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Effect of chlorine dioxide on cyanobacterial cell integrity, toxin degradation and disinfection by-product formation

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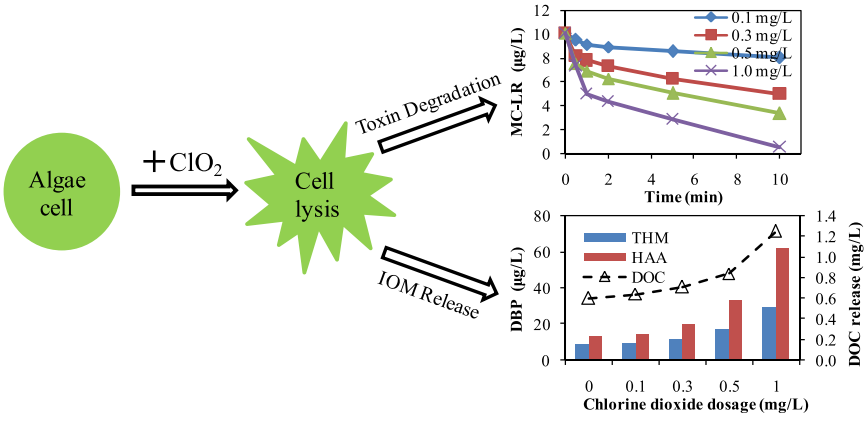
HIGHLIGHTS

- ClO_2 can inhibit photosynthetic capacity of *Microcystis aeruginosa*.
- Cell lysis and toxin degradation were observed after ClO_2 oxidation.
- DOC release contributed to the formation of THM and HAA.

GRAPHICAL ABSTRACT



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ABSTRACT

Bench scale tests were conducted to study the effect of chlorine dioxide (ClO_2) oxidation on cell integrity, toxin degradation and disinfection by-product formation of *Microcystis aeruginosa*. The simulated cyanobacterial suspension was prepared at a concentration of 1.0×10^6 cells/mL and the cell integrity was measured with flow cytometry. Results indicated that ClO_2 can inhibit the photosynthetic capacity of *M. aeruginosa* cells and almost no integral cells were left after oxidation at a ClO_2 dose of 1.0 mg/L. The total toxin was degraded more rapidly with the ClO_2 dosage increasing from 0.1 mg/L to 1.0 mg/L. Moreover, the damage on cell structure after oxidation resulted in released intracellular organic matter, which contributed to the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) as disinfection by-products. Therefore, the use of ClO_2 as an oxidant for treating algal-rich water should be carefully considered.

1. Introduction

Cyanobacterial blooms pose a series of problems for drinking water supplies. Among a variety of cyanobacteria, *Microcystis aeruginosa* is the most abundant and common species responsible for natural water bloom (Kemp and John, 2006; Yang et al., 2008; Zhang et al., 2010). Cyanobacterial cells in drinking water can deteriorate its quality by releasing toxins, causing taste and odor problems, and forming disinfection

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by-products (DBPs) (Fang et al., 2010a; Knappe et al., 2004; Lui et al., 2011). For example, microcystin-LR (MC-LR) produced by *M. aeruginosa* is highly hepatotoxic and the guideline limits for MC-LR is 1 μg/L according to the Chinese Sanitary Standards for Drinking Water (GB5748-2006). The undesirable taste and odor-causing crisis significantly degraded the quality of drinking water in Taihu Lake in China in 2007 (Zhang et al., 2010). Moreover, cyanobacterial cells and algal organic matters have been demonstrated to contribute to disinfection by-product (DBP) formation during subsequent chlorination and chloramination (Fang et al., 2010a; Li et al., 2012; Wert and Rosario-Ortiz, 2013).

Although traditional drinking water treatment processes such as coagulation, sedimentation and filtration are effective in removing intact cyanobacterial cells (Chow et al., 1999; Teixeira and Rosa, 2006), the massive presence of cyanobacterial cells will reduce the removal efficiency (Coral et al., 2013; Zamyadi et al., 2012). Pre-oxidation, especially with potassium permanganate, chlorine or ozone, is deemed effective in promoting the coagulation of cyanobacterial cells and their organic matters (Chen et al., 2009; Plummer and Edzwald, 2002; Xie et al., 2013). Chlorine dioxide (ClO_2) is another emerging water treatment oxidant due to its stronger oxidizing capacity than chlorine. ClO_2 has been proved to be effective in oxidative elimination of cyanotoxins (Kull et al., 2006; Rodriguez et al., 2007). Moreover, ClO_2 does not produce harmful trihalomethanes (THMs) in the reaction with natural organic matters (NOMs) and can reduce the formation of other halogenated organic by-products (Li et al., 1996; Yang et al., 2013). However, the application of ClO_2 can produce inorganic by-products such as chlorite (ClO_2^-) and chlorate (ClO_3^-). Due to the health concerns over chlorite and chlorate, it has been regulated at the threshold value of 0.7 mg/L for both chlorite and chlorate in the Chinese Sanitary Standards for Drinking Water (GB5748-2006). Therefore, ClO_2 is often used as a preoxidant combined with post-chlorination in drinking water treatment process. To date information about the effect of ClO_2 on cyanobacterial cell integrity and toxin degradation remains unclear.

The overall objectives of this study were: (1) to study the effects of ClO_2 oxidation on photosynthetic capacity and cell integrity of *M. aeruginosa*; (2) to investigate the toxin degradation and the release of algal organic matters; and (3) to evaluate the effects of ClO_2 on DBP formation during subsequent chlorination.

