Effect of Ozonation on the Removal of Cyanobacterial Toxins during Drinking Water Treatment

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Water treatment plants faced with toxic cyanobacteria have to be able to remove cyanotoxins from raw water. In this study we investigated the efficacy of ozonation coupled with various filtration steps under different cyanobacterial bloom conditions. Cyanobacteria were ozonated in a laboratory-scale batch reactor modeled on a system used by a modern waterworks, with subsequent activated carbon and sand filtration steps. The presence of cyanobacterial toxins (microcystins) was determined using the protein phosphatase inhibition assay. We found that ozone concentrations of at least 1.5 mg/L were required to provide enough oxidation potential to destroy the toxin present in 5 × 10^5 *Microcystis aeruginosa* cells/mL [total organic carbon (TOC), 1.56 mg/L]. High raw water TOC was shown to reduce the efficiency of free toxin oxidation and destruction. In addition, ozonation of raw waters containing high cyanobacteria cell densities will result in cell lysis and liberation of intracellular toxins. Thus, we emphasize that only regular and simultaneous monitoring of TOC/dissolved organic carbon and cyanobacterial cell densities, in conjunction with online residual O_3 concentration determination and efficient filtration steps, can ensure the provision of safe drinking water from surface waters contaminated with toxic cyanobacterial blooms. ***Key words*: cyanobacteria, microcystin, *Microcystis aeruginosa*, ozonation, *Planktothrix rubescens,* TOC, total organic carbon. *Environ Health Perspect* 110:1127–1132 (2002). [Online 23 September 2002]**

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The presence of toxic cyanobacterial blooms occurring in bodies of water used either as drinking water reservoirs or for recreational purposes may represent serious health risks for the human population. A large number of intoxications not only of cattle (Mez et al. 1997; Puschner et al. 1998; Van Halderen et al. 1995), dogs (DeVries et al. 1993; Harding et al. 1995), and waterfowl (Matsunaga et al. 1999; Wirsing et al. 1998) but also of humans has been reported. A high incidence of primary liver cancer in China has been attributed to drinking water contaminated with cyanobacterial toxins (Harada et al. 1996; Ueno et al. 1996a; Yu 1989), and the tragic deaths of 60 patients in a hemodialysis clinic in Brazil in 1996 was connected to the presence of cyanobacterial toxins in the water supply (Jochimsen et al. 1998; Pouria et al. 1998). The latter event highlights the importance of adequate water treatment techniques: inadequate bloom monitoring and water treatment by the city's water utility, in combination with insufficient maintenance of the clinic's filters, led to this disastrous event.

Cyanobacteria produce a variety of toxins that are usually defined by their chemical structure and fall into three groups: cyclic peptides (the hepatotoxic microcystins and nodularins), alkaloids (the neurotoxic saxitoxins and anatoxins, and the protein-synthesis–inhibiting cylindrospermopsin), and lipopolysaccharides. The cyanobacterial genera most often associated with toxicity are *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia,* and *Planktothrix* (Carmichael 1997; Codd et al. 1999). The cyclic peptide toxins are the most widespread freshwater cyanobacterial toxins and are important with respect to treatment of drinking water. Acute intoxications with microcystins result in fulminant liver damage (Runnegar and Falconer 1982). Microcystins and nodularins have also been shown to be tumor promoters (Fujiki and Suganuma 1999).

Concern regarding these potential health risks has prompted the World Health Organization (WHO) to adopt a provisional guideline value for microcystin-LR [MC-LR; containing leucine (L) and argenine (R) as the variable L-amino acids], the most common microcystin congener, of 1.0 µg/L drinking water (WHO 1998). The development of methods to effectively reduce the concentrations of the toxins as well as their potential breakdown products to below acceptable levels in drinking water has thus become an important focus of current research efforts (Hitzfeld et al. 2000). The destruction of cyanobacterial cells by chemical or mechanical means (coagulation/flocculation) is not only insufficient but may also enhance the release of toxins contained within the cells and thus mandates further treatment of the water for drinking purposes. Rapid filtration and slow sand filtration are also not efficient in removing cyanobacterial cells and, in the event of cell lysis on the filter, may even lead to release of toxins into the water. Adsorption via granular activated carbon (GAC) or powdered activated carbon (PAC) can be an efficient method, provided that an effective carbon type is employed, the carbon is unused, and the dosing of carbon is adequate. Chlorination has been shown in several studies to be inefficient in removing cyanobacterial toxins. Chlorination also has the inherent disadvantage that chlorination by-products are generated, which have been implicated in the subacute toxicity (progressive liver damage) seen in mice after intraperitoneal injection of chlorinated microcystin (Rositano et al. 1995). Only if a residual of ≥ 0.5 Cl_2 mg/L is present after 30 min of contact time is a destruction of cyclic peptides guaranteed. The combination of titanium dioxide, ultraviolet (UV) light, and hydrogen peroxide has been demonstrated to be a potentially viable technique for waterworks faced with microcystin contamination in raw water (Cornish et al. 2000). Micro- or ultrafiltration has so far revealed promising results in removing cyanobacterial cells and toxins.

