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Impact of chlorine on the cell integrity and toxin release and degradation of colonial *Microcystis*

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ABSTRACT

The occurrence of toxic cyanobacteria in drinking water sources is problematic for water authorities as they can impair drinking water quality. Chlorine as a commonly used oxidant in water treatment plants has shown the potential to lyse cyanobacterial cells, resulting in the release of secondary metabolites which are hard to be removed during conventional water treatment processes. The majority of cyanobacterial species such as *Microcystis*, often occur in colonial forms under natural conditions. However, previous studies have mainly focused on the influence of chlorination on individual cyanobacterial cells due to technique limitations. A syringe dispersion method combined with a fluorescence technique (SYTOX Green stain with flow cytometry), was successfully developed for the evaluation of cell integrity of colonial *Microcystis*. Chlorination of *Microcystis*-laden water was conducted at different chlorine dosages for different colonial sizes (<37, 37–270 and 270–550 μm). The results indicated that colonial *Microcystis* cells were more resistant to chlorine oxidation than individual cells, which may be attributed to protection from the cell-bound mucilage. There was a lag phase before cell rupture occurred and a Delayed Chick Watson Model describes the experimental data very well for the kinetics of cyanobacterial cell rupture. The growing colonial size caused increases in the lag phases but decreases in the cell lysis rates. Chlorination also induced the release of microcystins (MCs) from colonial *Microcystis* cells. In particular, increased levels of dissolved MCs were observed in Cheng Kung Lake (CKL) water. In summary, the reaction of chlorine with colonial cyanobacteria is more complicated than with individual cells. The efficiency of chlorine oxidation could be reduced by the cell-bound mucilage and natural water matrix. These observations may provide insights for water authorities to assess the risk to drinking water quality posed by chlorination under natural conditions.

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1. Introduction

Cyanobacterial (blue-green algae) blooms are one of the main critical problems which could affect aquatic ecosystems in many water bodies including marine and freshwater sources. Overgrowth of cyanobacteria can reduce the suitability of source water for drinking, sanitation and irrigation by increasing turbidity and production of unfavorable secondary metabolites. In particular, some metabolites could impart taste and odour (T&O) compounds, like geosmin and 2-methylisoborneol (Young et al., 1996; Watson, 2004) and cyanotoxins, such as microcystins (MCs) (Carmichael, 1992; Codd et al., 2005; Rapala et al., 2005), leading to an increasing risk of human health and public perception for water supplies.

Among the cyanobacterial family, *Microcystis* species are the most problematic and widespread genera. The hepatotoxic MCs associated with harmful blooms of *Microcystis* are implicated as one of the most common cyanotoxins with more than 75 variants, and microcystin-leucine arginine (MC-LR) is emerging as the most toxic analogues among the various isoforms of MCs (Chorus and Bartram, 1999; Svrcek and Smith, 2004). A provisional guideline value of 1.0 μg L^{-1} has been issued by the World Health Organization (WHO) for the toxicity of MC-LR (WHO, 1998). However, the toxicological evidence is insufficient to establish guidelines for other microcystin analogues such as microcystin-RR (MC-RR); consequently, a drinking water standard of 1.0 μg L^{-1} has been set in some countries such as Spain for the total MCs (BOE, 2003; Acero et al., 2005).

In general, technologies currently being used to treat

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cyanobacterial issues by water utilities include nutrient control, and physical, biological and chemical strategies with each of them having benefits and drawbacks. Chemical options like oxidants (e.g. chlorine, potassium permanganate, and ozone) have not only been widely used as efficient disinfectants in water treatment systems, but also been regarded as effective pre-oxidants which could improve the overall removal of cyanobacterial cells during successive conventional water treatment processes (Plummer and Edzwald, 2002; Chen and Yeh, 2005; Chen et al., 2009). However, some studies have indicated that chlorine treatment may destroy cyanobacterial cells resulting in the release of intracellular metabolites into the surrounding water (Daly et al., 2007; Lin et al., 2009). This unwanted consequence should be avoided due to the inability of conventional water treatment processes to remove dissolved toxins (Chow et al., 1999). Nevertheless, studies have also shown that MCs are susceptible to chemical oxidation, and chlorine can oxidise the resultant dissolved toxins efficiently if appropriately applied (e.g. sufficient chlorine residual amount and contact time) (Fan et al., 2014a; Zamyadi et al., 2013).

