Combined Application of Natural Sunlight and Hydrogen peroxide on the Removal of Harmful Cyanobacteria

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Abstract. This study provides an efficient and environmentally friendly advanced oxidation technique involving the combined application of natural sunlight and hydrogen peroxide for the removal of harmful cyanobacteria from lakes and reservoirs. In this paper, we collected water samples from Taihu Lake (Wuxi, China) in August 2016 when cyanobacterial blooms had occurred and then performed an outdoor experiment. Hydrogen peroxide at 0.6 mM had no obvious effect on the cyanobacterial inactivation in the dark, even stimulating cyanobacterial growth to some extent. Cyanobacteria were inactivated by higher concentrations of hydrogen peroxide (1.0 mM) in the dark, as well as 0.4 mM hydrogen peroxide under sunlight irradiation, indicating that natural sunlight significantly enhanced the effect of hydrogen peroxide on the removal of cyanobacteria. An experiment involving *Pseudanabaena* sp. (a harmful species) led to similar conclusions as the study using algae attained from Taihu Lake. This study provides a practical and effective method for controlling harmful cyanobacteria in natural water bodies.

1. Introduction

Cyanobacterial blooms are among the most threatening consequences of freshwater pollution [1] and have caused great disasters in some of the world's largest water bodies, including Taihu Lake, China [2]; Okeechobee, USA, and Kasumigaura, Japan [3]. Many bloom-forming cyanobacterial species can release toxins responsible for acute lethal, acute, chronic and subchronic toxicity in wild/domestic animals and humans [4]. Some metabolites of cyanobacterial species, especially 2-methyl isoborneol (2-MIB) and geosmin (GSM) [5], alter the taste and odor of drinking water and increase water treatment costs. Thus, the removal of harmful cyanobacteria from lakes and reservoirs is essential for environmental and human health.

Restricting nutrient inputs to a water body is believed to be an efficient approach to cyanobacterial bloom management [1, 6] but requires a long-term project and considerable investment, which local economies usually cannot afford. Kong F X and Gao G [7] posited that nutrients are not a limiting factor for the growth of certain cyanobacterial species since cyanobacterial blooms have occurred in many water bodies with low nitrogen and phosphorus concentrations. Following that concept, many short-term measures have been applied to directly reduce the biomass of blue-green algae in a water body, including copper sulfate addition [8], herbicide application[9], and potassium permanganate application [10]. The treatment of cyanobacteria with algaecides produces the desired effect instantly, but almost all algaecides cause secondary pollution, threatening water security and the ecological balance. Studies have also reported controlling cyanobacterial blooms using light shading [11-13], but an inexpensive

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and efficient light-screening material has not been reported to date. Most importantly, laboratory studies on cyanobacteria maintained in the dark have revealed a range of different results, from survival to death [14-16], indicating that the mechanism of cyanobacterial control by light shading is not fully understood.

Hydrogen peroxide, a non-polluting and strong oxidant, was reported to be a potential algaecide against the cyanobacterium *Oscillatoria rubescens* in 1986 [17]. Subsequently, Samuilov V D [18] found that hydrogen peroxide inhibits the oxygen-evolving complex and photosynthetic electron transfer and leads to the destruction of the photosynthetic apparatus. In addition, Ding Y's study [19] showed that hydrogen peroxide induces programmed cell death (PCD) in *Microcystis aeruginosa* in a dose-dependent manner. Recent studies have also found that cyanobacteria are more sensitive to hydrogen peroxide than other phytoplankton species [20, 21]. Thus, hydrogen peroxide is a potent and environmentally friendly algaecide.

