Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1180264

57 (2016) 28187–28194
December

Taylor & Francis
Taylor & Francis Group

Evaluation of microtoxicity and biodegradability of residual organic solvents in pharmaceutical wastewater by combined prediction-test system

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Received 31 December 2015; Accepted 14 April 2016

ABSTRACT

The wastewater generated in pharmaceutical process generally contains residual organic solvents (ROSs) which will cause the toxicity and inhibition of the micro-organisms in treating wastewater, thus affecting the treatment effect of wastewater. The aim of this study was to establish a system for the analysis and evaluation of microtoxicity and biodegradability of ROSs in pharmaceutical wastewater. The quantitative structure activity relationship models were used to predict the toxicity and biodegradability; meanwhile, the biological toxicity was tested by the method of dehydrogenase activity (DHA) as well as luminescent bacteria and biodegradability was measured by shaking experiment. The proposed system was applied to predict and evaluate toxicity and biodegradability of toluene, acetone, isopropanol and dichloromethane in pharmaceutical wastewater. The results showed that the actual measured values fitted well with the calculated values. The microtoxicity of toluene was the highest and the degradation was more difficult. Dichloromethane toxicity was the second highest after toluene and more easily degradable. Acetone and isopropanol were less toxic and had easy degradation. The fact indicated the system was reliable and easy to operate which could be extended to the screening and identification of highly toxic and difficult degradation components in industrial wastewater.

Keywords: Residual organic solvent; Pharmaceutical wastewater; Evaluation; Microtoxicity; Biodegradability

1. Introduction

The pharmaceutical manufacturing industry encapsulates the manufacture, extraction, processing, purification and packaging of chemical and biological materials, as solids and liquids to be used as medication of humans and animals. Water is a critical raw material in pharmaceutical manufacturing operations. The wastewater streams in a pharmaceutical manufacturing industry generated at the various outlets. The processes of synthesis and formulation of the drugs usually produce a lot of high concentration organic wastewater varying in character and quantity depending upon the products and related manufacturing processes [1]. The pharmaceutical wastewaters contain

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hazardous and refractory organic pollutants which can cause severe problems in the environment [2]. They must be treated to satisfy the water quality regulations and the demand for recycling of water. In the treatment of pharmaceutical wastewaters, it has always been troublesome to achieve more and more stringent effluent standards. Nowadays, the pharmaceutical wastewater treatments have become more difficult than before. The biological processes are commonly used for wastewater because these methods are economic and environmentally sound. Overall, degradability of pharmaceutical wastewater is quite low [3]. Thus, a number of recent studies on pharmaceutical wastewater are gradually turning to explore suitable technologies to treat pharmaceutical refractory residuals. The persistent existence of emerging micropollutants in effluent of wastewater treatment plants (WWTPs) has raised awareness and is a challenge to the global pharmaceutical industry, which is likely to be a significant issue of environmental and public health concerns in the near future.

Large amounts of solvents are used for the purification of the desired product. The water washing off crystallized cakes or precipitated solids from organic solvents leads to a considerable release of solvents into water. The residual organic solvents (ROSs) in the pharmaceutical process has certain toxic and inhibitory effects on micro-organisms [4–6], mainly because they can increase the permeability of cell membranes which can make the membrane protease inactivation and loss of basic functions [7,8]. No unit process was specifically designed to remove these pollutants. The activated sludge and secondary sedimentation in most WWTPs seem to not completely eliminate the ROSs, therefore they become one kind of the sticking components in the treatment of pharmaceutical wastewater [9]. An emerging task for WWTPs would be to act as a barrier for ROSs, preventing their emission into the aqueous environment. Biological toxicity to micro-organism and biodegradability are key properties in the environmental hazard and risk assessment of organic chemicals. However, these assessments are often hampered by the lack of consistent experimental biodegradation data. To develop suitable technologies for the evaluation of the biological toxicity and degradability of the ROSs during wastewater treatment and the mechanisms relevant for their removal need to be understood. However, in the absence of validated and unified evaluation methodologies, it is difficult to make a correct assessment about the microtoxicity and/or degradability of the ROSs in the wastewater. No such evaluation approach hitherto has yet been developed for biodegradability and toxicity assessment of industrial effluents.

