Inactivation Kinetics of the Cyanobacterial Toxin Microcystin-LR by Free Chlorine

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Abstract: Worldwide, the increasing occurrence of toxins produced by cyanobacteria in water bodies used as source waters for drinking water has become an important public health issue. Microcystin-LR is one of the most commonly found cyanotoxins. A detailed evaluation of the free chlorine induced inactivation kinetics of extracellular microcystin-LR is presented in this study. Rate constants needed for chlorine inactivation of the toxin were derived from the data. The effects of varied pH, chlorine dose, toxin concentration, and temperature on the rate of inactivation were evaluated. Batch chlorination experiments were run using carbonate-buffered Milli-Q water at three different initial toxin concentrations (1, 2, and 8 $\mu g/L$), three different chlorine doses (1, 3, and 9 mg/L), and three different pH values (6.0, 7.5, and 9.0) at 11, 20 and 29°C. The study showed that extracellular microcystin-LR was inactivated by free chlorine and the inactivation rate was affected by pH. The highest inactivation rates were observed at pH 6.0 and the lowest at pH 9.0.

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Introduction

Cyanobacteria, or blue–green algae, have long been known by water utility professionals for their adverse impacts on water quality. Until recently, these impacts were considered to be primarily aesthetic, producing tastes and odors and interfering with certain water treatment processes. As more and more source waters are being impacted by the intrusion of nutrients from human activities, the occurrence of cyanobacteria blooms has been increasing worldwide. An emerging problem associated with cyanobacteria is the production of cyanotoxins, such as microcystin-LR.

Microcystin-LR has been identified as one of the toxins of highest priority. In the United States, this prioritization was based on four criteria: human health effects, occurrence, susceptibility to drinking water treatment, and toxin stability (USEPA 2001). In 1998, the World Health Organization stressed the potential public health importance of the occurrence of microcystin in drinking water and adopted a provisional guideline value for microcystin-LR of 1.0 $\mu g/L$ (WHO 1998). Drinking water guidelines of 1.3 and 1.5 $\mu g/L$ have been proposed in Australia and Canada, respectively (Health Canada 1998; NHMRC and ARMCANZ 2001).

There are several structural variants of microcystins generated from cyanobacteria, with microcystin-LR being the most commonly observed. Microcystins are absorbed into the bloodstream and transported to the liver where damage can occur. When ingested by mammals, acute high doses of microcystins can even lead to death due to extensive liver hemorrhaging and liver failure. The median lethal dose for microcystin-LR has been estimated to be 50 μg toxin/kg body weight based on intraperitoneal injection studies with mice (Rinehart et al. 1994). In addition, when chronic sublethal doses were administered to mice through drinking water, increased mortality and chronic active liver disease were observed (Falconer et al. 1988).

Carmichael (2001) has shown that microcystins were detected in 80% of 677 raw water sources in the United States and Canada. He also showed that only 4.3% of source water samples had concentrations larger than 1 $\mu g/L$. For those waters that exceeded the guideline level, microcystin-LR concentrations of the plant influents ranged from 2 to 17 $\mu g/L$. For treated drinking water, similar detection levels were reported in Wisconsin (Karner et al. 2001). Microcystins are extracellular toxins, cells need to lyse in order for the microcystin toxin to be released into water. For

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example, lysis of microcystis cells occurs with $CuSO_4$ addition, which is often used as an oxidant in the case of algal blooms. Release of microcystin from lysed whole microcystis cells could raise microcystin concentrations well over the amounts reported in cell-free water.

Since drinking water consumption is a potentially significant route of exposure to microcystins, a number of water treatment technologies have been evaluated for their effectiveness at removing microcystin from drinking water supplies. Chlorination is one of these technologies. Chlorination is used extensively by water utilities in the United States (AWWA 2000) and therefore it can serve as an easily accessible means of microcystin inactivation.