2. Materials and methods

2.1. Materials and reagents

All chemicals were at least of analytical grade except as noted. A stock solution of free chlorine (HClO) was prepared from 5% liquid sodium hypochlorite (Sinopharm Chemical Reagent Co., Ltd., China) and standardized according to the diethyl-p-phenylene diamine (DPD) ferrous titration method. A stock solution of ClO_2 (150 mg/L) was freshly prepared from gaseous ClO_2 by slowly adding dilute H_2SO_4 to a sodium chlorite ($NaClO_2$) solution according to the standard method (APHA et al., 1998). MC-LR (purity > 95%) isolated from *M. aeruginosa* was obtained from Alexis biochemicals (Switzerland). THM and HAA standard solutions were purchased from Sigma-Aldrich (USA). Methyl tert-butyl ether (MTBE) and methanol were purchased from J.T. Baker (USA).

M. aeruginosa (FACHB-912) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and cultured in BG11 media (Rippka et al., 1979). The cultures were incubated at 25 °C in 1 L conical flasks, under a 12-h diurnal cycle every day. *M. aeruginosa* cells in the late exponential growth phase were harvested and centrifuged at 4500 rpm for 10 min. The cell pellets precipitated in the centrifuge tubes were rinsed with ultrapure water, followed by two additional cycles of centrifugation and supernatant removal. The simulated cyanobacterial suspension was prepared by adding *M. aeruginosa* cells to 0.8% NaCl solution to achieve a final cell density of 1.0×10^6 cells/mL. The total organic carbon (TOC) of the suspension was 4.0 mg/L and the dissolved organic carbon (DOC) in the suspension was about 0.6 mg/L.

2.2. Experimental procedures

Oxidation experiment was carried out with 500 mL of simulated suspension of *M. aeruginosa* in a 1000 mL glass beaker. Chlorine dioxide was added from the stock solution to obtain the desired doses of 0.1–1.0 mg/L. At each designated sampling time, 5 mL sample was harvested for immediate photosynthetic capacity analysis, and 2 mL sample was quenched with ascorbic acid and then subjected to three freeze/thaw cycles to analyze for total microcystin-LR. After 10 min reaction time, the remaining samples were divided into two subsamples. The first subsample was subjected to cell integrity tests within 30 min after the end of oxidation experiment. The second subsample was immediately filtered through 0.45 μm filter (Whatman, UK) to remove cellular materials, and then subjected to DOC analysis and chlorination.

The chlorination experiments were carried out using sealed 40 mL amber bottles at 25 °C in the dark. The mass ratio of chlorine to DOC was 5 mg Cl_2:1 mg C. Solution pH was buffered at 7.0 with phosphate solution (KH_2PO_4–NaOH). After 3 days, the samples were quenched with ascorbic acid for THM and HAA analysis.

2.3. Analytical methods

2.3.1. Photosynthetic capacity

Photosynthetic capacity was measured in vivo directly using the PHYTO-PAM phytoplankton analyzer (Walz, Germany) according the method of the previous study (Zhou et al., 2013). Without dark adaption, the effective quantum yield (Φ_e) can be calculated as:

$$\Phi_e = \Delta F / F_m' = \left(F_m' - F_s\right) / F_m' \quad (1)$$

where F_s and F_m' are the corresponding light-adapted steady-state and maximal chlorophyll-*a* fluorescence, respectively (Maxwell and Johnson, 2000).

2.3.2. Cell integrity test

Cyanobacterial cell viability before and after oxidation was monitored using a flow cytometer (Accuri C6, BD Biosciences, Franklin Lakes, USA) equipped with an argon laser emitting at a fixed wavelength of 488 nm for fluorescence measurement. Fluorescent filters and detectors collected green fluorescence in channel FL1 (535 nm) and red fluorescence in channel FL2 (585 nm). *M. aeruginosa* cell was double stained with fluorescein diacetate (FDA) and propidium iodide (PI) (Sigma-Aldrich St Louis, MO, USA) following the method of Xiao et al. (2011). Cells with an intact cell membrane or active esterase are stained green fluorescence by FDA in FL1, whereas cells with a damaged cell membrane are stained red fluorescence by PI in FL2.

2.3.3. Measurement of total MC-LR

MC-LR concentrations were determined by LC/MS/MS (Thermo TSQ Quantum Access MAX, USA) with a Basic-C18 HPLC column (100 mm × 2.1 mm, 5 μm, Thermo, USA). A mobile phase containing water (0.1% formic acid) and acetonitrile with a volume ratio at 65/35 was maintained for 10 min with a flow rate of 0.2 mL/min. Sample injection volumes were typically 10 μL. Positive electrospray ionization source (ESI) combined with the selected reaction monitoring (SRM) mode was used. LC/MS/MS parameters were as follows: ESI spray voltage at 4500 V, vaporizer temperature at 300 °C, sheath gas pressure at 45 a.u., aux gas pressure at 15 a.u., and capillary temperature at 270 °C. The detection limit is 0.1 μg/L.

2.3.4. Analysis of DBPs

THMs were quantified by liquid/liquid extraction with MTBE followed by a Thermo TSQ Quantum XLS Triple Quadruple GC–MS/MS (Thermo Fisher Scientific, CA, USA), based on USEPA Method 551.1 (U.S.EPA, 1995). HAAs were analyzed by liquid/liquid extraction