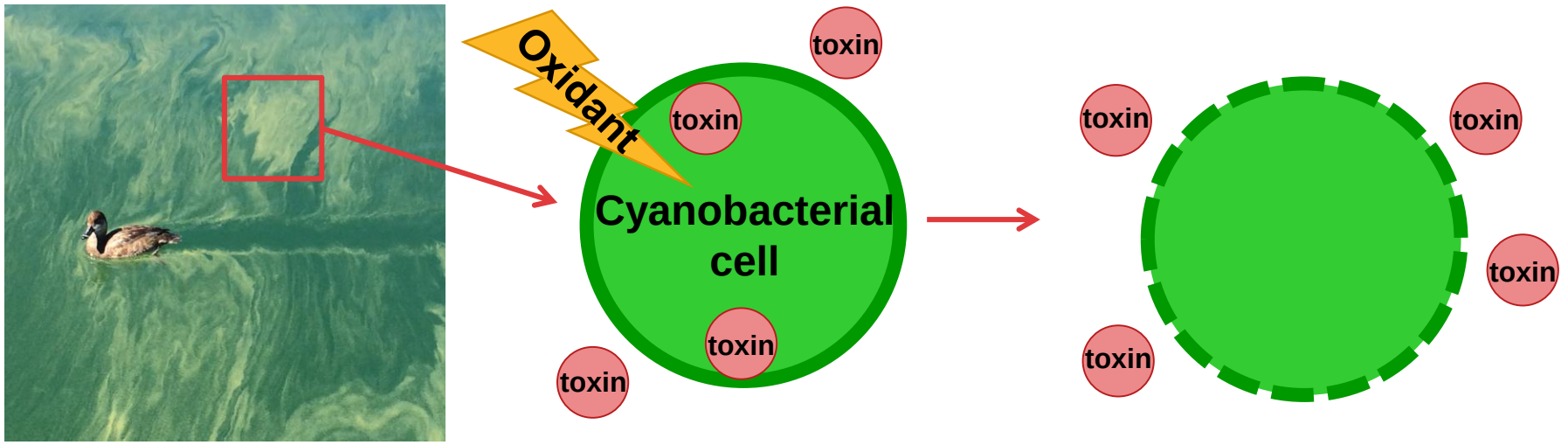
Release of Intracellular Cyanotoxins during Oxidation of Naturally Occurring and Lab-Cultured Cyanobacteria



Resilience -
Are you Ready?

A Seminar presented by the VA AWWA Drinking Water Quality and Research Commitee
in collaboration with the
Water Resources and Environment Committee

Wednesday, March 20, 2019
8:00 am - 4:00 pm

University of Richmond
Jepson Center
Richmond, VA

American Water Works Association
VirginiaSection

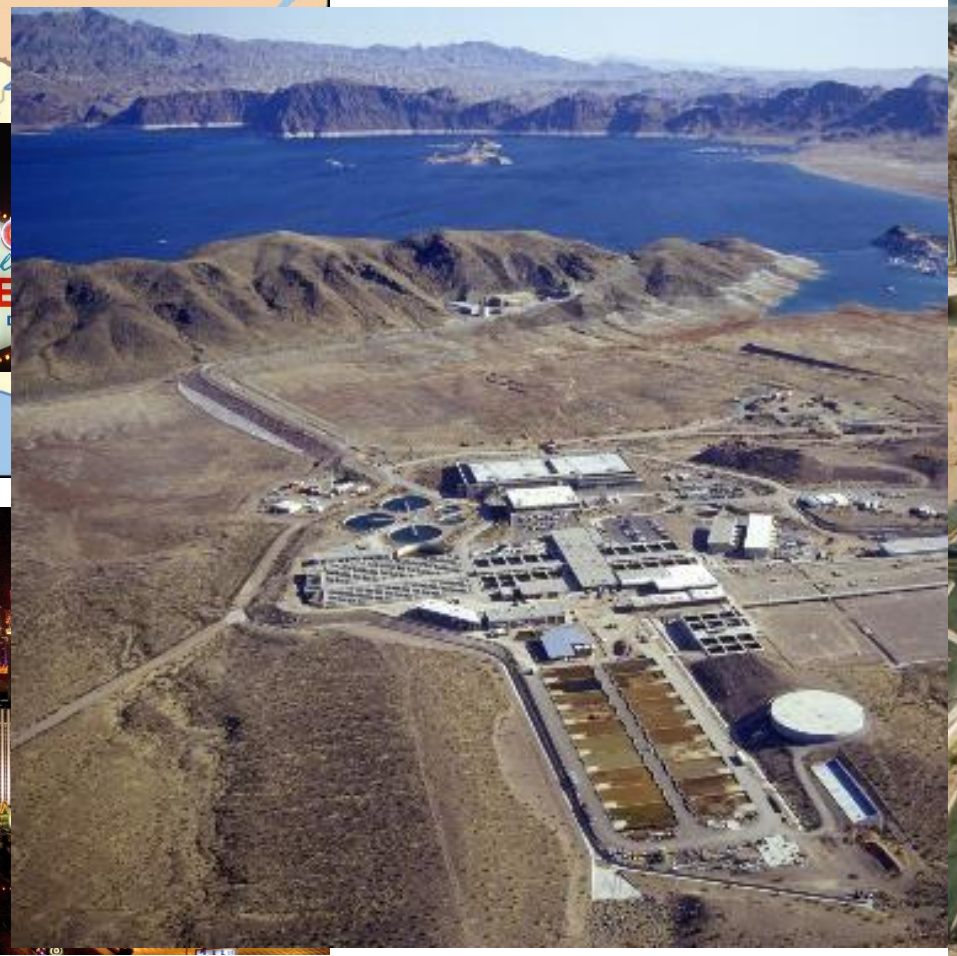
Katie Greenstein, PhD, PE

Water Quality Research and Development
SOUTHERN NEVADA WATER AUTHORITY

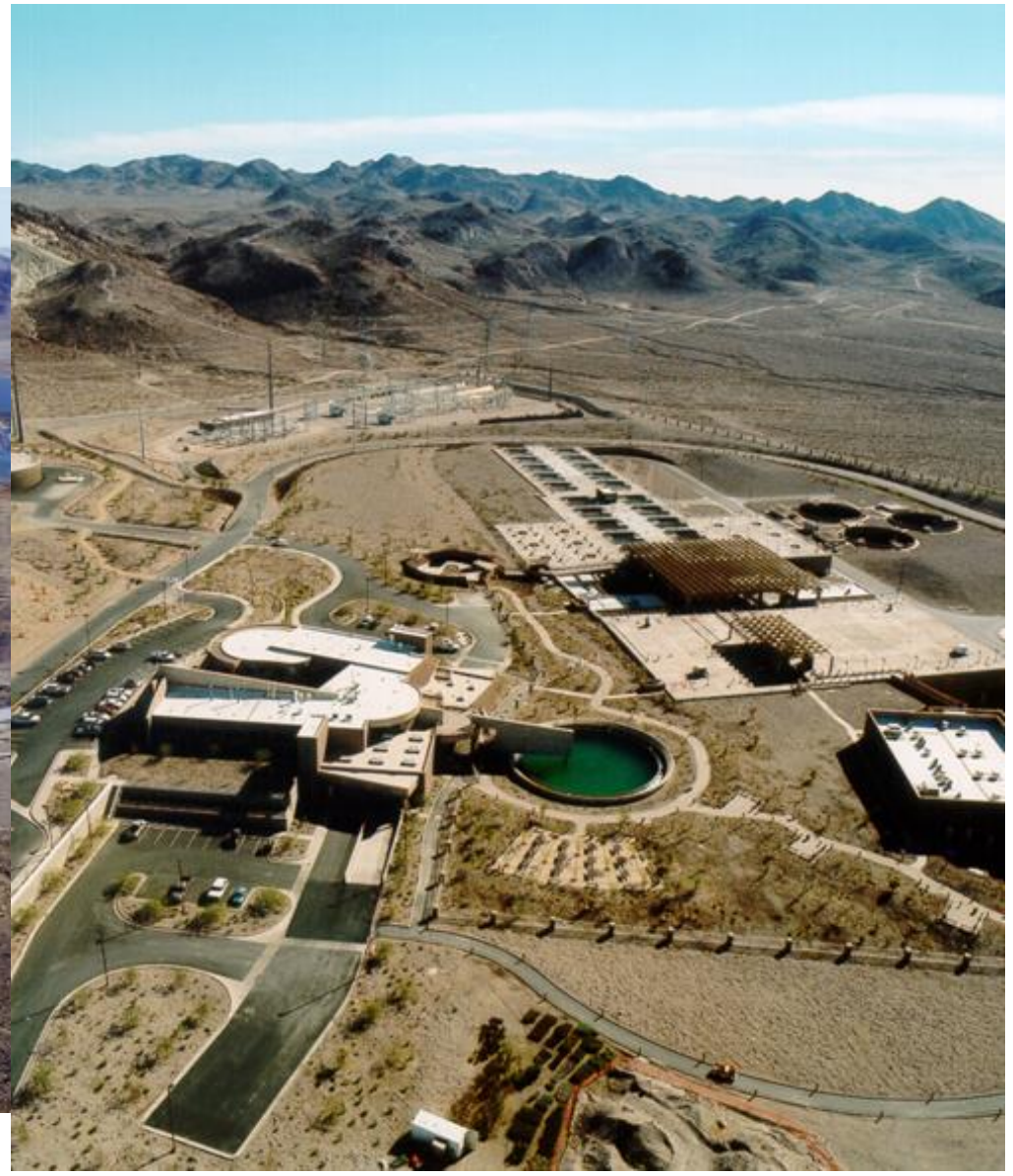
Des Moines Water Works
Water You Can Trust for Life

100 years 1919-2019

Southern Nevada Water Authority



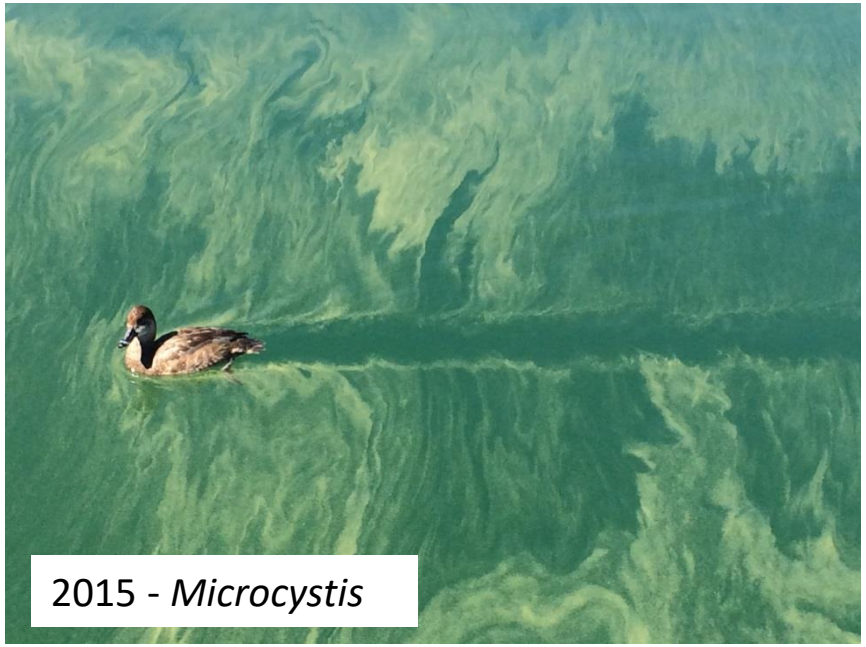
Alfred Merritt Smith Water Treatment Facility
600 Million Gallons/Day (MGD)



River Mountains Water Treatment Facility
(300 MGD)

Previous bloom events on Lake Mead

2001 – *Pyramichlamys*



2015 - *Microcystis*

Green algae

No cyanotoxins

No taste and odor

Blue-green algae or cyanobacteria

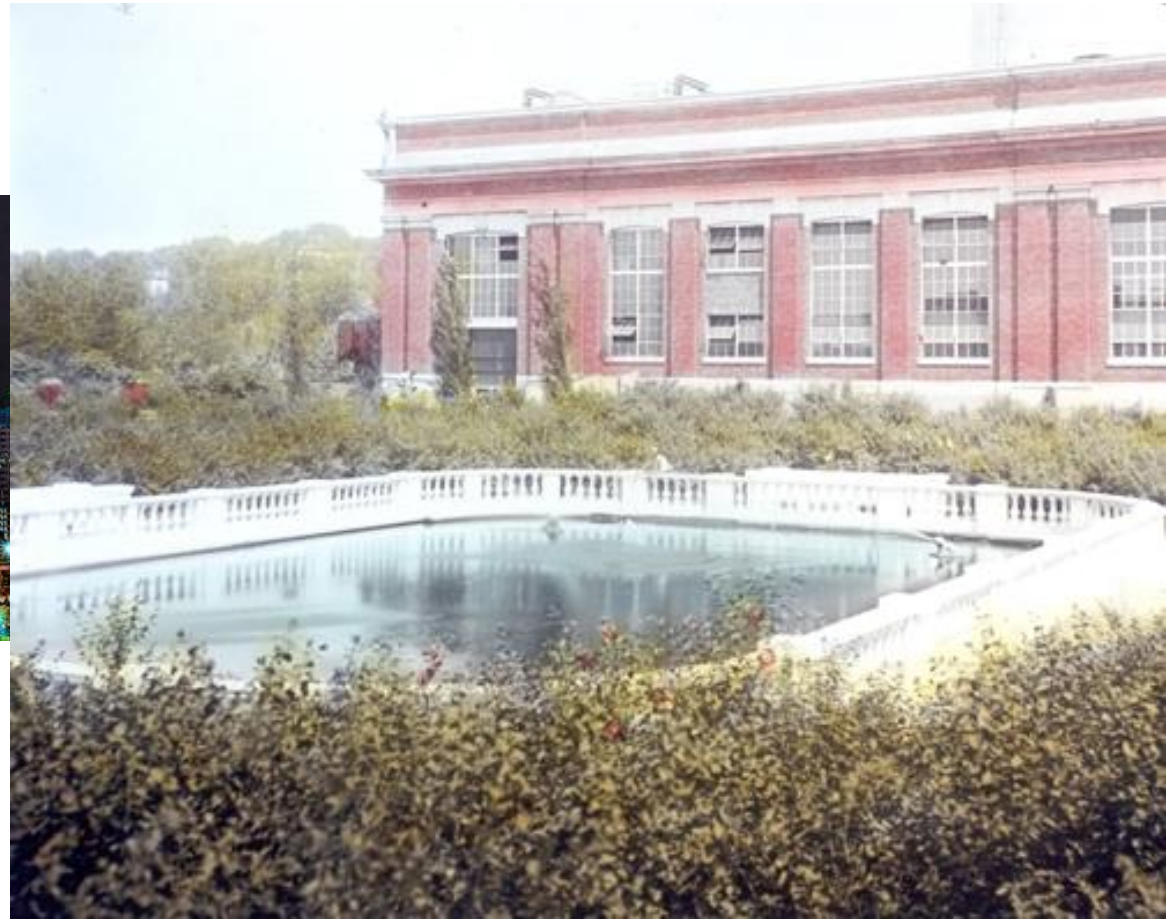
Can produce cyanotoxins

Can produce taste and odor compounds

Des Moines Water Works

Source: RAYGUN
(www.raygunsite.com)

3 water treatment plants; 100 MGD total capacity



Previous bloom event in Des Moines

Des Moines Water Works

News Release

Des Moines Water Works Detects Microcystin in Des Moines Water System

Wednesday, August 03, 2016

Drinking water samples analyzed by Des Moines Water Works show microcystin, a compound produced by cyanobacteria (or commonly referred to as blue-green algae), has been detected in the treated drinking water. At this time, there are no restrictions on water use. The United States Environmental Protection Agency has established national health advisory levels for microcystin when these compounds are detected in drinking water for at least 10 days. While Des Moines Water Works has detected elevated levels for only two days, and testing performed today shows results below advisory levels, the utility is exercising an abundance of caution in notifying customers of the detection of microcystin.

Des Moines Register

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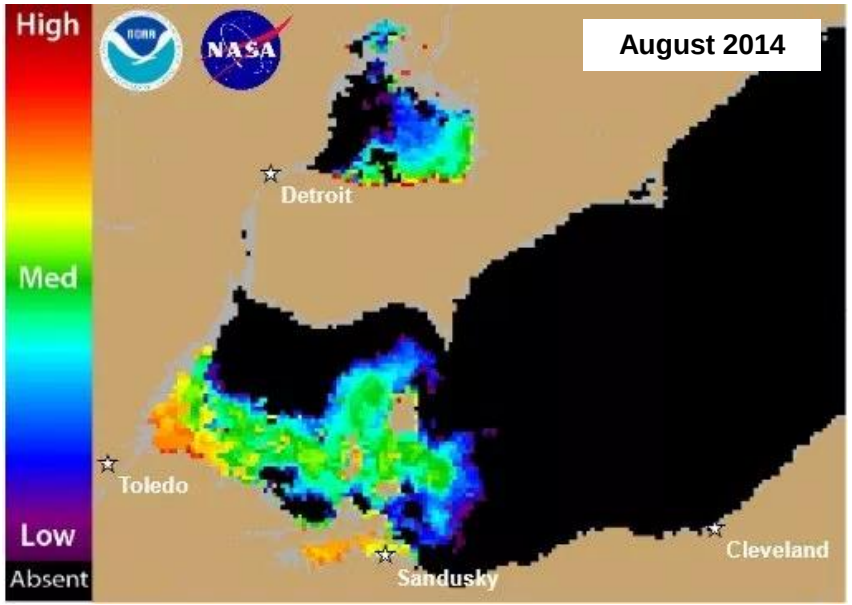
Greenfield won't be last Iowa town to have drinking water threatened by toxic blue-green algae, environmentalists say

Donnelle Eller, Des Moines Register Published 4:57 p.m. CT July 20, 2018



https://www.circleofblue.org/2014/great-lakes/lake-erie-algae-bloom-hits-pelee-island-moving-toward-sandusky/

Implementation of Ozone for Microcystin Treatment at the City of Toledo's Collins Park WTP

BUILDING A WORLD OF DIFFERENCE®

Bryan Townsend
Process Specialist
Water Technology Group

BLACK & VEATCH

EPA United States Environmental Protection Agency

Office of Water
820F15003
June 2015

2015 Drinking Water Health Advisories for Two Cyanobacterial Toxins

Summary

EPA has issued 10-Day Drinking Water Health Advisories (HAs) for the cyanobacterial toxins microcystins and cylindrospermopsin.

EPA recommends HA levels at or below 0.3 micrograms per liter for microcystins and 0.7 micrograms per liter for cylindrospermopsin in drinking water for children pre-school age and younger (less than six years old). For school-age children through adults, the recommended HA levels for drinking water are at or below 1.6 micrograms per liter for microcystins and 3.0 micrograms per liter for cylindrospermopsin. Young children are more susceptible than older children and adults as they consume more water relative to their body weight.

HAs are non-regulatory values that serve as informal technical guidance to assist federal, state and local officials, and managers of public or community water systems to protect public health from contaminants. EPA has also published health effects support documents for the cyanobacterial toxins microcystins and cylindrospermopsin. These documents contain the health effects basis for the development of HAs for the protection of human health. In addition, EPA has published a health effects support document for anatoxin-a but concluded that there was not adequate information to support a health advisory for this toxin.

(cyanobacterial toxins or "cyanotoxins") that are harmful to the environment, animals and human health. Winds and water currents can transport cyanobacterial blooms within proximity to drinking water intakes at treatment plants that, if not removed during treatment, can cause odor, taste and color problems in treated drinking water and can be harmful to human health.

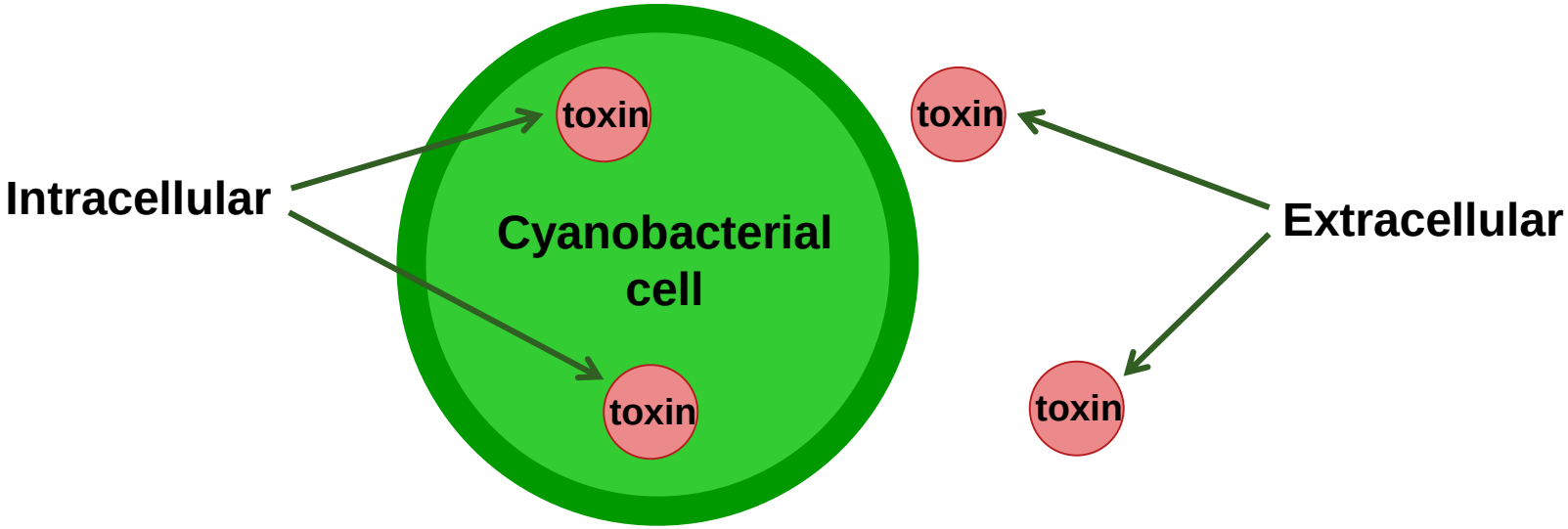
What is a health advisory?

