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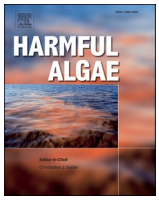

Effects of peroxide-based compounds on growth and toxicity of *Microcystis aeruginosa*

Miquel Lürling [a], Ziwei Liao [a], Li Kang [a], West M. Bishop [b], Maíra Mucci [a,c,*]

[a] *Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA, Wageningen, the Netherlands*
[b] *SePRO Research and Technology Campus, 16013 Watson Seed Farm Rd., Whitakers, NC, USA*
[c] *Limnological Solutions International Pty Ltd, Hamburg, Germany*

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ABSTRACT

Cyanobacterial blooms are increasing worldwide, compromising water bodies and ecosystem services. Among the possible management measures are curative methods, such as hydrogen peroxide, that can suppress cyanobacteria growth. However, the rapid breakdown of H_2O_2 could limit its effectiveness; thus new formulations (liquid and solid) that are, in theory, more stable have been developed. Here, we tested three new formulations (Oximycin™ P5, Phycomycin® SCP and Lake Guard® Oxy) and traditional peroxides (H_2O_2 and CaO_2) on their capacity to end *Microcystis aeruginosa* bloom and their effect on microcystin dynamics. In general, all the products controlled *M. aeruginosa* biomass. The products were comparable mainly in the tests with lower initial Chl-*a* (150 μg L^{-1}), with slightly less effectiveness of CaO_2. At a higher initial bloom concentration (600 μg Chl-a L^{-1}), all the products were effective in reducing Chl-*a* concentration to zero at a dose of 3 mg H_2O_2 L^{-1}, except Lake Guard® Oxy and CaO_2, which showed an increase in Chl-*a* by the end of the experiment. This suggests that Oximycin™ P5, Phycomycin® SCP, and liquid H_2O_2 may perform better at lower doses. Nonetheless, at the highest dose tested (10 mg H_2O_2 L^{-1}), all products were highly effective, with no signs of recovery. After one day of exposure, extracellular MC concentrations were higher in all treatments but reduced to lower levels than the control after nine days, pointing to the degradation of dissolved MCs. There is no obvious top choice in terms of overall efficiency. However, each product may be better suited for different purposes depending on the specific situation and strategy.

1. Introduction

Cyanobacterial blooms have been increasing globally, mainly driven by anthropogenic factors such as nutrient pollution of surface waters (i.e., eutrophication), climate change, and water use dynamics (Heisler et al., 2008; Kosten et al., 2012; Poikane et al., 2024; Smith and Schindler, 2009). Eutrophication in particular, may lead to harmful cyanobacterial blooms that impair freshwater ecosystems, incite economic damage, and compromise ecosystem services (Chislock et al., 2013; Huisman et al., 2018). Cyanobacterial blooms are in direct conflict with societal and legislative demands for safe and healthy surface water, creating an urgent need for water authorities to mitigate the problems associated with harmful cyanobacterial blooms.

The main causes of eutrophication are nutrient losses from wastewater and agriculture, which makes minimizing nutrient spills from these sources the most logical first management measure to control eutrophication and mitigate cyanobacterial blooms (Cooke et al., 2005; Tammeorg et al., 2023). However, high-income countries treat only about 70 % of their municipal and industrial wastewater, while in low-income countries, less than 10 % of the wastewater is treated (United Nations World Water Assessment Programme WWAP, 2017) and catchment measures to reduce diffuse nutrient pollution are even more challenging to implement (OECD, 2017; Steinman et al., 2018). Consequently, in systems such as lakes, ponds, and reservoirs, where sufficient external nutrient inflow control has not been realized or is not economically feasible, in-lake, effect-oriented or curative measures may provide the most favourable intervention to temporarily alleviate cyanobacterial nuisance (Lürling and Mucci, 2020).

* Corresponding author at: Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA, Wageningen, the Netherlands.
E-mail address: maira.mucci@wur.nl (M. Mucci).

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Curative methods should rapidly suppress the proliferation of cyanobacteria or terminate a bloom, leading to a fast recovery of hampered ecosystem services (Jančula and Maršálek, 2011). A whole suite of curative measures exists ranging from copper-based algaecides and herbicides to peroxides and coagulants (Jančula and Maršálek, 2011; Kang et al., 2022; Lürling and Mucci, 2020; Matthijs et al., 2016). The use of approved algaecides and herbicides may meet legislative and regulatory constraints (Jančula and Maršálek, 2011), whereas coagulants are primarily effective in deeper, stratified lakes (Lürling et al., 2020). Among algaecides, the use of peroxide compounds is one of the most promising emergency methods to eliminate cyanobacteria (Piel et al., 2024).

Hydrogen peroxide (H_2O_2) is a strong oxidant to which cyanobacteria are generally more sensitive than eukaryotic algae and most non-target animal species (Barroin and Feuillade, 1986; Drábková et al., 2007b; Weenink et al., 2015). In a recent meta-analysis on treatments to control freshwater algal blooms, H_2O_2 was among the few chemicals that significantly reduced cyanobacteria cell density (Anantapantula and Wilson, 2023). H_2O_2 was successfully applied to a *Planktothrix agardhii*-dominated lake in The Netherlands, where a mild dose of 2 mg H_2O_2 L^{-1} was sufficient to wipe out 99 % of the cyanobacteria (Matthijs et al., 2012). Peroxide-based algaecide treatments have also resulted in decreases in cyanotoxins following treatment (Barrington et al., 2013; Kinley-Baird et al., 2021; Matthijs et al., 2012; Sinha et al., 2018). An advantage of H_2O_2 over other algaecides is that it rapidly dissociates into water and oxygen, leaving no chemical residues in the environment (Barrington and Ghadouani, 2008; Matthijs et al., 2016). The decay rates of H_2O_2 are usually in the range of hours to days (Cooper and Zepp, 1990; Matthijs et al., 2012; Watts et al., 1999). Peroxide-based algaecides provide a scalable and rapid management technique to restore water use and can complement longer-term eutrophication control measures.

Cyanobacteria removal efficacy with peroxide depends on several factors, such as cell densities (Weenink et al., 2015), colony formation/size (Liu et al., 2017), abundance of green algae (Weenink et al., 2021), application dose (Weenink et al., 2022), and the duration of H_2O_2 exposure (Smit et al., 2008). Since the relatively rapid breakdown of H_2O_2 could limit the duration of its effectiveness and hamper the successful suppression of cyanobacterial blooms, alternative formulations have been tested and developed to expand the exposure time to H_2O_2. These include liquid formulations such as Oximycin™ P5, which consists of 26.5 % H_2O_2 and 4.9 % peroxyacetic acid (SePRO Corporation, USA), or granular forms, such as CaO_2, and sodium carbonate peroxyhydrate (SCP) formulations that more gradually release H_2O_2. SCP is a solid granular substance ($2Na_2CO_3 \bullet 3H_2O_2$) that, in water, dissociates into H_2O_2 and sodium carbonate (Knox Galwey and John Hood, 1982; Sinha et al., 2018). SCP may have advantages of safer storage, ease of transport and dispersion, and extended duration of H_2O_2 release (Sinha et al., 2018), although more information is required on the comparative effectiveness of peroxide formulations on controlling nuisance cyanobacteria.

CaO_2 has been tested to some extent on cyanobacteria cultured in the laboratory (Cho and Lee, 2002; Wang et al., 2021; Zhang et al., 2023) and cyanobacteria collected from the field (Gu et al., 2023; Noyma et al., 2016). Some SCP varieties have been tested (Phycomycin® SCP, Geer et al., 2016; PAK® 27, Sinha et al., 2018; GreenClean® PRO Calomeni et al., 2023), while for Lake Guard® Oxy, no peer-reviewed study is found, yet some reports of *in-situ* applications exist (Sukenik and Kaplan, 2021; Trainic et al., 2021). Oximycin™ P5 has been tested on a red tide alga (Hu et al., 2022) and only recently on cyanobacteria (Yun et al., 2024). Therefore, in this study, we evaluated the efficacy of Oximycin™ P5, Lake Guard® Oxy, as well as Phycomycin® SCP, CaO_2, and traditional liquid H_2O_2 in suppressing blooming densities of the cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*) and consequent toxin dynamics. We tested the hypothesis that all products would be equally effective based on peroxide concentration, regardless of formulation, and that at higher *M. aeruginosa* densities, the slow-release products would outperform H_2O_2.

2. Materials and methods

2.1. Materials

Five peroxide products were tested in this study, consisting of two more common cyano-bactericides (H_2O_2 and CaO_2) and three novel compounds (Lake Guard® Oxy, Phycomycin® SCP and Oximycin™ P5):

1) Hydrogen peroxide, 30 % liquid hydrogen peroxide (H_2O_2), (EMSURE® ISO Merck KGaA, 64271 Darmstadt, Germany).
2) Calcium peroxide, 65 % typically calcium peroxide powder (CaO_2), (Alfa Aesar, Thermo Fisher GmbH, Erlenbachweg 2, 76,870, Kandel, Germany).
3) Lake Guard® Oxy, 83.3 % Sodium percarbonate ($2Na_2CO_3 \bullet 3H_2O_2$), (BlueGreen US Water Technologies, Inc. 2100 West Loop South, Suite 1100 Houston, Texas, 77,027, U.S.A.).
4) Phycomycin® SCP, 85 % Sodium Carbonate Peroxyhydrate ($2Na_2CO_3 \bullet 3H_2O_2$), (SePRO Corporation, 11550 N. Meridian Street, Suite 600 Carmel, IN 46032, U.S.A.).
5) Oximycin™ P5, 26.5 % liquid hydrogen peroxide (H_2O_2); 4.9 % peroxyacetic acid (CH_3CO_3H), (SePRO Corporation, 11550 North Meridian Street, Suite 600 Carmel, IN 46032, U.S.A.).

