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Studies on characteristics of nanostructure of N-TiO_2 thin films and photo-bactericidal action*

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Abstract: *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) from Ming lake were decomposed by photocatalytic nanostructure N-TiO_2 thin films in a photo-reactor under UV irradiation. The different thickness nanostructure N-TiO_2 thin films coated on mesh grid were prepared by sol-gel method and immobilized at 500 °C (films A) or 350 °C (films B) for 1 h in a muffle furnace. The results showed that N-TiO_2 thin film B (8.18 nm thickness, 2.760 nm height and 25.15 nm diameter) has more uniform granular nanostructure and thinner flat texture than N-TiO_2 thin film A (12.17 nm thickness, 3.578 nm height and 27.50 nm diameter). The bactericidal action of N-TiO_2 thin film A and film B for *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis varniger* strain (AS1.439) were investigated in this work. More than 95% of photocatalytic bactericidal efficiency for *Pseudomonas aeruginosa* strain (AS1.50) and 75% for *Bacillus subtilis* strain (AS1.439) were achieved by using N-TiO_2 thin films-B for 70~80 min of irradiation during the photo-bactericidal experimental process. The results indicated that the photo-induced bactericidal efficiency of N-TiO_2 thin films probably depended on the characteristics of the films.

Key words: Photocatalytic degradation, Photo-bactericidal action, N-TiO_2 thin film
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INTRODUCTION

Matsunaga *et al*.(1985) first discovered the bactericidal activity of photocatalytic TiO_2 reactions. Using TiO_2 powder particles in the bactericidal action and photocatalytic degradation for the oxidation of organic and inorganic water pollutants were extensively studied (Michael *et al*., 1999). However, conventional TiO_2 powder catalysts were characterized by difficult separation and reproduction after reaction, so the application of titanium dioxide thin film has attracted much attention in recently years. We investigated the relationship between the antibacterial activity and the properties of the N-TiO_2 thin films, and found that the crystal structure (anatase, rutile) and morphology of particles size are important factors affecting the photocatalytic bactericidal action. The viability of *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) during the N-TiO_2 photocatalytic reactions are also discussed.

MATERIALS AND METHODS

Preparation of nanostructure N-TiO_2 thin film

The nanostructure N-TiO_2 thin film was prepared by using the sol-gel method (Fig.1). A certain amount of tetrabutylorthotitanate and acetylacetone were mixed together first, then ethanol was added with vigorous stirring for 1 h at room temperature. After that, distilled water and nitric acid were added under stirring into the above solution. The resultant alkoxide solution was further mixed with carbamide/chloroethylamine solution for 1 h and preserved at room temperature for full hydrolysis to form the N-TiO_2 colloidal sol.

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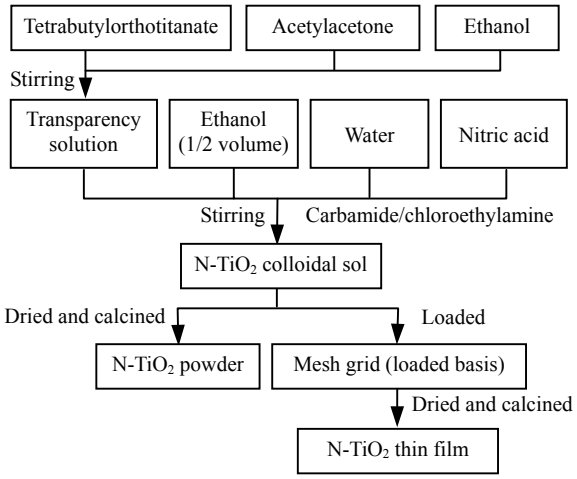


Fig.1 Preparation schematic of N-TiO_2 powder and films

The mesh grid (156.2 mm×25.4 mm×1.5 mm), which was pretreated as the loaded basis for thin films, was dipped into the N-TiO_2 colloidal sol solution for 2 min, then withdraw from the solution at rate of 1 mm/s. The film was formed on mesh grid and dried at ambient temperature for 24 h. The mesh grid coated with gel films was treated at different temperature (film A at 500 °C and film B at 350 °C/500 °C) for 1 h in a muffle furnace.

For film A, the mesh grid coated with the gel was sintered at 500 °C for 1 h with temperature raising rate of 3 °C/min in the muffle furnace, then repeatedly coated and calcined several times and finally sintered at 500 °C. For film B, the mesh grid coated with the gel films was sintered at 350 °C for 1 h, then repeatedly coated and calcined several times, with the last coating film being sintered at 500 °C for 1 h. Different thickness films were obtained by repeating the above process several times.

