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The Impacts of Potassium Permanganate and Powdered Activated Carbon on Cyanotoxin Release

Nicholas R. Dugan [Environmental Engineer],

U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, 513-569-7239, dugan.nicholas@epa.gov

Samantha Smith [Environmental Scientist], and

Pegasus Technical Services, 46 East Hollister Street, Cincinnati, OH 45219, 513-569-7681, smith.samantha@epa.gov

Toby T. Sanan [Physical Scientist]

U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, 513-569-7667, sanan.toby@epa.gov

Abstract

Bench-scale trials were performed to: (1) Expose *Microcystis aeruginosa* cells to potassium permanganate doses of 1, 3 and 5 mg/L, at contact times of 15, 30 and 90 minutes, pH levels of 7 and 9, and turbidities of 0.1, 5 and 20 NTU; (2) Compare the impacts of oxidation alone and oxidation plus powdered activated carbon for the final 60 minutes of contact time and (3) evaluate the impact of these treatment conditions on extracellular microcystins, extra- plus intracellular (combined) microcystins, cell membrane integrity and chlorophyll-*a* concentrations. Toxin releases from the cells were observed at both pH levels. The greatest toxin releases were observed at the lowest KMnO₄ doses. The toxin releases were accompanied by relatively stable total cell counts, increases in membrane compromised cells and decreases in chlorophyll-*a*. The application of 10 mg/L PAC resulted in extracellular toxin concentrations that were markedly lower than those observed in oxidant-only situations.

Introduction

Toxin producing cyanobacteria pose both particulate and dissolved contaminant control challenges for drinking water utilities. Cells with intact membranes propagate through the conventional treatment process (coagulation/flocculation/sedimentation/filtration) as particulates and can be effectively removed with minimal release of toxins (Drikas et al, 2001; Newcombe et al, 2015). Complications arise when cyanobacterial cells are exposed to

Corresponding author contact information: Nicholas R. Dugan, PE, Environmental Engineer, U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, 513-569-7239, dugan.nicholas@epa.gov.

Co-author contact information: Samantha Smith, Environmental Scientist, Pegasus Technical Services, 46 East Hollister Street, Cincinnati, OH 45219, 513-569-7681, smith.samantha@epa.gov, Toby T. Sanan, Physical Scientist, U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, 513-569-7667, sanan.toby@epa.gov

⁴Enzo, Farmingdale NY, USA

⁵Sigma, St. Louis MO, USA

a variety of environmental stressors that include salinity changes (Chen et al, 2015), pH changes (Qian et al, 2014), exposure to herbicides (Ye et al, 2014; Wu et al, 2016), sulfate (Chen et al, 2016), algicides (Fan et al, 2013a; Qian et al, 2010) and water treatment oxidants (Zamyadi et al, 2012; Korak et al, 2015). Exposure to these stressors is associated with detrimental changes in a suite of indicators that range from measures of photosynthetic efficiency to release of cellular contents, including toxins.

Of the water treatment oxidants, permanganate (MnO_4^-) is commonly applied in the earliest stages of the drinking water treatment process, where concentrations of intact cells and the concomitant potential for release of cellular contents is high. Permanganate is typically applied as a potassium salt (KMnO_4), sodium salt (NaMnO_4) or proprietary formulation, and is used to control mussels, taste- and odor-causing compounds, disinfection by-product precursors, and to oxidize reduced iron and manganese. The widespread use of permanganate (Singer and Reckhow, 2011), combined with the potential for toxin release, has motivated a number of studies that evaluated the release of intracellular material from cultured *Microcystis aeruginosa* (*M. aeruginosa*) cells as a function of permanganate dose and contact time. Results from these studies are summarized in Table 1.