The five products were tested for their cyanobactericidal effect using the cyanobacterium *Microcystis aeruginosa* (strain PCC 7820) as a test organism. This strain was acquired from the Pasteur Culture Collection of Cyanobacteria (PCC). *M. aeruginosa* was grown in a 2 L Erlenmeyer flask on modified WC-medium (Lürling and Beekman, 2006) at 20 °C and a light intensity of 31 µmol quanta $m^{-2}s^{-1}$ with a 16:8 h light-dark cycle. Once the culture reached the exponential growth phase, *M. aeruginosa* was adapted to the experimental conditions by adjusting the light intensity: first, the light intensity was increased to 62 µmol quanta $m^{-1}s^{-1}$ for two days, then to 81 µmol quanta $m^{-1}s^{-1}$ for two days and to 100 µmol quanta $m^{-2}s^{-1}$ for another two days.

2.2. Experimental design

To evaluate the effectiveness of the five peroxide compounds on laboratory-cultured *M. aeruginosa*, three series of nine or fifteen-day tests, differing the initial chlorophyll-*a* (Chl-*a*) concentration, were conducted in 300 mL Erlenmeyer flasks containing 200 mL of *M. aeruginosa* suspension and the test compound at its designated concentration.

In the first series, aliquots of *M. aeruginosa* from the homogenized stock culture were transferred to 300 mL Erlenmeyer flasks and diluted with sterile WC-medium to reach 200 mL suspensions with initial *M. aeruginosa* Chl-*a* concentrations of 150 µg L^{-1} ($\sim 6.61 \times 10^5$ cells mL^{-1}). Different amounts of the five peroxide compounds were added to the flasks to obtain a concentration range of 0, 1, 3, 5, 10, and 20 mg H_2O_2 L^{-1}. Each concentration had three replicates, and the flasks were closed with a cellulose plug. The flasks were incubated in a climate-controlled room for nine days with a 16:8 light-dark cycle, a temperature of 20 °C, and a light intensity of 100 µmol quanta $m^{-2}s^{-1}$ and were shaken manually daily. Aliquots of 2 mL were taken from each flask initially (before the material additions) and after 1, 3, 6, and 9 days to measure Chl-*a* concentration and the photosystem II efficiency (PSII efficiency) using a PHYTO-PAM analyzer (Heinz Walz GmbH, Effeltrich, Germany). H_2O_2 concentrations were measured by inserting peroxide Quantofix test sticks (0.5- 25 mg L^{-1}; Macherey–Merck, Darmstadt, Germany), while pH was measured using a WTW Inolab pH 7110 m In addition, after 1 and 3 days, from each treatment, a sample was taken that was filtered through a 0.45 µm filter unit (Whatman, Germany), and the Chl-*a* concentration and the PSII efficiency were determined.

At the start, after one day and after 9 days of the experiment, intracellular and extracellular microcystins (MCs) were measured. A 30 mL subsample was taken from each flask and filtered through a glass fiber filter (GF/C, Whatman®, VWR International B.V., Amsterdam, The Netherlands). The glass fiber filters were placed in Petri dishes and used for intracellular MC analysis. For extracellular MC analysis, 5 mL of the GF/C filtrate was transferred into 8 mL glass tubes. The filters and filtrate were wrapped in aluminium foil and stored at −20 °C until MC analysis using LC-MS/MS (Agilent 1200 LC and Agilent 6410A QQQ, Waldbronn, Germany) following the method described by Lürling and Faassen (2013). In short, the glass fiber filters were transferred into 10 mL glass tubes and dried for 24 h in a Freeze Dryer (Christ Alpha 1–2 LD, Martin Christ, Germany) at −55 °C; the filtrate was dried too in the freeze-drier. Then 2.5 mL of 75 % methanol/water was added to the glass tube with a filter, thoroughly stirred, mixed, and then heated in a water bath set to 60 °C for 30 min. Subsequently, the liquid was transferred into an 8 mL glass tube; the procedure was performed three times, yielding a total of 7.5 mL extract. The extract was placed in a SpeedVac concentrator (SavantTM SPD121P, Thermo Fisher Scientific, Asheville, NC, USA) at 50 °C until vaporization. Subsequently, sublimated extracellular and intracellular microcystin samples were reconstituted with 900 µL methanol (J.T. Baker®, 97 %, VWR International B.V., Amsterdam, The Netherlands). The samples were transferred to vials fitted with 0.22 µm cellulose-acetate spin centrifuge tube filters and centrifuged at 16,000 x g for 5 min. The filtrate was then moved to an amber glass vial and analyzed for the following microcystin variants; dmMC-RR, MC-RR, MC-YR, dmMC-LR, MC-LR, MC-LA, MC-LY, MC-LW, and MC-LF; and nodularin (NOD).

In the second series, the growth inhibition test was performed with an initial *M. aeruginosa* Chl-*a* concentration of 300 µg L^{-1}. The five peroxide compounds were tested in triplicate at 0, 1, 3, and 10 mg $H_2O_2L^{-1}$. The flasks were incubated under the same conditions as for the first series for fifteen days, and samples were taken at the start and after 1, 3, 6, 9 and 15 days of incubation. Samples were analysed for Chl-*a* concentration and PSII efficiency. In the third series, all conditions were similar to those in the second series except that the initial *M. aeruginosa* Chl-*a* concentration was 600 µg L^{-1}.

2.3. Data analysis

A two-way ANOVA was run on the biomass yield, which was calculated following the OECD algal and cyanobacteria growth inhibition test guideline 201 (OECD, 2011) as the biomass at the end of the test minus the starting biomass for each single vessel of controls and treatments. Growth rates were determined using linear regression on natural logarithm-transformed Chl-*a* data against time. A One-way ANOVA was performed to evaluate the difference in growth rates between the controls for each material. Spearman's rank correlation coefficient was applied to determine the relationship between pH and Chl-*a*.

3. Results

3.1. Effects of peroxides on Microcystis inoculated at 150 µg Chl-a L^{-1}

M. aeruginosa expressed good growth in the controls of each product (Fig. 1). A one-way ANOVA indicated growth rates in controls were different between products ($F_{4,13}$= 35.0; p< 0.001), which was mainly a result of very low within-series variability (Fig. S1). Growth rates in controls varied between 0.224 (±0.005) and 0.267 (±0.004) D^{-1} (Fig. S1). In contrast, *M. aeruginosa* was strongly suppressed when peroxide-based compounds were dosed at 1 or 3 mg H_2O_2 L^{-1} (Fig. 1). A two-way ANOVA on the yield, i.e., Chl-*a* concentrations after nine days minus Chl-*a* concentration at start, revealed a significant product effect ($F_{4,89}$= 13.3; p< 0.001), a significant dose effect (($F_{5,89}$= 645.7; p< 0.001), and a significant product × dose effect ($F_{20,89}$= 17.9; p< 0.001). The latter interaction effect can be explained by CaO_2 and Lake Guard® Oxy exerting no or limited effect when dosed at 1 mg H_2O_2 L^{-1} (Fig. 1C, E), while H_2O_2, Phycomycin® SCP, and Oximycin™ P5 already showed strong effects at 1 mg H_2O_2 L^{-1} (Fig. 1A,G,I).

The somewhat elevated Chl-*a* concentrations after one day in all the treatments are mostly a result of dissolved pigments detectable in filtered samples (Fig. S2). In all filtered samples, no PSII efficiencies were detected (Fig. S2).

PSII efficiencies were also strongly depressed when *M. aeruginosa* was exposed to the different peroxide compounds and concentrations (Fig. 1). Recovery of the PSII efficiencies occurred in all cultures that had been exposed to 1 mg H_2O_2 L^{-1} of the corresponding peroxide product. After nine days, recovery of PSII efficiency was complete in cultures exposed to a 1 mg H_2O_2 L^{-1} dose of CaO_2 (Fig. 1D) and Lake Guard® Oxy (Fig. 1F), it was partly recovered in H_2O_2 (Fig. 1B) and Phycomycin® SCP (Fig. 1H) and slightly recovered in Oximycin™ P5 (Fig. 1J). In addition, some recovery of the PSII efficiencies was observed after nine days in the 3 mg H_2O_2 L^{-1} treatments of CaO_2, Lake Guard® Oxy, and Phycomycin® SCP (Fig. 1D,F,H). At a higher dose than 3 mg H_2O_2 L^{-1}, no signs of PSII efficiency recovery were observed in any of the products. A two-way ANOVA on the PSII efficiencies determined after nine days of incubation indicated a significant product effect ($F_{4,89}$= 9.9; p< 0.001), a significant dose effect (concentrations applied; ($F_{5,89}$= 191.7; p< 0.001), and a significant product × dose effect ($F_{20,89}$= 5.0; p< 0.001).

