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Effects of Nano-TiO₂ Mediated Photocatalysis on *Microcystis aeruginosa* Cells

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Abstract: The effects of nano-TiO₂ mediated photocatalysis on *Microcystis aeruginosa*, a common species that causes algal bloom, were studied. The metabolic activity of the *M. aeruginosa* cells was inhibited by nano-TiO₂ mediated photocatalysis, as demonstrated by the significant decrease in metabolic heat with the increase in the time of photocatalysis. SEM images also showed that photocatalysis significantly altered the surface morphology of the cells, and the cell disruption was observed by treatment for 6 h. The nano-TiO₂ mediated photocatalysis decreased the negative charge on the cell surface because the hydrophilic carboxylic acid groups and ammonium groups in the proteins were modified by free radicals. Metal cations of different valence and charge density could neutralize the negative charges on the cell surface to varying degrees. The adsorption heat of metal cations on the cell surface was higher for the control cells than the cells by photocatalysis, because the surface of the latter was modified and had lower charge density.

Keywords: photocatalysis; *Microcystis aeruginosa*; metabolic heat; surface charge; adsorption heat

1. Introduction

In recent decades, algal blooms have occurred increasingly frequently in lakes and reservoirs around the world, and seriously threaten the supply of clean drinking water [1,2]. *Microcystis aeruginosa* is a common species that causes algal blooms and affects water quality [3,4]. It can also release microcystins, which are a class of toxins that endanger the health of animals and humans [5,6].

Advanced oxidation processes (AOPs) can efficiently degrade various pollutants. The AOPs produce highly reactive free radicals, mainly the hydroxyl radical (HO·), which can break down organic molecules. The hydroxyl radical can convert organic molecules into CO₂ and H₂O [7,8]. Free radicals can also damage algae cells, inhibit the growth of algae, and thus can be used to treat algal bloom. Among the AOPs, photocatalysis by nano-TiO₂ has attracted much attention, because the catalyst is inexpensive and highly stable. In photocatalytic oxidation, photons with greater energy than the band-gap energy excite the valence band electrons and expedite the breakdown of organic pollutants [9]. In addition, illuminating the catalyst active surface with sufficient energy can help create a positive hole (h⁺) in the valence band and produce an electron (e⁻) into the conduction band. The positive hole can then oxidize either the organic pollutant or H₂O and generate the hydroxyl radicals [10].

Coagulation is one of the most common methods to treat algal bloom. The coagulants can turn the algal bloom suspended in water into a gel and then form a clot. *M. aeruginosa* is known for its negatively charged surface, and can thus be aggregated through charge neutralization with

cationic metallic coagulants (e.g., inorganic metallic substances such as aluminum and iron salts) [11]. The mechanism of coagulation involves activation, adhesion, and aggregation of the algal bloom [12]. It must be noted that the cell surface plays an important role in the protecting cell. In this work, to demonstrate the biological effect of photocatalysis on *M. aeruginosa* with regard to metabolism, morphological change, and surface zeta potential, *M. aeruginosa* was treated with nano-TiO₂ upon UV irradiation. Specifically, metabolic heat was measured to evaluate the effect of AOPs on *M. aeruginosa*. The morphology of *M. aeruginosa* cells was observed to analyze the surface feature. Zeta potential and metallic adsorption were determined after nano-TiO₂ treatment to evaluate the role of metallic coagulant in damaging *M. aeruginosa*.

2. Materials and Methods

2.1. Cells and Reagents

M. aeruginosa (FACHB-905) was received from the Freshwater Algae Culture Collection in the Institute of Hydrobiology, Chinese Academy of Science. *M. aeruginosa* was grown in BG-11 nutrient solution (pH = 7.5). The algae culture was cultivated in an incubator at a constant temperature of 25 °C under 2000 lx with a light-dark cycle of 12 h/12 h. The cultures were harvested at 2×10^6 cells/mL in the exponential growth phase.

Nano-TiO₂ (P25) was purchased from Degussa AG (Essen, Germany). Other reagents were purchased from Shenshi Chem. Deionized water was used in all experiments.