The Safe Drinking Water Act provides the authority for EPA to publish health advisories for contaminants not subject to any national primary drinking water regulation. Health advisories describe non-regulatory concentrations of drinking water contaminants at or below which adverse health effects are not anticipated to occur over specific exposure durations (e.g., one-day, 10-days, several years, and a lifetime). They serve as informal technical guidance to assist federal, state and local officials, and managers of public or community water systems by providing information on the health effects of and methods to sample and treat cyanobacterial toxins in drinking water. HAs are not legally enforceable federal standards and are subject to change as new information becomes available.

Why has EPA taken this action?

There are no U.S. federal guidelines, water quality criteria, standards or regulations for cyanobacteria or cyanotoxins in drinking water under the Safe

What are intracellular and extracellular cyanotoxins?



10-day drinking water health advisories are relatively low

- **Microcystin: 0.3 µg/L (young children); 1.6 µg/L**
- **Cylindrospermopsin: 0.7 µg/L (young children); 3.0 µg/L**

Release of intracellular toxin is important in water treatment

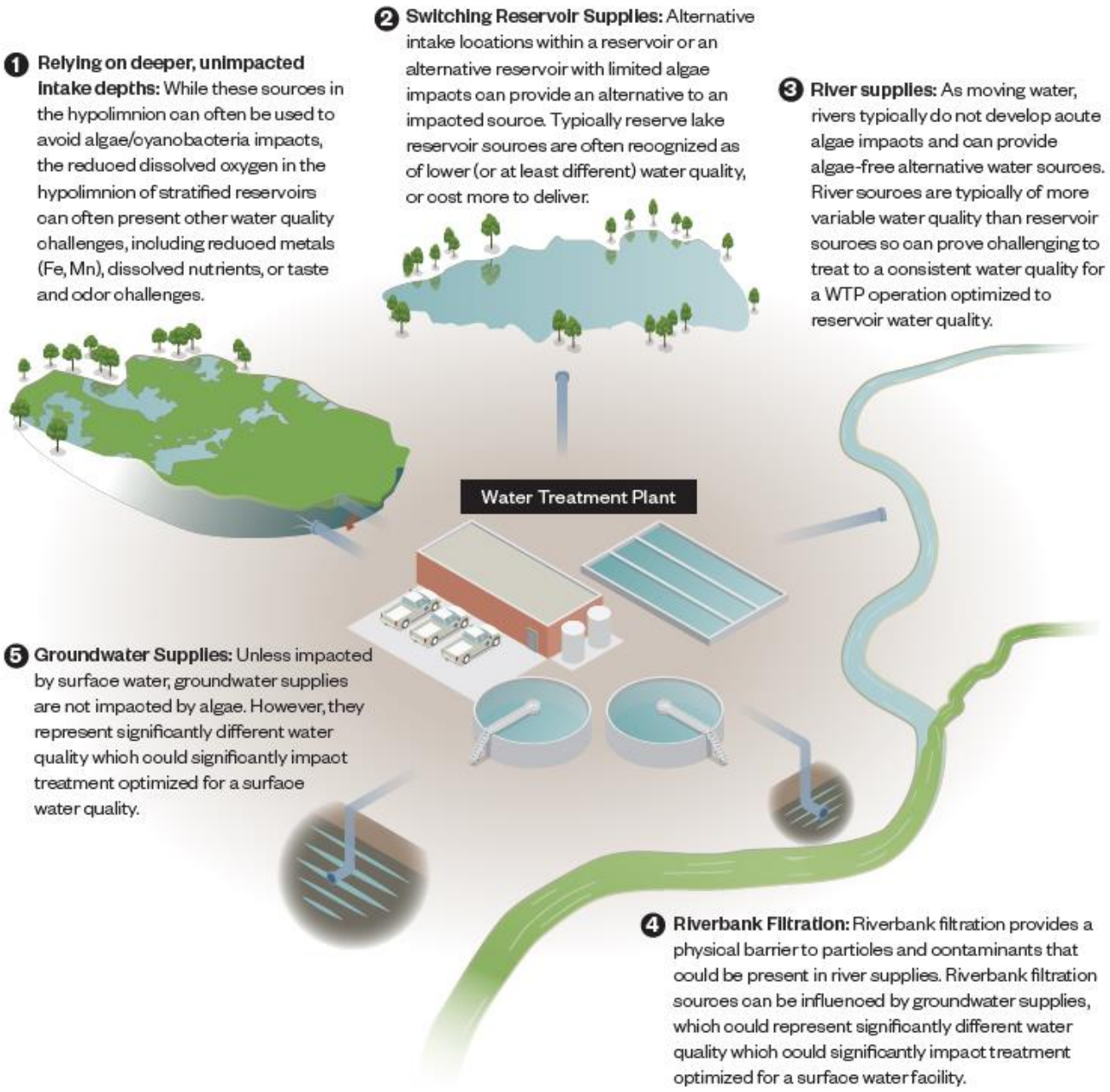
Treatment of extracellular toxins is well-studied

Oxidant	Microcystin	Cylindrospermopsin	Anatoxin A	Saxitoxin
Free chlorine	Moderate (f(pH))	Effective	No, slow	Effective
Monochloramine	Slow/no oxidation	No	No	?
Chlorine dioxide	Slow/no oxidation	No	No	?
Permanganate	Effective	No	Moderate	No
Ozone	Effective	Effective	Effective	No
AOP	Effective	Effective	Effective	?
UV	No	No	?	?

Treatment of intracellular toxins has also been studied

Consistent guidance across manuals….

Option A – Switch Sources



Option B – Remove Intact Cells

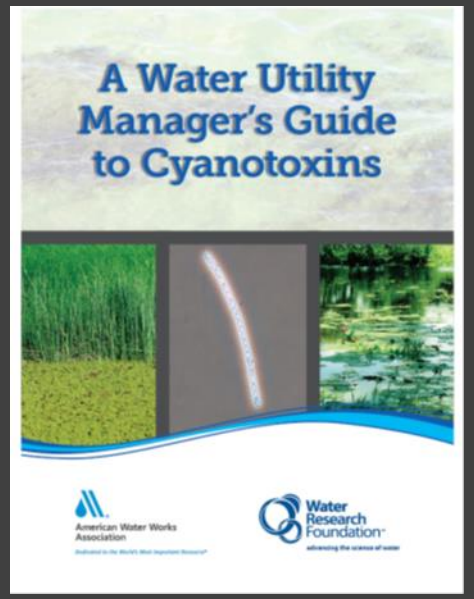


Table 5 Common cyanotoxin treatment practices and their relative effectiveness

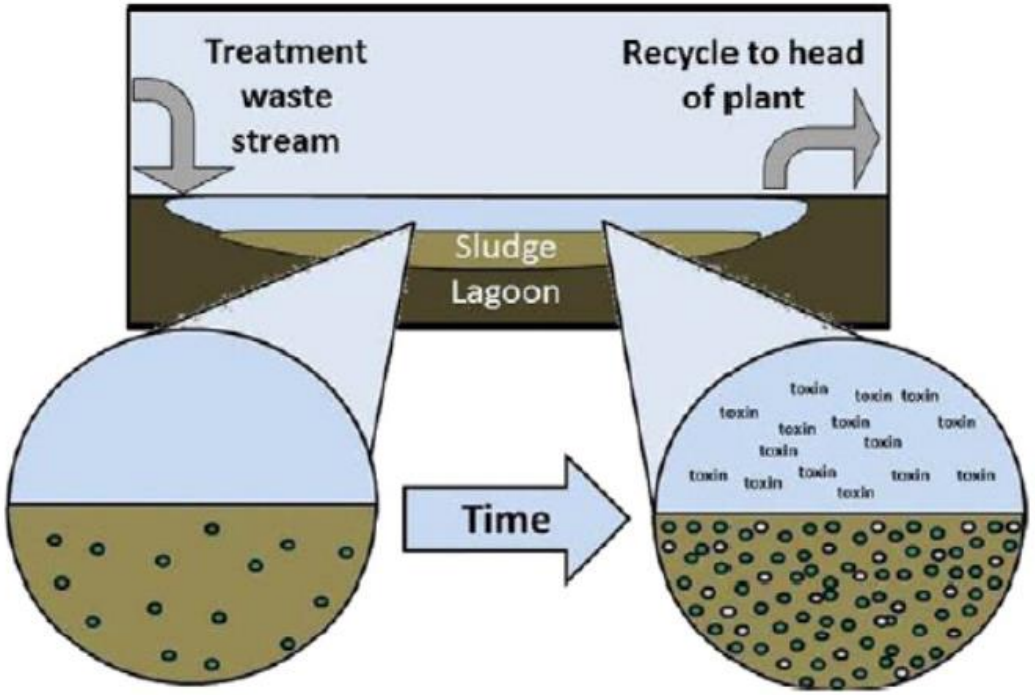
Treatment Process	Relative Effectiveness
Intracellular Cyanotoxins Removal (intact cells)	
Conventional coagulation, sedimentation, filtration	Effective for the removal of intracellular/particulate toxins by removing intact cells. Generally more cost effective than chemical inactivation/degradation, removes a higher fraction of intracellular taste and odor compounds, and easier to monitor.
Flotation (e.g., dissolved air flotation)	Effective for removal of intracellular cyanotoxins because many toxin-forming cyanobacteria are buoyant.
Pretreatment oxidation (oxidant addition prior to rapid mix)	Overall, can either assist or make treatment more difficult, depending on the situation. Pre-oxidation processes may lyse (cause dissolution or destruction of) cells, causing the cyanotoxins contained within to release the toxins. Ozone may be an exception (see "Ozone" row) because it both lyses cells and oxidizes the cyanotoxins.
Membranes (microfiltration or ultrafiltration)	Effective at removing intracellular/particulate toxins. Typically membranes require pretreatment.

Management and disposal of cells is critical

Filtration

Dissolved Air Flotation

Lagoons, Clarifiers, Drying Beds



Source: Water Research Foundation 4315 & 4523

Guidance currently states to avoid or minimize use of pre-oxidants to prevent intracellular release

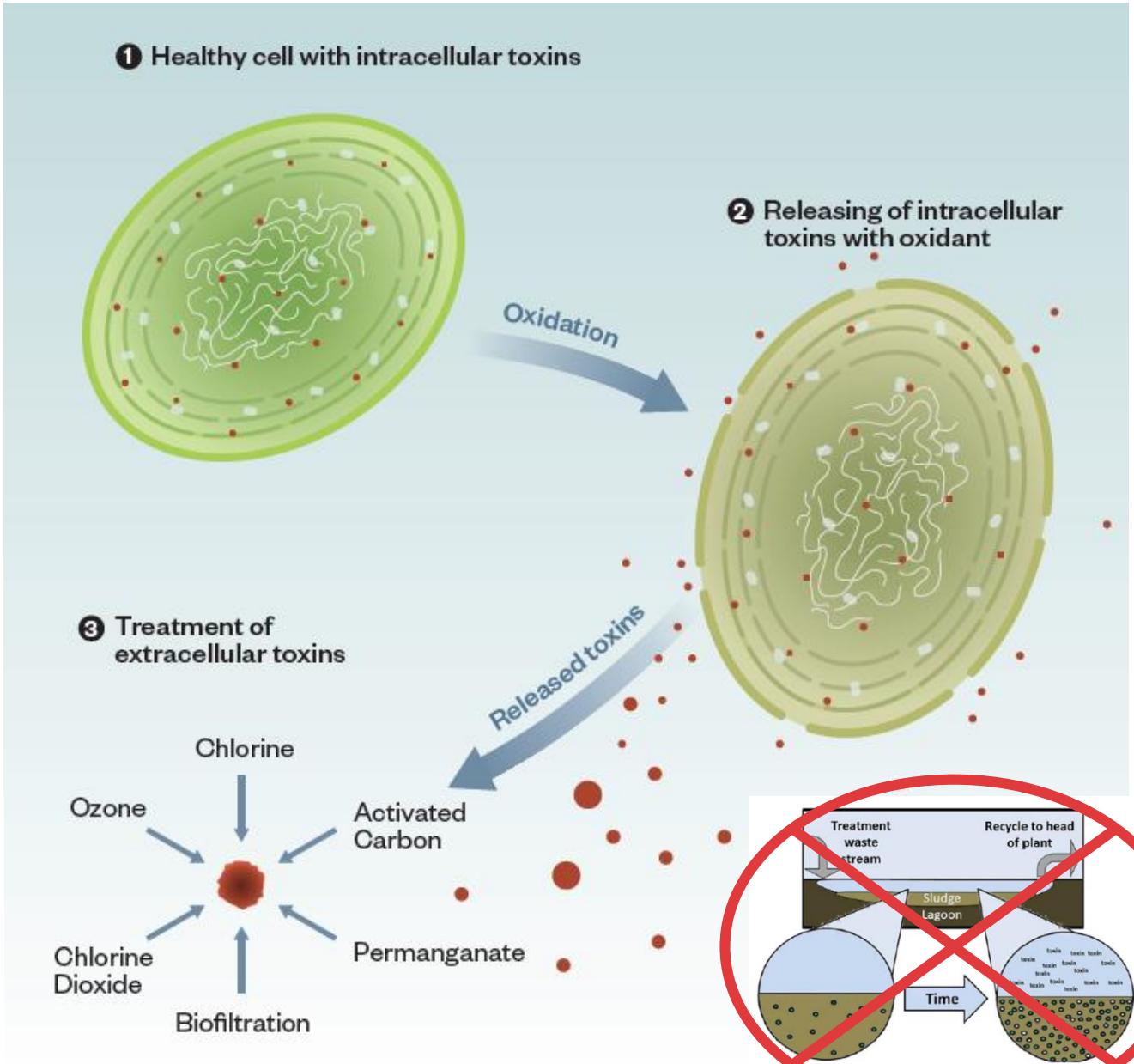
Pre-oxidation is common in drinking water treatment

- Disinfection
- Control of invasive species
  - Zebra/Quagga mussels
- Biofilm control on intake pipelines
- Inorganic contaminants
  - Iron/manganese
- Organic contaminants
  - Taste and odor compounds
  - Micropollutants

Turning off pre-oxidant is not always an option

Option C – Release and Treat Approach

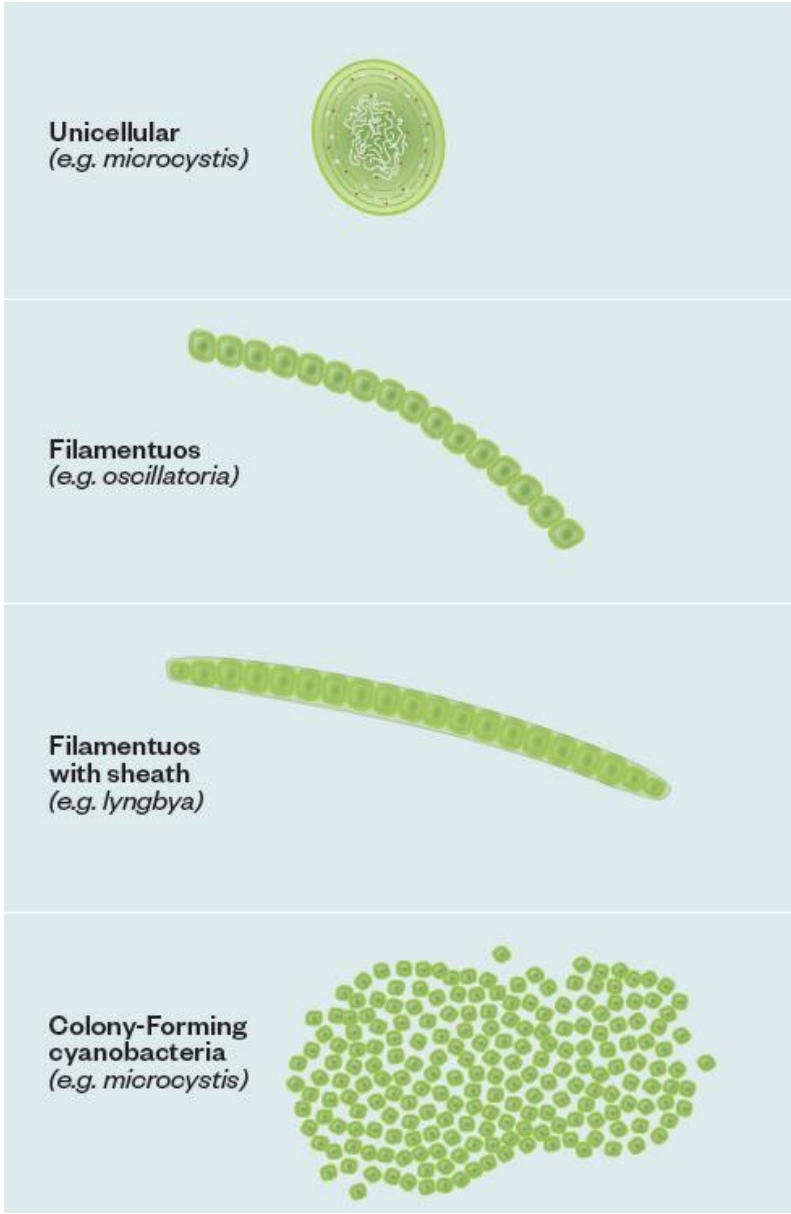
Oxidant	Microcystin	Cylindrospermopsin	Anatoxin A	Saxitoxin
Free chlorine	Moderate (f(pH))	Effective	No, slow	Effective
Monochloramine	Slow/no oxidation	No	No	?
Chlorine dioxide	Slow/no oxidation	No	No	?
Permanganate	Effective	No	Moderate	No
Ozone	Effective	Effective	Effective	No
AOP	Effective	Effective	Effective	?
UV	No	No	?	?



