Cyanotoxins and Drinking Water Quality: Treatment Options

Judy Westrick, PhD
Paul Zimba, PhD
David Szlag, PhD
Benjamin Southwell, MS
Overview Drinking Water Treatment

- Treatment to remove intracellular algal toxins
  - Conventional treatment
    - Filtration
    - Membrane technologies
- Treatment to remove extracellular algal toxins
  - Oxidation
  - Physical removal
  - Biologically active filters
Understanding microorganism and chemical removal/inactivation

• Living organisms
  – Nonviable
  – Removal

• Chemical Contaminants
  – Adsorption
  – High Pressure Membrane Filtration
  – Degradation/Biodegradation
Source Water

• Intracellular Toxin
  – Flushing
  – Harvesting
  – Diversion
  – Flocculants
  – Algaecides (low levels)
  – Ultrasound

• Extracellular Toxin
  – Awareness and get ready to treat

Photo courtesy of John Lehman, University of Michigan
Intake

• Intracellular Toxin
  – Adjustable Intake
  – Night vs Day

• Extracellular Toxin
  – Oxidants
  – Inline Powdered Activated Carbon (PAC)

• A conventional treatment plant will want to keep the cells intact.
Powdered Activated Carbon

- Wood-based PAC is more effective than coconut-based and bituminous PACS in the removal of microcystins.

- Jar Test

- Pre-chlorination is not recommended before the use of PAC.
Summary of Intact Algal Cell Removal Performance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intact Cell Removal</th>
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<tbody>
<tr>
<td>Coagulation/sedimentation or dissolve air flotation/rapid sand filtration</td>
<td>&gt; 99.5% auxiliary</td>
</tr>
<tr>
<td>Lime precipitation/sedimentation/rapid sand filtration</td>
<td>&gt; 99.5% ancillary</td>
</tr>
<tr>
<td>Microfiltration/Ultrafiltration</td>
<td>&gt; 75% (becoming auxiliary)</td>
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</tbody>
</table>
Coagulation/Sedimentation

- **Intracellular Toxin**
  - Oxidants (not often used, afraid of lysing cell)
  - Flocculent aides
  - Settled water with less than 100 units algae/mL

- **Extracellular Toxin**
  - Activated Carbon
    - Powder (PAC)
    - Granular (GAC)
  - Filtration
    - Conventional
    - Biologically Active

- **Monitoring Techniques to determine treatment**
  - Turbidimeter
  - Streaming current detector
  - Particle Counter
  - Chlorophyll-a
  - Cell counts
  - ELISA
    - Saxitoxin, Anatoxin-a, Cylindrospermopsin, Microcystin
    - Plate, Test tube kit, Dip Stick
Filtration

- Conventional
- Biologically Active
- GAC
- Low Pressure Membrane
Ultrasonic Technology Treatment

Before Ultrasound

After Ultrasound

Low power ultrasound

Commercial Sonic Solutions
LG Sonic

Typical operating parameters

average 18 W
28 kHz

George Hutchinson, Opflow April 2008

Low power ultrasound
Tunable (79 frequencies)
Critical resonance (gas vesicles)
Cyanobacteria – Microcystis, Anabaena, Lyngba (Sonic Solutions)
Biologically active filters

- INTRACELLULAR TOXIN
- MCY-LR, MCY-LA, cylindrospermopsin, and anatoxin-a can be removed by biologically active sand and GAC filters
- Empty bed contact times-- 5 to 15 minutes.
  - Slow filtration
  - Rapid filtration
- Saxitoxin - not removed
GAC filtration

- Effectiveness of GAC filtration against cyanotoxins is source water dependent
- Significant differences in adsorption between LA and LR
- Saxitoxins and anatoxin-a are more readily adsorbed than microcystins
Pore Size

- Equilibrium
  - Micropore
    - Taste and odor
    - Industry spills, solvents
    - Anatoxin-a
  - Mesopore
    - Microcystins
      - RR > YR > LR > LA
    - Cylindrospermopsin
    - Saxitoxin

- Kinetic <1 hour contact time
- Large pore volume seems to be more effective
## Summary of Oxidation Treatment Processes

