Preparation of double-doped Cu, N-nano-TiO$_2$ photocatalyst and photocatalytic inactivation of Escherichia coli in ballast water

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Abstract. With more and more international economic exchanges, ship transport becomes more and more frequent, which further increases the exchange capacity of ship ballast water in different sea areas, thus causing more and more serious marine problems of alien microbial invasion. Therefore, in this paper, on the basis of microbial inactivation of ship ballast water treated by uv irradiation, the influencing factors of its catalytic activity were explored by adding photocatalyst TiO$_2$ into the bacterial suspension, that is to change the catalytic activity of titanium dioxide noble metal and other modified substances and doping amount. Firstly, the UV-sterilization was used as a control, and the modified photocatalyst nano-TiO$_2$ was added. It was found that the sterilization rate increased greatly, indicating that the photocatalyst-modified nano-TiO$_2$ has a very strong reinforcing effect on ultraviolet sterilization. Secondly, the photocatalyst itself was changed. It was found that the amount of photocatalyst nano-TiO$_2$ had a great influence on UV sterilization. Within a certain range, the more the photocatalyst nano-TiO$_2$ is, the better the sterilization effect is. Finally, changing the external conditions of the photocatalyst, it was found that the photocatalyst nano-TiO$_2$ had the best activity at higher temperature (33 °C), alkaline (pH=8.5) or neutral (pH=7.5) and long-wave ultraviolet radiation.

1. Introduction

Energy consumption and environment sustainability is also getting more and more attention in the shipping industry[1,2], such as energy saving methods for refrigeration rooms [3], power consumption [4,5], energy conversion [6,7], especially for marine ship pollution [8]. At present, ocean transportation has become the mainstream way of trade and transportation between countries in the contemporary world, so it has developed rapidly. The ship's ballast water is indispensable for strengthening the ship's no load in contemporary ocean transportation. Ship ballast water is one of the basic guarantees for safe and stable navigation during the ocean sailing of contemporary ships. Every year, more than 80% of the
world's goods are transported by ocean, which brings about 10 billion tons of ballast water. Ship ballast water is the guarantee for the safe and efficient operation of modern ocean-going vessels, but the invasive spread of alien pests has become one of the four major threats facing the ocean and has become a worldwide environmental problem to be solved. Commonly used ballast water treatment technologies have certain shortcomings, and no method can meet the requirements of the International Maritime Organization for ballast water treatment.

In this study, the sterilization effect and loading mode of UV-catalyzed nano-TiO$_2$ in ship ballast water treatment technology were studied. The relevant experimental platform was built to complete the verification test, and the effects of different factors and operating parameters on the treatment efficiency were investigated.

2. Materials and methods

2.1. Cultivation of *Escherichia coli*

According to the D2 standard of ballast water treatment of the International Ocean Group, *Escherichia coli* was selected as our final experimental strain, which was purchased from the Microbiology Laboratory of Harbin Institute of Technology, and inoculated into the LB liquid medium at room temperature which has been autoclaved by using the inoculating ring. Medium (10g peptone, 5g yeast powder, 10g sodium chloride, 1000mL deionized water, pH=7.2~7.4), sealed with sterilized plastic film, 37°C, 130r/min constant temperature gas field oscillator was cultivated for 18 to 24 hours. After being shaken to the logarithmic growth phase, it is inoculated into the sterilized physiological saline, and after being placed in a certain concentration of the suspension, the sterilization experiment can be performed.

2.2. Detection of *Escherichia coli*

The original bacterial solution after inoculation of *Escherichia coli* was diluted with 9% physiological saline and diluted into 10 E. coli suspensions of $10^1$ to $10^{12}$ dilutions. The diluted suspensions were each taken 5 mL with a filter, and LB medium was used. After the culture medium was cultured for 18~24 hours, the number of bacteria was observed, and the dilution factor which can accurately count the number of bacteria was found, and the order of bacteria in the liquid solution per milliliter of the original liquid was pushed out. According to the literature, the order of E. coli is around $10^8$, and we have confirmed this by pushing back, so we can determine that the order of E. coli is around $10^8$.