Ozonation has been shown to be a very effective method for destroying microcystins and nodularins. Generally, ozonation is used as a single or multiple application in the water treatment plane as an early phase (preozonation) or late phase (intermediate ozonation) of the water treatment process. Pure MC-LR and nodularin can be oxidized within seconds to minutes (Rositano et al. 1998). However, because of the competition between the toxins and organic material in the raw water, the ozone present may be rapidly depleted, resulting in incomplete oxidation of the toxins (Shawwa and Smith 2001). Thus, a single ozonation step may not be sufficient, and additional ozonation (intermediate ozonation) is advised. Even if the

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water treatment train involves both preozonation and intermediate ozonation steps, major problems with toxin contamination may arise if the cyanobacterial biomass is not monitored at the level of water intake. In the case of a large cyanobacterial bloom (high cell numbers), treatment plants could be ill-prepared to meet the increased O_3 demand due to the high organic load. This could lead either to an increase in the soluble toxin concentration and/or to incomplete destruction of the cyanobacterial toxins. Furthermore, the issue of ozonation by-products of cyanobacterial toxins has so far not been adequately addressed. Indeed, one has to be aware of the fact that waterworks do not aim at complete oxidation of organic material to carbon dioxide and water. One can therefore expect semioxidation products to form. Such ozonation by-products have been found by high-performance liquid chromatography (HPLC) when cyanobacteria were ozonated, but neither their structure nor their toxicity has been characterized (James and Fawell 1991).

An additional confounding factor in the analysis of water treatment efficiency in cyanobacterial toxin destruction is the fact that the toxins were either "quantified" via the mouse bioassay, detecting only acutely toxic doses of microcystins or other cyanobacterial toxins, or by HPLC, which clearly does not detect all of the toxins (> 70 microcystin congeners, > 5 nodularin congeners and others) potentially present.

The objective of this study was to determine the potential limitation of a water treatment system having ozonation coupled with several filtration steps, when faced with varying bloom conditions. Toxin analyses were carried out via a biochemical detection technique [protein phosphatase inhibition assay (PPA)]. HPLC-UV was used to identify the predominant microcystin congeners in the cyanobacterial samples. To address these questions, an ozonation batch reactor was modeled according to the technically advanced system in the Lengg waterworks on Lake Zurich, Switzerland. MC-LR, an extract of *Planktothrix rubescens*, a cyanobacterium blooming regularly in Lake Zurich, and a culture of *Microcystis aeruginosa* [Pasteur Culture Collection (PCC) 7806] were oxidized using different O_3 concentrations. Filtration steps using fast filtration, activated carbon, and slow sand filtration were included to study the retention of toxins at every step.

Materials and Methods

Culture and extraction of cyanobacteria. *M. aeruginosa* (PCC 7806) was cultured in 10-L flasks using BG 11 medium (Stanier et al. 1971) (26°C, 0.003% CO_2, 24 hr light). Cells were harvested when a density of 10^7 cells/mL was reached.

P. rubescens was collected from a depth of 10 m from a bloom in Lake Zurich, Switzerland (August 1997), using a net sampler (45 µm) and concentrated via centrifugation (10,000 × *g*, 15 min). The concentrated material was lyophilized and stored at –20°C until extraction.

Extracts were obtained from 1 g (dry weight) of cyanobacteria samples. These samples, suspended in 25 mL 75% methanol, were sonicated for 60 min and centrifuged at 48,000 × *g* for 60 min, and the supernatants were collected. This procedure was repeated three times. The methanol was removed via rotary evaporation and the final extract resuspended in a defined volume of MilliQ water (MQ-H_2O; Millipore, Eschborn, Germany).

Biomass of *M. aeruginosa* was determined via cell counting using a Casy 1 (model TTC; Schaerfe System, Reutlingen, Germany) as well as in a Neubauer counting chamber (Roth, Karlsruhe, Germany).

Dissolved/total organic carbon determination. Cell culture (100 mL) and extract (100 mL of a 1:1,000 dilution) samples were filtered through glass microfiber filters (GF/F; Whatman, Göttingen, Germany). Dissolved organ carbon (DOC) in the filtrate was tested using a NCS 2500 elemental analyzer (Fisons Instruments, Beverly, MA, USA). Particulate organic carbon (POC) was determined in the filter using a TOC-5000 A analyzer (Shimadzu, Duisburg, Germany). Total organic carbon (TOC) was calculated by adding DOC and POC (precision ± 1%). The detection limit was approximately 0.2 µg carbon.