### Extracellular Toxins

<table>
<thead>
<tr>
<th>Treatment Process</th>
<th>Microcystin</th>
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<tbody>
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<td>Chlorine</td>
<td>Yes</td>
</tr>
<tr>
<td>Ozone</td>
<td>Yes</td>
</tr>
<tr>
<td>Chloramine</td>
<td>No</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>No</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>Yes</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>Yes</td>
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### Summary of Oxidation Treatment Processes

#### Extracellular Toxins

<table>
<thead>
<tr>
<th>Process</th>
<th>Anatoxin-a</th>
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<tr>
<td>Chlorine</td>
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<tr>
<td>Chloramine</td>
<td>No</td>
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<tr>
<td>Chlorine dioxide</td>
<td>No</td>
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<tr>
<td>Hydroxyl radical</td>
<td>Yes</td>
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<td>Potassium permanganate</td>
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### Summary of Oxidation Treatment Processes Extracellular Toxins

<table>
<thead>
<tr>
<th>Treatment Method</th>
<th>Cylindrospermopsin</th>
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<td>Ozone</td>
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<tr>
<td>Chloramine</td>
<td>No</td>
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<tr>
<td>Chlorine dioxide</td>
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<tr>
<td>Hydroxyl radical</td>
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<td>Potassium permanganate</td>
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## Summary of Oxidation Treatment Processes

### Extracellular Toxins

<table>
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<tr>
<th>Process</th>
<th>Reaction</th>
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<tr>
<td>Saxitoxin</td>
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<td>Chlorine</td>
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<tr>
<td>Ozone</td>
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</tr>
<tr>
<td>Chloramine</td>
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</tr>
<tr>
<td>Chlorine dioxide</td>
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<tr>
<td>Hydroxyl radical</td>
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<tr>
<td>Potassium permanganate</td>
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### PREDICITON of Oxidation Treatment Processes Extracellular Toxins

<table>
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<th>Predicted Effect</th>
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<tr>
<td>Chlorine dioxide</td>
<td>No?</td>
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<tr>
<td>Hydroxyl radical</td>
<td>Yes?</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>Yes?</td>
</tr>
</tbody>
</table>

The table above summarizes the predicted effectiveness of various oxidation treatment processes on extracellular toxins. The symbols "Yes?" and "No?" indicate the likelihood of successfully treating the respective toxins with the given process.

![Chemical Structure](image)
Chlorine CT values for reducing microcystin concentration to 1 ugl⁻¹ (Acero et al 2005)

<table>
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<tr>
<th>pH</th>
<th>[MCLR]₀</th>
<th>CT-values, mgL⁻¹min</th>
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<td></td>
<td>ugl⁻¹</td>
<td>10°C</td>
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<tr>
<td>6</td>
<td>50</td>
<td>46.6</td>
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<td>10</td>
<td>27.4</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>67.7</td>
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<td>8</td>
<td>50</td>
<td>187.2</td>
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<td>110.3</td>
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<td>9</td>
<td>50</td>
<td>617.2</td>
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<td></td>
<td>10</td>
<td>363.3</td>
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</tbody>
</table>

Compared to CT Values for Disinfectants to inactivate 99.9 (3-logs) of Giardia Lamblia cysts.
UV Treatment

• UV inactivation dose is about 40 mJ/cm$^2$ – inactivation of *Cryptosporidium parvum*.

• Photolytic destruction dose for microcystin, cylindrospermospin, anatoxin-a and saxitoxin is 1530 to 20,000 mJ/cm$^2$. 
Photolysis and Advanced Oxidation Processes

- Photolysis
- UV/H$_2$O$_2$
- Fenton Reagent
- Radiolysis
- Ultrasonic degradation
- TiO$_2$ photocatalysis
- Ferrate

Sharma et. al, Separation and Purification 91 (2012) 3-17
Ultrasonic Degradation

• Acoustic Cavitation
  – Formation and collapse of microbubbles
  – Transient high temperature (>5000 K) and pressure (>1000 atm)

• Generates reactive species (radicals)
  – Hydroxyl
  – Hydrogen
  – Oxygen
  – And more
TiO$_2$ Photocatalysis