This body of work, with one exception performed at neutral or near neutral pH, established that exposure of *M. aeruginosa* to KMnO_4 resulted in the loss of cellular membrane integrity, loss of cellular function and release of cellular contents, including toxins, into aqueous solution. The magnitude and timing of observed toxin releases varied significantly between studies. Not specifically addressed in the aforementioned work were the potential impacts of higher pH, suspended particulates or powdered activated carbon (PAC) addition. Western Lake Erie treatment facilities experience above-neutral influent pH. For example, the City of Toledo recorded 10th, 25th, 50th, 75th and 90th percentile influent pH values of 7.9, 8.0, 8.1, 8.3 and 8.6, respectively from May 1, 2016 through May 23, 2017 (National Oceanic and Atmospheric Administration, 2017). Data extracted from the National Lakes Assessment (USEPA, 2012) indicates median, 75th, 90th and 95th percentile pH values of 7.7, 8.4, 8.8 and 9.1, respectively. These pH results provided the motivation to incorporate pH values of 7 and 9 in the designs of experiments that investigated the oxidation of cyanobacterial cells. With regards to turbidity, several of the previously discussed studies used natural water or mixtures of growth media and natural water as the aqueous matrix in which *M. aeruginosa* cells were suspended. However, none of these studies formally incorporated turbidity variation into their experimental designs. Turbidity is a proxy for the concentration of suspended inorganic and organic particulate material, both of which have the potential to exert oxidant demand that competes with that of suspended cyanobacterial cells and their metabolic products. Turbidities, in the raw water itself or due to coagulant addition, are highest in the early stages of drinking water treatment, when KMnO_4 is typically added. Also, the early stages of drinking water treatment are often used as a point of PAC application. PAC has the potential to adsorb cyanobacterial toxins (Westrick et al, 2010) and neutralize KMnO_4 . However, the application of PAC will increase sludge production and sludge management costs. Finally, KMnO_4 contact times prior to coagulant addition vary widely, from just a few minutes, to more than 90 minutes in systems that feed KMnO_4 at lake intakes.

Cognizant of the potential benefits and complications of KMnO_4 and PAC, and charged with helping implement the Harmful Algal Bloom and Hypoxia Research and Control Amendment Act of 2014 (U.S. Congress, 2014), the U.S. Environmental Protection Agency (USEPA) undertook a series of bench-scale trials with the following objectives: (1) Expose suspensions of *M. aeruginosa* to KMnO_4 doses of 1, 3 and 5 mg/L, at contact times of 15, 30 and 90 minutes, pH levels of 7 and 9, and turbidities of 0.1, 5 and 20 NTU; (2) Compare the impacts of oxidation alone and oxidant plus PAC for the final 60 minutes of contact time at all the previously described experimental conditions and (3) evaluate the impact of these treatment conditions on extracellular MCs, extra- plus intracellular (combined) MCs, cell membrane integrity and chlorophyll-*a* concentrations.

Methods and Materials

Unless otherwise noted, all chemical solutions and PAC dosing slurries were prepared with laboratory grade water¹ and reagent grade constituents. Stock oxidant dosing solutions were prepared by dissolving crystalline KMnO_4 to achieve a concentration of 1 mg/mL. Concentrated phosphate and borate buffers were used to adjust pH to 7.0 ± 0.2 and 9.0 ± 0.2 , respectively. The phosphate buffer composition was 0.32 M potassium dihydrogen phosphate (KH_2PO_4) and 0.18 M sodium hydroxide (NaOH). The composition of the borate buffer solution was 1.0 M boric acid (H_3BO_3) and 0.4 M NaOH. PAC dosing slurries were prepared at a concentration of 1 mg/mL using a coal-based PAC² (iodine number = 950 mg/g, surface area = 950 m²/g, average pore size = 23 Å). All bench-scale trials were carried out using de-chlorinated tap water as the aqueous suspension medium. De-chlorinated tap water was prepared by passing City of Cincinnati tap water through a bed of granular activated carbon, and testing each batch for chlorine residual using N,N-diethyl-p-phenylenediamine salt (DPD) indicator³. Average water quality indicators for the de-chlorinated tap water prior to *M. aeruginosa* or turbidity addition were: turbidity < 0.1 NTU, total organic carbon (TOC) = 0.82 mg/L, copper (Cu) < 1.9 µg/L, total hardness = 110 mg/L as CaCO_3 .

The seed culture for the *M. aeruginosa* used in all trials was originally obtained from the University of Texas Algal Culture Collection (UTEX B2666) and propagated in BG-11 growth medium⁶ at 20 °C in 250-mL De Long flasks⁷. Illumination was provided by muted fluorescent lamps⁸ on 12 hour diurnal cycles at a light-phase illumination intensity totaling 5.4 µmol photons/m² x s across the 400 – 700 nm photosynthetically active radiation wavelength range⁹. Cells were harvested during the late stationary phase and were centrifuged and rinsed three times in de-chlorinated tap water. Each centrifugation cycle was performed for 15 minutes at 2900 × G and 20 °C¹⁰. Following the final resuspension in de-chlorinated tap water, the cell titer of the stock suspension was determined by flooding

¹Thermo-Barnstead, Waltham MA, USA

²Norit PAC 20B, Cabot, Boston MA, USA

³Hach, Loveland CO, USA

⁶Sigma, St. Louis MO, USA

⁷Bellco, Vineland NJ, USA

⁸Phillips - Natural Light, Somerset NJ, USA

⁹LI-190R, LI-COR, Lincoln NE, USA

¹⁰Thermo-Jouan, Waltham MA, USA

duplicate wells on a hemacytometer¹¹ and counting at 400X magnification¹² all cells that fluoresced red under green epi-fluorescent illumination using a 545 nm excitation/562 nm dichroic/570 nm emission optical filter set¹³. This technique was also used to quantify cell titers during jar tests. All cell counts obtained this way will be referred to in the remainder of the manuscript as “total cell counts.”