Domestic and international prediction on the toxicity and degradability of organic compounds is based on the quantitative structure activity relationship (QSAR) model which has been a very active international research focus since the 1990s [10]. QSAR has been invaluable for the prediction of environmental pollutants' toxicity [11–13] and degradation [14–16]. However, the theoretical prediction usually has many traps for unwary practitioners. The actual measurement is essential to a correct assessment of the activity. Various techniques have been developed for determining the biological toxicity including luminescent bacteria toxicity test (LBT) [17], dehydrogenase activity (DHA) [18], respiration rate [19], nitrification rate [20] and animal embryo toxicity assay [3]. Several methods have also been proposed to test the biodegradability, such as shake-flask test [21], carbon dioxide production measurement (Sturm test and modified Sturm test) [22,23], adenosine 5′-triphosphate (ATP) measurement [24] and so on. All of these methods have their limitations. There are few reports on prediction and analysis of ROSs combining of biological toxicity and degradation. Organic solvents used in the pharmaceutical process are up to dozens of species; furthermore, they have different degrees of toxicity to micro-organisms. It is necessary to establish a system for the prediction and evaluation of biological toxicity and biodegradability of ROSs in pharmaceutical wastewater focusing on the analysis of components of high toxicity and difficult to degrade. No evidence exists linking the microtoxicity and biodegradability of ROSs to WWTPs.

The objective of this study was to establish a combined prediction-test system for microtoxicity and biodegradability by QSAR model to predict, and with luminescent bacteria, DHA method and shake-flask test to determination of the toxicity and biodegradability for ROSs in the pharmaceutical wastewater.

2. Materials and methods

2.1. Sample collection

The wastewater samples were taken from influent of a WWTP and activated sludge collected from the oxidation ditch. The WWTP located in a pharmaceutical industrial park in Shijiazhuang, North China. The plant received effluent from all pharmaceutical companies in the park. After pretreatment by pharmaceutical companies, pharmaceutical wastewater was discharged into the WWTP to carry out comprehensive treatment. Water samples were collected in the 2-L brown glass bottles that had been successively washed with tap water, ultrapure water and hexane. The activated

sludge samples were stored in the 1-L Teflon bottles then kept at 4℃ in the dark for at most 2 d.

2.2. Materials

The ROS determination was carried by a gas chromatography (SHIMADZU GC 2010 Plus, Japan) with head space sampler (SHIMADZU HS-20, Japan). Total organic carbon (TOC) was conducted on a TOC analyzer (Elementar, Germany). The bioluminescence inhibition assay of the wastewater sample was conducted using a water quality toxicity analyzer (Beijing HAMAMATSU, China). The luminescent bacteria used were *Vibrio qinghaiensis* sp. Nov.-Q67, which was provided by the instrumental manufacturer. The main reagents contained dichloromethane, acetone, isopropanol and toluene (HPLC grade, China).

2.3. Experiment methods

2.3.1. Methods for determination of ROSs

In this study, ROSs in pharmaceutical wastewater were determined by headspace gas chromatography (column: PEG-20M, 30 m × 0.25 mm × 0.25 μm). Inlet temperature was 150℃ and the detector temperature was 250℃. The column temperature was 40℃ maintained for 6 min, then at the speed of 5℃/min raised to 200℃ maintained for 2 min. Headspace conditions included that equilibrium temperature was 80℃ and the equilibrium time was 40 min.

On the above conditions each sample was determined repeatedly for seven times and precision of the method was described by the relative standard deviation (RSD). Recovery studies were carried out for samples known concentration fortified at the 0.1, 1 and 10 mg/L levels. For each concentration six replicates were injected and the recovery was their average.