- Generates reactive species
  - Hydroxyl
  - Oxygen
- 254 UV light
- pH dependent
  - Surface pH
  - Toxin pI
• Intake
• Inline Chemical
• Coagulation/Flocculation/Sedimentation
• Storage Reservoir
• Filtration
• Carbon Adsorber
• Chlorine
Pilot and Plant Studies

• Early studies focused on the removal of intracellular toxins only

• Complete treatment gives 31%-99% removal

• Most of the published studies are microcystin removal
  – Algae: Source to Treatment 2010
  – Cyanobacterial Harmful Algal Blooms 2008
  – Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management 1999
  – Lambert et al 1996
  – Karner et al 2001
  – Schmidt et al 2002
  – Hoeger et al 2005
Assay and Analytical Methodologies
Sample Preparation

• In situ
  – No sample modification
  – Potential for greater matrix interference

• Cell Lyse
  – Sonication
  – Chemical
  – Freeze/thaw

• Filtration
  – Vacuum
  – Centrifugation

• Lyophilization
  – Sample Concentration
  – Analyte volatilization?

• Immunocolumn
  – Retention
  – Specificity

• Solid Phase Extraction
  – Analyte retention
Selectivity and Sensitivity Relationships between Analytical Methods for Microcystins

- NMR
- LC/MS
- HPLC
- MMPB
- ELISA
- PPIA
- Bioassay
- TLC

Biological and biochemical
Physio-chemical
Bioassay Based Detection

- Protein Phosphatase Inhibition Assay (PPIA)
  - No commercial kit available
  - End Point Kinetics

Comparison of microcystin-LR (MC-LR) equivalents determined by HPLC with DAD and PP1 inhibition

Bioassay Based Detection

• Enzyme Linked Immunosorbent Assay (ELISA)
  – Inexpensive to setup and to run
  – Screening (Care must be taken as to the data’s use)
  – Antibody Based
    • Microcystin
    • Cylindrospermopsin
    • Saxitoxin
  – Receptor Based
    • Anatoxin-a
Liquid Chromatography

• High Performance Liquid Chromatography (UPLC and HPLC) with various detectors

• Liquid Chromatography Mass Spectrometry (UPLC and HPLC)

• Analyte Verification
  – Standards
  – Surrogates
UPLC and HPLC

- Various detectors available (PDA and fluorescence most common)
- Variable analysis time
- Only approved method for Microcystin LR (ISO 20179:2005)
- Limitations include relying solely on retention time for identification and the inability to differentiate co-eluting peaks
Separation of the Cyanotoxins by HPLC-PDA

AU

Minutes

Toxic cyanobacteria in water 1999
LC/MS(MS)

- Analysis can be performed by both single and triple quadrupole instruments
- Allows for positive conformation of compound
- Variable analysis time
- No approved method
- Limitations can include variable analyte response and non-linear standard curves
Single analytical method discerns common MYC and other toxins
Microcystin-LR Mass Spectrum

LR_8201b 1567 (23.689) Cm (1565:1571-1585:1610)

1: Scan ES+ 2.50e6
   135.06
   265.15
   482.43
   498.47

2: Scan ES+ 2.49e6
   995.50
   996.47
   861.44
   862.37
   996.45
   997.54

M+H

Low V

Higher V
DNA Based Technologies

– Methodology allows for presence determination of a species and the presence of the toxin gene.
– Appropriate gene clusters have been determined for the toxins microcystin, cylindrospermopsin, anatoxin, and saxitoxin.
– Does not determine toxin concentrations.
Microcystin Gene
Cycling

1. Denaturation
2. Annealing
3. Elongation
Summary

• Intra vs extra cellular toxin
• Multi-barrier approach for each toxins
• One species can make multiple toxins
• More than one toxin may be present
• Understand the different analyses
  – Surrogate measurement
  – Semi-quantitative
  – Quantitative
• Have a contingency plan with in-house analyses to guide treatment
Development of a multiplex freshwater and marine method for cyanotoxin and euglenophycin detection

Judy Westrick, Wayne State University

Paul Zimba, Texas A&M University Corpus Christi

Collaborators
Brett Nielan
Tim Davis
Benjamin Southwell
David Szlag
**Toxins** (multiple!) can be produced by the same species e.g. microcystin and saxitoxin  
cylindrospermopsin and saxitoxin  
anatoxin and microcystin

This cocktail of toxins likely causes synergistic effects when present at levels below that known to cause mortality/visible impacts in low level exposures

**Detection methods**
Specific:
- toxin measurement analytically (HPLC, MS, MS/MS, NMR)
- toxin activity assessment (ELISA, aptamer)
- genomic analyses (PCR, multiplex possible?)

