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Inhibition mechanism of *Microcystis aeruginosa* under UV-C irradiation

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ABSTRACT

The inhibition of *Microcystis aeruginosa* in water with UV-C irradiation was investigated. Results revealed that 84% of *M. aeruginosa* were removed on the 5th day after UV-C irradiation at a dose of 2,117 mJ/cm^2. The SEM images of irradiated *M. aeruginosa* cells showed evidence of injury. However, the presence of CH_3OH, thiourea, and NO_3^- decreased the UV-C irradiation efficiency. Acidic conditions were more conducive to *M. aeruginosa* removal than neutral or alkaline conditions. H_2O_2 in the mM level was added into the *M. aeruginosa* solution to investigate the removal efficiency, and the results indicated that 94% of *M. aeruginosa* were removed. The phycocyanin photosynthetic pigment content in *M. aeruginosa* cells decreased with the increase of UV-C irradiation dose. Low UV-C irradiation doses enhanced the activity of superoxide dismutase (SOD) in the *M. aeruginosa* cells, whereas the SOD activity decreased with high doses.

Keywords: *Microcystis aeruginosa*; UV-C irradiation; Additive; Superoxide dismutase; Phycocyanin

1. Introduction

Cyanobacteria are widely distributed in the aquatic environment, especially in drinking water sources [1–3], and may cause drastic changes in turbidity, pH, dissolved oxygen, tastes, and odors [4]. The treatment processes of drinking water supply may be negatively affected by these variations in water quality. Consequently, drinking water production is facing a great challenge posed by cyanobacteria bloom [5,6].

The presence of algae in sources of drinking water can affect subsequent water treatment significantly. Algae can be divided into cyanophyta, chlorophyta, rhodophyta, chrysophyta, and so on. *Chlorella* sp., *Microcystis aeruginosa*, etc. are the main algae in eutrophic lake. Previous studies have analyzed the removal and application of *Chlorella* sp. [7–9]. *M. aeruginosa*, as a harmful cyanobacteria, can produce harmful hepatotoxic and neurotoxic compounds [10,11]. Microcystins (MCs), a series of hepatotoxin compounds produced by *M. aeruginosa*, are aqueous contaminants because of their acute poisoning and chronic cancer promotion potentials to human beings [12]. In addition, the death of *M. aeruginosa* generates algal residues and excretions that increase the level of natural organic matter, which is a precursor to by-products formed during disinfection [13]. Therefore, the inactivation of cyanobacteria is a significant strategy to control cyanobacterial bloom and to reduce cyanobacteria-induced emerging contaminants.

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In the past few years, new technologies have been introduced to address the removal of cyanobacterial cells or MCs in drinking water sources. Physical methods, such as coagulation, are potential technologies for the control of algal growth [14]. However, realizing the appealing effects with low cost of conventional coagulation is difficult because of their low specific density, motility, morphological characteristics, and negative surface charge [15]. Chemical methods with chlorine, ozone, chlorine dioxide, or chemical algaecides may have adverse effects on other organisms [16,17]. Ultraviolet (UV) irradiation, especially the shortwave ultraviolet (UV-C at 254 nm), has high cyanobacteria removal efficiency [18,19]. UV-C irradiation can cause critical damage on DNA, which blocks protein transcription and synthesis in bacteria [20]. DNA is the predominant target of UV irradiation in cyanobacteria [21]. Moreover, some researchers reported that H_2O_2 may be generated during the UV-C irradiation process [22] and that H_2O_2 may play an important role in cyanobacteria damage. Although UV-C irradiation can cause damage to DNA resulting in cell death, a suitable UV-C dose could be chosen to inactivate cyanobacteria. The UV-C dose ranging from 300 to 2,400 mJ/cm^2 that is typically used for disinfection can also be used to destroy cyanobacteria [23].