Microcystin-LR chlorination studies have been performed by several researchers. Nicholson et al. (1994) showed that a chlorine residual of at least 0.5 mg/L was required after a contact time of 30 min for complete microcystin destruction, as long as pH was maintained below pH 8. The researchers evaluated microcystin concentrations ranging from 130 to 300 μg/L, and chlorine doses ranging from 0 to 30 mg/L. The molar ratio of chlorine to microcystin ranged from 50 to approximately 3,500. Tsuji et al. (1997) demonstrated poorer removals than these observed by Nicholson et al. (1994). The researchers evaluated initial microcystin concentrations of 10 mg/L and applied chlorine doses ranging from 0.7 to 2.8 mg/L (chlorine to microcystin molar ratios of 1 to 4), at pH values less than pH 7.2. They identified that their chlorination by-products had low toxicity and that the chlorine attacked the Adda group of the microcystin molecule. Hart et al. (1998) tested various contact times using an initial toxin concentration of 6.9 μg/L and applied a chlorine dose of 1.7 mg/L (chlorine to microcystin molar ratio of 3,500). They observed microcystin-LR removal of greater than 93% at pH 5 and contact time of 30 min. At pH 9, the reduction in toxin concentration was significantly less, even after a contact time of 22 h. Bruchet et al. (1998) observed greater than 80% removal of microcystin-LR when a 1 mg/L chlorine dose was added to water with an initial toxin concentration of 500 μg/L (chlorine to microcystin molar ratio of 29) for 2 h of contact time.

To help engineers design and operate chlorination systems for microcystin-LR inactivation, the objective of this project was to conduct a detailed evaluation of the inactivation kinetics of extracellular microcystin-LR by free chlorine. The effects of pH, chlorine dose, microcystin-LR concentration, and temperature on the rate of inactivation were evaluated. The free chlorine to toxin molar ratios in this study varied approximately from 900 to 190,000 and the free chlorine to toxin mass ratios varied between 60 and 13,000. These ratios are consistent with the chlorine doses used in practice and the microcystin concentrations observed in North American drinking water supplies (AWWA 2000; Carmichael 2001). For example, when free chlorine ranges from 1–3 mg/L (doses that are typically used, AWWA 2000) and the toxin ranges from 1–17 μg/L (Carmichael 2001) the molar ratios range between 800 and 40,000. For microcystin concentrations as low as 0.2 μg/L, the ratio becomes as high as 190,000.

Methods

Inactivation Experiments

A total of 33 chlorination experiments were performed in 1 L amber glass bottles. Twenty-seven of the experiments were performed in an incubator at 11±1°C. All experiments were performed with $NaHCO_3$-buffered Milli-Q water by adjusting the alkalinity of the Milli-Q water to 100 mg/L as $CaCO_3$. The experiments were run at three different initial toxin concentrations (1, 2, and 8 μg/L), three different free chlorine doses (1, 3, and 9 mg/L), and three different pH values (6.0, 7.5, and 9.0).

Each experiment was performed at five chlorination contact times to allow for the estimation of kinetic parameters. The chlorine-toxin contact time never exceeded 100 min. At the beginning of each experiment, an appropriate amount of microcystin-LR stock solution and an appropriate dose of NaOCl stock solution were stirred in a 1500 mL glass beaker. This solution was then immediately transferred to a 1 L amber glass bottle with no head space. The amber bottle was incubated at 11°C for the target contact time. Two 130 mL amber glass bottles were filled with the remaining solution, head-space-free. These were also incubated at 11°C.

When the target contact time was reached, the two 130 mL amber bottles were immediately used for chlorine residual and pH analysis. At the target contact time, ascorbic acid (1.3 g) was added to the 1 L amber bottle to stop the chlorine reaction, and the temperature of the solution was recorded. This quenched sample was used for the analysis of microcystin-LR concentration. The experiments produced a total number of 135 samples with 13 of the experimental conditions replicated. The RSD (defined as the standard deviation over the mean times 100) in microcystin-LR concentration between replicate samples was 10%.

