Kinetic characteristics of oxidation of microcystin-LR at low concentration by chlorine and permanganate

Wei Li, Jinming Duan and Dennis Mulcahy

ABSTRACT

In response to increasing concerns in the literature about human toxicity of microcystin-LR (MC-LR) in drinking water at very low concentrations, this study investigated the destruction of MC-LR down to ng L^{-1} concentrations by chlorine and permanganate. Results showed that decomposition in the low concentration range (1–2 ng L^{-1}) exhibits a pseudo-first-order reaction with respect to both oxidants in pure water solutions. However, when the reaction proceeded down to a very low concentration (<10 ng L^{-1}) a much smaller rate constant dictates the kinetics. In the presence of humic acids, the reaction rates and the final concentration removal were affected. The extent of such influence varied between the two oxidants, likely due to the different reaction mechanisms involved. This creates some profound effects of the governance of an oxidant exposure CT (residual oxidant concentration (C) × contact time (T)) over MC-LR degradation between the two oxidants. This study indicates that chlorine and permanganate may be still effective for MC-LR decomposition down to a concentration of 10 ng L^{-1} or below. However, the influence of solution chemistry must be carefully examined before a feasible CT is determined for the control of this toxin in a water treatment system.

Key words | chlorine, humic acid, microcystins, permanganate, rate constants

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INTRODUCTION

Cyanobacterial blooms in surface waters are becoming a challenging issue in water treatment for safe drinking water supplies around the world. According to reports, 50–70% of cyanobacterial blooms produce highly acute toxins to human health (Dietrich & Hoeger 2005; Fang et al. 2010). Microcystins (MCs) are common toxins produced by the genera Microcystis, Anabaena, Planktothrix and Nostoc (Gupta et al. 2003; Dietrich & Hoeger 2005). Among nearly 80 variants that have been reported, microcystin-LR (MC-LR) has been identified as the most prevalent and most toxic (Dietrich & Hoeger 2005). Exposure to low levels of MC-LR leads to acute or chronic liver injury because MC-LR's accumulation in the liver inhibits protein phosphatase activities (Gupta et al. 2003; Xu et al. 2008). Evidence relates the consumption of drinking water contaminated by MCs to illness and death of wild and domestic animal (Francis 1878; Frazier et al. 1998; Sedan et al. 2010), primary liver cancer and fatalities of

humans (Ueno et al. 1996; Pouria et al. 1998). For public health protection against these harmful effects, the World Health Organization (WHO) recommends a guideline value 1 μg L^{-1} (or 0.04 μg kg^{-1} of body weight per day) for MC-LR in drinking water (WHO 2004). However, as the guideline value is based on the acute toxicity it does not take into account the effects of possible long-term exposure to MC-LR at a low concentration (Kull et al. 2006). In fact, recent studies indicate that there is a risk of health effects from chronic exposure to low concentrations of MC-LR. A recent study showed that about 0.39 μg L^{-1} MCs was determined in the blood serum of fishermen at Lake Chaohu in China, equivalent to an intake of about 2.2–3.9 μg MC-LR daily (Chen et al. 2009). Long-term persistence of the dissolved toxins has been reported around the world for up to 6 months or longer at low concentrations (e.g. 0.05–1.6 μg L^{-1} in China, 0.07–0.76 μg L^{-1} in Germany, 0.02–0.8 μg L^{-1} in Japan) (Chorus & Bartram 1999). To

safeguard public health against chronic harmful exposure to a low concentration of MC-LR, Ueno *et al.* (1999) recommended a value of 0.01 µg L^{-1} as a maximum acceptable level for MCs in drinking water. Likewise, Duy *et al.* (2000) proposed 0.07, 0.11 and 0.32 µg L^{-1} MC-LR in drinking water for infants, children and adults respectively. A limit of 0.006 and 0.003 µg kg^{-1} body weight per day (or 0.15 and 0.075 µg L^{-1} in drinking water, equivalent to WHO's definition) MC-LR for short-term and chronic exposure was recently proposed for consideration by the US EPA (Hudnell 2010).

Although conventional water treatment processes (i.e. coagulation, sedimentation and sand filtration) can achieve effective removal of cyanobacterial cells, they have no effect of removing the dissolved toxins (Chow *et al.* 1999; Newcombe & Nicholson 2004). MC-LR can be removed by activated carbon adsorption, reverse osmosis or nanofiltration in laboratory and pilot-scale experiments but with high costs (Chorus & Bartram 1999; WHO 2004; Upadhyayula *et al.* 2009). In comparison, oxidation by chlorine and permanganate, agents commonly used in water treatment, is considered a more practicable means of eliminating MC-LR from water (Nicholson *et al.* 1994; Acero *et al.* 2005; Rodríguez *et al.* 2007). Chlorination at the dose usually practised in water treatment for disinfection appeared to be effective in eliminating MC-LR to the limit 1 µg L^{-1}, though concern over disinfection by-products such as trihalomethanes may be raised if the chlorine dose or natural organic matter (NOM) content is too high (Nicholson *et al.* 1994; Ho *et al.* 2006; Xagoraraki *et al.* 2006; Rodríguez *et al.* 2007; Acero *et al.* 2008). Permanganate oxidation at a relatively low dose (<1.5 mg L^{-1}) can effectively decompose dissolved MC-LR in water (Rodríguez *et al.* 2007; Acero *et al.* 2008). Note that high doses of permanganate are not encouraged and it is normally dosed prior to the sedimentation/filtration processes (Chen *et al.* 2005; Rodríguez *et al.* 2007).

Extensive studies have been reported on the removal of MC-LR using chlorine and permanganate oxidation, mostly targeted at the present WHO provisional limit, 1 µg L^{-1} (Acero *et al.* 2005; Ho *et al.* 2006; Xagoraraki *et al.* 2006; Rodríguez *et al.* 2007; Acero *et al.* 2008). As evidence continues to accumulate of harmful effects of the long-term exposure to MCs, the present limit may need to be reviewed.

Therefore, it is useful to examine the degradation kinetics in a low MC-LR concentration range by the two oxidants to provide information on limits of removal and the relationship between oxidant exposure (measured as CT, where CT is residual oxidant concentration (C) × contact time (T), a measure of total exposure) and removal.

