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. 1997; Puschner et al. 1998; Van Halderen 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 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 O3 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.  
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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 plant 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 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 Cl2 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-ultrafiltration has so far revealed promising results in removing cyanobacterial cells and toxins.

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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-H₂O; Millipore, Eschborn, Germany).

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

Dissolved and 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 organic 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-5000A 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), 32P-adenosine triphosphate (kindly provided by Werner Hofer, University of Konstanz, and with MC-LR (Calbiochem), microcystin-RR (kindly provided by J. Meriluoto, Turku, Finland), 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-H₂O. Cyanobacterial extracts were applied to the cartridge and, after washing with 10 mL MQ-H₂O, eluted with 10 mL 100% methanol. The eluent was dried under a nitrogen atmosphere and subsequently 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. O₃ was produced in an O₃ generator (type LN 103 AT, kindly provided by Ozone, Duendendorf, Switzerland) by regulation of voltage (25–50 mA) and gas flow (166–208 cm³/min) with oxygen as substrate. MC-LR purified standard and extracts of P. rubescens and M. aeruginosa material culture 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%) (IC₅₀) 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 t-αmino 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-H₂O. Cyanobacterial extracts were applied to the cartridge and, after washing with 10 mL MQ-H₂O, eluted with 10 mL 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.

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were ozonated with different concentrations for 9 min of contact time (O₃ on) and 60 min of reaction time (O₃ 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 NaHCO₃, 30 mg/L KCl, 20 mg/L CaCl₂, 2H₂O, 60 mg/L CaSO₄ · 2H₂O, 154 mg/L MgSO₄ · 7 H₂O), 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-H₂O. The final concentrations were 0.5, 1.0, and 1.5 mg diluted O₃/L. The O₃ 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 O₃/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. O₃ contact time, and after 60 min of reaction time were collected before ozonation, after 9 min of contact time, and after 60 min of reaction time 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 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-H₂O), 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-H₂O to 3.54 mg/L for P. rubescens extract in MQ-H₂O (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 concurrence 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 O₃ 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 O₃ quickly destroyed the toxin within 9 min contact time (t₉; Table 2). No oxidation capacity (O₃ residual) was detectable 30 min after active ozonation (t₉₀; Figure 2A), whereas 50% of the initial O₃ was still detectable in the corresponding controls. In contrast, ozonation with either 1.0 or 1.5 mg/L left an O₃ residual of 0.27 ± 0.16 and 0.72 ± 0.03 mg/L, respectively, at 30 min after active ozonation (t₉₀; 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 O₃ was not sufficient to significantly reduce the PP-inhibiting capacity of this extract. Indeed, all the O₃ was consumed within 10 min after initial ozonation (t₉; Figure 2A). Using higher O₃ concentrations, the PP-inhibiting capacity could be reduced (Table 2). However, all of the O₃ was consumed to <0.2 mg/L within 10 min (t₉₀), and no O₃ residual could be detected after 20 min (t₅₀; 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 O₃ concentrations. As demonstrated in Table 2, ozonation of 1 × 10⁵ cells/mL M. aeruginosa with 0.5 mg/L O₃ was not sufficient to completely destroy the PP-inhibiting capacity after 9 min (t₉) and even after 69 min (t₅₀) of contact time. Indeed, ozonation with 0.5 mg/L and a cyanobacteria density of 1 × 10⁵

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

| Sample | Toxin concentration (µg/L) | Dominant toxin congener(s) (HPLC) | TOC (mg C/L) |
|--------|-----------------------------|-----------------------------------|--------------|
| MQ-H₂O | —                           | —                                 | 0.14         |
| Artificial lake water | —                           | —                                 | 0.36         |
| MR-LR (in MQ-H₂O) | 10 µg/L                      | MC-LR                             | 0.14         |
| P. rubescens extract (in MQ-H₂O) | 100 µg MC-LR equiv/L | MC-LR, desmethyl-MC-LR (> 80%) | 3.54         |
| M. aeruginosa 10⁵ cells/mL (in artificial lake water) | 12 µg MC-LR equiv/L | MC-LR (> 90%) | 0.6          |
| M. aeruginosa 5 × 10⁶ cells/mL (in artificial lake water) | 60 µg MC-LR equiv/L | MC-LR (> 90%) | 1.54         |

equiv, equivalent.

*0.14 for MQ-H₂O + 0.006 for microcystin (calculated, not measured).