The turbidity suspension was produced using particulate matter separated from raw Ohio River water. Twenty liters of river water was divided into aliquots in 250-mL centrifuge tubes¹⁴. The tubes were centrifuged for 15 minutes at 2900 × G at 20 °C. The supernatant was discarded and all pellets were floated and transferred to a single container until a volume of 250 mL was obtained. This particulate stock suspension was stored in the dark at 2 °C and used for all trials where turbidities were adjusted to 5 or 20 NTU.

During the bench-scale trials, residual and stock solution concentrations of KMnO₄ were determined spectrophotometrically and by sodium oxalate titration, respectively, according to Standard Method 4500-KMnO₄ (Standard Methods, 2017). Chlorophyll-*a* concentrations were determined by filtration through 0.7 µm nominal pore size glass fiber filters¹⁵, followed by fluorometric analysis¹⁶ according to EPA Method 445.0 (USEPA, 1997) with one modification: instead of disrupting filters with a tissue grinder, the glass fiber filters with retained cells were transferred to micro centrifuge tubes¹⁷, amended with 0.3 – 0.5 g glass beads¹⁸ and lysed on a bench-top homogenizer¹⁹ at 4 m/s for 20 seconds. Cell membrane integrity was determined by staining cell suspensions with a dead cell stain²⁰ at a ratio of 1µL of stain:mL of sample and incubating in the dark for 15 minutes. Cells with compromised membranes took up the stain, fluoresced green under blue epi-fluorescent illumination using a 480 nm excitation/510 nm dichroic/535 nm emission optical filter set²¹ and were counted in duplicate wells at 400X magnification on a hemacytometer. These green-fluorescing cells were defined as “SYTOX (+)”, after the brand name of the stain.

MC concentrations were determined by commercially available enzyme linked immuno-substrate assay (ELISA) 96-well plate kits²². For the remainder of this manuscript, the term “toxin” refers to MCs quantified by this method. The ELISA assays were performed according to the manufacturer’s instructions with the exception that extra blanks and positive control samples were added to each plate. The assay detected all MC congeners with the ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldecca-4,6-dienoic acid) functional group and was, as a result, a measure of total MCs plus nodularin. The ELISA assay was used to quantify extracellular and combined (extra- plus intra-cellular) toxins. Extracellular toxin samples were prepared by passing raw sample through 0.7 µm nominal pore size glass

¹¹Hausser, Horsham PA, USA

¹²Zeiss Axioskop, Zeiss, Thornwood NY, USA

¹³Chroma, Bellows Falls VT, USA

¹⁴Corning, Tewksbury MA, USA

¹⁵Whatman GF/F, GE-Whatman, Boston MA, USA

¹⁶Trilogy, Turner Designs, San Jose CA, USA

¹⁷Thermo-Fisher, Waltham MA, USA

¹⁸Sigma, St. Louis MO, USA

¹⁹FastPrep 24, MP Biomedicals, Solon OH, USA

²⁰SYTOX-Green, Thermo-Fisher, Waltham MA, USA

²¹Chroma, Bellows Falls VT, USA

²²Abraxis, Warminster PA, USA

fiber filters²³ mounted on a glass/stainless steel solid phase extraction manifold and quantifying toxin concentrations in the filtrate. Combined toxin samples were prepared by filling 15-mL glass centrifuge tubes²⁴ with 7 mL of sample, subjecting the tubes to three freeze/thaw cycles at - 20°C, centrifuging at 2900 × G for 15 minutes and collecting the supernatant for analysis.