This paper aims to explore the feasibility and mechanism of *M. aeruginosa* removal under UV-C irradiation. The effects of different conditions, such as irradiation doses, pH values, and various additives, were evaluated, and the effect of UV-C irradiation on chlorophyll-a (Chl-a), phycocyanin (PC), superoxide dismutase (SOD), and other enzymes in *M. aeruginosa* cells was analyzed. This study provides a theoretical basis for the large-scale application of UV-C irradiation in algae removal and a new idea for controlling cyanobacteria bloom.

2. Materials and methods

2.1. Materials and reagents

M. aeruginosa (FACHB-905) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). *M. aeruginosa* was cultivated in batches in BG11 [24] medium that had been adjusted to pH 8.0 by adding 0.1 M of $NaHCO_3$ or 0.1 M of HCl into 1 L conical flasks. Samples were incubated at 25 ± 2°C under incandescent light at 2,000 l× with a 12 h diurnal cycle [25]. The growth of *M. aeruginosa* followed an S-shaped curve. Exponential growth was attained after 5 d. Exponential growth continued for 10 d, after which it stabilized. All experiments were conducted using cells in the exponential growth stage. The reagents and solvents were of analytical grade, and the solutions were prepared with ultrapure water.

2.2. UV-C irradiation experiments

All the UV-C irradiation (at 254 nm) experiments were performed in a customized reactor containing a thermostatic magnetic stirrer and four removable low-pressure mercury lamps (TUV Philips, Holland). All the UV-C treatments were performed at the starting point with different irradiation times. The illuminating intensity in the reactor was 0.588 mW/cm^2, and the UV-C exposure dose was calculated as Eq. (1):

$$\text{Dose} = 0.588 \times t \quad (1)$$

where t is the irradiation time (s) and the exposure dosage unit is mJ/cm^2.

2.3. Effect of pH, H_2O_2, and additives study

The effect of the solution pH values (2.37, 3.94, 6.04, 7.49, and 9.76) on *M. aeruginosa* removal under the UV-C irradiation dose of 706 mJ/cm^2 was studied at 25 ± 2°C. After the treatment, the treated samples were kept in normal growth conditions and the chlorophyll-a concentrations were measured on the 1st day. The effect of H_2O_2 concentrations (0, 0.20, 0.40, 0.60, and 0.80 mM) in the solution was examined at 25 ± 2°C, and no UV-C irradiation was provided in the experiment. The samples were kept in normal growth conditions, and the chlorophyll-a concentrations were measured at the 60th h. The effect of the additives was studied, and the free radical scavengers (CH_3OH, thiourea, and NO_3^-) were added into the *M. aeruginosa* suspensions, which were irradiated by doses of 706 and 1,411 mJ/cm^2 (pH 8.0 at room temperature of 25 ± 2°C). The chlorophyll-a concentration was measured on the 1st day.

2.4. Measurement of M. aeruginosa

In this study, chlorophyll-a concentration was used to measure the change in the concentration of *M. aeruginosa* [26]. Initially, 10 mL of *M. aeruginosa* suspension was pipetted and centrifuged at 8,000 g for 10 min, and 10 mL of 95% (V/V) ethanol solution was added into the pellet. Subsequently, the samples were

incubated for 24 h in a darkened room at 4℃. After incubation, the samples were centrifuged at 8,000 g for 10 min, and the absorbance of the supernatant was measured at 665 and 649 nm. The chlorophyll-a concentration was calculated as Eq. (2) [27]:

$$C = A_{665} \times 13.7 - A_{649} \times 5.76 \quad (2)$$

where A_{665} and A_{649} are the absorbances measured at 665 and 649 nm, respectively, and C is the chlorophyll-a concentration (mg/L).

