

Research Article

TiO₂ and N-Doped TiO₂ Induced Photocatalytic Inactivation of *Staphylococcus aureus* under 405 nm LED Blue Light Irradiation

Hongfei Chen,¹ Zhong Xie,^{1,2} Xiujuan Jin,² Chao Luo,³ Chao You,^{1,2} Ying Tang,² Di Chen,² Zhengjia Li,² and Xiaohong Fan^{1,2}

¹ Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Hubei, WuHan 430074, China

² College of Optoelectronics Science and Engineering, Huazhong University of Science and Technology, Hubei, WuHan 430074, China

³ School of Life Science and Technology, Huazhong University of Science and Technology, Hubei, WuHan 430074, China

Correspondence should be addressed to Xiaohong Fan, xhfan@mail.hust.edu.cn

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Irradiation source has been a serious impediment to induce photocatalytic bacterial inactivation which was taken as an advanced indoor air purification technique. Here we reported the synergistic effects of 405 nm LED light and TiO₂ photocatalyst in inactivation process of *Staphylococcus aureus* (*S. aureus*). In this work, TiO₂ and N-doped TiO₂ particles were, respectively, suspended into the nutrient broth suspension with *S. aureus*. Then, the mixed system was exposed to a 405 nm LED light source with energy density of about 0.2 W/cm² for 3 hours. Irradiated suspension was then scanned by UV-vis spectrophotometer for bacteria survive/death rate statistics. Subsequently, the inactivation efficiency was calculated based on the difference of the absorption optical density between experimental and controlled suspensions. Results showed that both TiO₂ and N-doped TiO₂ particles exhibit potential bacterial inactivation effects under similar experimental conditions. Specifically, N-doped TiO₂ with the concentration of 5 g/L displayed enhanced inactivation efficiency against *S. aureus* under 405 nm LED light irradiation. Thus, it is a promising indoor air purification technique by using N-doped TiO₂ particles under the LED light irradiation.

1. Introduction

More and more attention has been paid to indoor air quality which is very important to human health [1]. Microbial contamination, especially from the superbug *S. aureus*, is very dangerous. Usually, *S. aureus* can cause local skin infections and even more serious infections in wounds, bones, lungs, and blood and *S. aureus* can be undetected and carried quite harmlessly until the one suffers [2]. Moreover, superbug *S. aureus* is even resistant to all commonly used antibiotics and infects people in both community and hospitals [3, 4]. As an important defensive strategy, indoor air purifying becomes increasingly popular since its inception more than a decade ago. Several different processes have been used to purify the indoor air, such as ultraviolet germicidal irradiation, purification traps filter, porous material-activated carbon adsorbing, polarized-media electronic, photocatalytic oxidation, ionizer purifiers, liquid ioniser purifiers, ozone generators, and titanium dioxide (TiO₂) technology [5]. Among these processes, heterogeneous photocatalytic oxidation (PCO)

is the most promising air purifying technique in outdoor conditions using TiO₂ particles activated under UV light irradiation. However, due to the shortage of UV light irradiation in indoor condition, there is still no effective indoor PCO air purifier in our daily life at present.

Chung et al. have reported photocatalytic inactivation of *S. aureus* with various light sources on titanium dioxide thin film. They found that the bactericidal effect on *S. aureus* under UV or fluorescent light irradiation was better than that under visible light irradiation [6]. The inactivation effect of modified TiO₂ sample activated under visible light (Vis) or LED light irradiation has been intensely studied recently. The valence band of TiO₂ can be changed by different dopants and the spectral response of TiO₂ can be extended from the UV into the visible light region [7–10]. Up to now, the visible-light-induced photocatalytic inactivation of human pathogens by modified TiO₂ has been studied deeply by many research groups around the world. Wong et al. have reported photocatalytic inactivation of *Escherichia coli* cell

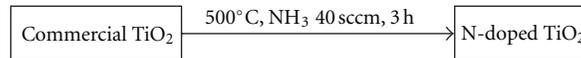


FIGURE 1: The manufacturing process of N-doped TiO₂.