The pH increased steadily over time in all controls as well as in the 1 mg H_2O_2 L^{-1} dose of CaO_2, whereas pH remained lower in the other treatments over time (Fig. 2). Initially, pH was elevated proportionally to the dose of CaO_2 applied, but pH gradually declined in these treatments (Fig. 2C). In all series, pH was strongly correlated with Chl-*a* concentrations (Fig. S3); H_2O_2 - ρ = 0.929, p< 0.001; CaO_2 - ρ = 0.829, p< 0.001; Lake Guard® Oxy - ρ = 0.876, p< 0.001; Phycomycin® SCP - ρ = 0.871, p< 0.001; Oximycin® P5 - ρ = 0.889, p< 0.001.

After one day, H_2O_2 concentrations were mostly below target nominal concentrations in the H_2O_2 treatments, whereafter they gradually declined until no H_2O_2 was present anymore after nine days in any of the flasks (Fig. 2B). In the CaO_2 series, H_2O_2 concentrations were lower than expected. After three days, H_2O_2 was no longer detectable in the CaO_2 doses with nominal H_2O_2 concentrations of 1 – 10 mg L^{-1}, and after six days also no H_2O_2 was measured in the highest CaO_2 dose (Fig. 2D). In Lake Guard® Oxy treatments, H_2O_2 declined in the 1 – 10 mg L^{-1} doses and was no longer detectable after 1 to 3 days in the dose, while H_2O_2 remained present at relatively high concentration in the highest dose of Lake Guard® Oxy (Fig. 2F). A similar pattern was observed in the Phycomycin® SCP treatments with H_2O_2 remaining at relatively high concentration in the highest dose applied (Fig. 2H). In the Oximycin™ P5 treatments, H_2O_2 concentrations gradually declined in all treatments with only in the highest Oximycin™ P5 dose remaining until the end of the experiment (Fig. 2J). After one day, measured H_2O_2 concentrations in the highest doses of the H_2O_2 treatments were still 90 % (± 11 %) of the initially added dose, 35 % (± 5 %) for CaO_2, 63 % (± 14 %) for Lake Guard® Oxy, 83 % (± 3 %), for Phycomycin® SCP and 78 % (± 3 %) for Oximycin™ P5 (Fig. 2B,D,F,H,J).

Six MC variants were detected in the start cultures of *M. aeruginosa* PCC 7820 of which dmMC-LR made up on average 3.7 % (± 0.6 %) of the total MC pool, MC-LR was the most abundant with 75.3 % (± 3.4 %), LY comprised 2.7 % (± 3.4 %), LW 8.7 % (± 1.3 %), LF 9.6 % (± 1.4 %), while LA was detected sporadically in trace amounts (Fig. 3). In all H_2O_2 treatments, total intracellular MC concentrations were significantly reduced after one day (H_5 = 16.0; p= 0.007) and after nine days (H_5 = 16.6; p= 0.005) compared to the control (Fig. 3A). In contrast, extracellular MC concentrations after one day were significantly elevated in H_2O_2 treatments (H_5 = 12.0; p= 0.035), but after nine days, total extracellular MC concentrations were highest in all the controls and gradually declined with higher H_2O_2 concentrations (Fig. 3B).

In the CaO_2 treatments, total intracellular MC concentrations were significantly different after one day (H_5 = 16.4; p= 0.006) and after nine days (H_5 = 15.4; p= 0.009), where MC concentrations were lower in the

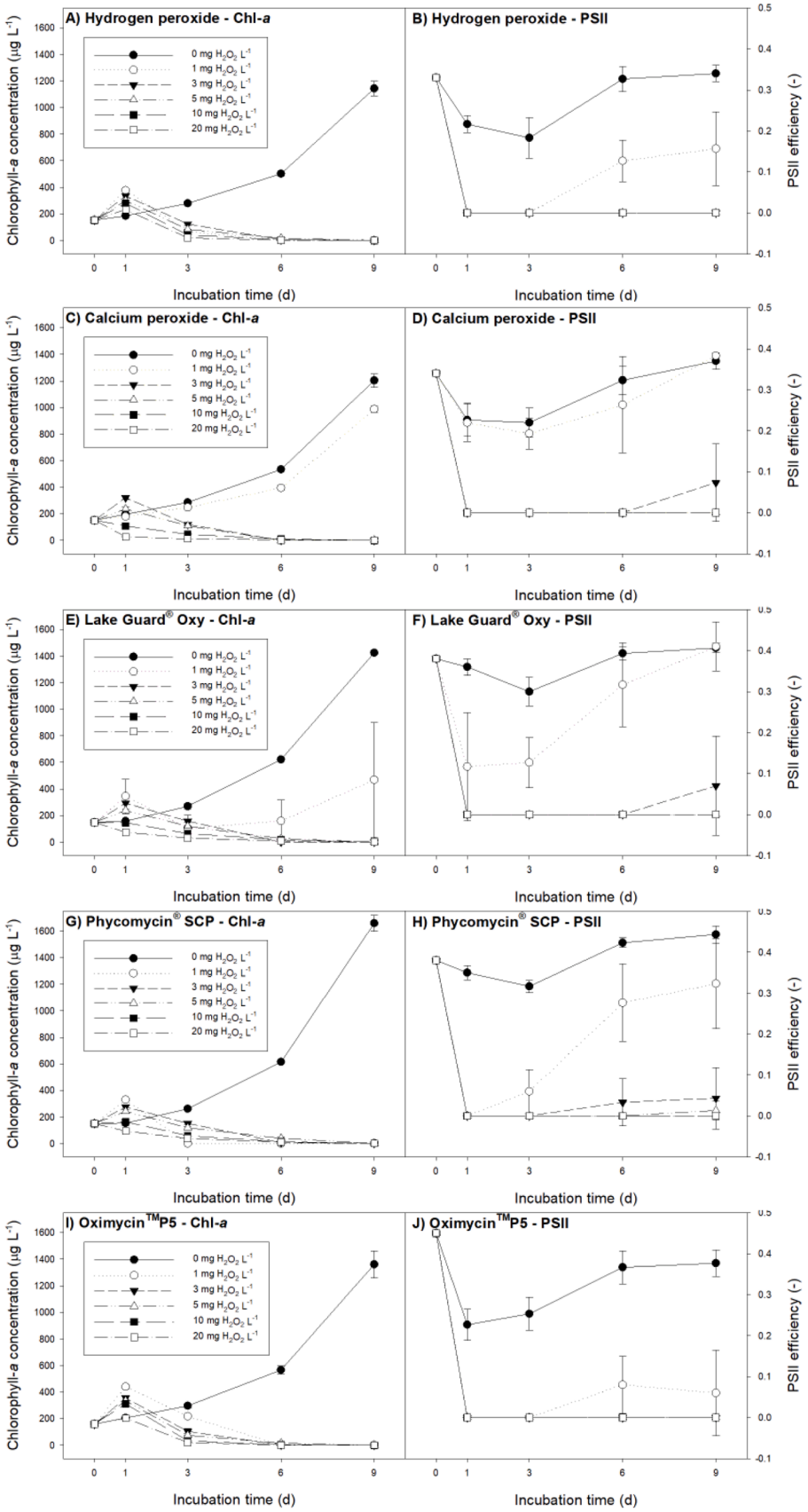

Fig. 1. Chlorophyll-a concentrations (left graphs) and PS II efficiency (right graphs) over time for all the products tested: Hydrogen peroxide (A and B), Calcium peroxide (C and D), Lake Guard® Oxy (E and F), Phycomycin® SCP (G and H), and Oximycin™ P5 (I and J) from the experiment with an initial Chl-a concentration of 150 µg Chl-a L^{-1}. Error bars represent one standard error.

