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Colonial cell disaggregation and intracellular microcystin release following chlorination of naturally occurring *Microcystis*

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ABSTRACT

Colonial cell disaggregation and release of intracellular microcystin were evaluated following chlorine treatment of naturally occurring *Microcystis*. Microscopic observations of water samples collected from Lake Mead, Nevada, USA, confirmed the presence of colonial *Microcystis* with cells protected by an outer sheath up to 30 μm thick. During chlorination, two stages of cell decomposition were observed, stage 1: colonial cell disaggregation, and stage 2: unicellular degradation. Following a $[Cl_2]_0$:DOC_0 ratio of 0.15 (t = 20 min, pH = 8.2–8.5) in unfiltered Lake Havasu samples, total particle count increased from $(1.0 \pm 0.11) \times 10^5$ to 4.2×10^5 particles/mL and fluorescent particle count increased from $(1.2 \pm 0.50) \times 10^4$ to 1.2×10^5 particles/mL, illustrating colonial cell disaggregation. Although total and fluorescent particles increased, the concentration of chlorophyll-a (Chl-a) decreased from 81 μg/L to 72 μg/L, and continued to decrease at higher $[Cl_2]_0$:DOC_0 ratios. The preliminary second order rate constant for the reaction between *Microcystis* and chlorine in natural waters was estimated using either Chl-a ($k = 15\ M^{-1}\ s^{-1}$) or fluorescence particle count ($k = 38\ M^{-1}\ s^{-1}$) as an indicator of cell damage following colonial disaggregation (i.e., at $[Cl_2]_0$:DOC_0 ratio ≥0.15). Complete release of intracellular microcystin-LR (MC-LR) was observed in both Lake Havasu and Lake Mead samples when applying a $[Cl_2]_0$:DOC_0 ratio of 0.30 (t = 20 min), which was equivalent to a chlorine exposure of 8 min-mg/L for Lake Havasu samples. With chlorination, DOC increased by 3–18% indicating release of either colony-bound or cell-bound DOC. The results demonstrated the ability of chlorine to disaggregate/inactivate natural *Microcystis* colonies, and identified oxidation conditions resulting in complete release of intracellular MC-LR.

1. Introduction

The occurrence of cyanobacterial blooms has been increasing in freshwaters around the world, which has raised public health concern by drinking water treatment utilities, because certain blooms are often accompanied with a production and release of toxic cyanobacterial metabolites called cyanotoxins. By far, microcystin-LR (MC-LR) is the most studied cyanotoxin and has also been considered the most potent among microcystin congeners (USEPA, 2015). In 1998, the World Health Organization established a guideline safety value of 1 μg/L for MC-LR in drinking water (WHO, 1998). In August 2014, the drinking water treatment plant in the City of Toledo, Ohio failed to completely remove MC-LR from its finished water and consequently, more than 400,000 residents in the affected area were advised not to drink or boil their tap water (Ohio 2014). In June 2015, the United States Environmental Protection Agency (USEPA) issued a health advisory level for total microcystins (although MC-LR was used as a surrogate for total MCs) of 0.3 μg/L for bottle-fed infants and pre-school age children ≤ 6 years old and 1.6 μg/L for school-age children through adults (USEPA, 2015). Due to the more stringent health advisories for microcystins in drinking water, additional information is needed regarding the fate of cyanobacteria cells and cyanotoxins in full-scale drinking water treatment facilities during natural bloom events.

Chlorine is the most commonly applied oxidant in water treatment plants. The reaction of free chlorine with *Microcystis aeruginosa* was proposed to initiate an instantaneous penetration of chlorine through slime layers, cell walls and membranes, followed by the chlorination of the intracellular organic contents (t = 20 min) and lastly cytoclasis (Ou et al., 2011). When cyanobacteria exist in colonies, both the kinetics and mechanism of cell

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damage could vary from the unicellular form. Colonies can be different in size and morphology and can disaggregate into unicellular cells when cultured in artificial growth media (Liu et al., 2016; Wang et al., 2015). These colonies are protected by a sheath, the complex composition of which has been studied from its identification, synthesis, to its ecological roles (Kehr et al., 2006; Ma et al., 2014; Pereira et al., 2009; Shahzad-ul-Hussan et al., 2011). Extracellular polymeric substances (or extracellular polysaccharides, or exopolysaccharides, abbreviated as EPS) that can loosely attach to the cell surfaces have been found to be the main constituents of the sheath (Pereira et al., 2009). EPS mainly consist of high-molecular-mass heteropolysaccharides, with elemental C, H, and O. The number of monosaccharides, and the dry weight percentage from uronic acids and deoxysugars can differ significantly from one cyanobacteria species/strain to another. In some cases, varied percentages of sulfate, pyruvate, acetate and even peptides can be found in the sheath material (Pereira et al., 2009). Wang et al. (2015) used GC/MS to identify the extracts of *Microcystis* cultures and found seven compounds to be exclusively present in colony samples. These included amides and phenols, which were proposed to be responsible for maintaining the mucilage of *Microcystis* colonies in lakes (Wang et al., 2015). Lectins are mono- or oligosaccharide binding proteins commonly found in microorganisms. Two or more carbohydrate binding sites of lectin molecules allow the cross-linking of the sugar moieties on cyanobacteria cell surfaces after the sugars are secreted from the cells (Kehr et al., 2006; Rastogi et al., 2014). Microvirin, for example, has been discovered to be an important lectin in *Microcystis aeruginosa* PCC7806 and responsible for the formation of *Microcystis* colonies (Kehr et al., 2006). Some other linking pigments include mycosporine-like amino acids and scytonemin (Rastogi et al., 2014). The different compositions of the sheath may present different resistance toward external chemical treatment based on the reaction kinetics of chlorine with organic compounds (Deborde and Von Gunten, 2008; Pereira et al., 2009).

