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Screening and estimating of toxicity formation with photobacterium bioassay during chlorine disinfection of wastewater

Li-Sha Wang[a], Dong-Bin Wei[a,b], Jie Wei[a], Hong-Ying Hu[a,*]

[a] *Environmental Simulation and Pollution Control State Key Joint Laboratory, Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, PR China*

[b] *Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama 240-8501, Japan*

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Abstract

Reclamation and reuse of wastewater is one of the most effective ways to alleviate water shortage. Disinfection plays a key role in killing the harmful pathogens in reclaimed water, while an unwanted side effect is the formation of disinfection by-products (DBPs). Recently, a number of researches have been conducted on the formation regularities of certain DBPs. However, with current physiochemical techniques, it is impossible to detect all the DBPs. In this study, photobacterium bioassay was used to measure the formation of DBPs and their toxic effect as a whole. The effects of water quality characteristics and operational conditions on the toxicity formation during wastewater chlorination disinfection process were evaluated. A statistical model, depending on chlorine disinfectant dosage, concentration of ammonia nitrogen, and concentration of dissolved organic carbon, was developed to quantitatively estimate the toxicity formation during the disinfection process. It was found that the toxicity of the wastewater samples was positively correlated with chlorine disinfectant dosage, concentration of dissolved organic carbon and UV absorbance at 254 nm, while negatively correlated with concentration of ammonia nitrogen.

Keywords: Photobacterium bioassay; Toxicity formation; Disinfection; Chlorination

1. Introduction

With the development of social production and improvement of human living standard, the shortage of water resources is becoming more and more serious in many countries. In recent years, water conservation and searching for new sustainable water resources has been identified as an urgent task. Among many options, wastewater reclamation and reuse is a viable and attractive method [1,2]. However, historically, people usually associate wastewater with "dirty" and "useless". For many people, it is hard to accept and use the reclaimed water. Water quality of reclaimed water becomes a prominent issue facing regulatory authorities. Generally speaking, the effect factors on water quality includes two parts: one is biological, which is related with the harmful pathogens in water; the other is ecological, which is related with the toxic chemicals in water [3,4].

Compared with drinking water and river water, there are more kinds of pathogens and chemicals in wastewater [3,5]. With the conventional secondary treatment, concentrations of chemical pollutants in wastewater can be reduced effectively, while the removal of pathogens is limited. Disinfection can kill the harmful pathogens, but the organic substances in wastewater can react with disinfectants to produce some toxic disinfection by-products (DBPs) [6,7].

Recently, the formation of certain DBPs, such as trihalomethanes (THMs), haloacetic acids (HAAs) has been studied by many researchers. However, with current physiochemical techniques, it is impossible to detect all the DBPs. Fortunately, some toxicity tests have been used to measure the whole toxicity of all pollutants in water in recent years [8,9]. Therefore, to screen the toxic chemicals in wastewater by using toxicity test is quite direct and necessary [10]. Among different toxicity tests, photobacterium bioassay has been used to measure the toxicity of chemicals and practical samples for many years, and its reliability has been accepted by more and more researchers [11,12].

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[*] Corresponding author. Tel.: +86 10 62794005; fax: +86 10 62771472.
E-mail address: hyhu@tsinghua.edu.cn (H.-Y. Hu).

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It is well known that disinfection of wastewater is a complex process because of the multi-components in water, complicated chemical reactions and various operational conditions, all of which will influence the formation of DBPs. During the past several decades, many researchers have paid their attentions to exploring the mechanisms involved in disinfection process, and have developed some predictive models for the formation of DBPs [13–16]. However, all those models focused on the studies of raw and treated drinking water. What's more, most models were developed to predict the formation of DBPs, and few researches have covered the formation of toxicity during chlorine disinfection.

The purpose of this study was to measure and evaluate the toxicity formation during chlorination disinfection process of biologically treated wastewater. Photobacterium bioassay test was used: to analyze the toxicity changes before and after disinfection under various water quality characteristics and operational conditions; to explore the relationships between toxicity formation and disinfection conditions, water quality parameters; and to provide basic information for optimizing disinfection processes in order to produce reclaimed water with high quality.

