

# *Study on Treatment of Cyanobacterial Bloom by UV-C/KMnO<sub>4</sub> Advanced Oxidation*

Long Sun, Wang Zhang, Binbin Li, Xin Tan, Zengguo Zhang, Wei Hong\*

Shandong Resources and Environment Construction Group Co., Jinan, Shandong, China

\*corresponding author

**Keywords:** UV-C/KMnO<sub>4</sub>, Advanced Oxidation, Cyanobacterial Bloom

**Abstract:** Based on the existing algae removal study, this paper uses the UV-C/KMnO<sub>4</sub> combined technology to efficiently remove cyanobacteria and algal toxins in water. The results show that the combination of UV-C/KMnO<sub>4</sub> can achieve efficient removal with a lower dose, the doses of 120mJ cm<sup>-2</sup>/0.7~1.0μmol L<sup>-1</sup> and 180mJ cm<sup>-2</sup>/0.4~1.0μmol L<sup>-1</sup> had better effect.

## 1. Introduction

The frequency and degree of harm of cyanobacterial blooms have increased year by year with the changes of climate and other factors<sup>[1]</sup>. Microcystins, the toxic metabolites produced by cyanobacteria in blooms, will threaten the safety of drinking water and some freshwater recreational projects in where they are located, and the accompanying problems such as hypoxia and odor may take years to eliminate<sup>[2,3]</sup>. So far, the control of cyanobacterial bloom mainly includes reducing nitrogen and phosphorus input, adding algaecide/coagulant, mechanical removal, microwave/ultraviolet irradiation and introducing allelopathic plants, etc<sup>[4]</sup>. Considering the current management of cyanobacterial bloom has been carried out for many years, the incidence of cyanobacterial bloom is still at a high value, suggesting the existence of unknown regulatory factors. Studies have shown that antibiotic contamination in water bodies will have a stimulative effect on prokaryotes (such as cyanobacteria)—hormesis<sup>[5]</sup>. And considering the status of antibiotics in modern medicine, the natural water pollution caused by their wide application and the induction of drug resistance/resistance to some microorganisms, more effective algae removal methods should be considered<sup>[6,7]</sup>.

Existing studies have shown that short-wave ultraviolet (UV-C) irradiation can damage the DNA of cyanobacteria, thereby affecting the growth of cyanobacteria and the synthesis of microcystins (MCs)<sup>[8]</sup>. The low concentration of KMnO<sub>4</sub> has a high removal rate for algae, while the residual manganese content in the water is far less than the national standard (0.1mg/L)<sup>[9]</sup>. Therefore, in this study, UV-C/KMnO<sub>4</sub> combined technology was used to treat *Microcystis aeruginosa* in the environment of antibiotics, and the feasibility of the combined technology to remove microcystins and its toxins was studied.

## 2. Materials and Method

### 2.1. Materials

*Microcystis aeruginosa* NIES-843 was purchased from the Freshwater Algae Seed Bank of the Chinese Academy of Sciences.  $\text{KMnO}_4$  was purchased from Shanghai Lianshi Chemical Reagent Co., Ltd. Test containers and related measuring tools were all sterilized at  $121^\circ\text{C}$  for 20 minutes. The antibiotics used in the experiment (amoxicillin, sulfamethoxazole, spiramycin, ciprofloxacin and tetracycline (99%)) were produced by Shanghai Sigma-Aldrich Company.

### 2.2. Method

*Microcystis aeruginosa* was pre-cultured in BG11 medium for two weeks under sterile conditions before the start of the experiment. The light source was a cool white fluorescent lamp, the light-dark cycle was 2:1 under the light intensity of 20 photons  $\text{m}^{-2} \text{s}^{-1}$ , and the 24-hour cycle was cultured, and the temperature was controlled at  $25 \pm 1^\circ\text{C}$ .

Before the UV-C/ $\text{KMnO}_4$  treatment experiment started, the algal cells entering the exponential growth phase were collected using a centrifuge with parameters of 4000g,  $25^\circ\text{C}$ , 10min and inoculated algal in BG11 medium containing  $100\text{ng L}^{-1}$  mixed antibiotics (amoxicillin : sulfamethoxazole : spiramycin : ciprofloxacin : tetracycline =1:1:1:1:1). The initial algal density was controlled to be  $2 \times 10^6$  cells  $\text{mL}^{-1}$ , and the continuous culture was carried out for 7 days under the same culture conditions as the pre-culture.