**Table 2.** PP activity (% control) before (t₀) and after ozonation (t₉₀) and after 60 min reaction time (O₃ off, t₉ₐ) with different concentrations (± SD, n = 3).

| O₃ (mg/L) | MC-LR (10 µg/L) | P. rubescens extract | M. aeruginosa 10⁵ cells/mL | M. aeruginosa 5 × 10⁶ cells/mL |
|-----------|-----------------|----------------------|---------------------------|-----------------------------|
| 0.5       |                  |                      |                           |                             |
| 1.0       |                  |                      |                           |                             |
| 1.5       |                  |                      |                           |                             |
| PP activity t₀ | 62.5 ± 1.9 | 68.3 ± 4.4 | 68.7 ± 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 |
| PP activity t₉₀ | 105.3 ± 2.0 | 87.6 ± 7.3 | 91.3 ± 5.1 | 6.2 ± 0.6 | 110.7 ± 12.3 | 87.7 ± 7.4 | 67.1 ± 4.1 | 98.3 ± 0.5 | 85.9 ± 6.0 | ND |
| PP activity t₉ₐ | 103.2 ± 14.9 | 94.1 ± 14.8 | 103.4 ± 10.4 | 8.8 ± 2.8 | 98.4 ± 13.9 | 95.5 ± 6.6 | 88.9 ± 13.7 | 101.9 ± 9.4 | 92.3 ± 2.9 | ND |

ND, not determined.
cells *M. aeruginosa* mL also consumed almost all of the O₃ within 19 min (*t₁/₂*), while the toxin content was still high (Figure 2A, Table 2). Although 1.0 mg/L O₃ was sufficient to cope with a “bloom” containing 1 × 10⁵ cells *M. aeruginosa* mL, this was not the case when cell densities were higher (i.e., 5 × 10⁵ cells *M. aeruginosa* mL). When 1 × 10⁵ or 5 × 10⁵ cells/mL *M. aeruginosa* were ozonated with 1.0 mg/L O₃, nearly all of the O₃ was consumed (< 0.2 mg/L) within 19 min of contact time (*t₁/₂*; Figure 2B, Table 2). However, although the amount of O₃ was sufficient to reduce the toxin in the sample with 1 × 10⁵ cell/mL (Table 2), a PP-inhibiting capacity of > 20% (0.37 µg MC-LR/L) remained after 69 min of contact time (t₉) in the sample containing 5 × 10⁵ cells/mL. In the latter case, a minimum of 1.5 mg/L O₃ 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 O₃ 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 × 10⁶ cells/mL) still displaying PP-inhibiting capacity after previous ozonation with 1.0 mg/L O₃ 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 O₃) *M. aeruginosa* sample (2 × 10⁶ 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 lyzed and rotted (Welker et al. 2000). Free MC-LR is oxidized rapidly by O₃ and has a half-life of 1 sec at 0.1–2.0 mg O₃/L (Shawwa and Smith 2001). More emphasis must therefore be placed on the efficiency of ozonation of raw water contaminated with high densities of cyanobacterial cells. *Microcystis* and *Planktothrix* can reach very high cell densities (10³–10⁶ 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 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 by-products), 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 O₃ 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 O₃/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 O₃/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 10⁵ cells/mL, an average of 0.5 mg O₃/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 O₃/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 O₃ concentrations, water treatment plants treat water with 1.0 mg O₃/L, monitor O₃ concentrations, and automatically add O₃ to the reaction basin when the residual O₃ concentration falls below 0.5 mg/L. This, however, may not ensure complete destruction of cyanobacterial toxins even of variations in

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**Figure 2.** Effects of ozonation on MC-LR (10 µg/L), 1 × 10⁶ cells/mL *M. aeruginosa*, 5 × 10⁵ cells/mL *M. aeruginosa*, and extract of *P. rubescens* on O₃ consumption compared with control. (A) 0.5 mg O₃/L; (B) 1.0 mg O₃/L; (C) 1.5 mg O₃/L. Abbreviations: ct, contact time; rt, reaction time (n = 3). Error bars indicate SD.
TOC composition and concentrations in raw water, resulting in O₃ 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 O₃ dose increases (Hozalski et al. 1999), but at routinely employed O₃ concentrations of > 1.2 mg O₃/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 O₃ 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 O₃.

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 O₃. During ozonation of extracts of toxic P. rubescens, a predominant phytoplankton species in many European lakes that regularly gives rise to toxic blooms (Michelleti et al. 1998; Walsby et al. 1998), O₃ 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 × 10⁶ 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 10⁴ and 10⁵ M. aeruginosa cells with 0.8 and 1.3 mg O₃/L, respectively, only about 60% of the toxin was destroyed and the O₃ was completely consumed (Carli 1994). Even ozonation with very high O₃ levels (3.7 mg/L, 5 min) is not sufficient to completely eliminate microcystin levels when high cell numbers (2 × 10⁶ 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. O₃ 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 ran their own experiments...
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