Chemosphere 92 (2013) 529–534

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Impact of potassium permanganate on cyanobacterial cell integrity and toxin release and degradation

Jiajia Fan [a], Robert Daly [b], Peter Hobson [b], Lionel Ho [a,b], Justin Brookes [a,*]

[a] Water Research Centre, The Environment Institute, The University of Adelaide, SA 5005, Australia
[b] Australian Water Quality Centre, SA Water Corporation, GPO Box 1751, SA 5001, Australia

HIGHLIGHTS

- $KMnO_4$ can degrade microcystins in a large range of CT values in *Microcystis* cultures.
- Kinetics modelling of cyanotoxins release and degradation was developed.
- A scenario was applied using the model to show its use as a tool for water supplies.

ARTICLE INFO

Article history:
Received 11 November 2012
Received in revised form 14 March 2013
Accepted 19 March 2013
Available online 7 May 2013

Keywords:
Cyanobacteria
Cyanotoxins
Potassium permanganate
Cell integrity
Pretreatment

ABSTRACT

Potassium permanganate ($KMnO_4$) is commonly used as a pre-treatment oxidant to remove soluble manganese (Mn) and iron (Fe) which can contribute to dirty water in drinking water supplies. Because Mn and Fe problems are commonly associated with thermal stratification in summer and autumn, they frequently coincide with the presence of cyanobacteria. The use of $KMnO_4$ as an oxidant for Mn and Fe control therefore needs to consider the potential impacts on cyanobacterial cell integrity and toxin release. This study aims to assess the effect of $KMnO_4$ on cyanobacteria cell integrity, toxin release and toxin oxidation. A toxic strain of *Microcystis aeruginosa* was exposed to various concentrations of $KMnO_4$ and the cell integrity of cyanobacteria was measured with flow cytometry. Further the intra- and extra-cellular toxin concentrations were quantified and it was apparent that $KMnO_4$ reduced both the intra- and extra-cellular toxins at low initial concentrations of 1 and 3 mg L^{-1} without complete cell lysis. However, the cell integrity of cyanobacteria was compromised at $KMnO_4$ concentrations of 5 mg L^{-1} and 10 mg L^{-1} and led to intracellular toxin release. In the 10 mg L^{-1} $KMnO_4$ treatment, the total toxin was oxidised after 7 h contact time. A model describing the two step process of release and degradation was developed and may provide a tool to assess the risk water quality posed by toxin release. Consequently, it may be possible to use $KMnO_4$ as a pre-treatment for Mn and Fe at concentrations <3 mg L^{-1} and short contact time when cyanobacteria are also present.

1. Introduction

Cyanobacteria in drinking water supplies can create a water quality hazard because they produce metabolites or toxins that are not readily removed by conventional water treatment processes (Himberg et al., 1989; Chow et al., 1999; Zamyadi et al., 2012). The preferred option for the removal of cyanobacterial toxins, taste and odours involves the removal of whole, intact cells to avoid release of intracellular metabolites into surrounding waters. However, sometimes other water quality objectives require the use of an oxidant as a pretreatment that can affect the cell integrity of cyanobacterial cells. For example, thermal stratification in reservoirs promotes the growth of cyanobacteria which are able to exploit the relative stability of the water column, these conditions also promote the release of Mn and Fe from sediments which can contribute to dirty water problems (Carlson and Knocke, 1999; Ellis et al., 2000). Potassium permanganate ($KMnO_4$) is a common pre-oxidant used for Mn and Fe control (Ellis et al., 2000; Roccaro et al., 2007). However, $KMnO_4$ can also oxidise cyanobacterial cells and induce leakage of intracellular metabolites which increases the concentration of dissolved metabolites in the surrounding water body (Chen and Yeh, 2005, 2006; Chen et al., 2009; Ho et al., 2009) although $KMnO_4$ can also degrade toxins (Rodriguez et al., 2007a,b).

