Rapid response of *Microcystis aeruginosa* bloom microbiome to high concentration hydrogen peroxide

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Abstract

Hydrogen peroxide has recently gained popularity as an environmentally friendly treatment for cyanobacterial harmful algal blooms (HABs). Low concentration applications in lentic systems have proven effective with desirable outcomes as the treatment degrades into water and oxygen, and does not harm non-target aquatic life. However, the general populace desires rapid removal of HABs. High concentration hydrogen peroxide can achieve this but may introduce an increased risk to the environment. Most susceptible is the microbial community, with hydrogen peroxide noted to have temporary but recoverable impacts at low concentration treatments. This is concerning as recently the importance of HAB-associated microorganisms has been highlighted, shown to directly impact HAB succession and nutrient modulation. Currently, there is a gap between small-scale laboratory experiments and real world field studies of high concentration applications and impacts to the microbial community. A mesocosm study provides the necessary bridge to further our understanding. In this study we used a high concentration hydrogen peroxide treatment of a *Microcystis aeruginosa* HAB to examine impacts on microbial community succession and the resulting effects on nutrient modulation and toxin degradation. Over the course of four days in May 2021, we monitored *M. aeruginosa* bloom biomass retrieved from Lake Okeechobee in six mesocosms filled with 300 L of sieved river water on the bank of the Caloosahatchee River at Franklin Lock and Dam. Our treatment group consisted of three mesocosms which received a hydrogen peroxide spray treatment of 130x the lethal dose for *M. aeruginosa* (theoretical concentration of 13 mM; 15.7 mM measured). This treatment successfully achieved rapid (< 24 hour) bloom collapse. The treated microbial community exhibited drastic change, with a steady increase in Planctomycetes (0.58% to 46.85%) and Gammaproteobacteria (0.81% to 17.38%) relative abundance post-treatment. The treatment group also saw extreme increases of ammonia (13.9x higher) and nitrate/nitrite (14.5x higher), and fluctuations in organic phosphorous when compared to the control. There was no clear evidence of cyanotoxin or hydrogen peroxide degradation beyond the expected increase of extracellular microcystin due to cell lysing from treatment. Our study identified microbial components of a South Florida *M. aeruginosa* bloom resilient to hydrogen peroxide. Theses taxa are different from previously reported hydrogen peroxide resilient bacteria and even show opposite trends in studies from other parts of the world, decreasing after hydrogen peroxide treatment. This further highlights the importance of understanding local systems and populations with respect to water resources and HAB management.

Introduction

- Hydrogen peroxide (HP) takes advantage of cyanobacterial sensitivity to treat Harmful algal blooms (HABs) at low concentrations which do not harm non-target organisms (Drábková et al., 2007).
- However, low concentration treatments work over the course of days or longer, even at a lethal dosage (0.12 mM) (Sukenik & Kaplan, 2021).
- We anticipate stakeholders will eventually look to achieve rapid results using higher concentration applications, introducing additional risk to the ecosystem.
- Most susceptible is the microbial community, which has been noted to experience limited impacts at even low concentrations (Piel et al., 2021).
- Recently the importance of HAB-associated microorganisms have been highlighted due to their direct effects on nutrient modulation, bloom succession and development (Wang et al., 2021), and cyanotoxin and HP degradation (Giannuzzi et al., 2021).
- While limited small-scale laboratory studies have examined the effects of high concentration hydrogen peroxide (Weenink et al., 2015), there exists a concerning gap before attempting real-world field trials.
- In this study we used a high concentration HP treatment for a freshwater cyanobacterial HAB species prevalent in southwest Florida, *Microcystis aeruginosa*, to examine microbial community impacts.
- We hypothesize treated microbial community abundance and succession will differ from the control and will disrupt nutrient modulation and cyanotoxin degradation.