Three batch chlorination experiments were performed at 20±1°C and three batch chlorination experiments were performed at 29±1°C. The procedure for the inactivation experiments run at 20 and 29°C were the same as above, except that they were incubated at the appropriate temperatures. The experiments were run at one initial toxin concentration (2 μg/L), one free chlorine dose (3 mg/L), and three different pH values (6.0, 7.5, and 9.0).

Microcystin-LR Determination

Microcystin-LR concentrations were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) as per manufacturer's directions (EnviroGard, Strategic Diagnostics Inc., Newark, Del., USA). Microcystin-LR was also quantified using high performance liquid chromatography (HPLC) (Agilent 1090, Wilmington, Del., coupled with photodiode array detection) on 19% of the samples. Analysis of microcystin-LR in water samples by HPLC was based on methods developed by Nicholson et al. (1994) and Moollan et al. (1996). Samples were analyzed with HPLC to provide additional confidence in the ELISA results. The results of the two methods were correlated with an $R^2=0.904$. There was no tendency observed for one of the methods (HPLC or ELISA) to read consistently higher or lower than the other.

pH, Free Chlorine, and Temperature

pH was measured with an Orion Model SA520 glass-bulb liquid-junction electrode. The pH meter was calibrated daily with pH 4, pH 7, and pH 10 standards according to standard methods (APHA 1996). Free chlorine concentration was quantified according to the DPD ferrous titrimetric method (Method 4500, APHA 1996). The temperature was measured with a digital thermometer.

Kinetic Analysis

A plausible rate law for the interaction of free chlorine and microcystin-LR is presented in Eq. (1). In Eq. (1), [free chlorine] is the concentration of free chlorine in milligrams per liter as Cl_2, [toxin] is the concentration of microcystin-LR in micrograms per liter, and $[H^+]$ is the concentration of protons in micromole per liter. The equation includes the rate constants for the reaction of hypochlorous acid with microcystin-LR (k_{HOCl}), and the reaction of hypochlorite with microcystin-LR (k_{OCl^-}). The equation also includes the equilibrium constant of the hypochlorous acid/hypochlorite system in μmol/L (K_a), and time (t)

$$\frac{d[\text{toxin}]}{dt} = -\left(\frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a}\right)[\text{free chlorine}]\,[\text{toxin}] \tag{1}$$

In experiments performed for this study, the molar free chlorine concentration exceeded the molar microcystin-LR concentration by 3 to 5 orders of magnitude. Because of this, the free chlorine concentration did not vary with time and Eq. (1) can be integrated to produce the pseudo-first-order rate law shown in Eq. (2). In Eq. (2), $[\text{toxin}]_0$ and $[\text{free chlorine}]_0$ are the initial toxin concentration and free chlorine dose, respectively. With Eq. (2), data from all 27 experiments at 11°C were simultaneously fit with nonlinear regression to obtain estimates for K_a, k_{HOCl}, and k_{OCl^-}

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp\left\{-\left(\frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a}\right)[\text{free chlorine}]_0\, t\right\} \tag{2}$$

Data from several experiments at one pH value and several chlorine doses cannot be used to estimate values for K_a, k_{HOCl}, or k_{OCl^-}. However, they can be fit with Eq. (3), where k is an apparent rate constant that is specific to the pH used in the experiment [see Eq. (4)]

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp\{-k[\text{free chlorine}]_0\, t\} \tag{3}$$

$$k = \frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a} \tag{4}$$

Data from a single experiment at one chlorine dose and one pH value can be fit with Eq. (5). The inactivation rate constant k' is an apparent rate constant that is specific to the pH and chlorine dose used in the experiment [see Eq. (6)]

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp\{-k' t\} \tag{5}$$

$$k' = k[\text{free chlorine}]_0 \tag{6}$$

In this study, Statistica 4.3 (StatSoft Inc., Tulsa, Okla.) was used to estimate values of k', k, K_a, k_{HOCl}, and k_{OCl^-} with nonlinear regression. Statistica 4.3 was also used to estimate the standard error and p value for each parameter estimate. Parameter estimates in this study are reported as the estimate ± the standard error.