Although the most widespread application is for the oxidation of Mn (Carlson and Knocke, 1999; Ellis et al., 2000; Lee et al., 2003; Roccaro et al., 2007), $KMnO_4$ is also used to fulfil a variety

* Corresponding author. Address: Benham Building, School of Earth and Environmental Sciences, The University of Adelaide, SA 5005, Australia. Tel.: +61 8 8313 3747; fax: +61 8 8313 6222.
E-mail address: justin.brookes@adelaide.edu.au (J. Brookes).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.03.022

of other objectives including control of algae and associated metabolites (Cherry, 1962; Chen and Yeh, 2005; Chen et al., 2005; Rodriguez et al., 2007b; Ho et al., 2009), antibiotics (Hu et al., 2010), colour (Liu et al., 2011), and disinfection by-products (Chu et al., 2011) (see Table 1, Supplementary material). To date, minimal studies have assessed the effects of $KMnO_4$ on cyanobacteria cells and the toxins systematically. Therefore, it is necessary to determine the appropriateness for pre-oxidation with $KMnO_4$ for instances when Mn and Fe and cyanobacteria are simultaneously present. In this study, the effects of $KMnO_4$ pre-treatment on cyanobacterial cell integrity, the concomitant toxin release and toxin degradation was investigated.

2. Experimental methods

2.1. Materials and reagents

A toxic strain of *Microcystis aeruginosa* Kutz. emend Elenkin (strain 338 from the Australian Water Quality Centre culture collection) was used in this study and routinely cultured in ASM-1 medium (Gorham et al., 1964) to maintain logarithmic growth. All cultures were incubated under constant cool-fluorescent light intensity 50 μmol m^{-2} s^{-1} on a 12 h:12 h light–dark cycle at a constant temperature of 20 ± 1 °C, and bubbled with air. Prior to use in experiments, the algal cultures were adjusted to pH 7.5 ± 0.1.

Samples for cell counts by microscopy were treated with Lugol's iodine, pressurised to 900 kPa for 2 min to collapse gas vesicles and then counted at 400× magnification (Brookes et al., 1994). Cultures with an initial cell density of 7.0×10^5 cells mL^{-1} were used in all experiments. All experiments were performed at room temperature 20 ± 2 °C. All chemicals and reagents used were analytical grade and solutions were made using ultra-pure water purified to 18 MΩ cm by a Milli-Q water purification system (Millipore Pty Ltd., USA).

Potassium permanganate stock solution (1.0 g L^{-1}) was prepared by dissolving crystal $KMnO_4$ in Milli-Q water. It was then standardised by titration with sodium oxalate and diluted as required for the experiments and kept under darkness at 4 °C. Sodium thiosulfate stock solution (4.0 g L^{-1}) was prepared by dissolving $Na_2S_2O_3{\cdot}5H_2O$ in Milli-Q water and used to quench the reaction of permanganate and arrest further oxidation of cyanobacterial cells. $KMnO_4$ concentrations in the samples were measured after filtration the sample through 0.2 μm nylon syringe filters (Whatman, UK) to remove manganese oxide. A standard curve was constructed by diluting the stock solution to generate a range of KMnO4 concentrations from 0 to 20 mg L^{-1} and compared to the linear relationship of absorbance at 525 nm in a 10 mm cuvette in a UV/VIS spectrophotometer (Thermo Scientific, UK).

2.2. Pre-oxidation experiments

Typically low concentrations of $KMnO_4$ (0.1–1.0 mg L^{-1}) were used in pre-treatment (Carlson and Knocke, 1999; Ho et al., 2009; Liu et al., 2011), however, high concentrations have been applied in some circumstances: e.g. 13 mg L^{-1} (Cherry, 1962); between 5 and 10 mg L^{-1} (Chen et al., 2005). The NSF/ANSI Standard 60 on drinking water treatment chemicals, recommends a maximum $KMnO_4$ concentration of 50 mg L^{-1} be used for disinfection and oxidation (NSF, 2002). Based on the $KMnO_4$ concentrations used to oxidise the suite of contaminants detailed above, concentrations between 1 and 10 mg L^{-1} were considered appropriate for use in this study.