2.5. Measurement of phycocyanin

PC was determined according to the method of Xing et al. [28]. First, the cell suspensions were lyzed by sonication using a probe tip (specify setting of the sonicator) for 10 min. The solutions were centrifuged for 10 min at 8,000 g. PC was extracted in 5 mL of phosphate buffer saline (PBS 0.05 mol/L, pH 7.6), and then, the samples were frozen for 24 h. The absorbances of transparent blue supernatant at 615 and 652 nm were recorded. The PC content was calculated using Eq. (3):

$$\text{Phycocyanin} = (A_{615} - 0.474A_{652})/5.43 \quad (3)$$

2.6. Enzyme activity analysis

The SOD activity was assayed based on the inhibitory effect of SOD on the spontaneous auto-oxidation of pyrogallol (the method in GB/T5009.171-2003, China). One activity unit refers to the amount of SOD required to inhibit the initial rate of pyrogallol auto-oxidation by 50%. The volume of the reaction mixture was 4.5 mL, composed of 1.5 mL of phosphate buffer saline, 2.4 mL of Tris–HCl solution (0.1 mol/L), 0.1 mL of pyrogallol solution (4 mmol/L), and 0.5 mL of enzyme extracts. The absorbance of the mixture at 325 nm was measured, and the absorbances were recorded once per minute. The activity of SOD was calculated as Eq. (4):

$$\text{SOD (U/mL)} = \frac{\frac{\Delta A_{325} - \Delta A'_{325}}{\Delta A_{325}} \times 100\%}{50\%} \times 4.5 \times \frac{1}{V} \times D \quad (4)$$

where ΔA_{325} is the auto-oxidation rate of pyrogallol, $\Delta A'_{325}$ is the auto-oxidation inhibitory rate of pyrogallol by the sample, V is the sample volume, and D is the dilution factor of the sample.

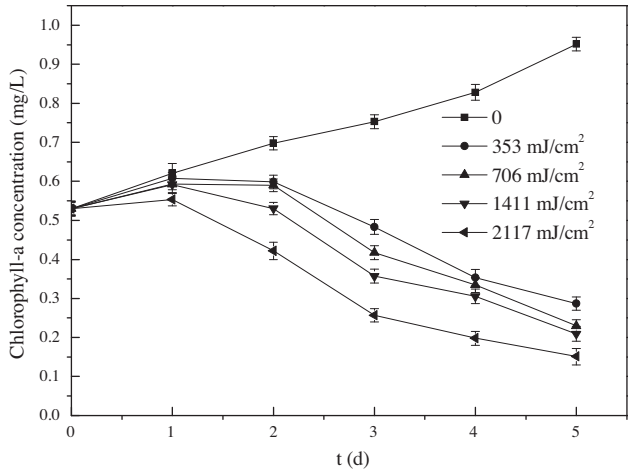

Fig. 1. Change in chlorophyll-a concentration with the irradiation dose and cultivation time.

3. Results and discussion

3.1. Effects of irradiation dose

Fig. 1 shows the change in chlorophyll-a concentration (pH 8.0, room temperature 25 ± 2℃) with the irradiation dose and time. The dose range 353 to 2,117 mJ/cm^2 was selected to investigate the dependence of *M. aeruginosa* removal on irradiation dose. The chlorophyll-a concentrations increased from 0.530 to 0.952 mg/L within 5 d in the control sample. However, under the irradiation doses of 353 and 706 mJ/cm^2, the concentrations of chlorophyll-a slightly increased within the first 2 d and then decreased to 0.287 and 0.230 mg/L in the subsequent 3 d, respectively. For the samples treated under the irradiation doses of 1,411 and 2,117 mJ/cm^2, the concentrations of chlorophyll-a increased within the 1st day and then dropped down rapidly to 0.209 and 0.151 mg/L, respectively. The removal efficiency of chlorophyll-a could reach 84% when a dose of 2,117 mJ/cm^2 is administered.

On the basis of the results observed (Fig. 1), the growth of *M. aeruginosa* in water could be inhibited by UV-C irradiation. Although chlorophyll-a was not significantly reduced immediately within 5 d at these four UV-C doses, it gradually disappeared in the subsequent days [29]. This finding might indicate a decline of the biomass of *M. aeruginosa* cells.