Characteristics of N-TiO_2 films

The surface morphology of the films and nanoparticles were determined by atomic force microscope (AFM, Dimension 3100, American DI). The crystallite size and phase of the N-TiO_2 thin films were determined by BDX3300 X-ray diffraction (XRD) measurements, carried out with Cu K_α radiation (λ=1.54 nm). The accelerating voltage and the applied current were 40 kV and 40 mA, respectively.

Setup of photocatalytic reactor system

Fig.2 is a schematic diagram of the experimental apparatus. The photo-reactor with cooling jacket was assembled with UV light (mercury lamp 20 W, 30 cm long at wavelength 365 nm), covered with 3 mm thick transparent quartz glass cannula and immersed in the reaction solution at a constant temperature, used as a source of UV radiation. Sterile compressed air was bubbled into the reactor through air line. The incident UV radiation for the reactor was measured by ultraviolet luminometer.

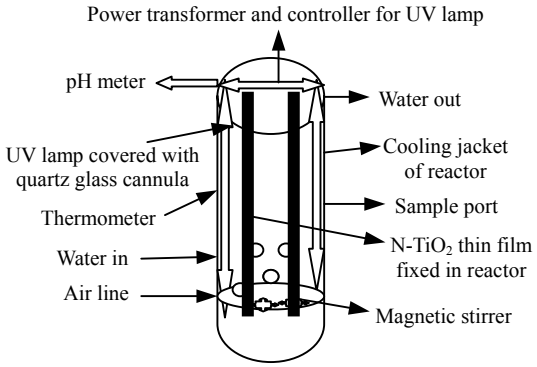


Fig.2 Diagram of photo-degradation reactor

Cultivation of microbial strain

Pseudomonas aeruginosa strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) were isolated from lake water and grown aerobically in 500 ml liquid nutrient broth at 37 °C on a rotary shaker (120 r/min) for 16 h. The medium was autoclaved at 121 °C for 30 min to ensure the sterility for testing. *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) cells were cultivated for 2 d and harvested by centrifugation at 8000×g for 10 min, washed and resuspended in the 1000 ml phosphate-buffer solution (0.2 mol/L, pH=7.0). The number of viable cells in the suspensions samples was counted by using the spread plate colony-counting method. Serial dilution of the cells was performed to obtain initial concentration of 10^6 colonies forming units per milliliter (CFU/ml) for photocatalytic bactericidal experiment.

Photocatalytic bactericidal action procedure and cell viability assay

The 750 ml cells-suspended solution with initial concentration of 10^6 CFU/ml in 1 L reactor was illuminated by irradiation of 20 WUV-Hg lamps (GGZ-20 W) with emitting spectral maximum at 365 nm peak wavelength. The light intensity reaching the

surface of the films fixed at the glass reactor was approximately 18 W/m^2, which was measured by UV-meter with the peak intensity at 365 nm (model J-1221, UVP Inc. USA). N-TiO_2 film A or N-TiO_2 film B was placed into the reactor. Aliquots of cells solution were taken at 15 min intervals after irradiation with continuous stirring magnetically for uniform distribution, were added into the tube containing phosphate buffer solution for CFU testing.

The CFU were determined by plating aliquots of serially diluted suspensions on duplicate nutrient agar plates. All plates were incubated for 24 h at 30 °C, the viable number of cells were counted. The loss of cell viability was examined by the viable count procedure. All experiments were performed at least in triplicate.

