Mitigation of *Microcystis sp.* with metallic peroxide granules: matrix effect on hydrogen peroxide release kinetics and toxicity study on invertebrates

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Abstract. An array of mitigation strategies has been applied over the years for toxic blue-green algae with the most recent one to be hydrogen peroxide (H_2O_2). Hydrogen peroxide has been widely used as an alternative to copper algaecides, and it is perceived as a more environmentally friendly option for treating surface waters. However, dense blooms demand high oxidant causing undesirable effects to the non-targeted organisms in the aquatic ecosystem. Slow releasing H_2O_2 metallic granules have been used in this study as an alternative approach to direct application of high-doses liquid H_2O_2 application. In this study, calcium peroxide (CaO_2) granules were applied in surface water matrix (Kouris Dam, Cyprus) to examine: (a) their H_2O_2 releasing properties with varying pH values, (b) their mitigation efficiency on *Microcystis* sp. bloom in comparison with liquid H_2O_2, and (c) their toxicity on *Gammarus* sp. in a range of concentrations. Results showed that in acidified environments granules have higher H_2O_2 releasing capacity. Moreover, treatment of *Microcystis* sp. with 0.5 – 2.0 g/L CaO_2 were efficient to eliminate the blooms and safe on zooplankton species. All of the above, are indicative towards the potential of CaO_2 treatment, however its application necessitates further investigation prior taking it to the field.

Keywords: cyanobacteria, hydrogen peroxide, metallic granules, mitigation, surface water

1. Introduction

Cyanobacteria (blue-green algae) are phototrophic microorganisms which can be found in almost every terrestrial and aquatic environment such as surface waters, oceans, soil and muds, lagoons, rocks and even icebergs. Their growth is favored in ponds, dams, and lakes in stagnant and turbidity-free waters. In combination with high loads of organic matter and nutrients in water, cyanobacteria can excessively grow and form “blooms” which can be harmful to humans, animals, and other living organisms. A wide range of physical, chemical, and biological techniques are applied to surface waters for the removal or treatment of cyanobacteria harmful algal blooms (cyano-HABs). These methods aim to prevent or eliminate the undesirable effects caused by cyano-HABs, and they vary in treatment efficiency, cost effectiveness and environmental friendliness. Even though both physical and chemical methods are used in water treatment processes, chemical methods are preferable for *in-situ* treatments as their application is easier, faster, more cost effective and usually have lasting effects. Hydrogen peroxide has been widely used the last decade as an alternative to copper sulfates (algicides) and it is considered as a more environmentally friendly method. Since the use of copper sulfate is highly regulated in EU due to its adverse environmental impact, hydrogen peroxide treatments in EU have been applied extensively over the last years. However, concerns over unregulated dosing and potential environmental effects have raised. Recent studies showed that some cyanobacterial species demand extremely high doses of oxidant for high treatment efficiency which may be harmful to the rest of the ecosystem.

In this study, metallic peroxide granules were examined as an alternative method to direct high-dose liquid hydrogen peroxide application for the mitigation of *Microcystis* sp.. The capacity of calcium peroxide granules to release H_2O_2 was performed in water collected from the Kouris Dam that was adjusted in various pH to investigate the pH effect on their ability to release hydrogen peroxide. Then, the same water matrix spiked with *Microcystis* sp. was treated with CaO_2 and H_2O_2 and their mitigation efficiency was monitored through cyanobacterial pigments fluorescence (Ft) and quantum yield of the Photosystem II (QY). Also, in order to verify their appropriateness for the *in-situ* mitigation, toxicity tests were performed on a zooplankton species, named *Echinogammarus veneris*, in a range of concentrations.

2. Methods

2.1. Matrix effect on the H_2O_2 release kinetics by CaO_2 granules

Experiments on the release kinetics of H_2O_2 were conducted in two different matrixes: (a) Milli-Q water and (b) surface water. Surface water was collected from Kouris Dam (Limassol, Cyprus) and CaO_2 granules used in concentrations of 1, 2 and 3 g/L to examine the maximum release of H_2O_2 by CaO_2 granules in each matrix. Samples were collected from each flask for H_2O_2 quantification in t= 0-6, 24-26, 28 hours, and quantifiedby the colorimetric reaction introduced by Sellers et al. (1980). Surface water was also collected, filtered for pH effect experiments. The pH of each flask adjusted to pH= 2, 4, 6, 8, 10 and 3 g/L CaO_2 granules were added in each flask. Samples were collected from each flask for H_2O_2 quantification, following the same method as before.

2.2. Toxicity study on invertebrates

Gammarus species were collected from a river in Cyprus with the kicking method and transferred to the laboratory in aqueous environment. CaO_2 granules in concentrations 0.2, 0.5, 1.0, 2.0 g/L and liquid H_2O_2 in concentrations 1, 3, 6 and 12 mg/L were added in each flask contained 200 mL of surface water free from cyanobacteria or other contaminants and 10 invertebrates. Mortality of invertebrates and residual oxidant concentration recorded in t=1-6, 24,26, 48, and 50 h after oxidant addition.