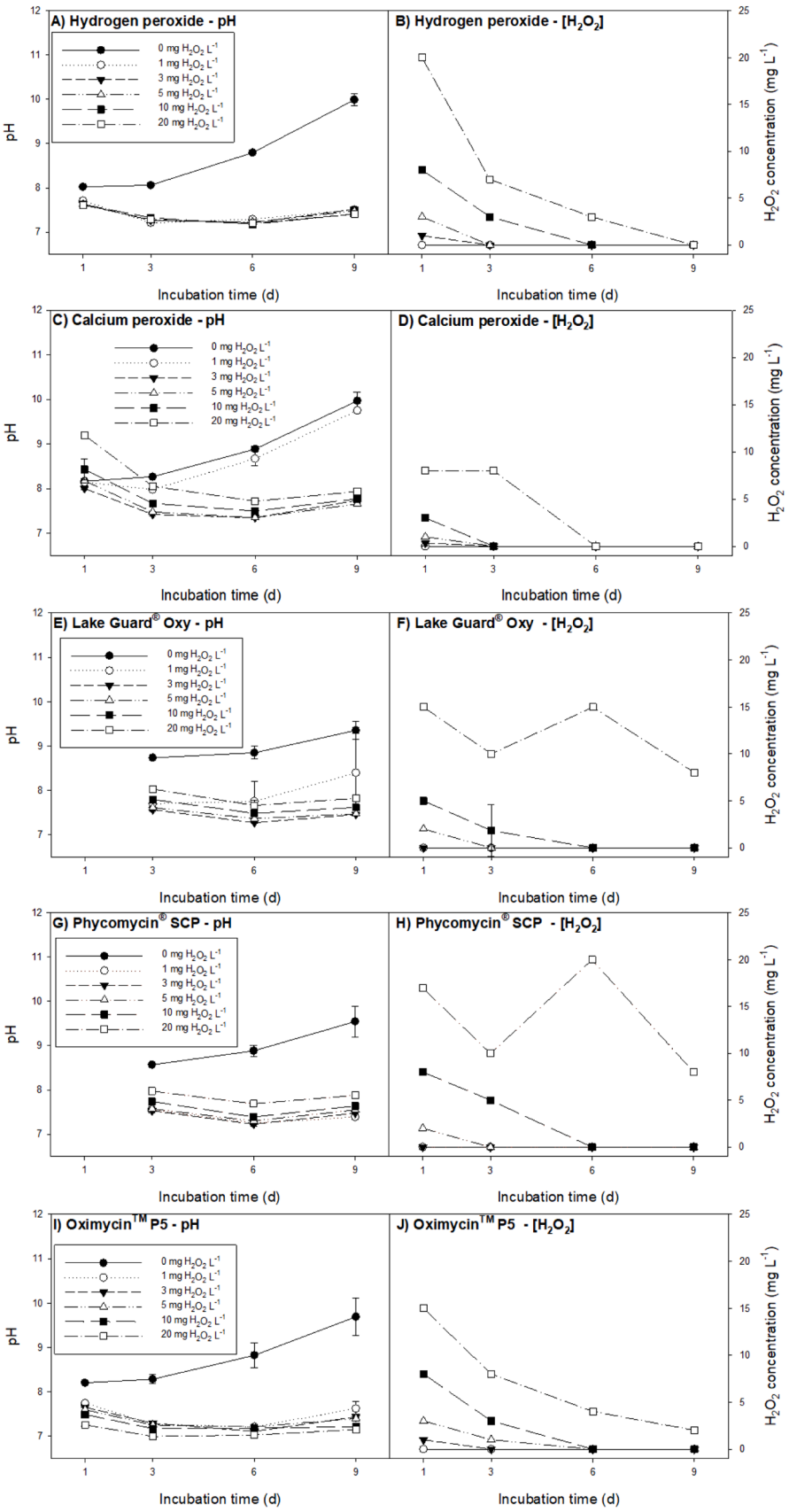

Fig. 2. pH (left graphs) and H_2O_2 concentration (right graphs) over time for all the products tested: Hydrogen peroxide (A and B), Calcium peroxide (C and D), Lake Guard® Oxy (E and F), Phycomycin® SCP (G and H), and Oximycin™ P5 (I and J) from the experiment with an initial Chl-a concentration of 150 µg Chl-a L^{-1}. Error bars represent one standard error.

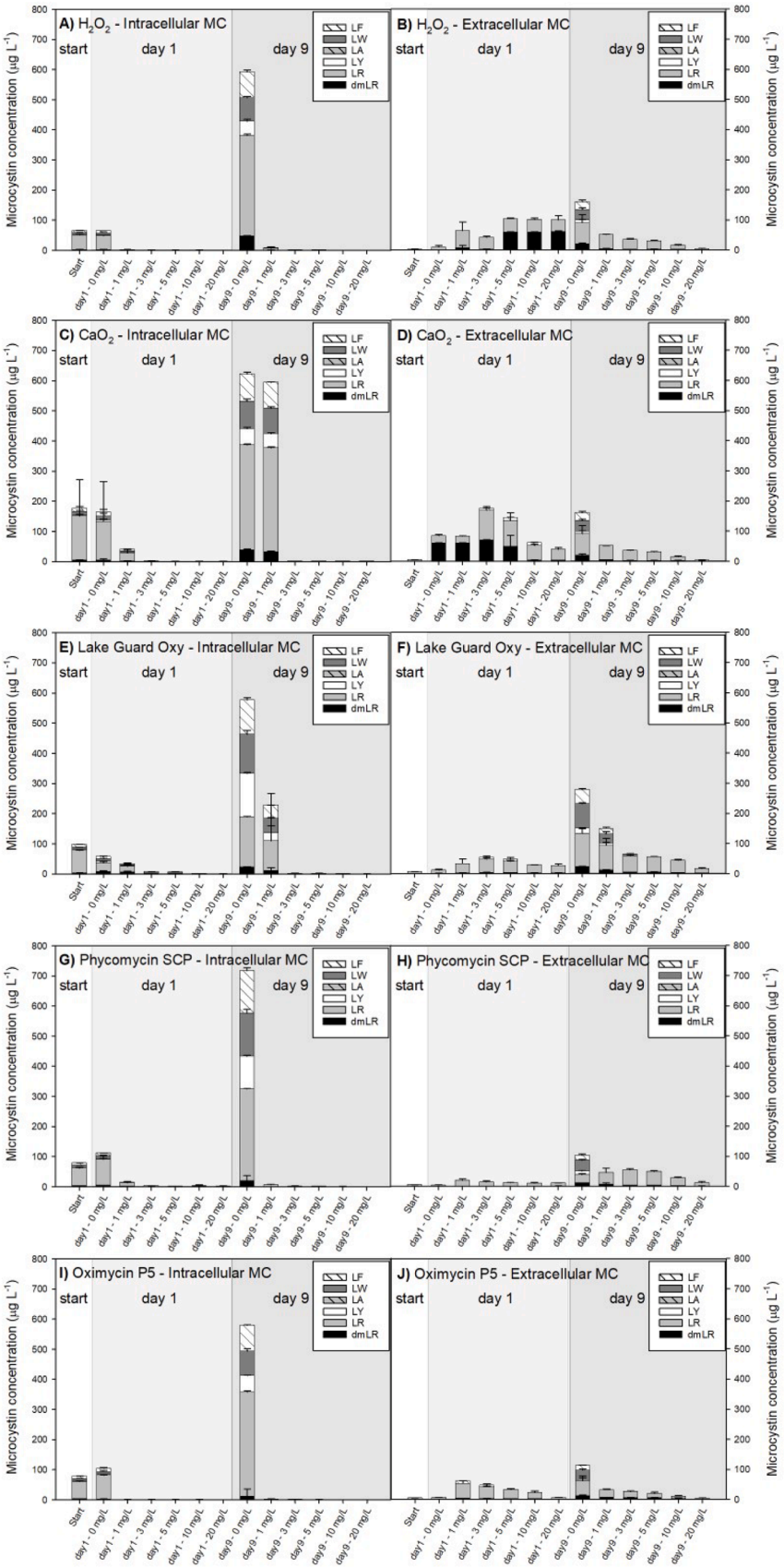

Fig. 3. Intracellular (left graphs) and extracellular (right graphs) microcystin concentrations at the start, on day one and after nine days of incubation for all the products tested: Hydrogen peroxide (A and B), Calcium peroxide (C and D), Lake Guard® Oxy (E and F), Phycomycin® SCP (G and H)), and Oximycin™ P5 (I and J) from the experiment with an initial Chl-a concentration of 150 µg Chl-a L^{-1}. Error bars represent one standard error.

3–20 mg H_2O_2 L^{-1} treatments compared to the control and 1 mg H_2O_2 L^{-1} treatment (Fig. 3C). Extracellular MC concentrations after one day were significantly elevated in the 3 and 5 mg H_2O_2 L^{-1} treatments ($H_5 = 14.1$; $p= 0.015$), while after nine days total extracellular MC concentrations were highest in the control and 1 mg H_2O_2 L^{-1} treatment and significantly ($F_{5,17}= 11.1$; $p< 0.001$) lower in the 3–20 mg H_2O_2 L^{-1} treatments (Fig. 3D).

In the Lake Guard® Oxy treatments, total intracellular MC concentrations were significantly different after one day ($H_5 = 15.3$; $p= 0.009$) and after nine days ($H_5 = 13.2$; $p= 0.021$), where MC concentrations were lower in the 1 mg $H_2O_2L^{-1}$ treatment compared to the control and the lowest in the 3–20 mg H_2O_2 L^{-1} treatments (Fig. 3E). Extracellular MC concentrations, after one day, were significantly elevated in the 3 and 5 mg H_2O_2 L^{-1} treatments compared to the control and the 20 mg H_2O_2 L^{-1} treatment ($F_{5,17}= 8.24$; $p= 0.001$), while after nine days total extracellular MC concentrations were highest in the control and gradually declined with Lake Guard® Oxy dose ($H_{5,17}= 16.6$; $p= 0.005$) (Fig. 3F).

Total intracellular MC concentrations in the Phycomycin® SCP treatments were significantly different after one day ($H_5 = 13.9$; $p= 0.016$) and after nine days ($H_5 = 15.3$; $p= 0.009$), where MC concentrations were higher in the control and lowest in all Phycomycin® SCP treatments (Fig. 3G). Extracellular MC concentrations after one day of exposure to Phycomycin® SCP were significantly higher in the 1, 3 and 5 mg H_2O_2 L^{-1} treatments compared to the control ($F_{5,17}= 10.9$; $p< 0.001$). However, after nine days, total extracellular MC concentrations were highest in the control, significantly reduced in the 1, 3 and 5 mg H_2O_2 L^{-1} treatments and lowest in the 10 and 20 mg H_2O_2 L^{-1} treatments ($F_{5,17}= 44.8$; $p< 0.001$) (Fig. 3H).

In *M. aeruginosa* cultures exposed to Oximycin™ P5 treatments virtually no intracellular MC was detected after one or nine days of incubation, whereas considerable amounts were measured in the controls (Fig. 3I). After one day, extracellular MC concentrations were similarly low in the control and 20 mg H_2O_2 L^{-1} treatment ($F_{5,17}= 60.2$; $p< 0.001$), and significantly different in other treatments being highest in the 1 mg H_2O_2 L^{-1} treatment and declining with increasing Oximycin™ P5 dose (Fig. 3J).