MATERIALS AND METHODS

Chemicals and materials

MC-LR (purity>95%) (for molecular structure see Figure 1) was purchased from Alexis Biochemicals (Lausen, Switzerland). Acetonitrile and methanol (HPLC grade) were purchased from Merck (Germany). Formic acid (HPLC grade), sodium hypochlorite (5% chlorine) and potassium permanganate (analytical grade) were from Kermel Corporation (Tianjin, China). Leucine enkephalin (>95%, as an internal standard (IS) for detection of MC-LR and humic acid (HA)) were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water (18.2 MΩ cm^{-1}, TOC≤1 µg L^{-1}) was produced by using an Elga Purelab Ultra Analytic system (UK). The solid-phase extraction cartridges (Supel™-Select HLB Resin, 60 mg/3 mL) were purchased from Supelco Analytical (Bellefonte, PA, USA).

Preparations of stock solutions

A stock solution of MC-LR at 25 mg L^{-1} was prepared in pure methanol. This solution was further diluted by 10% methanol solution to 0.25 mg L^{-1} as a working stock
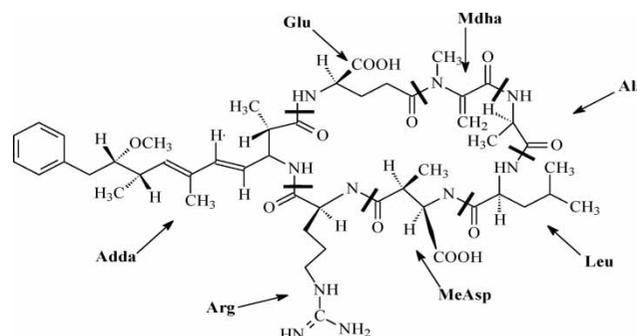

Figure 1 | Molecular structure of MC-LR.

solution. The stock solution of IS at $2.5 \, mg \, L^{-1}$ was prepared in 10% methanol solution. The commercial sodium hypochlorite solution was directly used as a stock solution without dilution and permanganate stock solution $(1 \, g \, L^{-1})$ was prepared by dissolving $KMnO_4$ in the ultrapure water. All of these solutions were stored in amber glass bottles at 4 °C.

Experimental procedures

Experiments were conducted in 250 mL amber glass flasks with stoppers at ambient temperature 20 ± 1 °C, and at four pH values: 5.3, 6.5, 7.2 and 8.0 (10 mM phosphate buffer). HA solutions of 0, 1, 2 or $5 \, mg \, L^{-1}$ were prepared using the buffered ultra-pure water solutions, which were then spiked with MC-LR to $1 \, \mu g \, L^{-1}$. In each oxidation test, a predetermined amount of chlorine or permanganate from the stock solution was added and immediately mixed into the prepared solution (250 mL) by shaking. At a predetermined reaction time, $100 \, \mu L$ of ascorbic acid solution $(100 \, g \, L^{-1})$ was pipetted into the flask to quench the residual oxidant.

Analytical methods

The concentration of chlorine was measured using the standard method 4500-Cl (DPD colorimetric method); the concentration of permanganate was determined using the standard method $4500\text{-}KMnO_4$ (APHA/AWWA/WEF 1999). The concentration of the HA was determined as dissolved organic carbon using a IL500 TOC analyzer (Hach, USA).

Detection of MC-LR in water samples was performed with addition of IS (i.e. leucine enkephalin). Samples of the MC-LR with the IS for analysis of MC-LR were pre-concentrated using solid phase extraction (SPE) performed in an extraction manifold (Waters). The pre-concentrated samples were then analyzed using ultra-performance liquid chromatography tandem mass spectrometry (Waters Corporation, Milford, MA, USA). The analytical column was the Waters Acquity UPLC BEH C_{18}. The length and internal diameter of the column were 100 and 2.1 mm respectively and the particle diameter was $1.7 \, \mu m$. The analytical procedures followed a well-documented method (Xu et al. 2008).

The SPE procedures were as follows. First, $100 \, \mu L$ of the IS stock solution was pipetted into 250 mL of the water sample to give a IS concentration of $100 \, ng \, L^{-1}$, followed by addition of 5 mL methanol. Before use, the SPE cartridges were conditioned with 10 mL methanol and then with 10 mL phosphate buffer solution at the same pH as the water sample. The sample was sucked via Teflon tubes at a flow rate about 1–2 drops per second to pass through the cartridge installed in the extraction manifold. The cartridge was then washed with 10 mL 10% methanol and dried by applying a strong vacuum for 20 min to remove residual moisture. MC-LR was eluted with 4 mL methanol: acetonitrile (50:50) through the cartridge at 1–2 drops per second into a borosilicate test tube and the eluate was evaporated with N_2 at 50 °C. The residue was redissolved in $250 \, \mu L$ methanol:ultrapure water (50:50). The resultant solution was transferred into a glass autosampler vial for analysis.

To assess the recovery of MC-LR in the SPE sample pretreatment and the limits of detection (LOD) of the analytical method, the following experiments were performed. MC-LR ultrapure water solutions at concentrations of 1, 5, 10, 50, 100, 250, 500 and $1,000 \, ng \, L^{-1}$ were prepared in 250 mL amber flasks. SPE was performed in triplicate. The results showed that all the recoveries were $\geq 85\%$. The LOD were $0.39 \, ng \, L^{-1}$ calculated according to the ratio S/N = 3 based on the measurement at $1 \, ng \, L^{-1}$ MC-LR. Besides, the presence of HA up to $5 \, mg \, L^{-1}$ showed almost no influence on the recovery of MC-LR, as reported previously (Kull et al. 2006).

Reaction kinetics

The reaction kinetics can be expressed as in Equation (1) below:

$$-\frac{d[\text{MC-LR}]}{dt} = k[\text{MC-LR}][\text{oxidant}] \tag{1}$$

It has been reported that when [oxidant]>>[MC-LR] in ultrapure water condition the reaction follows pseudo-first-order kinetics (Acero et al. 2005; Ho et al. 2006; Xagoraraki et al. 2006). Thus, Equation (1) may be expressed as:

$$-\frac{d[\text{MC-LR}]}{dt} = k[\text{MC-LR}][\text{oxidant}] = k_p[\text{MC-LR}] \tag{2}$$

where $k_p = k[\text{oxidant}]$; k_p is the pseudo-first-order rate constant. In the case of chlorine, [oxidant] is the sum of concentration of hypochlorous acid ([HOCl]) and hypochlorite concentration ([OCl⁻]).