2. Materials and methods

2.1. Water samples

Wastewater samples used in this study were collected from the effluent of different domestic wastewater treatment and reclamation plants before disinfection, in which activated sludge process (AS), biological filteration (BF), membrane bioreactor (MB) and anaerobic–anoxic–oxic process (A^2O) were used as the main treatment methods. The wastewater samples were immediately delivered to the laboratory and filtered to eliminate suspended solids. The principal conventional qualities of these water samples were analyzed in accordance with standard methods [17], and the results are shown in Table 1. Concentration of ammonia nitrogen ($C_{NH_3–N}$) was determined by colorimetry by using the Nesslerizatin method. Concentration of dissolved organic carbon (C_{DOC}) was detected with a TOC analyzer (Model: TOC-5000A, Shimadzu, Japan). UV absorbance at 254 nm (UV_{254}) was measured with a photospectrometer (Model: UV-2401, Shimadzu, Japan). In order to study the effect of ammonia nitrogen on the toxicity of disinfected wastewater, $(NH_4)_2SO_4$ was added to sample 7 to get different concentrations of ammonia nitrogen as samples 8 and 9.

Table 1
Water quality characteristics of wastewater samples used in this study

Sample	Source[a]	$C_{NH_3–N}$ (mg/L)	C_{DOC} (mg/L)	UV_{254} (m^{-1})
S1	BF	0.2	5.1	5.0
S2	BF	2.0	14.6	8.4
S3	MB	14.0	1.5	5.5
S4	AS	0.5	10.6	12.5
S5	AS	2.0	12.9	12.8
S6	AS	14.0	12.8	12.8
S7	AS	2.0	13.3	12.0
S8	AS	10.0	13.3	12.0
S9	AS	30.0	13.3	12.0
S10	AS	9.8	2.1	9.9
S11	AS	10.3	28.0	11.8
S12	AS	9.9	53.0	12.1
S13	AS	10.2	7.7	5.2
S14	AS	10.5	12.6	11.6
S15	AS	10.0	11.5	14.7

[a] BF, MB and AS show that the samples were collected from biological filteration, membrane bioreactor and activated sludge process, respectively.

2.2. Chlorine disinfection

Chlorine disinfection was conducted within 24 h after sampling. A series of 600 mL glass bottles with Teflon inner plugs were prepared, and each bottle was filled with about 580 mL of wastewater sample. The pH was adjusted to 7.0 ± 0.2 with 1 M H_2SO_4 or 2 M NaOH solution, then 12.5 mL phosphate buffer solution was added to keep stable pH in disinfection system. A series of different concentrations of available chlorine (0–50 mg/L) was added into corresponding reaction bottles by using NaClO solution with a concentration of 5 g available chlorine per liter. The bottles were sealed and kept in a dark isotherm chamber (20 °C) for 30 min, and then total residual chlorine was measured according to the standard method [17]. Based on the concentration of residual chlorine, different Na_2SO_3 was added to each bottle to remove residual chlorine to the level that did not inhibit photobacterium [18]. All of the chemical reagents used in this study were of analytical purity.

2.3. Toxicity measurement

Photobacterium bioassay method quantifies the decrease in light emission from the *Photobacterium phosphoreum* (*P. phosphoreum*) bacteria as a result of exposure under pollutants. Luminescence inhibition percentage after a 15-min exposure was standardized into equivalent concentration of Zn^{2+}, which was used to express the effect degree of pollutants on test bacteria [19]. For example, the higher the luminescence inhibition percentage is, the higher the equivalent concentration of Zn^{2+} should be. The test instrument (Model toxicity analyzer DXY-2) was made by the Institute of Soil Science, Chinese Academy Sciences, Nanjing, PR China. The bacteria *P. phosphoreum* Straus, was also provided as freeze-dried powder by the same Institute.