Determination and quantification of the toxins. **Protein phosphatase assay.** PPA was performed as described previously (Fischer and Dietrich 2000) using a phosphatase extracted from rape seed (*Brassicus napus*), ^{32}P-adenosine triphosphate (kindly provided by Werner Hofer, University of Konstanz), and with MC-LR (Calbiochem, Bad Soden, Germany) as a standard. In short, samples were preincubated with rape seed phosphatase (10 min, 30°C) and then incubated with ^{32}P-phosphorylase A (5 min, 30°C). The reaction was stopped with ice-cold 20% trichloroacetic acid and centrifugation. Free ^{32}P in the supernatant was extracted with acid molybdate, scintillation cocktail (Ready Safe; Beckman, Munich, Germany) was added, and radioactivity was counted in a liquid scintillation counter (LS 6500, Beckman). The degree of protein phosphatase inhibition (PP-inhibiting capacity) was calculated as a percentage of the phosphatase activity of the control.

The PPA was used to approximate the toxin contents of *P. rubescens* extracts and the *M. aeruginosa* cultures. The PP-inhibiting capacity of the latter samples was compared with a MC-LR standard curve and expressed as MC-LR equivalents. The detection limit of the radioactive PPA that we use in our laboratory is 0.05 µg MC-LR/L with a derived inhibitory concentration (50%) (IC_{50}) of 0.25 µg MC-LR/L. Because of the PPA-determined dilution factor (1:4), the WHO (1998) guideline of 1.0 µg/L corresponds to 50% inhibition in the assay (Figure 1).

HPLC. HPLC was used for determination of microcystin congeners in *M. aeruginosa* and *P. rubescens* samples. Toxins were analyzed according to the method B described by Meriluoto et al. (2000), with slight variations. Briefly, external standards were prepared for MC-LR (Calbiochem), microcystin-RR [MC-RR; containing two argenines (RR) as the variable L-amino acids] (Sigma, Deisenhofen, Germany), desmethyl MC-LR and desmethyl MC-RR (kindly provided by J. Meriluoto, Turku, Finland). Acetonitrile/0.0135 M ammonium acetate (27:73 volume/volume) was used as the mobile phase at a flow rate of 1 mL/min. Solid-phase extraction (SPE) of cyanobacterial extracts (1 mL each) was performed using Isolute C18 end-capped SPE cartridges (International Sorbent Technology, ICT, Bad Homburg, Germany) conditioned with 10 mL methanol and subsequently washed with 10 mL MQ-H_2O. Cyanobacterial extracts were applied to the cartridge and, after washing with 10 mL MQ-H_2O, eluted with 10 mL of 100% methanol. The eluent was dried under a nitrogen atmosphere and resuspended in 1 mL of the mobile phase (acetonitrile/0.0135 M ammonium acetate). If necessary, extracts were filtered through a 0.22 µm Millex-GV filter (Millipore). Extracts were injected into the HPLC (Beckman Autosampler 507e, Solvent Module 125, Programmable Detector Module 166, Beckman Ultrasphere ODS-Column, 250 × 4.6 mm, 5 µm) and peaks were compared with standards.

Ozonation. O_3 was produced in an O_3 generator (type LN 103 AT, kindly provided by Ozonia, Duebendorf, Switzerland) by regulation of voltage (25–50 mA) and gas flow (166–208 cm^3/min) with oxygen as substrate. MC-LR purified standard and extracts of *P. rubescens* and *M. aeruginosa* culture material

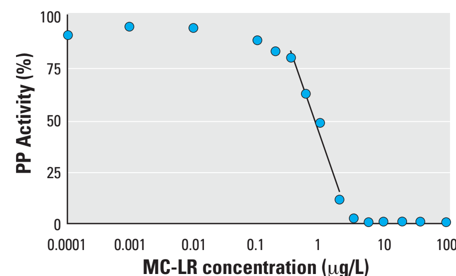

Figure 1. Inhibition of protein phosphatases by MC-LR (diluted 1:4). The IC_{50} extrapolated to 0.25 µg MC-LR/L. $y = –38.679\ \mathrm{Ln}(x) + 42.133$. $R^2 = 0.9765$.