Indirect/ambiguous:
- pigments
- cell counts
Project Aims:

1) Develop multiplex PCR method patterned after published research
2) Alter PCR method to detect anatoxin-a – replacing saxitoxin
3) Turn off euglenophycin production, RNA sequencing comparison to wild type
4) Add euglenophycin toxin-specific primer to PCR multiplex method
5) Apply new methods to field samples in several locales

Project partially funded in September 2012, fully funded January 2013
Demonstration a novel multiplex assay successfully generating specific products
Correlations of Microcystin analytical/PCR Mcy

R= 0.71, p. < 0.20

Correlations of CYL with CYR:

R=0.72, p. < 0.065

Correlations of Saxitoxin require more (+) samples

Microcystin: 34 positive by qPCR, 37 positive by LC/MS-MS (borderline qPCR concentrations)

Cylindrospermopsin: 9 positive by qPCR, 7 positive by LC/MS-MS
With a working q-PCR multiplex, we will

– Compare summer vs winter differences (3 sites).
  • Parameters- CqPCR, cyanotoxins, cyanobacteria identification/enumeration

– Multivariate toxin/evaluation (5 systems).
  • Parameters-CqPCR, cyanotoxins, cyanobacteria identification/enumeration, nutrients, water chemistries, trace metals for 9 weeks during summer-fall

– Drinking Water study (10 plants).
  • Parameters – CqPCR, cyanotoxins, cyanobacteria identification/enumeration
Work with Environment Canada
On 10 largest lake
Lake Winnipeg toxin load, Summer, Fall 2013

<table>
<thead>
<tr>
<th>Site</th>
<th>Collection date</th>
<th>Myc genes/ml</th>
<th>Cyr genes/ml</th>
<th>Sxt genes/ml</th>
<th>Site</th>
<th>Collection date</th>
<th>Mcy genes/ml</th>
<th>Cyr genes/ml</th>
<th>Sxt genes/ml</th>
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<tbody>
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<td>SUMMER CRUISE</td>
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<td>FALL CRUISE</td>
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<td>0.137</td>
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<td>40.375</td>
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<td>103.308</td>
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</table>
Natural Water Study: Causality

• Source water evaluated at 5 known cyanobacterial sites. (qPCR, cyanotoxins, cyanobacteria ID)
• Sampling requested weekly for nine weeks (7/8– 9/19/13)
• Water Quality Parameters
<table>
<thead>
<tr>
<th>Location</th>
<th>Cyanobacteria Species present</th>
<th>ANA</th>
<th>CYL</th>
<th>MC-RR</th>
<th>MC-YR</th>
<th>MC-LR</th>
<th>MC-LA</th>
<th>MC-LW</th>
<th>MC-LF</th>
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<tbody>
<tr>
<td>St. Johns River, FL Shand Pier</td>
<td><em>Pseudanabaena</em> Anabaena Aphanizomenon Microcystis</td>
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<td></td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Lake Huron Saginaw Bay Bay City, MI</td>
<td><em>Pseudanabaena</em> Anabaena Aphanizomenon Microcystis</td>
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<td>0.05</td>
<td></td>
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<td></td>
<td>(1)</td>
<td>0.07-</td>
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<tr>
<td>Grand Lake St. Marys Celina City, OH</td>
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<td>0.07-</td>
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<td>(7)</td>
<td>(7)</td>
<td>(5)</td>
</tr>
<tr>
<td>Western Basin, Lake Erie Toledo, OH</td>
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<td>.06</td>
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Questions?
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