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Removing cyanobacteria and associated toxins in aquaculture ponds

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Commercial algaecide effective at removing cyanobacterium *Planktothrix* sp.

Hypereutrophic ponds before the application of the granular-based H2O2 algaecide.

Most fish farmers operating in freshwater ponds will at some point encounter blooms of nuisance microalgae, which threaten the health and quality of their aquacultured products. Of particular concern are cyanobacteria because they produce toxins, as well as compounds (e.g., geosmin and 2-methyl isoborneol) that cause off-flavors in fish.

The main toxins are microcystins that can accumulate in the fish flesh, which poses safety concerns because these can be passed to human consumers. Preventive methods are perhaps the best management option, such as maintaining low nutrient levels – particularly phosphorus (P) – through water exchanges and/or the use of P-binding clay. However, some farmers observe that elevated temperatures often trigger nuisance microalgae blooms.

Off-flavors from nuisance microalgae cause serious economic losses to farmers due to the cost and delay involved to purge the fish prior to harvesting and/or strategies to remove cyanobacteria. Therefore, some farmers use algaecides, such as copper sulfate or alum. However, these tend to be less selective and can also cause toxicity to other beneficial microalgae and zooplankton.

The sudden die-off of the primary producers, including nuisance microalgae, can cause three problems. Firstly, without the main organisms to remove dissolved nitrogen, the ammonia and nitrite levels can subsequently spike. Secondly, the dead microalgae would contribute to increased biological oxygen demand that could strip oxygen out of the water. Thirdly, large amounts of microcystins would be released as the cyanobacteria die and are then taken up by animals.

A more selective compound to kill cyanobacteria includes using hydrogen peroxide (H_2O_2). Traditionally, this is added in liquid form but can pose safety concerns due to accidental spilling during transport, storage and application. Other H_2O_2-based products have emerged in the form of sodium carbonate peroxyhydrate (SCP) that, when added to water, is rapidly converted to H_2O_2 and sodium carbonate. Therefore, this is more stable, but largely untested in an aquaculture context.

Preparing the granular-based H2O2 algaecide. The mask was used as a precaution due to it being highly oxidizing.

In this article – adapted and summarized from the original publication (*Science of the Total Environment* (DOI:10.1016/j.scitotenv.2018.05.023) – we summarize results of a study to test a novel SCP-based product within small- and large-scale hypereutrophic tanks and ponds, respectively, that were both dominated with cyanobacteria. The small-scale trial was first conducted to narrow down the most appropriate doses to kill cyanobacteria without potentially harming beneficial microalgae and zooplankton. Based on these results, two optimal doses (2.5 and 4.0 mg/l H_2O_2) were tested within commercial ponds (large-scale trial) and over six weeks the cyanobacterial abundance, plankton abundance, microcystins and persistence of H_2O_2 levels were monitored.

Experimental design

Nutrients (inorganic fertilizer and de-oiled rice bran) were added, as needed, to pond water until hypereutrophic, cyanobacteria-dominated conditions were obtained (first image at the top). Identical nitrogen to total P conditions were used and the dominant cyanobacterium was *Planktothrix* sp.

In both trials, a granular SCP based algaecide (PAK® 27) was used, which is a USEPA-approved compound (SePRO Corporation, Carmel, IN, U.S.A.). Different dosages of H_2O_2 were prepared by dissolving the algaecide (active ingredient ~ 27 percent H_2O_2) granules in moderately hard water (~ 187 mg/l as $CaCO_3$) analogous to local water conditions.

Small-scale trial

Three circular, 75-L barrels were installed in each of the six hypereutrophic ponds and filled with this water. The algaecide was added at 0, 5.56, 7.41, 9.26, 11.11, 12.96, 14.81, 18.52 and 29.63 mg/L to achieve final concentrations of 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 8.0 mg/L H_2O_2, respectively. Samples were daily taken between 10:00 and 12:00 over 10 days to monitor changes in cyanobacteria, eukaryotic microalgae and zooplankton abundance. Dissolved oxygen (DO), conductivity (µS/cm), pH and water temperature (degrees-C) were measured using a digital probe. Turbidity was measured using a Secchi disk and total phosphorus, ammonia nitrogen, nitrite-N, nitrate-N, total alkalinity and total hardness using commercial kits.

The average concentration of *Planktothrix* sp. prior to the algaecide applications was 1.08×10^6 cells/mL, while the combined taxa of various microalgae were 5.24×10^4 cells/mL. Among the tested concentrations, 2.5 mg/L H_2O_2 was the lowest dose that significantly reduced *Planktothrix* sp., and higher concentrations led to no further significant improvement at reducing cyanobacteria. At 4.0 mg/L H_2O_2 there was also no adverse effect to the plankton. However, concentrations of ≥ 4.0 mg/L adversely impacted the abundance of the non-targeted plankton community that included *Synedra*, *Spirogyra* and *Cladophora* sp. Therefore, 2.5 and 4.0 mg/L H_2O_2 were selected for testing in the large-scale trial.

Large-scale trial

A total of four ponds were randomly selected for the two H_2O_2 treatments, while the remaining two ponds were used as reference (control) ponds. Paddlewheels were used to facilitate an even dispersal of the algaecide. Similarly, as in the small-scale trial, the cyanobacterial / plankton abundance and water quality were measured along with assessing microcystins, chlorophyll-*a* and persistence of H_2O_2 over six weeks.