were ozonated with different concentrations for 9 min of contact time (O_3 on) and 60 min of reaction time (O_3 off) in a 3.2-L batch reaction vessel equipped with a fritted glass sparger. Culture and extract were diluted in artificial lake water (625 mg/L NaCl, 962 mg/L $NaHCO_3$, 30 mg/L KCl, 20 mg/L $CaCl_2 \cdot 2H_2O$, 60 mg/L $CaSO_4 \cdot 2H_2O$, 154 mg/L $MgSO_4 \cdot 7\ H_2O$), ozonated at constant pH (8.0) and constant temperature (6–8°C). Artificial lake water was used to obtain a standardized medium, which tap water cannot provide, and to reflect lake conditions. MC-LR was ozonated in MQ-H_2O. The final concentrations were 0.5, 1.0, and 1.5 mg diluted O_3/L. The O_3 concentrations could not be adjusted exactly after 9 min of contact time and varied therefore around the aim concentrations of 0.5, 1.0, and 1.5 mg diluted O_3/L. Samples for toxicity determination via PPA were collected before ozonation, after 9 min of contact time, and after 60 min of reaction time and stored at –20°C. O_3 concentration was determined at times t_9 (at 9 min after O_3 contact time), t_{14}, t_{19}, $t_{29, \ldots,}$ t_{69} by the Indigo method (Bader and Hoigné 1981).

Filtration. To test the efficiency of the filtration steps typically used in a technically advanced water treatment system to remove or retain cyanobacterial toxins and/or their ozonation by-products, a laboratory-scale model filter system was set up that was composed of three columns. The columns used for filtration were 80 cm long with an internal diameter of 9 cm: *a*) Column 1: rapid sand filtration with 40% (1,780 cm^3) pumice/60% (2,800 cm^3) quartz sand, average flow of rate 425 mL/min; *b*) Column 2: activated carbon filtration with 75% (3,436 cm^3) GAC/25% (1,145 cm^3) quartz sand, average flow rate of 475 mL/min; and *c*) Column 3: slow sand filtration with 100% slow sand (4,580 cm^3), average flow rate of 125 mL/min.

The efficiency of the filtration steps was tested with extract of a *P. rubescens* bloom and with a toxic *M. aeruginosa* culture. Previously ozonated (0.5 mg O_3/L) samples (2.5 L), which still had showed phosphatase inhibitory activity after ozonation, were filtered successively through these three columns. After each filtration step, samples were collected and stored at –20°C for PPA. In experiments with *P. rubescens* extract (100 µg MC-RR/L and desmethyl MC-RR/L combined), the filtration materials were changed after every filtration step. In the second experiment with the *M. aeruginosa* culture (MC-LR equivalents between 25 and 250 µg/L), filtration materials were reused to determine the effects of cyanobacterial preloading on the removal/retention capabilities of the filtration materials.

Results

To mimic different cyanobacterial bloom situations resulting either in high cell concentrations and high TOC concentrations or in high concentration of free toxin in the water, pure MC-LR (in MQ-H_2O), extract of a *P. rubescens* bloom, and *M. aeruginosa* cells at different cell densities (both in artificial lake water) were ozonated in a batch reactor system. TOC concentrations ranged from < 0.14 mg/L for MQ-H_2O to 3.54 mg/L for *P. rubescens* extract in MQ-H_2O (Table 1). Initial toxin concentrations in the samples (*M. aeruginosa* cells and *P. rubescens* extract), determined using PPA, were between 12 and 100 µg/L, expressed as MC-LR equivalents. The concurrent analysis of these samples via HPLC demonstrated the presence of MC-LR in the *M. aeruginosa* cells and MC-RR and desmethyl MC-RR in the *P. rubescens* extract. Ozonation of these samples with 0.5, 1.0, and 1.5 mg/L O_3 led to a decrease in the PP-inhibiting capacity of the samples (Table 2). This reduction appeared largely dependent on the respective TOC of the samples as well as on the presence of intact cells and the respective cell numbers present.

Ozonation of MC-LR. Ozonation of 10 µg/L MC-LR with 0.5 mg/L O_3 quickly destroyed the toxin within 9 min contact time (t_0–t_9; Table 2). No oxidation capacity (O_3 residual) was detectable 30 min after active ozonation (t_{39}, Figure 2A), whereas 50% of the initial O_3 was still detectable in the corresponding controls. In contrast, ozonation with either 1.0 or 1.5 mg/L left an O_3 residual of 0.27 ± 0.16 and 0.72 ± 0.03 mg/L, respectively, at 30 min after active ozonation (t_{39}; Figure 2B,C).

Ozonation of* P. rubescens *extract. One of the main problems associated with oxidation reactions is that numerous particles and organic and inorganic compounds can compete for the oxidative capacity during ozonation. This is exemplified with extract from a toxic *P. rubescens* bloom, which has high concentrations of TOC and toxin (Table 2, Figure 2A). As shown in Table 2, 0.5 mg/L O_3 was not sufficient to significantly reduce the PP-inhibiting capacity of this extract. Indeed, all the O_3 was consumed within 10 min after initial ozonation (t_{19}; Figure 2A). Using higher O_3 concentrations, the PP-inhibiting capacity could be reduced (Table 2). However, all of the O_3 was consumed to < 0.2 mg/L within 10 min (t_{19}), and no O_3 residual could be detected after 20 min (t_{29}; Figure 2B,C).

