J. of the Environmental Sciences
Vol. 11, No. 11, 1157~1163(2002. 11)

Effect of Chlorination on Removal of Cyanobacterial Microcystins

Jong-Mun Jung[1,2*], Hong-Ki Park[2], You-Jung Lee[1,2], Eun-Young Jung[2], Ki-Won Kwon[2], Pan-Se Shin[2], and Gea-Jae Joo[1]

[1]*Dept. of Biology, Pusan National University, Busan, S. Korea, 609-735*
[2]*Busan Water Quality Institute, Busan Waterworks HQ, Kyoungnam, S. Korea, 621-813*

(Manuscript received 16 October, 2002 ; accepted 2 November, 2002)

The effective removal of microcystins by chlorination was investigated on a laboratory scale. With an initial chl.*a* concentration of more than 1,000 $\mu g/\ell$, the required chlorine dose for the effective removal of microcystins from the raw water was more than 8.0 mg/ ℓ. Whereas, a chlorine dose of 3.0 mg/ ℓ could effectively remove microcystins from raw water containing a chl.*a* concentration of less than 1,000 $\mu g/\ell$. The microcystin removal was more effective below pH 8.0, plus the optimum pH range was unrelated to the concentration of toxic algal material. Although chlorination is one of the most effective methods for reducing the toxin from blue-green algae, it causes cell lysis and toxin release. However, it was demonstrated that the released cell lysates and toxins could be effectively removed by a higher dose of the oxidant. The highest removal efficiency of dissolved microcystins(initial concentration: 280 $\mu g\ L^{-1}$) was with a chlorine dose of 5.0 mg/ ℓ.

Key words : cyanobacteria, *Microcystis aeruginosa*, microcystin-RR, microcystin-LR, chlorine

1. Introduction

Increased nutrient levels or eutrophication of freshwater can cause massive cyanobacterial blooms, and the bloom of blue-green algae can give rise to the production of toxins that may contaminate freshwater as a source for drinking water. Recently, there have been several reports of toxic algal blooms, and in some cases the ingestion of the algal scum has caused animal deaths[1,3,4]. Various genera of cyanobacteria are known to produce three types of intracellular toxins : cyclic hepatotoxins (microcystin, nodularin ; *Microcystis, Oscillatoria, Nostoc, Nodularia*), alkaloid neurotoxins(anatoxin, saxitoxin, neosaxitoxin ; *Anabaena, Aphanizomenon, Lyngbya, Oscillatoria, Trichodesmium*), and alkaloid cytotoxins(cylindrospermopsin ; *Cylindrospermopsis raciborskii, Umezakia natans*[1,4,7]. The most frequently occurring cyanobacterial toxin in freshwater is the hepatotoxin called microcystin. The microcystin produced by *Microcystis aeruginosa* has been identified as the cause of several poisonings of domestic animals and wildlife around the world, and also poses a health hazard for humans through the use of contaminated water for drinking and recreation.

The general structure of microcystins(microcystin-XZ) is cyclo(-D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha-) where X and Z are variable L-amino acids, D-MeAsp is D-erythro-β-methyl aspartic acid, and Mdha is *N*-methyldehydroalanine. Adda is a 20-carbon amino acid that is important for the toxicity of these compounds. Microcystins have been reported to inhibit protein phosphatase 1 and 2A, and act as a tumor promoter[3,12]. Therefore, if water supplies become contaminated with blue-green algae and water treatment leaves a displeasing odor, unpleasant taste, and DBPs(disinfection by-products), this results in consumer complaints[4]. Consequently,

Corresponding author ; Jong-Mun Jung, Busan Water Quality Institute, Busan Waterworks HQ, Kyoungnam, S. Korea, 621-813
Phone : +82-55-323-4718
E-mail : jmjung4356@empal.com

the presence of microcystins in drinking water sources has attracted increased attention[13].