2.2. Photocatalysis

M. aeruginosa cells were washed with PBS buffer before exposure to nano-TiO₂ and UV irradiation. In a typical procedure, a suspension of *M. aeruginosa* was centrifuged at 4000× rpm for 5 min, and the pellets were then resuspended in 0.05 mol/L PBS buffer. This operation was repeated three times to thoroughly remove the culture. Afterwards, *M. aeruginosa* was suspended in PBS buffer at 2×10^6 cells/mL.

The concentration of nano-TiO₂ was 100 mg/L, which was the optimum value tested. Beyond this value, nano-TiO₂ would coagulate and adversely affect the light transmission. After ultrasonic dispersion in PBS buffer for 15 min, the nano-TiO₂ was mixed with *M. aeruginosa* and stirred at 1000 r/min for 30 min avoiding light. The photocatalysis was carried out by irradiating the mixture with monochromatic light (wavelength 340–380 nm) under a 150 W mercury lamp for 0, 1, 2, 4, and 6 h, respectively (Figure 1). The distance between the lamp and the *M. aeruginosa* suspension was about 20 cm. Agitation speed can influence the degradation rate. When the agitation speed is too slow, the uniform distribution of nano-TiO₂ cannot be maintained. When the agitation speed is too high, the active site of TiO₂ would have too short of a contact time with *M. aeruginosa* cells, which will reduce the reaction rate. Therefore, the mixture was stirred at 1000 r/min in our study.

Figure 1. Schematic diagram of the photocatalytic reactor.

2.3. Measurement of Metabolic Heat

After photocatalysis, the metabolic thermogenic curve of *M. aeruginosa* was recorded at 26 °C in real time on a TAM Air system (Thermometric AB, Sweden) as follows. The suspension of *M. aeruginosa* (5 mL) was put into the sample cell, and distilled water (5 mL) was put into the reference cell. The TAM Air system recorded the metabolic process of *M. aeruginosa*. When calculating the metabolic parameter values, the interference signal data were deleted in the initial phase of the measurement.

2.4. Morphology

The morphology of *M. aeruginosa* was observed after nano-TiO₂ mediated photocatalysis for different times. Glutaraldehyde and osmium tetroxide (OsO₄) were used to fix the cells and dehydrate the protein (or lipid). The cells were then dehydrated with ethanol of gradually increasing concentration (50%, 60%, 70%, 80%, 90%, 95%, and 100%), and finally examined under a Hitachi S-4800 scanning electron microscope (SEM).

2.5. Charge Density on the *M. aeruginosa* Surface

The *M. aeruginosa* cells were subject to microelectrophoresis on a JS94G+ zeta potential analyzer (Shanghai Powereach Digital Technology Equipment Co., Ltd., Shanghai, China). The zeta potential was calculated by measuring the migration distance of colloid under an electric field. *M. aeruginosa* cells were considered as colloids because their particle size and charge density resembled those of colloids [13,14].

One sample group was quantified to assess the influence of nano-TiO₂ mediated photocatalysis on the surface charges of *M. aeruginosa* cells. Other sample groups were quantified to assess the variation of charge density with the addition of metal ions (LiCl, NaCl, KCl, MgCl₂, CaCl₂, SrCl₂, BaCl₂, AlCl₃, and FeCl₃) at pH = 7.55.

2.6. Adsorption Heat

The adsorption heat of cations on the surface of *M. aeruginosa* was measured on an isoperibol calorimeter (Figure 2). The calorimeter was completely surrounded by a jacket of constant and uniform temperature. In the isothermally jacketed calorimeter, some but not all of the heat of reaction is transferred to an outer isothermal heat jacket. The leakage modulus should be constant, and heat transfer due to convection should be kept as small as possible. The heat was calculated from the temperature-time measurements.

Figure 2. Schematic diagram of the isoperibol calorimeter used for adsorption heat measurement.