3.2. Effects of solution pH

Fig. 2 shows the effect of solution pH on *M. aeruginosa* removal at an irradiation dose of 706 mJ/cm^2. The results indicated that pH is an important factor in the

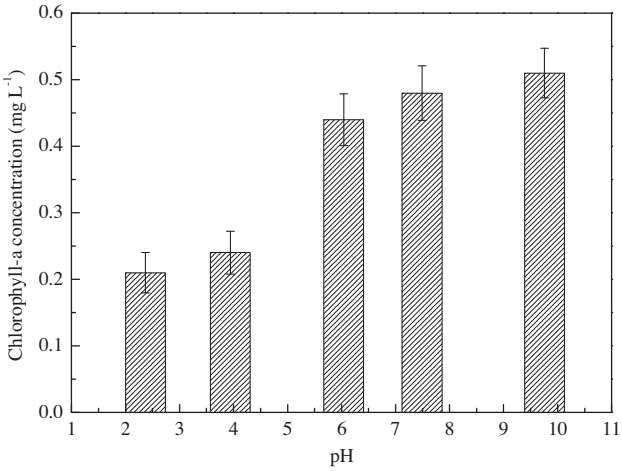

Fig. 2. Effects of pH on UV-C irradiation.

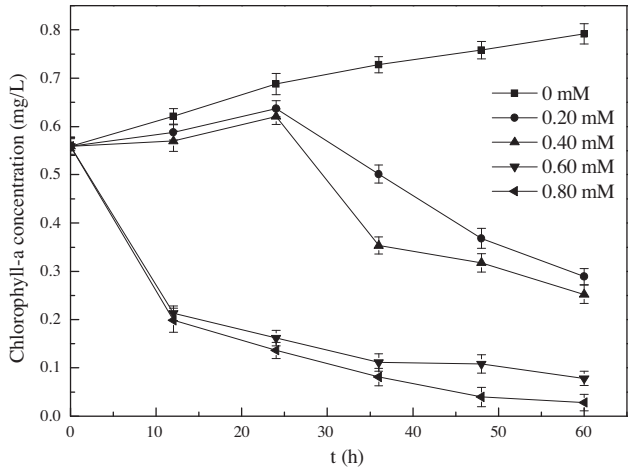

Fig. 3. Effects of H_2O_2 concentration on chlorophyll-a in *M. aeruginosa*.

removal of *M. aeruginosa* by UV-C irradiation. The concentration of chlorophyll-a increased from 0.21 to 0.51 mg/L with the increase of pH. The removal efficiency under an acidic condition was higher than that under a neutral or alkaline condition. The observation results might be interpreted as follows: during the irradiation process, a small amount of reactive species (·OH, e^-_{aq} and H·) was generated, and the changes in H^+ and OH^- concentrations in the solution affected the radical composition generation. In acidic conditions, the concentration of H· was relatively high, as shown in Eq. (5), and the growth of *M. aeruginosa* was inhibited. However, H· could react with OH^- under alkaline conditions to generate e^-_{aq} (Eq. (6)), which might cause the combination of ·OH and e^-_{aq} and reducing the effective radical concentrations, as shown in Eq. (7) [30]. The removal of *M. aeruginosa* decreased at high pH and increased at low pH because of these processes.

$$e^-_{aq} + H^+ = H \quad (5)$$

$$H + OH^- = e^-_{aq} + H_2O \quad (6)$$

$$OH + e^-_{aq} = OH^- \quad (7)$$

3.3. Effects of H_2O_2

H_2O_2 was produced during UV-C irradiation [18]. To investigate the removal efficiency of *M. aeruginosa*, a small amount of H_2O_2 was added into the solutions with *M. aeruginosa*. H_2O_2 in the mM level is toxic to algae [31]. Therefore, five concentration levels (0, 0.20, 0.40, 0.60, and 0.80 mM) of H_2O_2 were selected in this study, and no UV-C irradiation was provided in the experiment. Fig. 3 shows the change in chlorophyll-a concentrations with H_2O_2 concentrations and time (0–60 h). The concentrations of chlorophyll-a in *M. aeruginosa* suspensions with the addition of 0.20 and 0.40 mM of H_2O_2, respectively, increased to 0.637 and 0.621 mg/L within the first 24 h and, respectively, decreased to 0.289 and 0.252 mg/L within the subsequent 36 h. However, the chlorophyll-a concentrations of the *M. aeruginosa* suspensions with the addition of 0.60 and 0.80 mM of H_2O_2, respectively, decreased to 0.213 and 0.199 mg/L within 12 h and gradually disappeared within the subsequent 48 h. Under the addition of 0.80 mM of H_2O_2, the removal efficiency of *M. aeruginosa* could reach 94%. An appropriate amount (0.20–0.80 mM) of H_2O_2 accelerated the suppression of *M. aeruginosa* growth and produced ·OH in the medium, as shown in Eq. (8) [30].