Ozonation of* M. aeruginosa *cells. Oxidation of intact cyanobacteria most closely mimics the situation when a cyanobacterial bloom enters the water intake of a water treatment plant. Ozonation of intact cells, while consuming a large proportion of the oxidation capacity, will induce cell lysis and thus could provide for increased toxin concentrations in the treated water, this being largely dependent on the cell density of the respective bloom. Therefore, different cell densities of toxic *M. aeruginosa* were subjected to ozonation at three different O_3 concentrations. As demonstrated in Table 2, ozonation of 1 × 10^5 cells/mL *M. aeruginosa* with 0.5 mg/L O_3 was not sufficient to completely destroy the PP-inhibiting capacity after 9 min (t_9) and even after 69 min (t_{69}) of contact time. Indeed, ozonation with 0.5 mg/L and a cyanobacteria density of 1 × 10^5

Table 1. TOC and concentration and congener composition of toxins and of ozonated samples.

Sample	Toxin concentration (PPA)	Dominant toxin congener(s) (HPLC)	TOC (mg C/L)
MQ-H_2O	—	—	0.14
Artificial lake water	—	—	0.36
MR-LR (in MQ-H_2O)	10 µg/L	MC-LR	0.146[a]
P. rubescens extract (in MQ-H_2O)	100 µg MC-LR equiv/L	MC-RR, desmethyl-MC-RR (> 80%)	3.54
M. aeruginosa 10^5 cells/mL (in artificial lake water)	12 µg MC-LR equiv/L	MC-LR (> 90%)	0.6
M. aeruginosa 5 × 10^5 cells/mL (in artificial lake water)	60 µg MC-LR equiv/L	MC-LR (> 90%)	1.54

equiv, equivalent.
[a]0.14 for MQ-H_2O + 0.006 for microcystin (calculated, not measured).

Table 2. PP activity (% control) before (t_0) and after ozonation (t_9), and after 60 min reaction time (O_3 off, t_{69}) with different concentrations (± SD, n = 3).

	MC-LR (10 µg/L)			*P. rubescens* extract			*M. aeruginosa* 10^5 cells /mL			*M. aeruginosa* 5 × 10^5 cells/mL		
O_3 (mg/L)	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
PP activity t_0	62.5 ± 1.9	69.3 ± 4.4	62.7 ± 4 .2	3.7 ± 1.6	3.4 ± 2.6	3.3 ± 1.1	35.7 ± 11.9	36.8 ± 0.4	34.6 ± 7.8	ND	0.9 ± 1.6	0.5 ± 0.2
PP activity t_9	105.3 ± 2.0	87.6 ± 7.3	91.3 ± 5.1	6.2 ± 6.1	110.7 ± 12.3	87.7 ± 7.4	67.1 ± 4.1	98.3 ± 0.5	85.8 ± 6.0	ND	39.2 ± 20.7	81.8 ± 13.9
PP activity t_{69}	103.2 ± 14.9	94 ± 14.8	103.4 ± 10.4	8.8 ± 2.8	99.4 ± 13.9	95.5 ± 6.6	88.9 ± 13.7	101.9 ± 9.4	92.3 ± 2.9	ND	77.9 ± 20.9	92.6 ± 2.9

ND, not determined.

cells *M. aeruginosa*/mL also consumed almost all of the O_3 within 19 min (t_{19}), while the toxin content was still high (Figure 2A, Table 2). Although 1.0 mg/L O_3 was sufficient to cope with a "bloom" containing 1 × 10^5 cells *M. aeruginosa*/mL, this was not the case when cell densities were higher (i.e., 5 × 10^5 cells *M. aeruginosa*/mL). When 1 × 10^5 or 5 × 10^5 cells/mL *M. aeruginosa* were ozonated with 1.0 mg/L O_3, nearly all of the O_3 was consumed (< 0.2 mg/L) within 19 min of contact time (t_{19}; Figure 2B, Table 2). However, although the amount of O_3 was sufficient to reduce the toxin in the sample with 1 × 10^5 cell/mL (Table 2), a PP-inhibiting capacity of > 20% (~0.37 µg MC-LR/L) remained after 69 min of contact time ($t69$) in the sample containing 5 × 10^5 cells/mL. In the latter case, a minimum of 1.5 mg/L O_3 was required to significantly reduce the PP-inhibiting capacity of the cyanobacteria (Table 2).