If reactions proceed in real water (e.g. tap water) conditions, the oxidant concentration (chlorine or permanganate) may decrease with time due to competitive reactions of the reactant with some other reactant present in the water (Acero *et al.* 2005; Ho *et al.* 2006; Kull *et al.* 2006). The reaction rate was viewed as second-order kinetics, depending on both the concentration of the oxidant and that of MC-LR as described in Equation (1). By integration of Equation (1) we obtain:

$$\ln \frac{[\text{MC-LR}]}{[\text{MC-LR}]_0} = -k \int_0^t [\text{oxidant}] \mathrm{d}t \qquad (3)$$

or

$$\ln \frac{[\text{MC-LR}]}{[\text{MC-LR}]_0} = -k\text{CT} \qquad (4)$$

where $[\text{MC-LR}]_0$ is the concentration of the MC-LR at time $t = 0$ and the oxidant exposure $\text{CT} = \int_0^t [\text{oxidant}] \mathrm{d}t$.

RESULTS AND DISCUSSION

Removal of MC-LR at low concentrations by chlorine

The degradation of MC-LR in the concentration range from $1\,\mu g\,L^{-1}$ (or 1 nM) to a few $ng\,L^{-1}$ was examined at pH 7.2 and initial chorine concentrations 1, 2.5 and $5\,mg\,L^{-1}$ (14.1, 35.2 and 70.4 μM respectively). The results are presented in Figure 2. In the pure water condition, degradation of MC-LR exhibited second-order reaction kinetics with respect to MC-LR and chlorine as described by Equation (4), and also pseudo-first-order reaction kinetics at each chlorine dose (Equation (2); inset in Figure 2) as reported previously (Acero *et al.* 2005; Ho *et al.* 2006). The pseudo-first-order rate constant and the second-order rate constant varied with initial chlorine concentration (Figure 2). The higher the initial chlorine concentration, the greater the reaction rate constant.
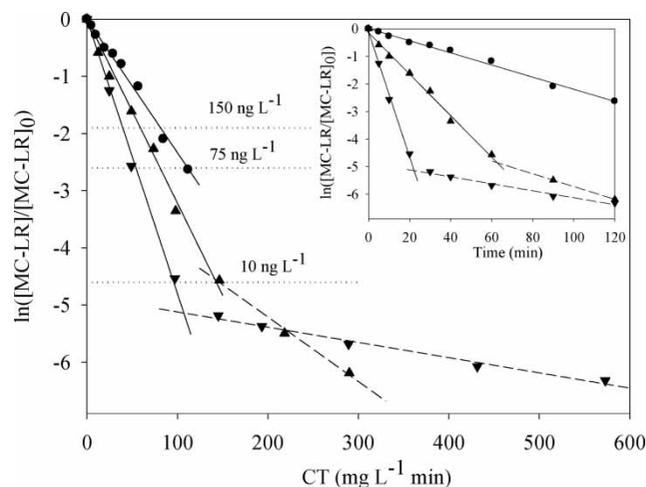
Figure 2 │ Second-order kinetic plot for the chlorination of MC-LR versus chlorine exposure CT at different dose of chlorine: (•) 1 mg L⁻¹ (14.1 μM); (▲) 2.5 mg L⁻¹ (35.2 μM); (▼) 5.0 mg L⁻¹ (70.4 μM) at pH 7.2. The solid line and dashed line are the linear least-squares regression of the data for the fast and slow kinetic concentration ranges respectively. Inset: pseudo-first-order kinetic plot versus reaction time for the same data.

Moreover, below a very low MC-LR concentration (i.e. approximately $<10\,ng\,L^{-1}$) the reaction rate constants changed and reaction proceeded under a lower kinetic reaction 'regime'. We suggest two possible explanations for the change of reaction constant at low concentration. (1) Reaction products of MC-LR formed in the course of reaction compete for the oxidants, and this influence becomes more prominent in its later stages. It has been reported that generation of intermediates in solution retards the reaction rate constant in a photolytic reaction system due to their competition for reactants (Lau *et al.* 2005). (2) At the same time, the presence of reaction products in solution may also reduce the fraction of the effective collisions between the two reacting molecules, thus decreasing the reaction rate from the point of view of the collision theory of reaction rates (Missen *et al.* 1999).

The rate constant in the low concentration range is much smaller than that in the high concentration range. For example, at the initial chlorine concentration (5 mg L⁻¹), the ratio of the rate constant in the fast reaction phase (k_1) to that in the slow reaction phase (k_2) is 17.3 (Table 1). A low concentration removal down to $2\,ng\,L^{-1}$ could be achieved at the neutral and acidic pH values when the chlorine concentration is higher than $2.5\,mg\,L^{-1}$. The chlorine exposure CT for the proposed

Table 1 | Second-order reaction constants (fast k_1 and slow k_2) of chlorination of MC-LR at different pH and initial chlorine concentrations

pH	[MC-LR]$_0$ (nM)	[Cl$_2$]$_0$ (μM)	k_1 (M^{-1}s^{-1})	Conc. range (ng L^{-1})	k_2 (M^{-1}s^{-1})	Conc. range (ng L^{-1})
7.2	1	14.1	28.0	1,000–71.8	–	–
7.2	1	35.2	36.5	1,000–10.5	13.4	10.5–2.1
7.2	1	70.4	55.3	1,000–5.9	3.2	5.9–2.0
5.3	1	35.2	496.3	1,000–3.1	5.7	3.1–2.1
6.5	1	35.2	77.8	1,000–8.4	7.1	8.4–2.0
8.0	1	35.2	17.8	1,000–330	10.9	330–44

limits of 150, 75 and 10 ng L^{-1} (Ueno et al. 1996; Hudnell 2010) was calculated and is shown in Table 2.