3.2. Effects of peroxides on Microcystis inoculated at 300 μg L^{-1}

In the controls of each series, *M. aeruginosa* expressed good growth over the course of the experiment (Fig. 4). Growth rates were similar in controls among series ($F_{4,14}= 2.33$; $p= 0.127$) and were on average 0.19 (± 0.01) d^{-1}. A two-way ANOVA on the yield, i.e., Chl-*a* concentrations after 15 days minus Chl-*a* concentration at the start, revealed a significant product effect ($F_{4,59}= 12.4$; $p< 0.001$), a significant dose effect ($F_{3,59}= 1446.5$; $p< 0.001$), and a significant product × dose effect ($F_{12,59}= 12.9$; $p< 0.001$). The latter interaction effect can be explained by H_2O_2 and CaO_2 having similar yields in control and the 1 mg H_2O_2 L^{-1} treatments (Fig. 4A,C), Lake Guard® Oxy and Phycomycin® SCP having a slightly higher yield in the 1 mg H_2O_2 L^{-1} treatments compared to control (Fig. 4E,G), while in the Oximycin™ P5 series the yield was lower in the 1 mg H_2O_2 L^{-1} treatment compared to the control (Fig. 4I). In all 3 and 10 mg H_2O_2 L^{-1} treatments chlorophyll-*a* concentrations remained very low or zero until the end of the experiment (Fig. 4A,C,E,G,I).

PSII efficiencies were depressed when *M. aeruginosa* was exposed to the different peroxide compounds, but this depended on the concentration of the product applied (Fig. 4). In the H_2O_2 and CaO_2 series, PSII efficiencies were similar in control and the 1 mg H_2O_2 L^{-1} treatments (Fig. 4B,D). Full recovery of the PSII efficiencies occurred in the H_2O_2 and CaO_2 series that had been exposed to 3 mg H_2O_2 L^{-1} (Fig 4B,D). In 1 mg H_2O_2 L^{-1} doses of Lake Guard® Oxy (Fig. 4F), Phycomycin® SCP (Fig. 4H), and Oximycin™ P5 (Fig. 4J), the PSII efficiencies were partly reduced compared to the controls but recovered towards the end of the experiment. Some recovery of the PSII efficiencies in the 3 mg H_2O_2 L^{-1} treatments of Lake Guard® Oxy, Phycomycin® SCP and Oximycin™ P5 was noted (Fig. 4F,H,J). A two-way ANOVA on the PSII efficiencies determined after 15 days of incubation indicated a significant product effect ($F_{4,59}= 18.8$; $p< 0.001$), a significant dose effect (($F_{3,59}= 80.8$; $p< 0.001$), and a significant product x dose effect ($F_{12,59}= 9.70$; $p< 0.001$).

3.3. Effects of peroxides on Microcystis inoculated at 600 μg L^{-1}

M. aeruginosa expressed good growth throughout the experiment in the controls of each product (Fig. 5). The growth rates in controls were similar among series ($F_{4,14}= 1.42$; $p= 0.296$) and were on average 0.11 (± 0.01) d^{-1}. A two-way ANOVA on the yield, i.e., Chl-*a* concentrations after 15 days minus Chl-*a* concentration at the start, revealed a significant product effect ($F_{4,59}= 3.85$; $p= 0.010$), a significant dose effect ($F_{3,59}= 165.7$; $p< 0.001$), and a significant product × dose effect ($F_{12,59}= 7.26$; $p< 0.001$). The interaction effect can be explained by H_2O_2 and Phycomycin® SCP having similar yield in control and the 1 mg H_2O_2 L^{-1} treatments and no detectable Chl-*a* in the 3 and 10 mg H_2O_2 L^{-1} treatments (Fig. 5A,G), CaO_2 having similar yield in the control, 1 and 3 mg H_2O_2 L^{-1} treatments (Fig. 5C), Lake Guard® Oxy having a higher yield in the 3 mg H_2O_2 L^{-1} treatment than in the 10 mg H_2O_2 L^{-1} treatment (Fig. 5E), and Oximycin™ P5 series showing the highest yield in the 1 mg H_2O_2 L^{-1} treatment (Fig. 5I). In all 10 mg H_2O_2 L^{-1} treatments Chl-*a* concentrations remained very low or zero until the end of the experiment (Fig. 5A,C,E,G,I), also PSII efficiencies remained zero until the end of the experiment in the 10 mg H_2O_2 L^{-1} treatments (Fig. 5B,D,F,H,J).

In the treatments with H_2O_2, Phycomycin® SCP and Oximycin™ P5, the course of PSII efficiencies was similar in control and 1 mg H_2O_2 L^{-1} treatment and those were significantly higher (Holm-Sidak post-hoc comparison test) than in the 3 and 10 mg H_2O_2 L^{-1} treatments, which remained non-detect throughout the exposure duration (Fig. 5B,H,J). In *M. aeruginosa* cultures exposed to CaO_2 or Lake Guard® Oxy, only in the dose corresponding with 10 mg H_2O_2 L^{-1} were PSII efficiencies significantly lower than in control, 1 and 3 mg H_2O_2 L^{-1} treatments (Fig. 5D,F).

4. Discussion

The results of our study are in line with the hypothesis that all five peroxide-based compounds – Oximycin™ P5, Lake Guard® Oxy, Phycomycin® SCP, CaO_2, and traditional H_2O_2 – would be effective in reducing growth and toxicity of the cyanobacterium *Microcystis aeruginosa*. At an initial bloom concentration of 150 μg Chl-*a* L^{-1}, Oximycin™ P5, Phycomycin® SCP, and traditional H_2O_2 were already effective at a dose of 1 mg H_2O_2 L^{-1}, whereas for CaO_2 and Lake Guard® Oxy doses corresponding with a nominal 3 mg H_2O_2 L^{-1} were the dose sufficient to keep Chl-*a* concentrations undetectable until the end of the experiment. At an initial bloom concentration of 300 μg Chl-*a* L^{-1}, all the products were effective in reducing Chl-*a* concentration to zero until the end of the experiment at a higher dose than when the bloom was less dense (e.g. > 3 mg H_2O_2 L^{-1}). Similarly, at a high initial bloom concentration of 600 μg Chl-*a* L^{-1}, all the products were effective in reducing Chl-*a* concentration to zero until the end of the experiment at a dose of 3 mg H_2O_2 L^{-1}, except Lake Guard® Oxy and CaO_2, which showed an increase in Chl-*a* by the end of the experiment. These results aligned with other studies reporting the sensitivity of *M. aeruginosa* to H_2O_2 (Drábková et al., 2007a, b; Weenink et al., 2022), as well as with findings that suggest a higher dose of H_2O_2 may be required when denser blooms are present (Buley et al., 2023).

The compounds tested were dosed based on their chemical composition, with the expectation that similar H_2O_2 concentrations would be achieved across doses. After one day, measured H_2O_2 concentrations in the highest doses remained at 80–90 % of the expected values for H_2O_2, Phycomycin® SCP and Oximycin™ P5 treatments. In contrast, concentrations were slightly over 60 % for Lake Guard® Oxy, and only 35 % for CaO_2. These somewhat lower-than-expected H_2O_2 concentrations may