Effects of filtration. Modern water treatment plants routinely employ different filtration steps after ozonation. For example, the Lengg water treatment plant uses quartz sand/pumice, activated carbon/quartz sand, and then slow sand. To study the effect of the filtration steps on the reduction of the PP-inhibiting capacity, *P. rubescens* extract still displaying toxic activity after ozonation with 0.5 mg/L O_3 was filtered in the laboratory-scale model filter system. The filtration medium was changed after each experiment. Although quartz sand/pumice filtration removed most of the PP-inhibiting capacity, activated carbon/quartz sand and slow sand filtration was necessary to completely reduce the remaining PP-inhibiting capacity (Figure 3). However, water treatment plants normally do not change the filtration materials for several years (10–15 years on average at the Zurich Water Works, Zurich, Switzerland). Therefore, the danger of repeated overloading of the filtration materials resulting from bloom events theoretically exists. During back-washing of filter material, release of toxic material may also occur. To mimic the latter situation, an *M. aeruginosa* sample (2 × 10^6 cells/mL) still displaying PP-inhibiting capacity after previous ozonation with 1.0 mg/L O_3 was filtered through the filter columns of the lab-scale model filter system. As expected, activated carbon/quartz sand filtration removed most of the PP-inhibiting capacity (Figure 4). However, when an ozonated (1.0 mg/L O_3) *M. aeruginosa* sample (2 × 10^5 cells/mL) was filtered with the same columns (i.e., without changing the filter materials) an increased PP-inhibiting capacity was observed (Figure 4). Because the PP-inhibiting capacity of the second sample had been almost completely removed by ozonation, this increased inhibiting capacity could not stem from this sample. Most likely, this inhibiting capacity resulted from toxins retained in the filters during the first filtration experiment and then released from the quartz sand/pumice filters during the second filtration experiment. Subsequent activated carbon/quartz sand filtration, however, was able to remove the majority of the observed PP-inhibiting capacity (Figure 4).

Discussion

Ozonation has previously been shown to be an effective method to reduce the cyclic peptide toxin concentration of waterborne cyanobacteria (Rositano et al. 1998; Shawwa and Smith 2001). This study confirms those findings. The data presented here, however, emphasize that it is essential to investigate the conditions under which toxin destruction is optimal.

The importance of free toxin regarding drinking water contamination is moderated by the fact that, in most cases, the free toxin levels rarely exceed 10 µg/L or < 10% of the total toxin present in the actual cyanobacterial bloom (Fromme et al. 2000; Hart et al. 1998; Ueno et al. 1996b). Microcystin levels > 70 µg dissolved microcystin/L have only been measured in a case of a thick surface scum, which lysed and rotted (Welker et al. 2000). Free MC-LR is oxidized rapidly by O_3 and has a half-life of 1 sec at 0.1–2.0 mg O_3/L (Shawwa and Smith 2001). More emphasis must therefore be placed on the efficacy of ozonation of raw water contaminated with high densities of cyanobacterial cells. *Microcystis* and *Planktothrix* can reach very high cell densities (10^3–10^6 cells/mL) in water reservoirs [Domingos et al. 1999; James et al. 1994; Kotak et al. 1994, 1995; Repavich et al. 1990; Sivonen et al. 1990; UK Water Industry Research Limited (UKWIR) 1997; Vasconcelos 1999]. Treatment of raw water from such contaminated bodies of water is often the only choice because alternative water sources may not be available or the depth of the water body may be too low to use alternative water intake levels.

The aim of water treatment plants is to eliminate color, bad taste, infectious organisms, and known toxic compounds such as pesticides. The intention is usually not the destruction of cyanobacterial cells by ozonation (Geering 1999), but rather the removal of intact cells by flocculation and filtration. Destruction of cells may lead to an increase of macromolecular compounds (disinfection byproducts), which are difficult to remove during water treatment (Plummer and Edzwald 1998). However, on a daily basis, it is not always possible to apply an effective O_3 concentration that does not lead to destruction of cyanobacterial cells.

