



## Effect of several factors on pseudo-kinetics in chlorine disinfection of phage MS2

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### ABSTRACT

The effect of pH, temperature, particulate matter, organic matter, and NH<sub>3</sub> on phage MS2 inactivation using chlorine in pure water, synthetic water and filtered water samples was studied. And four possible models, including the Chick, Chick–Watson, Hom, and delayed Chick–Watson models were attempted to fit the experimental data. The virus inactivation achieved 4 logs at a CT value of 2.8 mg min/L, which met the United States Environmental Protection Agency standards. However, over 2 mg/L chlorine was needed to achieve the same goal in filtered water originated from two local water treatment plants. Furthermore, organic matter, pH, and temperature as well as the presence of NH<sub>3</sub> and particulate matter strongly affected the chlorine inactivation of MS2. Among four possible models, delayed Chick–Watson model was found to provide the most suitable description of MS2 inactivation by chlorine. Furthermore, the equation of the inactivation rate was obtained. And the MS2 inactivation rate predicted by this equation well correlated with the measured results in both simulated and filtered water samples.

*Keywords:* Chlorine inactivation; MS2; Influence factors; Kinetics; Delayed Chick–Watson model

### 1. Introduction

Owing to the rapid development of human society, the water sources in many countries are polluted to various degrees, which have posed severe threat and harm to human health. Over 20 microbial diseases could spread through drinking water, such as typhoid, cholera, dysentery, and hepatitis [1]. Another Indian study of hepatitis E indicated that ~70% of the cases were caused by polluted water [2]. Moreover, in the

past few years, waterborne gastroenteritis induced by noroviruses has outbreaked for several times in America, China, and some other countries [3]. Consequently, more and more attention is paid to the safety of drinking water, and it has become an urgent concern to disinfect the drinking water properly and effectively.

Chlorine is a common valid disinfectant in drinking water treatment due to its strong oxidizing capacity. At present, most of Chinese water plants disinfected with chlorine and chloramine. According to statistics, above 90% water plants in Shanghai used

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chlorine and chloramine as disinfectant. Previous researches confirmed that chlorine could effectively inactivate bacteria and viruses in water [4,5], and the CT (concentration of disinfectant by contact time) of chlorine to inactivate 99.99% of the f2 phage is 6 min mg/L. Further studies illustrated that pH [1,6,7], temperature [5,8], particulate matter [9], organic matter [7,9,10], and other ions [6,8,11–13] affected the disinfection of bacteria, viruses, and protozoans by chlorine in varying degrees. However, most of the above studies on chlorine disinfection only focus on one or two influential factors, and few of them have evaluated the performance of chlorine disinfection in filtered water.

To better learn and describe the inactivation of viruses by disinfectants, several pseudo-kinetic models have been successively proposed, such as the Chick [14] and Chick–Watson [15] models. However, neither of them could accurately fit the inactivation curve, which deviates from first-order pseudo-kinetics. The Hom [16] and delayed Chick–Watson [17] models were further put forward to account for the observed deviations. Nevertheless, only a few studies have evaluated the appropriateness of these two models for fitting the inactivation curve of filtered water samples.

Based on the above background, this article studied the effect of several factors, including pH, temperature, particulate matter, organic matter, and ion concentrations, on virus inactivation (using chlorine as disinfectant in pure and filtered water). MS2 phages were selected as indicator, as it is a frequently used

model or surrogate for enteric viruses because of similar morphology. The pseudo-kinetics of MS2 inactivation with chlorine in simulated and filtered water samples was also investigated. It was expected that the obtained model would provide reliable reference for the effective disinfection of viruses in drinking water treatment plants.

