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The combined effects of UV-C radiation and H_2O_2 on *Microcystis aeruginosa*, a bloom-forming cyanobacterium

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HIGHLIGHTS

- Responses of photosynthetic activities to UVC and H_2O_2 were investigated.
- Changing of ultrastructure was screened under UVC and H_2O_2 treatments.
- Aggregation of the D1 protein was caused by H_2O_2.
- H_2O_2 had a stronger damage on PSI than UVC.
- Synergetic effects of UVC and H_2O_2 on membrane systems and photosystems were studied.

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ABSTRACT

In order to get insight into the impacts of UVC/H_2O_2 on *Microcystis aeruginosa*, physiological and morphological changes as well as toxicity were detected under different UVC/H_2O_2 treatments. In the presence of sole UVC or H_2O_2, the net oxygen evolution rate decreased significantly ($p < 0.05$) since activity of photosystem II (PSII) was inhibited. Meanwhile, increase of intracellular reactive oxygen species (ROS), degradation of microcystin (MC) and ultrastructure destructions were observed. Under sole UVC treatment, no changes happened in the activity of photosystem I (PSI), but the degradation of D1 protein was observed. Under sole H_2O_2 treatment, an increase of malondialdehyde, aggregation of D1 protein and deformation of the thylakoid membrane were observed. ROS content under H_2O_2 treatment was about 5 times than that under UVC treatment. Combined use of UVC and H_2O_2, as well as 20 mJ cm^{-2} UVC and 60 μM H_2O_2, showed high synergetic effects. Obvious damage to membrane systems, the marked degradation of MC and inhibition of the photosystems were observed. It could be deduced that UVC worked on intracellular membrane components directly and the damaged oxygen-evolving complex, which was followed by the D1 protein degradation. H_2O_2 oxidised the membrane lipids via an ROS-mediated process, with thylakoid injury and the aggregation of D1 protein being the lethal mechanisms, and both PSII and PSI being the attacking targets. With regard towards the effective inactivation of *M. aeruginosa* and high removal of MC, UVC/H_2O_2 proposed a novel practical method in controlling cyanobacterial blooms.

1. Introduction

Cyanobacterial bloom was first reported 130 years ago (Francis, 1878). To date, it has occurred frequently in both fresh and marine water (Paerl and Otten, 2013; Domingues et al., 2014), with a large accumulation of cyanobacterial cells floating on the surface, covering beaches with biomass or foam, and depleting oxygen levels through excessive respiration or decomposition (Sellner et al., 2003). Alternatively, about 50–75% of cyanobacterial blooms were

Abbreviations: ROS, reactive oxygen species; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; MDA, malondialdehyde; TCA, trichloroacetic acid; TBA, thiobarbituric acid; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; PBS, phosphate buffer saline; MC, microcystin; MC-LR, microcystin leucine arginine; HPLC, high-performance chromatography; PAR, photosynthetically active radiation; SP, saturation pulse; PSII, photosystem II; PSI, photosystem I; Fv/Fm, the maximal photochemical quantum yield; ETRII, the electron transport rate; Y(II), the photochemical quantum yield; ETRI, the electron transport rate of PSI; Y(I), the photochemical quantum yield of PSI; OEC, oxygen-evolving complex.

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detected to be toxic, which led to serious problems in the environment and the health of mammals (including humans) (Paerl and Otten, 2013).

In order to prevent cyanobacterial bloom, many methods have been developed. Among them, physical methods and chemical methods were considered as the highly efficient methods. Altering the hydro-physical conditions could favour other algal species and inhibit the growth of predominant species. Although it was recognised as a useful physical method for cyanobacterial bloom control, much more energy was required (Barrington and Ghadouani, 2008). Chemicals, such as aluminium and copper, have been used as alternatives for bloom control (Matthijs et al., 2012). Aluminium salt was used as a preventive treatment of water bloom through the chemical inactivation of phosphorus (Hullebusch et al., 2002), but its effects was short-term (Huser and Köhler, 2012). Copper sulphate has been practiced effectively in bloom control, which resulted in the accumulation of Cu in ecosystems and led to the second pollution (Hullebusch et al., 2003). Besides these treatments, UVC as a physical method and hydrogen peroxide as a chemical method have been paid much more attention.

Ultraviolet irradiation at 254 nm (UVC) was an important method to prevent cyanobacterial blooms (Alam et al., 2001; Sakai et al., 2007b, 2009). Although natural UVC is totally absorbed by the atmosphere (Holzinger and Lütz, 2006), artificial UVC, such as boats equipped with UV-lamps, was considered as a useful method to control algal growth in the eutrophic lakes. UVC irradiation treatment did not involve the addition of any harmful chemical to the water and could produce long-lasting residual effects (Alam et al., 2001). Ou et al. (2011) found that UVC inactivated and decomposed *Microcystis aeruginosa* via direct photo-degradation and indirect oxidation by reactive oxygen species (ROS). Meanwhile, UVC provided high removal of Microcystin Leucine Arginine (MC-LR) and dissolved organic matter in suspension. It was reported that UVC at 20–200 mJ cm^{-2} could suppress the growth of *M. aeruginosa* (Tao et al., 2010) and do harm to the expression of *psbA* and *cpc* gene; UVC (>50 mJ cm^{-2}) could induce the remarkable decreases in the photochemical efficiency (Tao et al., 2013). Although it was shown that the mechanisms of UVC irradiation for elimination of *M. aeruginosa* included the destruction of photosystems (Sakai et al., 2007a), the exact mechanism of photodamage by UVC was still a controversial issue as there are two possible targets for UV irradiation: the D1 protein (Wu et al., 2011) and manganese cluster (Hakala et al., 2005). To date, no data could be obtained to illustrate the exact lethal mechanism of UVC on cyanobacteria.