3. Results and discussion

3.1. Effect of chlorine dosage on toxicity formation

Fig. 1 shows the toxicity changes of six different wastewater samples after chlorine disinfection. It was noted that the toxicity of the wastewater increased with the addition of chlorine disinfectant. As the residual chlorine in the disinfected wastewater had been diminished during the dechlorination process in this study, the significant toxicity values suggested the formation of toxic by-products. It can also be seen from Fig. 1 that, at the same chlorine dosage, the toxicity differed with different wastewater samples, which suggested that the toxicity values depended on the characteristics of water quality, and this will be discussed later in this article.

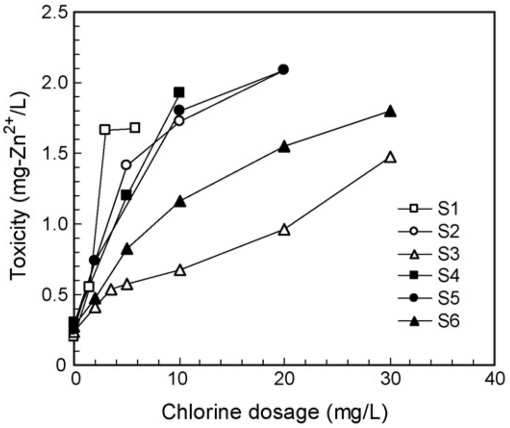

Fig. 1. Toxicity changes after chlorine disinfection (by Microsoft Office Excel).

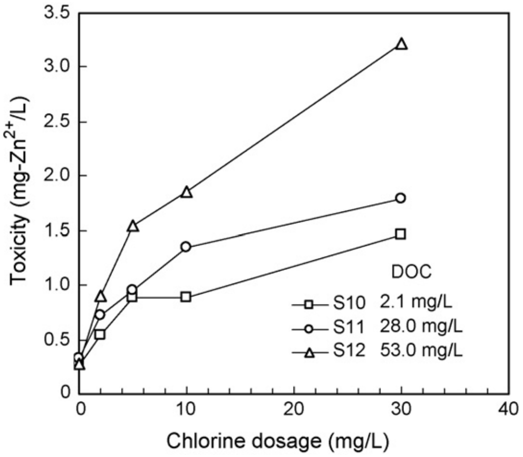

Fig. 3. Effect of DOC on the toxicity formation of the wastewater after chlorine disinfection (by Microsoft Office Excel).

3.2. *Effects of water quality characteristics on toxicity formation*

In this study, water characteristics such as $C_{\mathrm{NH_3-N}}$, C_{DOC} and UV_{254}, were selected to investigate their effects on toxicity formation during chlorine disinfection process according to the previous researches [20–22]. The water quality parameters are also listed in Table 1 and the results are shown as Figs. 2–4.

The effect of $C_{\mathrm{NH_3-N}}$ in wastewater on toxicity formation was studied and the results (Fig. 2) showed that when $C_{\mathrm{NH_3-N}}$ increased from 2 to 20 mg/L and 30 mg/L, the toxicity of disinfected water decreased obviously. This indicated ammonia nitrogen could reduce the toxicity of disinfected water, and this was agreeable with previous researches on disinfection by-products, which ammonia nitrogen will react with free chlorine and change it to combined chlorine (chloramine), and decrease the formation potential of toxic by-products because the reactivity of combined chlorine is lower than free chlorine. For example, Nissinen detected lower concentrations of DBPs after chloramination compared to chlorination in Finnish drinking waters [23]. Karen also reported that among 16 drinking waters from around Australia, most DBPs concentrations were low in chloraminated water [24]. Rebhun compared DBPs formation between non-nitrified and nitrified secondary effluents and found that the concentrations of THMs and HAAs produced in ammonia-containing effluents (non-nitrified) were less than that in nitrified effluents [25].

The effect of C_{DOC} on toxicity formation was also discussed, and the results are shown in Fig. 3. It was found that the water samples with higher C_{DOC} would form higher toxicity after chlorine disinfection. This is easy to understand because DOC is typically used as an aggregate measure of the content of organic matter which reacts with chlorine to form toxic by-products [26] and many researchers have developed positive relationships between DOC and the formation of THMs and HAAs in drinking water [27,28].