The irradiance intensity is one of the most significant factors impacting the toxicity of hydrogen peroxide [20, 22], and high-energy optical radiation such as UV (ultraviolet) radiation can multiply the toxicity of hydrogen peroxide [23]. Natural sunlight comprises a broad spectrum of wavelengths, including the UV range, and the irradiance intensity lies between 10000-80000 lx in direct sunlight in September (Shanghai, China). Studies tested the effect of diverse irradiance intensities on the toxicity of hydrogen peroxide [24, 25], but the irradiance was provided by fluorescent lamps, which do not emit UV radiation. A similar study carried out by Drabkova M [20] used high-pressure mercury lamps as a light source, which includes the UV range but fundamentally differs from natural daylight in terms of the wavelengths and irradiance intensity. Only one study [21] has tested the effect of hydrogen peroxide addition on the suppression of cyanobacterial blooms in a natural water body, but little information about the sunlight conditions was provided. Therefore, the impact of natural sunlight on hydrogen peroxide toxicity has not been properly studied, prompting the present study.

Taihu Lake, the third largest freshwater lake in China, is located in Jiangsu Province. The entire lake is at a medium level of eutrophication, according to the latest water resources bulletin of the Ministry of Water Resources, People's Republic of China. Several large-scale cyanobacterial blooms have occurred in Taihu Lake; of note is the bloom that occurred in 2007, which interrupted the drinking water supply to the city of Wuxi for at least a week. In this paper, we collected water samples in Taihu Lake (Wuxi, China) in August 2016 when cyanobacterial blooms had occurred and tested the effects of diverse hydrogen peroxide concentrations on the removal of cyanobacteria under direct sunlight and darkness. We then studied the regular inactivation pattern of *Pseudanabaena* sp. (a harmful species of cyanobacteria) experiencing combined exposure to hydrogen peroxide and natural sunlight. The factors impacting the toxicity of hydrogen peroxide are discussed, and the optimal hydrogen peroxide concentration was determined.

2. Materials and methods

2.1. Water sample cultures

We collected water samples from the northern area of Taihu Lake, Wuxi, in August 2016, when cyanobacterial blooms had occurred. The samples were cultured in BG-11 medium at 25°C under 1500 lx in an illumination incubator with a 12:12 hour light:dark cycle.

2.2. Paeudanabaena sp. cultures

Pseudanabaena sp. (FACHB-1277) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and grown in BG-11 medium at 25°C under 1500 lx in an illumination incubator with a 12:12 hour light:dark cycle.

2.3. Water sample treatment

For preadaptation to the outdoor environment, the water samples were inoculated into 1 L beakers that were placed outside at a location without direct sunlight and a temperature of 26-32°C. After one day, the initial photosynthetic activity (F_V/F_M) and chlorophyll concentrations were measured with a pulse-

amplitude–modulated fluorescence monitoring system (PAM, Walz, Effeltrich, Germany) [19] and recorded as $(F_V/F_M)_0$ and $(\text{Chlorophyll})_0$. The samples were distributed into ten 100 mL beakers if the cyanobacteria grew well. Hydrogen peroxide was added to seven of the beakers for final concentrations of 0.0 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, and 1.2 mM, and the beakers were placed outside under direct sunlight (irradiance intensity of 30000-80000 lx) at 27-31°C. Hydrogen peroxide at final concentrations of 0.0 mM, 0.6 mM, and 1.0 mM was added to three of the beakers, which were then placed in the dark at the same temperature as the other seven beakers outside. All steps were completed by 13:00 that day.

The photosynthetic activity (F_V/F_M) and chlorophyll concentrations were measured every hour before 18:00 and recorded as $(F_V/F_M)_{i,j}$ and $(\text{Chlorophyll})_{i,j}$ (i=1, 2, 3…10; j=1, 2, 3, 4, and 5). Then, the ten beakers were moved to a room and stored in the dark at 25°C. The morning of the second day, the beakers were placed back outside, and the photosynthetic activity (F_V/F_M) and chlorophyll concentrations were measured at 19:00 (30 hours after the addition of hydrogen peroxide) and recorded as $(F_V/F_M)_{i,j}$ and $(\text{Chlorophyll})_{i,j}$ (i=1, 2, 3…10; j=30).