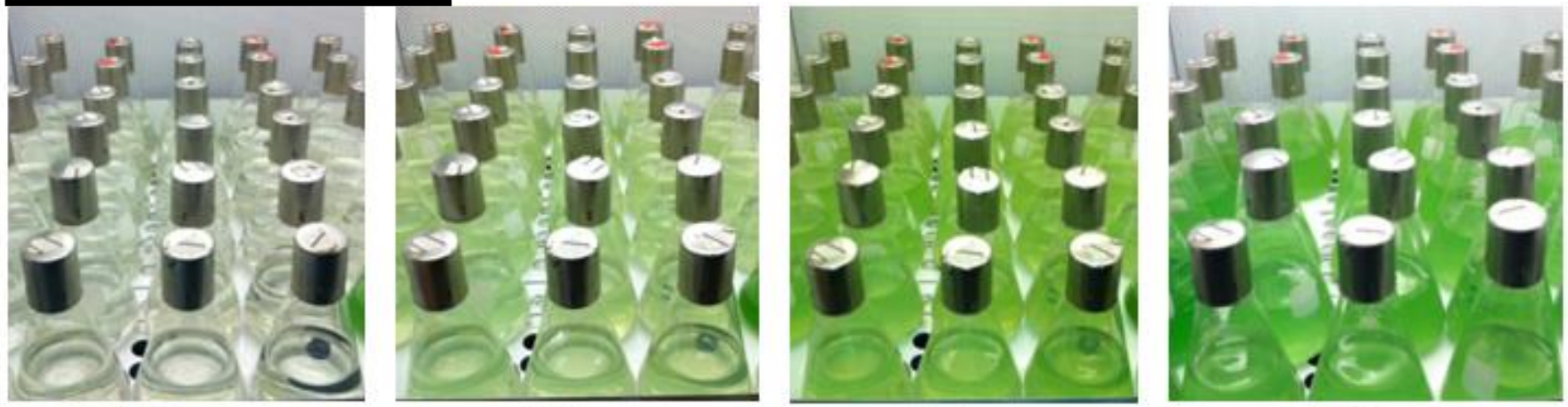
Cyanobacteria introduce a LOT of variables

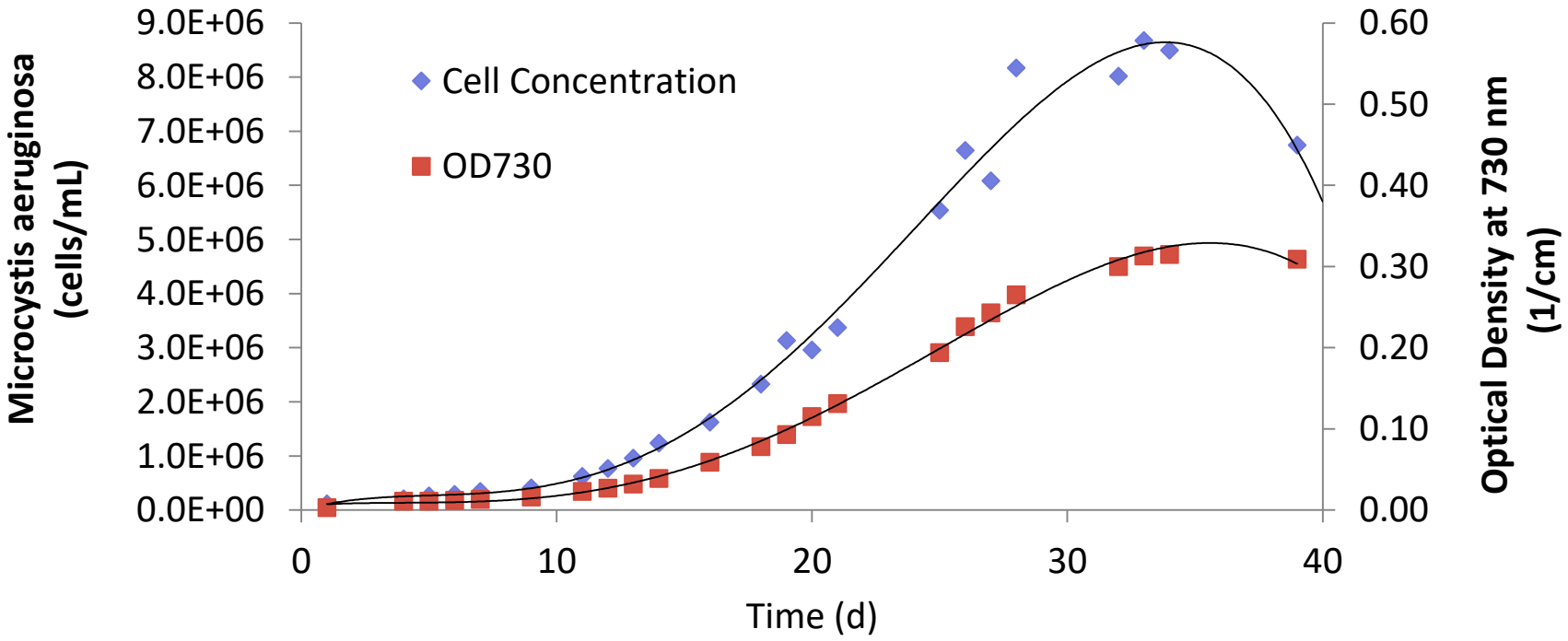
Cultured versus natural cells

Morphology & mixtures



Growth phase

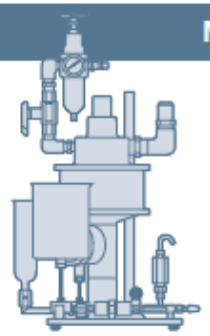




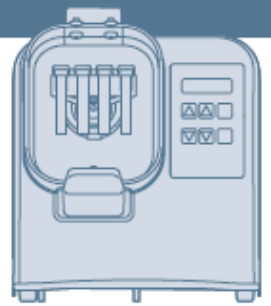
Cell lysis methods

MECHANICAL

High-pressure homogenizer
Cells in media are forced through a narrow nozzle using high pressure. The cell membrane is disrupted because of the change from compression to expansion.



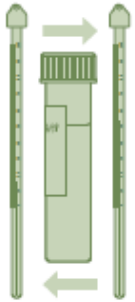
Bead beating
Also known as the bead mill method, bead beating agitates cells using tiny beads of glass, steel or caramic. The high-speed collisions disrupt the cell membrane and release intracellular components.



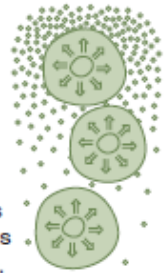
NON-MECHANICAL

PHYSICAL

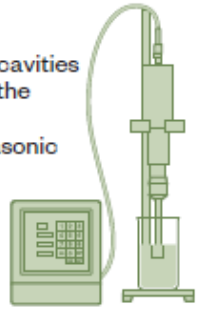
Freeze/thaw
Through repeated freezing and thawing cycles, ice forms on the cell membrane and breaks down due to osmotic stress.



Osmotic shock
By altering the concentration of salt surrounding a cell, the osmotic pressure suddenly changes, rupturing the cell membrane. Water enters the cell and the cell swells and subsequently bursts.

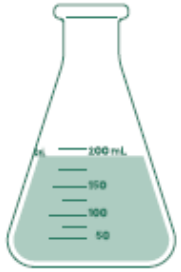


Cavitation
This technique creates cavities or bubbles by reducing the local pressure through increased velocity, ultrasonic vibration, etc. As the cavities/bubbles collapse, the energy disintegrates the cell membrane.



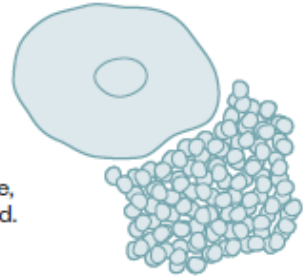
CHEMICAL

Alkali and detergents
Chemically changing the pH levels ruptures cell membranes and allows proteins to leak through the cell wall. Chemical lysis can be classified as alkaline or detergent.



BIOLOGICAL

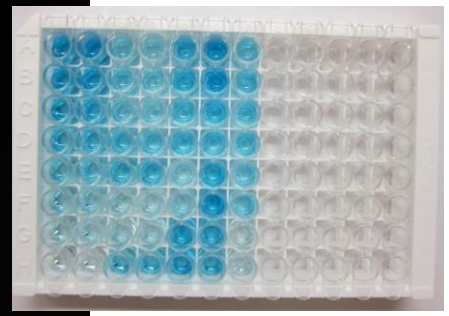
Enzyme
Mixing lytic enzymes with bacterial cells in the solution results in adsorbtion and break down of cell surfaces. Lysozyme, lysostaphin, zymoly vase, cellulose, protease or glycanase can be used.



Source: *A Review on Macroscale and Microscale Cell Lysis Methods by* Mohammed Shehadul Islam, Aditya Aryasomayajula and Ponnambalam Ravi Selvaganapathy

Cyanotoxin analytical methods

ELISA (Total Toxin)



Liquid Chromatography/ Mass Spectrometry (Congener Specific)

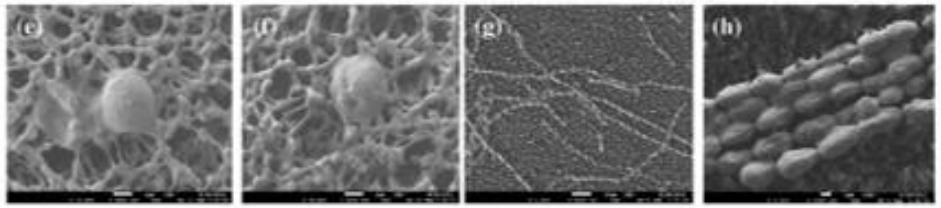
Cell damage

Chlorophyll-a

Digital Flow Cytometry (FlowCAM)

Scanning Electron Microscopy (SEM)

Ref: Coral, et. al. (2013)



Cell staining

Ref: Coral, et. al. (2013)

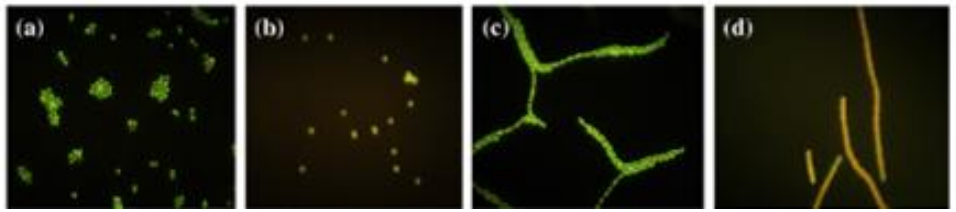
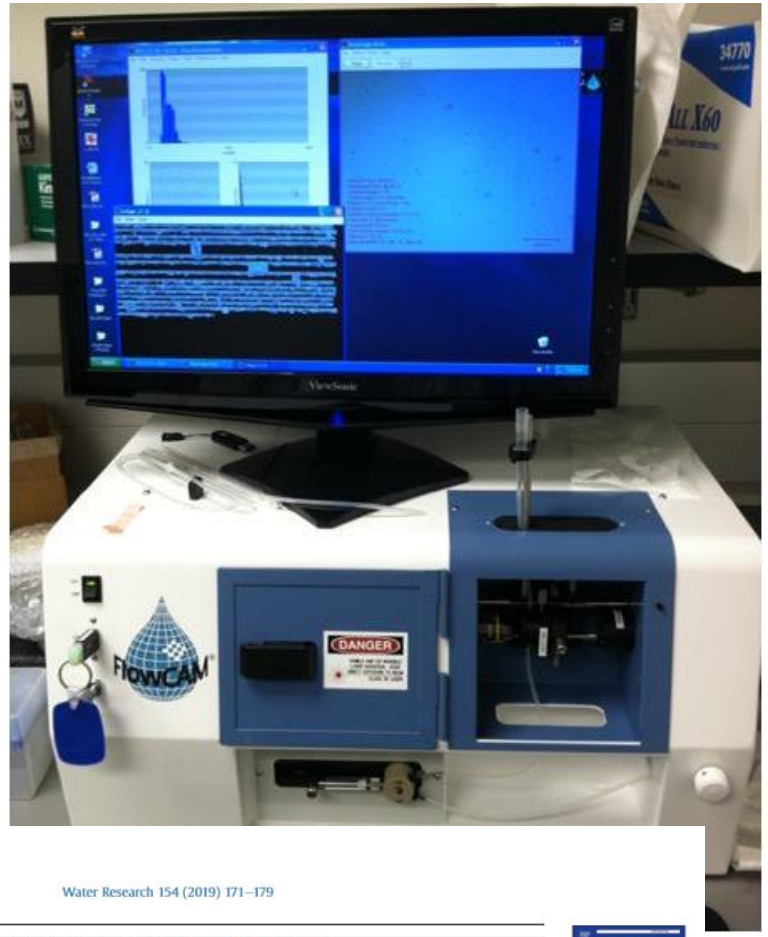


Photo: FlowCAM



Now: ATP

Water Research 154 (2019) 171–179

Contents lists available at ScienceDirect

Water Research

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

Using rapid quantification of adenosine triphosphate (ATP) as an indicator for early detection and treatment of cyanobacterial blooms

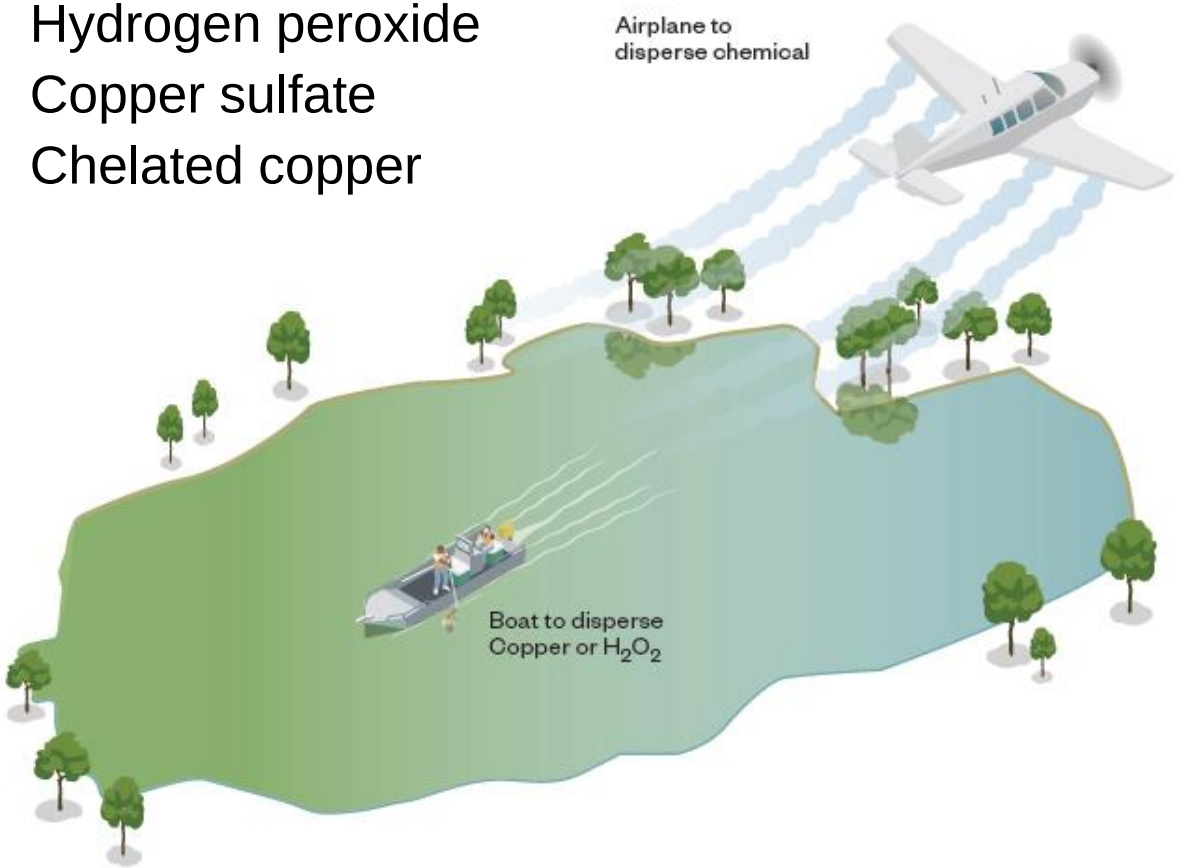
Katherine E. Greenstein, Eric C. Wert*

Southern Nevada Water Authority (SNWA), P.O. Box 99954, Las Vegas, NV, 89193-9954, United States

Many different chemicals are used for reservoir and drinking water treatment

Algaecides

- Hydrogen peroxide
- Copper sulfate
- Chelated copper

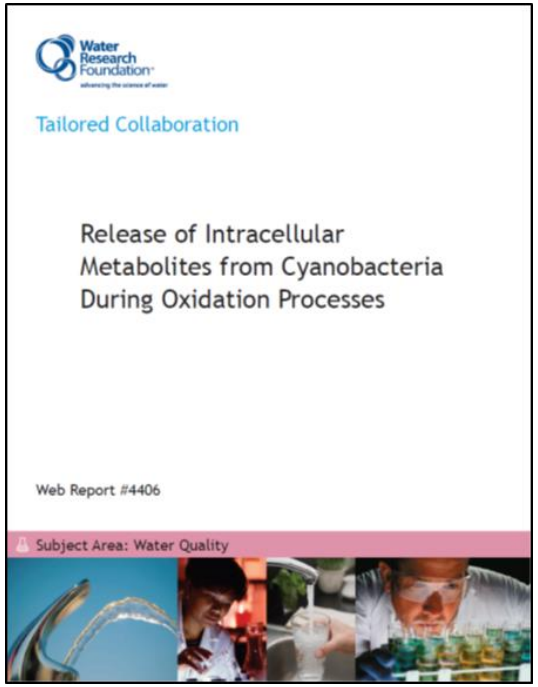


Oxidants

- Chlorine
- Chloramine
- Chlorine dioxide
- Ozone
- Permanganate
- Advanced Oxidation Process

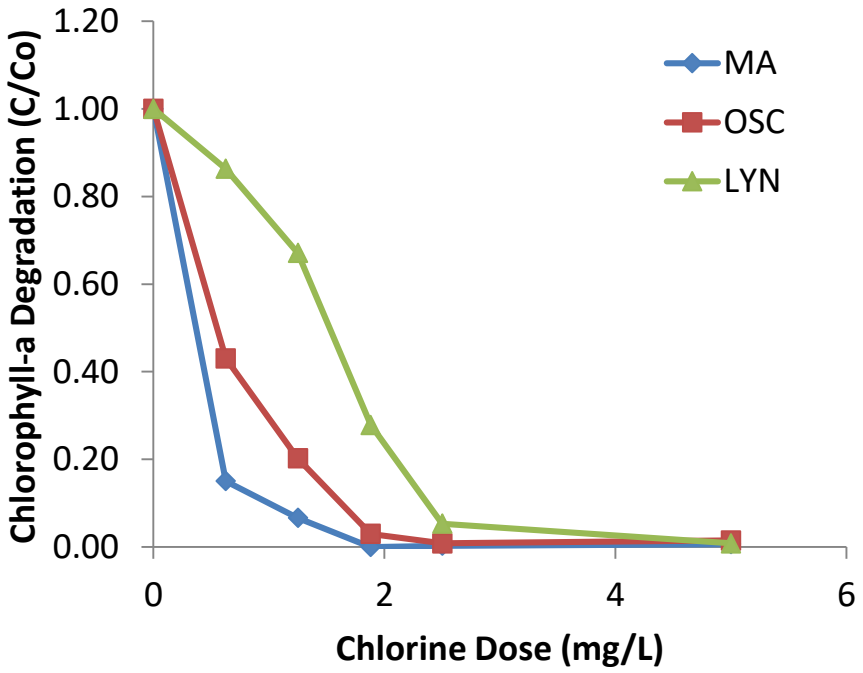
Background: Water Research Foundation (WRF) #4406

- 3 laboratory cultured strains of cyanobacteria
  - *Microcystis aeruginosa, Oscillatoria, Lyngbya*
- 4 Oxidants were evaluated
  - Ozone, chlorine, chlorine dioxide and chloramine
  - Oxidant:DOC mass ratio = 0, 0.25, 0.50, 0.75, 1.0, 2.0
  - Time = 24 hours
  - Measured oxidant exposure (i.e. concentration over time)

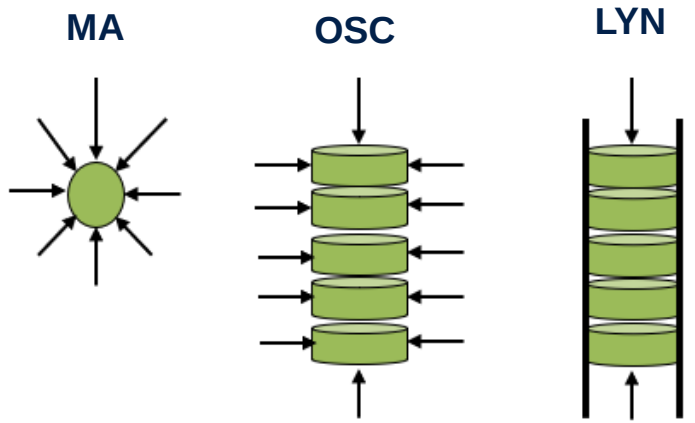


- Measured cell damage using chlorophyll and flow cytometry (FlowCAM)
- Measured microcystin-LR release using LC-MS/MS