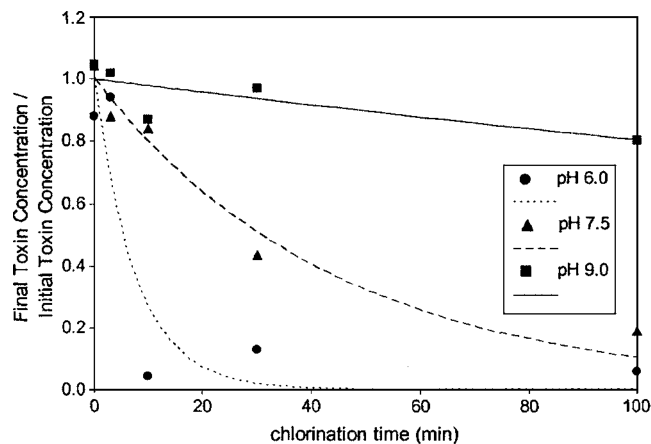

Fig. 1. Effect of pH and contact time on microcystin-LR inactivation for 1.1 μg/L initial toxin concentration and 1.1 mg/L free chlorine dose. Symbols show experimental data and lines show model analysis [using Eq. (5)].

Results and Discussion

Overall Results

Twenty-seven chlorine inactivation experiments using microcystin-LR were performed at five contact times each at 11°C with carbonate-buffered Milli-Q water. Each experiment produced a microcystin-LR inactivation curve and three of these curves are shown in Fig. 1 for pH 6.0, pH 7.5, and pH 9.0. The initial toxin concentration in the three experiments was 1.1 μg/L with a standard deviation of 0.4 μg/L, and the free chlorine dose was 1.1 mg/L with a standard deviation of 0.4 mg/L. The experimental data were fit with Eq. (5). The figure shows that the inactivation rate of microcystin-LR was highest at pH 6.0 and lowest at pH 9.0.

All 27 curves showed the same trend of increasing microcystin-LR inactivation rate with decreasing pH. Comparisons between the 27 inactivation curves revealed that the inactivation rate of microcystin-LR increased when chlorine dose increased. The trend was observed at all pH values, as was expected. Further comparisons indicated that the initial toxin concentration had no effect on inactivation rates under any condition.

For the 27 experiments fit with Eq. (5), the estimated values of k' ranged from 0.002 to 1.4 min^{-1}. The corresponding p values ranged from 0.0001 to 0.1622, indicating that the probability these inactivation rate constants were greater than zero was at least 84%. Table 1 shows the estimated k' values and their corresponding standard errors.

Eq. (2) was used to fit the data from all 27 experiments and obtain estimates for k_{HOCl}, k_{OCl^-} and K_a. The estimates for the toxin inactivation rate constants k_{HOCl} and k_{OCl^-} were 0.077 ± 0.010 L/(mg min) and 0.0036 ± 0.0005 L/(mg min), respectively. Therefore, microcystin-LR was estimated to react with HOCl more than 20 times faster than with OCl^-. The chlorine dissociation equilibrium constant, K_a, was estimated to be 0.176 ± 0.044 μmol/L ($pK_a = 6.8 \pm 0.1$). The estimated pK_a value is lower than the published value of 7.7 for pK_a at 11.2°C (Morris 1966). This difference may be attributed to the fact that only three pH values were tested in our research. A refined estimate of K_a could be obtained by testing more pH values between 6 and 9.

