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Performance and mechanism of standard nano-TiO_2 (P-25) in photocatalytic disinfection of foodborne microorganisms – *Salmonella typhimurium* and *Listeria monocytogenes*

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ABSTRACT

Under UV light, nano-TiO_2 is effective in photocatalytic disinfection. In this paper, we studied the disinfection effects of nano-TiO_2 on the two typical food-borne microorganisms, Gram-negative bacterium-*Salmonella typhimurium* and Gram-positive bacterium-*Listeria monocytogenes*, in meat products. Results show that nano-TiO_2 had a strong disinfecting activity against both Gram-negative and Gram-positive pathogens in a suspension under UV light. *L. monocytogenes* was more resistant to nano-TiO_2 treatment than *Salmonella* under UV light. Nano-TiO_2 concentrations and initial bacteria populations had significant influence on the photocatalytic disinfection effectiveness against *S. typhimurium*. The optimum concentration (1.0 g/L) was between 0.2 g/L and 1.5 g/L. Increasing *S. typhimurium* population from 10^4 to 10^7 CFU/mL resulted in reduced photocatalytic disinfecting effectiveness by nano-TiO_2. Electron microscope images revealed that nano-TiO_2 photocatalytic disinfection starts with damaging the cell walls of bacteria. With serious destructions of cell walls, cell components released or defused out of cell from the damaged areas, and finally the cells completely lost their integrity and dissolved. These results demonstrate that nano-TiO_2 is very effective against pathogens that can grow well on meat products and the effectiveness can be significantly influenced by nano-TiO_2 contents and pathogen populations. The findings by these experiments provide the essential information for further developing a nano-metal-based, antimicrobial packaging system to improve safety of meat products.

1. Introduction

TiO_2 in the anatase crystal form is a semiconductor with a band gap of 3.2 eV or more (Maness et al., 1999). Upon excitation by UV light, it can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals (•OH), or reduce O_2 to produce superoxide ions. Other reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and singlet oxygen, have also been detected in the presence of UV light and TiO_2 (Carp, Huisman, & Reller, 2004). All of the ROS can cause damages to live cells. In 1985, Matsunaga et al. firstly reported that nano-TiO_2 could disinfect *Escherichia coli* through photocatalysis (Matsunga 1985; Matsunaga, Tomoda, Nakajima, & Wake, 1985). Since then many studies have been conducted to evaluate photocatalytic disinfection activities of nano-TiO_2. In 1997, Mills et al. concluded that nano-TiO_2 under UV light could result in formation of reactive oxygen species (ROS) and effectively disinfect microorganisms (Mills & Le Hunte, 1997). Cho et al. found that hydroxyl radicals (•OH) generated by nano-TiO_2 under UV light were more effective in inactivating *E. coli* than chemical disinfectants like chlorine and ozone (Cho, Chung, Choi, & Yoon, 2004, 2005). More and more experimental data demonstrate that photocatalysis by nano-TiO_2 can effectively disinfect microbes, including fungi (Hur et al., 2005), bacteria (Rincón & Pulgarin, 2004a, 2004b) and virus (Sjogren & Sierka, 1994) in both water and air. It has been now applied in water sterilization (Chong, Jin, Chow, & Saint, 2010) and in production of self-cleaning glasses (Gamage & Zhang, 2010).

Salmonella and *Listeria* are responsible for many outbreaks and commonly contaminate raw and ready-to-eat meat products. *Salmonella typhimurium* is a Gram-negative, rod-shaped bacterium. The most common risk factor for *S. typhimurium* is ill prepared food, such as undercooked meat. Handling uncooked

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meat may contaminant hands, plates, kitchenware, and grounding the meat may also cause multiplication of the bacteria. More than 80% of the salmonellosis occurred globally results from *S. Typhimurium* and *Salmonella Enteritidis*. *Listeria monocytogenes* is a Gram-positive, non-spore forming, and facultative rod bacterium which grows between −0.4 and 50 °C. *L. monocytogenes* can be found in a variety of raw foods, including uncooked meats, as well as in processed meat that become contaminated after processing. It has been recognized as a human pathogen for 60 years. In the United States, an estimated 2000 persons become seriously ill with listeriosis each year. Of these, 500 die. To ensure food safety, various antimicrobial interventions have been approved and are being applied in meat processing to reduce the populations and restrict the growth of these pathogens. However, no single method has been proven to be fully effective and can result in the total elimination of *Salmonella* and *Listeria* in meat products during meat processing. Therefore, there is a tremendous need for additional interventions to use along with existing preventive measures. The use of antimicrobial packaging appears to be one of the most promising interventions.