In the UV-C/ $\text{KMnO}_4$  treatment experiment of *M. aeruginosa*, they were exposed to different doses of UV-C and  $\text{KMnO}_4$ .  $\text{KMnO}_4$  was injected directly into the medium at the beginning of the experiment, and UV-C was treated with parallel irradiation. UV-C is set to  $120 \text{mJ cm}^{-2}$  or  $180\text{mJ cm}^{-2}$  in two groups respectively.  $\text{KMnO}_4$  in both treatment groups was set at  $0.4 \mu\text{mol L}^{-1}$ ,  $0.7 \mu\text{mol L}^{-1}$  and  $1.0 \mu\text{mol L}^{-1}$ . Each group of UV-C/ $\text{KMnO}_4$  doses were set with a control group with or without antibiotics. At the same time, a blank group without any treatment and only dosed with the same dose of antibiotics as the treatment group was also set. The treatment conditions were set as shown in Table 1. Three parallel experiments were set for each group.

Table 1: UV-C/ $\text{KMnO}_4$  dose and antibiotic concentration in each experimental group.

Group settings	Dose of UV-C/ $\text{KMnO}_4$ treatment	Antibiotic concentration
Blank control	0	0
Antibiotics treated control	0	$100 \text{ng L}^{-1}$
UV-C/ $\text{KMnO}_4$ treatment group without antibiotics	$0.4 \sim 1.0 \mu\text{mol L}^{-1} / 120\text{mJ cm}^{-2}$ $0.4 \sim 1.0 \mu\text{mol L}^{-1} / 180\text{mJ cm}^{-2}$	0
UV-C/ $\text{KMnO}_4$ treatment group with antibiotics	$0.4 \sim 1.0 \mu\text{mol L}^{-1} / 120\text{mJ cm}^{-2}$ $0.4 \sim 1.0 \mu\text{mol L}^{-1} / 180\text{mJ cm}^{-2}$	$100 \text{ng L}^{-1}$

### 2.3. Cell Response Analysis

#### 2.3.1. Cell Growth Analysis

Take 1mL daily at regular intervals, and the algal cells were counted under a binocular microscope at a magnification of 400. Chlorophyll (Chl-a) content of algal cells was measured on the 4th and 7th day of UV-C/ $\text{KMnO}_4$  treatment. Use a centrifuge (5000g, 10min) to collect *Microcystis aeruginosa* cells, and extract with 3mL of (90%) ethanol solution for 30min with the aid of ultrasound, the extract was centrifuged again to remove impurities, and then the Chl-a content was determined by ultraviolet

spectrophotometry.

### 2.3.2. Microcystins Concentration Analysis

The microcystins MC-RR, MC-YR and MC-LR with high detection rate in natural water were selected as targets, and the Whatman GF/B filter was used to separate the cells from the culture medium. Oasis HLB extraction cartridge (200 mg, 6 mL) was used for extraction to determine the content of extracellular MCs. Add (50%) methanol aqueous solution to the filtered algal cells, ultrasonically extract for 2 hours, and wash with Oasis HLB extraction cartridge (200 mg 6 mL) to determine the content of intracellular MCs.

## 3. Results and Analysis

### 3.1. Inhibitory effect of cell growth

During the test period, the growth of algal cells in each group on the 1st to 2nd day showed a slow state. The untreated control group without antibiotics and the antibiotic control group entered the logarithmic growth phase after the lag period, and were in the logarithmic growth state during the remaining test period, as shown in Figures 1 and 2. In the UV-C/ $\text{KMnO}_4$  treatment group, under the condition of UV-C treatment dose of  $120\text{mJ cm}^{-2}$ , the growth of algal cells was significantly inhibited, the lag period was prolonged, and the growth rate showed a downward trend with the increase of  $\text{KMnO}_4$  concentration. The results also shown that the cell density of the treated group with antibiotics increased compared with that of the group without antibiotics, which were  $5.75 \times 10^6$  cells  $\text{mL}^{-1}$ ,  $3.11 \times 10^6$  cells  $\text{mL}^{-1}$ , and  $2.26 \times 10^6$  cells  $\text{mL}^{-1}$  and  $5.04 \times 10^6$  cells  $\text{mL}^{-1}$ ,  $2.94 \times 10^6$  cells  $\text{mL}^{-1}$ ,  $2.26 \times 10^6$  cells  $\text{mL}^{-1}$  on the seventh day, respectively. Under the condition of UV-C treatment dose of  $180\text{mJ cm}^{-2}$ , the growth of algal cells was more strongly inhibited, which was showing that a similar treatment effect was achieved with a lower concentration of  $\text{KMnO}_4$  and a higher dose of  $\text{KMnO}_4$  at  $120\text{mJ cm}^{-2}$ .