Three chlorine inactivation experiments using microcystin-LR were performed at five contact times at 20°C and three at 29°C

Table 1. Inactivation Rate Constants for 27 Experiments at 11°C with Carbonate-Buffered Milli-Q Water

Initial toxin concentration (ng/L)	Average free chlorine concentration (mg/L)	Average pH	Inactivation rate constant (k_1') (min^{-1})	Standard error
758	2.7	6.0	0.24	0.03
885	0.9	6.0	0.13	0.07
941	8.1	6.1	0.50	0.14
1,644	1.0	6.0	0.03	0.00
1,758	3.5	6.0	0.25	0.02
2,115	9.2	6.0	0.51	0.11
5,827	9.0	6.0	1.42	0.13
11,072	2.9	6.0	0.18	0.03
11,769	0.7	6.0	0.02	0.01
751	9.9	7.5	0.12	0.01
967	0.9	7.5	0.02	0.01
1,031	2.8	7.5	0.03	0.01
2,603	9.2	7.6	0.17	0.02
2,661	1.0	7.5	0.01	0.00
2,838	2.3	7.4	0.02	0.00
6,163	2.6	7.5	0.03	0.01
7,257	0.8	7.5	0.02	0.01
7,924	8.7	7.5	0.10	0.00
1,093	2.4	9.0	0.01	0.00
1,342	10.2	9.0	0.03	0.01
1,576	1.4	9.0	0.00	0.00
1,834	0.9	9.0	0.01	0.00
1,869	9.5	9.0	0.02	0.01
2,859	2.5	9.0	0.01	0.01
6,597	2.6	9.0	0.02	0.01
6,821	1.0	9.0	0.01	0.00
12,011	8.6	9.0	0.03	0.01

with carbonate-buffered Milli-Q water. Each of the six experiments produced a microcystin-LR inactivation curve. The curves were compared with the corresponding curves at 11°C. The comparison revealed that the microcystin inactivation rate increased with increasing temperature, as expected. Table 2 shows the estimated k' values and their corresponding standard errors. The estimated values of k' ranged from 0.01 to 0.62 min^{-1}. The corresponding p values ranged from 0.002 to 0.087, indicating that the probability these inactivation rate constants were greater than zero was at least 91%.

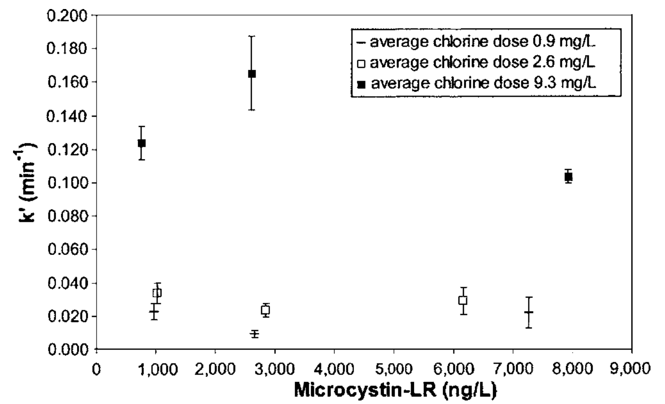

Fig. 2. Relationship between inactivation rate constant and initial toxin concentration at pH 7.5

Factors Affecting the Inactivation Rate Constant

Influence of Toxin Concentration

The relationship between inactivation rate constant k' and initial microcystin-LR concentration at pH 7.5 is shown in Fig. 2. The inactivation rate constants were estimated with Eq. (5). The figure presents the inactivation rate constants versus initial toxin concentration, for three chlorine doses. For each chlorine dose, the inactivation rate constants did not exhibit any increasing or decreasing trend with increasing initial toxin concentrations. The differences between inactivation rate constant at different initial toxin concentrations and the same chlorine dose were not statistically significant. The figure shows that the initial toxin concentration had no effect on k'. Similar results were obtained for other pH values. The results suggest that the assumption of a pseudo-first-order model was reasonable.