2.3.2. Prediction methods for toxicity and degradation

Molecular connectivity index (MCI) is based on the theory of QSAR and used to quantitatively describe the molecular space structure. It plays a significant role in QSAR studies for some of the molecular information needed to derive a candidate molecule. The MCI is hidden from organic molecules hydrogen skeleton produced, which reflects the size of the molecule, branched, and hetero atom bond type information, etc. MCI was calculated by Molecular Modeling Pro v6.3.3 program. Using half luminescence inhibition concentration (EC_{50}) of luminous bacterial as toxicity index of organic compounds, the relationship between the ${}^1X^V$ and luminescent bacteria EC_{50} values was analyzed. Results showed that $\lg EC_{50}$ and ${}^1X^V$ were significantly negatively correlated as follow:

$$-\lg EC_{50} = -1.330 + 0.90624\ {}^1X^V \quad (1)$$

The toxicity consisted of four grades. Classification criterions were as follows: $EC_{50} < 1$ mg/L, extreme toxicity; $1 \le EC_{50} < 10$ mg/L, high toxicity; $10 \le EC_{50} < 100$ mg/L, medium toxicity; $EC_{50} \ge 100$ mg/L, low toxicity.

The final product of biodegradation of organic matter is carbon dioxide and water. Therefore, production of carbon dioxide (PCD) can be used to characterize the degradation degree of organic pollutants. The larger the PCD value indicates it is more easy for the organics to degrade. The relationship between PCD and MCI was summarized as:

$$PCD = 53.18 - 4.537^3X_P - 20.73^3X_P^V + 13.78^4X_{PC}^V \quad (2)$$

The prediction biodegradability consisted of three grades. Standards for the biodegradability classification of organics were as follows: PCD ≥ 8 mmol/L, easy degradation; 1 < PCD < 8 mmol/L, medium degradation and PCD ≤ 0 mmol/L, difficult degradation.

2.3.3. Biological toxicity test

In this study, both DHA and luminescent bacteria test were used to measure biological toxicity.

DHA test was based on the redox dye 2,3,5-three phenyl tetrazolium chloride (TTC) as an indicator when there is microbial cell biological dehydrogenation reaction, then TTC would accept the hydrogen atom is reduced to red triphenyl formazone (TF). DHA test was referenced the method proposed by Aragon et al. [25].

Acute toxicity test was carried out using luminescent bacteria strain Vibrio Qinghai Q67 and taking resuscitation dilution added to the bacterial lyophilized vial equilibration at room temperature. The sample with the osmotic pressure regulating solution was in 19:1 ratio, formulating as the sample solution and added to the sample solution to a clean tube. Each tube was added with lyophilized powder solution sequentially, then shook till thoroughly mixed, finally measured luminescence value placed after 15 min.

2.3.4. Determination of biodegradability

Shake-flask test was used to emulate the aerobic treatment unit in practical engineering and determined

the concentration of activated sludge from the activated sludge of domestication. Pure water was added to phosphate buffer solution, magnesium sulfate, ferric chloride and calcium chloride solution and configured to dilute water. The activated sludge was collected from a WWTP mainly dealing with pharmaceutical wastewater. Four kinds of ROSs were formulated into a TOC value of about 100 mg/L used as samples. The samples were injected into conical flasks with activated sludge and shook culture for 7 d called training time simultaneously the diluted water added into the activated sludge as the blank group. Because of the characteristics of organic solvents, the control group was set up in order to reflect the true degradation of organic solvent. The control group added no activated sludge, while the reagent amount and the operation condition were the same as the experimental group. The supernatant was determined TOC in seven consecutive days through the 0.45-μm microporous membrane filtration.

As a result of the domestication of micro-organisms had been adapted to the ROSs and the biodegradation of the test sample was completely within 7 d, so the residue of TOC in 7 d after treatment was similar to the hard degradation COD (HD-COD). HD-TOC was used as the standard for the evaluation of organic degradation of TOC.

The tested biodegradability consisted of four grades. Classification criterions were as follows: when the ratio of HD-TOC/TOC was in the range of 0–20, 20–50, 50–80, and 80–100%, the corresponding biodegradability was easy biodegradability, possible biodegradability, medium biodegradability and difficult biodegradability, respectively.