The data were normalized by setting the initial values, $(F_V/F_M)_0$ and $(\text{Chlorophyll})_0$, to 1, and the remaining data were calculated using formulas (1) and (2), respectively.

$$F_V/F_{M\,i,j} = \frac{\left(F_V/F_M\right)_{i,j}}{\left(F_V/F_M\right)_0} \tag{1}$$

$$Chlorophyll_{i,j} = \frac{\left(Chlorophyll\right)_{i,j}}{\left(Chlorophyll\right)_0} \tag{2}$$

The values of $F_V/F_{M\,i,j}$ and $\text{Chlorophyll}_{i,j}$ (i=1, 2, 3…10; j=1, 2, 3, 4, 5…30) represent the photosynthetic activity and chlorophyll concentrations of cyanobacteria at different time points.

2.4. Treatments of Pseudanabaena sp.

A culture of *Pseudanabaena* sp. in the exponential growth phase was diluted with fresh BG-11 medium to achieve an initial concentration within the range of concentrations accurately detectable by the pulse-amplitude–modulated fluorescence monitoring system (PAM, Walz, Effeltrich, Germany) [19]. The solution of *Pseudanabaena* sp. was distributed into five 250 mL beakers, and every 250 mL sample was treated with a different hydrogen peroxide concentration (0, 10, 30, 60, and 100 μM). The five samples were placed in direct sunlight on the morning of a sunny day, with a temperature range of 28-29.5°C and a light intensity range of 60000-70000 lx during the experiment. The photosynthetic activity and chlorophyll concentrations were measured several times within a period of 2 hours.

2.5. Precision

All tests were conducted at least in duplicate if not otherwise noted. The relative standard deviations (RSD) for different tests were <15%. RSD of the detection equipment was <5%.

3. Results

3.1. Toxicity of hydrogen peroxide under sunlight and darkness

In direct sunlight, there was a short-term decrease in the photosynthetic activity, followed by recovery (Figure 1), and the chlorophyll concentration increased slowly and was remarkably higher than that detected after 30 hours (Figure 2), indicating that the cyanobacteria proliferated normally after adaption to direct sunlight. In the dark, there was no significant change in photosynthetic activity (Figure 1), and the chlorophyll concentration declined from 8 μg/L to 5.05 μg/L at 5 hours, which was possibly due to the change in the environment, but did not significantly change after 5 hours, finally stabilizing at

approximately 5.03 μg/L. In addition, the addition of 0.6 mM hydrogen peroxide in the dark caused a slight decrease in the photosynthetic activity, with subsequent recovery (Figure 1), and the chlorophyll concentration decreased slightly but was higher than that in the treatment without added hydrogen peroxide under darkness (Figure 2). The addition of 1.0 mM hydrogen peroxide in the dark slowly inactivated the cyanobacteria (Figure 1), but the chlorophyll concentration was slightly higher than that in the treatment without added hydrogen peroxide under darkness (Figure 2). The addition of 0.6 mM hydrogen peroxide under direct sunlight decreased the photosynthetic activity to zero in one hour (Figure 1) and decreased the chlorophyll concentration to 2 percent of the initial concentration (Figure 2), and these results were quite different from those obtained at the same hydrogen peroxide concentration under darkness. All of these results showed that sunlight significantly enhanced the effect of hydrogen peroxide on the removal of cyanobacteria.

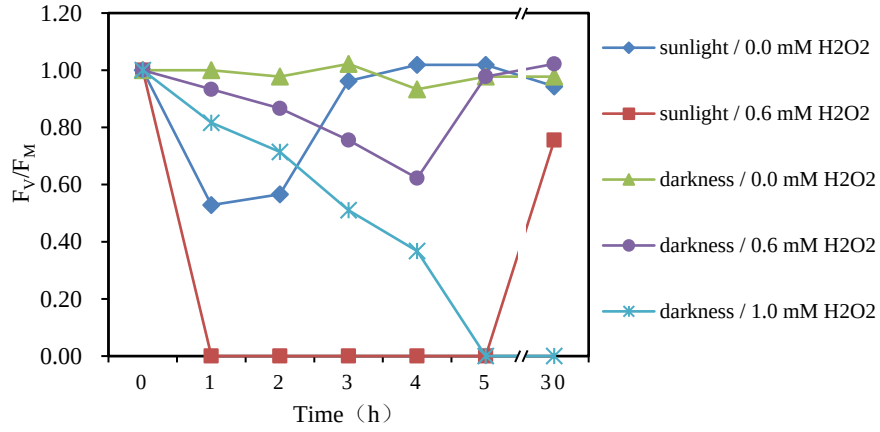


Figure 1. Variation in the photosynthetic activity after the addition of different hydrogen peroxide concentrations (0.0, 0.6, and 1.0 mM) under sunlight and darkness.