Microscopic observations during the experiments in this study showed that the majority of *Microcystis* cells lyse at concentrations of 1 mg O_3/L, a concentration routinely used in water treatment plants. This contradicts the observations of Plummer and Edzwald (1998), who reported lysis of cyanobacterial cells only at concentrations > 3 mg O_3/L. Consequently, oxidation of intact cyanobacterial cells often leads to cell lysis and subsequent release of toxins. Therefore, sufficient oxidation capacity has to be provided to destroy both the cells and the toxins. Indeed, as shown in this study in cases where cell numbers exceed 10^5 cells/mL, an average of 0.5 mg O_3/L will not suffice to guarantee complete toxin destruction (Table 2). Only single pulse (9 min of contact time) of ozonation with 1.5 mg O_3/L provides enough oxidation capacity to ensure the destruction of the PP-inhibiting toxins after 60 min of reaction time (Table 2). As an alternative to average O_3 concentrations, water treatment plants treat water with 1.0 mg O_3/L, monitor O_3 concentrations, and automatically add O_3 to the reaction basin when the residual O_3 concentrations fall below 0.5 mg/L. This, however, may not ensure complete destruction of cyanobacterial toxins ecause of variations in

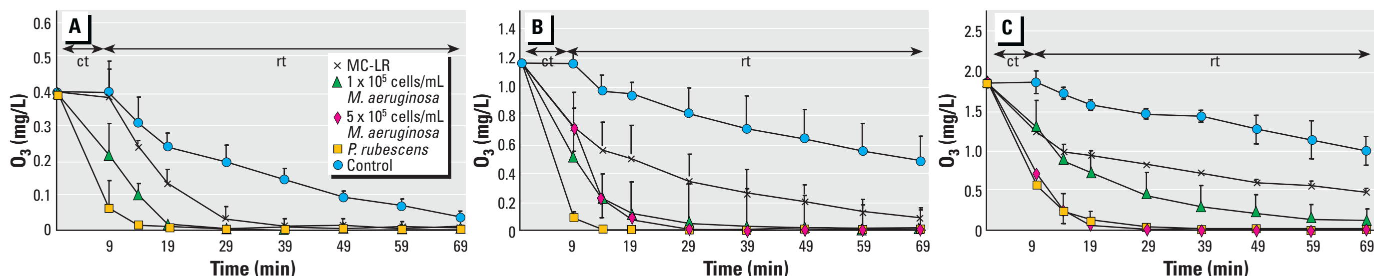

Figure 2. Effects of ozonation on MC-LR (10 µg/L), 1 × 10^5 cells/mL *M. aeruginosa,* 5 × 10^5 cells/mL *M. aeruginosa,* and extract of *P. rubescens* on O_3 consumption compared with control. (*A*) 0.5 mg O_3/L; (*B*) 1.0 mg O_3/L; (*C*) 1.5 mg O_3/L. Abbreviations: ct, contact time; rt, reaction time (*n* = 3). Error bars indicate SD.

TOC composition and concentrations in raw water, resulting in O_3 concentrations falling below 0.5 mg/L for certain time periods and thus allowing cyanobacterial cells to enter the next filtration step.

One of the most important factors influencing ozonation capacity is TOC or DOC (Bruchet et al. 1998; Hart and Stott 1993; Rositano et al. 1998; Shawwa and Smith 2001). The extent and rate of TOC removal typically increase as O_3 dose increases (Hozalski et al. 1999), but at routinely employed O_3 concentrations of > 1.2 mg O_3/mg C, TOC is not decisively reduced (~10–20%) (Amirsardari et al. 1997; Plummer and Edzwald 1998; Tuhkanen et al. 1994). Nevertheless, if the TOC is high in raw water, the efficacy of O_3 in destroying free cyanobacterial toxins is dramatically reduced, as exemplified by free MC-RR/desmethyl MC-RR in this study. Existing data suggest that the cyanobacterial toxins, a subset of the natural organic compounds in raw water, are oxidized proportionally to their fraction of the TOC. Consequently, it appears vital that the intra- and extracellular toxin concentrations as well as the TOC are known before raw water is treated with O_3.

The results presented here are important in consideration of the fact that raw water may contain varying and often higher TOC values than the ones employed in the present study (Lengg water treatment plant, 1996–2000, DOC 1.1–1.4 mg C/L) (Wasserversorgung Zürich 1996–2000). Because of variation of TOC and therefore variation of substances that can be oxidized (e.g., compound structures containing double bonds), a generalized statement cannot be made. In contrast to the situation in waterworks, in this study cyanobacteria were the exclusive source of the organic material. However, Shawwa and Smith (2001) show that TOC generally influences the kinetics of MC-LR oxidation by O_3. During ozonation of extracts of toxic *P. rubescens*, a predominant phytoplankton species in many European lakes that regularly gives rise to toxic blooms (Micheletti et al. 1998; Walsby et al. 1998), O_3 concentrations < 1.0 mg/L are insufficient to completely destroy the toxins when TOC levels rise above 3.0 mg/L (Tables 1 and 2). A comparison of the experiments using *P. rubescens* extracts with those using *M. aeruginosa* at a density of 5×10^5 cells/mL demonstrates the difference between free toxin in TOC-rich water and cell-bound toxin. Despite a lower TOC content and lower toxin concentrations, phosphatase inhibition could still be detected in the experiments using *M. aeruginosa* cells but not in the *P. rubescens* extracts. Similar results were obtained in the only comparable study, where during a 10-min ozonation of 10^4 and 10^5 *M. aeruginosa* cells with 0.8 and 1.3 mg O_3/L, respectively, only about 60% of the toxin was destroyed and the O_3 was completely consumed (Carlile 1994). Even ozonation with very high O_3 levels (3.7 mg/L, 5 min) is not sufficient to completely eliminate microcystin levels when high cell numbers (2×10^6 cells/mL) are present and when dealing with high TOC levels (8–11.4 mg/L) (Rositano et al. 1998). Ozonation of toxin-free cyanobacteria was not performed in the present study or, to our knowledge, by other investigators. Therefore, we cannot exclude the possibility that other cyanobacterial compounds show a PP-inhibiting activity after ozonation of whole cells. Our experiments were carried out at 6–8°C and at pH 8. O_3 capacity of water decreases with increasing temperature [this investigation (data not shown) and Langlais et al. (1991)] and decreasing pH (Langlais et al. 1991; Rositano et al. 1998). These parameters have to be kept in mind when different waterworks (with different raw waters) and different investigations are compared with each other.