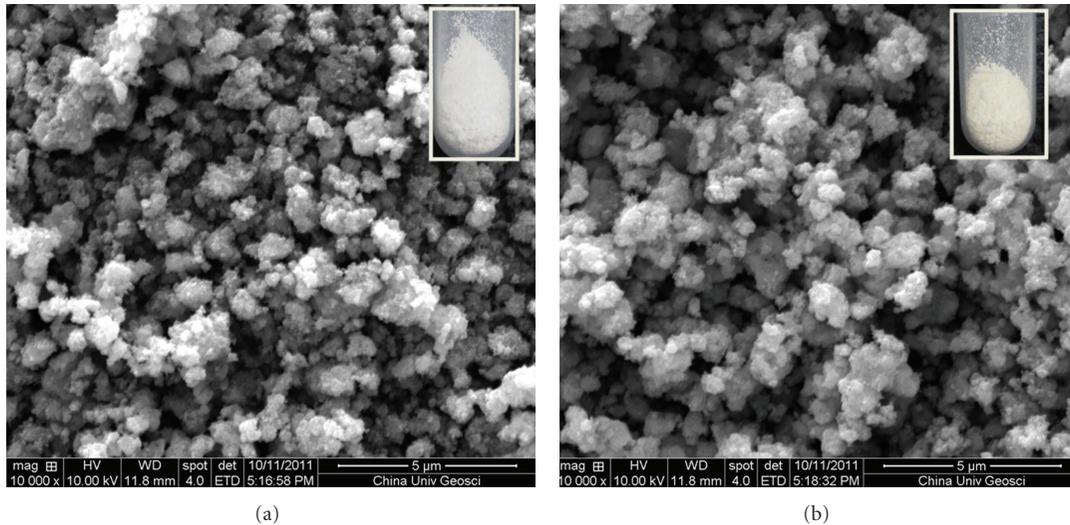


FIGURE 2: SEM image of commercial TiO₂ (a) and N-doped TiO₂ (b) samples.

using N-doped TiO₂ under an incandescent lamp [11]. However, incandescent lamp, with limited working time and low electrooptical conversion efficiency, produces a continuous spectrum of light from near ultraviolet to deep into the infrared. Enwemeka et al. have reported the photo-destroys methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro* using visible 405 nm LED [12]. Those reports directed our attentions to LED light, which may enhance the catalytic efficiency of TiO₂ and has many unique advantages, such as narrow bandwidth, long life span, incredible reliability, and high electrooptical conversion efficiency over incandescent lamp and other visible light source. In addition, bactericidal effect of 405 nm light exposure have been evidenced in many other studies [13, 14], recently.

In this work, we demonstrated the photocatalytic inactivation of the target organisms, *S. aureus* in nutrient broth suspension, using commercial and modified TiO₂ nanoparticles activated under 405 nm LED blue light. Experimental results confirmed that modified TiO₂ nanoparticles activated under 405 nm LED blue light is an ideal indoor purification method.

2. Experimental

2.1. Preparation of N-Doped TiO₂ Nanoparticles and Experimental Suspensions. N-doped TiO₂ was prepared from the vapor phase process (illustrated in Figure 1), using commercial anatase TiO₂ nanoparticles with diameter of 60 nm as the precursor.

Yellow colony of *S. aureus* on a nutrient broth agar plate, cultured at 37°C for 24 hours, was diluted into 500 mL nutrient broth suspension and broken up into 50 equal bacteria suspension samples. Those samples were divided

into five groups equally. Then certain weight of TiO₂ or N-doped TiO₂ nanoparticles will be suspended into the samples of each group. At last, we obtained five different groups with concentration of 1 g/L TiO₂, 5 g/L TiO₂, 1 g/L N-TiO₂, 5 g/L N-TiO₂ and 0 g/L, there were 10 samples in each group. Nutrient broth suspensions with the same concentration of bacteria in the absence of photocatalyst were prepared for contrast experiments.

2.2. Photocatalytic Inactivation Experiment. The obtained suspensions with various concentrations of suspended nanoparticles were transferred into cell culture plate for the photocatalytic inactivation experiments. Cell culture plate was directly exposed to the 405 nm LED light, which was vertically fastened right above and cooled with circulating water. The power density of the LED light irradiation was about 0.2 W/cm². After 3 hours exposure to LED light, the suspensions were collected and characterized by UV-vis spectrophotometer. The absorption optical density of each sample was measured at wavelength region from 240 nm to 800 nm, while the evaluation of activities of TiO₂ and N-doped TiO₂ at inactivation of *S. aureus* was the decrease in OD₆₀₀ values. In practice, nutrient broth suspensions with the same concentration of photocatalyst in the absence of bacteria were prepared for baseline liquid to eliminate the influence of light scattering by nanoparticles suspending in experimental suspensions.