Fig. 4 shows the wastewater samples with higher UV_{254} would produce higher toxicity under the same disinfection conditions. UV_{254} mainly reflects the content of unsaturated aromatic organic chemicals in water, such as unsaturated –C=C– band, –C=C– band and so on [29,30]. These kinds of organic compounds are active to react with chlorine disinfectant and form toxic disinfection by-products. UV_{254}, as an indicator of

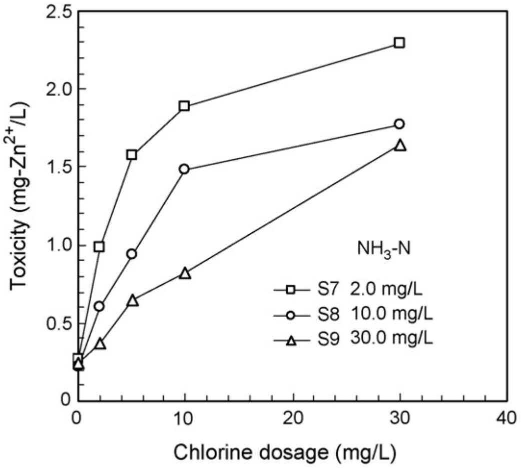

Fig. 2. Effect of $\mathrm{NH_3}$–N on the toxicity formation of the wastewater after chlorine disinfection (by Microsoft Office Excel).

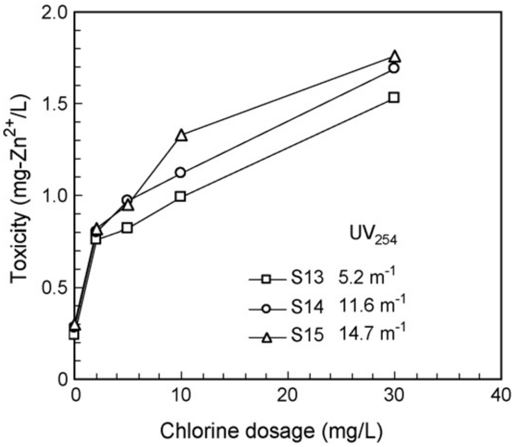

Fig. 4. Effect of UV_{254} on the toxicity formation of the wastewater after chlorine disinfection (by Microsoft Office Excel).

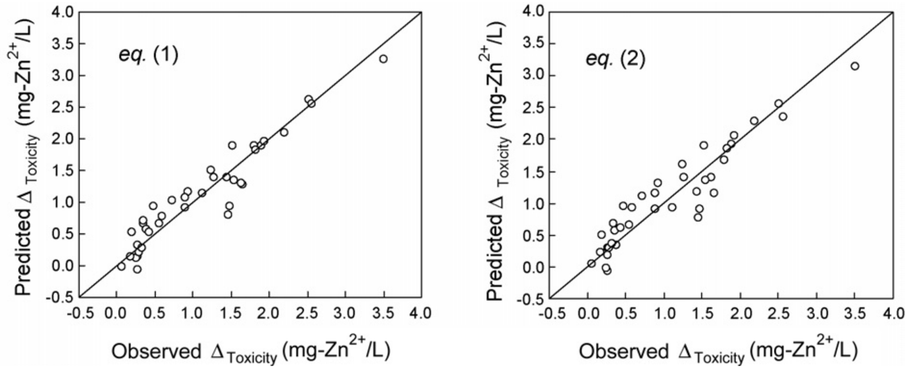

Fig. 5. Relationships between observed toxicity and predicted toxicity with Eqs. (1) and (2), respectively (by Microsoft Office Excel).