The set-up for the bench-scale trials is shown in Figure 1. Each trial used three 2-L glass jars arranged on a multi-position jar test stirring machine²⁵ operating at 30 revolutions per minute (rpm). All trials were carried out at temperatures ranging from 20 to 22 °C. Jar 1, containing oxidant and de-chlorinated tap water, was used to control for background oxidant demand. Jar 2 contained cell suspension and served as the control for Jar 3, which contained cell suspension and oxidant. Trials were performed at pH 7 and 9, using KMnO₄ doses of 1, 2.5, and 5 mg/L, at turbidities of <0.1, 5, and 20 NTU. The pH in all three jars was adjusted to the target value prior to the addition of oxidant, cells or turbidity suspensions. The turbidity of < 0.1 NTU was achieved by using de-chlorinated tap water with no extra particulate suspension added. Particulate suspension was added to achieve turbidities of 5 and 20 NTU, as measured on a nephelometric turbidity meter²⁶. Finally, stock cell suspension was added to achieve target titers of 1 × 10⁶ cells/mL in Jars 2 and 3. The system was sampled immediately prior to oxidant addition and at 15, 30, and 90 minutes following oxidant addition. The sample collected immediately prior to oxidant addition was defined as the 0 minute (min) sample. Immediately following the 30 min sample collection, in trials where PAC was added, 100-mL aliquots were transferred from Jars 2 and 3 to Erlenmeyer flasks, set stirring at 30 rpm on separate stir plates, and dosed with PAC slurry to achieve a target PAC dose of 10 mg/L. These flasks, along with the oxidant jars, were sampled at 90 min. Allowing PAC adsorption to take place in separate flasks made it possible to simultaneously evaluate the impacts of oxidant and oxidant plus PAC. Glass vials were used for the collection of raw (prior to processing) chlorophyll-*a*, cell membrane integrity, and toxin samples from the oxidant and control jars. All sample vials contained a volume of 20 g/L sodium ascorbate (C₆H₇NaO₆) oxidant quenching solution calculated to yield a final concentration of 0.125 mg/mL when the vials were filled. The results for all analytes immediately prior to oxidant addition have been summarized in supplementary Table S1.

An initial set of trials, without PAC addition, was carried out at pH 7 and 9, 0.1 NTU turbidity, and KMnO₄ doses of 1, 2.5, and 5 mg/L. A second set of trials at pH 7, 0.1 NTU turbidity, and KMnO₄ doses of 1, 2.5, and 5 mg/L was carried out with 2.5 mg/L PAC added at 30 min. Based on the adsorption results of the trials with 2.5 mg/L PAC, it was decided to perform all subsequent PAC trials with doses of 10 mg/L. This decision led to a final set of trials at pH 7 and 9, 0.1–20 NTU turbidity, 1–5 mg/L KMnO₄, and the addition of 10 mg/L PAC at 30 min. The toxin adsorption results for 10 mg/L PAC are reported in this manuscript. The toxin release data generated during the 2.5 mg/L PAC trials are reported in this manuscript along with the toxin release data generated during the 0 and 10 mg/L PAC

²³Whatman GF/F, GE-Whatman, Boston MA, USA

²⁴Corning, Tewksbury MA, USA

²⁵Phipps & Bird, Richmond VA, USA

²⁶Hach 2100N, Loveland CO, USA

trials. This experimental progression resulted in replication of toxin release results for all combinations of pH and KMnO_4 doses. Interactions between experimental factors were analyzed by three-way analysis of variance (ANOVA)²⁷.

Results and Discussion

The impact of turbidity.

Increases in turbidity were associated with increases in oxidant demand at both pH levels. At pH 7, increasing turbidities from 0.1 to 20 NTU was associated with 45, 51, and 19 percent decreases in 90 min CT values following KMnO_4 doses of 1, 2.5, and 5 mg/L, respectively. At pH 9, the same turbidity increase was associated with 36, 24 and 2.9 percent decreases in 90 min CT values following the same progression of KMnO_4 doses. However, a 3-way ANOVA analysis did not indicate a statistically significant ($p < 0.05$) association between turbidity, alone or in combination with oxidant dose or pH, and relative changes (C_t/C_0) in the concentration of any analyte. Consequently, the remainder of the discussion focuses on the impacts of pH and oxidant dose, with the results of trials conducted at different turbidities, but the same oxidant dose and pH, binned together.

Toxin dynamics.

A typical set of results for a single trial at pH 7 is shown in Figure 2. Summary results as a function of time at pH 7 are presented in Figure 3. Individual trial results as a function of CT are presented in supplemental figures S1, S2, and S3. Oxidant jar extracellular toxin concentrations varied markedly as a function of time for all KMnO_4 doses. Control jar extracellular and combined toxin concentrations did not exhibit systematic changes during the trials (supplementary Figure S3).

Fundamentally, extracellular toxin concentrations were a balance between release and oxidative destruction. At a 1 mg/L dose, there was sufficient KMnO_4 to stimulate toxin release, but not enough to degrade what had been released. At the 2.5 and 5 mg/L doses, it appears that KMnO_4 concentrations were sufficient to stimulate toxin release and degrade the released toxin.