With most of the reported studies on the treatment of cyanobacteria by chlorine conducted using unicellular laboratory cultured cyanobacteria (Coral et al., 2013; Wert et al., 2013, 2014; Wert and Rosario-Ortiz, 2013; Zamyadi et al. 2012, 2013), there is a strong scientific gap on the treatment of naturally occurring cyanobacteria colonies that are protected by a colonial sheath or slime layer, as observed in a localized cyanobacterial bloom in Lake Mead, Nevada, USA (Fig. 1). A research study was subsequently performed to investigate the effect of chlorine oxidation on naturally occurring colonial *Microcystis*. The main objectives were to evaluate the inactivation of *Microcystis* colonies by chlorine and investigate the fate of extracellular, intracellular, and total MC-LR.

2. Materials and methods

2.1. Sample collection and preparation

On March 17, 2015, water samples containing naturally occurring cyanobacteria cells were collected from the Hemenway Harbor shorelines at Lake Mead, Nevada in 10 L plastic cubitainers. Microscopic analysis of similar samples from the bloom confirmed the cells were predominately colonial *Microcystis* and were surrounded by a mucilaginous sheath extending approximately 30 μm beyond the cell margins (Fig. 1). The samples contained a significant amount of floating cells and debris that had a white appearance, indicating the samples might have reached the end of the stationary growth phase or the decay period. The samples were allowed to settle until there was an obvious separation of the floating cells from water (Fig. S1, in supporting information, SI). The lower layer with fewer cells was filtered through a glass microfiber filter (1.5 μm, Whatman®, Marlborough, MA, USA) by a vacuum pump and the filtrate was defined as a filtered Lake Mead sample (LM-F) for the evaluation of extracellular toxin removal. The upper layer with dense cells was completely mixed and used as an unfiltered Lake Mead sample (LM-UF) to assess the inactivation of colonial *Microcystis* cells, the potential release of organic contents, and the subsequent degradation of MC-LR. On March 25, 2015, another sample was collected from the London Bridge area at Lake Havasu, Arizona. This sample appeared to contain heathier cells than the Lake Mead sample based on its green appearance (Fig. S2). The existence of *Microcystis* colonies in this sample was demonstrated by the digital flow cytometry analysis and the result is shown in Fig. S3. The original samples were completely mixed and used directly as an unfiltered Lake Havasu sample (LH-UF).

2.2. Experimental methods

Oxidation experiments were performed as batch processes at room temperature (20–25 °C) and ambient pH (Accumet® AP110, Fisher Scientific). Chlorination experiments were performed using a stock solution generated from 5.6% sodium hypochlorite (NaOCl,

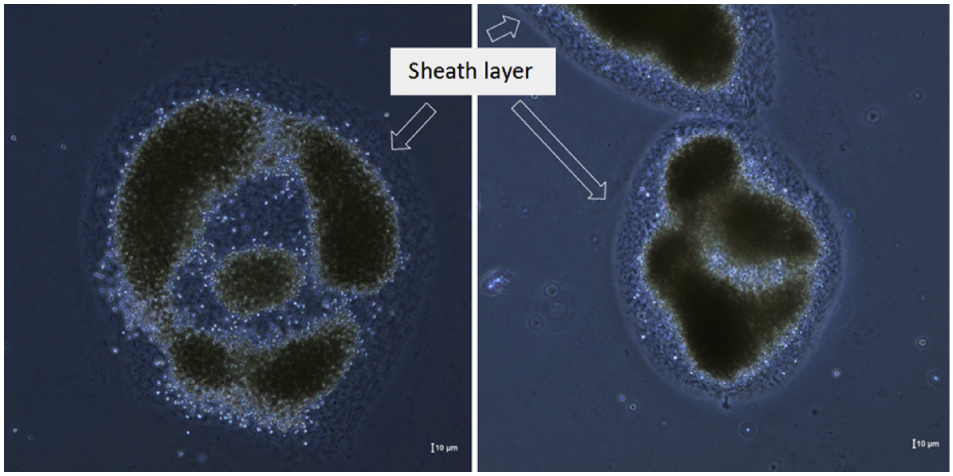

Fig. 1. Microscopic image of cyanobacteria colonies in Lake Mead sample. Dimensions: cells (average 4.5 μm); sheath extending an average of 30 μm beyond cell margins (Photo courtesy of Ann St. Amond, PhycoTech, Inc., St. Joseph, Michigan, USA).