## 2. Materials and methods

### 2.1. Materials

Three kinds of water samples were used in this study: Pure water sample, simulated water samples (S1–S19), and filtered water sample. Filtered water was sampled from two water treatment plants, and therefore named filtered water A and B. All 19 types of simulated water samples (S1–S19), as shown in Table 1 were formed by adjusting different parameters of the pure water. The pH was adjusted by adding 0.1 mol/L NaOH and/or 0.1 mol/L HCl. Kaolinite was added to produce the targeted turbidity, although the turbidity in natural waters is not always composed to kaolinite (100% inorganic).  $\text{NH}_3\text{Cl}$  and humic acid were quantifiably dosed to produce different ammonia ( $\text{NH}_3$ ) and chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) values, respectively. The filtered water A was collected from the sand filter effluent of a reservoir in a southern city of China. The filtered water B was obtained from the sand filter effluent of a river in the same city. The

Table 1  
Water quality parameters of synthetic water

Synthetic water	pH	Temperature (°C)	Turbidity (NTU)	$\text{NH}_3$ (mg/L)	$\text{COD}_{\text{Mn}}$ (mg/L)
S1	6.5	20	0	0	0
S2	7.0	20	0	0	0
S3	7.5	20	0	0	0
S4	8.5	20	0	0	0
S5	6.5	15	0	0	0
S6	6.5	10	0	0	0
S7	6.5	5	0	0	0
S8	6.5	20	2	0	0
S9	6.5	20	5	0	0
S10	6.5	20	10	0	0
S11	6.5	20	20	0	0
S12	6.5	20	0	0.5	0
S13	6.5	20	0	1	0
S14	6.5	20	0	2	0
S15	6.5	20	0	0	1
S16	6.5	20	0	0	2
S17	6.5	20	0	0	3
S18	6.5	20	0	0	6
S19	6.5	20	0	0	8

Table 2  
Water quality parameters of pure water, filtered water A and B

	pH	Turbidity (NTU)	NH <sub>3</sub> (mg/L)	COD <sub>Mn</sub> (mg/L)	Br <sup>-</sup> (mg/L)	I <sup>-</sup> (mg/L)
Pure water	6.5	0	0	0	0	0
Filtered water A	7	0.32	0.84	2.11	0.06	0
Filtered water B	7.5	0.69	1.09	4.79	0.12	0.02

water quality of pure water, filtered water A and B is shown in Table 2.

Besides, chlorine stock solutions were prepared by dosing quantities of sodium hypochlorite (14% wt/vol) to chlorine demand-free water.

## 2.2. Methods

According to the double-agar-layer plaque method of ISO 10705-1 [18], *Escherichia coli* K-12 Hfr strain (ATCC 23631) was used as the host to propagate and titrate the MS2 phages (ATCC 15597-B1). The range of MS2 stock cultures was between  $5 \times 10^{10}$  and  $3 \times 10^{11}$  PFU/mL.

The experiments were conducted in 2 L batch reactors. For each kind of water sample, five parallel reactors were simultaneously operated. Firstly, 2 L of test water and 0.5 mL of MS2 stock cultures were sequentially added to each reactor. The initial chlorine concentrations of the five parallel reactors were controlled to 0.5, 1.0, 1.5, 2.0, and 5.0 mg/L, respectively. The batch reactors were continuously mixed using a magnetic stirrer at 300 rpm for 120 min. The reaction temperature was controlled by water bath. At each predetermined time point (5, 10, 15, 30, 60, 120, 300, 600, 1,800, and 3,600 s), 1 mL water samples were collected from the middle part of reactor. These samples were transferred to a 5 mL centrifuge tube, which contains 1 mL of sodium thiosulfate (0.35%, w/w) to remove the residual chlorine. Afterward, the MS2 concentration of each water sample was determined. In addition, 100 mL water samples were withdrawn at

certain time points (0.5, 1, 5, 10, 15, 30, 60, and 120 min) to measure the residual chlorine in the reactors. All experiments were conducted thrice. The results were averaged and presented in this study.

## 2.3. Kinetic modeling

To better describe the virus inactivation curve, the Chick, Chick–Watson, Hom, and delayed Chick–Watson models (Table 3) were attempted to fit the experimental data. And the most suitable one (delayed Chick–Watson models in this study) was selected to describe the simulated and filtered water samples.

