0.025	−252.164
13	2,4-Dichlorophenol		0.200	0.663	−0.342	−506.005
14	m-Phenylenediamine	23.000	1.611	2.469	0.222	−225.885
15	2,4-Diaminotoluene	10.686	4.996	1.238	0.073	−205.378
16	Aniline	4.269	2.783	2.487	0.118	−195.024
17	o-Toluidine	2.663	1.836	1.949	0.022	−186.041
18	p-Toluidine	2.228	1.515	2.138	0.111	−194.947
19	m-Toluidine	2.372	1.558	2.282	0.046	−194.291
20	o-Phenylenediamine		2.259	3.340	0.067	−208.878
21	m-Nitroaniline		0.162	7.933	−1.144	−390.467
22	p-Nitroaniline		0.112	11.354	−0.980	−396.145
23	o-Nitroaniline		0.208	7.632	−1.023	−370.894
24	2,4-Dinitroaniline		0.100	10.969	−1.040	−568.980

were items of 2–5, 9–12, and 16–19 in Table 3.

$$\log(IC_{50\text{-M}}) = -0.655 \log K_{ow} - 0.126\mu + 1.538$$
$$n = 12, \quad R^2(\text{adj.}) = 0.9730, \quad \text{SE} = 0.0739, \quad F = 181.3312 \tag{4}$$

$$\log(IC_{50\text{-S}}) = -0.286 \log K_{ow} - 0.68\mu + 0.846$$
$$n = 12, \quad R^2(\text{adj.}) = 0.9627, \quad \text{SE} = 0.1728, \quad F = 129.9078 \tag{5}$$

The correlation parameters of the equations were perfect and confirm the reliability of the models. Model of short-term toxicity presented a relatively high and negative coefficient of μ (−0.680). Meanwhile, model of mid-term toxicity still presented better accuracy than short-term toxicity.

3.4.3. Multiple linear QSARs analysis of IC_{50} by K_{ow} and core–core repulsion

The CCR is E_N (A, B)=$Z_A Z_B/R_{AB}$, where Z_A and Z_B are core charges (nuclear point charges minus inner shell electrons) from electrostatics. The QSARs models were developed based on the relationship of CCR and K_{ow}. Eqs. (6) and (7) presented the obtained results on mid and short-term toxicity, respectively. The chemicals used to establish model were items of 4, 6–7, 10–14, 16–18, and 23–24 in Table 3.

$$\log(IC_{50\text{-M}}) = -0.3859 \log K_{ow} - 0.01589\text{CCR} + 1.251$$
$$n = 12, \quad R^2(\text{adj.}) = 0.8024, \quad \text{SE} = 0.0978, \quad F = 19.2753 \tag{6}$$

$$\log(IC_{50\text{-S}}) = -0.0959 \log K_{ow} - 0.0027\text{CCR} + 0.8991$$
$$n = 12, \quad R^2(\text{adj.}) = 0.8623, \quad \text{SE} = 0.1510, \quad F = 35.4355 \tag{7}$$

It was observed that the CCR did not play a leading impact on toxicity of chemicals from the obtained coefficients in above two equations. The internal validation parameters R^2 of CCR model is obviously smaller than those from above models of E_{lumo} and μ.

3.4.4. Validation of the predictive capacity of the models based on molecular descriptors

The above three models are used to predict the toxicity of the remaining chemicals and the prediction results were presented in Fig. 4. The errors between predicted results and experimental toxicity ranged from 5% to 44%, averaging 15%. This range of accuracy was acceptable and the established models could be applied to predict the influence of toxicity to activated sludge process. It is important to note that the predicting errors of $IC_{50\text{-M}}$ were almost smaller than that of $IC_{50\text{-S}}$, which reconfirmed that the mid-term toxicity was more reliable and stable. The comparison of both correlation coefficients and prediction errors between single linear model and multiple linear model, based on K_{ow} as well as molecular descriptors, demonstrated that the multiple model shows better reliability. This indicated that molecular descriptors were more directly related to the reactivity of toxicant. Comparing the values of coefficients in each equation, it can be predicted that among these molecular descriptors, E_{lumo} is the most important impact factor for mid-term system and μ is the most effective factor for short-term system.