It can be seen that for even a fixed limit the CT value required would be different at different initial chlorine doses: the higher the chlorine dose, the lower the CT value required. For example, to achieve a limit of 150 ng L^{-1}, at a chlorine dose of 2.5 mg L^{-1} the required CT value is 59 mg L^{-1} min, whereas at a chlorine dose of 5 mg L^{-1} the required CT is only 40 mg L^{-1} min. This value for chlorine dose 2.5 mg L^{-1} is close to that required for 3-log inactivation of Giardia cysts (CT = 65 mg L^{-1} min) in similar water conditions (US EPA 2003). However, elimination of MC-LR down to the limit of 10 ng L^{-1} requires a much high CT value (153 mg L^{-1} min), and the reaction is moved to the slow reaction kinetic regime

(Figure 2). From the experimental results, the lowest removal concentration reached 2 ng L^{-1} by chlorination with the initial concentration 5 mg L^{-1} and a CT value of 570 mg L^{-1} min (Figure 2).

As shown in Figure 3, effects of pH on the chlorination kinetics of MC-LR were examined at pH values 5.3, 6.5, 7.2 and 8.0, with initial MC-LR concentration 1 μg L^{-1} and chlorine concentration 2.5 mg L^{-1}. The second-order reaction constants (k) were calculated according to Equation (4) and are shown in Table 1. From the results, it can be seen that rate of degradation of MC-LR was increased as the pH value was reduced from 8.0 to 5.3, which accords with previous studies (Acero et al. 2005; Ho et al. 2006; Acero et al. 2008). From previous studies, the explanation for pH dependence in chlorination of organic substances involves: (1) speciation

Table 2 | Values of chlorine and permanganate exposure CT required for the proposed limits of MC-LR removal in the literature under the experimental conditions

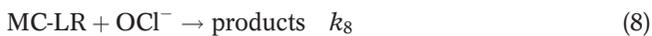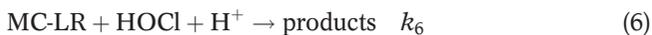| Oxidant exposure		CT (mg L^{-1} min)										
		MC-LR v chlorine					MC-LR v permanganate					
				MC-LR limits (ng L^{-1})					MC-LR limits (ng L^{-1})		
Humic acid (mg L^{-1})	pH	Initial chlorine (mg L^{-1})	Final MC-LR (ng L^{-1})	150	75	10	Initial permanganate (mg L^{-1})	Final MC-LR (ng L^{-1})	150	75	10
0	7.2	1.0	71.8	81	110	197	0.5	6.4	23	32	56
0	7.2	2.5	2.1	59	80	153	1.0	2.2	21	29	51
0	7.2	5.0	1.8	40	54	96	1.5	1.2	20	27	48
0	5.3	2.5	2.0	5	6	11	1.0	2.2	21	29	51
0	6.5	2.5	2.1	25	33	60	1.0	2.2	21	29	51
0	8.0	2.5	44.1	156	232	–[a]	1.0	2.4	21	29	52
1.0	7.2	2.5	2.6	50	68	121	1.0	5.1	27	37	82
2.5	7.2	2.5	3.6	34	47	86	1.0	19.6	38	51	91
5.0	7.2	2.5	5.3	21	29	52	1.0	345.4	–[a]	–[a]	–[a]

Note: [a]The concentration limit of MC-LR could not be achieved.
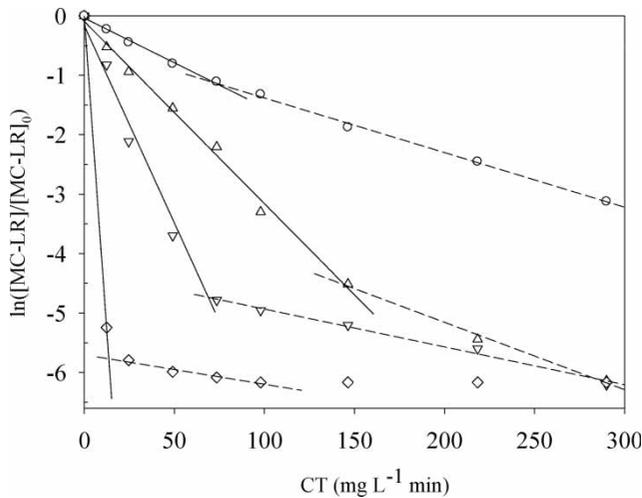
Figure 3 | Influence of solution pH on the second-order degradation kinetics of MC-LR versus CT at a chlorine dose 2.5 mg L^{-1} (35.2 μM): (◇) pH 5.3; (▼) pH 6.5; (▲) pH 7.2; (○) pH 8.0.

of chlorine and a reactant; (2) the acid-catalyzed reaction of HOCl (Gallard & von Gunten 2002; Acero *et al.* 2005).

The pH-dependence of the reaction kinetics was described by considering the dissociation of hypochlorous acid HOCl, reactions of MC-LR with HOCl and hypochlorite ion OCl$^-$, including an acid-catalyzed reaction with HOCl (Acero *et al.* 2005):

$$HOCl \leftrightarrow OCl^- + H^+ \qquad (5)$$

$$MC\text{-}LR + HOCl + H^+ \rightarrow products \quad k_6 \qquad (6)$$

$$MC\text{-}LR + HOCl \rightarrow products \quad k_7 \qquad (7)$$

$$MC\text{-}LR + OCl^- \rightarrow products \quad k_8 \qquad (8)$$

$$[Cl_2]_t = [HOCl] + [OCl^-] \qquad (9)$$

In reaction 5, pK_a = 7.5 (Snoeyink & Jenkins 1980). [Cl$_2$]$_t$ is the total concentration of oxidants in solution. The total degradation of MC-LR can be expressed as follows according to Equations (6)–(8):

$$-\frac{d[MC\text{-}LR]}{dt} = (k_6[HOCl][H^+] + k_7[HOCl] + k_8[OCl^-])$$
$$\times [MC - LR] \qquad (10)$$

Combining Equations (5), (9) and (10), it is expressed by Equation (11) (see also Acero *et al.* 2005).

$$-\frac{d[MC\text{-}LR]}{dt} = \left[k_6\left(\frac{[H^+]^2}{[H^+]+K_a}\right) + k_7\left(\frac{[H^+]}{[H^+]+K_a}\right) \right.$$
$$\left. + k_8\left(\frac{K_a}{[H^+]+K_a}\right) \right][MC\text{-}LR][Cl_2]_t \qquad (11)$$