3. Results and discussion

3.1. Determination results of four kinds of ROSs

The mixed standard solution was determined in the former condition and a qualitative analysis based on the retention time of the single standard under the same conditions. Linear regression was performed with the chromatographic peak area and mass concentration, and the measurement results are shown in Table 1.

As shown in the above results, the ROSs concentration reduction rates were 37.1, 51.0, 70.8, and 81.5% after treatments for toluene, dichloromethane, acetone and isopropanol, respectively. The results showed that the structure of toluene was stable and had stronger biological toxicity comparing the other three kinds of ROSs. In addition, toluene could make the microbial protein denaturation and the cell lose its activity, which was difficult to be degraded under the existing conditions. On the contrary, the concentration reduction rates of acetone and isopropanol were both above 70%, which indicated that it was easy for them to be degraded under the present conditions.

3.2. Toxicity and biodegradability predictions

In this study, the PCD and EC_{50} values of four kinds of ROSs were calculated by two QSAR models. The results are shown in Table 2.

Table 2 shows that the toluene was medium toxicity and difficult to degrade, while the others were low toxicity and easy degradation. According to prediction results of EC_{50}, the toxicity of the ROSs was in the order of toluene, dichloromethane, acetone and isopropanol. According to the results of PCD, the difficult degree of biodegradability followed the order of toluene > dichloromethane > isopropanol > acetone. Symmetry and stability of the benzene ring made it difficult to be oxidized and decomposed. Studies [26] showed that non-cyclic compounds than single phenyl ring compounds are easy degradation, which contains compounds of the benzene ring is not conducive to biological degradation.

3.3. Results of biological toxicity test

3.3.1. Toxicity analysis of activated sludge DHA

Fig. 1 shows that the ROSs concentration and DHA inhibition rate equation was proposed by Sigmoidal synthetic curve. From Fig. 1, it was indicated that with the increase in the concentration of organic pollutants, the DHA was decreased and the inhibition rate of DHA increased. When the concentration of pollutants increased to a certain extent, the inhibition rate of the pollutant to DHA tended to be stable. Expression of Sigmoidal curve was calculated using the following equation. The parameters in the curves are listed in Table 3:

$$y = A_2 + (A_1 - A_2)/(1 + \exp(x - x_0)/\mathrm{d}x) \quad (3)$$

Dose effect regression analysis was shown in Table 3 also. Table 3 shows that the toxicity in sequence of toluene > dichloromethane > isopropanol > acetone.

3.3.2. Analysis of toxicity to luminescent bacteria

Fig. 2 reflected the impact of the four kinds of ROSs on luminescent bacteria inhibition respectively.

Table 1
Determination results of four kinds of ROSs concentrations

Compound	Linear range (mg/L)	r	Measurement result (mg/L)		Recovery (%)	RSD (%)
			Influent	Effluent		
Toluene	0.05–5	0.9992	0.259	0.163	94.3	0.85
Acetone	0.05–5	0.9992	0.751	0.219	92.4	1.33
Isopropanol	0.05–5	0.9991	0.637	0.118	87.7	1.47
Dichloromethane	0.05–5	0.9996	1.627	0.798	89.5	1.11

Table 2
QSAR analysis of biodegradation and toxicity

Compound	PCD (mmol/L)	EC_{50} (mg/L)	Analysis result	
			Biodegradability	Toxicity
Toluene	−0.441	34.35	Difficult	Medium
Dichloromethane	37.412	22,879	Easy	Low
Acetone	47.708	33,530	Easy	Low
Isopropanol	37.622	35,199	Easy	Low

