Impact of Growth Conditions and Suspension Time on Toxin Release from *M. aeruginosa* Upon Exposure to Potassium Permanganate

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Background

- The increased influx of nutrients into various bodies of water around the world has caused an increase in Harmful algal blooms (HABs).
- HABS are often dominated by cyanobacteria- photosynthetic bacteria with the ability to synthesize toxic compounds that can end up in water used for drinking and recreation.
- Potassium Permanganate ($KMnO_4$) has shown success in degrading cyanotoxins in water treatment plants.
- It is possible that $KMnO_4$ oxidation may compromise cell integrity of cyanobacteria and cause the release of intracellular toxin.

Objectives

The objective of this work was to assess the effects of $KMnO_4$ on pure cultures of cyanobacteria (*Microcystis aeruginosa)* in a jar test. Of particular interest was the impact this oxidant has on the release of intracellular toxin from cells as a function of growth conditions in culture, pH (7 or 9) of cell suspension during the test, and suspension time prior to oxidant exposure. Pre-oxidant addition suspension times of 30 minutes and 24 hours were evaluated.

Experimental Set-up

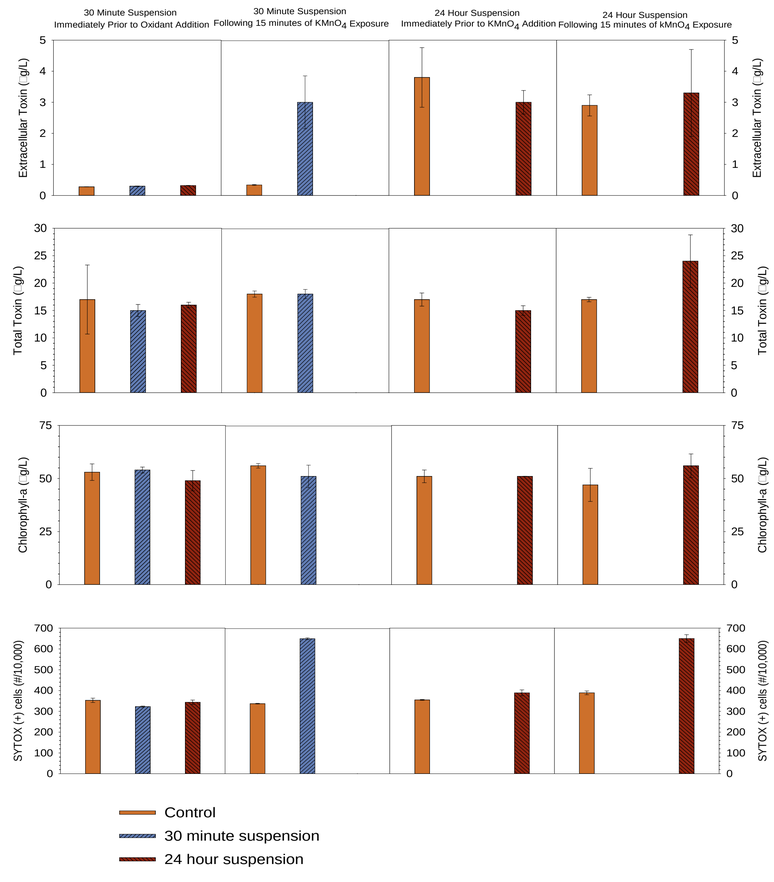
Jar Test set up including duplicate control, 30 minute suspension and 24 hour suspension jars.

M. Aeruginosa treatment groups. Left to right: 76.5:1 N:P low, moderate, high light; 10:1 N:P low, moderate, high light.

Materials and Methods

Microcystis aeruginosa cells were cultured under three light levels (5, 20 and 60 µmol photons / m^2 s) with two initial nitrogen to phosphorus ratios (76.5:1, 10:1) at each light level. All growth media used was either BG-11 blue green algae media with baseline nitrogen (76.5:1 N:P) or a modified BG-11 media with reduced N:P (10:1). A culture of 76.5:1 N:P grown under moderate light was selected for the trial shown here. The impact of these growth conditions on toxin release was assessed by exposing the cell suspension (pH 9.0 dechlorinated tap water) to a 1 mg/L dose of $KMnO_4$ for 15 minutes. Also, to assess the effects of suspension time prior to oxidant addition on toxin release, all experiments performed included a 30 minute and a 24 hour suspension prior to $KMnO_4$ addition. In addition to extracellular toxin concentration, total toxin concentration (intracellular + extracellular), levels of chlorophyll-*a* (extracted), and cell membrane integrity were measured throughout the experiment. Toxin concentrations were measured using enzyme-linked immunosorbent assay (ELISA).

Results



Discussion

- Results showed major increases in extracellular toxin after $KMnO_4$ exposure in the 30 minute suspension, signifying intracellular toxin release from oxidation.
- There were also large increases in extracellular toxin after 24 hours of suspension immediately prior to oxidant addition, potentially due to cell stress caused by a lack of available nutrients in the cell suspension.
- Levels of chlorophyll-a appeared to remain relatively constant throughout the experiment.
- Both oxidant additions corresponded to increases in the number of cells that tested positive for the Sytox Green stain. This was direct evidence of the impact of $KMnO_4$ oxidation on the cyanobacteria cell membranes. It should be noted, however, that the cell membrane damage seen in the 24 hour suspension did not correspond to toxin release.

Acknowledgments

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References

1. Ou, H.; Gao, N.; Wei, C.; Deng, Y.; Qiao, J., Immediate and long-term impacts of potassium permanganate on photosynthetic activity, survival and microcystin-LR release risk of Microcystis aeruginosa. *Journal of hazardous materials* **2012,** *219-220*, 267-75.

2. Fan, J.; Daly, R.; Hobson, P.; Ho, L.; Brookes, J., Impact of potassium permanganate on cyanobacterial cell integrity and toxin release and degradation. *Chemosphere* **2013,** *92*, (5), 529-34.

3. Rodríguez, E.; Majado, M. E.; Meriluoto, J.; Acero, J. L., Oxidation of microcystins by permanganate: Reaction kinetics and implications for water treatment. *Water Research* **2007,** *41*, (1), 102-110.