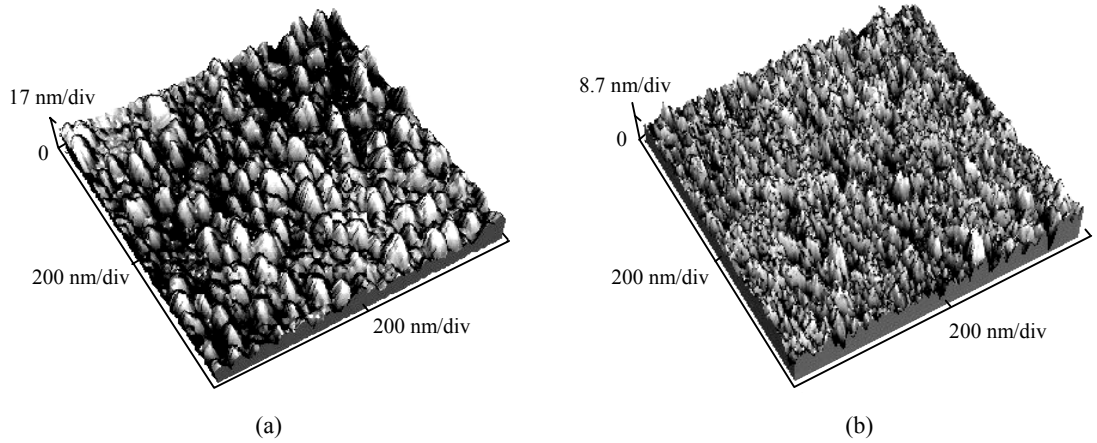
RESULTS AND DISCUSSION

Characteristics of the nanostructure N-TiO_2 thin films

It is well known that the films surface morphology is dependent on the calcining treatment temperature. Different size particles on the N-TiO_2 films will be obtained at different sintering temperature for the immobilized films treatment. The film styles formed were divided into film A and film B based on a different sintering temperature. The results are shown in Fig.3.

Fig.3 is the AFM images of the N-TiO_2 thin films with five layers in film A and film B. N-TiO_2 film B has more uniform granular microstructure and thinner flat texture than N-TiO_2 film A (Fig.3). The thickness and the average diameter of the particle size of the film were 12.17 nm and 27.50 nm for film A, 8.18 nm and 25.15 nm for film B respectively determined by AFM and XRD technique. The granular microstructure is the key factor influencing the photocatalytic activity (Jang *et al*., 2001). In the N-TiO_2 film B, the mass fraction of crystallitic anatase present in granular nanostructure are mostly up to 94.8% higher than 65.2% of mass fraction in the N-TiO_2 film A.

Zarzychi *et al*.(1982) explained the mechanism of the particles growth of gel films in the calcining process. From Fig.4 shows that during calcining treatment for the film A process, there are many H_2O moleculars and hydroxyl groups forming hydrogen bonds on the sol-gel surface of the N-TiO_2 film coated on mesh grid, when N-TiO_2 gel films were treated at 500 °C five times, hydrogen bonds between the two molecules would turn into chemical covalent bond during sintering process, then link close N-TiO_2 crystallitic particles tightly, result in the particles growing and solid cluster conglomeration, consequently, lead to the rapidly increasing number of larger diameter particles. In film B, N-TiO_2 thin film (8.18 nm thickness, 2.760 nm height and 25.15 nm average nanoparticle diameter) has more uniform granular microstructure and thinner flat texture than N-TiO_2 thin film A (12.17 nm thickness, 3.578 nm height and 27.50 nm average nanoparticle diameter) because low sintering temperature decreases the coagulation and growth rate of particles, hence, the mass



(a) (b)

Fig.3 AFM images of the N-TiO_2 thin films. (a) Film A (at 500 °C, five layers); (b) Film B (at 350 °C/500 °C, five layers)

fraction of N-TiO_2 anatase nanostructure has more reactive center in N-TiO_2 thin film B than in N-TiO_2 thin film A, which is probably due to the smaller size of anatase nanoparticle.

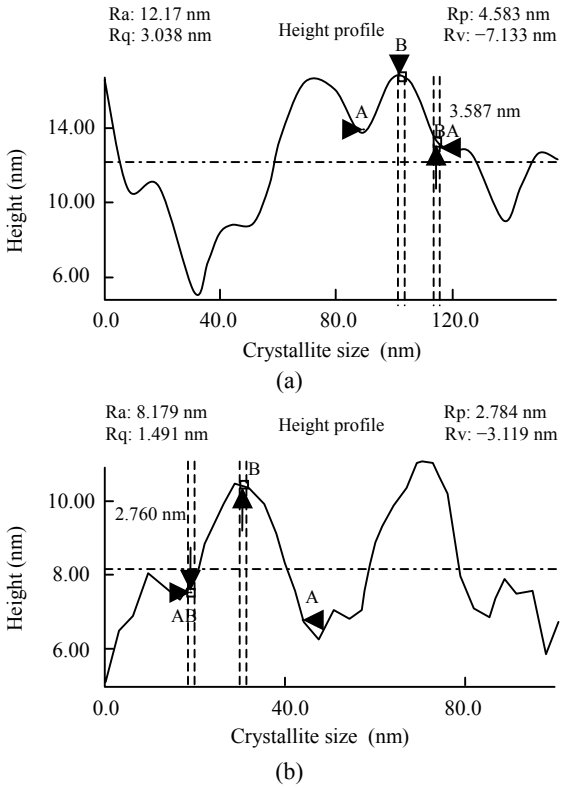


Fig.4 The size of nanoparticle on N-TiO_2 thin film for (a) film A (at 500 °C, five layers) and (b) film B (at 350 °C/500 °C, five layers)