When all > 0 min oxidant jar extracellular toxin concentrations were plotted as a function of CT (supplementary Figure S1), the relationships between KMnO_4 dose, KMnO_4 residuals, and toxin concentrations manifested themselves as a general decline in extracellular toxin concentrations with increasing CT.

The balance between toxin release and oxidative destruction was also reflected in the ratios of extracellular to combined (extra- plus intracellular) toxins (Figure 3B). Over the first 15 minutes, the extracellular/combined toxin ratios increased at all KMnO_4 doses, as toxins were released. Following the 1 mg/L dose, the ratio remained constant, on average, for the remaining 75 min. This was most likely due to the low concentration of residual oxidant. At 2.5 mg/L, residual oxidant concentrations were higher, yet the extracellular/combined toxin ratio remained relatively stable from 15 to 90 min. This was most likely due to the fact that

²⁷Sigmaplot, SYSTAT Software, San Jose CA, USA

extracellular and combined toxin concentrations were decreasing at approximately the same rates over that time period (supplementary Table S2). The decline in the extracellular/combined toxin ratio at the 5 mg/L KMnO_4 dose reflects extracellular toxin concentrations decreasing more rapidly than combined toxins (supplementary Table S2).

Summary results for pH 9 as a function of time are shown in Figure 4. Individual results as a function of CT are presented in supplementary Figures S4, S5 and S6. As at pH 7, oxidant jar extracellular toxin concentrations varied with time, while control jar concentrations did not vary and were never greater than 2 $\mu\text{g/L}$ (supplementary Figure S6). The balance between toxin release and oxidation was apparent over the first 15 minutes. Toxin releases were observed at all three KMnO_4 doses, with the largest releases at 1 mg/L and the smallest at 5 mg/L. One principal difference versus pH 7 was the lower relative magnitude of toxin releases over the first 15 minutes. The other major difference was that, at 5 mg/L KMnO_4 , extracellular toxin concentrations increased progressively over the course of 90 minutes. These increases in extracellular toxins were also reflected in progressive increases in the extracellular/combined toxin ratio at 5 mg/L (Figure 4B). Combined toxin concentrations did not vary significantly at any oxidant dose (supplementary Table S3). It is unclear if the increases in extracellular toxin concentrations at pH 9 and high KMnO_4 doses occurred because KMnO_4 stimulated toxin release at rates that exceeded the oxidation rate of toxins in aqueous solution, or if KMnO_4 was reacting preferentially with non-toxin cellular material. The differences in net toxin releases at pH 9 versus pH 7 indicate the possibility that reactions between KMnO_4 and toxins, or KMnO_4 and cellular constituents, proceeded at different rates or via different mechanisms at the two pH levels.

Changes in cell counts, cell integrity, and pigment concentrations.

Although this work was not designed to explicitly assess the rates and mechanisms of KMnO_4 reaction with cell constituents or toxin producing mechanisms, decreases in chlorophyll-*a* concentrations and increases in the concentrations of SYTOX (+) cells indicated that KMnO_4 affected not only cell membranes but also aspects of internal cellular function. The SYTOX stain used in these studies penetrated only membranes that had been compromised in some way. The *in vitro* chlorophyll-*a* extraction and analysis procedure was applied to cells that had been retained on a filter immediately after sampling, ensuring that any chlorophyll-*a* quantified during the procedure originated from inside cells and not from aqueous solution. As a result, observed changes in chlorophyll-*a* concentrations served as an indicator of changes in internal cellular function. Relative changes in oxidant jar concentrations of chlorophyll-*a*, SYTOX (+) cells, and KMnO_4 residuals, binned by KMnO_4 dose, are summarized in Figures 5 and 6. The oxidant jar concentrations of SYTOX (+) cells and chlorophyll-*a* as a function of CT in all individual trials are presented in supplementary Figures S7 and S8. Control jar concentrations of these two analytes as a function of CT are presented in supplementary Figures S9 and S10. The concentrations of chlorophyll-*a* and SYTOX (+) cells in the control jar did not change significantly as a function of time, oxidant dose, or oxidant concentration at either pH level.