Influence of Chlorine Dose

The relationship between inactivation rate constant k' and chlorine dose at pH 7.5 is shown in Fig. 3. The inactivation rate constants were estimated with Eq. (5) and the fitted line in Fig. 3 was estimated with Eq. (6). The figure shows that the inactivation rate constant k' increased linearly with increasing chlorine dose at pH 7.5. This trend was consistent with expectations since an increase in chlorine concentration increases the likelihood of chlorine and toxin interaction. The linearity of the trend also suggests that the pseudo-first-order modeling approach was appropriate for describing microcystin-LR inactivation by free chlorine under the conditions tested. The same was observed at pH 6.0. However, when pH was 9.0, the inactivation rate constant did not change

Table 2. Inactivation Rate Constants for 27 Experiments at 20 and 29°C with Carbonate-Buffered Milli-Q Water

Initial toxin concentration (ng/L)	Average free chlorine concentration (mg/L)	Average pH	Inactivation rate constant (k_1') (min^{-1})	Standard error	Average temperature (°C)
1,831	2.8	6.0	0.25	0.02	21
2,038	3.1	6.0	0.62	0.09	29
1,867	2.9	7.5	0.05	0.01	20
1,634	2.7	7.5	0.08	0.02	29
1,615	3.0	9.0	0.01	0.00	20
2,354	2.9	9.1	0.02	0.01	29

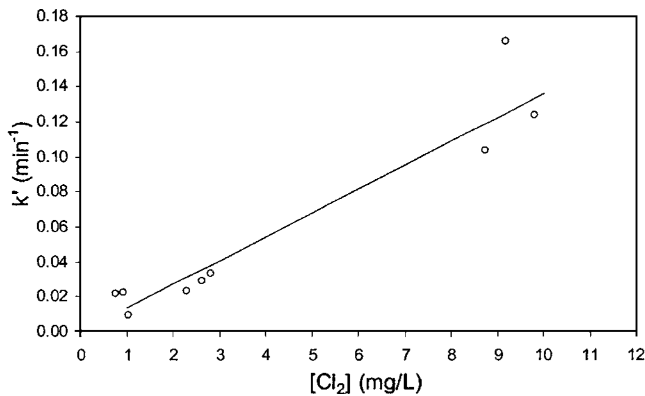

Fig. 3. Relationship between inactivation rate constant and chlorine dose at pH 7.5. Symbols show estimated inactivation rate constants and line shows linear regression.

significantly with increasing chlorine dose. The influence of chlorine dose on microcystin inactivation was also demonstrated by Nicholson et al. (1994) and Tsuji et al. (1997).

Influence of pH

The relationship between microcystin-LR inactivation rate constant k and pH at all chlorine doses tested is shown in Fig. 4. The figure shows the microcystin-LR inactivation rate constant k for experimental data at the three different pH values tested in this study. The inactivation rate constants k for each pH were estimated with Eq. (3). The fitted line was estimated with Eq. (4), using all data at all pH values. The figure shows that pH affected the microcystin-LR inactivation rate constant k. The highest inactivation rates were observed at pH 6.0 and the lowest at pH 9.0. In general, as pH increased to pH 9.0, the inactivation rates decreased and less degradation of microcystin-LR occurred.

The values of k in Eq. (4) were estimated to be 0.065 ± 0.007 L/(mg min), 0.014 ± 0.001 L/(mg min), and 0.005 ± 0.001 L/(mg min) for pH 6.0, pH 7.5, and pH 9.0, respectively. With this analysis, we can definitely conclude that the inactivation of microcystin-LR by free chlorine increased with decreasing pH. The reason for this observation may be explained by the fact that when pH decreases, the chlorine dissociation equilibrium shifts toward hypochlorous acid (White 1986). It appears