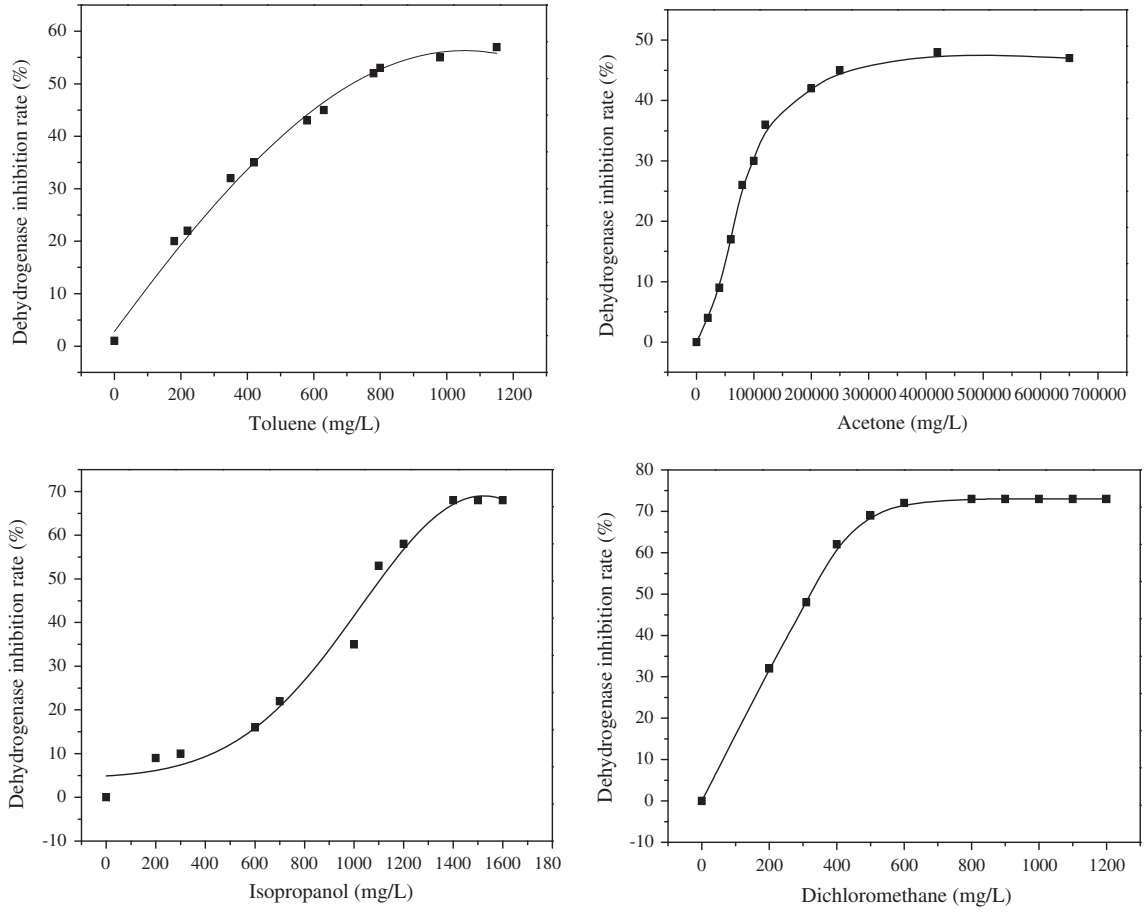

Fig. 1. Effects of organic solvents on the DHA.

Table 3
Regression analysis of dose effect concentration and inhibition rate of DHA

Compound	r	Parameter			EC_{50} (mg/L)
		A_1	A_2	x_0	
Toluene	0.9985	−417.6	65.7	−955.7	293.9
Dichloromethane	0.9899	−15.7	79.84	197.23	663.2
Acetone	0.9980	−543.9	50.18	−195,899	470,927
Isopropanol	0.9950	0.732	69.76	815.97	1,309.9

The luminescent intensity of the luminescent bacteria was significantly correlated with their concentration namely that with the increase of their concentration, the inhibition rate of the luminescent bacteria increased; when the concentration of the toxic substance increased to a certain degree, the inhibition rate of the toxic substances to the luminescent bacteria was stable. In the case of a certain concentration, the luminescence intensity of the luminescent bacteria could be calculated according to the dose effect regression equation, as shown in Table 4.