## 2.4. Analytical methods

The pH, temperature, turbidity, as well as the concentrations of COD<sub>Mn</sub>, NH<sub>3</sub>, and chlorine (including chlorine residuals) were analyzed according to Standard Methods [19]. The pH was measured with PHS-25 (Leici), while temperature with thermometer, COD, and chlorine with titrimetry according to their instructions. And turbidity and NH<sub>3</sub> were determined with DR2800 (Hach).

## 3. Results and discussion

### 3.1. Chlorine disinfection of MS2 in pure water

It was found in pure water that 1 mg/L chlorine inactivated the MS2 with a 4 log reduction within 60 s,

Table 3  
Applied kinetic models

Equation*	Model
$\ln N/N_0 = kCt = -kC_0/k' \times (1 - e^{-k't})$	Chick [14]
$\ln N/N_0 = kC^n t = -kC_0^n/nk' \times (1 - e^{-nk't})$	Chick–Watson [15]
$\ln N/N_0 = kC^n t^m = -kC_0^n t^m \times [(1 - e^{-nk't/m})/(-nk't/m)]^m$	Hom [16]
$N/N_0 = \left(\frac{N_{1,0}}{N_0}\right)e^{-k_1 Ct} + \left(\frac{N_{2,0}}{N_0}\right)e^{-k_2 Ct}$	Delayed Chick–Watson [17]

\*N: Number of infective organisms at any given time; N<sub>0</sub>: Initial number of infective organisms; N<sub>1,0</sub> and N<sub>2,0</sub>: Initial number of infective organisms for rapid and slow period; k, k<sub>1</sub>, k<sub>2</sub>: Inactivation rate constant; k': Disinfectant decay constant; C<sub>0</sub>: Disinfectant concentration; t: Time; n, m: Coefficient of dilution.

which could satisfy the inactivation requirement of the United States Environmental Protection Agency (USEPA) for enteroviruses. The inactivation effect increased with the increasing chlorine concentrations. For example, when 0.5 mg/L chlorine was added to pure water, the MS2 titers only reduced by about 2.4 log after 3,600 s. Moreover, 1.5 mg/L of chlorine achieved a 4 log reduction of MS2 within 30 s. The corresponding CT value of 99.99% MS2 inactivation in this study was 2.8 min mg/L (20°C, pH 6.5).

Then, the pseudo-kinetic parameters of MS2 inactivation in pure water were fitted using the Chick, Chick–Watson, Hom, and delayed Chick–Watson models. The results are shown in Table 4. The correlation coefficients ( $R^2$ ) of the Chick and Chick–Watson models were 0.827 and 0.831, respectively. By comparison, both of the Hom and delayed Chick–Watson models could achieve a  $R^2$  value of over 0.960. Obviously, the Chick and Chick–Watson models cannot accurately fit the inactivation curve, which may be caused by the fact that the inactivation curve deviates from first-order pseudo-kinetics. Meanwhile, the standard deviation (STDEV) of the Hom model (23.957) was much higher than that of the delayed Chick–Watson model (10.024), which favored that the delayed Chick–Watson model was more suitable for simulating MS2 inactivation by chlorine than the Hom model.

### 3.2. Effect of several factors on MS2 inactivation

In Fig. 1, the reduction of MS2 at different chlorine CT values under various factors (i.e. pH, temperature, turbidity, organic matter, and  $\text{NH}_3$ ) is compared. As shown in Fig. 1(a), the reduction of MS2 decreased with the increasing of pH under the same chlorine CT value. For different CT values, the reduction of MS2 under pH 6.5 was 1.42 log (0.08 min mg/L), 2.40 log (0.15 min mg/L), 4.01 log (0.25 min mg/L), 4.88 log (0.5