So, by comparing Equations (1) and (11), the second-order rate constant k at each pH is:

$$k = \frac{k_6[H^+]^2 + k_7[H^+] + k_8K_a}{(K_a + [H^+])} \qquad (12)$$

Now the rate constants of k_6, k_7, k_8 in the first stage of reactions can be calculated by nonlinear least-squares regression of the experimental data of k (or k_1, Table 1) versus pH using Equation (12). The calculated values for k_6, k_7 and k_8 were 8.89×10^7, 53.79 and 4.05, respectively with a regression coefficient (r) > 0.999. These values are comparable with those obtained by Acero *et al.* (2005). In Figure 4, the solid line denotes the second-order reaction constant k calculated using Equation (12) based on the values of k_6, k_7, k_8. The agreement between the experimental data (symbols) and the calculated values further
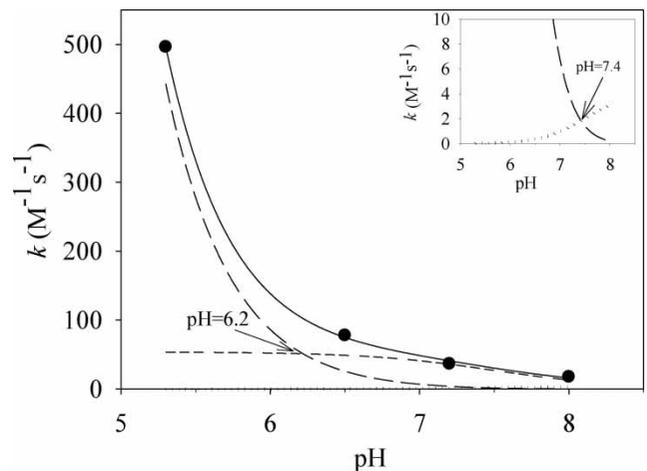
Figure 4 | The second-order rate constant k in the first stage of reaction versus pH: (1) ● calculated from the experimental results shown in Figure 3; (2) solid line: values calculated using Equation (12); (3) dashed and dotted lines: contributions of Equations (6)–(8) to the total second reaction rate k respectively.

validated the previously proposed reaction equations listed above. As observed by Acero *et al.* (2005), reaction rate constant, k, decreases with increase of pH in the pH range studied.

In addition, contributions of each of the three different reaction mechanisms represented by Equations (6)–(8) to the total k as a function of pH were also calculated and illustrated in Figure 4. Similarly, the contributions at several selected experimental pHs were calculated and are shown in Table 3. From Figure 4 and Table 3, at pH 5.3 the reaction is predominantly dependent on the acid-catalyzed HClO reactions; while at neutral pH (=7.2), the acid-catalyzed effect become small (approximately 9%) and the HClO oxidation become the major reaction mechanism. At pH 8.0, the oxidant HClO still dominates the reaction mechanisms, although the contribution of OCl⁻ becomes quite noticeable (approximately 19%). It should be noticed that at pH 8.0, although concentration of hypochlorite ion (OCl⁻) in solution is very much greater than hypochlorous acid (HOCl) (Snoeyink & Jenkins 1980), its contribution is relatively small because its reaction constant k_8 is very small compared with k_7. HOCl⁻ is a stronger oxidant than its deprotonated form OCl⁻ (Letterman 1999; Acero *et al.* 2005). Besides, it is argued that the low reaction rate at high pH may also be due to the reduction of the concentration of more reactive deprotonated species of MC-LR under the solution condition (Ho *et al.* 2006). Note that calculations using the second-order reaction constants k in the second (or slow) reaction stage (k_2, Table 1) indicated that at the low concentrations the reaction rate constants did not follow the proposed reaction mechanisms, likely for the reasons mentioned before.

As NOM such as HA exists ubiquitously in surface water, the presence of HA competes with other reactants

in water for chlorine, which affects the kinetics of oxidation of MC-LR by chlorine (Kull *et al.* 2006). To examine such influences, experiments were conducted at different HA concentrations (1, 2.5 and 5 mg L⁻¹) at a chlorine dose of 2.5 mg L⁻¹ and pH 7.2. The chlorine depletion versus the reaction time is shown in Figure 5(a). Note that due to the large variation of chlorine concentration in solution during the reaction duration pseudo-first-order kinetics did not hold. However, second-order reaction kinetics still hold and calculations were carried out accordingly (Equation (4)). The log plot of MC-LR concentration removal versus the exposure (CT) is presented in Figure 5(b), with the corresponding rate constants (k) shown in Table 4. It can be observed from the results that, two-stage second-order
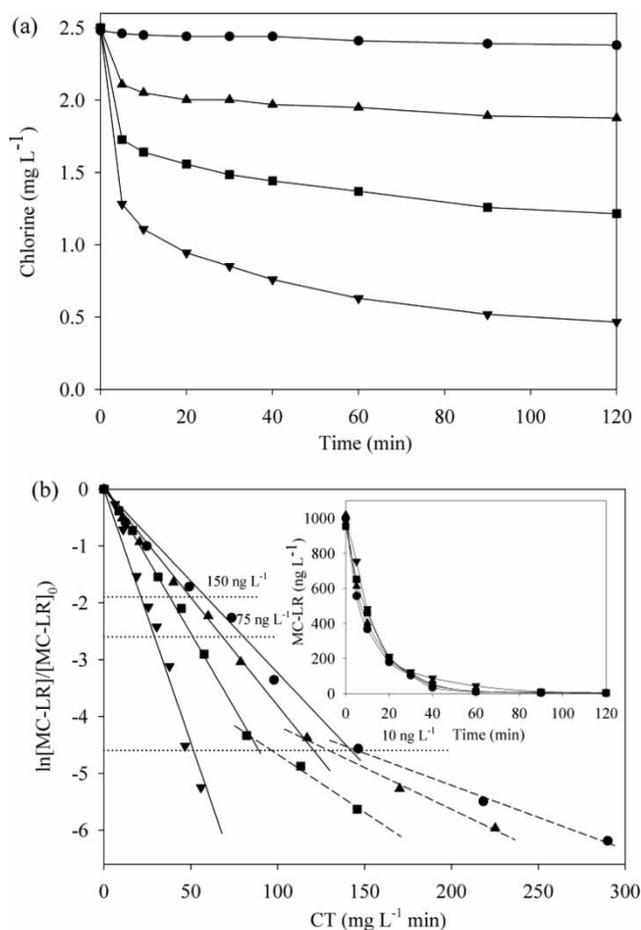
Figure 5 | (a) Influence of HA on chlorine depletion in solutions as a function of time; (b) Influence of HA on second-order reaction kinetics of MC-LR versus CT (Inset: the same data, presented as MC-LR concentration versus time). HA concentrations added: (●) 0 mg L⁻¹; (▲) 1.0 mg L⁻¹; (■) 2.5 mg L⁻¹; (▼) 5.0 mg L⁻¹.