2.3. Microcystis sp. treatment with CaO_2 granules and liquid H_2O_2

The collected surface water was spiked with *Microcystis sp.* to simulate a cyano-HABs blooming event. CaO_2 granules in concentrations 0.2, 0.5, and 1.0 g/L and liquid H_2O_2 in concentrations 1, 3, and 6 mg/L were added in each flask cyanobacterial water. For determining the efficiency of oxidants on mitigating the bloom; photosynthetic changes associated with H_2O_2 additions, including instantaneous fluorescence and PSII efficiency were monitored in both wavelengths (450, 620 nm) at 1-6, 24, 48, 120 h following oxidant addition.

3. Results & Discussion

Release kinetics showed higher release of H_2O_2 concentration in surface water in comparison with MQ-water. For example, 1 g/L CaO_2 granules released 2.9 mg/L H_2O_2 in pure water while 5.0 mg/L in surface water. The matrix load favors the activation of granules, leading faster to the products side of the reaction. Lack of organic and inorganic load is limiting the releasing capacity of CaO_2 granules.

Testing the release in various pH showed that CaO_2 granules are releasing faster H_2O_2 in acidic pH which complies with the fact that CaO_2 granules solubility increases when pH decreases. Even though reaction rates differ in each pH, the accumulative release of H_2O_2 at 4 hours after CaO_2 addition is the same in all tested pH (up to pH=10; p>0,05). Surface water pH is expected between 7 - 9 which indicates a release rate same as pH= 8.

The next objective of this study was to examine the toxicity of the oxidants on a freshwater amphipod specie, the *Echinogammarus veneris* species. An 100% mortality was recorded from the first 6 hours of exposure to 2.0 g/L CaO_2 but in concentrations < 2.0 g/L mortality was close to or less than 50%.

Treatment of surface water spiked with *Microcystis* sp. utilizing 1, 3, 6 and 12 mg/L of liquid H_2O_2, and 0.2, 0.5, 1.0 and 2.0 g/L of CaO_2 result in various efficiencies. After 120 hours of treatment, all concentrations (except 1 mg/L H_2O_2) effectively decreased the phycocyanin content and quantum yield of treated samples confirming inhibition of PSII and thus cell destruction equal to bloom mitigation (Figure 1).

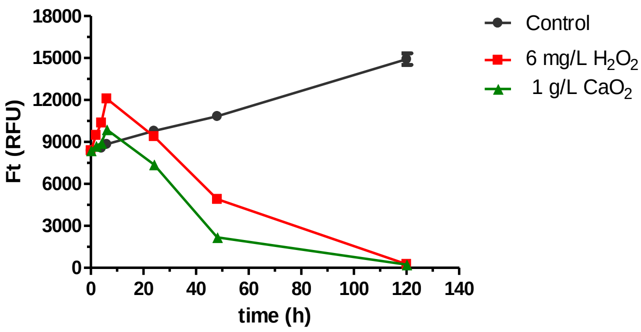

Figure 1. Instantaneous fluorescence of phycocyanin (λ=620 nm) during 120 hours of treatment with 6mg/L liquid H_2O_2 and 1 g/L CaO_2 granules.

4. Conclusions

Release of H_2O_2 is favoured in natural waters (surface waters) which are loaded with nutrients, organic and inorganic matter which caused higher released concentrations than in MQ-water. The pH does not affect the overall released concentration but the initial release rates. As explained, acidic pH accelerates H_2O_2 release while basic pH slows down the release of H_2O_2 by granules. Treatment with CaO_2 granules ≤ 1.0 g/L does not affect the wellness of zooplankton communities as tested on *Echinogammarus veneris* species and results in high treatment efficiency on removing dense single species *Microcystis* sp. blooms. Concentrations of ≤1 g/L CaO_2 granules are preferred for treatment than the direct application of up to 6 mg/L H_2O_2, due to high efficiency and low impact on zooplankton communities in comparison with H_2O_2 treatment.

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References

Lusty, M.W., Gobler, C.J. (2020), The Efficacy of Hydrogen Peroxide in Mitigating Cyanobacterial Blooms and Altering Microbial Communities across Four Lakes in NY, USA, *Toxins*, ***12***, 428.

Lu S., Zhang X., Xue Y. (2017) Application of calcium peroxide in water and soil treatment: A review, *Journal of Hazardous Materials*, **337**, 163-177.

Matthijs H., Visser P., Reeze B., Meeuse J., Slot P., Wijn G., Talens R., Huisman J. (2012), Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide, *Water Research*, **46**, 5, 1460-1472.

Wang H., Zhao Y., Li T., Chen Z., Wang Y., Qin C. (2016), Properties of calcium peroxide for release of hydrogen peroxide and oxygen: A kinetics study, *Chemical Engineering Journal*, **303**, 450-457