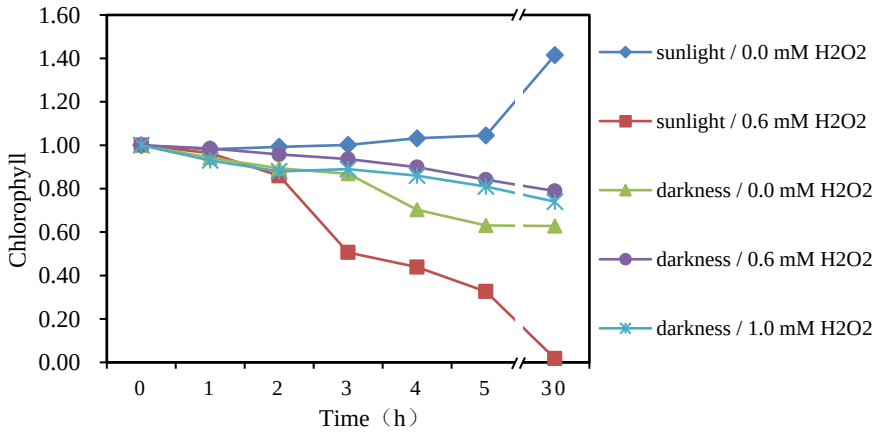


Figure 2. Variation in the chlorophyll concentration under different hydrogen peroxide concentrations (0.0, 0.6, and 1.0 mM) under sunlight and darkness.

3.2. Water sample treatments with different hydrogen peroxide concentrations

In direct sunlight, the effect of hydrogen peroxide on the removal of cyanobacteria was proportional to the dosage. Seventy percent of the decrease in photosynthetic activity was observed with the addition of 0.2 mM hydrogen peroxide, but the activity ultimately recovered (Figure 3); the chlorophyll concentration decreased slowly, and the final concentration was 46 percent of the initial value (Figure 4), indicating that 0.2 mM hydrogen peroxide significantly inhibited the growth of cyanobacteria. Concentrations of 0.4 mM, 0.6 mM and 0.8 mM hydrogen peroxide caused the inactivation of cyanobacteria in 1-3 hours, but recovery was observed after 30 hours (Figure 3); and the chlorophyll concentration decreased to 3 percent, 2 percent and 1 percent of the initial value, respectively (Figure 4). Addition of 1.0 mM hydrogen peroxide caused the inactivation of cyanobacteria, and no recovery was observed. Thus, the optimal hydrogen peroxide concentration in direct sunlight was 1.0 mM in the present study.

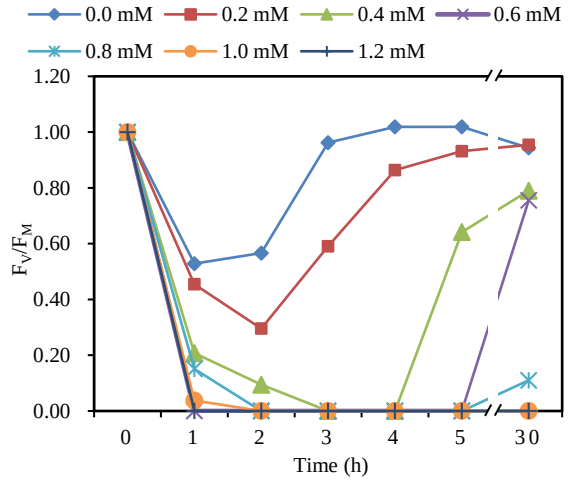


Figure 3. Variation in the photosynthetic activity under different hydrogen peroxide concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mM).

