Environmental Pollution 240 (2018) 590–598

Contents lists available at ScienceDirect

Environmental Pollution

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

Hydrogen peroxide treatment promotes chlorophytes over toxic cyanobacteria in a hyper-eutrophic aquaculture pond☆

Zhen Yang [a, b], Riley P. Buley [a], Edna G. Fernandez-Figueroa [a], Mario U.G. Barros [a], Soorya Rajendran [a], Alan E. Wilson [a, *]

[a] Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL 36849, USA
[b] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China

ARTICLE INFO

Article history:
Received 15 December 2017
Received in revised form
2 May 2018
Accepted 3 May 2018

Keywords:
Algaecide
Diversity
Green algae
Microcystin
Phytoplankton
Zooplankton

ABSTRACT

Controlling blooms of toxigenic phytoplankton, including cyanobacteria, is a high priority for managers of aquatic systems that are used for drinking water, recreation, and aquaculture production. Although a variety of treatment approaches exist, hydrogen peroxide (H_2O_2) has the potential to be an effective and ecofriendly algaecide given that this compound may select against cyanobacteria while not producing harmful residues. To broadly evaluate the effectiveness of H_2O_2 on toxigenic phytoplankton, we tested multiple concentrations of H_2O_2 on (1) four cyanobacterial cultures, including filamentous *Anabaena*, *Cylindrospermopsis*, and *Planktothrix*, and unicellular *Microcystis*, in a 5-day laboratory experiment and (2) a dense cyanobacterial bloom in a 7-day field experiment conducted in a nutrient-rich aquaculture pond. In the laboratory experiment, half-maximal effective concentrations (EC_{50}) were similar for *Anabaena*, *Cylindrospermopsis*, and *Planktothrix* (average $EC_{50} = 0.41\ mg\ L^{-1}$) but were ~10x lower than observed for *Microcystis* ($EC_{50} = 5.06\ mg\ L^{-1}$). Results from a field experiment in an aquaculture pond showed that ≥ 1.3 and $\geq 6.7\ mg\ L^{-1}$ of H_2O_2 effectively eliminated *Planktothrix* and *Microcystis*, respectively. Moreover, $6.7\ mg\ L^{-1}$ of H_2O_2 reduced microcystin and enhanced phytoplankton diversity, while causing relatively small negative effects on zooplankton abundance. In contrast, $20\ mg\ L^{-1}$ of H_2O_2 showed the greatest negative effect on zooplankton. Our results demonstrate that H_2O_2 can be an effective, rapid algaecide for controlling toxigenic cyanobacteria when properly dosed.

1. Introduction

Eutrophication of freshwater systems is a global predicament that has important ramifications for the health of aquatic food webs, animals, and people through the promotion of cyanobacterial blooms, including taxa known to produce toxic secondary metabolites such as microcystins and saxitoxins (Neilan et al., 2013; Ibelings et al., 2014). Moreover, some cyanobacteria produce taste and odor compounds, such as geosmin and 2-Methylisoborneol (MIB), that have no known negative human health consequences but impart unpleasant musty flavors and odors in drinking water and aquaculture products (Zhang et al., 2011; Olsen et al., 2016).

Undoubtedly, controlling nutrient concentrations in aquatic systems is necessary for the long-term elimination of harmful cyanobacterial blooms; however, minimizing runoff inputs and managing both sedimentation and internal loading is challenging. Nutrient control in aquaculture is especially difficult given the need to regularly feed farmed fish. Although most water resource managers understand that reducing cyanobacterial blooms should be a long-term goal, most are keen to find solutions that create quick and noticeable improvements in water quality. Consequently, multiple methods have been developed aimed at reducing phytoplankton density or inhibiting their growth, including ultrasonication (Ahn et al., 2007a; Lürling et al., 2014), modified clays (Copetti et al., 2016), and bacterial or chemical agents (Cornish et al., 2000; Marsalek et al., 2012; Iredale et al., 2012; Greenfield et al., 2014). Although these techniques can be effective, some can harm non-target organisms or lead to chemical residual accumulation in treated ecosystems while others only produce short-lived effects (Matthijs et al., 2016). These drawbacks have

☆ This paper has been recommended for acceptance by Sarah Harmon.
* Corresponding author.
E-mail address: wilson@auburn.edu (A.E. Wilson).

https://doi.org/10.1016/j.envpol.2018.05.012
0269-7491/

prevented their popularization as control methods for cyanobacterial blooms.