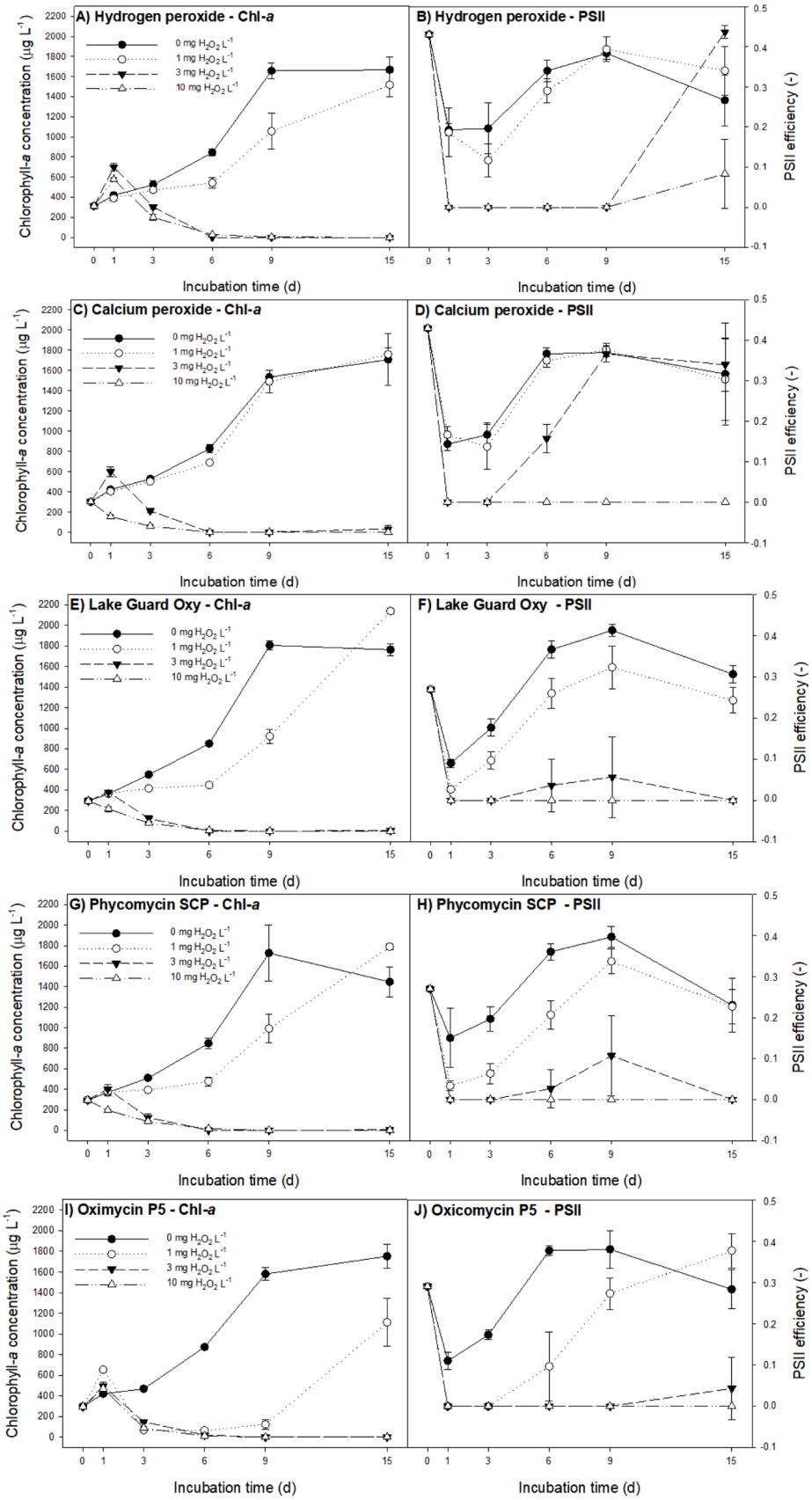

Fig. 4. Chlorophyll-a concentrations (left graphs) and PS II efficiency (right graphs) over time for all the products tested: Hydrogen peroxide (A and B), Calcium peroxide (C and D), Lake Guard® Oxy (E and F), Phycomycin® SCP (G and H)), and Oximycin™ P5 (I and J) from the experiment with an initial Chl-a concentration of 300 µg Chl-a L^{-1}. Error bars represent one standard error.

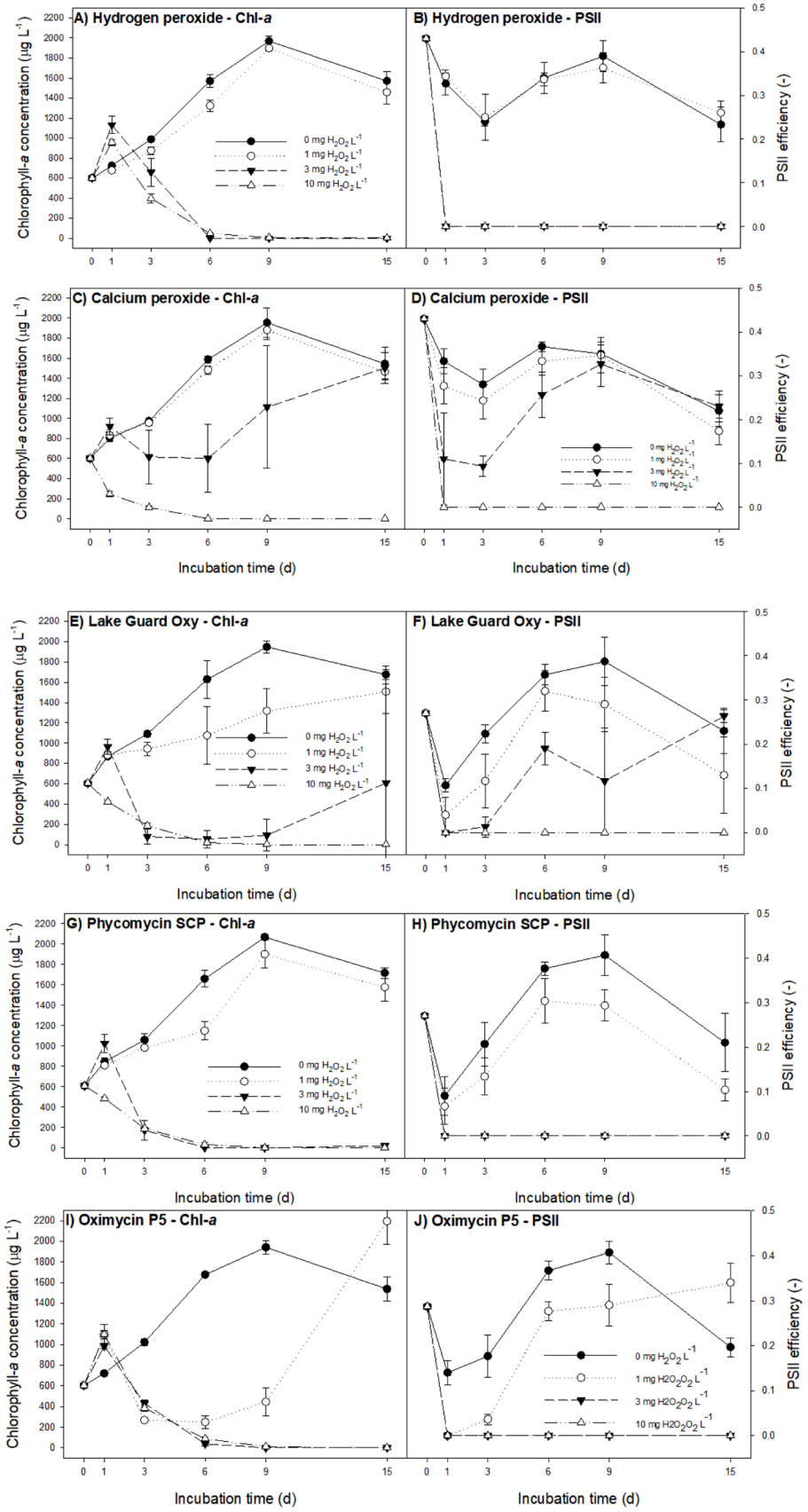

Fig. 5. Chlorophyll-a concentrations (left graphs) and PS II efficiency (right graphs) over time for all the products tested: Hydrogen peroxide (A and B), Calcium peroxide (C and D), Lake Guard® Oxy (E and F), Phycomycin® SCP (G and H)), and Oximycin™ P5 (I and J) from the experiment with an initial Chl-a concentration of 600 µg Chl-a L^{-1}. Error bars represent one standard error.

be partly due to the reactivity and thus decay of H_2O_2. However, an 80–90 % retention of the initial calculated concentration was expected for all the high-dose treatments except CaO_2. The considerably lower H_2O_2 concentration present in the 1 mg H_2O_2 L^{-1} treatment of the CaO_2 series caused no or less depression of *M. aeruginosa*. Theoretically, each gram of CaO_2 can release up to 0.47 g H_2O_2 (Wang et al., 2016). However, when CaO_2 was added to water, two reactions occurred simultaneously with respect to O_2 and H_2O_2 production. The release of O_2 via $CaO_2 + H_2O \rightarrow Ca(OH)_2 + ½\ O_2$, producing O_2 and $Ca(OH)_2$, while H_2O_2 is not an indispensable intermediate in the conversion of CaO_2 to O_2 (Wang et al., 2016), the competition between the generation of H_2O_2 and O_2 may lead to lower H_2O_2 release. The release of H_2O_2 via $CaO_2 + 2H_2O \rightarrow Ca(OH)_2 + H_2O_2$, generating both H_2O_2 and calcium hydroxide ($Ca(OH)_2$), raises pH. This pH increase leads to a reduction in H_2O_2 production (Wang et al., 2016), which drops to nearly zero at pH 12 (Northup and Cassidy, 2008). As the pH was initially elevated in a CaO_2 dose-dependent manner, the reduced H_2O_2 release from CaO_2 seems most likely a result of this pH increase. While it is possible that the lower H_2O_2 concentrations were due to an underdosing during the experiment—since the expected H_2O_2 concentrations were never reached in the CaO_2 treatment—this may also be explained by the slower release of H_2O_2 from solid materials, such as CaO_2, compared to traditional liquid H_2O_2. Several studies that reported a detrimental effect of CaO_2 on cyanobacteria applied relatively high concentrations of 100 mg CaO_2 L^{-1} or more, and consequently at high pH and low H_2O_2 concentrations (Gu et al., 2023; Wang et al., 2021; Zhang et al., 2023). Noyma et al. (2016) reported effectiveness at a much lower concentration of 4 mg CaO_2 L^{-1}, but also in that study pH rose above pH 10 at 4 mg CaO_2 L^{-1} and above pH 11 at 64 mg CaO_2 L^{-1}. A positive effect of such high pH following CaO_2 treatment is the reduction of phosphate (Wang et al., 2021), which is caused by Ca-P precipitation (Recillas et al., 2012). Although not many studies have evaluated the effect of alkaline pH on the viability and membrane integrity of cyanobacteria, no direct effect of high pH on *M. aeruginosa* is expected as cyanobacteria like *M. aeruginosa* were reported to grow at pH >10 (Reynolds, 1986), and also in our study in control series pH 10 was reached with actively growing *M. aeruginosa*.