Methods

Fig 1. *M. aeruginosa* surface scum biomass collected from a toxic Lake Okeechobee bloom was acclimated for three days before distributed (~0.75 L) across six mesocosms. Each was filled with 300 L of sieved Caloosahatchee River water from the upstream side of Franklin Lock and Dam, the study location (A). For each of the three treatment mesocosms, four liters of 3% HP was directly applied via a spray applicator (B). The force of the spray output effectively mixed the mesocosms, resulting in a theoretical diluted HP concentration of 13 mM, supported by our measurements (15.7 mM). Mesocosms were monitored one hour after treatment and every 24 hours for the next three days.

Results

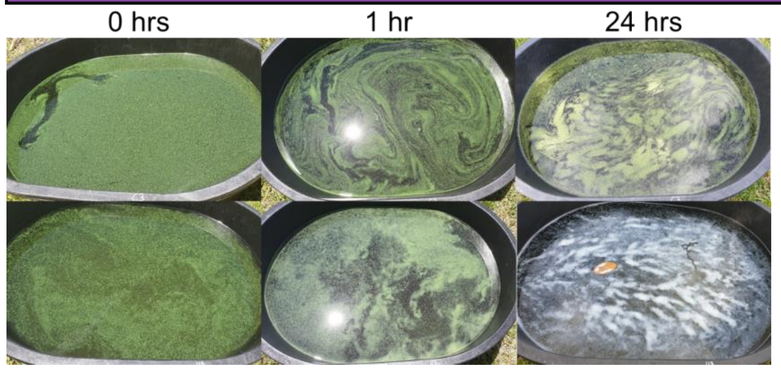

Fig. 2. Photographs of a control (top) and HP treatment (bottom) mesocosm over time. After one hour, treated *M. aeruginosa* surface scum visually began to bleach and fully bleached within 24 hours. Surface scum in the control mesocosms did not exhibit such dramatic changes but did dissipate over time.

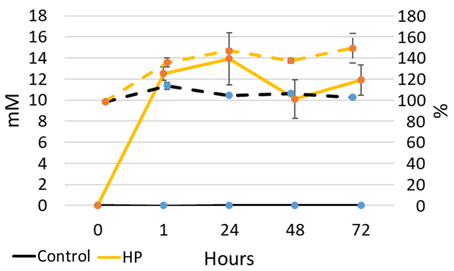

Fig. 3. Mean HP concentration (solid, left) and dissolved oxygen (DO) (dashed, right) of mesocosms over time with standard error (SE) bars (n = 3). Mesocosms treated with HP saw elevated DO levels throughout the experiment.

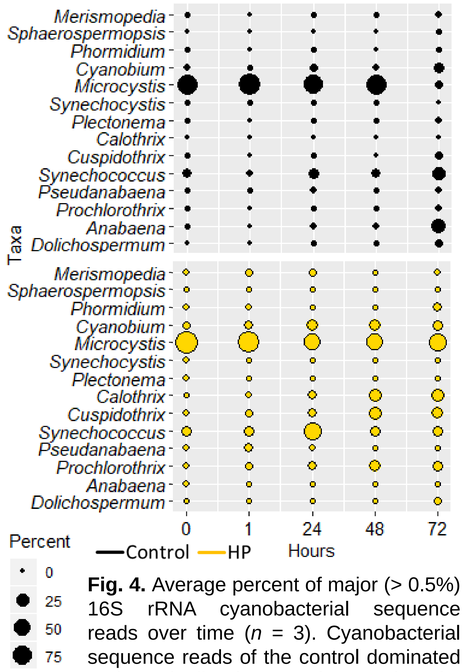

Fig. 4. Average percent of major (> 0.5%) 16S rRNA cyanobacterial sequence reads over time (n = 3). Cyanobacterial sequence reads of the control dominated and gradually decreased over time by 33.5% whereas the treatment group decreased by 99% after one hour.

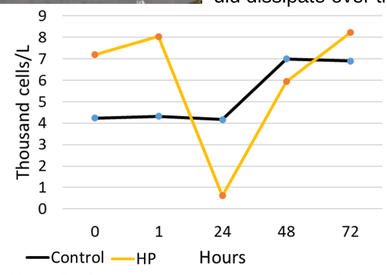

Fig. 5. Initial microscopy count of bacteria cells (excluding cyanobacteria) over time (n = 1). The treated mesocosm saw a 92.4% decrease after 24 hours post-treatment before a swift recovery over the next two days.