Microcystis generally occurs in colonies of different sizes under natural conditions (Reynolds, 2007; Wu et al., 2007). *Microcystis* forms colonies with mucilage or sheaths which can increase buoyancy and floating velocity, in addition to defense the environmental stress including zooplankton grazing, bacterial attack and nutrient deficiencies (Cyr and Curtis, 1999; Kearns and Hunter, 2001; Yamamoto et al., 2011; Ma et al., 2014). Recent literature have reported that apparent variations were found between individual and colonial *Microcystis* cells in morphology, and physical and biochemical characteristics (Otsuka et al., 2000; Zhang et al., 2007). However, most of the previous laboratory studies have mainly focused on the influence of water treatment processes on individual cyanobacterial cells. For instance, the quantitative measurements of the viability of *Microcystis* cells and fate of MCs during oxidation treatments are limited to individual cells (Lin et al., 2009) with the following reasons. First, the large irregularly shaped *Microcystis* colonies may have tens of thousands of cells per colony in a three-dimensional matrix and it is very difficult to determine their exact cell numbers using microscopy. Also, flow cytometry as an efficient equipment (Daly et al., 2007) used for cyanobacterial viability evaluation is limited to events with small sizes (~0.1–50 μm) and is not suitable for large colonies. Moreover, laboratory cultures of *Microcystis* predominantly exist as individual cells after long-term cultivation due to the favorable growth condition (Reynolds et al., 1981). There are doubts as to whether the water treatment parameters targeted in laboratory cultures with individual cells are suitable to the real cyanobacterial blooms with colonial cells. Approaches to the colonial cyanobacteria assessments are rare in the literature due to the above-mentioned list of limitations. Joung et al. (2006) attempted to break cyanobacterial colonies via vortexing, sonication and boiling in order to measure the natural cyanobacterial samples; some of them have shown the promise to break cyanobacterial colonies. However, whether these techniques could destroy the cyanobacterial membranes and induce cell lysis had not been assessed. Therefore, it is necessary to develop an effective method to measure the natural colonial *Microcystis* samples without affecting membrane integrity of the cells.

Climate change and nutrient loads into source waters continue to favour cyanobacterial proliferation increasing the shortage of drinking water supply (Paerl and Huisman, 2008; Carey et al., 2012). Chlorine is likely to remain in use because of its high efficiency applied either as a pretreatment oxidant or disinfectant (Fan et al., 2013b, 2014b). Therefore, it is of great importance to determine the appropriateness of the cyanobacteria oxidation with chlorine. To date, no studies have systematically assessed the impacts of chlorination on colonial *Microcystis* cells and associated metabolites in the publication domain. Consequently, the aims of the present study were to: (1) develop a technique to assess the membrane integrity of colonial *Microcystis* cells; (2) investigate and compare the impacts of chlorination on the *Microcystis* with various colonial sizes; (3) evaluate the effect of chlorination on the fate of the major metabolites, MCs, of colonial *Microcystis*.

2. Materials and methods

2.1. Water source and quality

Water was sourced from Cheng Kung Lake (CKL) (cyanobacterial blooms occurred there historically), National Cheng Kung University Campus, Tainan, Taiwan and then filtered through the following filters to remove the particles and microorganisms present in the water: 1 μm glass fiber filter (GB-140, Advantec, Japan), 0.45 μm and then 0.2 μm cellulose acetate filters (Sartorius Stedim Biotech, Germany). The filtered CKL water was then stored in a refrigerator (4 °C) and used up in one week for each batch experiment. A total organic carbon (TOC) concentration ranging from 4.85 to 7.12 mg L^{-1} and pH of 7.8–8.8 was detected from the filtered water.

2.2. Materials and reagents

Colonial *Microcystis aeruginosa* (strain TWNCKU12) samples were collected from the Moodan Reservoir, located at Pingdong, Taiwan, as *M. aeruginosa* blooms occur frequently at that site. The samples were then isolated and purified by a micropipette method (Andersen, 2005). The mentioned strain was identified to produce MCs (MC-LR:MC-RR = 1:6). The purified *M. aeruginosa* cells were cultured in ASM-1 media (see Table S1, supplementary material) and routinely sub-cultured to maintain growth in logarithmic phase (Gorham et al., 1964). Cultures were incubated under a constant cool-fluorescent light flux (1500 lux) on a 12 h:12 h light-dark cycle at a constant temperature of 25 ± 1 °C to achieve healthy condition with high cell densities. Then the *M. aeruginosa* cultures were separated by sequential screening through 30 mesh (550 μm), 50 mesh (270 μm) and 400 mesh (37 μm) sieves (Bunsekifurui Kuang Yang, Taiwan). The largest colonies could pass through the 30 mesh screen completely but got trapped by the 50 mesh screen. Therefore, the colonial cells were classified into three groups with sizes <37, 37–270 and 270–550 μm. The meshes/filters with cells were immediately washed with either ASM-1 media or CKL water (depending on the experimental media used in the oxidation experiments) for several times to transfer all of the cells into a beaker, and then the cells were diluted with the ASM-1 media or CKL water to achieve an initial cell density of 100,000 cells mL^{-1} for the following experiments. The algal cultures were adjusted to pH 7.6 ± 0.1 using either 0.1 M sterile filtered hydrochloric acid or sodium hydroxide. All experiments were performed at room temperature of 25 ± 1 °C. All chemicals and reagents used in this study were analytical grade and solutions were made using ultra-pure water purified by a Milli-Q water purification system (Millipore Pty Ltd, USA).

2.3. Oxidation experiments

For chlorine oxidation experiments, one-liter glass vessels were applied as reactors and the *M. aeruginosa* laden water samples were mixed with a Teflon-coated magnetic stirrer at a low speed continuously. The samples were dosed with sodium hypochlorite (Riedel-de Haen, Germany) to achieve the desired chlorine concentrations (0.3, 0.5, 1.0 and 2.0 mg L^{-1}) and incubated in darkness.

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