Table 4 shows that four kinds of ROSs in descending order of the degree of inhibition of luminous bacteria were: toluene, dichloromethane, isopropanol and acetone. Referring to the acute toxicity grading standards of Table 1, the results showed that although all belonged to the range of low toxicity, the EC50 value of toluene was close to 100 mg/L

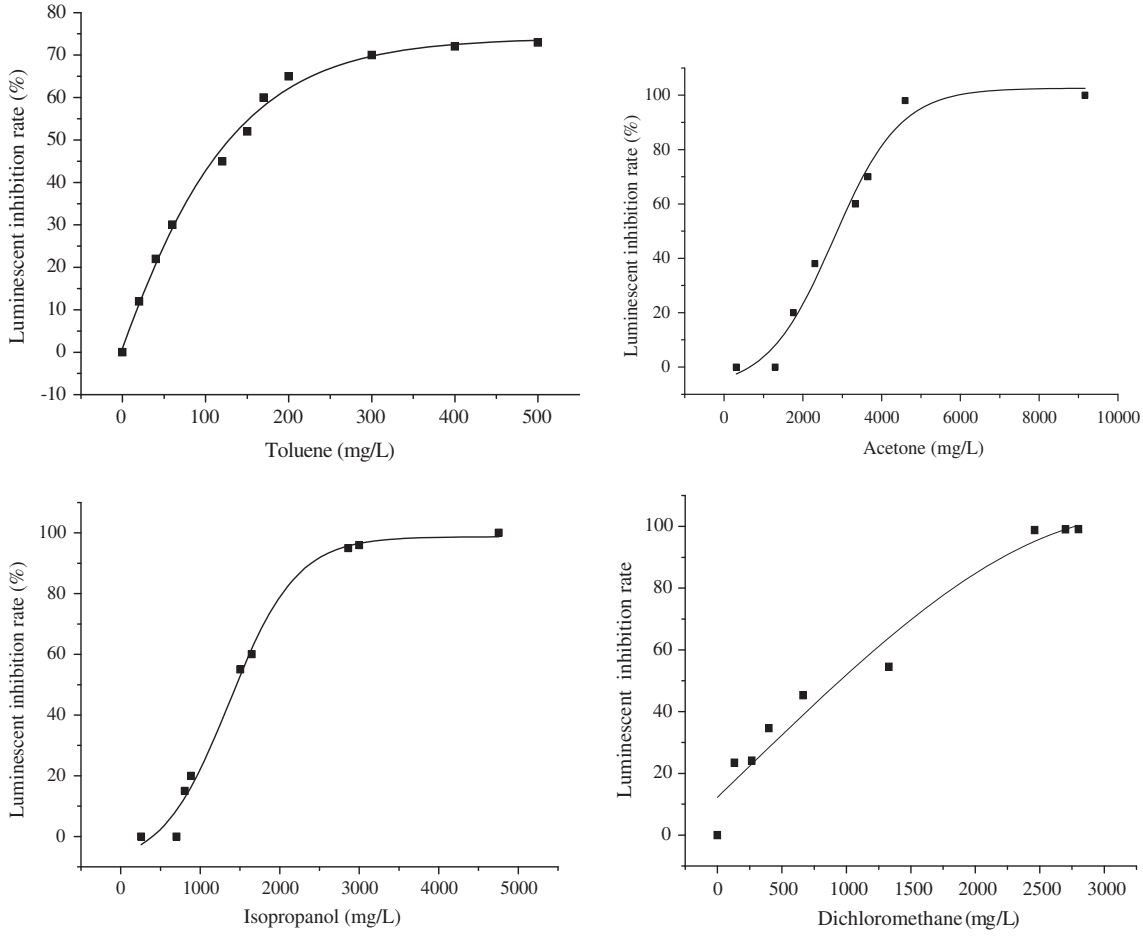

Fig. 2. Effect of organic solvents on the luminescent bacteria.