Compared with the above experimental data, the results were also showing that antibiotics can promote the growth of *Microcystis aeruginosa*, and their existence will affect the treatment effect of UV-C/ $\text{KMnO}_4$ . But this effect will weaken with the increase of UV-C/ $\text{KMnO}_4$  dosage, which was especially obvious when the UV-C irradiation dose is  $180\text{mJ cm}^{-2}$ .

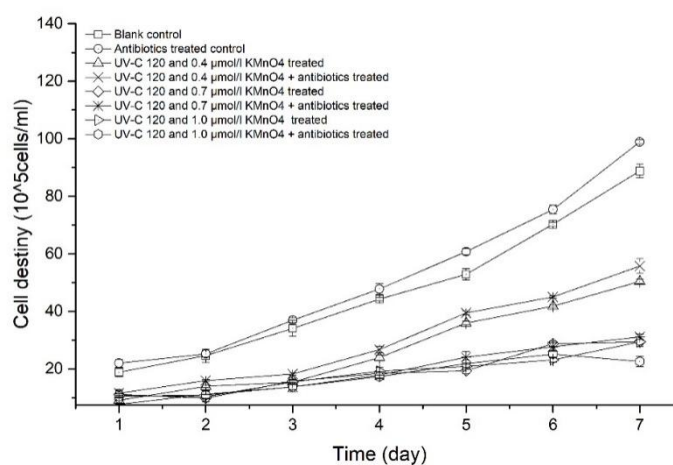


Figure 1: Growth curve of *Microcystis aeruginosa* under UV-C/ $\text{KMnO}_4$  treatment dose of  $120\text{mJ cm}^{-2}/0.4\sim 1.0\mu\text{mol L}^{-1}$ .

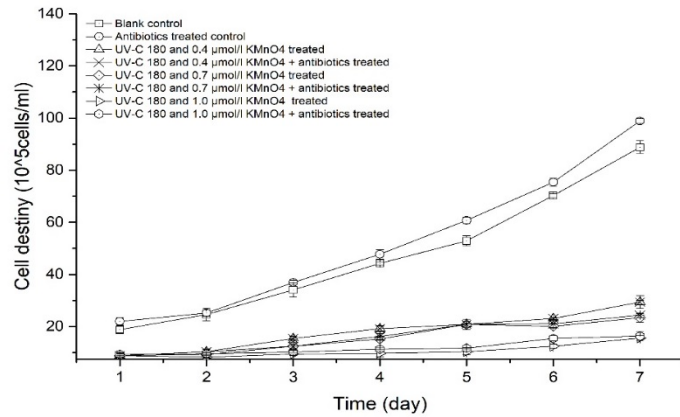


Figure 2: Growth curve of *Microcystis aeruginosa* under UV-C/ $\text{KMnO}_4$  treatment dose of  $180\text{mJ cm}^{-2}/0.4\sim 1.0\mu\text{mol L}^{-1}$ .

### 3.2. Cellular Response

Figures 3 and 4 show the changes of chlorophyll a concentration in different treatment groups under different doses of UV-C/ $\text{KMnO}_4$  treatment. The research showed that the concentration of chlorophyll a presents the same situation as the change of algal cell density, and was inversely proportional to the treatment dosage. In the case of a fixed dose of UV-C or  $\text{KMnO}_4$ , the concentration of chlorophyll a decreased with the increase of another factor, and the concentration of chlorophyll a also increased in the treatment groups with antibiotics. Existing studies have shown that UV-C can damage the photosynthetic system by inhibiting the expression of photosynthesis-related genes<sup>[10]</sup>. Combined with the change of chlorophyll concentration, it is suggested that UV-C/ $\text{KMnO}_4$  treatment can inhibit the growth of *Microcystis aeruginosa* by killing the photosynthetic system.