Before the test, the instrument was first calibrated by measuring the dissolution of KCl in water at 298.15 K. A suspension of *M. aeruginosa* (100 mL, 2×10^6 cells/mL) was put into a Dewar bottle and allowed to arrive at the equilibrium temperature. The sample box was then opened to release the solution of the cations (2 mL, 1 mol/L) into the cell suspension (see Ref. [15] for more procedural details), and the generated heat was recorded. Since the cations were in excess, the heat of dilution of the cations in 100 mL of water was calculated. The adsorption heat of the cations on the cell surface was finally obtained by subtracting the heat of dilution from the generated heat.

3. Results and Discussion

3.1. Metabolic Heat of *M. aeruginosa* Cells

The growth of microorganisms includes the lag stage, the log stage, the stationary stage, and the decline stage [16,17]. Figure 3 shows the metabolic heat of *M. aeruginosa* cells after treatment with nano-TiO₂ mediated photocatalysis and without nutrients from the culture. On the whole, the metabolic heat declined gradually and ceased finally.

Figure 3. The metabolic heat of *M. aeruginosa* after nano-TiO₂ mediated photocatalysis.

M. aeruginosa makes use of nitrogen, phosphorus, and other nutrients to maintain its metabolism. Without nutrition from external sources, the cells can still live on endogenous nutrients for some time. Glycogen is the energy and carbon source stored in the cells. During photosynthesis, a large amount of optical energy is converted into chemical energy and stored in glycogen.

Compared with the control *M. aeruginosa* cells, the nano-TiO₂ photocatalyzed cells experienced inhibited metabolism. The inhibition was more severe when the nano-TiO₂ mediated photocatalysis lasted longer.

Table 1 lists the metabolic parameters for the *M. aeruginosa* cells that were treated with nano-TiO₂. In the table, P_{max} is the measured maximum output power during metabolism, and Q_T is the total heat produced for the entire metabolic process. Clearly, both P_{max} and Q_T decreased upon prolonged nano-TiO₂ mediated photocatalysis. In particular, the cells that underwent nano-TiO₂ mediated photocatalysis for 6 h generated negligible metabolic heat.

Table 1. Metabolic parameters for the *M. aeruginosa* cells by nano-TiO₂ mediated photocatalysis.

Sample	P_{max} (mW)	Q_T (J)
Control	0.22 ± 0.012	36.21 ± 1.87
1 h treated Cells	0.12 ± 0.051	19.32 ± 1.05
2 h treated Cells	0.08 ± 0.0054	13.88 ± 0.95
4 h treated Cells	0.05 ± 0.0023	10.65 ± 0.56
6 h treated Cells	0.02 ± 0.0010	0.89 ± 0.067

A simple photolysis test suggested that the illumination without nano-TiO₂ showed little action on *M. aeruginosa*. Nano-TiO₂, particularly in the anatase form, is a photocatalyst. Upon irradiation with UV light, TiO₂ can produce electrons (e[−]) and holes (h⁺) on its surface. The holes are strongly oxidative and can oxidize water to create hydroxyl radicals, and can also oxidize organic molecules directly [18,19].

The HO[·], O₂[·], and HO₂[·] radicals produced in the photocatalytic process are reactive enough to attack many organic bonds, such as C–C, C–H, C–N, C–O, and H–O [20,21]. Thus, the free radicals produced by nano-TiO₂ can efficiently modify the molecules in microorganisms. As a result, nano-TiO₂ mediated photocatalysis can inhibit the metabolic activity and even damage the cells. The band gap of nano-TiO₂ is 3.2 eV, so the wavelength of light should be less than or equal to 387.5 nm. Therefore, in our study, a monochromatic lamp was used for the photocatalysis with high efficiency.

3.2. Morphology of the *M. aeruginosa* Cells

Since the cell wall and membrane were exposed to the free radicals in the medium, we also investigated the damage on the outermost layer of *M. aeruginosa* cells caused by nano-TiO₂ mediated photocatalysis. Figure 4 shows the morphology of the *M. aeruginosa* cells after nano-TiO₂ mediated photocatalysis. The control cells exhibited regular sphericity with a diameter of 2.5 μm, and regular wrinkles appeared on the cell surface at the nano-level (Figure 4A). After nano-TiO₂ mediated photocatalysis for 1 h and 2 h, the surface became much rougher than that of the control cells (Figure 4B,C). Photocatalysis for 4 h deformed the surface morphology of the *M. aeruginosa* cells (Figure 4D). After photocatalysis for 6 h, the cells were disrupted (Figure 4E).