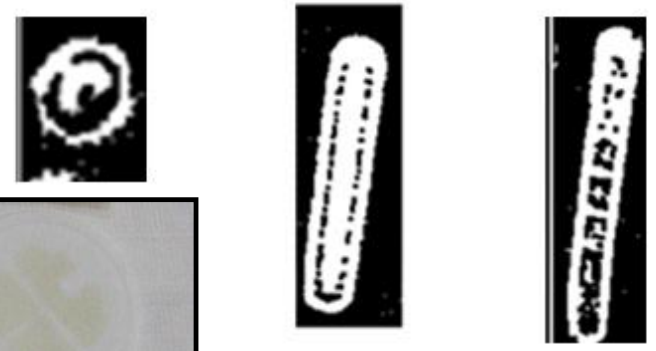
Morphology effects cell damage



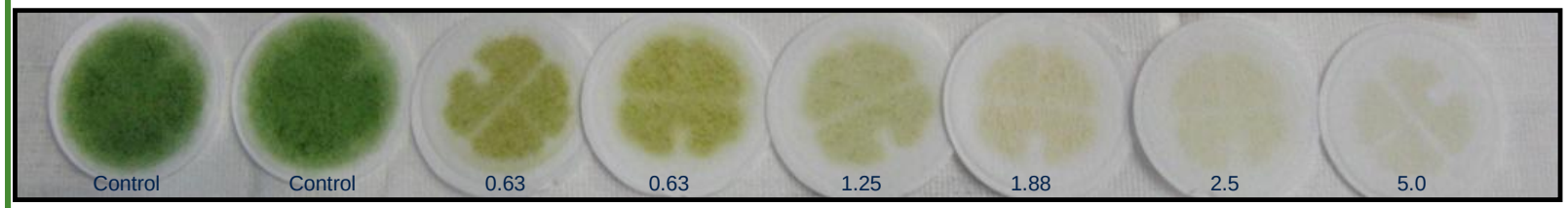
Consider exposed surface area



Theory supported with FlowCAM images



Cl_2

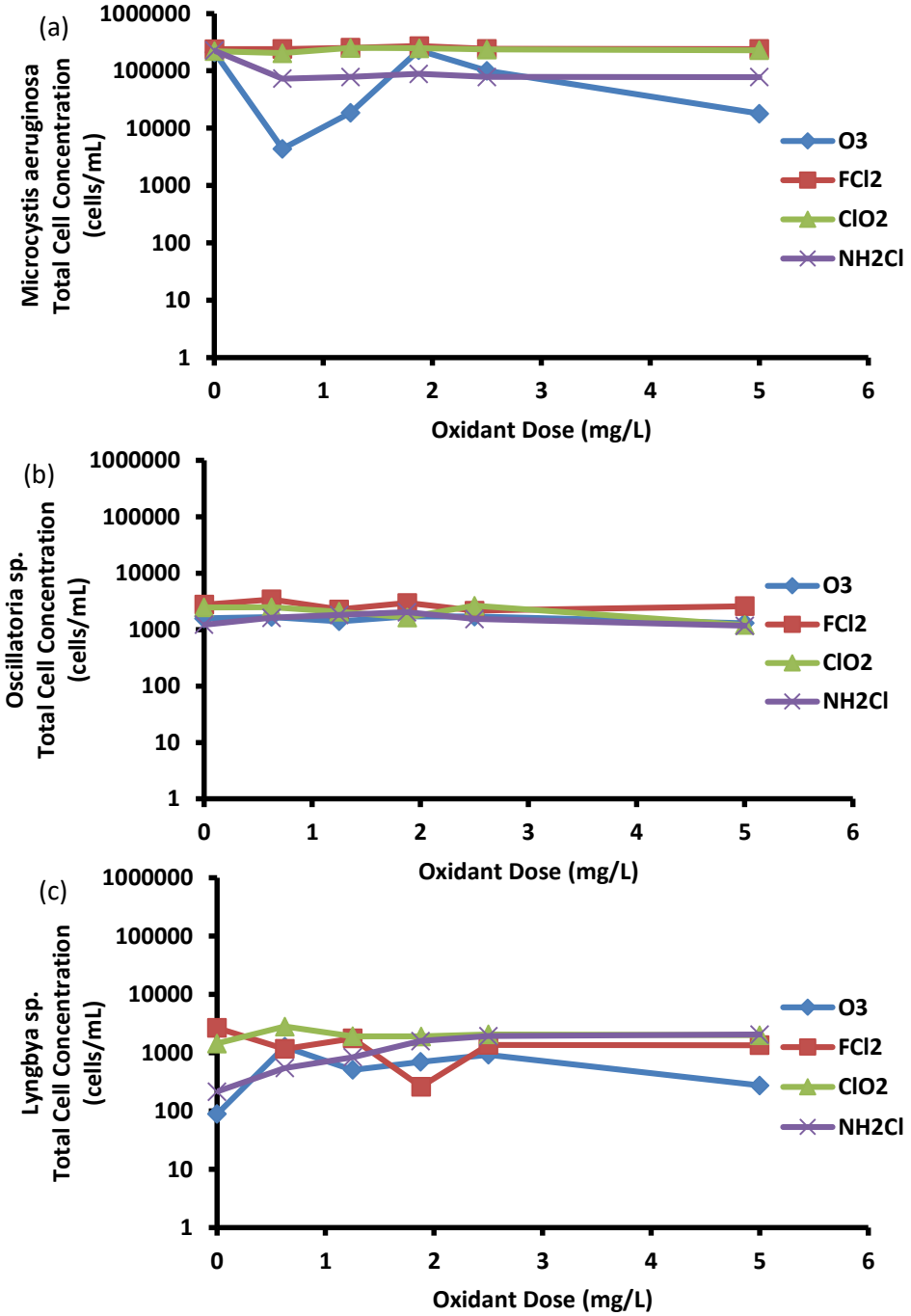


Cell lysis ≠ total destruction

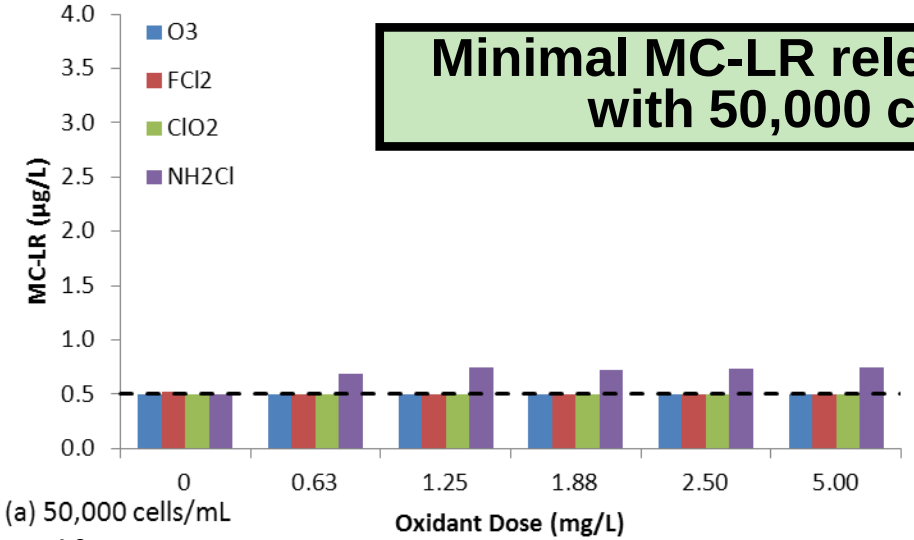
- Assessment
  - All particles

- Results indicate cells are damaged without resulting in complete lysis or fragmentation

Ref: Wert, E.C., et al., Water Research (2013)

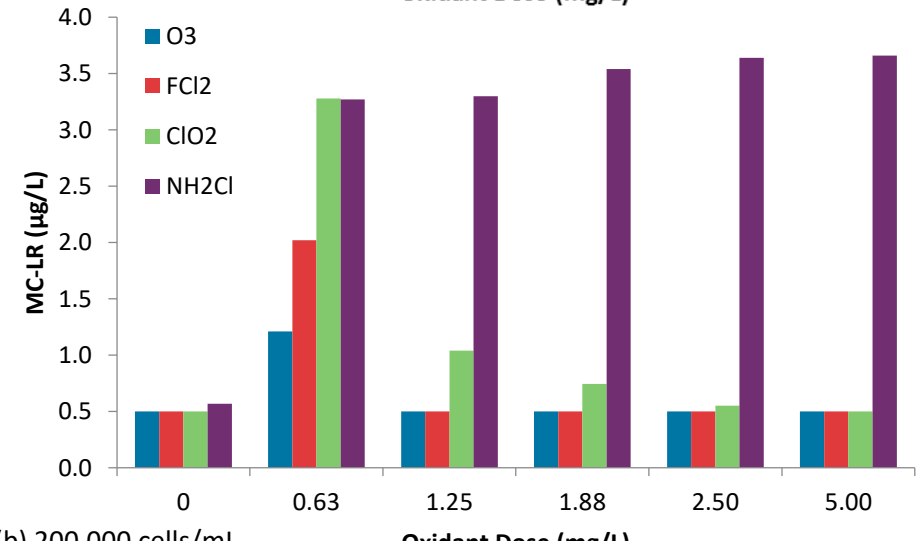


Microcystin was released in 24 h experiments



(a) 50,000 cells/mL

Minimal MC-LR release occurred with 50,000 cells/mL



(b) 200,000 cells/mL

Majority of release occurred at dosages < 0.63 mg/L

Oxidation of released extracellular MC-LR followed literature

WATER RESEARCH 52 (2014) 251–259

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/watres

Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species

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ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form
30 October 2013
Accepted 1 November 2013
Available online 12 November 2013

Keywords:
Drinking water
Cyanobacteria
Cyanotoxin
2-Methylisoborneol (MIB)
Geosmin
Microcystin-LR
Intracellular organic matter (IOM)
Fluorescence

ABSTRACT

The release of intracellular microcystin-LR (MC-LR), 2-methylisoborneol (MIB), and geosmin was investigated after the oxidation of three cyanobacteria (*Microcystis aeruginosa* (MA), *Oscillatoria* sp. (OSC), and *Lyngbya* sp. (LYN)). During the oxidation of 200,000 cells/mL of MA, release of intracellular MC-LR exceeded the World Health Organization (WHO) guideline of 1 µg/L during the lowest oxidant exposures (CT) tested: ozone (0 mg-min/L, below the ozone demand), chlorine (<40 mg-min/L), chlorine dioxide (<560 mg-min/L), and chloramine (<640 mg-min/L). As the CT increased, ozone, chlorine, and chlorine dioxide were able to oxidize the released MC-LR. During the oxidation of OSC (2800 cells/mL) and LYN (1600 cells/mL), release of intracellular MIB and geosmin exceeded reported threshold odor values after exposure to chlorine, chlorine dioxide, and chloramine, which have low reactivity with these taste and odor compounds. Ozone oxidation of OSC yielded an increase in MIB concentration at lower exposures (≤2.9 mg-min/L), likely due to insufficient oxidation by hydroxyl radicals. The release of intracellular organic matter (IOM) was also measured to determine the potential of bulk measurements to act as a surrogate for cyanotoxins and metabolite release. In all cases, the dissolved organic carbon (DOC) release was less than 0.25 mg/L, which lacked the sensitivity to indicate the release of MC-LR, MIB, or geosmin. The fluorescence index proved to be a more sensitive indicator of intracellular organic matter release than DOC for MA. These results illustrate that toxic or odorous compounds may be released from cyanobacteria cells during oxidation processes with minimal changes in the DOC concentration.

1. Introduction

Due to the effects of climate change, the frequency and intensity of harmful cyanobacteria blooms is expected to increase globally (Paerl and Paul, 2012). These blooms can directly impact the quality of drinking water through the production of toxic metabolites, odorous metabolites or both. Several cyanotoxins including microcystin, anatoxin-a, saxitoxin, cylindrospermopsin, and nodularin are known to be generated by

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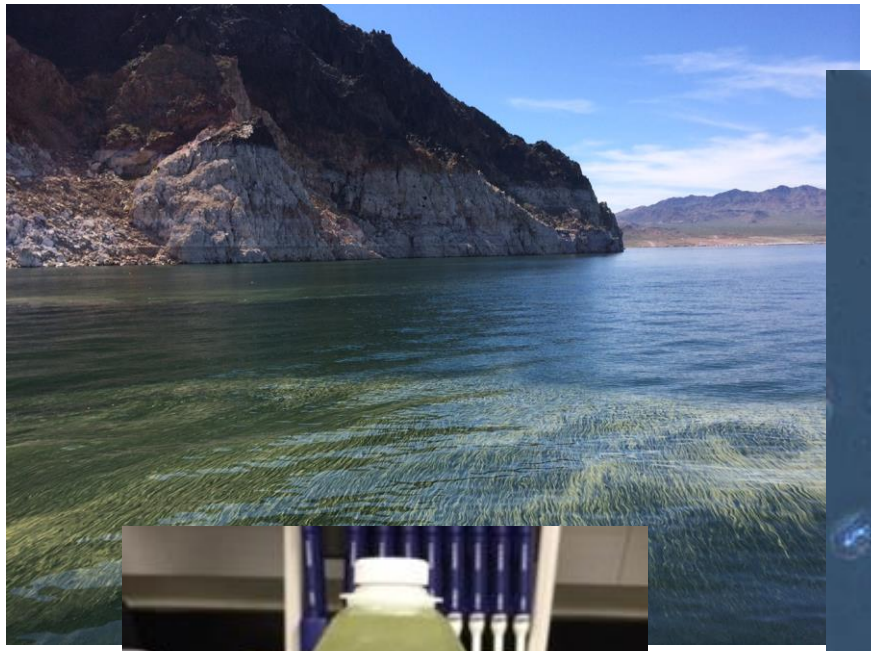
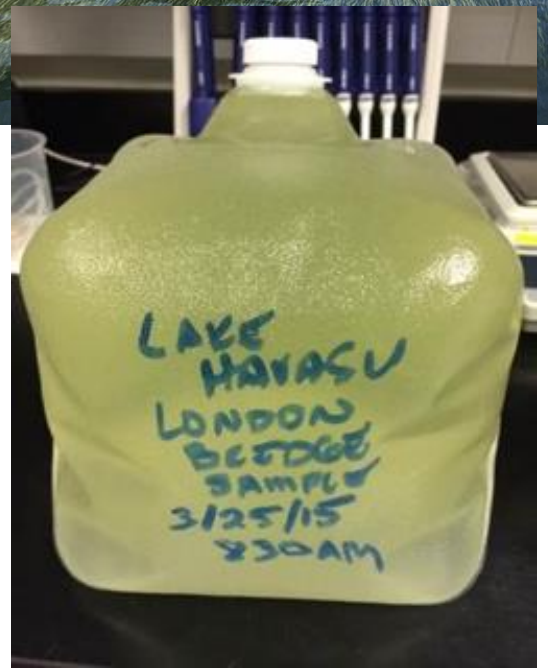
http://dx.doi.org/10.1016/j.watres.2013.11.001

Oxidation of natural *Microcystis* was examined





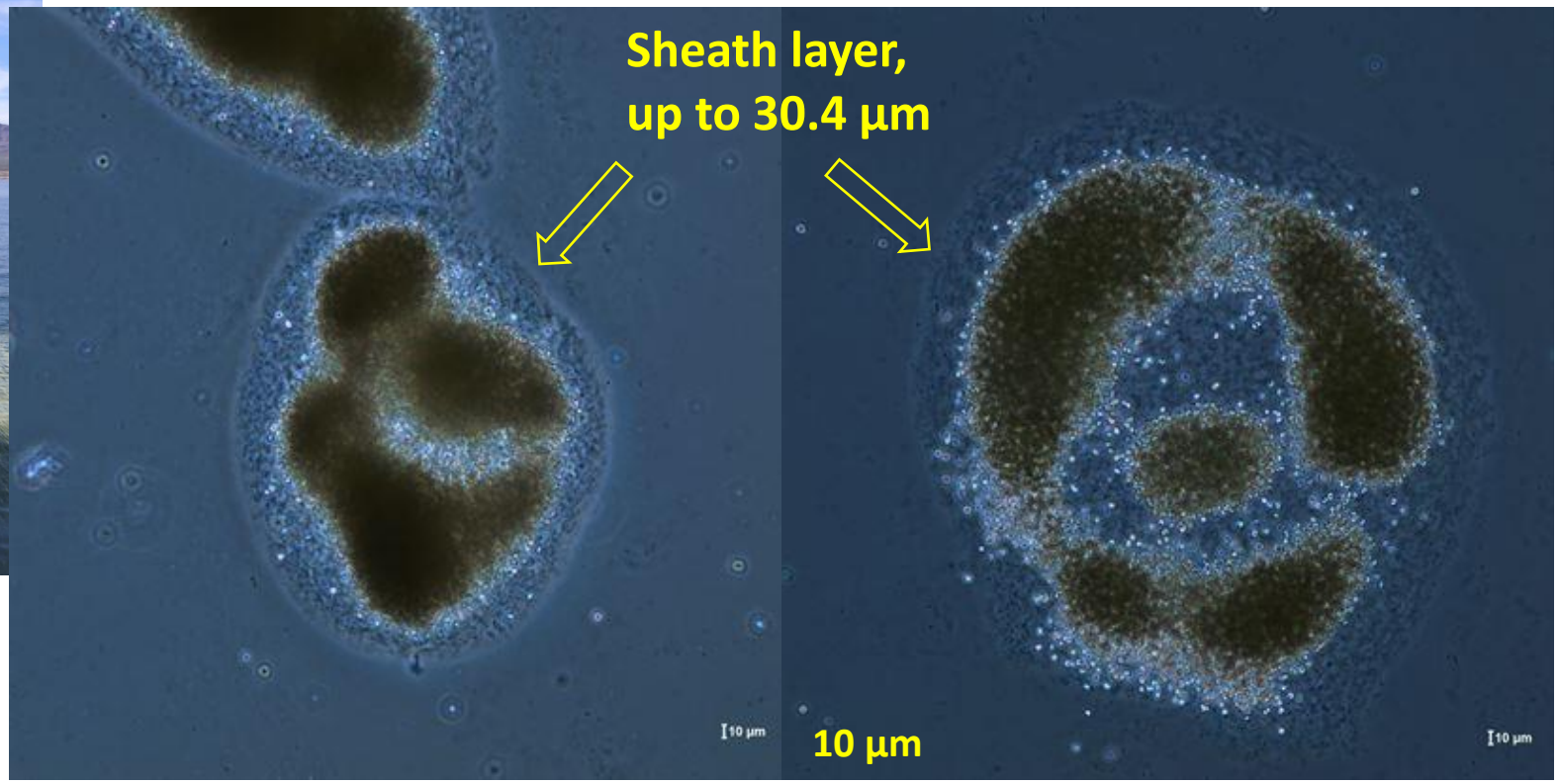
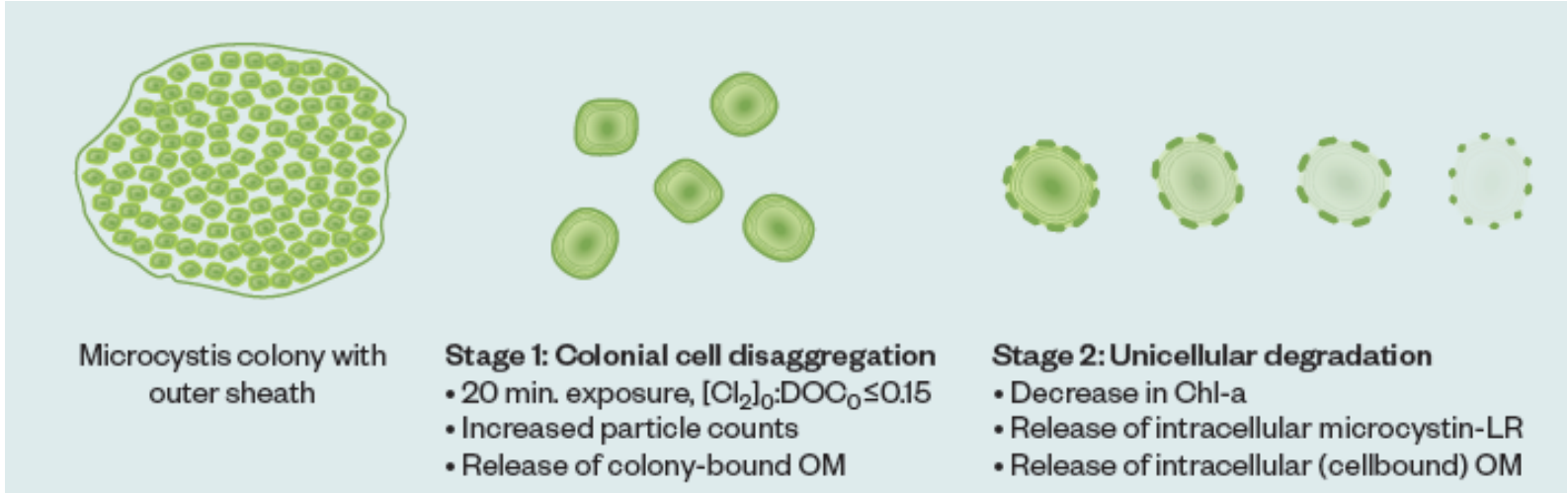


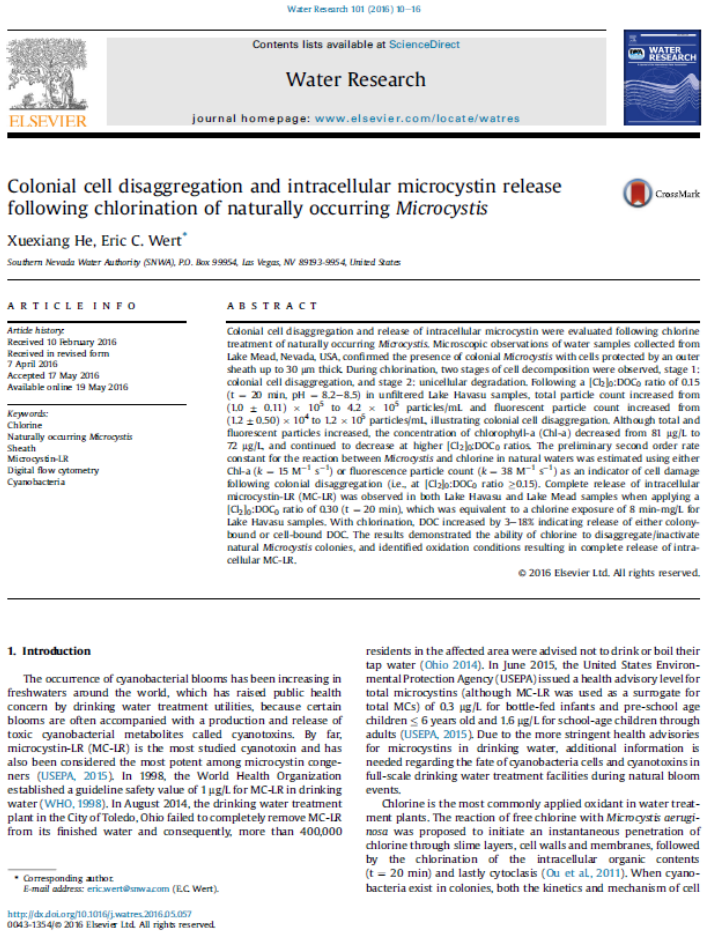
Image credit: Ann St. Amand (PhycoTech, Inc., St. Joseph, MI)