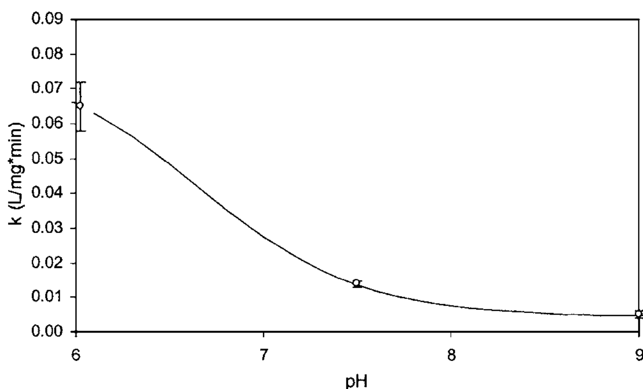

Fig. 4. Relationship between inactivation rate constant and pH at 11°C and all chlorine doses tested (bars indicate standard errors). Symbols show inactivation rate constants for each pH [Eq. (3)] and line shows model analysis [using Eq. (4)].

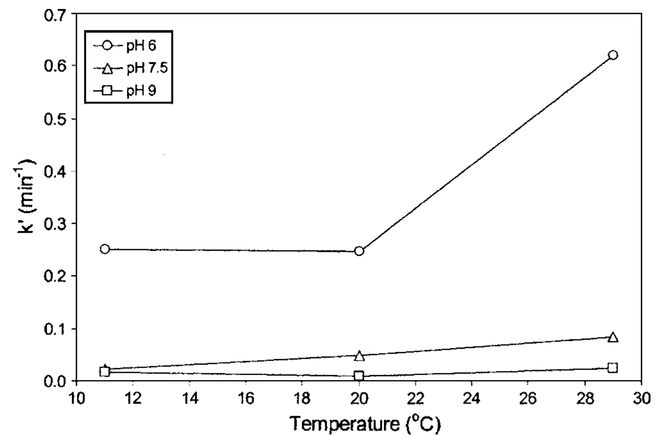

Fig. 5. Relationship between inactivation rate constant and temperature

from the data that hypochlorous acid is more effective than hypochlorite in inactivating microcystin-LR. These results are consistent with trends published elsewhere. The influence of pH on microcystin inactivation by free chlorine was also qualitatively demonstrated by Nicholson et al. (1994) and Hart et al. (1998).

Influence of Temperature

The onset of favorable cyanobacteria growth conditions is indicated by a rise in surface water temperature above 15°C, with maximum growth rates attained by most cyanobacteria at temperatures above 25°C (Reynolds 1984; Robarts and Zohary 1987; Yoo et al. 1995). Algal toxins in natural systems are not expected to be at significant concentrations when temperatures drop to 10–12°C. Because chemical reaction rates increase with increasing temperature, the results presented above for 11°C are expected to be conservative results and represent the lowest expected toxin degradation rates. To check this expectation and to quantitatively analyze the effect of temperature on the interaction between chlorine and microcystin-LR, six batch chlorination experiments were performed in 1 L amber glass bottles at 20 and 29°C.

The results of these experiments were compared with the results of three chlorination experiments conducted at 11°C at similar conditions. These were the experiments conducted with a toxin concentration of 1.7 μg/L and a chlorine dose of 3.5 mg/L at pH 6.0, a toxin concentration of 2.8 μg/L and a chlorine dose of 2.3 mg/L at pH 7.4, and a toxin concentration of 2.9 μg/L and a chlorine dose of 2.5 at pH 9.0. The comparison is shown in Fig. 5. The figure shows that microcystin-LR inactivation rates increased as temperature increased.

Conclusions

Our study found that extracellular microcystin-LR was inactivated by free chlorine. The rate of inactivation was affected by pH. The highest inactivation rates were observed at pH 6.0 and the lowest at pH 9.0. In addition, the rate of inactivation increased linearly with increasing chlorine dose and increasing temperature. In general, as pH increased to pH 9.0 and free chlorine concentration decreased to 1.0 mg/L, the inactivation rates decreased and less degradation of microcystin-LR occurred. Inactivation rate constants are developed for all conditions tested and the results of this study are quantitative. The inactivation rates did not

depend on initial toxin concentrations. In this study, microcystin-LR was estimated to react with HOCl more than 20 times faster than with OCl^-.

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