Both doses of 2.5 and 4.0 mg/L H_2O_2 significantly decreased *Planktothrix* sp. concentrations, but at different times of seven and five days post-application, respectively (Fig. 1). There was also a notable change in the appearance of the water after three days, where the cyanobacteria were dying and the water appeared to have a more greenish-brown coloration. A gradual recovery of *Planktothrix* sp. populations became apparent between weeks 5 and 6, although abundances were still far less than both the control or during the pre-application phase. In the 2.5 mg/L H_2O_2 treatment, the cyanobacterial decrease coincided with increased eukaryotic phytoplankton, particularly diatoms and green algae. Moreover, herbivorous zooplankton that included *Brachionus* sp., *Daphnia* sp., and calanoid and cyclopoid copepods were unaffected. In contrast, 4.0 mg/L H_2O_2 suppressed these phyto- and zooplankton, which was in contrast to the small-scale trial.

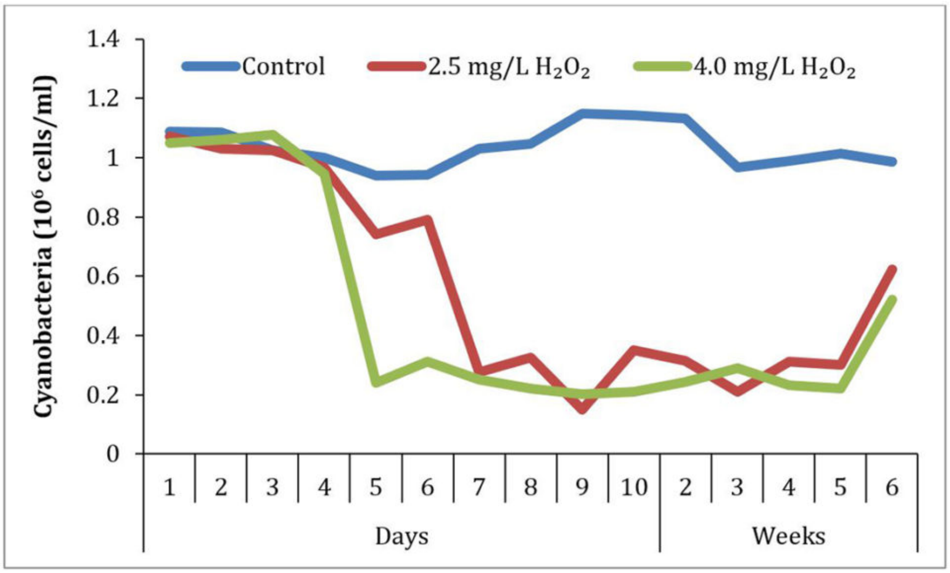

Fig. 1: Mean cyanobacteria (106 cells/mL) in ponds after adding increasing concentrations of the granular-based H2O2 algaecide.

Three days after the application of the granular-based H2O2 algaecide.

Both doses of 2.5 and 4.0 mg/L H_2O_2 significantly reduced total microcystins from seven and five days, respectively, until the end of the trial (Fig. 2). The added H_2O_2 rapidly degraded to below detectable levels after days 3 and 4, respectively (Fig. 3). The dissolved oxygen remained relatively stable over this study (Fig. 4a). The pH was lower in both H_2O_2 ponds but was more stable over time (Fig. 4b). There was a spike in ammonia within 2.5 and 4.0 mg/L H_2O_2 ponds on days 7 and 8, respectively, but these were within the tolerance of freshwater animals and decreased after week 3 (Fig. 4c). On the other hand, nitrite remained low and stable in all ponds (Fig. 4d).

Fig. 2: Mean total microcystins (ppb) in ponds after adding increasing concentrations of the granular-based H2O2 algaecide.

Fig.3: Degradation of 2.5 and 4.0 mg/L H2O2 after applying the granular-based H2O2 algaecide.

Fig. 4: Mean dissolved oxygen (a), pH (b), ammonia (c) and nitrite (d) in ponds treated with increasing concentrations of the granular-based H2O2 algaecide.

Overall conclusions

Issues with extermination of cyanobacteria with traditional algaecides may include temporary deteriorations in water quality, unintended toxicity to beneficial plankton or high levels of microcystins. However, these were substantially mitigated by H_2O_2 at 2.5 mg/L in the form of SCP. In fact, this was slightly beneficial to zooplankton, likely due to the reemergence of eukaryotic microalgae as the cyanobacteria died. Moreover, the use of this product left no long-term traces of H_2O_2 in the water.

It is important to point out that, while the small-scale trial was an important step in this study, there were some discrepancies that included a concentration of 4.0 mg/L of the algaecide negatively affecting the plankton community within the ponds. Thus, caution should be used when small-scale trials are used to predict those in a large-scale commercial setting.

Overall, our results demonstrate that the granular SCP-based algaecide (PAK® 27) –corresponding to 2.5 mg/L H_2O_2 – can be recommended as an eco-friendly strategy to effectively remove populations of the cyanobacterium *Planktothrix* sp. without compromising water quality or other plankton communities.

Future research and perspectives

A limitation of this was study was not assessing the potential toxicity of H_2O_2 to fish. There are indications this would be safe, based on no apparent toxic effects to zooplankton at 2.5 mg/L and limited persistence in water; however, this will be tested in the future on juvenile channel catfish and largemouth bass.

This research will also extend to other cyanobacterial species as well as any potential effect this could have on compounds that cause “off-flavors” (e.g. geosmin and MIB), often a side-effect of hypereutrophic ponds that causes substantial economic losses to farmers.

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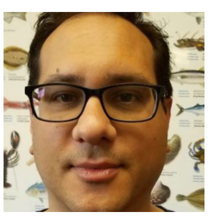

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