Table 2
Water quality characteristics and toxicity data of wastewater samples used in this study

Samples	Sources[a]	D_{Cl} (mg/L)	$C_{\mathrm{NH_3–N}}$ (mg/L)	C_{DOC} (mg/L)	UV_{254} (m^{-1})	$\Delta_{\mathrm{Toxicity}}$ (mg Zn^{2+}/L)		
						Observed	Predicted Eq. (1)	Predicted Eq. (2)
1	AS	10.0	44.7	15.4	16.6	0.28	−0.08	−0.07
2	AS	10.0	8.0	17.6	13.3	1.66	1.27	1.15
3	AS	10.0	23.8	15.6	13.3	0.36	0.66	0.57
4	AS	10.0	32.9	5.3	8.9	0.06	−0.01	0.06
5	A^2O	10.0	0.4	6.4	10.6	0.93	1.16	1.32
6	MB	10.0	32.5	22.1	15.9	0.39	0.57	0.35
7	MB	10.0	30.4	6.8	12.4	0.27	0.13	0.29
8	AS	10.0	38.7	14.8	12.6	0.25	0.11	−0.01
9	AS	10.0	16.0	10.8	14.9	0.59	0.77	0.92
10	AS	10.0	31.9	13.8	11.1	0.28	0.31	0.18
11	AS	50.0	44.7	15.4	16.6	1.90	1.88	1.91
12	AS	50.0	8.0	17.6	13.3	3.51	3.23	3.13
13	AS	50.0	23.8	15.6	13.3	2.52	2.61	2.55
14	AS	50.0	32.9	5.3	8.9	1.93	1.95	2.04
15	MB	50.0	32.5	22.1	15.9	2.57	2.53	2.34
16	MB	50.0	30.4	6.8	12.4	2.20	2.09	2.27
17	BF	1.5	0.2	5.1	5.0	0.35	0.71	0.68
18	BF	3.1	0.2	5.1	5.0	1.46	0.79	0.76
19	BF	5.8	0.2	5.1	5.0	1.48	0.92	0.89
20	BF	5.0	2.0	14.6	8.4	1.13	1.14	0.93
21	BF	10.0	2.0	14.6	8.4	1.44	1.38	1.17
22	BF	20.0	2.0	14.6	8.4	1.81	1.87	1.67
23	MB	2.0	14.0	1.5	5.5	0.18	0.13	0.22
24	MB	3.5	14.0	1.5	5.5	0.29	0.21	0.29
25	MB	5.0	14.0	1.5	5.5	0.33	0.28	0.37
26	MB	10.0	14.0	1.5	5.5	0.43	0.52	0.61
27	MB	20.0	14.0	1.5	5.5	0.73	1.01	1.11
28	MB	30.0	14.0	1.5	5.5	1.24	1.50	1.60
29	AS	5.0	0.5	10.6	12.5	0.90	1.05	1.15
30	AS	10.0	0.5	10.6	12.5	1.63	1.30	1.39
31	AS	2.0	2.0	12.9	12.8	0.48	0.93	0.95
32	AS	10.0	2.0	12.9	12.8	1.55	1.32	1.35
33	AS	20.0	2.0	12.9	12.8	1.83	1.81	1.85
34	AS	2.0	14.0	12.8	12.8	0.20	0.51	0.51
35	AS	5.0	14.0	12.8	12.8	0.55	0.66	0.66
36	AS	10.0	14.0	12.8	12.8	0.89	0.90	0.91
37	AS	20.0	14.0	12.8	12.8	1.27	1.39	1.40
38	AS	30.0	14.0	12.8	12.8	1.53	1.88	1.90

[a] BF, MB, AS and A^2O show that the sample were collected from biological filteration, membrane bioreactor, activated sludge process and anaerobic–anoxic–oxic process, respectively.

organic halide, has been reported to have linear relationships with the formation potentials of THMs and HAAs in Alaskan surface water [22].