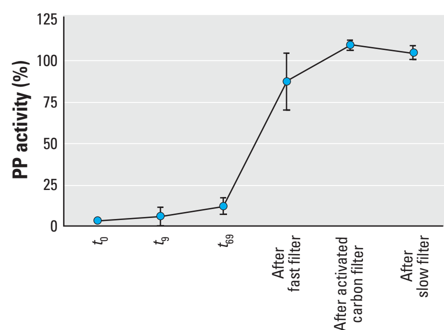

Figure 3. Filtration of a toxic *P. rubescens* extract through quartz sand/pumice (fast filter), activated carbon/quartz sand (activated carbon filter), and then slow sand after the ozonation step (0.5 mg O_3/L). PP activity is shown as percentage of control (n = 3). Error bars indicate SD.

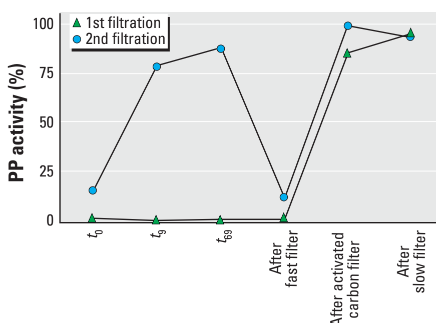

Figure 4. Results of repeat use of filtration materials [quartz sand/pumice (fast filter), activated carbon/quartz sand (activated carbon filter), and slow sand]. In the first filtration, *M. aeruginosa* cells (2×10^6/mL; still displaying PP activity after ozonation with 1.0 mg/L O_3) were filtered through the three filters after the ozonation step. In the second filtration, 2×10^5 cells/mL *M. aeruginosa* (no toxicity after ozonation with 1.0 mg/L) were filtered through the same material. A breakthrough of PP-inhibiting compounds are present after the first filtration. PP activity is shown as percentage of control of a representative experiment.

The Lengg water treatment plant at Lake Zurich employs one filtration step between preozonation and the main intermediate ozonation step and two filtration steps before drinking-water release. This situation was modeled in the present study and was shown to be very efficient in removing remaining phosphatase inhibitory activity of *P. rubescens* extracts when using fresh filter materials (Figure 2). In water treatment plants, however, such filter materials may be in use for several years and are used as a substrate for a microbial biofilm rather than for filtration/adsorption purposes. This can therefore significantly impair the ability of the activated carbon filter to adsorb toxins (Falconer et al. 1989; Lambert et al. 1996), whereas biodegradation on the biofilm does not seem to occur (Lambert et al. 1996). Frequent changes of filter materials are obviously also very costly and thus rarely carried out. As the present study suggests, there could potentially be a danger of a breakthrough of cyanobacterial toxins even when quartz sand/pumice filter materials are used only once (Figure 3). Therefore, only the efficient destruction of the cells and toxins via high concentrations of O_3 potentially offers the necessary protection from microcystin contamination of drinking water.

In light of the tumor-promoting activity of microcystins, an exposure of the general public even to very low microcystin concentrations should be avoided. However, because ozonolysis by-products have not been characterized regarding their structure and toxicity, the reliability of even a very efficient method such as ozonation during cyanobacterial bloom events should be treated with caution.

In conclusion, despite the uncertainties described above, a modern water treatment plant using pre- and intermediate ozonation steps in conjunction with online O_3 measurements and different filtration steps, and regular monitoring of TOC/DOC and cyanobacterial cell densities in the raw water, should be able to provide safe drinking water.

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