According to the relevant literature, the bacterial suspension before sterilization is selected as $10^8$ cells/mL, and 1 mL of the original solution is diluted into a solution having a concentration of 10 cells/mL and a volume of 10 mL, and divided into 5 mL parallel samples for suction filtration. The remaining suspension was taken to the reactor for UV sterilization experiments. The sterilization time is set to 5s, 10s, 15s, and 20s, respectively. Each time the bacteria were killed, 1 mL of the bacterial solution was diluted to an appropriate multiple and then filtered by two 5 mL parallel samples. After pumping out the filter, the bacteria in the filter membrane were trapped on the medium face up and placed upside down in a 37°C constant temperature gas domain oscillator for culture for 18~24h. After that, the bacteria in the filter membrane were removed and counted and analyzed.

2.3. Modification treatment of photocatalyst

Preparation of Cu, N double-doped nano-titanium dioxide: 18 mL of tetrabutyl titanate was weighed and slowly stirred into 84 mL of absolute ethanol for 10 minutes with magnetic stirring to obtain a mixed solution A. 42.0 mL of absolute ethanol, 6.0 mL of deionized water, 12.0 mL of glacial acetic acid, a certain amount of copper nitrate (0.1917 g) and urea (0.4065 g) were uniformly mixed, and the pH was adjusted to be not more than 3 to obtain a mixed solution B. The solution A in the separatory funnel was slowly added dropwise to the solution B which was heated and stirred in a water bath at 30°C to obtain a sol-gel solution C. The treated stainless steel wire piece was immersed in the sol-gel solution C. It is first placed in a dry box for drying, then placed in a muffle furnace for calcination and heat preservation. Finally, different loadings of Cu, N-doped nano-TiO$_2$ were obtained.
2.4. Sterilization method
The continuous flow experiment was carried out on the sterilization performance of titanium dioxide doped with Cu and N under ultraviolet conditions. The main part of the reactor of the experimental device is a closed cylindrical structure. Two parallel ultraviolet tubes can be installed at the top of the reactor. In order to reduce the damage caused by ultraviolet light to the human body, we package the periphery, top and bottom of the reactor with tin foil paper. The tail of the reactor is equipped with an adjustable valve, which is mainly used to open the flowing water and adjust the flow rate. The flow rate can be calculated by the numerical value of the liquid flow meter. After comparison, the flow rate is determined to be 6.9 L/min.

With UV radiation dose and UV lamp ambient temperature as fixed invariants, sterilization experiments were carried out during the reaction period under the conditions of only UV irradiation without catalyst, UV irradiation without catalyst doping and UV irradiation with catalyst doping different dopants such as Cu and N respectively.

3. Results and analysis

3.1. Study on UV sterilization performance of photocatalyst

3.1.1. Effect of modified nano-TiO$_2$ photocatalyst on UV sterilization. After the photocatalyst titanium dioxide was prepared and modified, the photocatalytic ultraviolet sterilization experiment of E. coli was first carried out. According to the concentration of E. coli in the ship's ballast water, the concentration is about $10^8$ mL per mL of the stock solution (E. coli suspension), and after the sterilization experiment, the actual number of E. coli is detected according to the experimental method described in 2.2. In order to compare and observe the effect of photocatalysis on killing Escherichia coli, this experiment first carried out the sterilization experiment under ultraviolet only condition, and obtained the sterilization amount and sterilization rate under the single action as a blank control experiment.

The effect of photocatalyst TiO$_2$ on UV sterilization can be seen directly from Figure 1. The horizontal comparison of the two curves shows that as time increases, the number of E. coli decreases and the sterilization rate continues to increase. Especially the first 5s effect is the most obvious, the sterilization effect is relatively flat in 5~20s time, and the sterilization effect has a small increase in 20~25s time, indicating that UV sterilization has persistence, not instantaneous completion. From the vertical comparison of the two curves, we can clearly see the difference in the numerical values of the two curves. The pure ultraviolet sterilization rate is below 45%, but after adding the modified TiO$_2$ doped with Cu and N elements, the sterilization rate With a substantial increase, the lowest point in Figure 1 is maintained above 90%, which indicates that the photocatalyst TiO$_2$ has a significant enhancement effect on UV sterilization, which can greatly improve the UV sterilization rate.