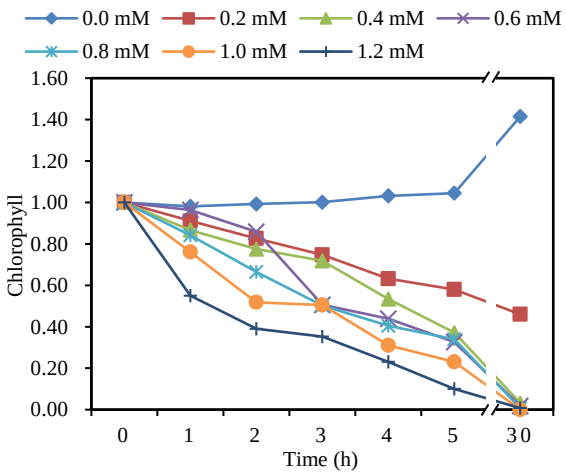


Figure 4. Variation in the chlorophyll concentration under different hydrogen peroxide concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mM).

3.3. Pseudanabaena sp. treatments

The treatments with the water samples from Taihu Lake showed that combined exposure to natural sunlight and hydrogen peroxide was effective for algal inactivation. Further study was performed with a specific cyanobacteria, *Pseudanabaena* sp., an odorous, filamentous species, to verify the conclusions from the first experiment. *Pseudanabaena* sp. was inactivated in 20 minutes, even in the absence of hydrogen peroxide (Figure 5), indicating that *Pseudanabaena* sp. cannot survive under such strong irradiance. Nevertheless, the process of inactivation was more rapid with the addition of hydrogen peroxide, and higher concentrations led to more rapid rates. The chlorophyll concentrations decreased significantly in all samples, but the addition of hydrogen peroxide accelerated the process and resulted a higher rate of decrease (Figure 6). These results demonstrate that the combination of natural sunlight and hydrogen peroxide is an efficient method to remove these harmful cyanobacteria, and low hydrogen peroxide concentrations (10 μM) can significantly alter the inactivation of *Pseudanabaena* sp. under direct sunlight.

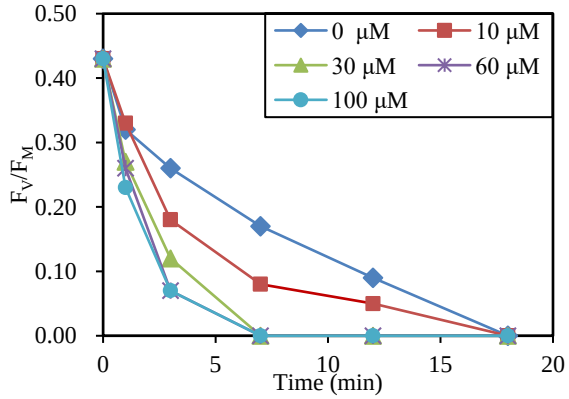


Figure 5. Variation in the photosynthetic activity under different hydrogen peroxide concentrations (0, 10, 30, 60, and 100 μM).

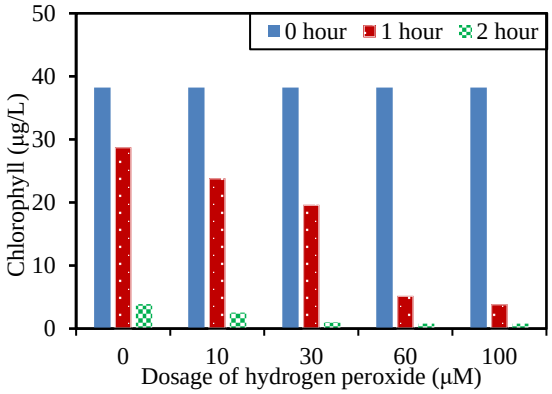


Figure 6. Variation in the chlorophyll concentration under different hydrogen peroxide concentrations (0, 10, 30, 60, and 100 μM).