Effect of N-TiO_2 nanoparticles of thin film on photocatalytic bactericidal activity

Pseudomonas aeruginosa strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) cells inactivation was found to be rapid in the presence of nanostructured N-TiO_2 films during UV irradiation.

Fig.5 shows the effect of illuminated N-TiO_2 thin films B (8.18 nm thickness) on the sterilization of *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis* strain (AS1.439) in comparison with N-TiO_2 thin films A (12.17 nm thickness) under the conditions of initial concentration of 10^6 CFU/ml, 18 W/m^2 of UV intensity. The viability of cells is determined by colonies counting. The survival curves are shown in Fig.5 for *Pseudomonas aeruginosa* strain (AS1.50) and *Bacillus subtilis* strain (AS1.439), respectively.

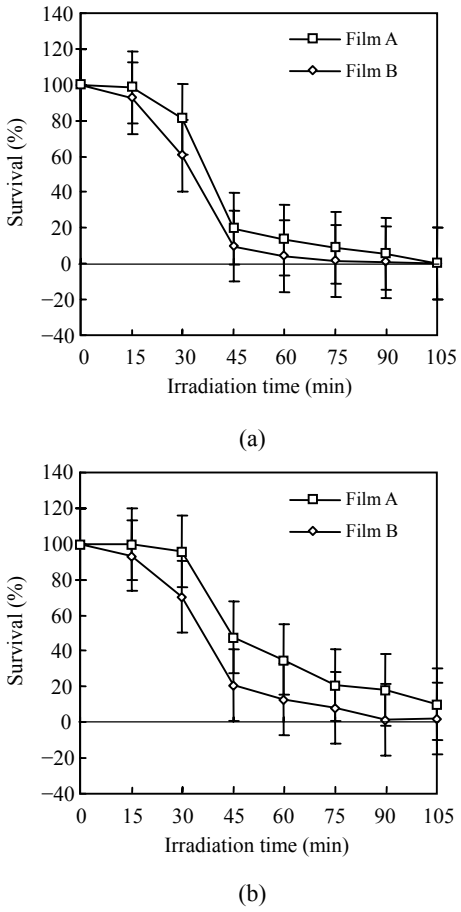


Fig.5 Effect of the N-TiO_2 films on the viability of (a) *Pseudomonas aeruginosa* strain (AS1.50) and (b) *Bacillus subtilis* strain (AS1.43)

More than 95% photocatalytic sterilization rate for *Pseudomonas aeruginosa* strain (AS1.50) and 75% for *Bacillus subtilis* strain (AS1.43) were achieved by using N-TiO_2 thin films B for 70~80 min of irradiation during the photo-bactericidal experimental process. The results indicated that the anti-bactericidal effect of N-TiO_2 film was associated directly with the thickness of films (Trapalis *et al*., 2003). The photo-bactericidal action no *Bacillus subtilis* strain (AS1.439) and *Pseudomonas aeruginosa* strain (AS1.50) treated by N-TiO_2 thin films B was shown to be more effectively than N-TiO_2 thin films A. *Bacillus subtilis* strain (AS1.439) showed stronger resistance to photocatalytic sterilization than the *Pseudomonas aeruginosa* strain (AS1.50) (Fig.5).

The photocatalytic chemical reaction mainly occurs on the surface of the films, indicating that the photocatalytic reaction mainly occurs on the specific

particles size, the photocatalytic activity is enhanced with decreasing nanoparticles diameter and increasing contact area between the photocatalyst and target material (Maness *et al*., 1999). In addition, the higher temperature treatment could not only cause the aggregation of N-TiO_2 particles, but also partially change crystalline phase from the anatase to rutile which probably result in losing some active sites of the films for photocatalytic reaction during different thermal treatment (Trapalis *et al*., 2003). The experimental result suggested photo-induced bactericidal efficiency of N-TiO_2 was affected by the morphology and thickness of N-TiO_2 films, the grain size of nanoparticles, surface area, mass anatase composition of N-TiO_2.

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