Hydrogen peroxide is a well-known agent with a strong oxidising capability, which was employed in cyanobacteria control (Barrington and Ghadouani, 2008; Qian et al., 2010). Some studies indicated that cyanobacteria were more sensitive to H_2O_2 than other phototrophs. For example, 1.75 mg/L H_2O_2 had a deleterious effect on *Planktothrix rubescens* (Barroin and Feuillade, 1986; Drábková et al., 2007). Experiment in Lake Koetshuis (in Netherlands) demonstrated low concentrations of H_2O_2 (60 μM or 2 mg/L) worked selectively against cyanobacteria without affecting other biota (Matthijs et al., 2012). Admittedly, the successful application of H_2O_2 in cyanobacterial bloom control depended on the oxidative damage to photosystems (Matthijs et al., 2012). The main oxidising damage to photosystem II (PSII) was damage to the D1 protein, which was a key component of the PSII complex. Some documents indicated that H_2O_2 induced cleavage of the D1 protein directly *in vitro*. The generation of the intensive 20 kDa product was the result of 0.2 M H_2O_2 treatment on the D1 protein isolated from *Synechocystis* PCC 6803, while 10 mM H_2O_2 also resulted in the generation of specific fragments (Lupínková and Komenda, 2004). However, Nishiyama et al. (2001) found that H_2O_2 (0.5 mM) stimulated the damage of PSII in *Synechocystis* PCC 6803 *in vivo* by inhibiting repair of the D1 protein and not by destroying PSII directly. The inhibiting pathway of D1 protein repair was the following: H_2O_2 inhibits the translation of *psbA* gene, which encodes the D1 protein precursor. Lack of the D1 protein precursor was the inhibition mechanism of the repair of D1 protein. All studies mentioned above focused on the damage mechanism of short-term treatment with high-concentration H_2O_2 on cyanobacteria. Apparently, taking the precious advantage of H_2O_2, which selectively harmed cyanobacteria with very low concentration, will be of valuable. Furthermore, as another photosystem involving in the dark reactions, photosystem I (PSI) were also very important to cyanobacteria, and the effects of H_2O_2 on PSI should be screened. Due to the practicability of the low concentration of H_2O_2, it was worthy of the further study *in situ*.

As mentioned above, both UVC and H_2O_2 damaged the photosystems of cyanobacteria. Our hypothesis was that when UVC was used in conjunction with H_2O_2, the efficiency of bloom control might be higher than the sole utilisation. If so, the combined use of UVC/H_2O_2 would have a synergistic effect on cyanobacterium, which would be a good choice for cyanobacterial bloom control. In order to verify our hypothesis and determine the lethal mechanisms of UVC/H_2O_2 on cyanobacteria, *M. aeruginosa*, a bloom-forming species, was selected as the delegate and used to estimate different treatments of UVC irradiation and H_2O_2. The reduction of photosynthetic activity, ROS and MDA contents, injury of the D1 protein, deformation of the ultrastructure and toxicity effects were demonstrated in this study. The synergistic effects of UVC irradiation and H_2O_2 on cyanobacterium were observed. Meanwhile, it suggested that the combined use of UVC irradiation and low-concentrations of H_2O_2 was an optimal choice to control the cyanobacterial bloom.

2. Materials and methods

2.1. Cultivation of algae

Axenic cultures of *M. aeruginosa* (FACHB 905) were obtained from the Culture Collection of the Freshwater Algae of the Institute Hydrobiology (FACHB-Collection; Wuhan, China). Cells were incubated in BG-11 (Stanier et al., 1971) at 25 °C. Fluorescent lamps (Philips) were used as the light source with an automated light/dark cycle of 12 h/12 h. Light intensity was 50 ± 5 μmol photons m^{-2} s^{-1}.

2.2. Treatments

Cells in the exponential growth phase were diluted with fresh medium to achieve an initial concentration of 10^6 cells mL^{-1}. Every 40 mL sample was treated with different concentrations of H_2O_2 (0, 60, 150, 240, and 300 μM).

With UVC irradiation treatments, each 40 mL sample was irradiated in petri dishes (diameter = 9 cm, depth = 1.5 cm) vertical to the low-pressure mercury lamp (253.7 nm, 20 W, Jiangsu Junguang Photoelectric Technology Co., Ltd. China) for 20, 40, 80 and 120 s, respectively. Magnetic stirrer kept the suspension mixed (Tao et al., 2010). The intensity of UVC at the surface of the suspension was 1 mW cm^{-2}, which was measured by a spectroradiometer (Spectronics, XF-1000, XS-254, USA), UVC dose were 20, 40, 80 and 120 mJ cm^{-2}, respectively.

Referring to the growth of *M. aeruginosa* under different treatments, the dosages of UVC irradiation and H_2O_2 that inhibited the growth rather than killed the cells were chosen to assess the synergic effect. Here, 60 μM and 150 μM H_2O_2 combined with 0–120 mJ cm^{-2} UVC irradiation were used to suppress *M. aeruginosa*. All treatments were conducted in sequence; samples were exposed