Figure 4. Cont.

Figure 4. SEM images of *M. aeruginosa* cells after nano-TiO₂ mediated photocatalysis. (A1,A2): the control; (B1,B2): 1 h treatment; (C1,C2): 2 h treatment; (D1,D2): 4 h treatment; (E1,E2): 6 h treatment.

The cell wall and cell membrane is the outermost layer of the *M. aeruginosa* cells. They consist of extracellular polymeric substances, and they are responsible for the adsorption of nutrients and the defense against environmental toxins [22]. When the cells were exposed to UV irradiation in the presence of nano-TiO₂ for 4 h, the cell wall and cell membrane were seriously damaged, but the cells still remained spherical and did not show major morphological changes. However, when the irradiation was extended to 6 h, the cells were erupted, most likely due to the failure of the cell wall. A previous report found that the main mechanism of degradation involves hydroxyl substitution, which could occur repeatedly [23]. When *M. aeruginosa* cells were exposed to free radicals generated by nano-TiO₂, hydroxyl substitution of extracellular polymeric substances may also have occurred repeatedly, which ultimately led to the photolysis of organic molecules.

The nano-TiO₂ also experienced changes during the photocatalytic process. At the beginning, nano-TiO₂ particles were suspended well in cell solution. After some time, nano-TiO₂ particles tended to coagulate, but there were no Ti⁴⁺ ions detected in solution. The nano-TiO₂ particles showed a particle size of 25 nm and were easy to aggregate due to high surface energy. In the photocatalytic process, the particle size increased gradually. However, nano-TiO₂ is chemically stable and Ti⁴⁺ ions would not be released from nano-TiO₂ particles into solution.

3.3. Charge Density on the *M. aeruginosa* Cell Surface

Figure 5 shows how the charge density of the *M. aeruginosa* cells varies with the time of nano-TiO₂ mediated photocatalysis. The zeta potential of the control *M. aeruginosa* cell surface was -30.20 ± 0.32 mV. *M. aeruginosa* was characterized with negative charges, which resulted from the molecular groups on the surface. Both the cell wall and the cell membrane contain plenty of proteins, which consist of amine (–NH₂) and carboxylic acid (–COOH) functional groups that are joined orderly through peptide bonds. The carboxylic acid groups (–COOH) can be deprotonated to become the negatively charged carboxylates (–COO[−]), and the amino groups (–NH₂) can be protonated to become the positively charge ammonium groups (–NH₃⁺). When the pH of the cell suspension exceeded the isoelectric point, the cells would carry negative charge. Generally, the pH of the cell suspension is higher than the isoelectric point of bacteria. As a result, the *M. aeruginosa* cells were characterized with negative charges [23,24].

Figure 5. Zeta potential of *M. aeruginosa* cells after nano-TiO₂ mediated photocatalysis.

After the *M. aeruginosa* cells were subject to nano-TiO₂ mediated photocatalysis, their zeta potential decreased; that is, the negative charges on the cell surface decreased. When the time of nano-TiO₂ mediated photocatalysis was increased to 6 h, the zeta potential could no longer be measured. Both the carboxylic acid groups and the ammonium groups in the proteins are hydrophilic. The proteins are inserted into the phospholipids bilayer and extracellular polymeric substances in the cell wall and cell membrane, and the hydrophilic groups (–COOH and –NH₃⁺) are exposed to the external

environment and easily damaged by free radicals during nano-TiO₂ mediated photocatalysis [24]. Therefore, the charge density decreased significantly due to the damages of the hydrophilic groups.