$$H_2O_2 + e^-_{aq} = OH + HO^- \quad (8)$$

3.4. Effects of additives

Several primary reactive species, such as ·OH, e^-_{aq}, and H·, and molecular products, such as H_2 and H_2O_2, were generated because of the energy absorption by water when *M. aeruginosa* suspensions were irradiated. To investigate the role of the primary reactive species (·OH, e^-_{aq}, and H·) in the *M. aeruginosa* removal, the free radical scavengers were added into the *M. aeruginosa* suspensions. Table 1 shows the reaction rate constants between the primary species and the additives [32–35].

Table 1
Reaction rate constants for primary species and various additives in aqueous solution

	Rate constants (M^{-1} s^{-1})		
Ion species	CH_3OH	Thiourea	NO_3^-
·OH	$0.8–1.0 \times 10^9$	$3.9–5.3 \times 10^9$	–
e_{aq}^-	$2.4–3.0 \times 10^6$	$0.01–6.0 \times 10^9$	–
H·	$<1.0 \times 10^4$	$2.9–4.3 \times 10^9$	$7.7–8.9 \times 10^9$

In the various experiments with various additions of CH_3OH, thiourea, and NO_3^-, the concentrations of chlorophyll-a were obtained and are shown in Table 2. The chlorophyll-a concentrations in the solutions decreased when the irradiation doses increased (706 and 1,411 mJ/cm^2) in the presence or absence of additives. As the different additives concentrations increased, the chlorophyll-a concentrations increased and the removal efficiency of *M. aeruginosa* decreased. An increase in the CH_3OH concentration (experiments 2–4 and 12–14) produced an increase in chlorophyll-a concentration and a decrease in the *M. aeruginosa* removal efficiency. The experimental results of the thiourea addition (experiments 5–7 and 15–17) indicated that chlorophyll-a increased with the increase of the thiourea concentration. Chlorophyll-a increased with the increase of the NO_3^- concentration in the experiments of NO_3^- addition (experiments 8–10 and 18–20).

Moreover, as radical scavengers, CH_3OH, thiourea, and NO_3^- restricted the UV-C irradiation process. The experiments of CH_3OH addition indicated that ·OH played an important role in *M. aeruginosa* removal. CH_3OH reacts more rapidly with ·OH radicals than with e_{aq}^- and H· (Eq. (9)). Thiourea is a very strong scavenger, and the experimental results of thiourea addition indicated that its addition, a stronger e_{aq}^- and H· radical scavenger [36], participated in the removal of *M. aeruginosa*. The experiments of NO_3^- addition indicated that e_{aq}^- radicals also participated in the *M. aeruginosa* removal because an increase in NO_3^- concentration resulted in a slight increase in chlorophyll-a concentration.

$$OH + CH_3OH \rightarrow H_2O + CH_2OH \quad (9)$$

3.5. Changes in phycocyanin and SOD

UV-C irradiation had an adverse effect on photosynthetic pigments, such as PC in *M. aeruginosa* cells. When the UV-C irradiation dose was increased from 353 to 2,117 mJ/cm^2, PC changed (Fig. 4). The ratio of PC in the treated sample to PC in the control sample decreased from 30.81 to 9.06%. Relatively low UV-C