As blooms of toxic cyanobacteria cannot always be prevented or controlled in water bodies, and the removal of intact algal cells including the toxins is not easy, new methods for removing toxins from water are of great importance[15]. Chlorination has been previously reported to have no effect on algal toxins[10,11], yet chlorine can be effective in destroying toxins when a sufficient dose is used[17]. With initial toxin concentrations of 130~300 μg/ℓ, aqueous chlorine and calcium hypochlorite can remove over 95 % of the toxins within 30 minutes when using a dose of 1.0 mg/ℓ or more, while sodium hypochlorite can only remove approximately 40 % when using a dose of 1.0 mg/ℓ, and only 70~80% when using a dose of 5.0 mg/ℓ or more[15]. In addition, chlorine can remove over 74 % of microcystins within 30 minutes when using a dose of 2.5 mg/ℓ [9]. In particular, the effect of chlorine on microcystins is dependent on the pH, which needs to be below pH 8.0 to ensure adequate toxin destruction[15]. After using copper sulfate to treat *Microcystis aeruginosa*, the concentration of microcystins measured in the water was still 990 μg/ℓ [18]. Thus, a pretreatment process is required for removing microcystins from drinking water.

Accordingly, the current study investigated the effect of chlorination on the removal of microcystins and dissolved toxins, plus the effect of pH on the removal of microcystins.

2. Materials and Methods

2.1 Cyanobacterial materials and experimental design

Blue-green algal(cyanobacteria) samples were collected on 21 June, 2000 from a freshwater bloom in the West-Nakdong River. Different concentrations of the cyanobacterial materials were then mixed with distilled water. Each sample was analyzed for its chl. *a* concentration. The samples used to analyze the chl. *a* concentrations were filtered through a glass microfiber filter(GF/C, Whatman, England) and the chlorophyll extracted by 10 mℓ acetone(90 %, v/v). The optical densities of the extracts at 664, 645, 630, and 750 nm were determined using a UV/Vis spectrophotometer (Cary-100, Varian, Australia). The chl. *a* concentrations were then determined based on the trichromatic method[20]. Using 500 mL erlenmayer flasks with a cap, the samples were initially chlorinated with doses of 2.0 mg/ℓ, 5.0 mg/ℓ, 8.0 mg/ℓ, 12 mg/ℓ, and 20 mg/ℓ, followed by a second dose of 1.0 mg/ℓ, 2.0 mg/ℓ, 3.0 mg/ℓ, 4.0 mg/ℓ, 5.0 mg/ℓ and 8.0 mg/ℓ, with subsequent stirring for 30 minutes. Another set of samples was chlorinated after adjusting the pH to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 with 0.1 M HCl and 0.1 M NaOH. The experiments all were repeated three times.

2.2 Sample preservation for removal of dissolved microcystins

The freeze-dried bloom materials(10 g) were mixed with distilled water, then the microcystins were dissolved in distilled water by sonication three times for 30 seconds, and stirring for one day. The concentration of microcystins measured after filtering through a GF/C was determined as the initial value. The filtrate was then chlorinated with doses of 2.0, 5.0, 8.0, 12.0, and 20.0 mg/ℓ followed by 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 mg/ℓ, respectively. The contact time was 30 minutes and the samples adjusted to pH 7.0. The experiments were all repeated three times.

2.3 Microcystin analysis

The suspensions were filtered through a GF/C filter(Whatman, 47mm Ø, England) to remove any cell debris, then the filters were washed with 5% acetic acid and the samples added to preconditioning ODS cartridges(Sep-Pak C_{18}, Waters, U.S.A.). The cartridges were washed with water and 10% methanol(v/v), then the toxins were eluted with 100 % methanol(HPLC grade, Merck, Germany). Next, the eluate was evaporated under reduced pressure and the residue dissolved in 1.0 mℓ of 20 % methanol[8]. Finally, the solution was filtered using a 0.2 μm membrane filter(Acrodisc Syringe Filters, PALL Gelman, U.S.A) (Fig. 1).