4. Discussion

Studies of cyanobacteria responses to dark conditions have yielded different results, from survival to death. In the study by Popels L C et al. [14], *Aureococcus anophagefferens* (a harmful bloom-forming species) survived under conditions of low temperature and darkness, and no change was detected in the intracellular chlorophyll concentrations. The same result was reported by Wu Z X et al. [26], while Furusato E et al. [16] found that the abundance of *Microcystis aeruginosa* decreased markedly with the length of the darkness period after experiencing 20 days of darkness. In the present study, the chlorophyll concentration initially decreased slightly, possibly due to the change in environment, but no significant change was observed after 30 hours. Meanwhile, the photosynthetic activity remained high from beginning to end of the experiment. All of these results indicate that the cyanobacteria were possibly able to survive in the dark, in agreement with the findings of Popels L C et al., Wu Z X et al. and Bouchard J N et al. [14, 15, 26]. Both Popels L C et al. and Wu Z X et al. found that the surviving algae remaining after prolonged periods of darkness resumed growth quickly when returned to light. This phenomenon may explain why cyanobacteria sink to the bottom of freshwater lakes in the winter and then reappear when environmental conditions are favorable [7].

Hydrogen peroxide is photochemically generated from the organic constituents present in water [27]. When natural surface and groundwater are exposed to sunlight, the hydrogen peroxide concentration rapidly increases [27]. Thus, hydrogen peroxide commonly occurs in the freshwater environment, and concentrations of up to 800 nM have been detected in freshwater lakes [28]. Exposing cyanobacterial cells to sunlight can cause internal oxidative stress [20]. During photosynthesis, superoxide radicals are produced by the photoreduction of oxygen and further converted to hydrogen peroxide and oxygen in a reaction catalyzed by superoxide dismutase (Mehler reaction). Next, the hydrogen peroxide is detoxified to water via the ascorbate peroxidase pathway [20]. In direct sunlight, the process is interrupted because of high irradiance intensity, and excessive intracellular hydrogen peroxide and superoxide radicals cannot be scavenged in a timely manner, causing physiological changes in cyanobacterial cells. In the present study, inactivation was observed in the treatment of pure *Pseudanabaena* sp. under direct sunlight; meanwhile, the treatment of the water samples showed a sharp decrease in photosynthetic activity under direct sunlight that subsequently recovered. The recovery of photosynthetic activity in the water samples was possibly due to the adaption to sunlight and the rebalancing of the production and scavenging of hydrogen peroxide and superoxide radicals. The effect of low concentrations of extracellular hydrogen peroxide on cyanobacterial inhibition was not significant in some studies [19, 29] possibly because of the intracellular hydrogen peroxide scavenging system and extracellular polymeric substances (EPS), which play an important role in buffering against the adverse effect of hydrogen peroxide [30]. In the present study, even 0.6 mM hydrogen peroxide increased the photosynthetic activity and chlorophyll concentrations slightly under darkness. The same result was reported by Wang Z C et al., who found that addition of 10 mg/L hydrogen peroxide slightly increased the photosynthetic activity of cyanobacteria [29]. This phenomenon is possibly related to the role of hydrogen peroxide as a signaling messenger [31-33], activating related enzymes. The treatment of cyanobacteria with higher concentrations (1.0 mM) of hydrogen peroxide under darkness decreased the photosynthetic activity to zero, but contrary to our expectations, the chlorophyll concentration was slightly higher than that in the treatment without hydrogen peroxide under darkness, indicating that hydrogen peroxide does not degrade chlorophyll in the dark. Wang Z C et al. [29] demonstrated that hydrogen peroxide degraded phycobilisomes (light-harvesting pigments) but had no significant effect on chlorophyll degradation.