3.1.2. Influence of intrinsic factors on photocatalyst performance. 1. The effect of the amount of photocatalyst on UV sterilization: In this experiment, stainless steel wire was used as the carrier of photocatalyst TiO$_2$. In order to ensure the integrity of the experimental results, each piece of stainless steel wire is used in a size of 25 cm$^2$. Since the amount of photocatalyst cannot be directly controlled, the number of photocatalysts is expressed side by side with the number of carrier stainless steel wires.

The effect of the amount of photocatalyst on UV sterilization can be seen directly from Figure 2. In the interval of 8~24p stainless steel wire pieces (carrier area 200cm$^2$~600cm$^2$), as the number of photocatalysts increases, the UV sterilization efficiency becomes higher and higher. When the number of carriers is 24p (the carrier area is 600cm$^2$), the ultraviolet The sterilization effect reached the best point, the sterilization rate in 5s exceeded 91%, and the 25s time point was close to 95%. As the number
of photocatalysts increases, the efficiency of UV sterilization gradually decreases, and the reduction is not too obvious. Looking at the literature, we suspect that this is because there are too many electron holes, causing some free electrons to return directly to the holes without collision in water. The oxidation of hydroxyl groups in the water is reduced, which in turn inhibits the activity of a part of the photocatalyst, resulting in sterilization. The effect is reduced. In summary, when the number of carrier stainless steel wire pieces reaches 24p (the carrier area is 600cm$^2$), the ultraviolet sterilization effect is the best.

![Figure 1. Comparison of sterilization rate before and after adding catalyst](image1)

![Figure 2. Effect of the amount of photocatalyst on UV sterilization](image2)

2. The effect of photocatalyst reuse rate on UV sterilization: This experiment belongs to the practical application experiment, and the experimental results are finally applied to the actual ship, so we use the continuous flow experiment, that is, the dynamic experiment. At the same time, in the course of the experiment, we consider that the carrier stainless steel wire piece is under the continuous scouring environment, which will cause some photocatalyst TiO$_2$ to be wasted by the water flow due to insufficient load, which will cause material waste, which will affect the experimental results. Therefore, for practical applications, we conducted experiments on the photocatalytic reuse rate.

From Figure 3, we can see the effect of photocatalyst re-use rate on UV sterilization. In the first three sets of data, the difference of each fold line can be almost ignored, and the photocatalyst activity is very good, which has obvious enhancement effect on UV sterilization. As the experiment progressed, we found that from the fourth group, there was a very significant decrease in UV sterilization efficiency, and the UV sterilization rate barely remained at around 75%. Through the longitudinal comparison with the first three groups, this shows that some photocatalysts are washed away by the water flow due to insufficient load, but even then, we found that the sterilization efficiency of the third group, the fifth group, and the sixth group are still can maintain a high level, and remain above 70%. When the experiment was carried out to the seventh group, the ultraviolet sterilization efficiency had a cliff-type drop compared with the first six groups, which was only better than the pure ultraviolet sterilization effect. This is because most of the photocatalyst has been washed away by the water stream, and only a small part of the nano-TiO$_2$ is still playing a catalytic effect. Therefore, when the photocatalyst nano-TiO$_2$ is loaded on the stainless steel wire, the action time should not exceed 150 s, and the effect of the operation is negligible.

3.1.3. Influence of external factors on photocatalyst performance. In the previous groups of experiments, we considered more about the effect of internal factors of photocatalyst on the overall UV sterilization results, but in actual marine applications, external conditions vary widely. Therefore, we next explore the effects of changes in external conditions on the performance of photocatalysts.
1. Influence of external temperature on photocatalyst: Temperature is one of the most common changing conditions for ship's ballast water. We were unable to test the photocatalytic activity at each temperature, except for the three common temperature conditions of 13 °C, 23 °C and 33 °C.