Traditional H_2O_2 undergoes rapid decay into water and oxygen and thus has a limited reaction time (Matthijs et al., 2012; Weenink et al., 2015). Prolonged reaction with cyanobacteria and a stronger impact could be achieved using higher doses of H_2O_2, but this is not recommended because of negative effects on zooplankton non-target organisms (Weenink et al., 2022). The novel liquid cyanobactericide Oximycin™ P5, which is a liquid formulation of H_2O_2 and peroxyacetic acid (PAA), could be an alternative as PAA itself may exert some oxidation effects on *M. aeruginosa* (Zheng et al., 2024), can kill *M. aeruginosa* effectively at lower pH (Alnahas et al., 2024), and can also generate H_2O_2 under certain conditions (Zhao et al., 2007). Moreover, Oximycin™ P5 was the most stable of the three peroxide-based compounds tested, with a half-life of one week, while two other formulations (e.g. PAK® 27 and GreenClean® Liquid 5.0) had a half-life of half a day and one day (Hu et al., 2022). Lower extracellular toxin and fewer toxin variants were found in Oximycin™ P5 treatments compared to comparative Lake Guard® Oxy treatments. Oximycin™ P5 at 10 mg H_2O_2 L^{-1} would result in 1.85 mg PAA L^{-1}; this could also contribute to some direct toxin oxidation. Juárez et al. (2020) found 18 % degradation of microcystin following 1.5 mg PAA L^{-1} treatment for 72 hours. Yun et al. (2024) similarly found fewer extracellular toxins in Oximycin™ P5 treatments compared with an SCP formulation. PAA could decrease the pH of the water, slowing down the decomposition of H_2O_2 (Watts et al., 1999). However, pH in our experiment was similar between Oximycin™ P5 and H_2O_2 treatments (*see* Figs. 2A,I) and compared with the H_2O_2 treatments, H_2O_2 only prevailed longer in the highest dose of Oximycin™ P5 (*see* Figs. 2B,J). Overall, the effects of Oximycin™ P5 and H_2O_2 on *M. aeruginosa* were somewhat similar for all three blooming densities tested in this study though Oximycin™ P5 performed initially better at 1 mg H_2O_2 L^{-1} before recovery (Figs. 4,5B,J), and had less recovery of treated cells in treatment concentrations ≥ 3 mg H_2O_2 L^{-1} (Fig. 4B,J).

To promote prolonged release of low concentrations of H_2O_2, Wang et al. (2021) embedded CaO_2 in alginate beads. However, they encapsulated 0.057 g CaO_2 in 1 g alginate beads and applied doses of 40 to 400 mg CaO_2 L^{-1}, implying 0.7 to 7 g alginate beads L^{-1}, making large-scale lake applications unfeasible. To facilitate the gradual release of H_2O_2 over extended periods, sodium carbonate peroxyhydrate (SCP) or sodium percarbonate formulations have been developed. Sodium percarbonate yields hydrogen peroxide according to the reaction $2Na_2CO_3{\cdot}3H_2O_2 \rightarrow 4Na^+\ 2CO_3^{2-} + 3H_2O_2$, meaning that one gram of sodium percarbonate produces 0.325 g H_2O_2 maximally. In this study, two SCP products were tested: Phycomycin® SCP and Lake Guard® Oxy.

Phycomycin® SCP was highly effective in reducing *M. aeruginosa* and in keeping Chl-*a* concentrations virtually at zero when dosed at 3 mg H_2O_2 L^{-1} or higher, even at high blooming densities of 300 and 600 µg Chl-*a* L^{-1}. These findings are in line with a study that reported a Phycomycin® SCP EC_{50} to *M. aeruginosa* of 0.9–1.0 mg H_2O_2 L^{-1} (Geer et al., 2016). Another study that compared Phycomycin® SCP with three copper-based algaecides found Phycomycin® SCP was more effective in killing the cyanobacterium *Lyngbya magnifica* than the copper-based algaecides and when applied *in-situ,* Phycomycin® SCP was also able to minimize regrowth for several months (Bishop and Rodgers, 2011). However, in another study, Phycomycin® SCP was not effective in killing the cyanobacterium *Lyngbya wollei* (Calomeni et al., 2015) and did not affect *Microcystis* (mostly *M. wesenbergii)* up to the highest dose of 10 mg H_2O_2 L^{-1} tested (Lefler et al., 2022). Hence, the effectiveness of Phycomycin® SCP seems to depend on cyanobacteria species or strain and underpins that before applying it in the field, laboratory experiments are needed to identify an effective treatment (Bishop and Rodgers, 2011).

The other SCP used in our study, Lake Guard® Oxy, was also effective in controlling *M. aeruginosa* at the blooming densities tested. When adding the granules, we observed them floating, which aligns with the product information (BlueGreen Water Technologies 2021). This floating capacity makes Lake Guard® Oxy particularly useful for treating surface-accumulated cyanobacteria. Trainic et al. (2021) mentioned that Lake Guard® Oxy is "composed of 98 % (w/w) sodium percarbonate, and 2 % (w/w) of an inert, biodegradable encapsulating agent that floats and time-releases the active ingredient on the water surface." We used the product information of the sample provided, which is also available at the manufacturer's website (BlueGreen Water Technologies 2021), i.e., 83.3 % sodium percarbonate and 16.7 % other ingredients. We did not find peer-reviewed literature on Lake Guard® Oxy, but there are several mentions of its application in aquatic systems with limited details of the treatment amounts and exposure conditions, such as in the C-43 Canal/ Caloosahatchee River (South Florida, U.S.A.) (Trainic et al., 2021), Chippewa Lake (Ohio, U.S.A.), Nanhu Lake (Yueyang, China) (BlueGreen Water Technologies, 2021), Lake Minneola (South Florida, U.S.A.), where during 21 weeks monitoring no cyanobacteria blooms reoccurred (URL4). It was also applied to Roodeplaat reservoir (South Africa) in 2020, leading to an almost complete wipeout of the *Microcystis* bloom (Sukenik and Kaplan, 2021), followed by a relapse of blooming cyanobacteria (Lukhele and Msagati, 2024). The latter was also visible in our 1 mg L^{-1} H_2O_2 treatment with low starting biomass, where Chl-*a* concentrations started to rise again after 6 days, and PSII efficiencies had fully recovered by the end of the experiment, whilst PSII efficiency started to recover in the 3 mg L^{-1} H_2O_2 treatment after 9 days (Figs. 1E, F).

PSII efficiency, or photosynthetic yield, indicates the photosynthetic vitality and physiological state of phytoplankton and is used broadly as an indicator of oxidative damage to cells (Drábková et al., 2007b, 2007a; Lürling et al., 2014; Weenink et al., 2021). H_2O_2 has a high oxidizing capacity, producing reactive oxygen species (ROS) with free radicals, which can cause a chain reaction that destroys biological molecules (Thoo et al., 2020). H_2O_2 is converted into hydroxyl radicals (OH•) by UV irradiation or by the Fenton reaction in the presence of iron and

manganese (Korytowski et al., 1987; Zepp et al., 1992). Accumulation of these ROS can cause oxidative stress in cells (Mittler, 2002), which further leads to the disruption of cellular information transmission and ultimately cell death (Apel and Hirt, 2004). OH• produced by H_2O_2 can inhibit the electron transfer of PSII (Zhou et al., 2013). Consequently, in several treatments, PSII efficiency dropped to zero. With increasing concentrations of the five peroxide-based products tested, increased ROS is expected, ultimately leading to permanent inactivation of the cells (Ding et al., 2012). In the highest doses applied, no Chl-*a* or PSII efficiency could be measured until the end of the experiments, which spanned initial blooming densities of 150 to 600 μg Chl-*a* L^{-1}. This is consistent with findings from other studies (Chen et al., 2021; Ding et al., 2012; Zhou et al., 2018). At lower doses, a (partial) recovery of PSII efficiency was observed without a concomitant increase in chlorophyll-*a* concentrations (*see* Figs. 1B,H,J and Figs. 3B,D,F,H,J). This can be attributed to a portion of cells remaining intact (Fan et al., 2013), or active, even though the photosynthetic activity is completely inhibited. This is followed by a period of repair of damaged cells, rather than cell division of the surviving cells (Luo et al., 2024) *M. aeruginosa* can enhance both enzymatic and non-enzymatic mechanisms to improve its antioxidant and detoxification capacity (Wang and Xie, 2007). Schuurmans et al. (2018) found that after H_2O_2 addition, a toxic *Microcystis* strain changed the expression of 547 genes (11.7 % of the transcriptome) within 2 h. In particular, it upregulated the expression of multiple genes (e.g. t2prx and trxA) that can act as antioxidants and protect cells from reactive oxygen species, specifically catalyzing the reduction of H_2O_2 to non-active forms (Latifi et al., 2007). Cell division begins later, once damaged membranes and photosystem components have been repaired (Luo et al., 2024). Therefore, sublethal exposures to peroxide are not recommended as they could impact the ability to control the bloom and potentially enhance toxin production, as microcystin has been indicated in oxidative stress response (Zilliges et al., 2011). The inhibitory mechanism of H_2O_2 on *M. aeruginosa* includes both extracellular oxidation and intracellular attacks (Zhou et al., 2018), which results in damage to various cell components, such as cell membranes, proteins, and lipids, eventually leading to cell apoptosis or even lysis (Rezayian et al., 2019). Membrane deformation and partial disintegration of thylakoids induced by H_2O_2 in *M. aeruginosa* can cause pigment leakage, leading to an overestimation of Chl-*a* concentrations (Kang et al., 2022). The subsequent decline in Chl-*a* concentrations observed towards the end of the experiment at higher doses reflects the degradation of released pigments.