Table 3 | Percentage of contributions calculated by Equations (6)–(8) to the total second-order reaction rate constants, k, at different pH values

pH	% of contributions to k		
	By Equation (6) (%)	By Equation (7) (%)	By Equation (8) (%)
5.3	89.22	10.77	0.01
6.5	34.04	65.46	0.50
7.2	8.96	87.67	3.37
8.0	1.32	79.71	18.97

Table 4 | The second-order rate constant (fast k_1 and slow k_2) of chlorination of MC-LR (chlorine: 35.2 μM; MC-LR: 1 nM) in presence of humic acid

Humic acid (mg L^{-1})	k_1 (M^{-1}s^{-1})	Conc. range ng L^{-1}	r_1	k_2 (M^{-1}s^{-1})	Conc. range ng L^{-1}	r_2
0	36.5	1,000–10.5	0.993	13.4	10.5–2.1	0.996
1.0	43.5	1,000–12.7	0.998	17.4	12.7–2.5	0.994
2.5	62.2	1,000–12.8	0.997	24.1	12.8–3.7	0.998
5.0	114.9	1,000–5.4	0.992	–	–	–

reaction kinetics applied. That is, when the oxidant went down to a certain concentration (e.g. 12.7 ng L^{-1} for HA = 1.0 mg L^{-1}) and below, the reaction rate constant decreased. For example, for HA = 1.0 mg L^{-1} the ratio of the fast rate constants (k_1) to the slow rate constants (k_2) is 2.5. Interestingly the second-order reaction rate constant (especially in the fast stage) is greater at a higher HA concentration. The rate constant (k) for pure water conditions is much smaller than that in the presence of HA (Figure 5, Table 4). Xagoraraki *et al.* (2006) reported that MC-LR removal (C/C_0) was faster in natural waters than in pure waters. Ho *et al.* (2006) also observed this phenomenon and proposed that the enhanced reaction rate with presence of higher HA is due to certain NOM-microcystin interactions or the formation of highly reactive quaternary chloramines on NOM moieties. Certainly, from the plot of MC-LR concentration versus reaction time (inset, Figure 5 (b)), the chlorine depletion at higher HA in solution did not significantly compromise its removal of MC-LR with respect to the reaction time. Certain interactions between MC-LR and HA must occur to compensate for the negative influence of chlorine reduction in solutions. However, such interactions do not occur in the oxidation of MC-LR with the presence of HA when permanganate is applied (see later).

In addition, the results indicate that at chlorine 2.5 mg L^{-1} and in the presence of HA, removal of MC-LR to the magnitude of ng L^{-1} can still be achieved, though the CT is different at different concentrations of HA (see Table 2). The MC-LR concentration (ng L^{-1}) in solution can be expressed as a linear function of HA concentration (mg L^{-1}) with a high correlation ($r = 0.998$) as observed previously (Kull *et al.* 2006):

$$[\text{MC-LR}] = 0.62[\text{humic acid}] + 1.99 \qquad (13)$$

Finally, the kinetics of the two-stage reaction regime may be expressed as:

$$\ln\frac{[\text{MC-LR}]}{[\text{MC-LR}]_0} = -k_1 Ct$$
$$[\text{MC-LR}]_0 \geq [\text{MC-LR}] > [\text{MC-LR}]_1$$
$$\ln\frac{[\text{MC-LR}]}{[\text{MC-LR}]_0} = y_0 - k_2 Ct$$
$$[\text{MC-LR}]_2 < [\text{MC-LR}] \leq [\text{MC-LR}]_1 \qquad (14)$$

here k_1 is the second-order rate constant in the higher MC-LR concentration range, while k_2 is the rate constant in the lower range (i.e. 2–10 ng L^{-1}).

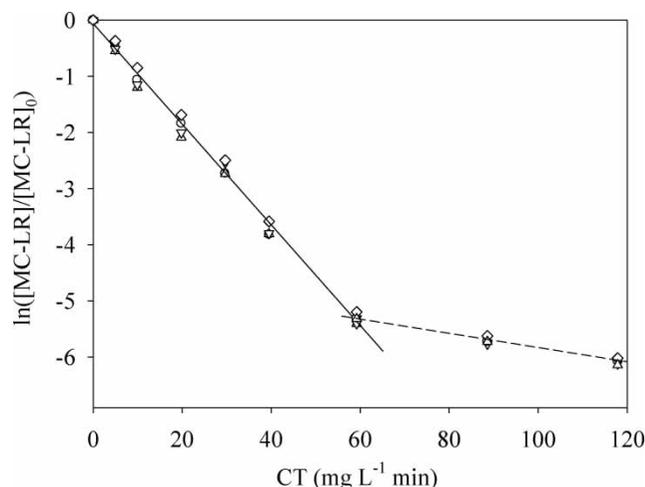## The removal of MC-LR at low concentrations by permanganate

Figure 6 demonstrates the results of oxidation of MC-LR at an initial concentration of 1 μg L^{-1} by permanganate concentrations of 0.5, 1.0 and 1.5 mg L^{-1} (or 3.17, 6.33 and 9.49 μM), under pure water solution conditions (pH = 7.2).