From Fig. 4, we can see that the photocatalyst nano-TiO$_2$ has the best catalytic effect on UV sterilization at 33 °C. The sterilization efficiency is close to 92% in 5s, and the sterilization efficiency is breaking through 95% in 25s, indicating that the catalytic activity of photocatalyst nano-TiO$_2$ is improved to some extent at 33 °C. The curve at room temperature can be seen that at 23°C, the UV sterilization rate is lower than that at 33 °C, but the decrease is not very obvious, especially in the 15s ~ 25s time, the sterilization rate is improved. However, when the temperature drops to 13 °C, we can clearly see the relative decrease in the relative degree of sterilization, but the UV sterilization rate is still close to 90% at the 25s time point, indicating that a small part of the photocatalyst is low temperature inhibits catalytic activity, resulting in a decrease in UV sterilization rate.

Through the comparison of these three curves, the temperature change generally has little effect on the photocatalytic activity, but when the temperature rises, the catalyst activity will increase slightly, and when the temperature is lower than room temperature, the catalyst activity is slightly inhibited.

2. The effect of pH on photocatalyst: In actual life, due to the doping of various substances, the pH value of the aqueous solution is constantly changing, not fixed, so we choose several common pH environments to explore the catalytic activity of photocatalysts in these environments.

From Fig. 5, we can see that macroscopically, the pH change does not have much influence on the photocatalytic activity, and the lowest point of each curve exceeds 90%, and the sterilization rate is still

![Figure 3](image1.png)  **Figure 3.** Effect of photocatalyst reuse rate on UV sterilization

![Figure 4](image2.png)  **Figure 4.** Effect of temperature change on photocatalytic performance

![Figure 5](image3.png)  **Figure 5.** Effect of pH change on photocatalytic performance
Maintained at a high level. However, from the microscopic comparison, we can see that the photocatalytic activity is slightly inhibited under acidic conditions. Under neutral or alkaline conditions, the activity of the catalyst is slightly increased, especially at pH=8.5, 5s time. The sterilization rate of the spot is close to 92%, and the photocatalyst has the best catalytic effect. When the pH value is continuously increased, the catalytic activity will be reduced. In the longitudinal comparison of the four curves, we can know that the photocatalytic nano-TiO$_2$ has enhanced catalytic activity under neutral or alkaline conditions, but the enhancement is not large. Under acidic or higher alkaline conditions, the photocatalytic activity will decrease.

4. Conclusion
In this study, the sterilization effect and loading mode of UV-catalyzed nano-TiO$_2$ in ship ballast water treatment technology were mainly studied. Based on the UV-treated ballast water technology, a fixed type with modified substances such as Cu and N was prepared. Photocatalyst titanium dioxide was added to Escherichia coli suspension for investigation of sterilization efficiency. This paper draws the following conclusions:

When Escherichia coli is killed for 25s under ultraviolet irradiation, the sterilization efficiency can reach about 45%, and the sterilization rate is increased after adding the double-doped modified photocatalyst titanium dioxide, and the highest is close to 91%. It shows that the modified photocatalytic titanium dioxide has a great effect on UV sterilization.

When Escherichia coli was killed for 25s under the ultraviolet irradiation condition of the photocatalyst, it was found that within a certain range, the more the amount of photocatalyst, the higher the sterilization rate. However, when the limit is exceeded, the amount of the catalyst increases, which in turn suppresses the photocatalytic activity.

Under the ultraviolet irradiation condition of adding photocatalyst to kill escherichia coli for 25s, when the temperature is low, the photocatalytic activity is inhibited. With the gradual increase of temperature, the photocatalytic activity is gradually increased. It indicated that the photocatalytic activity was extremely high at this time. When the pH of the aqueous solution is changed, the photocatalytic activity is also affected. Under acidic and basic conditions, the photocatalytic activity is gradually reduced.

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