Revised Cell Degradation Model



- A $[Cl_2]_0$:DOC_0 ratio of 0.30 (t = 20 min) was found to completely release intracellular MC-LR
  - With further verification, may be used as an indicator of intracellular toxin release when treating naturally occurring *Microcystis*

Develop "Release and Treat" Guidance



Water Research 101 (2016) 10–16

Contents lists available at ScienceDirect

Water Research

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

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

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ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form
7 April 2016
Accepted 17 May 2016
Available online 19 May 2016

Keywords:
Chlorine
Naturally occurring Microcystis
Sheath
Microcystin-LR
Digital flow cytometry
Cyanobacteria

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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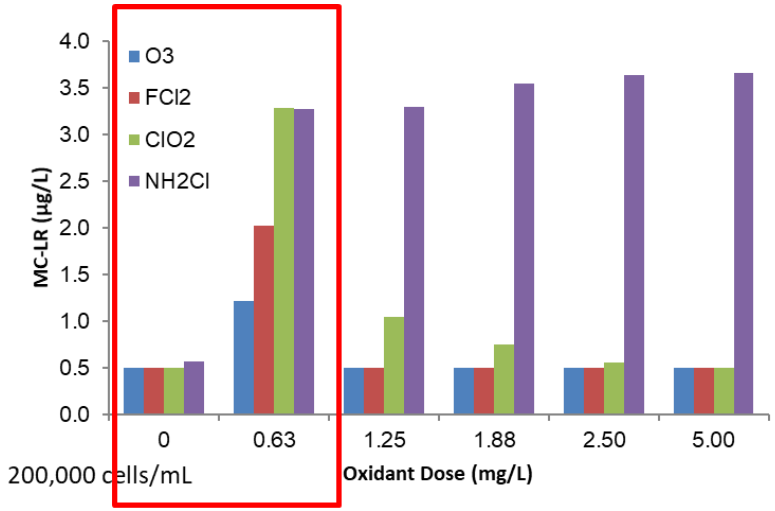
http://dx.doi.org/10.1016/j.watres.2016.05.057
0043-1354/

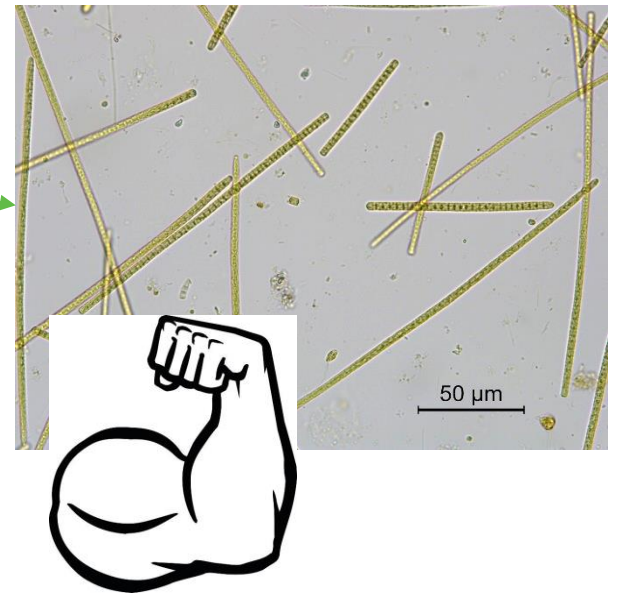
Response – WRF #4692 looks at pre-oxidation exposures and lab + natural cells

- **Oxidants:** O_3, Cl_2, NH_2Cl, ClO_2, $KMnO_4$
- **Reservoir treatment chemicals**: $CuSO_4$, H_2O_2
- Exposure times ≤20 min*, oxidant:DOC ratios less than demand
  - Normalized doses by DOC
  - **24 h for reservoir treatment chemicals*
- Refine utility guidance for intracellular release



3 cell suspensions/water matrices were examined

- **Lab-cultured** 1.0x10^{6} cells/mL *Microcystis aeruginosa* in Colorado River water (CRW; DOC 2.5 mg/L, pH 8)
- **U.S. bloom:** 2.7x10^{6} cells/mL *Planktothrix agardhii-suspensa* in Grand Lake St. Mary's (GLSM; DOC 9.4 mg/L, pH 8)
- **Canadian bloom:** 1.6x10^{5} cells/mL *Anabaena spiroides*, 1.0x10^{5} cells/mL *Aphanothece clathrata brevis*, and 4.0x10^{4} cells/mL *Microcystis aeruginosa* in Lake Champlain (DOC 6.1 mg/L, pH 7.9)

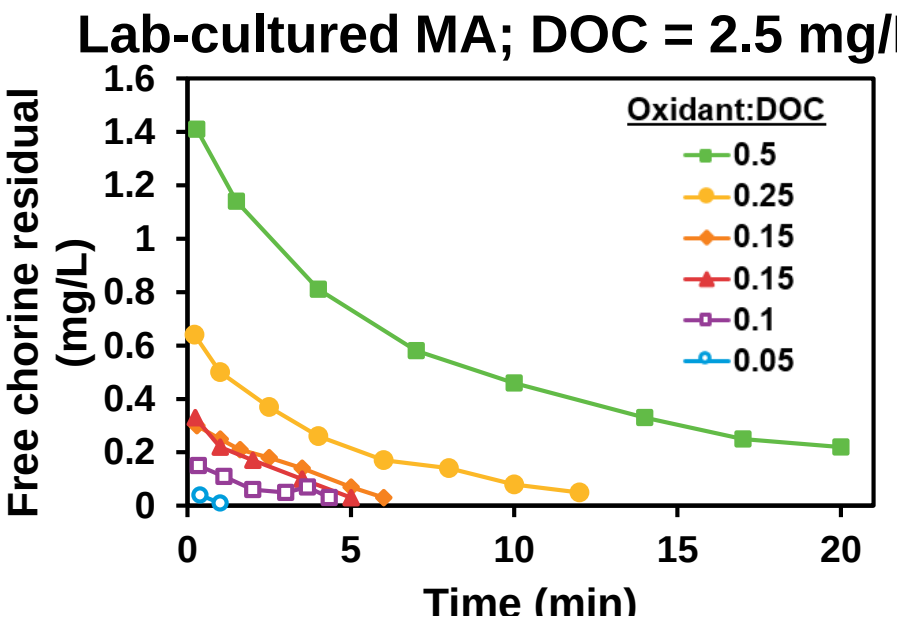


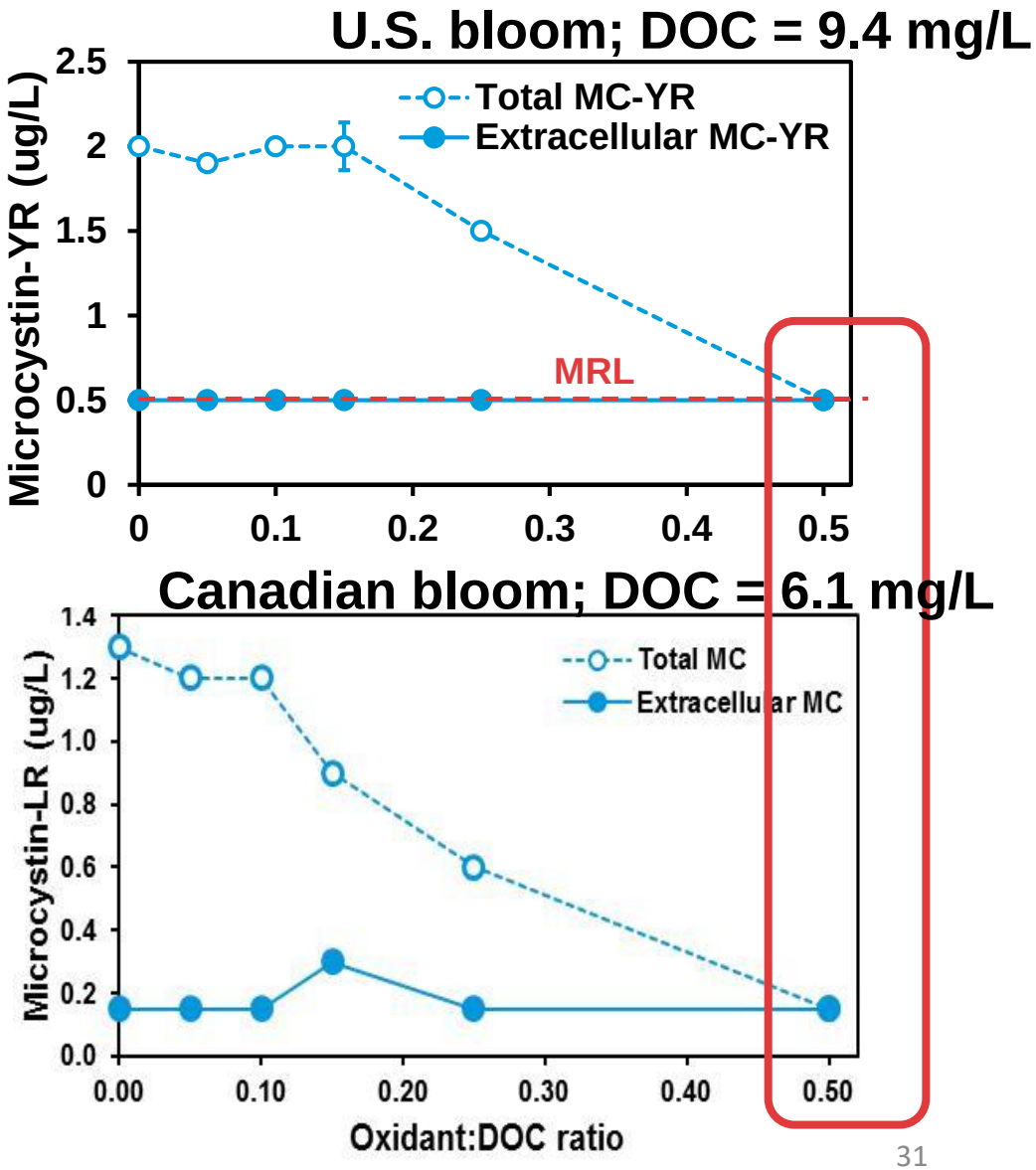
Cell suspensions were screened for microcystins

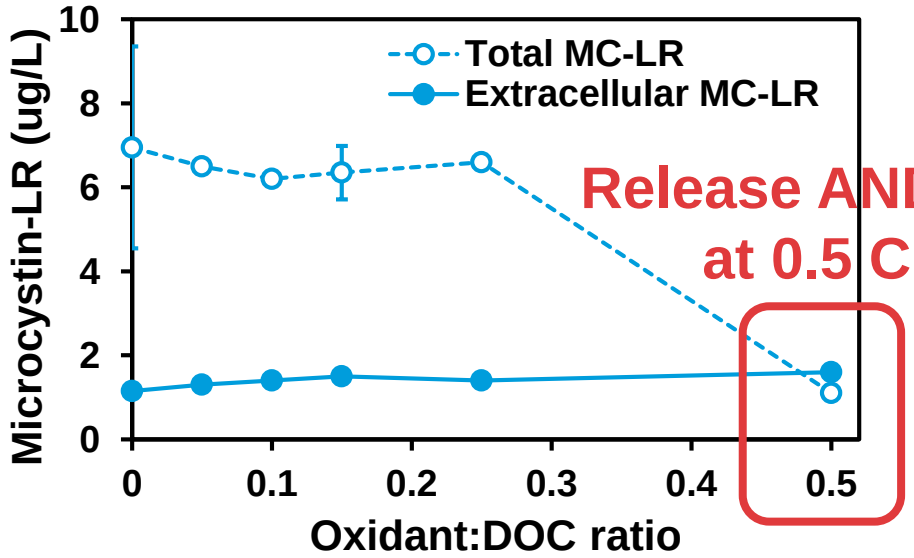
- LC-MS/MS used to screen for 8 different MC congeners
  - -LA, LF, LR, LW, LY, RR, WR, YR
  - **U.S. bloom → only MC-YR found (~2 ppb)**
  - **Lab-cultured cells/Canadian bloom → only MC-LR found (~6-10 ppb)**
- Suspensions were also screened with ELISA
  - Higher concentrations than just MC-YR in GLSM
  - MC-LR only confirmed for lab-cultured cells
- **Here, results focusing on MC-LR and MC-YR will be presented**

What happens within 20 minutes or less with low oxidant exposures?

Cl_2 oxidized MCs (time ≤ 20 min)

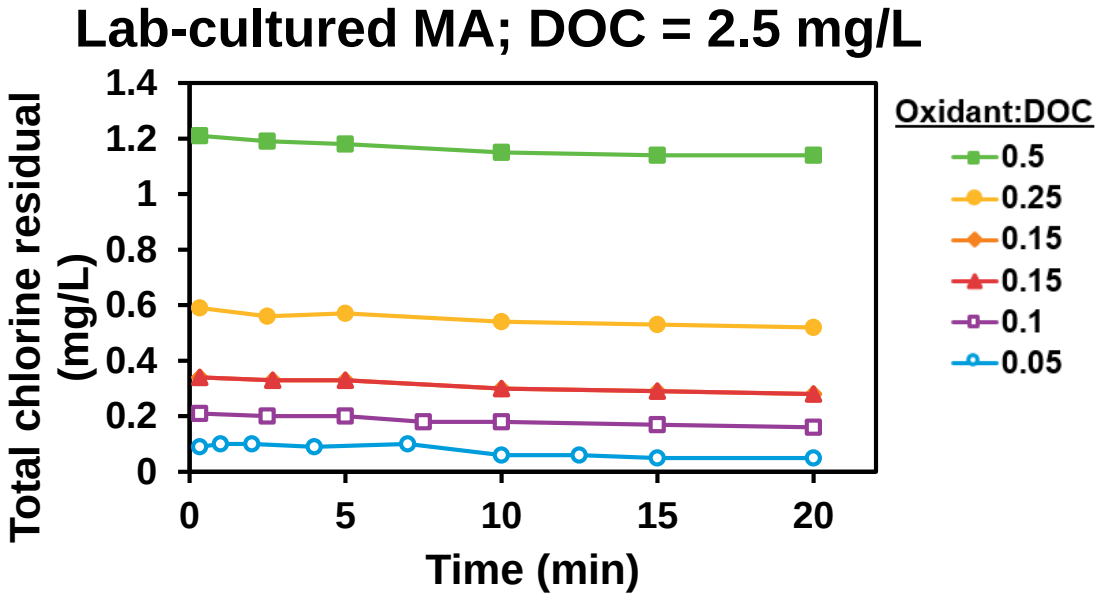


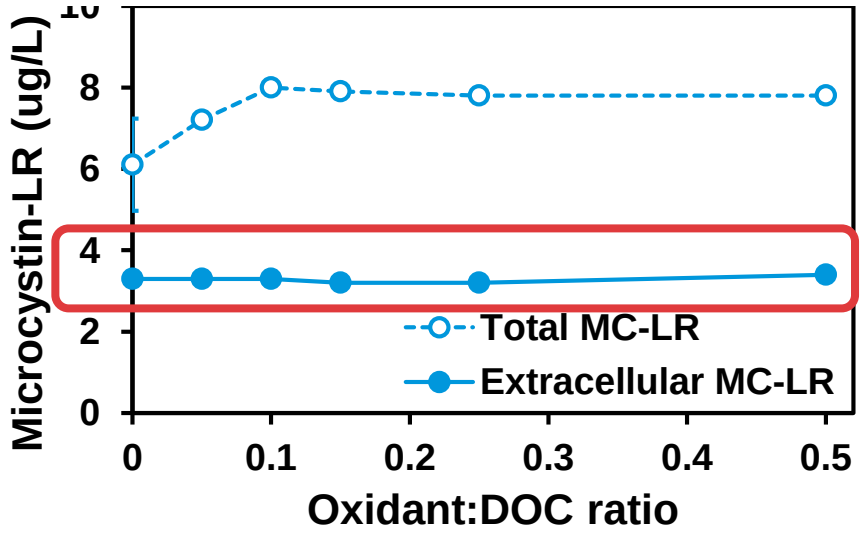


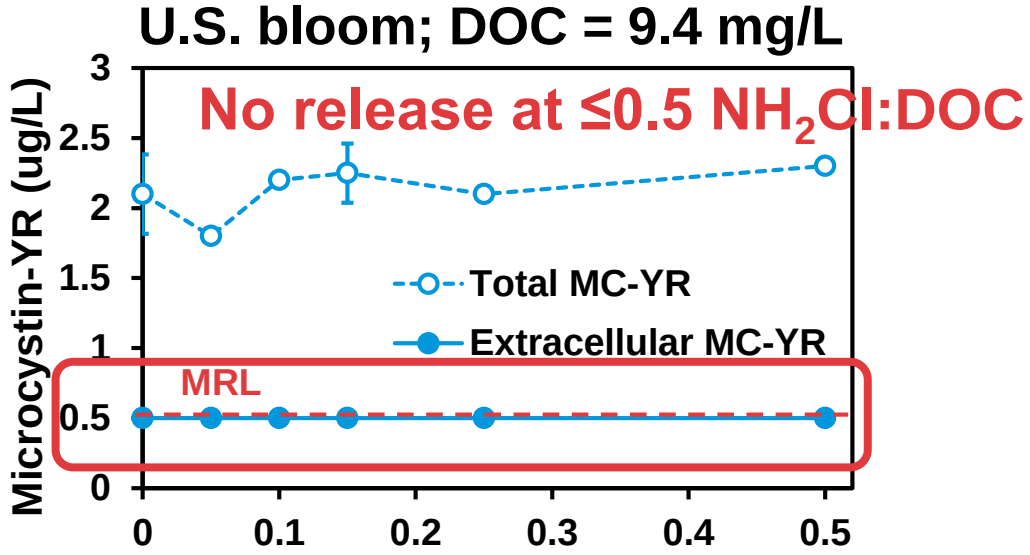


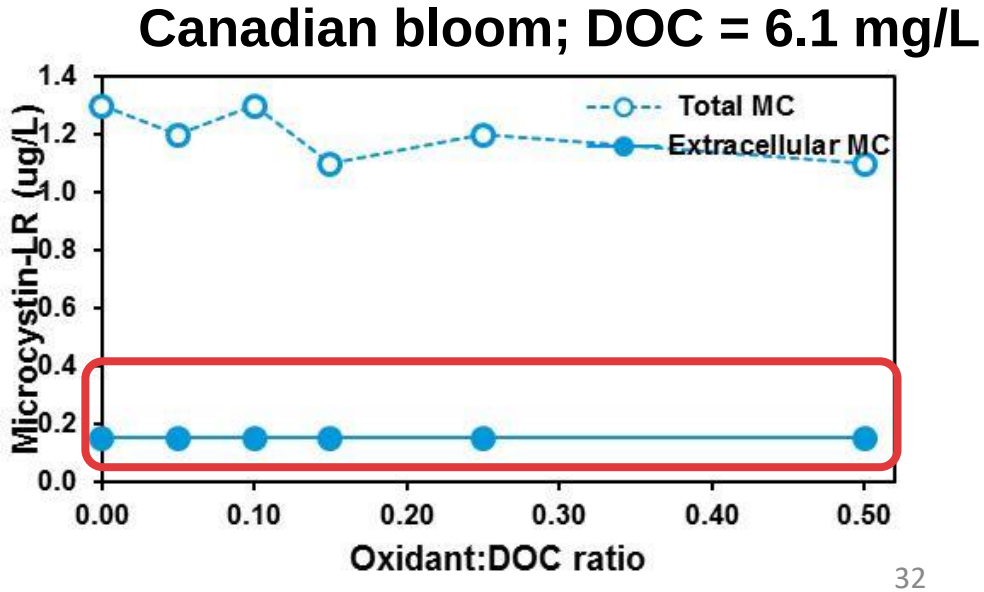
Release AND oxidation at 0.5 Cl_2:DOC

NH_2Cl did not release MCs (time ≤ 20 min)