2.3. Characterization. The obtained samples were characterized by scanning electron microscopy (SEM) and UV-vis spectrophotometer (UV-2500, Shimadzu). As shown in Figure 2, commercial TiO₂ powder is white while N-doped

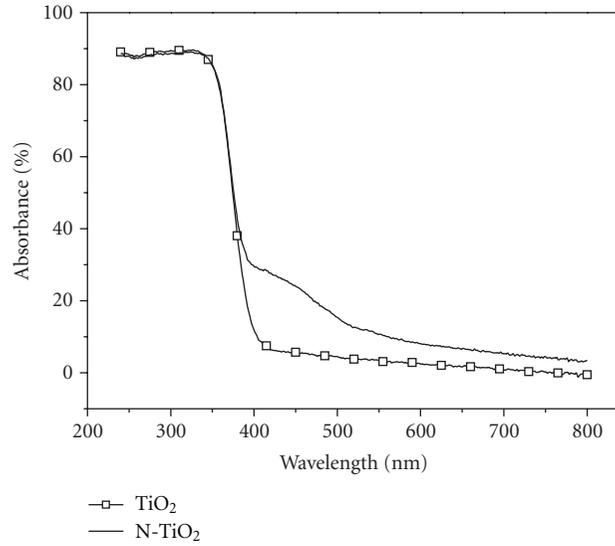


FIGURE 3: UV-vis absorbance spectra of commercial TiO₂ and N-doped TiO₂ samples.

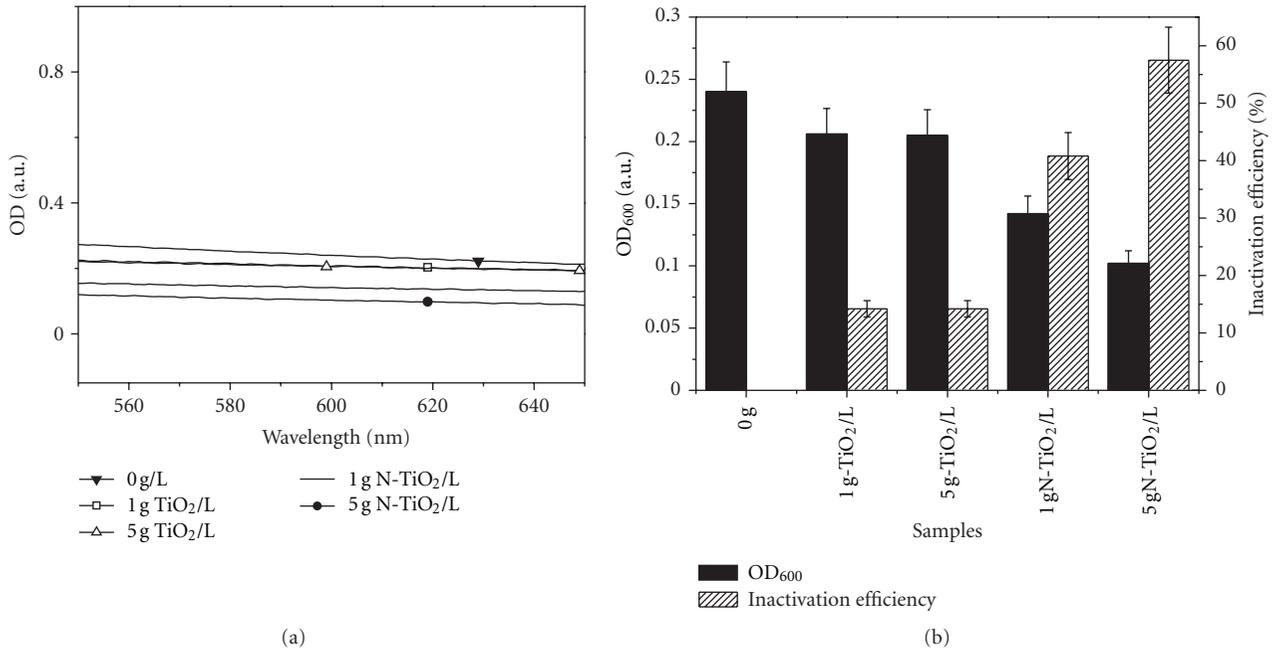


FIGURE 4: Inactivation efficiency of commercial TiO₂ and N-doped TiO₂.

TiO₂ power has a pale yellow color, and both samples are anatase crystalline phase.

