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Biodegradation of microcystins in Lake Erie source waters and sand filters from drinking-water plants

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Addressing cyanoHABs requires multiple strategies

1. Reduce sources and causes
2. Monitor for and predict cyanoHAB occurrence and toxin concentrations
3. Water treatment and bloom control strategies to reduce or eliminate toxins

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Microcystin removal in water

- Chemical and physical methods for removal of MCs have efficiency and cost limitations
  - Cell bound—coagulation, sedimentation, filtration
  - Extracellular—activated carbon, chlorination, ozonation, permanganate, UV, membranes
- Biodegradation is environmentally friendly and could augment existing treatments

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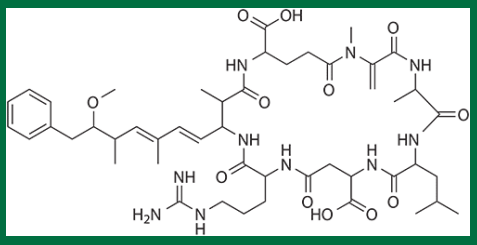
Microcystin biodegradation

- Microcystins are relatively stable compounds and resistant to abiotic degradation
  - Decomposition of 10 µg/L MC's took >27 days in deionized water, but only 7 days in lake water[1].



[1]Cousins et al., 1996, Water Res. 30 (2), 481.

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Microcystin biodegradation studies

- Indigenous MC-degrading bacteria identified in lakes, rivers, and water-treatment plant biofilms
  - Studies in the 1990's in Australia and Japan
  - Identified as *Pseudomonas sp*[3] and later reclassified as *Sphingomonas sp*[4]
  - Other genera identified in Europe, Australia[2], and Asia
  - Bacteria may contain the *mlrA* gene[5]

[2]Ho, L. et al., 2006, Water Research 40, 768
[3]Jones et al., 1994, Nat. Toxins 2 (4), 228
[4]Bourne et al., 1996, Appl. Environ. Microbiol. 62 (11), 4086
[5]Saito et al., 2003, FEMS Microbiol. Lett. 229 (2), 271.
Sphingomonas from Wikepedia.

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U.S. MC biodegradation studies

- Los Angeles/Lake Mead[6]
  - Samples from the LA Aqueduct Filtration Plant anthracite filter and Lake Mead
  - The addition of a carbon source (acetate) to laboratory bioreactors reduced MC biodegradation
  - Identified the MC biodegrader as *Morganella morganii*

[6]Eleuterio and Batista, 2010, Toxicon 55, 1434
Picture of Lake Mead from earthobservatory.nasa.gov

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U.S. MC biodegradation studies

- Lake Okeechobee[7]
  - Phosphorus levels did not affect biodegradation.
  - Identified *Micobacterium* and *Rhizobium gallicum* as MC biodegraders
- Lake Erie[8]
  - Offshore samples
  - Used metagenomics to identify a diverse array of bacterial phyla as potential MC biodegraders

[7]Ramani et al., 2011, Biodegradation 23 (1), 35.

[8]Mou et al., 2013, Appl. Environ. Microbiol. 75(21), 6924.

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MC biodegradation—some conclusions

- MC biodegradation is widespread, can be facilitated by various species of bacteria, and is not limited to one route of degradation[9].
- If active biofilters are used, bioaugmentation may not be needed; however, work is needed to determine the presence of MC degraders in more biofilters[6]
- Efficient biodegradation appears to be site specific[10]

[9]Gagala, H., and Mankiewicz-Boczek, 2012, Pol. J. Environ. Stud. 21 (5), 1125.

[6]Eleuterio and Batista, 2010, Toxicon 55, 1434.

[10]Ho, L. et al., 2012, Water Research 46, 1536.

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USGS Western Lake Erie study Objectives, 2015–17

- Identify naturally occurring microcystin-degrading bacteria from source waters and sand filters in drinking-water plants in the Western Basin of Lake Erie and watershed.
- Identify whether a molecular target, such as the *mlrA* gene, can be used to quantify biodegradation of microcystins.
- Compare rates to those of a known microcystin biodegrader.

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Timeline

- **Aug–Dec, 2015 and July–Aug, 2016**—Samples from source waters and sand filters
- **Aug 2015–Sept 2016**—Laboratory work
  - Microcosms to determine biodegradation potential
  - Isolate and identify biodegraders
  - Compare the rate of biodegradation
- **2017**—Data analysis and report

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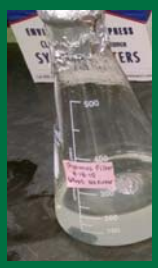
Laboratory microcosms

A. MC + unfiltered source water
B. MC + 5-μm filtered source water
C. MC + 0.2-μm filtered source water (abiotic control)
D. Unfiltered source water (background control)