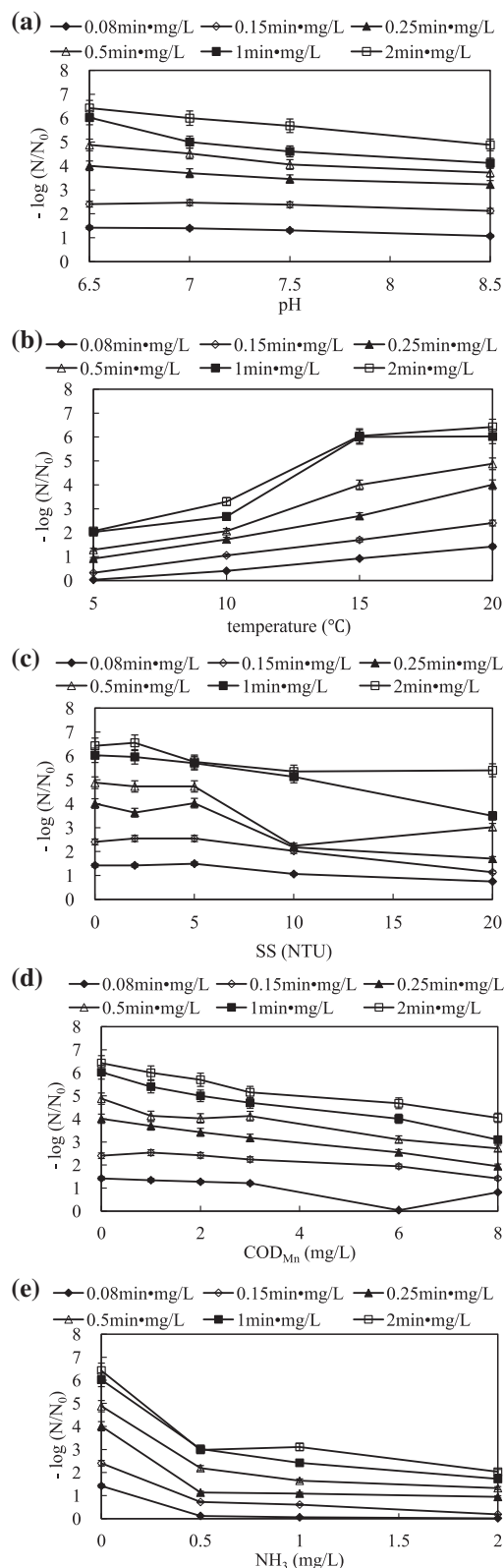


Table 4  
Pseudo-kinetic parameters in the process of MS2 inactivation in pure water

Model	$k$ (or $k_1$ )	STDEV	$n$	$m$	$k_2$	$R^2$
Chick	44.850	39.094	– <sup>a</sup>	– <sup>a</sup>	– <sup>a</sup>	0.765
Chick– Watson	57.624	49.471	1.478	– <sup>a</sup>	– <sup>a</sup>	0.796
Hom	41.519	23.957	4.589	0.494	– <sup>a</sup>	0.913
Delayed Chick– Watson	37.559	10.024	– <sup>a</sup>	– <sup>a</sup>	0.418	0.997

<sup>a</sup>“–”: This parameter does not exist in this model.

Fig. 1. The reduction of MS2 ( $-\log(N/N_0)$ ) at different chlorine CT values under various factors: (a) pH (S1 to S4); (b) temperature (S1, S5 to S7); (c) turbidity (S1, S8 to S11); (d)  $\text{NH}_3$  (S1, S12 to S14); and (e)  $\text{COD}_{\text{Mn}}$  (S1, S15 to S19).

min mg/L), 6.03 log (1 min mg/L), and 6.42 log (2 min mg/L), respectively. When the pH was adjusted to 8.5, the corresponding reduction of MS2 decreased to 1.07 log, 2.12 log, 3.23 log, 3.72 log, 4.12 log, and 4.88 log, respectively. This variation trend was consistent with those reported by Sun and Zhong [1] and Wang et al. [7]. The decrease was probably due to the following reasons: (1) Chlorine disinfection mainly relies on HOCl. The concentration of HOCl rises with the decreasing pH. (2) The surface charge of microorganism is negative under pH 8.5. It is hard for HOCl to adhere to or get through the cell membrane with negative charge.