As in the chlorination of MC-LR, the reaction exhibited two kinetic stages, a fast followed by a slow process within the whole concentration range from 1 μg L^{-1} down to a few ng L^{-1}. The reaction also obeys second-order kinetics with respect to the two reactants (Figure 6), and pseudo-first-order kinetics with respect to MC-LR under pure water solution conditions ($r > 0.99$) (inset in Figure 6). For example, at a permanganate dose of 1.0 mg L^{-1}, the ratio of the fast rate constant (k_1) at higher MC-LR concentrations (6–1,000 ng L^{-1}) to the slow rate constant (k_2) at low MC-LR concentrations (2.2–6 ng L^{-1}) is 6.6 (Table 5). The final MC-LR concentrations under the conditions are shown in Table 2. The lowest concentration of MC-LR achieved is approximately 1.2 ng L^{-1} at a permanganate dose of
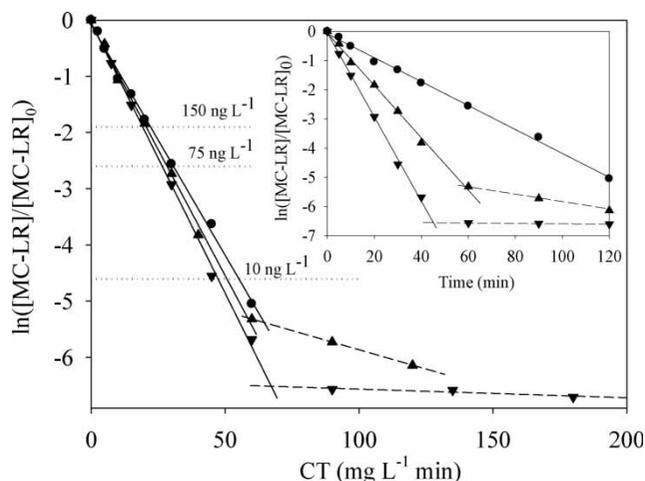
Figure 6 | Second-order kinetic plot for MnO_4^- oxidation of MC-LR at initial concentration 1 μg L^{-1} (nM), at different doses of the oxidant (pH 7.2). The solid and dotted lines are linear least-squares regressions of the data for the two concentration reaction regimes. Permanganate dose: (•) 0.5 mg L^{-1} (3.17 μM), (▲) 1.0 mg L^{-1} (6.33 μM), (▼) 1.5 mg L^{-1} (9.49 μM).

Figure 7 | Influence of pH on permanganate oxidation of MC-LR at a dose of 1.0 mg L^{-1} KMnO$_4$: (○) pH 5.3; (▲) pH 6.5; (▼) pH 7.2; (◇) pH 8.0.

1.5 mg L^{-1}. The magnitude of the fast reaction constants is comparable with those observed previously (Rodríguez et al. 2007; Acero et al. 2008). The difference in the fast reaction constants between this and previous studies may be caused by the low MC-LR and the larger ratio of [KMnO$_4$]$_0$/[MC-LR]$_0$ used in this study. In pure water conditions, a comparison of MC-LR oxidation kinetics between the two oxidants reveals that, the initial permanganate concentrations produced a much smaller influence on MC-LR removal as function of the CT (Figure 6) than the initial chlorine concentration did (Figure 2). That is, in permanganate oxidation of MC-LR, a CT value indicates a level of MC-LR concentration removal regardless of the initial concentrations used, whereas in the case of chlorination a CT value but with different initial chlorine concentration

indicates very different MC-LR removal. It is proposed that permanganate attacks on the conjugate double bond of the Adda group of the MC-LR molecule and chlorine does the same in the oxidation process (Ho et al. 2006; Rodríguez et al. 2007).

The effect of solution pH on oxidation by permangante of MC-LR at low concentrations was examined at pH 5.3, 6.5, 7.2 and 8.0. The results are presented in Figure 7. They indicate that unlike the case of chlorination, pH did not affect the reaction kinetics of MC-LR by permanganate. This observation agrees with a previous study under similar solution conditions (Rodríguez et al. 2007). This is due to the fact that solution pH does not affect the speciation of permanganate in water as it does in case of chlorine (as mentioned earlier). One practical indication is that when permanganate is applied in water treatment, no pH

Table 5 | The second-order rate constants (fast k_1 and slow k_2) of permanganate oxidation of MC-LR at different pH

pH	[MC-LR]$_0$ (nM)	[KmnO4]$_0$ (μM)	k_1 (M^{-1}s^{-1})	Conc. range (ng L^{-1})	k_2 (M^{-1}s^{-1})	Conc. range (ng L^{-1})
7.2	1	3.17	215.9	1,000−6.4	–	–
7.2	1	6.33	236	1,000−5.1	36	5.1−2.2
7.2	1	9.49	253	1,000−3.6	77.7	3.6−1.5
5.3	1	6.33	238.3	1,000−4.9	33.4	4.9−2.1
6.5	1	6.33	237.3	1,000−5.1	32.1	5.1−2.2
8.0	1	6.33	234.1	1,000−5.3	36.9	5.3−2.3
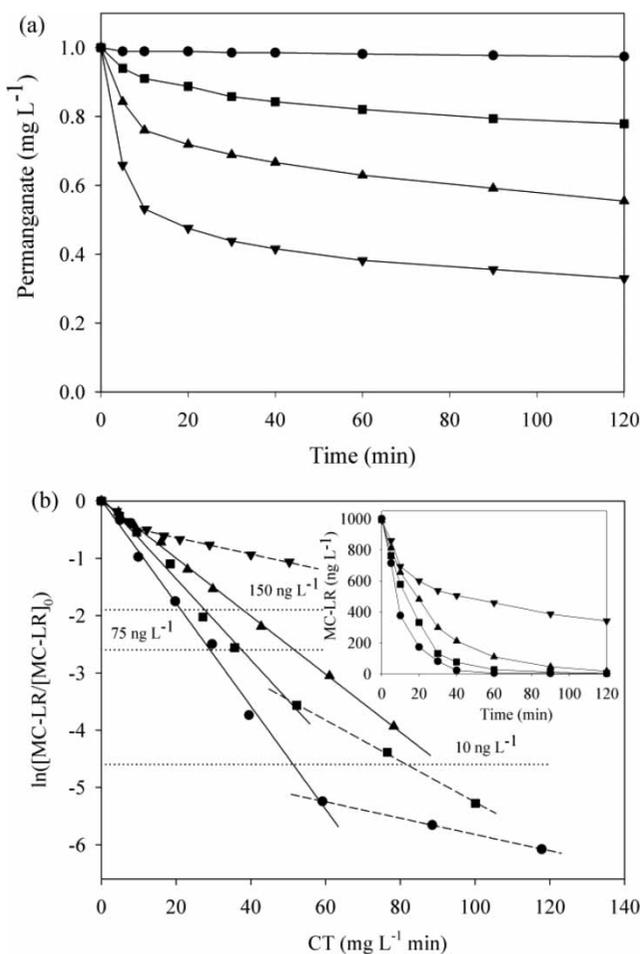
Figure 8 | Oxidation of MC-LR by MnO_4^{-1} in presence of HA (permanganate dose 1.0 mg L^{-1}, pH 7.2). (a) Influence of HA on depletion of permanganate concentration with time; (b) Influence of HA on second-order reaction kinetics of MC-LR versus CT (Inset: same data, presented as MC-LR concentration versus time). Initial HA concentrations: (•) 0 mg L^{-1}; (■) 1.0 mg L^{-1}; (▲) 2.5 mg L^{-1}; (▼) 5.0 mg L^{-1}.