Hydrogen peroxide (H_2O_2) is a strong oxidant that is widely used for disinfection in water treatment and is on the U.S. Food and Drug Administration (FDA) approved aquaculture drugs list for aquaculture (U.S. FDA Approved Aquaculture Drugs, assessed 11 November 2017). Since H_2O_2 rapidly decomposes to H_2O and O_2 via biological, chemical, and photochemical mechanisms during oxidation, it does not leave harmful residues in the environment. Its strong oxidizing ability also promotes algal cell mortality by producing hydroxyl radicals under light exposure, which destroys proteins, lipids, and DNA (Latifi et al., 2009). More importantly, cyanobacteria are more sensitive than other phototrophs to H_2O_2 because of their unique cellular structure (Drábková et al., 2007). Thus, H_2O_2 is expected to be a selective algaecidal compound to control cyanobacterial blooms and may be a sensible alternative to controlling cyanobacterial blooms.

Prior studies have highlighted the potential of H_2O_2 for controlling cyanobacteria while also promoting non-toxic phytoplankton. For example, Barroin and Feuillade (1986) found the toxicity threshold of *Oscillatoria* (renamed to *Planktothrix*) under laboratory conditions to be 1.75 mg L^{-1} H_2O_2, whereas the dominant chlorophyte, *Pandorina* sp, was unaffected at a 10x higher H_2O_2 dose. Moreover, Drábková et al. (2007) also showed that cyanobacteria were negatively affected by H_2O_2 at concentrations 10 times less than that of green algae and diatoms, and that high light enhanced this effect across phytoplankton taxa. Lastly, Matthijs et al. (2012) performed an unreplicated experiment in a mesotrophic lake to test if H_2O_2 can be used to selectively suppress cyanobacteria in natural waters without affecting other organisms. In that study, *Planktothrix* was reduced by 99% only 3 d after the addition of 2 mg L^{-1} of H_2O_2, while eukaryotic phytoplankton and zooplankton remained largely unaffected. More recently, a field experiment confirmed that H_2O_2, used as sodium carbonate peroxyhydrate (a granular source of H_2O_2), caused a decline of phycocyanin concentrations and cell densities but did not affect chlorophyll *a* concentrations (Geer et al., 2017). Clearly, hydrogen peroxide has potential for controlling cyanobacteria in diverse systems.

Since freshwater algal blooms can be dominated by one or many cyanobacterial genera, including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis, Microcystis,* and *Planktothrix*, it would be useful to know if interspecific variation exists in H_2O_2 toxicity thresholds across important bloom-forming genera. Past efforts aimed at using H_2O_2 to control cyanobacterial blooms have focused on *Planktothrix* and *Microcystis*. Few studies exist documenting the effect of H_2O_2 on *Anabaena*, *Aphanizomenon*, and *Cylindrospermopsis*. Across these studies, H_2O_2 toxicity thresholds span several orders of magnitude from 0.33 to 60 mg L^{-1} (Barrington et al., 2013; Bauza et al., 2014; Wang et al., 2012).

In this study, we assessed the toxicity of H_2O_2 on three filamentous and one currently unicellular but originally colonial cyanobacterial genera under laboratory conditions. Based on results from the laboratory study, we conducted a replicated, field enclosure experiment in a hyper-eutrophic aquaculture pond to investigate the effect of four different H_2O_2 concentrations on the plankton community, which included a dense cyanobacterial bloom dominated by toxic, filamentous *Planktothrix* and colonial *Microcystis,* as well as the associated zooplankton community.