Table 2
Effects of radical scavengers on UV-C irradiation

Exp.	Dose (mJ/cm^2)	CH_3OH (mM)	Thiourea (mM)	NO_3^- (mM)	Chl-a[a] (mg/L)
1	706	0	0	0	0.590
2	706	2.5	0	0	0.601
3	706	5	0	0	0.617
4	706	10	0	0	0.624
5	706	0	2.5	0	0.621
6	706	0	5	0	0.635
7	706	0	10	0	0.646
8	706	0	0	2.5	0.595
9	706	0	0	5	0.601
10	706	0	0	10	0.613
11	1,411	0	0	0	0.530
12	1,411	2.5	0	0	0.551
13	1,411	5	0	0	0.559
14	1,411	10	0	0	0.565
15	1,411	0	2.5	0	0.558
16	1,411	0	5	0	0.573
17	1,411	0	10	0	0.596
18	1,411	0	0	2.5	0.541
19	1,411	0	0	5	0.553
20	1,411	0	0	10	0.561

[a]Values were measured on the 1st day after irradiation.

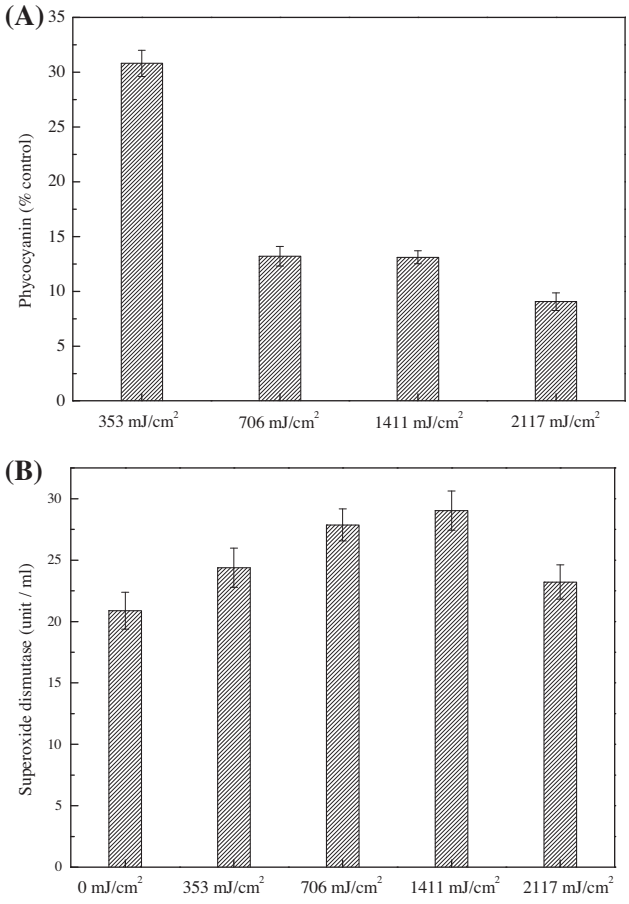

Fig. 4. Effects of UV-C irradiation on phycocyanin (A) and SOD (B). Values were measured on the 1st day after irradiation.

irradiation doses (353–1,411 mJ/cm^2) increased the activity of SOD from 20.88 to 29.04 unit/mL. However, when the UV-C irradiation dose was increased to 2,117 mJ/cm^2, the activity of SOD decreased to 23.21 unit/mL.

The PC in *M. aeruginosa* decreased when the irradiation dose increased. The observation results could be interpreted as follows: UV-C irradiation may directly act on the PC on the outer surface of the thylakoid membrane. Furthermore, the reactive oxygen species (ROS) produced during UV-C irradiation caused protein peroxidation, which had an adverse effect on PC [37].

As the UV-C irradiation dose increased, ROS increased in the *M. aeruginosa* cells and suspensions and triggered the activity of several antioxidative enzymes, such as SOD. These antioxidative enzymes play an important role in the protection against ROS [38]. The activity of SOD increased from 20.88 to 29.04 unit/mL and then decreased to 23.21 unit/mL. Low UV-C irradiation dose (353–1,411 mJ/cm^2) could enhance the activity of SOD in *M. aeruginosa* cells; whereas when the irradiation dose increased from 1,411 to 2,117 mJ/cm^2, the activity of SOD in *M. aeruginosa* cells declined, indicating that the enzyme system in *M. aeruginosa* cells was destroyed by excessive ROS and lost its protection capability against ROS [39].