The HPLC(Waters 2690, Waters 996 Photodiode Array Detector, U.S.A.) was equipped with a constant-flow pump and variable-wavelength UV detector operated at 238 nm. The separation was performed on a Capcellpak C_{18}(4.6×150 mm, Ø

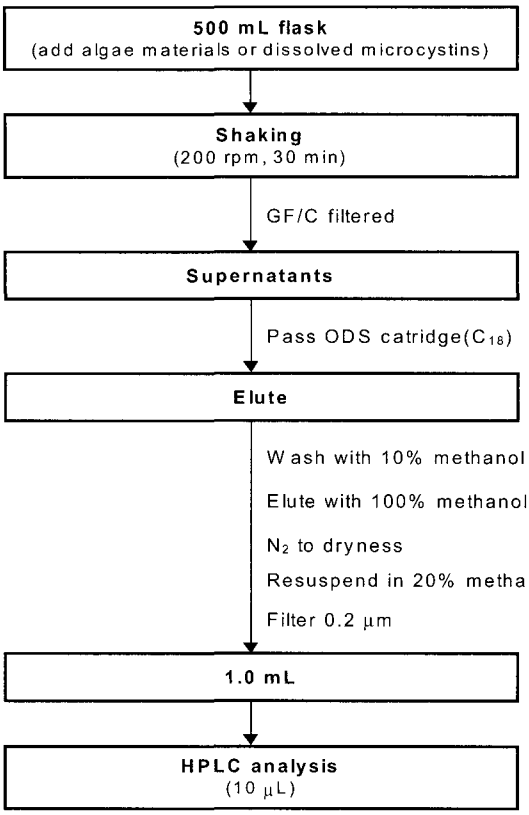

Fig. 1. Analysis procedure of microcystins in water samples.

5.0, Shiseido, Japan) reverse-phase column and the mobile phase was a methanol-0.05 mol/ ℓ phosphate buffer(58:42, pH 3.0) at a flow rate of 1.0 ml/min. The microcystins were identified based on their UV spectra and retention times, and by spiking the sample with a purified standard of microcystin-RR(M-1537, Sigma, U.S.A.) and microcystin-LR(M-2912, Sigma, U.S.A.). The detection limit for microcystins in the cell material was 0.1 μg/g. The microcystin concentrations were expressed in gravimetric terms(i.e. mg/toxin g dry weight), the currently accepted method for presenting information on the toxigenesis of hepatotoxic cyanobacteria[14].

3. Results and Discussion

3.1 Effect of chlorination on removal of microcystin content

The cyanobacterial microcystins were not completed removed by chlorination, yet only a low concentration of microcystins was released from the cyanobacteria cells. When chlorination is used to treat cyanobacteria and organic materials, THMs and HAAs are formed as disinfection by-products in the resulting drinking water[2,6,15,19]. In the current study, when the concentration of chl. *a* in the spiked water was 4,100 μg/ ℓ and the chlorine dose was 12.0 mg/ ℓ , the microcystin-RR and -LR was 28.7 μg/ ℓ and 48.3 μg/ ℓ , respectively, making a total microcystin concentration of 77.0 μg/ ℓ , which was more detectable. Yet, this decreased with a chlorine dose below 12.0 mg/ ℓ . When the concentration of chl. *a* was 1,800 μg/ ℓ and the chlorine dose was 8.0 mg/ ℓ , the microcystin-RR and -LR was 8.9 μg/ ℓ and 21.6 μg/ ℓ , respectively, which was also more detectable(Fig. 2).