2. Materials and methods

2.1. Laboratory experiment

The unicellular *Microcystis aeruginosa* (UTEX 2667) used for the laboratory experiment study was obtained from the University of Texas at Austin culture collection. Three filamentous cyanobacterial cultures, including *Anabaena flos-aquae* (also called *Dolichospermum flos-aquae*) clone R5 (AU pond R5; isolated 15 August 2010), *Planktothrix agardhii* clone G24 (AU pond E24; isolated 15 August 2010), and *Cylindrospermopsis raciborskii* clone R2 (AU pond R2; isolated 29 August 2010), were originally isolated from aquaculture ponds located at the E. W. Shell Fisheries Center of Auburn University in Auburn, Alabama. Axenic batch cultures of each strain were diluted with 60 ml of BG11 medium to achieve similar initial phycocyanin concentrations (filter blank corrected; data reported as raw fluorescence units (RFU); ~3000 RFU) with the CyanoFluor (Turner Designs, CA, USA) and added to 100 ml glass flasks at 25 °C on a12:12 h light:dark cycle at 40 μmol m^{-2} s^{-1} photosynthetically active radiation. Hydrogen peroxide (Baker Analyzed, 30%) was added into each flask to achieve the follow concentrations: 0 (control), 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}. Each treatment was replicated three times. Photosynthetic changes associated with H_2O_2 additions, including photosynthetic activity and phycocyanin pigments, were monitored before the treatments as well as 1, 2, and 5 d after H_2O_2 was added to each flask. Due to sample volume constraints, samples were collected from each flask using sterile pipet tips and analyzed for quantum efficiency (measured as F_v/F_m, where F_v is the maximal variable fluorescence and F_m is the maximal fluorescence intensity) using the Aquapen C100 (Photon Systems Instruments, Brno, Czech Republic) after placing the samples in the dark for 5 min at room temperature. Flask subsamples were also analyzed for phycocyanin concentration with the CyanoFluor.

2.2. Field experiment

The mesocosm experiment was conducted in a small (1.5 hectare), shallow (maximum depth = 2.7m), hyper-productive (~1400 μg L^{-1} total nitrogen and 122 μg L^{-1} total phosphorus) catfish aquaculture pond (S9; Boyd and Shelton, 1984) located at the E. W. Shell Fisheries Center of Auburn University during May 2017. At the start of the experiment, there was a toxic cyanobacterial bloom dominated by *Planktothrix* and *Microcystis*. Twelve greenhouse plastic limnocorrals (1500 L) that were sealed at the bottom and open at the top were suspended from a floating PVC frame in the pond and filled by pumping pond water through a sieve (500 μm) to remove small fish. To increase the biomass of *Microcystis* colonies in the enclosures, we collected plankton with a 100 μm net and then added similar volumes of the concentrated plankton homogenate into each enclosure. All enclosures were fertilized with KNO_3 and K_2HPO_4 to reach the target nutrient levels (4 mg L^{-1} for total nitrogen and 0.2 mg L^{-1} for total phosphorus) and sampled at 9:00 am on day 0 before establishing the four H_2O_2 treatments (0, 1.3, 6.7, and 20 mg L^{-1} H_2O_2, single application; three replicates per treatment). One enclosure was damaged during the experiment and data collected from this enclosure was not used for later analyses. Each enclosure was mixed thoroughly prior to collecting integrated samples (surface to 1 m depth) with a rigid tube sampler at 9:00 am 1, 3, 5, and 7 days after the H_2O_2 addition. Samples were returned to the lab and processed for phytoplankton and zooplankton diversity and abundance, two algal pigments (chlorophyll *a* and phycocyanin), and the hepatotoxin microcystin (both intracellular (particulate) and extracellular (dissolved)).

Phytoplankton samples were preserved with 1% Lugol's iodine solution before settling a small volume (180 μl) in a Palmer chamber and enumerating all phytoplankton taxa observed in 10 fields under 100x magnification (Olsen et al., 2016). Nikon image software was used to estimate the biovolume for each taxon. Zooplankton were collected using a 60 μm filter and preserved in