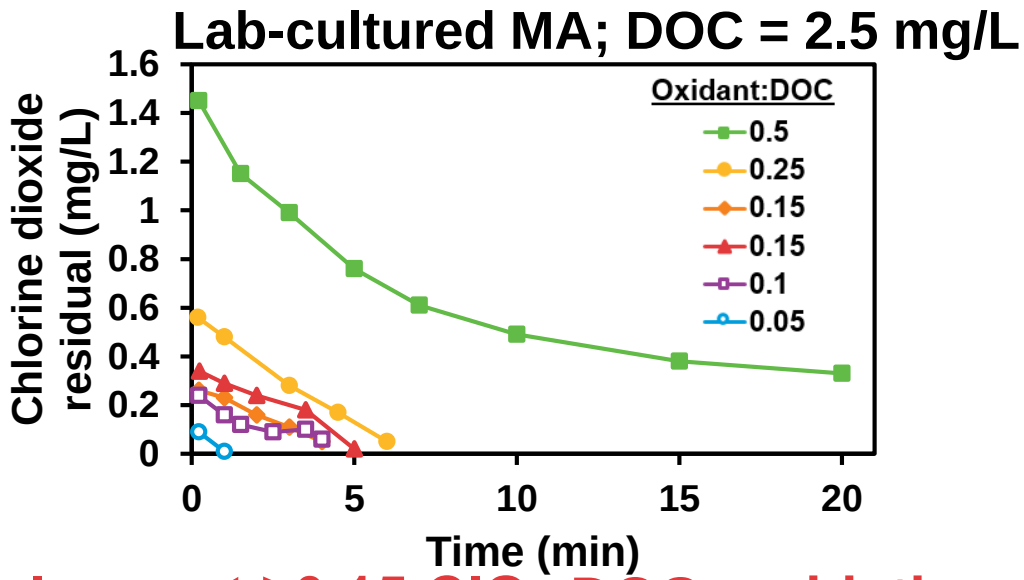


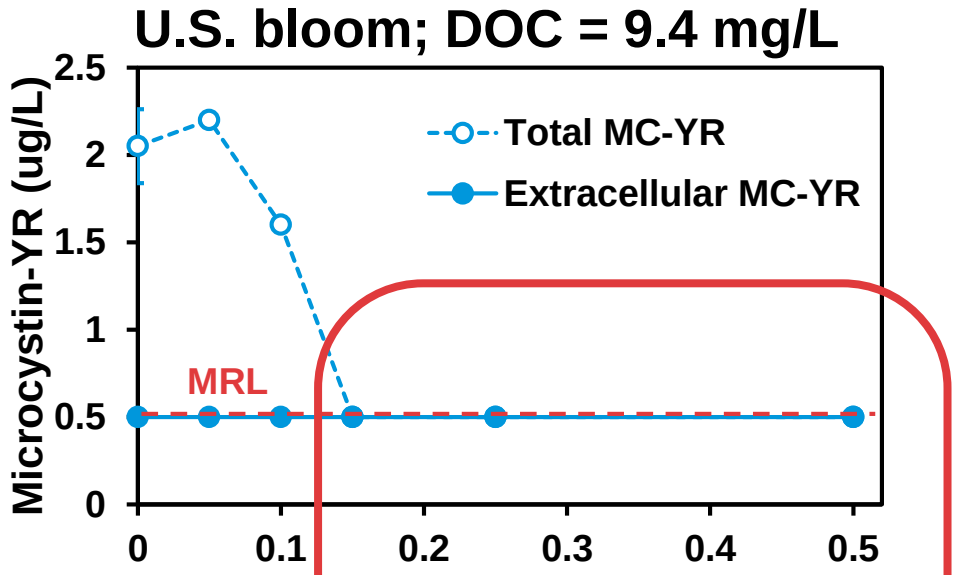




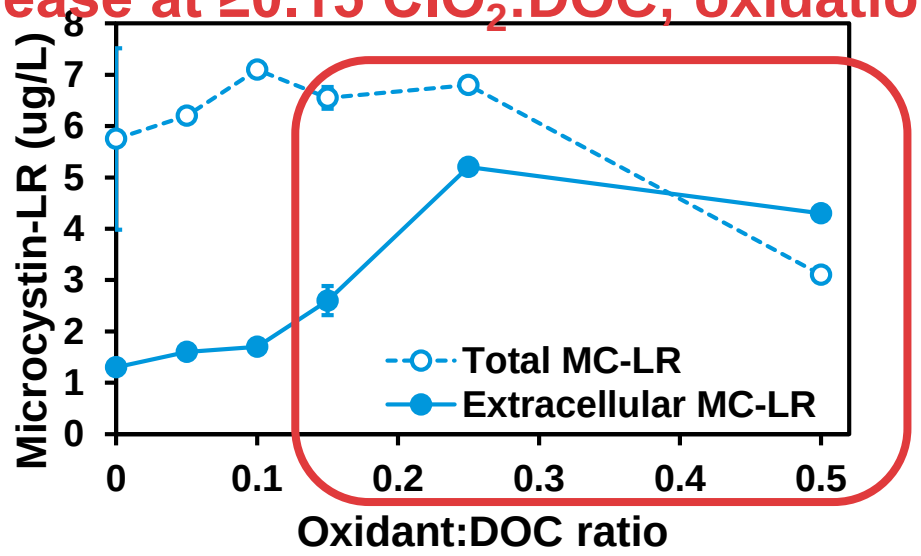


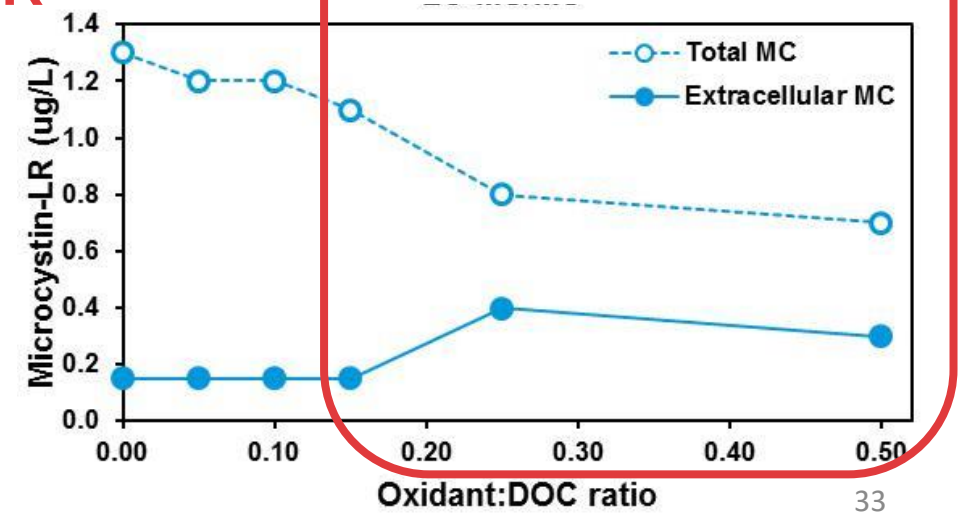
ClO_2 oxidized MC-YR, released MC-LR (time ≤ 20 min)



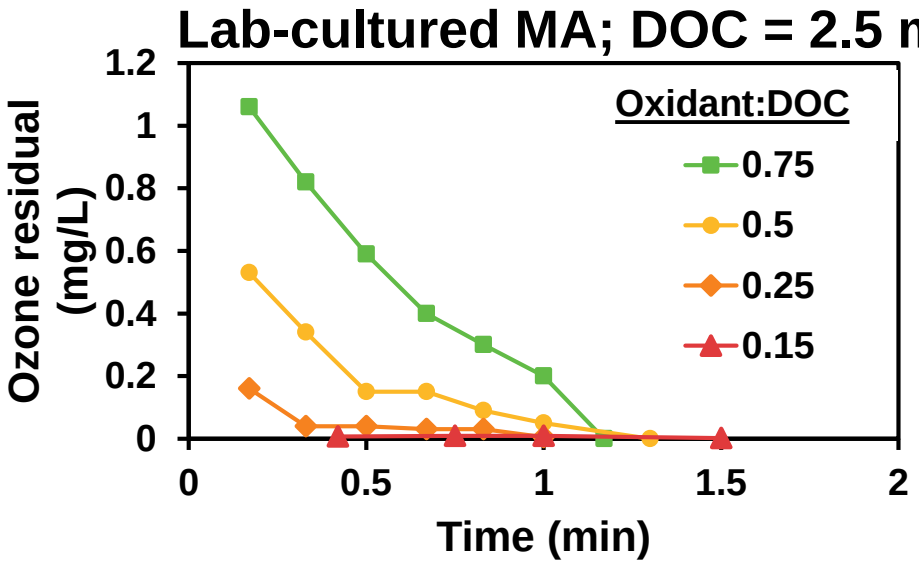


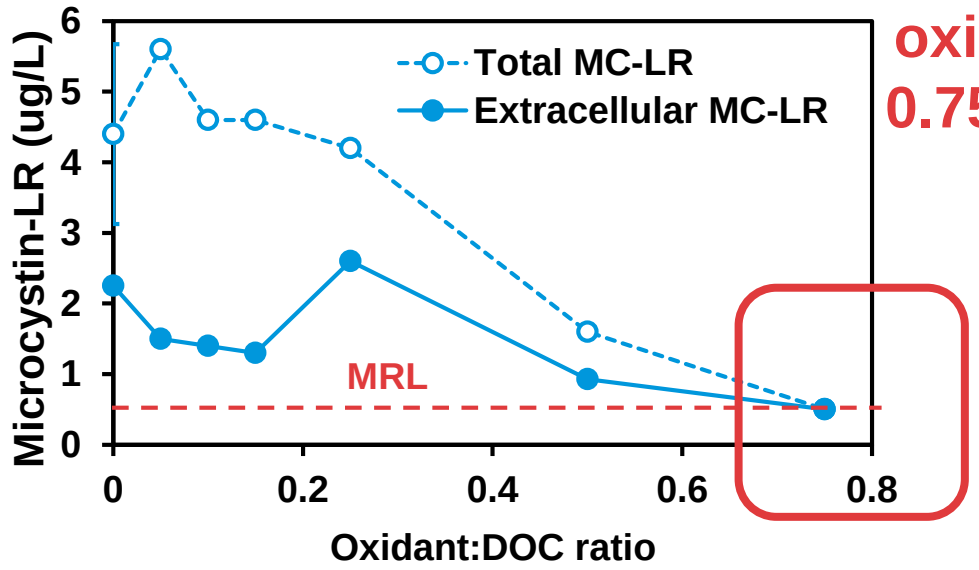
Release at ≥0.15 ClO_2:DOC; oxidation of MC-YR



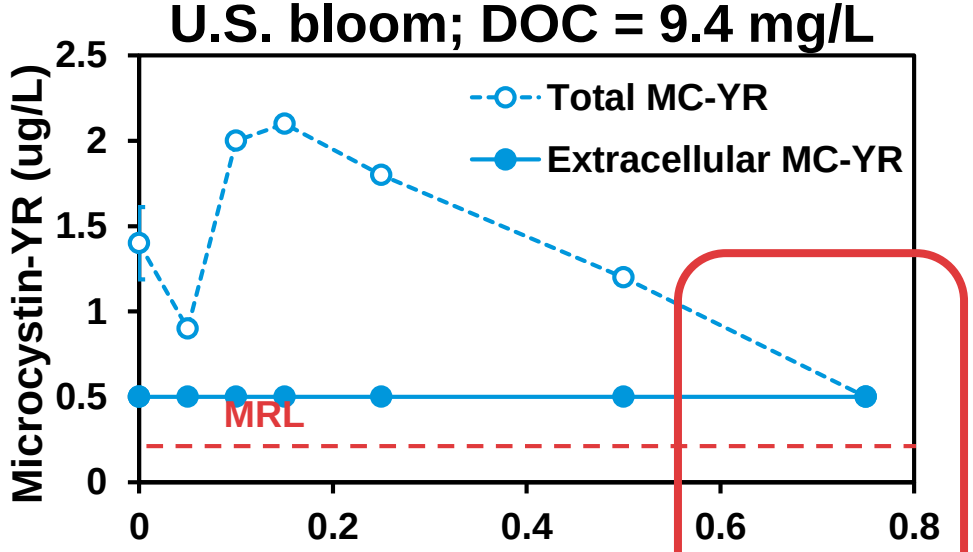


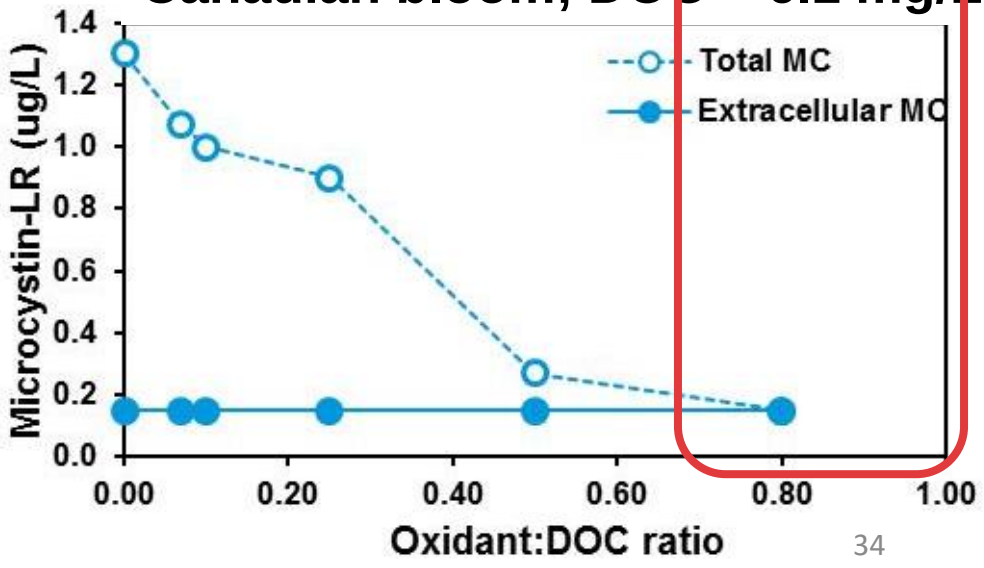
O_3 oxidized MCs with <2 min exposure



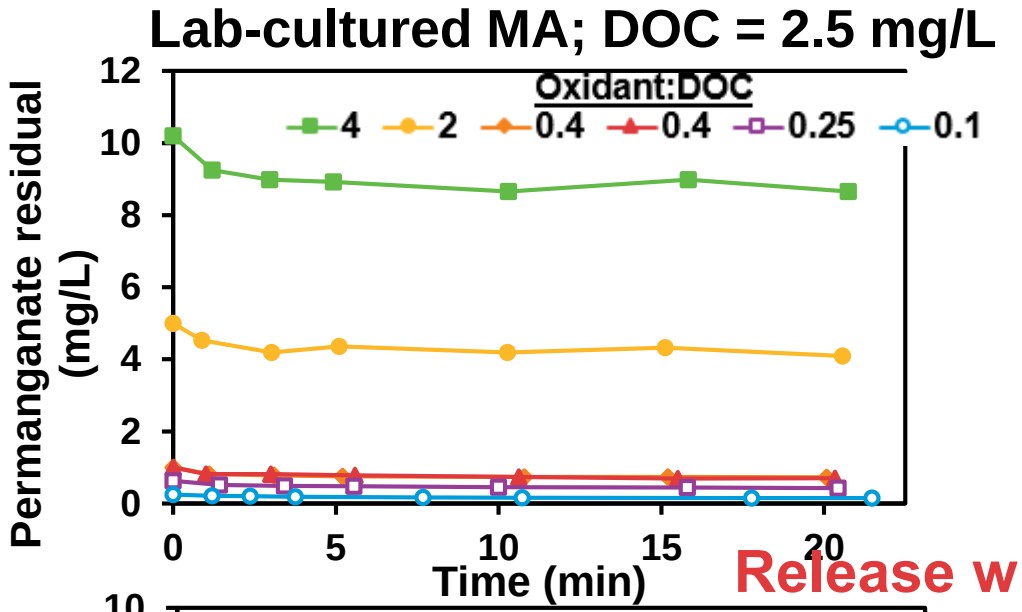


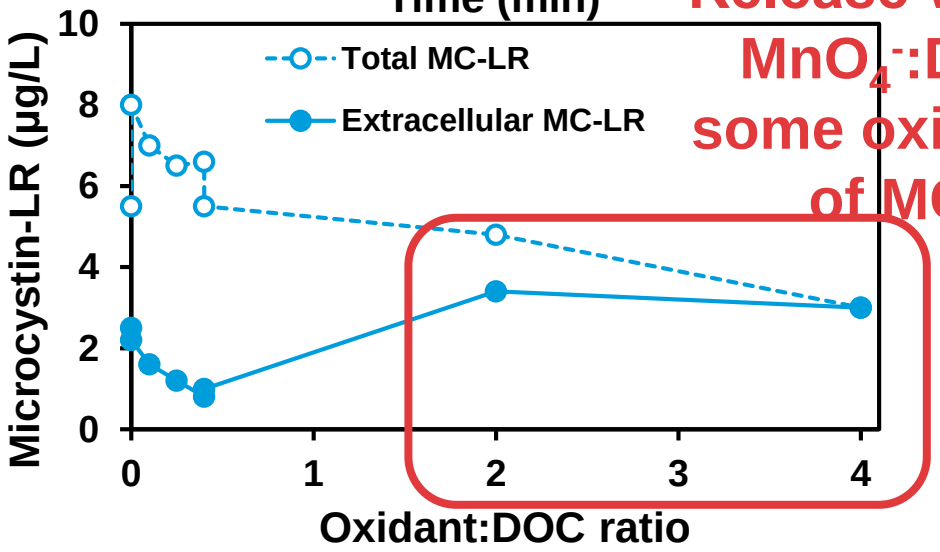
Release AND oxidation at 0.75 O_3:DOC



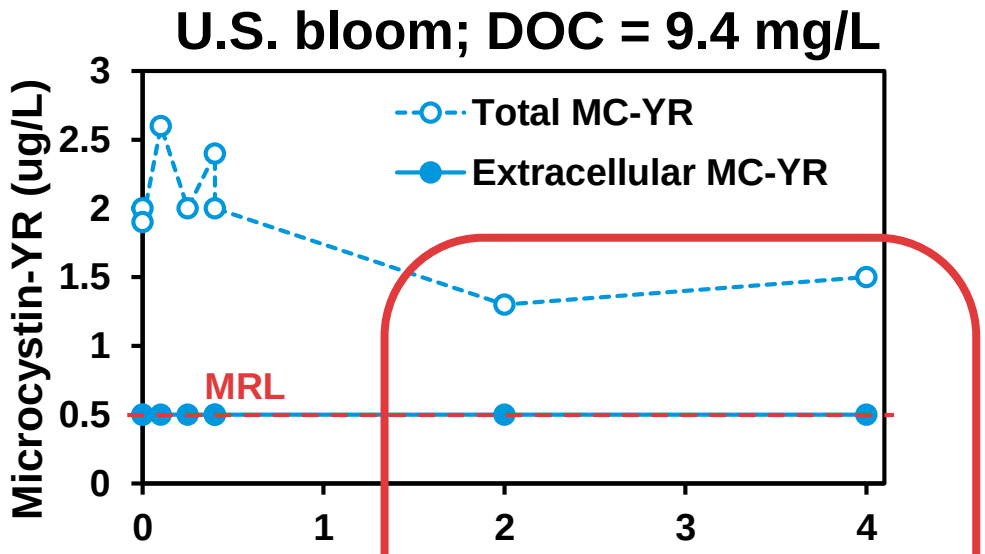


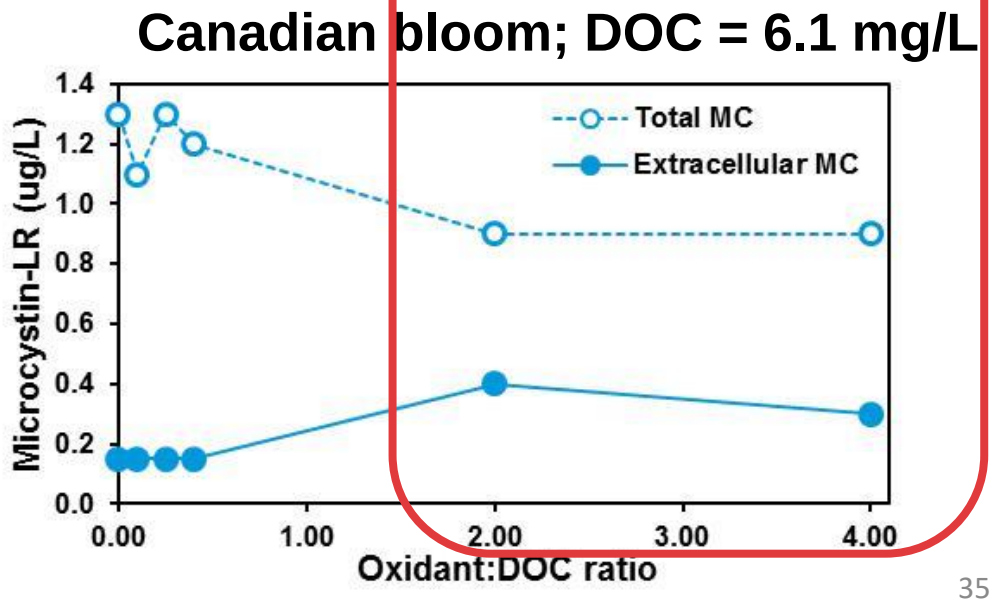
MnO_4^- released and oxidized some MCs (time ≤ 20 min)