Fig. 1(b) presents the reduction of MS2 at various chlorine CT values under different temperatures. When the temperature decreased from 20 to 5°C, the reduction of MS2 also decreased. The maximum and minimum decrease ranges were 2.07 log and 0.03 log, respectively, at the CT values of 2 and 0.08 min mg/L. The results implied that under low temperature (10°C or even below), to achieve the same inactivation efficacy as that in room temperature, a higher CT value was needed. Thus, the increase in temperature can improve chlorine effectiveness. This results were consistent with those of Lim et al. [5], who reported that the CT value needed to inactivate *Murine norovirus* by 3 log at 5°C (0.38 min mg/L) is much higher than that at 20°C (0.18 min mg/L). Generally, the temperature change affects the reaction percentages in two opposite ways. On one hand, the increase in temperature accelerates the Brownian motion of molecules, and thereby improves the reaction rates between microorganisms and chlorine. On the other hand, the increase in temperature would decrease the solubility of chlorine in water and leads to the decline of reaction rate. This study and that of Rennecker et al. [17] implied that the integrated effect of the above two opposite processes ultimately improves the effectiveness of disinfection.

Fig. 1(c) shows the effect of turbidity on the reduction of MS2 at various chlorine CT values. When the turbidity was raised from 0 to 20 NTU under the CT values of 2 and 0.08 min mg/L, the reduction of MS2 was reduced by 1.03 log and 0.67 log, respectively. This result is consistent with that of Winward et al. [9]. Particulate matter could combine with the microorganisms in a certain degree and thereby protects them from being inactivated.

The reduction of MS2 at various chlorine CT values under different concentrations of organic matter is compared in Fig. 1(d). With the increase in concentrations of organic matter, the reduction of MS2 gradually decreased. For instance, at the CT value of 0.08 min mg/L, the reduction of MS2 decreased by 2.37 log under the effect of 8 mg/L COD<sub>Mn</sub>. Wang et al. [7] obtained similar

results that raising the serum concentration from 0 to 15% evidently decreases the inactivation percentage from 78 to 21% in 5 min. The reason may be that organic matter can act as reducing substance and consume a considerable amount of chlorine, then further induce a distinct decrease in the reduction of MS2.

Besides, Fig. 1(e) shows the effect of NH<sub>3</sub> concentrations on the reduction of MS2 at various chlorine CT values. The reduction of MS2 decreased rapidly with the increase in NH<sub>3</sub> concentrations. For example, it was reduced by 1.41 log and 4.41 log when NH<sub>3</sub> concentrations was raised from 0 to 2 mg/L, respectively, under a CT values of 2 and 0.08 min mg/L. This is because chlorine can react with NH<sub>3</sub> to generate chloramine. And the disinfection of chloramine is worse than chlorine.

Among all the above influential factors, it can be found that NH<sub>3</sub> had the greatest effect on the reduction of MS2 of phage MS2 using chlorine, followed by temperature, turbidity, organic matter, and pH.

### 3.3. Pseudo-kinetic fitting

According to the delayed Chick–Watson model, the observed inactivation rate equaled the inactivation rate of rapidly infected organisms and slowly infected organisms. As a result, the inactivation rate of rapidly infected organisms could be written as Eqs. (1)–(3):

$$N/N_0 = \frac{N_1}{N_0} + \frac{N_2}{N_0} = \left(\frac{N_{1,0}}{N_0}\right)e^{-k_1 Ct} + \left(\frac{N_{2,0}}{N_0}\right)e^{-k_2 Ct} \quad (1)$$

$$\ln N_1/N_{1,0} = -k_1 Ct \quad (2)$$

$$r_A = -k_1 C \quad (3)$$

With  $N_1$  and  $N_2$  are the numbers of rapidly infected and slow infected organisms at any given time, respectively, whereas  $r_A$  is the inactivation rates of rapidly infected and slow infected organisms.