Over the first 15 minutes, changes in SYTOX (+) cells were greater at pH 7 than at pH 9, increasing by multiples of 3.0 to 3.3 at pH 7 compared to 1.5 to 2.8 at pH 9. Concentrations

of these membrane compromised cells continued to increase over the final 75 minutes, reaching multiples of 4.4 to 6.4 and 3.0 to 6.2 at pH 7 and 9, respectively. The increases in membrane compromised cells were accompanied by decreases in chlorophyll-*a* concentrations at both pH levels over 90 minutes (supplementary Tables S4 and S5), although at pH 9, chlorophyll changes took longer to manifest. At pH 7, over the first 15 minutes, chlorophyll-*a* concentration changes of -2 to -10 percent were observed, while at pH 9, chlorophyll concentrations remained unchanged over that time period. Over the final 75 minutes, chlorophyll changes of -13 to -34 percent and -8 to -15 percent were observed at pH 7 and 9, respectively. In contrast to the large increases in SYTOX (+) cells and steady decreases in chlorophyll-*a* concentrations, total cell counts at both pH levels remained relatively stable, changing by 0 to +10 percent over 90 minutes at pH 7 and -7 to +10 percent at pH 9. The relative stability of total cell counts, combined with increases in SYTOX (+) cell counts, increases in extracellular toxin concentrations, and decreases in chlorophyll-*a* concentrations form a body of evidence indicating that KMnO₄ application affected the integrity of cyanobacterial cell membranes, which in turn was associated with the release of toxins into solution and decreases in the concentration of intracellular photopigments.

These changes in cell integrity and composition occurred at KMnO₄ CTs of 8.7 – 380 mg x min/L and 11 – 300 mg x min/L at pH 7 and 9, respectively. How do these results compare with the impacts of *M. aeruginosa* exposure to chlorine (Cl₂) and ozone (O₃)? Ding et al (2010) exposed 2×10^6 cells/mL to Cl₂ and O₃ at pH 7.5. They observed a 50 – 85 percent decrease in viable cells at Cl₂ CTs of 45 – 75 mg x min/L and complete elimination of viable cells at 150 mg x min/L. They also observed a 25 percent reduction in viable cells at O₃ CTs of 7.4 – 12 mg x min/L. Zamyadi et al (2012) chlorinated 2×10^5 cells/mL and observed 60 percent reduction in viable cells at 3.5 mg x min/L and complete elimination of viable cells at 31 mg x min/L.

Impact of PAC addition.

Typical jar test results at pH 9, showing the impact of PAC addition at 30 min, are shown in Figure 7. The addition of PAC resulted in 90 min oxidant+PAC jar extracellular toxin concentrations that were lower than 90 min oxidant jar extracellular toxin concentrations at all combinations of pH, turbidity and KMnO₄ dose. ANOVA analysis did not indicate a statistically significant ($p < 0.05$) association between turbidity and differences in oxidant versus oxidant+PAC jar extracellular toxin concentrations. Consequently, the results of trials at identical combinations of pH and KMnO₄ dose but different turbidities have been averaged together. At pH 7, 90 min oxidant+PAC jar extracellular toxin concentrations were, on average, 50% (SD = 21%), 50% (SD = 20%), and 25% (SD = 35%) lower than 90 min oxidant jar concentrations at initial KMnO₄ doses of 1, 2.5, and 5 mg/L, respectively. At pH 9, the relative differences were 74% (SD = 1.7%), 81% (SD = 8.0%), and 86% (SD = 6.9%) at KMnO₄ doses of 1, 2.5, and 5 mg/L, respectively.

The results for all PAC trials are summarized graphically in Figure 8. The differences between oxidant and oxidant+PAC jar 90 min extracellular toxin concentrations correlated roughly with the concentrations of extracellular toxins in solution at 30 min immediately prior to PAC addition. The highest 30 min extracellular toxin concentrations in turn were

observed in conjunction with the lowest KMnO_4 residuals. In cases of low 30 min KMnO_4 residuals, the changes in extracellular toxin concentrations in the oxidant+PAC versus oxidant-only jars would have been due mainly to the adsorptive capacity of the PAC. Because PAC was dosed at a constant 10 mg/L, the oxidant+PAC versus oxidant-only concentration differences provide a rough estimate of toxin loading onto the PAC. The lowest 30 min extracellular toxin concentrations were observed when KMnO_4 residuals were highest. In these situations, the net changes in extracellular toxin concentrations in the oxidant+PAC jars would have been a function of KMnO_4 -stimulated toxin release, toxin consumption by KMnO_4 and toxin adsorption onto PAC.

By way of comparison, Park et al (2017) observed approximately 92% removal when 10 mg/L PAC of unspecified origin was applied for 60 minutes to river water spiked with 5 $\mu\text{g/L}$ MC-LR. They also tested adsorption at pH values ranging from 3.2 to 8 and found no significant impacts. Drogui et al (2012), starting with an initial MC-LR concentration of 22 $\mu\text{g/L}$, observed approximately 41% removal after 15 minutes of contact time with 10 mg/L PAC of unspecified origin. Ho et al (2011) observed 30–60% removal of total MCs following 60 minutes of contact time with 10 mg/L coal-based PAC. Cook and Newcombe (2008) observed 15–38% congener-specific MC removal when 8.0–8.9 $\mu\text{g/L}$ of toxin in surface water was treated with 15 mg/L of wood-based PAC for 30 minutes. Finally, Cook and Newcombe (2002) observed 20–65% congener-specific MC removal when surface water was spiked with 14–16 $\mu\text{g/L}$ of MC and treated with 15 mg/L of wood-based PAC for 60 minutes.