Microcystis aeruginosa samples were treated with the desired $KMnO_4$ concentrations (1, 3, 5 and 10 mg L^{-1}) and mixed with a magnetic stirrer at a low speed. Samples were withdrawn at intervals, over 24 h, and immediately analysed to determine $KMnO_4$ residual. At each time interval, 1 mL of each sample was taken for cell integrity measurement and 2 mL for cell microscopic counts. Samples with a volume of 200 mL were quenched with $Na_2S_2O_3{\cdot}5H_2O$ and used for microcystin analysis. These samples were divided in two subsamples: 100 mL sample was immediately filtered through a GFC filter (Whatman, UK) to remove extracellular materials and concentrate cells on the filter for intracellular microcystin analysis. The remaining 100 mL sample was freeze-dried for total microcystin determination (both the intracellular and extracellular toxin).

2.3. Microcystin extraction and analysis

The filter from the 100 mL for intracellular toxin analysis described above and the freeze-dried cells for total toxin measurement were added to a 5 mL solution made with 47.5% of Milli-Q water, 47.5% of methanol and 5% of formic acid. The solution was kept overnight and then sonicated on ice using Virsonic 475 (VirTis, US) sonicator for 4 min, with 0.5 s bursts punctuated with no pulsation for 0.5 s. The solutions were diluted with Milli-Q water to a final volume of 100 mL and filtered with a 0.45 μm membrane filter (Advantec MFS Inc., USA). Microcystins (>90% MC-LR plus <10% MC-LA) were concentrated from water samples by C18 solid-phase extraction according to the methods described by (Nicholson et al., 1994) prior to toxin analysis. A high-performance liquid chromatography (HPLC) system, consisting of a 600 pump controller, 717plus autosampler, 996 photodiode array detector (Waters Pty Ltd., Australia) and a 150 × 4.6 mm Luna C18 column (Phenomenex, Australia), was used to analyse the toxin samples using procedures and conditions previously documented by Ho et al. (2006).

2.4. Flow cytometry

A FACSCalibur flow cytometer (Becton Dickinson, USA) equipped with an air cooled 15 mW argon laser emitting at a fixed wavelength of 488 nm was used for measurement. Fluorescent filters and detectors were all standard with green fluorescence collected in channel FL1 (530 nm) and red fluorescence collected in channel FL3 (670 nm). Probe fluorescence, chlorophyll *a* fluorescence, forward scatter- FSC (cell size) and side scatter- SSC (cell granularity) (Brookes et al., 2000) data was collected and analysed using CellQuest software (Becton Dickinson, USA) and Cyflogic (CyFlo Ltd., Finland). FSC and FL3 were used to identify and gate the cyanobacterial cells. FL1 was used to quantify the fluorescent intensity of Sytox Green nucleic acid stain (Molecular Probes, USA). Sytox Green was used to determine the percentage of viable (Sytox negative) to nonviable (Sytox positive) cells in a sample as described by Regel et al. (2004) and Daly et al. (2007). Sytox Green was added to achieve a final concentration of 0.2 μM and measured on the flow cytometer after 10 min incubation. Unstained control was also measured to compare with treated samples.

2.5. Modelling

The kinetics of dissolved toxin degradation has been shown to follow a first order decay model using CT of $KMnO_4$ (similar to Eq. (2)) (Rodriguez et al., 2007a). Similarly, the CT concept is also applied to cell inactivation in water treatment using the Chick-Watson equation (AWWA, 1999). If these assumptions are valid then process of toxin release and degradation may be considered as consecutive reactions (Eq. (1)), where the concentration of intracellular (A) and extracellular (B) toxin are described by Eq. (2) and (3) (Jones, 1970; Zamyadi et al., 2013).