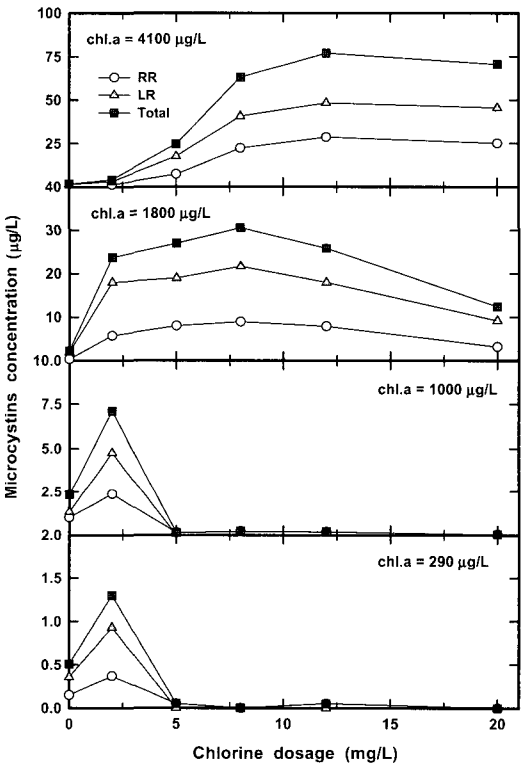

Fig. 2. Effect of chlorine dose on microcystin-RR and -LR.

When the biomass of cyanobacteria in the raw water increased, requiring a higher dose of chlorine, the residual microcystin concentration in the chlorine-treated water also increased. When the

blue-green algae were inserted into distilled water, the concentrations of chl. *a* were 1,000 μg/ℓ and the most effective removal was with a chlorine dose below 3.0 mg/ℓ. However, the appropriate chlorine dose changed according to the concentration of organic material in the raw water.

Figure 3 shows the microcystin concentrations resulting from 30 minutes contact between an initial chlorine dose of 1.0~8.0 mg/ℓ and chl. *a* concentrations of 400 μg/ℓ and 240 μg/ℓ When the concentration of chl. *a* was 400 μg/ℓ, the microcystin-RR and -LR increased to a maximum of 41 μg/ℓ with a chlorine dose of 2.0 mg/ℓ, yet when the chlorine dose was above 3 mg/ℓ, the microcystin-RR and -LR decreased. When the concentration of chl. *a* was 240 μg/ℓ, the microcystin-RR and -LR increased to a maximum of 9.5 μg/ℓ with a chlorine dose of 1.0 mg/ℓ, decreased with a chlorine dose above 2.0 mg/ℓ chlorine dose, and were undetected with a chlorine dose of 5.0 mg/ℓ.

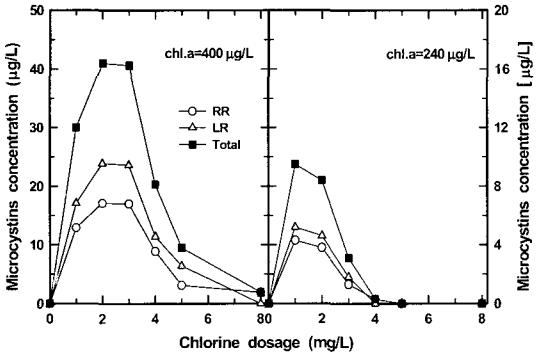

Fig. 3. Effect of chlorination dose on microcystin-RR and -LR.

The initial chlorine dose gradually decreased the microcystin concentration, indicating that the microcystins released with the low chlorine dose were oxidized. However, more chlorine was then required to remove the organic materials, except for the cyanobacteria, in the raw water. In South Korea, the main microcystins that have been detected are microcystin-RR, YR, and LR. In the current study, microcystin-RR and -LR were detected, and the microcystin-RR concentrations were two times higher than the -LR concentrations[16]. The removal of microcystin-RR was more effective than the removal of microcystin-LR, presumably because, among the two amino acids in the microcystin residues, the chlorination oxidized arginine more than leucine.