It is important to note that the MC removals reported by other research groups were obtained using different PACs, and were observed in environments where oxidants were not present and where no extracellular toxins were added after PAC application. In contrast, the PAC toxin removal data generated in this work was obtained in an environment that incorporated an oxidant residual at the time of PAC application as well as cyanobacterial cells that were capable of releasing toxins during the PAC contact period. Taken as a whole, all of the PAC toxin removal data discussed in this section indicated that PAC is an effective adsorbent of MCs under a wide variety of conditions. From a drinking water treatment perspective, the ability to add PAC is a critical tool in minimizing the possibility of toxin propagation to consumers' taps.

Conclusions

The lines of evidence developed in this manuscript point to the marked impact of KMnO_4 on the integrity and function of *M. aeruginosa* cells. The concentration of SYTOX (+) cells, an indicator of compromised membranes, increased after KMnO_4 application at all doses and pH levels. The concentrations of chlorophyll-*a*, a photopigment that serves as a component of the cellular system that converts light energy into chemical energy, decreased at all KMnO_4 doses and pH levels. The SYTOX and chlorophyll-*a* results are in broad agreement with data generated by other researchers that document the negative changes in various measures of *M. aeruginosa* cell integrity and metabolism upon exposure to KMnO_4 . The increases in membrane-compromised cells were accompanied by increased toxin releases. These releases of toxins were observed at both pH levels and all three KMnO_4 doses. The

release of toxins from *M. aeruginosa* and accumulation of toxins in aqueous solution was also documented by Fan et al (2013b) and Li et al (2014), albeit at higher KMnO_4 concentrations and longer contact times. It is possible that these differences were influenced by variations in *M. aeruginosa* strain type, strain age, and culturing conditions employed by different laboratories. The observations of toxin release in this study also agree with the observations of general intracellular material release, measured by indicators such as DOC and UV_{254} , that have been reported in the peer-reviewed literature and discussed in the Introduction section. The differences in the time progression of toxin release at pH 9 versus pH 7 are difficult to evaluate in the context of other published work because, to the best of the authors' knowledge, the laboratory trials described in this manuscript are the first to evaluate the impact of KMnO_4 on *M. aeruginosa* cells at pH 9. These observed differences as a function of pH do, however, highlight the need to investigate reactions between KMnO_4 and *M. aeruginosa* cellular constituents as a function of pH. The application of PAC for 60 minutes at 10 mg/L was shown to consistently decrease the concentrations of toxins in solution; an observation that fits with other published work in the area of MC adsorption.

The work presented here, along with other studies discussed in the literature review, provide evidence that intracellular material, including cyanobacterial toxins, may be released from *M. aeruginosa* upon KMnO_4 exposure. However, the magnitude and time progression of release, as well as the fate of the released material, will be dependent on site-specific factors such as cell concentration, age, stress history, species of cyanobacteria, oxidant dose, contact time, pH, and the concentration of other organic and inorganic water quality constituents. When these factors are balanced against the proven utility of KMnO_4 in achieving a variety of water treatment objectives, the prudent course of action is to maintain a posture of vigilance wherever KMnO_4 is used. Such a posture requires constant monitoring of cyanobacterial biomass, extracellular toxins and combined toxins in the source water and throughout the treatment plant. With this knowledge, it should be possible to optimize the treatment process to take advantage of KMnO_4 while guarding against the risk of cyanobacterial toxin passage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Bench-scale test setup

Figure 2:

Time progression of extracellular and combined toxin concentrations, oxidant versus control jars, pH 7, KMnO_4 dose = 1 mg/L, turbidity < 0.1 NTU; ■ = combined toxin concentration (oxidant jar), □ = extracellular toxin concentration (oxidant jar), ▲ = combined toxin concentration (control jar), △ = extracellular toxin concentration (control jar).

Figure 3:

(A) Changes in oxidant jar extracellular toxin concentrations as a function of time at pH 7.

(B) Changes in oxidant jar extracellular/combined toxin ratios as a function of time at pH 7.

Data points are averaged by initial oxidant dose: $\square$ = 1 mg/L KMnO_4 , $\blacksquare$ = 2.5 mg/L KMnO_4 , $\blacksquare$ = 5 mg/L KMnO_4 . Error bars represent one standard deviation.