The incorporation of TiO_2 nanoparticles into (ethylene-vinyl alcohol)-based food packaging copolymers affords an opportunity to synthesize polymer-based nanocomposite materials with novel and powerful antimicrobial and photodegradability properties. Experiments have shown that the materials displayed an unprecedented performance in the killing of both Gram positive (*Enterococcus faecalis*) and negative bacteria (*Pseudomonas aeruginosa*) without the necessity of being release to the medium (Kubacka et al., 2007). The objective of this study was to investigate effects of UV exposure time, nano-TiO_2 concentrations, and initial bacteria populations on nano-TiO_2 photocatalytic disinfection against Gram-negative pathogen bacteria *S. typhimurium* and Gram-positive bacteria *L. monocytogenes*, the two most typical pathogens found in meat products. The effect of nano-TiO_2 on individual bacterial cells was also examined with TEM (transmission electron microscope) to help further understanding antimicrobial mechanism by TiO_2 under UV light. This study is the first step of a series of studies that will lead to establishment of nano-metal-based, antimicrobial packaging system for meat products.

2. Materials and methods

2.1. Materials

Standard crystal phase nano-TiO_2 powder is Degussa P25 (Degussa Ltd., Germany; 87% anatase, 13% rutile-phase, 20–30 nm average size, 50 ± 15 m^2/g surface area).

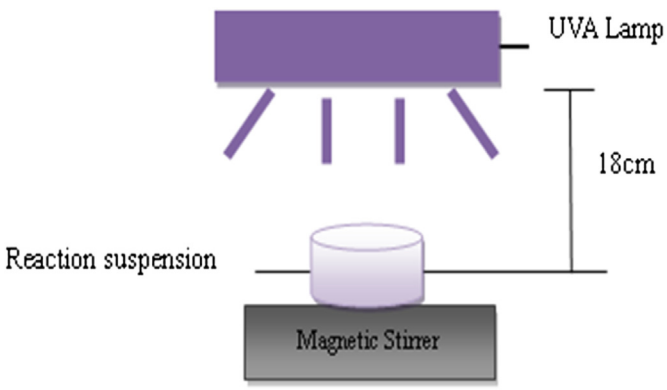

Fig. 1. Diagram of nano-TiO_2 photocatalytic disinfection system.

2.2. Bacterial strains

S. typhimurium (CMCC50115) was purchased from GangDong Engineer and Technology Research and Development Center of Microbial and *L. monocytogenes* (ATCC19114) was purchased from QingDao Hope Bio-Technology Co. LTD.

2.3. Medium

TSB (tryptic soy broth) liquid medium, containing 1.5% tryptone (g/100 ml), 0.5% soy peptone (g/100 ml) and 0.5% NaCl (g/100 ml), was purchased from Sigma.

TSA (tryptic soy agar) solid medium, containing 1.5% tryptone (g/100 ml), 0.5% soy peptone (g/100 ml), 0.5% NaCl (g/100 ml) and 1.3% agars (g/100 ml), was purchased from Sigma.

3. Methods

3.1. Culture of bacterial strains

Individual strains (0.1–0.2 mL of suspensions) were cultivated aerobically in TSB liquid medium in bed temperature incubator at 37 °C for 18 h and each culture was harvested by centrifugation (3000 *g* for 10 min at 4 °C).

3.2. Preparation of TiO_2 suspension

Certain amounts of Nano-TiO_2 powders were weighed and added in 0.9% NaCl aqueous solution to make TiO_2 suspensions with the concentrations specified in the experimental design. The suspensions were autoclaved at 121 °C for 20 min before used for photocatalytically disinfecting treatment.

3.3. Photocatalytic disinfection of pathogen strains by nano-TiO_2

Cultured bacterial strains were mixed with the nano-TiO_2 suspension in 80 mL beaker in dark based on the experimental design for different treatments. For UV light control, 0.9% NaCl aqueous solution only was used. The reaction suspensions were laced on the thermostatic magnetic stirrer and were 18 cm away from an UV lamp Philips, LEA-180B, 365 nm, 8W) (the setting is shown in Fig. 1). This setting was put on a dark chamber in our laboratory. Before exposed to UV light, the suspensions were mixed for 20 min by magnetically stirring to ensure the suspending solution to be formed. The samples of 1 mL were transferred into the 1.5 mL EP tube at each specified times. We investigated the influence of treatment duration, nano-TiO_2 concentrations, and initial bacterial populations on nano-TiO_2 photocatalytic disinfection.

3.4. Microbial analysis

Serial dilutions were prepared in 9 mL of 0.9% NaCl solution. From each dilution, 100 μL of aliquots were withdrawn and surface-plated onto TBA plates. The plates were incubated at 37 °C for 18 h before typical colonies of *Salmonella* and *Listeria* were counted. All microbial counts were reported as CFU/mL values.

3.5. Transmission electron microscopy analysis

Samples of *S. typhimurium* and *L. monocytogenes* were removed from the reaction suspensions at different times (0–180 min) during photocatalytic treatment, and were to be preprocessed according to the standard sample preparation procedure for TEM (Philips, Tecnai 12, CM120 kV, The Netherlands). Bacterial cells were examined with TEM.