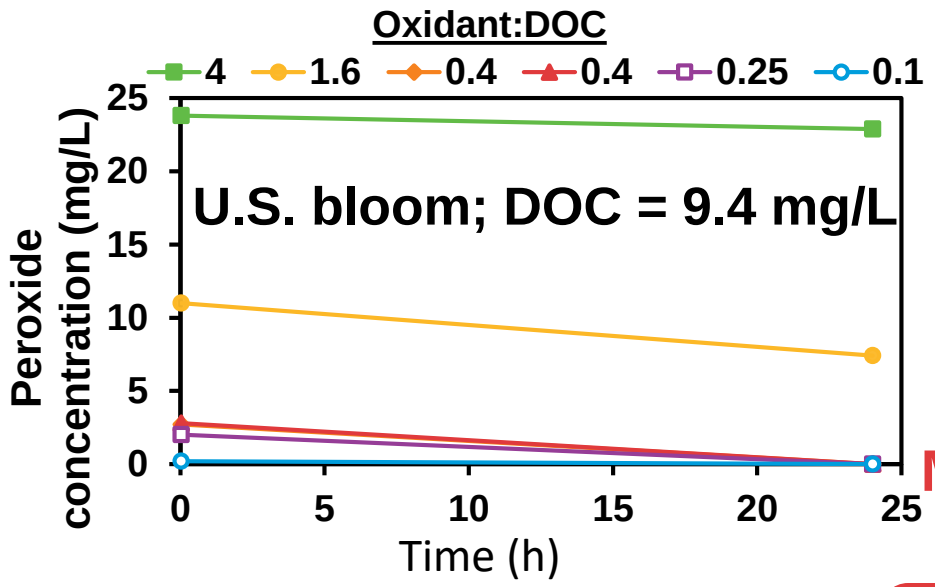


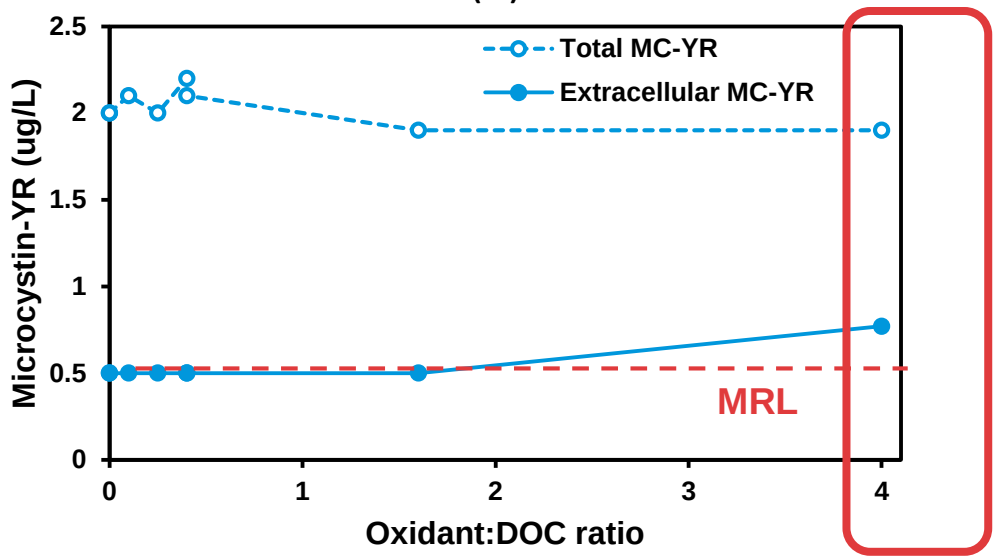
Release with ≥2 MnO_4^-:DOC; some oxidation of MCs





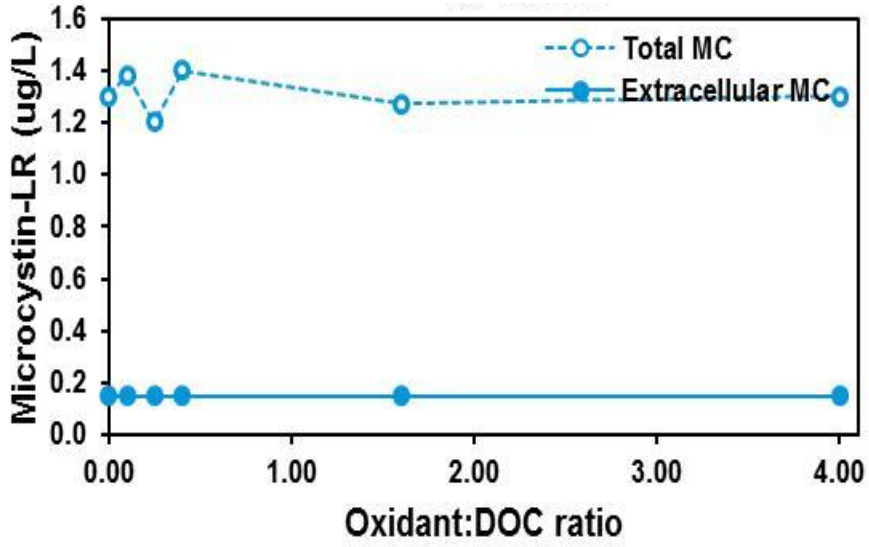
Hydrogen peroxide released minimal to no MCs within 24 hours



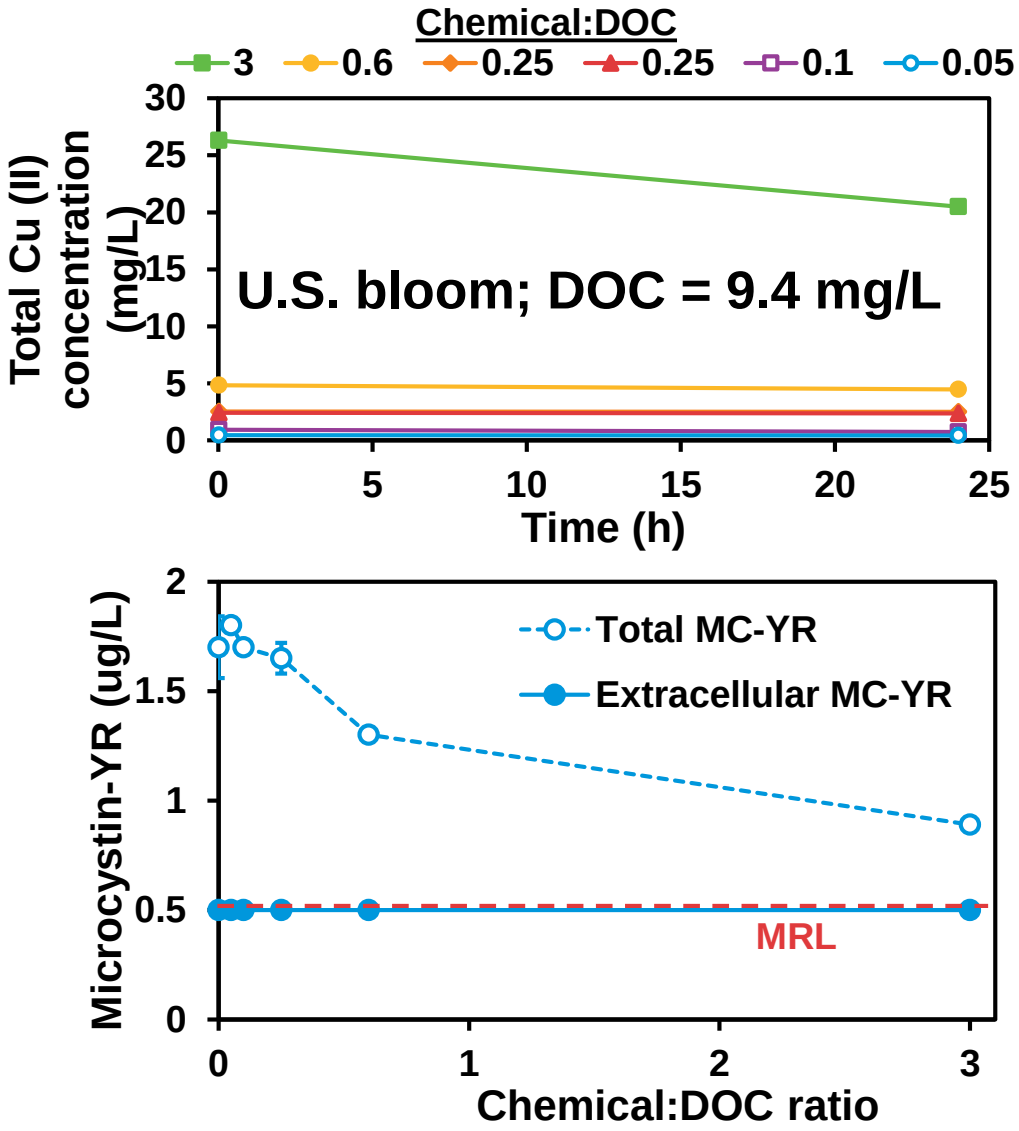


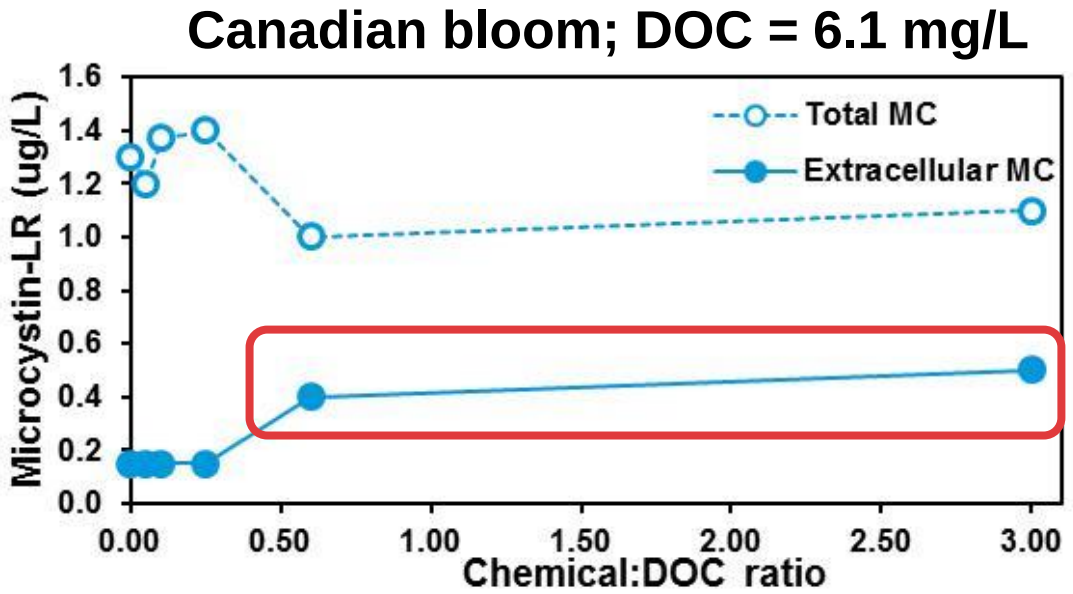
Minimal release at 4 H_2O_2:DOC

Canadian bloom; DOC = 6.1 mg/L



Copper released minimal to no MCs within 24 hours





Minimal release at ≥0.6 Cu(II):DOC

Preliminary guidance on effect of CT

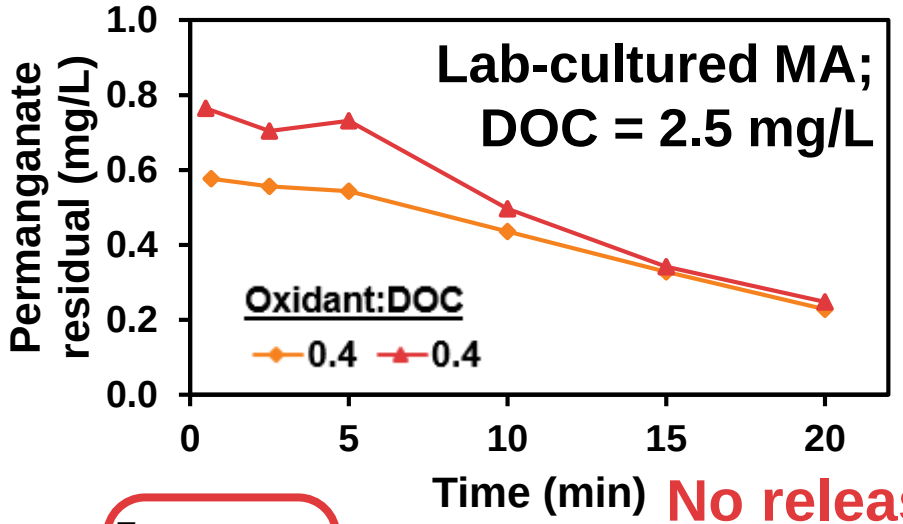
Effect of CT summary table

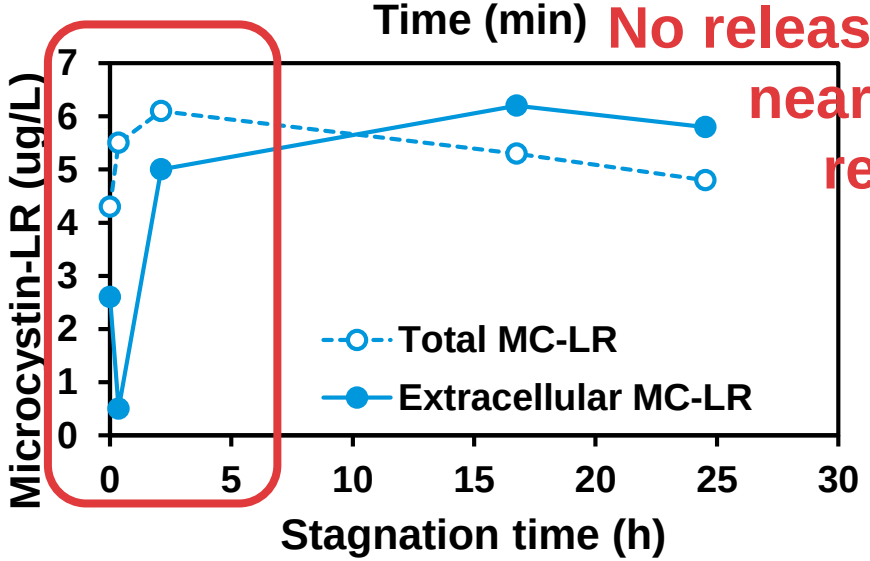
Oxidant:DOC ratios resulting in complete release of microcystins						
≤ 20 min oxidant exposure					24 h allowed for exposure	
Free chlorine	Monochloramine	Chlorine dioxide	Ozone	Permanganate	Hydrogen peroxide	Copper (II)
0.5 Cl_2:DOC	No release (≤0.5 NH_2Cl:DOC)	0.5 ClO_2:DOC	0.75 O_3:DOC	4 MnO_4-:DOC (for some water matrices/cells)	Minimal release (≤4 H_2O_2:DOC; for some water matrices/ cells)	Some release (0.6-3 Cu:DOC; for some water matrices/cells)

- Results were consistent with previous work
- Goal was to provide some general framework regarding release
- Additional research needed to further validate/revise these conditions

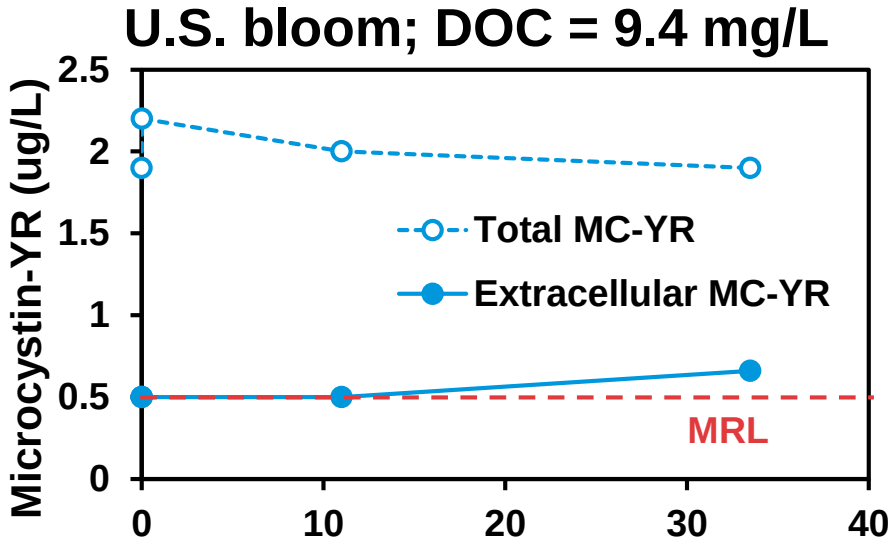
What happens with longer stagnation times following 20 min low oxidant exposures?

Example: MnO_4^- caused MC-LR release (lab cells)





No release at 20 min; nearly complete release at 2 h



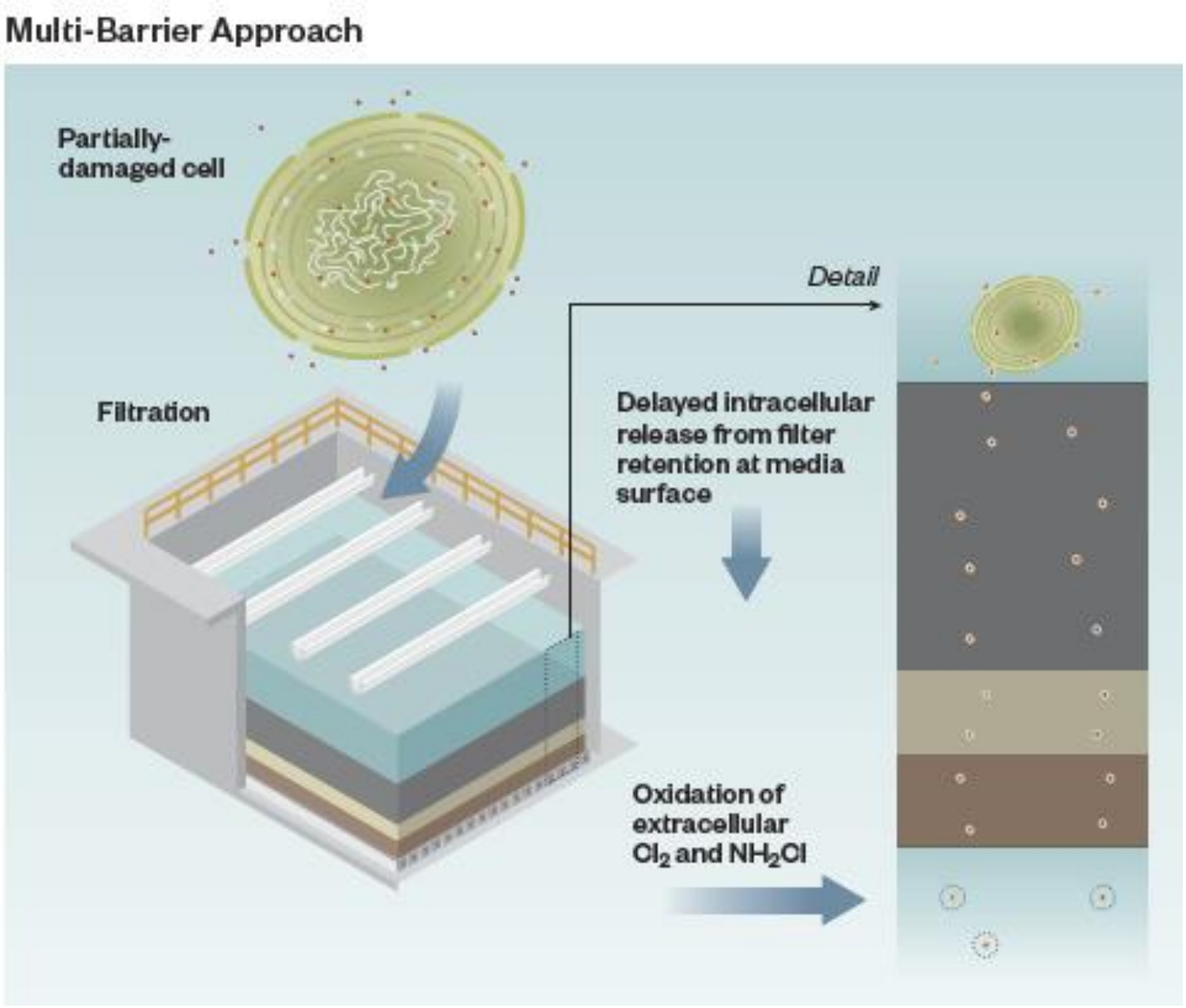
*Controls did not exhibit MC release

Preliminary guidance on stagnation

Effect of stagnation time summary table

Oxidant:DOC ratios/exposure times and stagnation times resulting in delayed release of microcystins						
0.15 Cl_2:DOC, 20 min	0.15 NH_2Cl:DOC, 20 min	0.15 ClO_2:DOC, 20 min	0.15 O3:DOC, 20 min	0.4 MnO_4-:DOC, 20 min	0.4 H_2O_2:DOC, 24 h	0.25 Cu:DOC throughout experiment
Free chlorine	Monochloramine	Chlorine dioxide	Ozone	Permanganate	Hydrogen peroxide	Copper (II)
Release at ≥2 h	Release at ≥12 h	Release at ≥20 min	Release at ≥8 h	Release at ≥2 h	Release at ≥24 h	Release at ≥24 h