3.2 Effect of chlorination dose and pH on microcystins

The removal of microcystins by chlorination was affected by both the chlorine dose and the pH. The removal of microcystins was most efficient at pH 5.0. Although the removal was the same at pH 7.0~9.0, more contact time was required[5]. Plus, with the same contact time, the microcystin removal became less efficient above pH 8.0. This result also concurred with that of Nicholson *et al.*,(1994). When a chlorine dose of 1.5 mg/ℓ was mixed with raw water for 30 minutes and the range of pH adjusted between 3.0~10.0, the microcystin removal was most efficient below pH 8.0. The microcystin concentration decreased when the pH was below 8.0 with chl. *a* concentrations of 110 μg/ℓ and 420 μg/ℓ in the raw water, then sharply decreased when the pH was below 6.0 with a chl. *a* concentration of 420 μg/ℓ in the raw water (Table 1).

As shown in Fig. 4, the initial concentration of total dissolved microcystins was 280 μg/ℓ. Chlorination within a range of pH 3.0~10.0 was evaluated. The removal efficiency was the highest at pH 3.0~5.0. No microcystins were detected at pH 3.0, and the removal efficiency was 99.3 % and 62.0 % at pH 5.0 and 6.0, respectively. The residual concentration of microcystins was 210 μg/ℓ and decreased at pH 9.0 and 10.0. A similar result is shown in Table 1 with a chl. *a* concentration of 420 μg/ℓ in distilled water(Fig. 4). Hart *et al.*,(1997) also reported results of microcystin-LR removal experiments with an NaOCl dose of 1.7 μg/ℓ and contact time of 30 minutes. The initial microcystin-LR concentration was 6.9 μg/ℓ at pH 7.0 and the removal efficiency was 93 %, 6 %, and 19 % at pH 5.0, 7.0, and 9.0, respectively. Thus the highest degree of toxin removal was achieved below pH 8.0[10].

3.3 Effect of chlorination dose on removal of dissolved microcystins

The results of experiments with dissolved microcystins have demonstrated that chlorination

Table 1. Effect of chlorination dose and pH on microcystin-RR and -LR

chl. *a* conc. (μg/ ℓ)	pH	microcystins (μg/ ℓ)		
		-RR	-LR	Total
110	3	0.00	0.00	0.00
	4	0.00	0.00	0.00
	5	0.00	0.00	0.00
	6	0.00	0.00	0.00
	7	0.00	0.00	0.00
	8	0.11	0.00	0.11
	9	0.20	0.05	0.25
	10	0.35	1.40	1.75
230	3	0.00	0.00	0.00
	4	0.00	0.69	0.69
	5	0.00	0.21	1.21
	6	1.70	1.02	2.72
	7	0.49	2.00	2.49
	8	0.58	1.05	1.63
	9	2.06	3.94	6.00
	10	2.45	5.12	7.57
420	3	0.31	0.00	0.31
	4	1.91	0.00	1.91
	5	3.09	3.12	6.21
	6	10.61	7.54	18.15
	7	7.88	11.46	19.34
	8	10.65	17.32	27.97
	9	8.38	16.19	24.57
	10	3.11	11.78	14.89

※ contact time : 30 minutes, chlorine dose : 1.5 mg/ ℓ

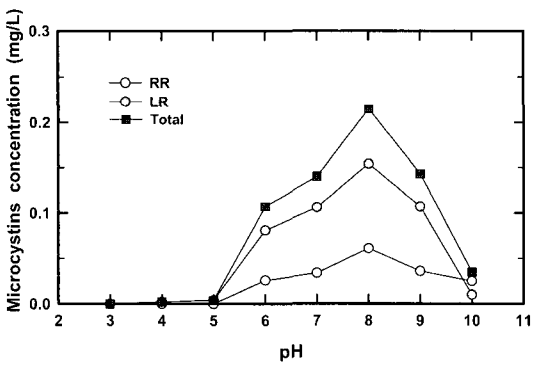

Fig. 4. Effect of chlorination dose(1.5 mg/ ℓ) and pH on dissolved microcystin-RR and microcystin-LR.

can be an effective means of removing toxins. However, if chlorination is applied to water containing toxic algal cells, it is possible that the oxidant can be consumed while reacting with the algal cells, thereby causing cell lysis and toxin release[10].