Figure 4:

(A) Changes in oxidant jar extracellular toxin concentrations as a function of time at pH 9.

(B) Changes in oxidant jar extracellular/combined toxin ratios as a function of time at pH 9.

Data points are averaged by initial oxidant dose: $\square$ = 1 mg/L KMnO_4 , $\blacksquare$ = 2.5 mg/L KMnO_4 , $\blacksquare$ = 5 mg/L KMnO_4 . Error bars represent one standard deviation.

Figure 5: Changes in oxidant jar (A) chlorophyll-a concentrations, (B) SYTOX(+) cell counts and (C) KMnO₄ residuals at pH 7. Data points are averaged by initial oxidant dose: □ = 1 mg/L KMnO₄, ■ = 2.5 mg/L KMnO₄, ■ = 5 mg/L KMnO₄. Error bars represent one standard deviation.

Figure 6: Changes in oxidant jar (A) chlorophyll-*a* concentrations, (B) SYTOX(+) cell counts and (C) KMnO₄ residuals at pH 9. Data points are averaged by initial oxidant dose: □ = 1 mg/L KMnO₄, ▨ = 2.5 mg/L KMnO₄, ■ = 5 mg/L KMnO₄. Error bars represent one standard deviation.

Figure 7:
Impact of PAC on the time progression of oxidant jar extracellular toxin concentrations at pH 9, turbidity = 0.1 NTU: (A) KMnO_4 dose = 1 mg/L, (B) KMnO_4 dose = 5 mg/L. ■ = oxidant only; ■ = oxidant + PAC.

Figure 8:

Impact of PAC addition versus extracellular toxin concentrations at the time of PAC application; □ = pH 7, 1 mg/L KMnO₄ dose; ▨ = pH 7, 2.5 mg/L KMnO₄ dose; ■ = pH 7, 5 mg/L KMnO₄ dose; ◻ = pH 9, 1 mg/L KMnO₄ dose; △ = pH 9, 2.5 mg/L KMnO₄ dose; ▲ = pH 9, 5 mg/L KMnO₄ dose. Each symbol represents values averaged across turbidities of 0.1, 5 and 20 NTU; error bars represent one standard deviation; numbers in parentheses represent average 30 min KMnO₄ residuals (mg/L) immediately prior to PAC application.

Table 1:
Previous studies investigating the impacts of KMnO_4 exposure on *Microcystis aeruginosa* cells

Reference	Cell titer (#/mL) and suspension medium	pH	Temperature (°C)	KMnO_4 dose (mg/L)	Exposure time or CT (min, hrs or mg x min/L)	Cell response
Ding et al (2010)	2×10^6 , buffer solution	7.6	22	NA	0 – 600 mg x min/L	100% elimination of cells with non-compromised membranes. No net accumulation of extracellular microcystin-LR (MC-LR)
Ma et al (2012)	1×10^6 , BG-11 ^a	8.4	23 – 25	0 – 3	0 – 30 min.	Extracellular UV ₂₅₄ increased by 32%
Wang et al (2013)	2.3×10^6 , 0.5% NaCl solution	NA	NA	0 – 10	1 – 4 hrs.	Extracellular DOC increased by 8 – 9 mg/L
Ou et al (2012)	4×10^6 , BG-11 ^a	7	NA	0 – 20	0 – 6 days	After 2 hrs exposure time following 2 and 5 mg/L doses, chlorophyll- <i>a</i> decreased by 3.6 and 14%, respectively; effective quantum yields ^b decreased by 13 and 58%, respectively
Xie et al (2013)	1×10^6 , Simulated drinking water	7	NA	0 – 2	10 min.	21% increase in extracellular DOC
Fan et al (2013a)	7×10^5 , ASM-1 ^c	7.5	20	1 – 10	0 – 6 hrs.	0 – 100% elimination of cells with non-compromised membranes
Fan et al (2013b)	7×10^5 , ASM-1 ^c	7.5	20	1 – 10	0 – 2500 mg x min/L	50% increase in extracellular MC-LR concentration over CT = 0 – 500 following 5 mg/L dose; doubling of extracellular MC-LR concentration over CT = 0 – 125 following 10 mg/L dose
Li et al (2014)	6.4×10^6 , BG-11 ^a	7.5	NA	0 – 60	0 – 24 hrs.	Decrease in extracellular microcystins 0 – 60 min.; increase in extracellular microcystins from 60 min. to 24 hrs. following 5 – 40 mg/L doses

^a (Allen and Stanier, 1968)

^b A measure of photosynthetic production

^c (Gorham, 1964)