Eq. (3) is a well-recognized first-order equation. The inactivation rate constant is just relative to temperature, pH, and turbidity. Nevertheless, in filtered water, the presence of organic matter and NH<sub>3</sub> would also affect the inactivation rate. Hence, the Eq. (3) should be rewritten as follows:

$$\begin{aligned} r_A &= -k'_1 C_A C = -M(\alpha)^{\text{pH}}(\beta)^{\text{T}}(\gamma)^{\text{SS}} C_A C \\ &= -R(\alpha)^{\text{pH}}(\beta)^{\text{T}}(\gamma)^{\text{SS}} (e)^{a \cdot \text{COD} + b \cdot \text{NH}_3} C \end{aligned} \quad (4)$$

$$k'_1 = M(\alpha)^{\text{pH}}(\beta)^{\text{T}}(\gamma)^{\text{SS}} \quad (5)$$

where  $k'_1$  is the inactivation rate constant; and  $M$ ,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $a$ ,  $b$ ,  $C$  are corresponding coefficients.

The change in inactivation rate constants under different factors is presented in (Fig. 2). This constant decreased with the decrease in temperature and increase in pH. The concentration of kaolinite (turbidity) also affected the observed inactivation rate constant  $k'_1$  to a certain extent. For instance, kaolinite could combine with MS2 and thereby inhibit the disinfection efficiency and reduce the  $k'_1$ . Then,  $k'_1$  was fitted with SPSS 19 and the inactivation rate constant was obtained as follows:

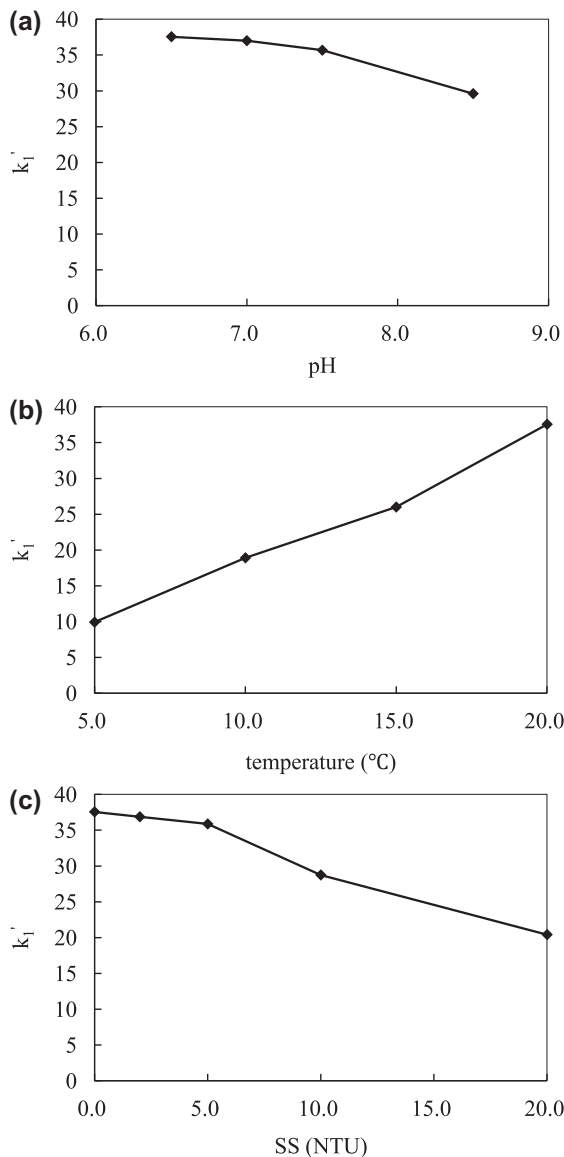


Fig. 2. Observed inactivation rate constant  $k'_1$  (fit by the delayed Chick–Watson model) at different factors: (a) pH; (b) temperature; and (c) turbidity.

$$k'_1 = 4.071 \times 10^{-9} (0.884)^{\text{pH}} (1.085)^T (0.970)^{\text{SS}} \quad (6)$$