Two times more dissolved microcystin-RR was detected than microcystin-LR. With an initial total microcystin concentration of 5,050 μg/ ℓ, the removal efficiency was 8.8 %, 30.5 %, and 98 % with a chlorine dose of 2.0 mg/ ℓ, 8.0 mg/ ℓ, and 20.0 mg/ ℓ, respectively, (Fig. 5-[A]). With an initial total microcystin concentration of 670 μg/ ℓ, the removal efficiency was 10.6 %, 28.5 %, 85.1 %, and 100 % with a chlorine dose of 2.0 mg/ ℓ, 8.0 mg/ ℓ, 12.0 mg/ ℓ, and 20.0 mg/ ℓ, respectively(Fig. 5-[B]). With an initial total microcystin concentration of 279 μg/ ℓ, the removal efficiency was 23 %, 90 %, and 100 % with a chlorine dose of 5.0 mg/ ℓ, 8.0 mg/ ℓ, and 12.0 mg/ ℓ, respectively. Accordingly, the higher the microcystin concentration, the higher the chlorine dose required(Fig. 5-[C]).

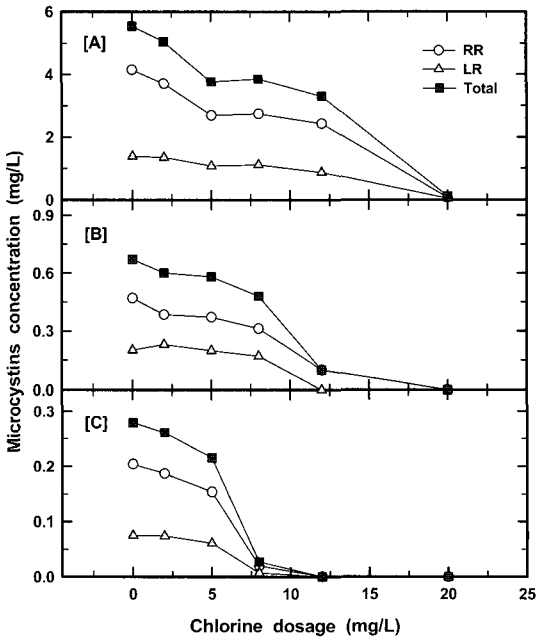

Fig. 5. Effect of chlorination dose on removal of dissolved microcystin-RR and microcystin-LR.
[A] : initial concentration of microcystin-RR (4.15 mg/ ℓ) and -LR(1.39 mg/ ℓ)
[B] : initial concentration of microcystin-RR (0.47 mg/ ℓ) and -LR(0.20 mg/ ℓ)
[C] : initial concentration of microcystin-RR (0.20 mg/ ℓ) and -LR(0.08 mg/ ℓ)

4. Conclusions

The removal of microcystins by chlorination was investigated in the laboratory. The effective removal of microcystins required a chlorine concentration of more than 8.0 mg/ℓ in raw water with a chl. *a* concentration of more than 1,000 μg/ℓ. For raw water with a chl. *a* concentration below 1,000 μg/ℓ, the removal efficiency was high with a chlorine concentration of 3.0 mg/ℓ. The microcystin removal was more effective below pH 8.0, and a lower pH removed more microcystins. When the initial concentration of microcystins was 5,540 μg/ℓ, the removal efficiency of dissolved microcystins by chlorination was the highest with a chlorine dose of 20.0 mg/ℓ. When the initial concentration of microcystins was 670 μg/ℓ, the highest removal efficiency was with a chlorine dose of 12.0 mg/ℓ. When the initial concentration of microcystins was 279 μg/ℓ, the highest removal efficiency was with a chlorine dose of 8.0 mg/ℓ. No microcystins were detected with a chlorine dose above 12.0 mg/ℓ. Accordingly, chlorination was found to be effective in reducing the toxicity of blue-green algae, yet caused cell lysis and toxin release. However, when the oxidant dose was high enough, the dissolved toxins could still be effectively removed.

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