For sand filter experiments, used buffered water with 20 g sand



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Laboratory microcosms

- Incubated in a water-bath shaker at 25ºC for 14 days.
- Aliquots removed at 0, 1, 2, 4, 8, and 14 days.
  - Analyzed for microcystins by enzyme-linked immunosorbent assay (ELISA)
  - Selected aliquots to be cultured to identify MC degraders and the presence of the mlrA gene

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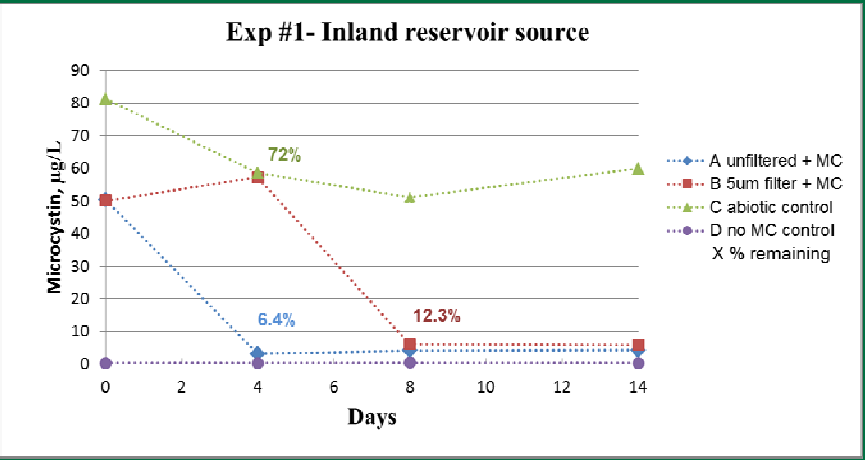
Preliminary results—August 17



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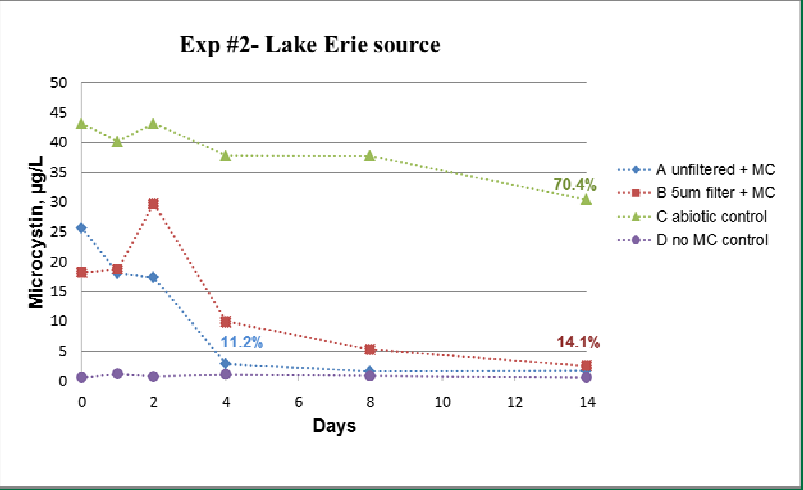
Preliminary results—October 5



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Preliminary results – Culture Work

- Samples cultured on R2A media at time 0 and at the time when degradation was seen (ie: time 4 and time 8 from flasks A and B from Exp #1)
- Colony morphologies that appeared to be enriched at the later time points were selected for further analysis
- Three-phase streaks done on LB media to generate pure cultures
- Glycerol stocks of each isolate are made for further testing and analysis.

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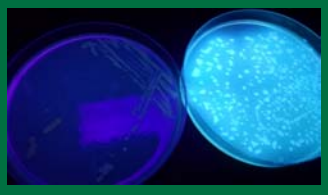
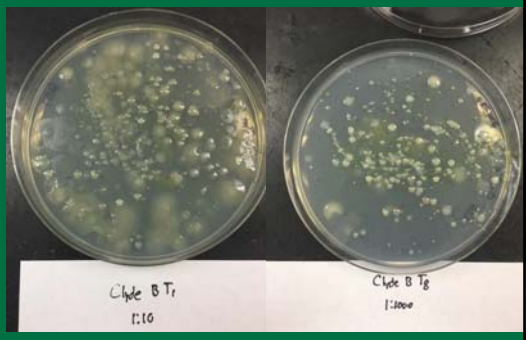
Preliminary results – Culture Work



Isolate from sand filter at time 0 viewed under UV light (right) and a non-fluorescent isolate (left)



Spread plates prepared from glycerol stocks collected during microcosm incubation. Isolated colonies with varying morphologies collected from T8 for pure cultures.

Three-phase streak of an isolate

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Preliminary results – Culture Work

Microcystin Minimal Media
Growth is seen for 2 of 4 isolates

Glucose Minimal Media
Growth is seen for 3 of 4 isolates

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Next steps

- Continue to isolate and identify MC-biodegraders from water and sand samples
  - Compare rates to a known MC-biodegrader.
- Identify the presence of the *mlrA* gene
- Collect samples from sand filters at several drinking water plants
  - Do water treatment strategies affect the presence of MC-biodegraders in the filters?

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