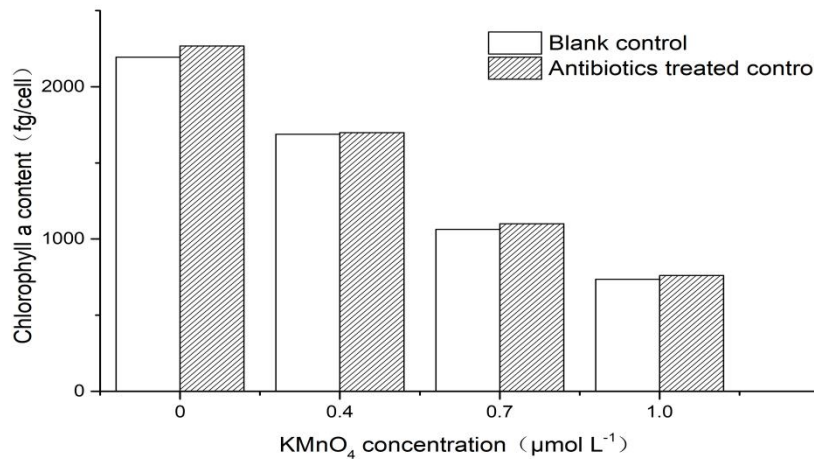


Figure 3: The concentration of chlorophyll a under UV-C/ $\text{KMnO}_4$  treatment dose of  $120\text{mJ cm}^{-2}/0.4\sim 1.0\mu\text{mol L}^{-1}$ .

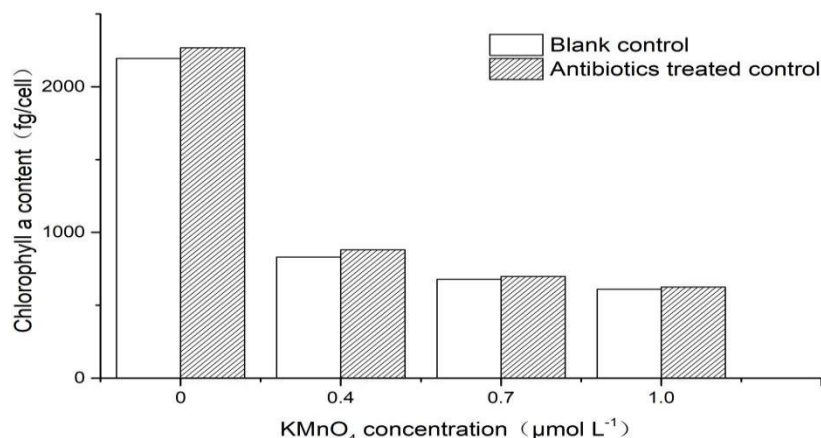


Figure 4: The concentration of chlorophyll a under UV-C/KMnO<sub>4</sub> treatment dose of 180mJ cm<sup>-2</sup>/0.4~1.0µmol L<sup>-1</sup>.

### 3.3. Microcystins Removal

As shown in Figures 5 and 6, the concentration of microcystins will gradually increase with the growth of *Microcystis aeruginosa* under normal circumstances, and reach a peak on the 7th day of the test cycle. At the same time, the presence of antibiotics also stimulated the synthesis of microcystins. In the blank control group, the concentrations of algal toxins in the medium without antibiotics and those with antibiotics on the 7th day were 180.16 µg L<sup>-1</sup> and 199.87 µg L<sup>-1</sup> respectively. In the UV-C/KMnO<sub>4</sub> treatment group, when the treatment dosage was lower, the concentration of microcystins still increased with the increase of test time, but when the dosage of UV-C reaches 180mJ cm<sup>-2</sup> or the concentration of KMnO<sub>4</sub> reaches 0.7µmol L<sup>-1</sup>, the concentration of cyanotoxins will decrease with time. The study by Park et al. found that high doses of UV-C alone had a high removal rate of microcystins<sup>[11]</sup>, and the combined use of potassium permanganate reduces the applicable dose of UV-C, and achieves an obvious treatment effect.

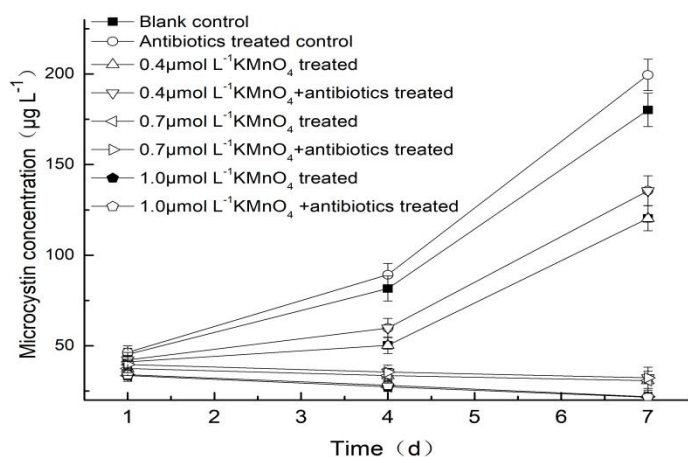


Figure 5: Microcystin concentration under UV-C/KMnO<sub>4</sub> treatment dose of 120mJ cm<sup>-2</sup>/0.4~1.0µmol L<sup>-1</sup>.