The UV-vis absorption spectra of two samples were shown in Figure 3. Clearly, pure TiO₂ particle showed only a strong absorption in UV light region, while the absorption of N-doped TiO₂ particle extended into visible light region.

3. Results and Discussions

3.1. Results. The absorption optical density of experimental and control suspensions were measured in the wavelength ranged from 550 nm to 650 nm and showed in Figure 4(a). The OD₆₀₀ values of suspensions were the evaluation of

activities of commercial TiO₂ and N-doped TiO₂ photocatalyst in inactivation of bacteria. Depended upon the principle of nephelometry [15], the photocatalytic inactivation efficiency (sterilization efficiency) is determined by following formula.

$$R = \frac{A_0 - A_x}{A_0} * 100\%, \tag{1}$$

where R is the photocatalytic inactivation efficiency, A_0 is the OD₆₀₀ value of the control suspension in absence of photocatalyst, and A_x is the OD₆₀₀ of each experimental suspension with photocatalyst. Here in order to eliminate the influencing of light scattering of nanoparticles, A_x was the

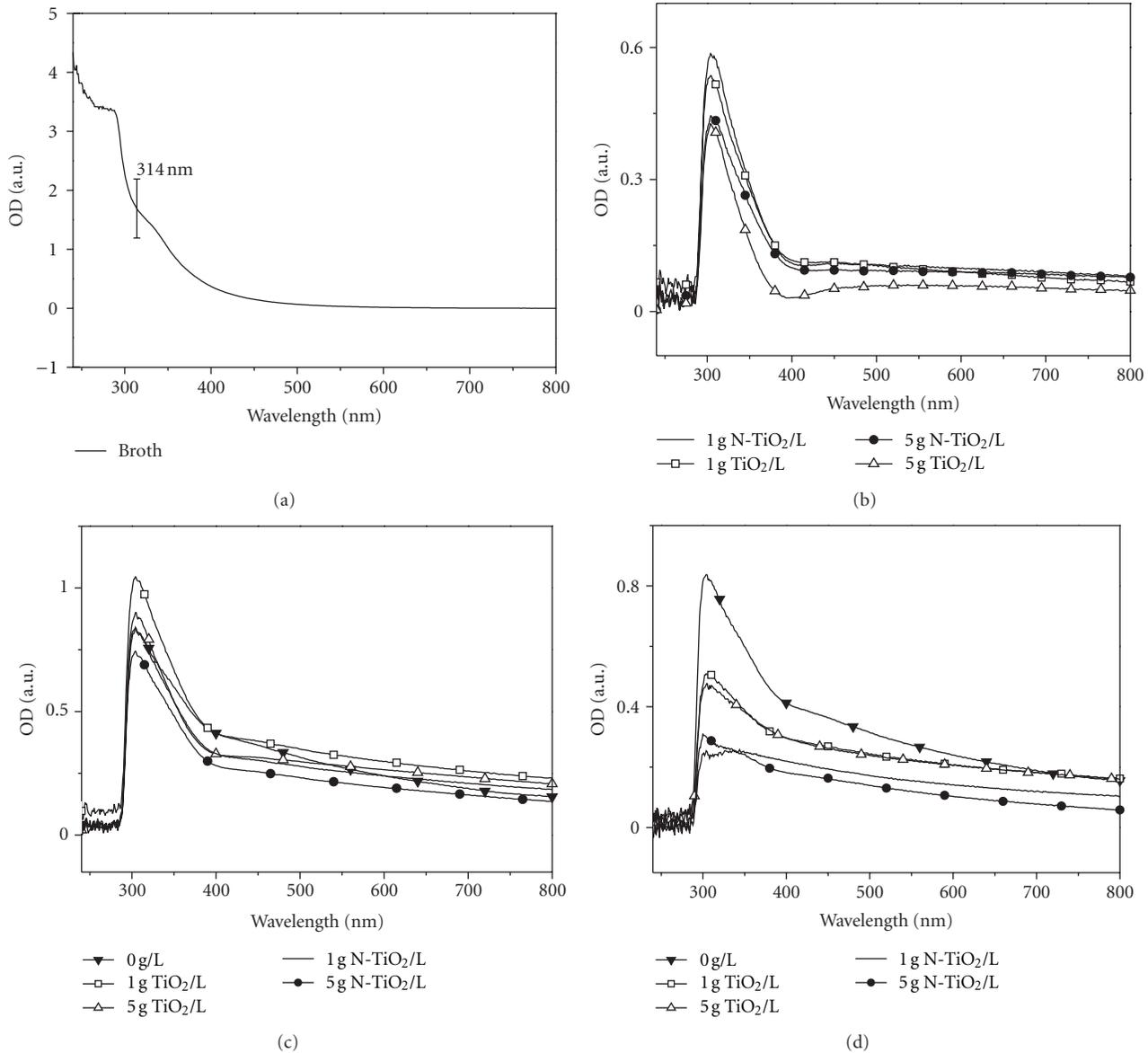


FIGURE 5: The optical density of nutrient broth (a), nutrient with photocatalyst (b), and experiment suspensions (c) exposed to 405 nm LED (0.2 W/cm^2) for 3 hours (employed bare nutrient broth for reference axis), (d) is the difference value between (b) and (c).

difference between the OD_{600} values of each experimental sample and the nutrient broth suspension with suspended nanoparticles at corresponding concentration but in absence of bacteria.

The inactivation efficiency of both commercial TiO₂ and N-doped TiO₂ at different concentrations was shown in Figure 4(b) ($P < 0.05$). According to Figure 4(b), N-doped TiO₂ nanoparticles suspended at 5 g/L exhibits the best inactivation effect, with an inactivation efficiency of about 57.5%, on *S. aureus* under 405 nm LED light irradiation for 3 hours in this work. It's visible that the efficiency was enhanced as the concentration changed from 1 g/L (about 40.8%) to 5 g/L (about 57.5%). Refer to Figure 4(b), the commercial TiO₂ nanoparticles also exhibit inactivation effect on *S. aureus* but with a limited and much lower inactivation efficiency of about 14.2% than that of N-doped

TiO₂ under the same experiment condition. It is due to the different absorption of TiO₂ and N-doped TiO₂ nanoparticles at wavelength 405 nm, where TiO₂ has no absorption but N-doped TiO₂ has (see Figure 3). Although 5 g/L N-doped TiO₂ displayed a very good inactivation effect with an efficiency of about 57.5% in this case, it is still far from the requirement of application in air-purifying and needed to be improved. Nevertheless, energy-efficient LED light source photocatalysis is promising.