Fig. 3 further presents the  $k'_1 C_A$  value at different concentrations of reducing substances (organic matter and  $\text{NH}_3$ ). According to the fitting results, the inactivation rate was written as follows:

$$r_A = -4.071 \times 10^{-9} (0.884)^{\text{pH}} (1.085)^T (0.970)^{\text{SS}} e^{-0.121 \text{ COD} - 1.191 \text{ NH}_3} C \quad (7)$$

$$\ln N_1/N_{1,0} = -4.071 \times 10^{-9} (0.884)^{\text{pH}} (1.085)^T (0.970)^{\text{SS}} e^{-0.121 \text{ COD} - 1.191 \text{ NH}_3} C \cdot t \quad (8)$$

Meanwhile,  $k_2$ ,  $N_{1,0}$ , and  $N_{2,0}$  exhibited little effect on the different factors in the fitting process, with average values calculated as 0.67,  $6.31 \times 10^7$ , and 9.8, respectively. These average values were further substituted into the model as follows:

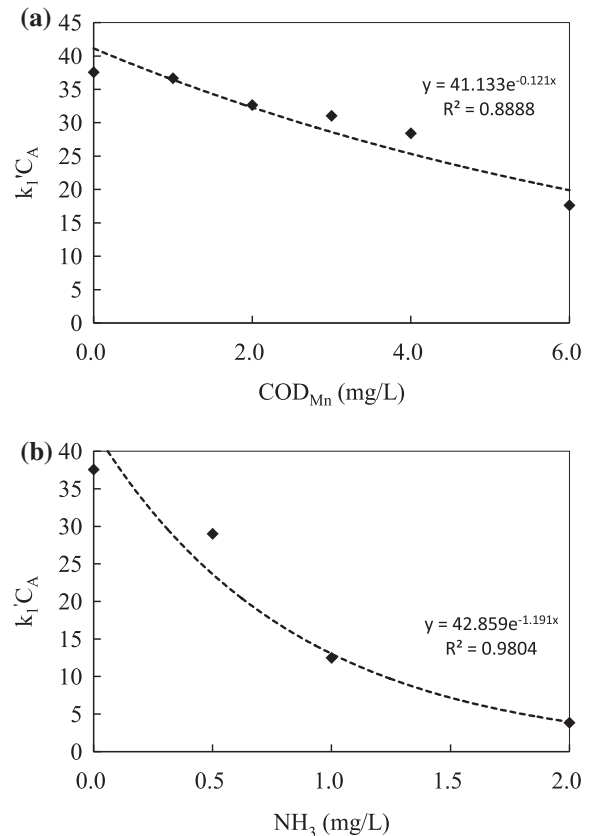


Fig. 3.  $k'_1 C_A$  value at different concentrations of reducing matter: (a)  $\text{COD}_{\text{Mn}}$  and (b)  $\text{NH}_3$ .

$$N/N_0 = \left( \frac{6.31 \times 10^7}{N_0} \right) e^{-k_1 Ct} + \left( \frac{9.8}{N_0} \right) e^{-0.67Ct} \quad (9)$$

where  $k_1 = -4.071 \times 10^{-9} (0.884)^{\text{pH}} (1.085)^T (0.970)^{\text{SS}} e^{-0.121 \text{COD} - 1.191 \text{NH}_3}$ .

Fig. 4 illustrates a comparison between the measured MS2 inactivation rates in simulated water samples and the calculated values based on Eq. (9). Theoretically, if the obtained equation could perfectly reflect the filtered inactivation conditions, the data points should exactly locate on the 45° line. In (Fig. 4), the two datasets were found to be well correlated, with a  $R^2$  value of 0.97. Moreover, the mean difference between the measured inactivation rates and predicted ones was relatively low (0.11 log).

### 3.4. Chlorine disinfection of MS2 in filtered water

In filtered water, 2 and 2.5 mg/L of chlorine were found to be needed in inactivating 4 log MS2 in filtered water A and B, respectively, which was much higher than that in pure water (1 mg/L chlorine). The temperature for both filter waters samples was around 20°C while the turbidity of both samples was lower than 1 NTU. Besides, the pH values for filtered water A and B were 7 and 7.5, respectively. According to the results in Section 3.2, the present temperature, turbidity, and pH would not significantly affect the disinfection efficiency. Therefore, the rises of  $\text{COD}_{\text{Mn}}$  and  $\text{NH}_3$  concentrations were considered as the main reason for increased chlorine dosage required. Furthermore, the  $\text{COD}_{\text{Mn}}$  and  $\text{NH}_3$  concentration in filtered water B was 7.5 and 4.66 mg/L, respectively, which exceeds that in filtered water A (7.00 and 2.28 mg/L).