Cell membrane damage, as indicated by increased Chl-*a* concentrations (Mucci et al., 2017), will also lead to the release of MCs (Kang et al., 2022; Lürling et al., 2014; Sandrini et al., 2020). After one day of exposure to H_2O_2, extracellular MC concentrations were higher in all treatments compared to the controls (*see* Fig. 3). After nine days, extracellular MC concentrations were lower than in controls and declined with higher doses of peroxide compounds pointing to degradation of dissolved MCs after the lysis of cyanobacterial cells (Huo et al., 2015). The degradation of the extracellular MC may result from the attack of OH• on the Adda benzene ring, which destroys the Adda structure (Song et al., 2006)(Song et al., 2006). In addition, due to cell lysis and growth stagnation, intracellular MC concentrations were much lower in H_2O_2 treatments compared to the controls. These results are comparable to other studies that also reported reduced intracellular MC concentrations in *M. aeruginosa* cultures treated with H_2O_2 (Fan et al., 2014; Kang et al., 2022; Lürling et al., 2014). Besides the fact that OH• generated from H_2O_2 can react with nearly every molecule in the cell (Bayr, 2005), leading to the breakdown of MCs, H_2O_2 may also inhibit MC synthesis (Qian et al., 2010). Peroxide-based products seem to be effective in reducing cyanobacterial biomass at moderate (150 μg Chl-*a* L^{-1}) to high (600 μg Chl-*a* L^{-1}) blooming densities, and at higher doses, with sustained peroxide present, they also contribute to the breakdown of released MCs.

In general, all five peroxide-based products could elicit control of *M. aeruginosa* in these studies. The products were comparable mainly in the tests with lower initial Chl-*a* (150 and 300 μg Chl-*a* L^{-1}), with slightly less effectiveness of CaO_2 and slightly increased effectiveness of Oxymycin™ P5 resulting in rapid control and minimal recovery at the lowest concentration (1 mg H_2O_2 L^{-1}) compared to other products. However, in the experiment with higher initial Chl-*a* concentrations (600 μg Chl-*a* L^{-1}), measurements of *M. aeruginosa* recovery were observed at a dose of 3 mg H_2O_2 L^{-1} for Lake Guard® Oxy and CaO_2, while no recovery of PS II efficiency occurred with the liquid H_2O_2, Phycomycin®SCP, and Oxymycin™ P5 treatments. This suggests that the latter products may perform better at lower doses, including when high-density blooms are present. Nonetheless, at the highest dose tested (10 mg H_2O_2 L^{-1}), all products were highly effective, with no signs of recovery. Hence, it is advisable to perform short-term laboratory experiments with the natural target community to determine the most effective dose in field applications.

We also expected that at higher *M. aeruginosa* densities, the slow-release SCP products would outperform H_2O_2, but we did not observe this in our experiments. Nonetheless, H_2O_2 remained present in the highest doses of the SCP products, and to a lesser extent in the highest concentration of the liquid formulation Oxymycin™ P5. Since the rapid degradation of H_2O_2 can limit its effectiveness, slow-release H_2O_2 compounds may prolong exposure duration. However, the desired concentrations of H_2O_2 necessary to elicit control may not be achieved due to the slow release. For example, even though the initial dose was calculated to be 20 mg H_2O_2 L^{-1}, the solid materials may not be released all at once, but rather gradually over time (see Fig 2D, F and H). The release rate of H_2O_2 from SPC products and its half-life in water are important factors to consider when determining the optimum dose for the materials. Some studies have attempted to correlate the required amount of H_2O_2 with the Chl-*a* concentration present, which can provide useful insights into the general range of H_2O_2 needed (Buley et al., 2023; Lusty and Gobler, 2023). However, factors such as pH, light, temperature, presence of organic matter and metals, different species, strains and colony size play a critical role in the release and decay rate of H_2O_2, ultimately influencing the optimum dosing (Buley et al., 2023; Häkkinen et al., 2004; Schuurmans et al., 2018; Wang et al., 2016). For instance, Liu et al. (2017) observed that a four times higher dose was required to damage *M. aeruginosa* colonies bigger than 25 μm compared to smaller ones. Large colonies contain high contents of extracellular polymeric substances, which help protect cyanobacterial cells from the algaecide effect of H_2O_2 and other algaecides (Wang et al., 2013; Wu et al., 2007). Therefore, laboratory experiments with laboratory-cultured cyanobacteria, often growing as single cells, should not be the basis for determining the optimum dose for field application, nor should the same dose be assumed effective across different species. Once more, the best approach is to test a range of doses with the actual community before applying treatments on a larger scale.

One factor not considered here is the price of each product, which may vary based on the required dose, geographical location, and time, as prices fluctuate yearly. Since cost is a key factor in product selection, we recommend contacting each provider for accurate pricing. All the products tested were effective in killing *M. aeruginosa*; there is no clear 'winner' in terms of overall efficiency. However, each product may be better suited for different purposes depending on the specific situation, strategy and price. Having a diverse toolbox of treatments allows for flexibility in addressing various cyanobacterial challenges. For instance, Phycomycin® SCP, a sink solid material, could be applied in cases where cyanobacteria are dispersed throughout the water column or for treating benthic mats. In support, SCP (as PAK® 27) was recommended as the most appropriate to eliminate dense blooms of harmful cyanobacteria (Sinha et al., 2018). Additionally, another SCP (GreenClean® Pro, PeroxiSolid) was reported as the most suitable algaecide for the treatment of overwintering cells in sediments from the Gathering Pond (Calomeni et al., 2023). The floating Lake Guard® Oxy, on the other hand, seems useful if most cyanobacteria have accumulated at the water's surface.

Although, as many cyanobacteria (even scum formers) may be present throughout the water column depending on environmental conditions (Reynolds and Walsby, 1975), or form accumulations at specific depths or in benthic zones (Erratt et al., 2022; Fallon and Brock, 1981; Saker and Griffiths, 2001), the ability to surface spray or inject a liquid product like Oximycin® P5 could assist in targeting the specific bloom location.

To ensure the right tools are selected for each scenario, conducting a system analysis is essential. Additionally, upscaled experiments *in-situ* enclosures are highly recommended, including evaluation of effects on nontarget organisms. This approach will provide the scientific evidence needed for water authorities to make informed decisions when selecting emergency agents to combat cyanobacteria blooms.

Conclusions

- The five peroxide-based products - Oximycin™ P5, Phycomycin® SCP, Lake Guard® Oxy, liquid H_2O_2 and solid CaO_2 - effectively reduced the growth and toxicity of the cyanobacterium *Microcystis aeruginosa* at different doses.
- At an initial bloom concentration of 150 µg Chl-*a* L^{-1}, Oximycin™ P5, Phycomycin® SCP, and traditional H_2O_2 were effective at a dose of 1 mg H_2O_2 L^{-1}, while CaO_2 and Lake Guard® Oxy required 3 mg H_2O_2 L^{-1} to keep Chl-*a* undetectable.
- At 300 µg Chl-*a* L^{-1}, all products reduced Chl-*a* to zero at 3 mg H_2O_2 L^{-1} until the end of the experiment.
- At 600 µg Chl-*a* L^{-1} all products except Lake Guard® Oxy and CaO_2 reduced Chl-*a* to zero at 3 mg H_2O_2 L^{-1}, the increase in Chl-*a* L^{-1} for these two suggests Oximycin™ P5, Phycomycin® SCP and liquid H_2O_2 may be more effective at lower doses; however, at 10 mg H_2O_2 L^{-1} all products were highly effective with no signs of recovery.
- Initially, extracellular MC concentrations were higher in all treatments but dropped below control levels after nine days, indicating dissolved MCs degradation.
- No single product stands out as the best overall. Each may be better suited for different applications depending on the situation.

CRediT authorship contribution statement

Miquel Lürling: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Formal analysis. **Ziwei Liao:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Li Kang:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization. **West M. Bishop:** Writing – review & editing, Methodology, Conceptualization. **Maíra Mucci:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The co-author, Dr. West Bishop, works as an Algae Scientist and Water Quality Research Manager in the Research and Development department at SePRO. He did not receive additional compensation for this research, as conducting research is part of his role at SePRO. Dr. Maíra Mucci works part-time as a Scientific Consultant for Limnological Solutions International (LSI) and part-time as a Researcher at Wageningen University (WUR). She did not receive extra compensation for this research, as research and development are integral to her roles at both LSI and WUR. Ziwei Lao did not receive any financial compensation for the research. Dr. Li Kang received a scholarship to support her research time, funded through LSI's unrestricted research grant. All institutions provided full autonomy in selecting the research topic and did not interfere with the experimental design, results, or conclusions. The authors are responsible for the scientific findings, conclusions, and opinions expressed in this work. Any mention of trademarks, products, or vendors is solely for the purpose of providing scientific information and does not imply endorsement, recommendation, or warranty. The research was conducted in accordance with the Netherlands Code of Conduct for Research Integrity.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2025.102930.

Data availability

Data will be made available on request.

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