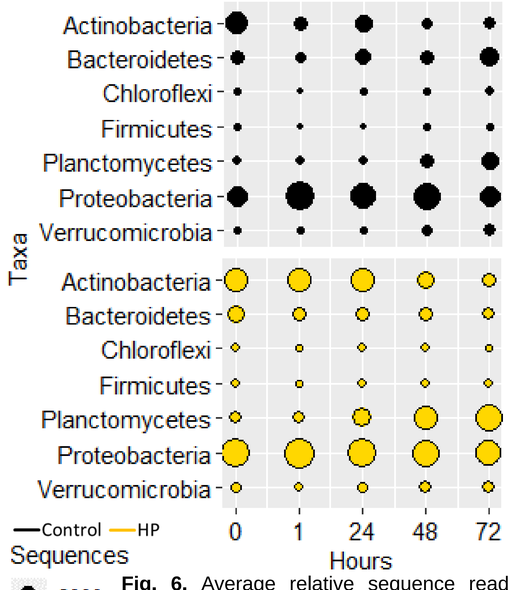

Fig. 6. Average relative sequence read abundance (standardized to 10,000 reads) of major (> 0.5%) 16S rRNA bacterial (cyanobacteria excluded) sequence reads over time (n = 3). Planctomycetes greatly increased in the treated mesocosms and became the dominant taxa. Within Proteobacteria, Gammaproteobacteria also increased while all other taxa decreased.

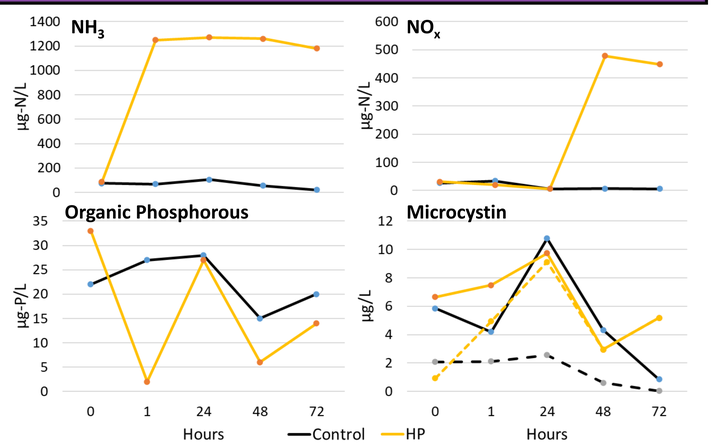

Fig. 7. Surface level nutrients and microcystin toxin overtime (n = 1). After one hour, ammonia (A) in the treatment mesocosm increased by 13.9x, and after one day nitrate and nitrite (B) increased by 14.5x, presumably due to nitrification, whereas the control only experienced minor fluctuations. Organic phosphorous (C) experienced minor fluctuations in both groups. Microcystin (D) levels were similar, although extracellular (dashed) levels were higher in the treatment mesocosm due to cells lysing upon death.

Discussion

- Hypothesis accepted; high concentration HP treatment influenced the microbial community. Nutrients differed, but not cyanotoxins.
  - Typically, HP results in increased cyanotoxin production, but here cells may have died before they could react.
- The treated microbial community experienced a dramatic reduction followed by quick growth, during this time the Planctomycetes phyla and Gammaproteobacteria class increased in relative abundance.
- Findings are contrary to field studies, where Planctomycetes was found as negatively impacted by HP (Piel et al., 2021).
  - However, industrial studies of water purification have noted Planctomycetes to be resilient to HP, noting its novel cell compartmentalization offering resistance (Want et al., 2018).
- We plan to continue this study by examining how the microbial community reacted physiologically (nutrient modulation specifically) via transcriptome analysis from collected RNA samples.
  - Should confirm nitrification is responsible for lagged No_x increase.

Acknowledgements

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