adjustment needs to be considered in relation to its reactivity with organics.

The influence of HA on reaction kinetics of MC-LR at low concentrations down to 5 ng L^{-1} by permanganate

(1 mg L^{-1}) was conducted at HA concentrations of 1, 2.5 and 5 mg L^{-1} at pH 7.2. The variation of MnO_4^- concentration versus reaction time is shown in Figure 8(a). The second-order kinetics of the MC-LR degradation is presented in Figure 8(b). The values of the second-order rate constants (k) under the experimental conditions are summarized in Table 6. From the results, permanganate depletion rate was significantly greater in the early stage of reaction at the higher HA concentration. For example, at HA concentration 5 mg L^{-1}, within less than 10 min, MnO_4^- concentration dropped from 1 mg L^{-1} to approximately 0.5 mg L^{-1}, indicating that HA is a very competitive reactant for MnO_4^-. More importantly, the presence of HA greatly affected the second-order reaction constants Figure 8(b) and the final removal of MC-LR (Table 2). Interestingly, unlike the case of chlorine oxidation, as the HA concentration in solution increased, the second order reaction rate constant (k) decreased greatly. This may be a result of differences in reaction mechanisms between the two oxidants in such solutions. Consequently, the final removal of MC-LR via permanganate oxidation also decreased. At the HA concentration of 1 mg L^{-1}, the reaction rate constant was 186.4 $M^{-1} s^{-1}$ (Table 6) and MC-LR was degraded from 1,000 to 10 ng L^{-1} at a CT of 82 mg L^{-1} min (Table 2). However, at the HA concentration of 5 mg L^{-1}, the rate constant was reduced to 130.2 $M^{-1} s^{-1}$ (Table 6) with a final removal of MC-LR greater than 150 ng L^{-1} (Table 2). This indicated that permanganate is not a good choice for achieving a low removal limit of MC-LR when the HA concentration is high.

CONCLUSIONS

Degradation of MC-LR by chlorine and permanganate exhibits two kinetic stages from 1 μg L^{-1} down to a few ng L^{-1}.

Table 6 | The second-order rate constants (fast k1 and slow k2) of permanganate oxidation of MC-LR (permanganate: 6.33 μM; MC-LR: 1 nM) in presence of different humic acid concentrations

Humic acid (mg L^{-1})	k_1 ($M^{-1}s^{-1}$)	Scale (ng L^{-1})	r_1	k_2 ($M^{-1}s^{-1}$)	Scale (ng L^{-1})	r_2
0	236	1,000–5.4	0.997	36	5.4–2.3	0.999
1.0	186.4	1,000–31.1	0.997	94.2	31.1–5.1	0.999
2.5	133.3	1,000–19.4	0.999	–	–	–
5.0	130.2	1,000–637.6	0.992	37.7	637.6–343	0.992

In pure water second-order reaction kinetics hold with respect to MC-LR and each of the oxidants, and pseudo-first-order kinetics to MC-LR at a fixed dose of each of the two oxidants. In comparison, in the presence of HA in solution, only the overall second-order reaction kinetics hold. The concentration ranges for the transition from the fast reaction regime to the slow reaction regime in the lower concentration range vary according to the oxidants and the solution conditions. In the case of chlorine, pH greatly affected the kinetic concentration range with a very scattered transition range from 330 ng L^{-1} to a few ng L^{-1}, whereas the HA concentration has a relatively negligible effect on it with a low transition concentration of 10 ng L^{-1}. In contrast, for permanganate, pH has almost no influence on the reaction kinetics and the kinetics exhibits a transition point at around 5 ng L^{-1}; however, the HA produced a very great influence on the reaction kinetics and the transition concentration range was then from 650 to 5 ng L^{-1}. The existence of HA in solution increased the second-order reaction constants (especially in the fast reaction stage) of chlorine oxidation, whereas it greatly suppressed the reaction constants of permanganate oxidation of MC-LR, especially at a high HA concentration. The different influences caused by HA on the reaction kinetics reflect the different reaction mechanisms between the two oxidants and MC-LR in this reaction system. For the same reasons, the initial concentrations of the oxidants, pH and HA concentration affect the CT governance over the degree of removal of the MC-LR; thus under different conditions, different CT values may be required for a fixed MC-LR concentration removal. Therefore, the criteria of CT for MC-LR removal can only be validated under each specific water condition. Under the experimental conditions, the achievable final removal concentrations of MC-LR are about 10 ng L^{-1} for chlorine and 5 ng L^{-1} for permanganate in the fast stage, and 2 ng L^{-1} for chlorine and 1.5 ng L^{-1} for permanganate in the slow stage. As HA has a detrimental influence on permanganate oxidation of MC-LR, permanganate is a less effective agent for removing MC-LR from water. However, chlorine appeared to be effective for removal of MC-LR down to low concentration levels of ng L^{-1} even in the presence of HA. Final removal of MC-LR down to a concentration of 150 ng L^{-1} would require a CT value equivalent to 3-log inactivation of *Giardia* cysts (CT = 65 mg L^{-1} min).

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