Hydroxyl radical (·OH), the strongest oxidant, can irreversibly damage cyanobacterial cells. The study carried out by Yu H J et al. [34] showed that the hydroxyl radicals generated in advanced oxidation processes (AOPs) can destroy the skeletal structure (porphin) of chlorophyll, further arresting the metabolism and protein synthesis of chlorophyll and ultimately inactivating cyanobacterial cells. Drabkova M et al. [20] used high-pressure mercury lamps as a light source to determine the effect of the irradiance intensity on hydrogen peroxide toxicity to *Microcystis aeruginosa* and found that the toxicity of hydrogen peroxide was proportional to the irradiance—higher irradiances caused higher toxicity. However, Drabkova M et al. [20] added Fe^{2+} ions to a microalgal suspension, and the photo-Fenton

reaction [35] of hydrogen peroxide with the Fe^{2+} ions enhanced the generation of hydroxyl radicals. Additionally, UV radiation enhanced the decomposition of hydrogen peroxide into hydroxyl radical. The irradiance from high-pressure mercury lamps includes UV rays, so hydroxyl radicals may have contributed to both the photo-Fenton reaction and the effect of UV radiation in Drabkova M et al. [20]. To test the effect of a single source of natural irradiance on the toxicity of hydrogen peroxide, we exposed water samples to hydrogen peroxide under direct sunlight and found that 0.6 mM hydrogen peroxide decreased the photosynthetic activity to zero in one hour and decreased the chlorophyll concentration to 2 percent of its initial concentration. The toxicity of 0.6 mM hydrogen peroxide was different under sunlight and darkness, indicating that sunlight greatly enhanced the effect of hydrogen peroxide on the removal of cyanobacteria. Meanwhile, the treatments of *Pseudanabaena* sp. in the present study showed that this species was more sensitive to sunlight and hydrogen peroxide. The rapid inactivation was possibly due to the filamentous characteristics of *Pseudanabaena* sp. because filamentous algae are more sensitive to hydroxyl radicals, hydrogen peroxide and other oxidants generated under direct sunlight. Nevertheless, the overall behavioral pattern of *Pseudanabaena* sp. was similar to that of the algae obtained from Taihu Lake, verifying that the combined application of natural sunlight and hydrogen peroxide is an efficient method for removing harmful cyanobacteria from natural water bodies.

Hydrogen peroxide decomposes rapidly in water, especially under direct sunlight [1]; therefore, the effect on cyanobacteria is brief, and hydrogen peroxide can only be applied as an emergency algaecide. Thus, determining the optimal concentrations needed to inactivate cyanobacteria in a short period of time is significant. In the present study, the treatments of the water samples from Taihu Lake revealed that 1.0 mM hydrogen peroxide under direct sunlight decreased the photosynthetic activity to zero in one hour and decreased the chlorophyll content to extremely low levels, indicating that this concentration irreversibly damages cyanobacterial cells under direct sunlight. However, high concentrations could also severely affect other aquatic organisms, while low concentrations cannot inhibit cyanobacteria in natural water bodies. Therefore, further studies are required to optimize the application of hydrogen peroxide under direct sunlight in natural water bodies.

5. Conclusion

The cyanobacteria collected from Taihu Lake survived under both high-intensity sunlight and darkness. The effect of hydrogen peroxide in the dark on the removal of cyanobacteria depended on the dosage. A low dosage did not inhibit the cyanobacteria and even stimulated growth to some extent, which was possibly related to the activation of intracellular enzymes by hydrogen peroxide. Compared with the application of hydrogen peroxide in the dark, the combined application of natural sunlight and hydrogen peroxide yielded different results. In direct sunlight, hydroxyl radicals (·OH) are generated from hydrogen peroxide and irreversibly damage cyanobacterial cells. The toxicity of hydrogen peroxide under direct sunlight is proportional to the dosage. The addition of 1.0 mM hydrogen peroxide under direct sunlight caused the inactivation of cyanobacterial cells collected from Taihu Lake, and no recovery was observed. Thus, the combined application of natural sunlight and hydrogen peroxide can significantly reduce the abundance of harmful cyanobacteria, but further studies are required for the application of this method in natural water bodies.

Acknowledgments

This research was financially supported by the 2016's National Undergraduate Innovation Program (0400107163/001), the National Natural Science Foundation of China (51678420). We thank Nellie S. for her help editing the language of this paper.

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