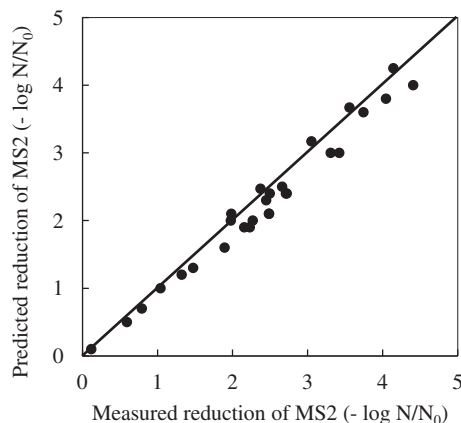


Fig. 4. Comparison between the measured inactivation rate in simulated water samples and the predicted one based on Eq. (9).

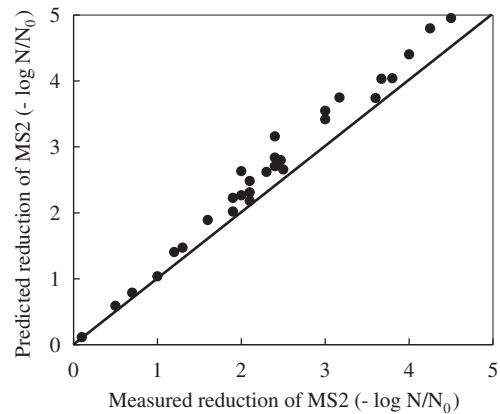


Fig. 5. Comparison between the measured reduction of MS2 in filtered water and the predicted one based on Eq. (9).

These differences well explain why a higher chlorine dosage was needed to inactivate 4 log MS2 in filtered water B than A.

As shown in Fig. 5, the measured MS2 inactivation rates in filtered water were also compared with the prediction ones based on Eq. (9). The mean difference between the two inactivation rates was 0.29 log, with a  $R^2$  value of 0.875. The inactivation rates in filtered water were a little lower than the predictions. This difference may be induced by the presence of other reducing substances and their synergistic effects in the filtered water. As the amount of reducing substances in source water for drinking water treatment plant was commonly comparatively limited, and the obtained equation has taken the main reducing substances (organic matter and  $\text{NH}_3$ ) into account, Eq. (9) could also be used in predicting the inactivation rates of MS2 in filtered water. By this model, it is possible for the drinking water plants to forecast, determine, and adjust technical parameters timely and conveniently in the process of chlorine inactivation of viruses. If substantive reducing substances were found in the filtered water, Eq. (9) should be further adjusted accordingly.

## 4. Conclusions

Chlorine showed an obvious effect on MS2 inactivation. Just 1 mg/L of chlorine could achieve the USEPA standards with a CT value of 2.8 mg min/L in pure water. While in filtered water, more than 2 mg/L chlorine was demanded to decrease 4 log of MS2 (99.99% inactivation). The presence of organic matter,  $\text{NH}_3$ , particulate matter, as well as the change in pH and temperature, was found to have distinct effect on

the inactivation of MS2 by chlorine. Moreover,  $\text{NH}_3$  had the greatest negative effect on the inactivation percentages of phage MS2 using chlorine, followed by turbidity, organic matter, and pH. And temperature had some positive effect on phage MS2 inactivation. Besides, the delayed Chick–Watson model was proved to be the most suitable model in describing the chlorine inactivation of MS2. Then an applicable equation of the model was obtained on filtered water, the reduction of MS2 predicted by which showed a positive correlation with the measured values. The obtained equation might provide some reference for the effective disinfection of viruses in drinking water treatment plants. Of course, further studies should be done in order to justify that this equation provides a reliable reference to disinfection of other viruses, because different viruses might behave differently.

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