- **Significance of these results?**
  - **Important to filter samples immediately after quenching**
  - **Relevant to methods used in previous research studies**
  - **Partially damaged cells may have applied impacts**



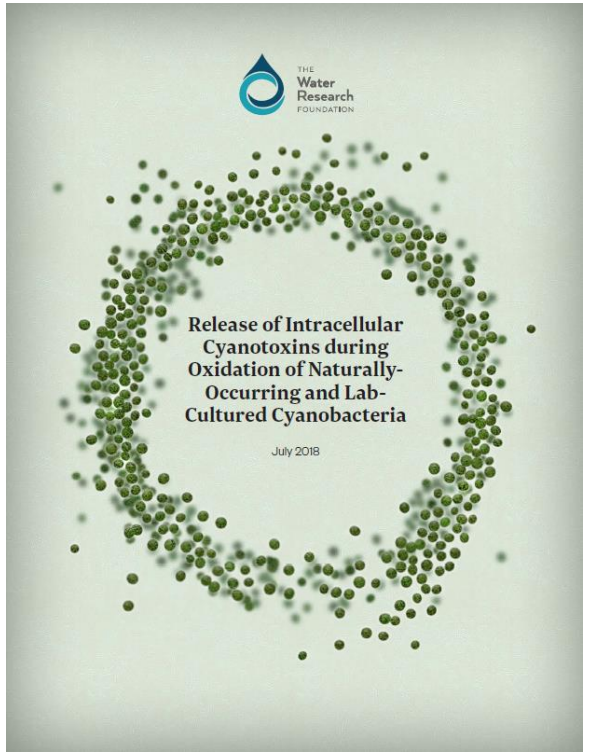
Minimal cyanotoxin breakthrough can impact a utility's ability to meet the Health Advisory

Recommendations:

- Multi-Barrier Approach
- Effective Post-Filter Oxidant
- More Frequent Backwashing

Final report and guidance document (~May 2019)

- **Water Research Foundation Project #4692**
  - Project manager: Djanette Khiari
  - PAC: Zaid Chowdhury, Sarah Page, Barry Rosen, Carol Walczyk
- **Southern Nevada Water Authority (SNWA)**
  - **Eric Wert**, Brett Vanderford, Beck Trenholm, Janie Holady, Brittney Stipanov, Glen de Vera, Julia Lew, Shandra Staker, Yesika Otano-Alonso, James Park, Mary Murphy
- **Polytechnique Montreal -** Arash Zamyadi, Caitlin Glover
- **Saint Louis University** – Craig Adams
- **Hazen and Sawyer** - Erik Rosenfeldt & Graphics Team!



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SAINT LOUIS UNIVERSITY

Hazen

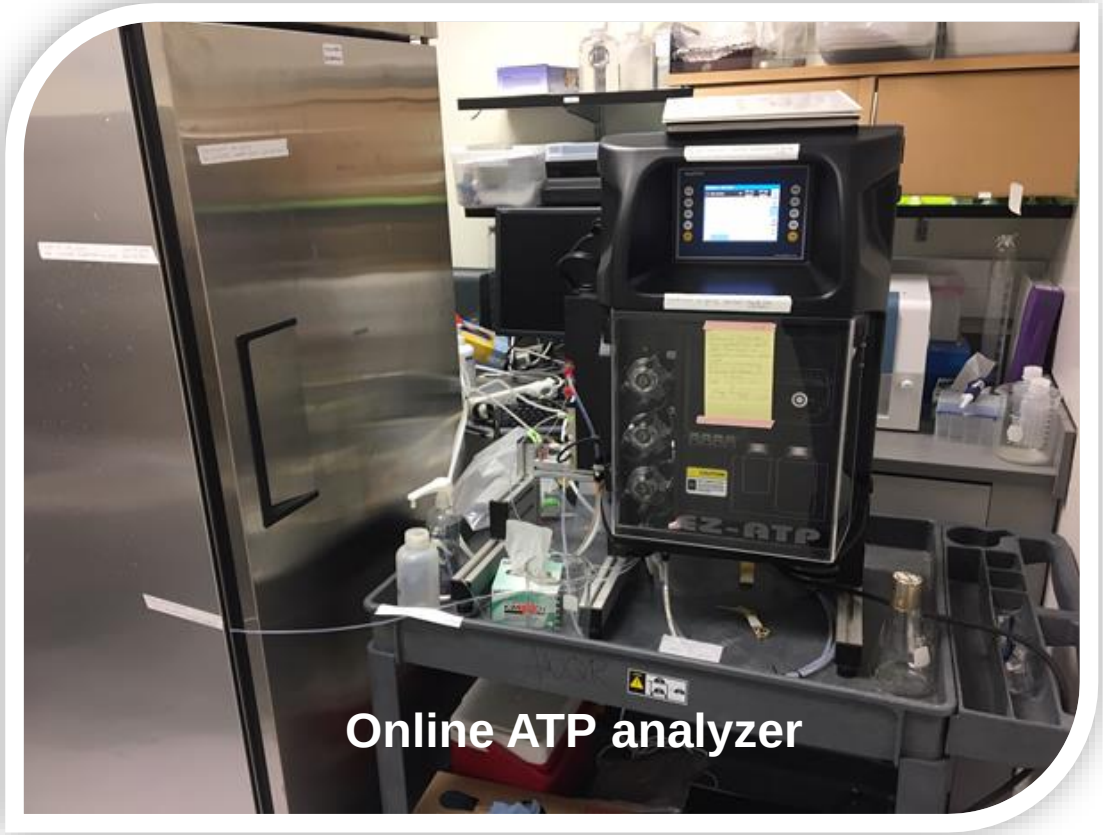
Future research on “Release and Treat”

Variables

- Growth phase
- Morphology
- Water matrix
- Mixtures

Full-scale systems

- Validation of oxidant:DOC ratios
- Threshold cell concentration
- Process control strategies for cell lysis



Online ATP analyzer

Future research: WRF #4912

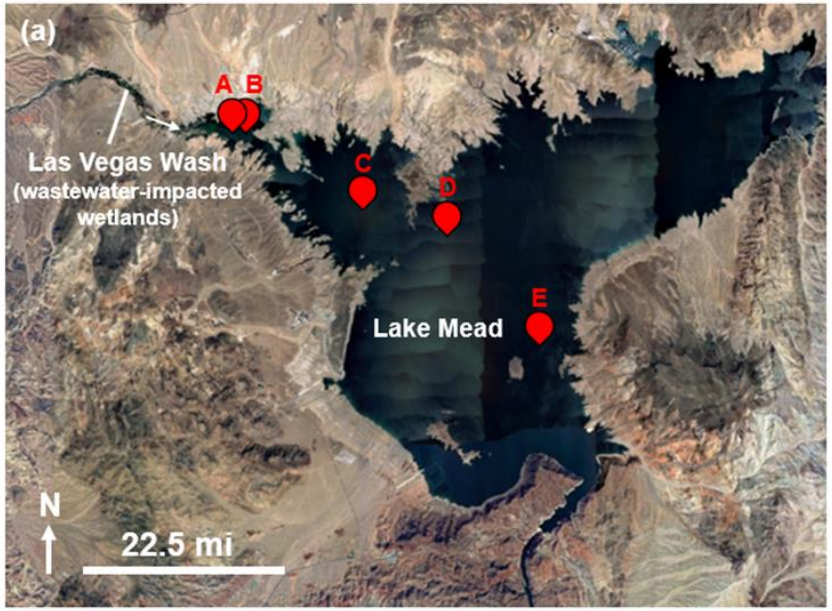
- **“Developing Guidance for Assessment and Evaluation of Harmful Algal Blooms, and Implementation of Control Strategies in Source Water”**

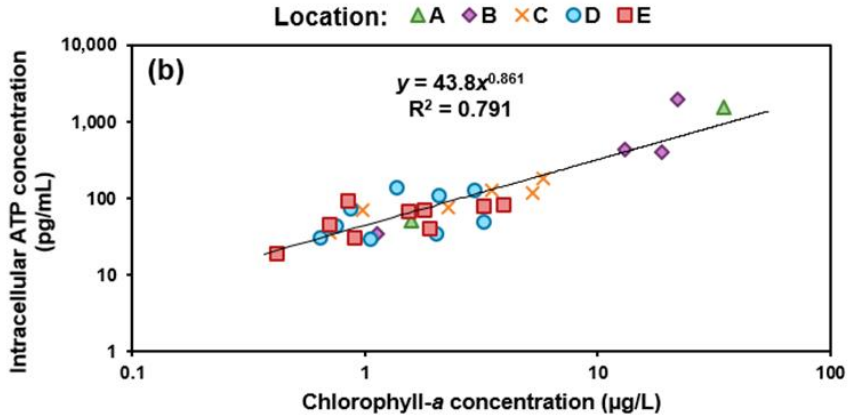
- **Project Team**
  - **Southern Nevada Water Authority –** Eric Wert
  - **Polytechnique Montreal –** Arash Zamyadi
  - **University of Adelaide –** Virginie Gaget
  - **Hazen and Sawyer –** Christine Owen

- **Utility and Technology Participants from US, Canada, Europe and Australia**

Future research: WRF #4912

- **Research approach**
  - **Critical review of early warning systems and source water control strategies**
  - **Summarize utility practices**
  - **Innovation evaluations**
    - **ATP-based approaches**
    - **Treatment of benthic cyanobacteria**
  - **Guidance document and decision trees**

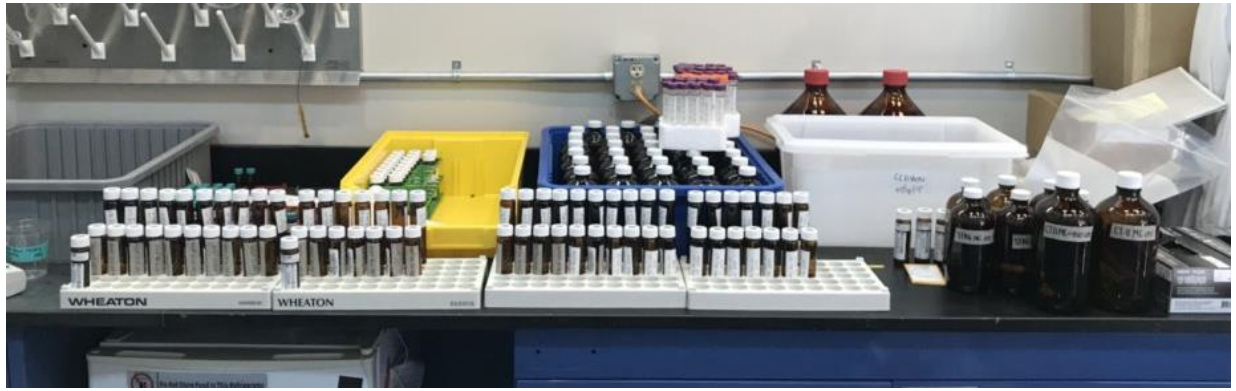




Ref: Greenstein and Wert, Water Research (2019)


WHEATON
WHEATON


SunX
SUNSCREEN
SPF 30+
WHEATON
WHEATON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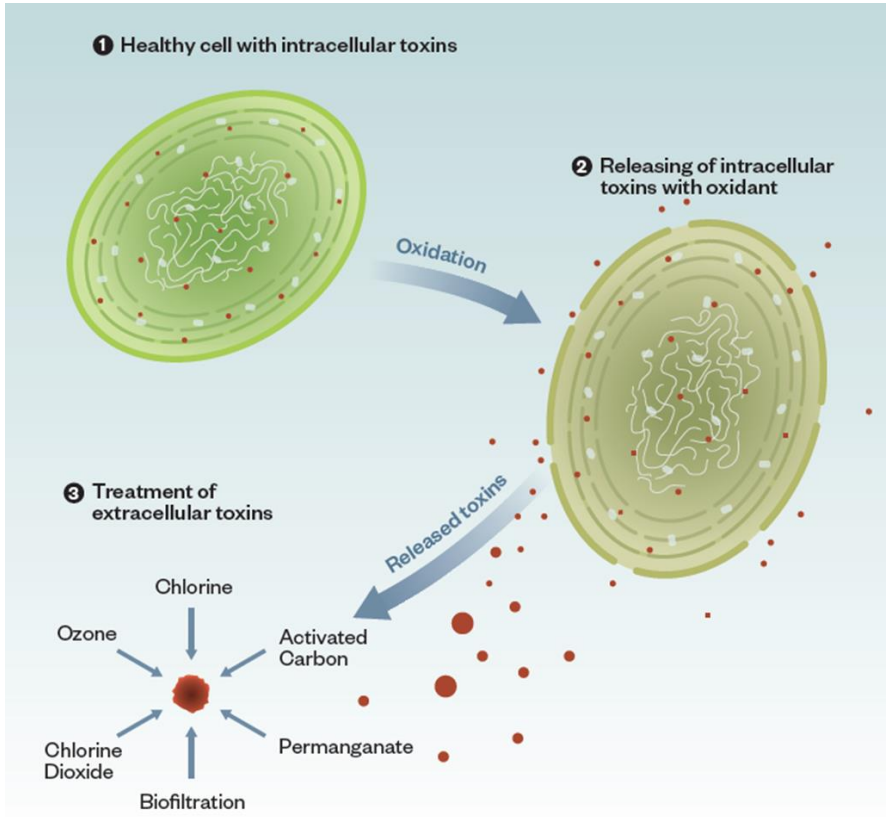

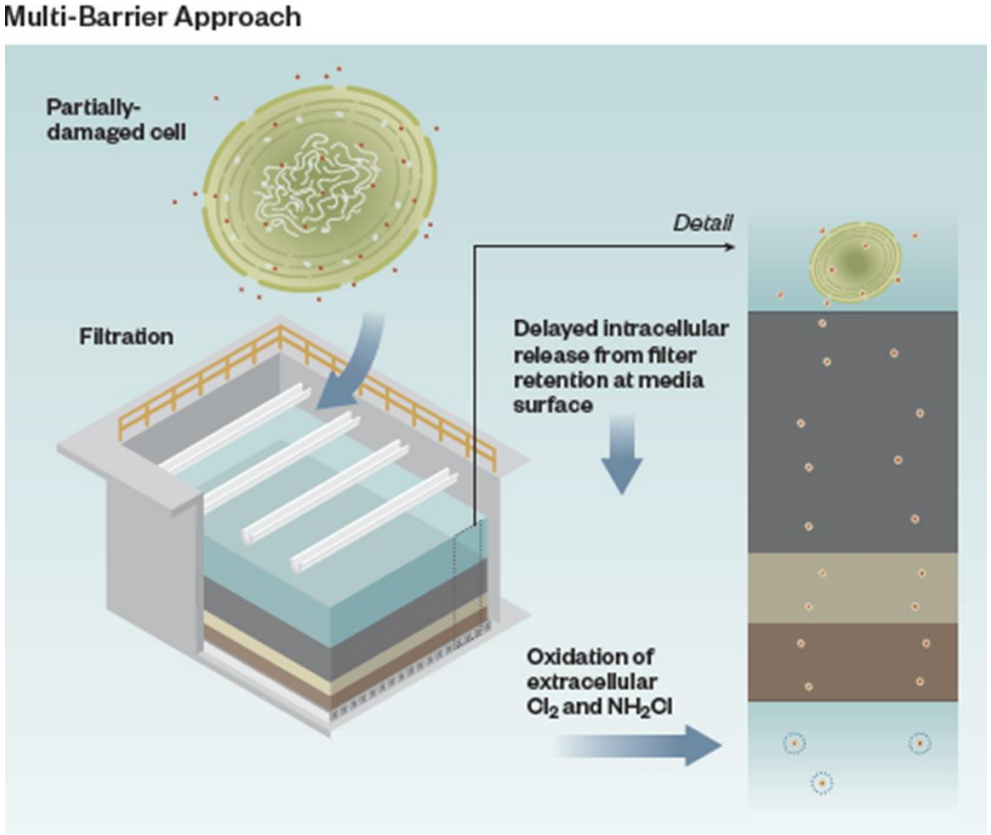

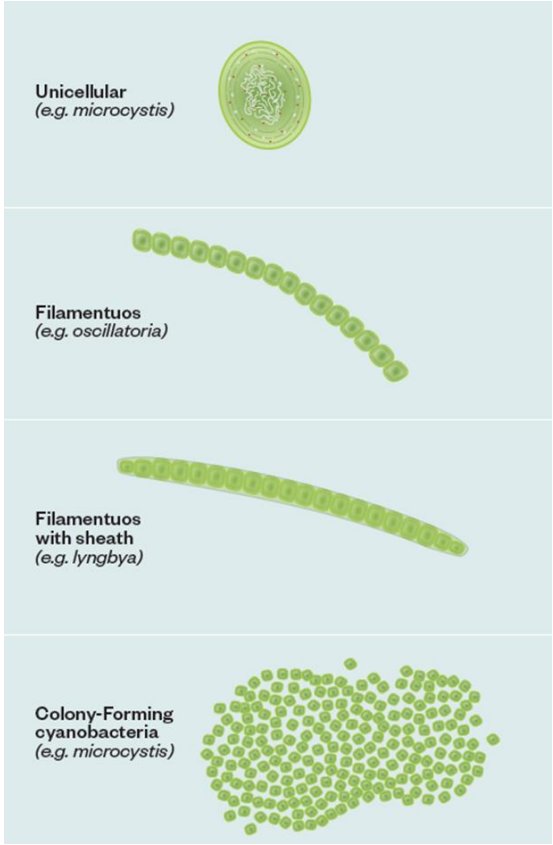

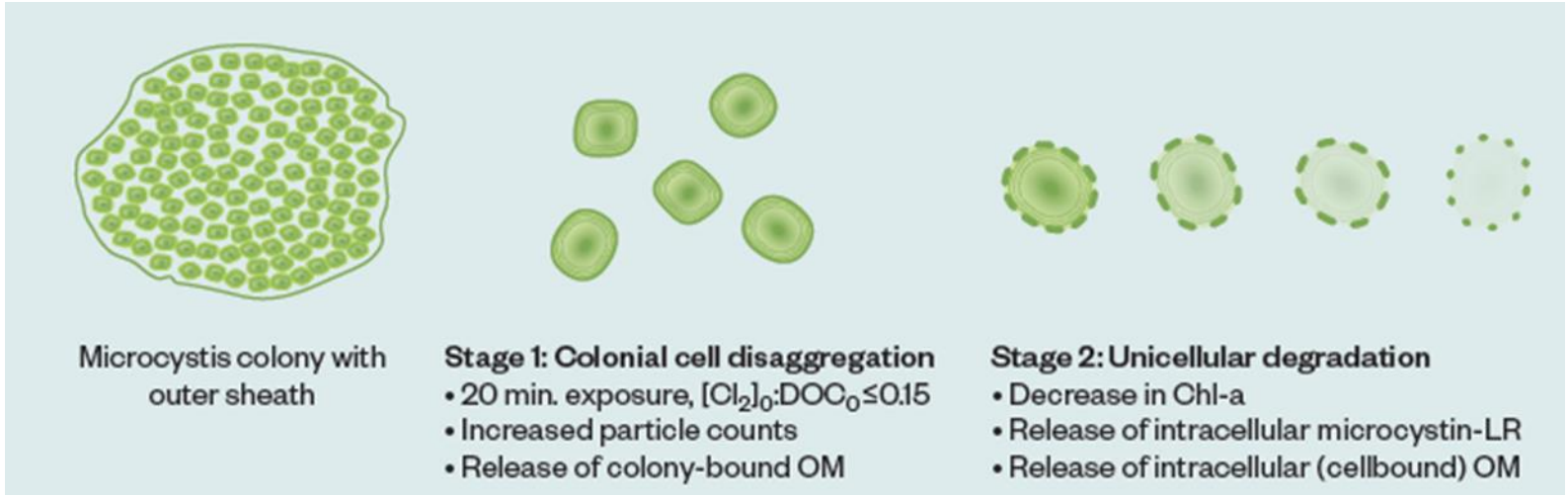

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