3.3. Quantitative prediction of toxicity formation

On the basis of the discussions in Sections 3.1 and 3.2 in this paper, it can be considered that both operational conditions and water quality characteristics will affect the toxicity formation during wastewater chlorine disinfection. Therefore, quantitative prediction relationships between D_{Cl}, $C_{\mathrm{NH_3{-}N}}$, C_{DOC}, UV_{254} and toxicity formation were developed with statistical method according to the experiments in this study and are shown as followings:

$$\Delta_{\mathrm{Toxicity}} = 0.471 + 0.049D_{\mathrm{Cl}} - 0.035C_{\mathrm{NH_3{-}N}} + 0.034C_{\mathrm{DOC}},\\ N = 38,\quad R = 0.952,\quad R^2 = 0.906,\quad R^2_{\mathrm{adj}} = 0.898,\\ \mathrm{S.D.} = 0.262,\quad F = 109.689,\quad p < 0.001 \tag{1}$$

$$\Delta_{\mathrm{Toxicity}} = 0.411 + 0.050D_{\mathrm{Cl}} - 0.037C_{\mathrm{NH_3{-}N}} + 0.040\mathrm{UV}_{254},\\ N = 38,\quad R = 0.938,\quad R^2 = 0.879,\quad R^2_{\mathrm{adj}} = 0.869,\\ \mathrm{S.D.} = 0.300,\quad F = 82.508,\quad p < 0.001 \tag{2}$$

where $\Delta_{\mathrm{Toxicity}}$ is the gap of toxicity after and before disinfection of the wastewater, which was expressed as equivalent concentration of Zn^{2+} (mg/L). N is the number of samples, R is the correlation coefficient, R^2 is the multiple correlation coefficient, R^2_{adj} is the adjusted multiple correlation coefficient, S.D. is the standard deviation, F is the result of F-test, and p is the significance level.

It can be seen from Eqs. (1) and (2) that besides D_{Cl} and $C_{\mathrm{NH_3{-}N}}$, which appear in both Eqs. (1) and (2), the important water quality parameters C_{DOC} and UV_{254} are also involved in Eqs. (1) and (2), respectively. The toxicity formation during disinfection process is positively correlated with D_{Cl}, C_{DOC} and UV_{254}, and negatively correlated with $C_{\mathrm{NH_3{-}N}}$. By analyzing the DBPs formation models mentioned in the previous studies on drinking waters [13–16], it would be found that D_{Cl}, C_{DOC} and UV_{254} are frequently involved in those various models and their relationships with DBPs formation are always positive which is similar to the present results. As for $C_{\mathrm{NH_3{-}N}}$, it is not involved in those models because the range of $C_{\mathrm{NH_3{-}N}}$ in drinking waters is too low to affect DBPs formation remarkably. However, considering the wide range of $C_{\mathrm{NH_3{-}N}}$ in biologically treated wastewater and its reaction with free chlorine to the formation of combined chlorine, it was reasonable for $C_{\mathrm{NH_3{-}N}}$ to be an important parameter to affect DBPs formation and to be involved in toxicity formation models for wastewater.

The reliability and robustness of Eqs. (1) and (2) were tested and the results indicated that there were no significant collinearity among those parameters involved in the equations, and both of the two equations were robust, that is, they can be used to estimate the toxicity formation during chlorine disinfection process. However, by comparing the results of statistical test on Eqs. (1) and (2), significance level of independent variable C_{DOC} is found to be relatively higher than that of UV_{254}. This implies that variable C_{DOC} may be more suitable than UV_{254} in predicting the toxicity formation during chlorine disinfection. The intrinsic mechanism about this is still ambiguous, which requires further investigation. The relationships between observed and predicted toxicities are plotted in Fig. 5, and the related data are listed in Table 2.

4. Conclusions

(1) Toxicity of the wastewater samples significantly increased after chlorine disinfection with the formation of toxic DBPs.
(2) Both ammonia and organic carbon may react with chlorine disinfectant, the former reduces the reactivity of chlorine due to the formation of combined chlorine, while the latter produces toxic chemicals and decreases the quality of disinfected water.
(3) The statistical analyses indicated that the toxicity formation significantly correlated with chlorine dosage, ammonia and organic carbon concentrations. The obtained equations are robust and can be used to predict the toxicity formation during wastewater chlorine disinfection process.

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