3.2. Discussions. The absorption optical density of nutrient broth (Figure 5(a)), nutrient broth suspension with only photocatalyst (Figure 5(b)), and nutrient broth bacteria suspensions (Figure 5(c)) measured from 240 nm to 800 nm was illustrated in Figure 5. Figure 5(d) was the difference between the value of photocatalyst suspension with bacteria

(Figure 5(c)) and without bacteria (Figure 5(b)), in order to eliminate the influence of light scattering of nanoparticles in experiment suspension. According to Figures 5(a)–5(d), all nutrient broth suspension measured showed an absorption peak in 315 nm. The OD value of the nutrient broth suspensions was bigger than 1 even up to 4 at the UV region (refer to Figure 5(a)), which was far beyond the liner region (0–0.4), and both TiO₂ and N-doped nanoparticles have high absorption in UV region (Figure 3). In addition, the nutrient broth was used as the baseline liquid of measurement and there were nanoparticles in the experimental suspension, the high OD value in UV region may influence the measurement accuracy. Thus the OD value in UV region cannot be the evaluation. There were crosses between curves according to Figure 5(c), and the crosses were eliminated in Figure 5(d). Thus, it is necessary to use OD value in Figure 5(d) for inactivation efficiency calculation.

At inactivation of *S. aureus* under 405 nm LED light in our case, the N-doped TiO₂ is much better than TiO₂ but the inactivation efficiency of which is still far from the requirement of application in air-purifying. Although it is feasible to enhance photocatalyst inactivation efficiency of N-doped TiO₂ under 405 nm irradiation by increasing the concentration of photocatalytic, it is wasteful and not environment-friendly to use too much nanoparticles. There is also another way to enhance the photocatalyst inactivation efficiency, which is to increase the N-doped concentration [10]. Of course, there are other factors, such as types of the crystal surfaces [16], types of photocatalytic, and strains of bacteria [17], influencing the inactivation efficiency besides the photocatalytic activity.

4. Conclusions

In summary, we investigated the inactivation effects of pure TiO₂ and N-doped TiO₂ products over *S. aureus* under the irradiation of 405 nm LED light. Under similar conditions, N-doped TiO₂ powder with the increased absorption ability to light shows enhanced activities for inactivation of *S. aureus* than pure TiO₂. Thus, using photocatalytic process with the assistance of LED light is a promising air purification technique in future.

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