3.4. Adsorption Heat of Cations on the *M. aeruginosa* Cells

Figure 6 shows the zeta potential as a function of the addition of cations (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, and Fe³⁺). Both the charge density and the zeta potential decreased significantly upon the addition of cations. The surface of *M. aeruginosa* cells carried negative charges and adsorbed cations as a result of electrostatic attraction. Among the cations of the first main group, the ability to neutralize negative charges ranked as K⁺ > Na⁺ > Li⁺. Similarly, the cations of the second main group ranked as Ba²⁺ > Sr²⁺ > Ca²⁺ > Mg²⁺. Within each group, the metal ion with higher charge density had stronger ability to neutralize negative charges. Cations of the second main group are divalent, and thus showed stronger ability to neutralize negative charges than those of the first main group. Furthermore, Al³⁺ and Fe³⁺ had the strongest ability to neutralize negative charges and changed the zeta potential the most dramatically. The results suggested that in neutralizing negative charges, the valence of the metal ion was the main driver, and the charge density of the metal ion also affected the strength of the impact. Among all tested metal ions, Al³⁺ and Fe³⁺ showed the strongest ability in changing the surface charges of the *M. aeruginosa* cells.

Figure 6. Zeta potential of *M. aeruginosa* cells after addition of different cations.

Table 2 shows the heat released when the cations bound to the cell surface. An exothermal process was observed for all tested cations. Theoretically, the binding reaction is accompanied with the formation of a chemical bond that is weaker than the covalent bond and similar to the hydrogen bond. During binding, the energy of the cation–cell system was reduced and heat was released. This heat depended on the valence and the charge density of the added cations, and ranked as K⁺ > Na⁺ > Li⁺, Ba²⁺ > Sr²⁺ > Ca²⁺ > Mg²⁺, and Al³⁺ > Ba²⁺ > K⁺. This order is reasonable because the valence and the charge density of the cation affected the bond strength and bond energy [25], which in turn determined the amount of heat released. The exothermal process indicated a negative enthalpy change. Besides, during electrostatic interaction, the cations migrated from the solution and were bound to the cell surface. The degree of order increased and the entropy of the system decreased, which in fact impeded the adsorption process. Nevertheless, the negative enthalpy change was strong enough to overcome the entropy factor and realize the binding process. Therefore, the electrostatic adsorption on the cell surface was “enthalpy-driven”.

Table 2. Adsorption heat of cations on *M. aeruginosa* cells after the addition of cations.

Adsorption Heat (J)	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Sr ²⁺	Ba ²⁺	Al ³⁺	Fe ³⁺
Control cells	1.24	1.86	2.78	3.12	3.45	3.66	3.98	5.83	4.65
Treated cells	0.18	0.32	0.44	1.02	1.30	1.35	1.41	2.08	1.95

The adsorption heat of cations was lower for the treated cells than the native cells. As outlined before, the amine (–NH₂) groups were modified by free radicals during photocatalysis. Consequently, the charge density on the cell surface decreased, which led to weaker binding between cations and the cells.

4. Conclusions

The stress of nano-TiO₂ mediated photocatalysis on *Microcystis aeruginosa*, a common species that causes algal bloom, was studied by several methods. The metabolic activity of *M. aeruginosa* was damaged by free radicals that were produced by nano-TiO₂ mediated photocatalysis, which reacted with the hydrophilic groups (–COOH and –NH₃⁺) on the cell surface. The metabolic heat of *M. aeruginosa* decreased upon prolonged treatment of nano-TiO₂ mediated photocatalysis, indicating the metabolic activity of the cells was inhibited. The SEM images showed how the surface morphology of the cells deteriorated along with nano-TiO₂ mediated photocatalysis, and cell disruption was observed after the photocatalysis was carried out for 6 h. The surface of the *M. aeruginosa* cells were negatively charged due to the presence of proteins. Nano-TiO₂ mediated photocatalysis decreased the negative charges on the cell surface by damaging the hydrophilic carboxylic acid groups and ammonium groups with free radicals. The density of negative charges on the cell surface decreased with the addition of cations. The valence of the cation was the primary factor that affected the neutralization of negative charges on the cell surface, and the charge density of the cation was the secondary factor in this process. The nano-TiO₂ can damage *Microcystis aeruginosa* cells, which may be used in the algae bloom control, as a promising candidate for coagulant of water pollution treatment.

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