Table 4
Dose effect regression analysis on ROSs concentration and the EC_{50}

Compound	Dose-effect regression equation	r	EC_{50} (mg/L)
Toluene	$y = 88.9 + (-1{,}699 - 99.631)/(1 + \exp((x + 427.7)/224.337))$	0.9992	121.4
Dichloromethane	$y = 0.0283x + 14.877$	0.9544	1,240
Acetone	$y = 98.75 + (-9.94 - 98.75)/(1 + \exp((x - 4.1368)/114.989))$	0.9909	1,454
Isopropanol	$y = 102.58 + (-8.15 - 102.58)/(1 + \exp((x - 2{,}785.6)/276.179))$	0.9960	2,872

and close to that of the toxic medium range. Through dose effect regression equations of the DHA and luminescent bacteria acute toxic it was seen that among the four kinds of ROSs, the biological toxicity of toluene was the highest. Toluene was a non-polar narcotic compound whose toxic effect was shown as the breakthrough of the biological membrane [27], then through a variety of processes and reaction with biological activity [28] so as to show high biological toxicity. Toxicity of dichloromethane was followed after toluene once which entering into the microbial organism, it could be transformed dependent mixed function cytochrome enzymes metabolize Paso and lead protein molecules of cells irreversible change and the occurrence of cancer [29]. Toxicity of acetone and isopropanol was relatively low which was basically consistent with the results predicted by the QSAR model.

3.4. Determination results of biological degradation

The results of four kinds of ROSs biodegradation tests are shown in Fig. 3. In the experiment, four kinds of organic solvents were significantly reduced, which was due to the degradation of organic solvents as the sole carbon source by micro-organisms. The measured concentration changes with time and the curve could objectively reflect the biological degradation.

Acetone and isopropanol were rapidly biodegradable and their degradation rates were more than 70% in the former 4d. HD-TOC/TOC values of toluene, dichloromethane, acetone and isopropanol were 58.7, 30.5, 13.1, and 11.7%, respectively which showed that toluene was more difficult to degrade referring to Table 3, while dichloromethane was easily degraded and isopropanol as well as acetone were susceptible to degradation. This result was consistent with the result of concentration measurement and the prediction of QSAR model.

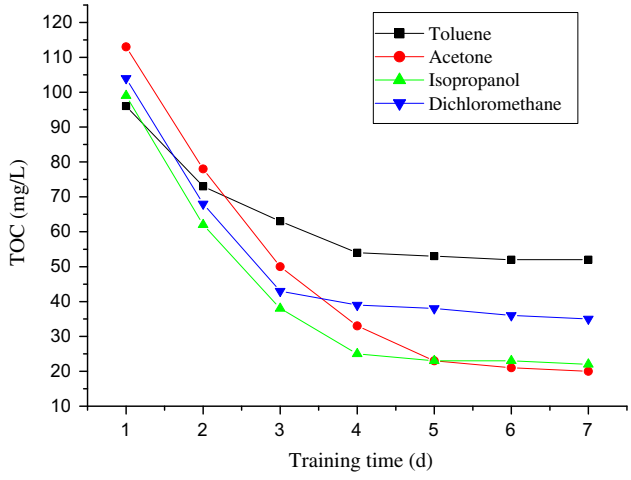

Fig. 3. Degradation curves of organic solvents.

4. Conclusions

In this paper, a combined prediction-test system for microtoxicity and biodegradability was established. This system had been applied to predict and evaluate the toxicity and degradation of four kinds of ROSs in pharmaceutical wastewater. The results showed that the prediction system was practical and reliable, and the predicted results were basically consistent with the measured results. This system could be further extended to a screening study of toxic and difficult degradation characteristic pollutants of industrial wastewater and would provide valuable information in developing appropriate treatment for organic compounds.

Acknowledgments

This work is supported by the National 12th Five-year Major Science and Technology Project on Control and Prevention of Water Pollution (2012ZX07203-003) and the Natural Science Foundation of Hebei Province (B2014201175).

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