3.6. SEM study

In the SEM images of *M. aeruginosa* cells (Fig. 5), the *M. aeruginosa* without UV-C irradiation maintained a round shape (Fig. 5A), and the surfaces of *M. aeruginosa*

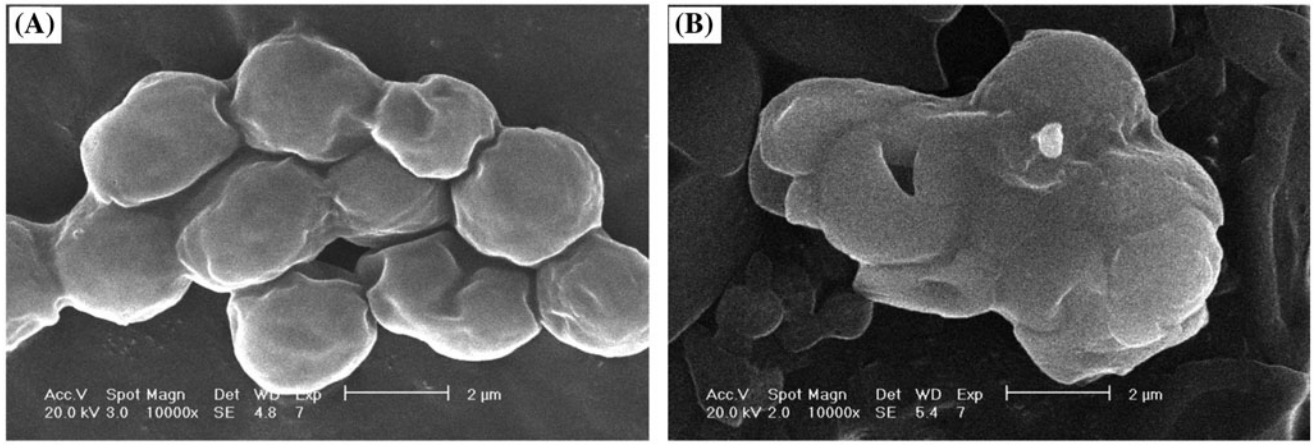

Fig. 5. SEM images of *M. aeruginosa* cells. (A) Untreated *M. aeruginosa* and (B) *M. aeruginosa* irradiated with the UV-C irradiation dose of 2,117 mJ/cm^2.

treated with UV-C irradiation dose of 2,117 mJ/cm^2 were damaged (Fig. 5B). According to the SEM images, the cell surface of irradiated *M. aeruginosa* showed a damaged cell wall and numerous depressions. The difference indicated that the cell wall might have been destroyed by the UV-C irradiation and that the growth of *M. aeruginosa* was inhibited.

4. Conclusion

In conclusion, *M. aeruginosa* in water could be injured by UV-C irradiation. When the irradiation dose was increased from 353 to 2,117 mJ/cm^2, the highest removal efficiency of *M. aeruginosa* reached approximately 84%. In addition, the removal of *M. aeruginosa* was influenced by pH, and the removal efficiency under an acidic condition was higher than that under a neutral or alkaline condition. Under the addition of H_2O_2 at the mM level, the removal efficiency of *M. aeruginosa* could reach 94% under 0.80 mM H_2O_2. In addition, as radical scavengers, CH_3OH, thiourea, and NO_3^- restricted the UV-C irradiation process. According to the SEM images, the cell surface of the irradiated *M. aeruginosa* showed a damaged cell wall and numerous depressions. The PC in *M. aeruginosa* decreased when the irradiation dose increased. Low UV-C irradiation dose (353–1,411 mJ/cm^2) could enhance the activity of SOD in *M. aeruginosa* cells; whereas when the irradiation dose increased from 1,411 to 2,117 mJ/cm^2, the activity of SOD in *M. aeruginosa* cells declined.

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