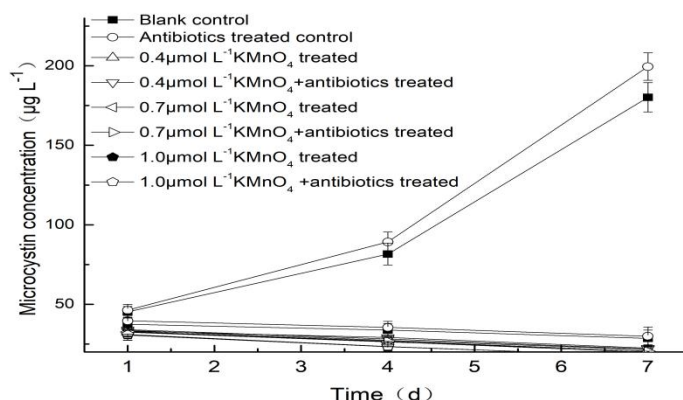


Figure 6: Microcystin concentration under UV-C/KMnO<sub>4</sub> treatment dose of 180mJ cm<sup>-2</sup>/0.4~1.0µmol L<sup>-1</sup>.

#### 4. Conclusions

UV-C/KMnO<sub>4</sub> treatment can inhibit the growth of *Microcystis aeruginosa* at a lower dosage, the doses of 120mJ cm<sup>-2</sup>/0.7~1.0µmol L<sup>-1</sup> and 180mJ cm<sup>-2</sup>/0.4~1.0µmol L<sup>-1</sup> had better effect. The presence of antibiotics has an inhibitory effect on the treatment effect of UV-C/KMnO<sub>4</sub>, and the inhibitory effect is negatively correlated with the treatment dose. The treatment of UV-C/KMnO<sub>4</sub> has a good effect on the removal of microcystins in water, which is beneficial to reduce the harmful events of microcystins caused by enrichment in the food chain. The inhibitory effect of UV-C/KMnO<sub>4</sub> on the growth of *Microcystis aeruginosa* is achieved through the inhibition of photosynthesis-related genes.

#### References

- [1] MAO F, LI W, SIM Z Y, et al. (2022) Phycocyanin-rich *Synechococcus* dominates the blooms in a tropical estuary lake. *Environ Manage*, 311(114889).
- [2] PENN K, WANG J, FERNANDO S C, et al. (2014) Secondary metabolite gene expression and interplay of bacterial functions in a tropical freshwater cyanobacterial bloom. *The ISME Journal*, 8(9): 1866-78.
- [3] BLOM J F, BISTER B, BISCHOFF D, et al. (2003) Oscillapeptin J. (2003) a New Grazer Toxin of the Freshwater Cyanobacterium *Planktothrix rubescens*. *Journal of Natural Products*, 66(3): 431-4.
- [4] SUN L. (2020) Study on treatment of *Microcystis aeruginosa* and low-concentration antibiotics by UV-C/H<sub>2</sub>O<sub>2</sub>. Ji Nan, Shandong University.
- [5] DANNER M-C, ROBERTSON A, BEHREND S V, et al. (2019) Antibiotic pollution in surface fresh waters: Occurrence and effects. *Science of the Total Environment*, 664(793-804).
- [6] STOKES J M, LOPATKIN A J, LOBRITZ M A, et al. (2019) Bacterial Metabolism and Antibiotic Efficacy. *Cell Metabolism*, 30(2): 251-9.
- [7] ZHOU L-J, LI J, ZHANG Y, et al. (2019) Trends in the occurrence and risk assessment of antibiotics in shallow lakes in the lower-middle reaches of the Yangtze River basin, China. *Ecotoxicology and Environmental Safety*, 183(109511).
- [8] LIU Y, GAO B, YUE Q, et al. (2012) Influences of two antibiotic contaminants on the production, release and toxicity of microcystins. *Ecotoxicology and Environmental Safety*, 77(79-87).
- [9] ZHOU L-J, LI J, ZHANG Y, et al. (2016) Experimental Study on Algae Removal by Potassium Permanganate and Chlorine Dioxide. *City and Town Water Supply*, 03): 38-4.
- [10] LI J-G, MA L, ZHOU Z-Y, et al. (2013) Mechanisms of photosynthetic inactivation on growth suppression of *Microcystis aeruginosa* under UV-C stress. *Chemosphere*, 93(4): 637-44.
- [11] PARK J-A, YANG B, JANG M, et al. (2019) Oxidation and molecular properties of microcystin-LR, microcystin-RR and anatoxin-a using UV-light-emitting diodes at 255nm in combination with H<sub>2</sub>O<sub>2</sub>. *Chemical Engineering Journal*, 366(423-32).