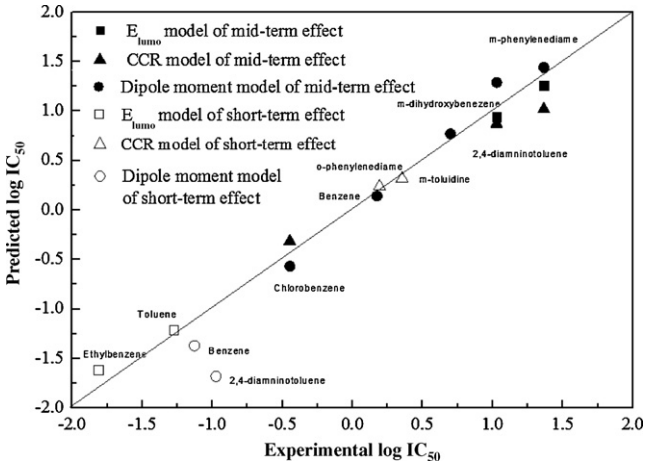

Fig. 4. Experimental toxicity concentration versus predicted toxicity concentration.

The high error (23.8–41.2%) for some compounds during prediction was observed and was contributed to the following reasons. Firstly, physical and chemical characteristics of compounds will impact the accuracy of the tested results. For example, limited solubility, i.e. 2,4-dinitroaniline (41.2% for short-term dipole moment model) could lead to outliers of the model. Secondly, the database used in this study is limited, which also impact the accuracy of predictive model. In addition, the QSARs models in this study were constructed with limited descriptors, octanol–water partition coefficient, and energy of lowest unoccupied molecular orbital, dipole moment, and core–core repulsion. Besides these descriptors, other molecular structural parameters may impact the toxicity of these chemicals, such as energy of highest occupied molecular orbital, heat of formation, electrical energy, ionization potential and etc. Thus the toxicity of certain chemicals with high error might be largely influenced by other descriptors which are not included in the models. Limited by the data set of model, we cannot include all the descriptors in the model. Further study is needed to improve the reliability of the model.

4. Conclusion

In this study, models of QSARs especially for toxicant disposed from petrochemical industry were established to predict their toxicity to activated sludge system. The toxicity of 24 chemicals (benzenes, phenols and anilines) on activated sludge was measured with the BI-2000 and YSI 5100 equipments for mid-term and short-term respiration rate, respectively. The values of $IC_{50\text{-}M}$ were observed to be higher than that of $IC_{50\text{-}S}$, indicating short-term toxicity is more acute than long-term toxicity. Based on the obtained data set, QSARs models for the prediction of $IC_{50\text{-}M}$ and $IC_{50\text{-}S}$ of activated sludge system were successfully developed based on physico-chemical parameter K_{ow} and molecular orbit descriptors E_{lumo}, μ, CCR. The models of $IC_{50\text{-}M}$ were more stable and accurate than that of $IC_{50\text{-}S}$. The multiple linear regression models based on K_{ow} and E_{lumo}, μ, CCR presented better internal and external validation than single regression model based on K_{ow} alone. The toxicity of several chemicals was predicted by using the established QSARs model and the errors were around 15% on an average. Among these molecular descriptors, E_{lumo} was the most important impact factor for mid-term toxicity evaluation.

These models can help predict the toxic effect of these contaminants with various concentration levels on activated sludge, once the physico–chemical properties and concentration of these toxicants are known. If the contaminants in the influent of WWTP will disorder the activated sludge system, pretreatments or dilution are required before the influent is discharged into biological reactor. Such prediction by model calculation could provide a simple and effective method for the operation and management of WWTP. However, the data set of model is not large enough for perfect QSARs model, and further research is still needed.

Acknowledgment

This work was supported by Shanghai Chemical Industry Park Sino French Development Co. Ltd., and in part by National Natural Science Foundation of China (50608056). They are gratefully acknowledged.

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