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Synergistic interaction of ultraviolet light and zinc oxide photosensitizer for enhanced microbial inactivation in simulated wash-water

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ABSTRACT

Synergistic interaction of ultraviolet light (UV-A) and zinc oxide (ZnO) was investigated for enhanced inactivation of *Escherichia coli* BL21 and T7 bacteriophage in simulated wash-water. In the absence of organic content, UV-A (9.53 J/cm^2) and 1 mM ZnO alone caused 3.9 and 0.7 log CFU/mL reductions respectively in logarithmic phase bacteria after 60 min, while a combined treatment caused 6 log CFU/mL reductions. Stationary-phase bacteria were more resistant and a combined treatment caused only 3.5 log CFU/mL reductions. Organic matter in the wash-water lowered the inactivation rates. Nevertheless, approximately 2-log reductions were observed at the highest organic load. T7 bacteriophage was not sensitive to UV-A alone. However, 1 mM photo-irradiated ZnO caused 6.00 log PFU/mL reductions after 60 min. Bacteriophage inactivation was also significantly lowered by organic matter. The reactive oxygen species generated from photo-irradiated ZnO were responsible for the microbial inactivation. UV-A irradiated ZnO is an attractive sanitation approach for fresh-produce washing.
Industrial relevance: Chlorine-based sanitizers that are conventionally used for washing fresh-produce suffer significant limitations including occupational hazard for workers from over-exposure to chlorine and safety hazards to the population due to formation of chlorinated organic matter. This study highlights that UV-A irradiated ZnO is a promising alternative to sanitize wash-water and fresh-produce and reduce the risk of bacterial as well as viral cross-contamination. Future studies are needed to optimize and scale-up this process for industrial use.

1. Introduction

Food safety is a significant public health concern in the world. In the US alone, each year approximately 1 out of 6 person gets sick, 128,000 are hospitalized, and 3000 die of foodborne diseases (CDC, 2011). The most common causes of foodborne illness are viral or bacterial contamination of foods (Pigott, 2008). Raw fruits and vegetables are the second leading cause of food-borne illnesses, which also costs the US economy $ 6.9 billion in the loss of productivity and medical expenses per year (Luksiene & Brovko, 2013).

Decontamination of fresh-produce is typically carried out during the washing operation by incorporation of chemical sanitizing agents such as chlorine, iodophors, and quaternary ammonium compounds (Luksiene & Brovko, 2013). However, chemical treatment is not environmentally friendly and has been associated with occupational and operational hazards. The fresh-cut produce industry commonly uses sodium hypochlorite as sanitizing agent, but by-products such as trihalometanes and chloramines formed by the interaction between the organic matter in the wash-water and sodium hypochlorite are potentially harmful for humans (Ramaswamy, Ahn, Balasubramaniam, Rodriguez Saona, & Yousef, 2013; Tirpanalan, Zunabovic, Domig, & Kneifel, 2011; Luksiene & Brovko, 2013). Moreover, the efficacy of chlorine decreases in the presence of organic content through binding with organic materials (Tirpanalan et al., 2011; Betts & Everis, 2005). For example, while Handojo, Lee, Hipp, and Pascall (2009) found that 100 ppm of chlorine can inactivate *Escherichia coli* K-12 and *Staphylococcus epidermidis* by 5 log CFU/mL in the absence of organic content, another study reported only 1.2 log CFU/mL reductions in *E. coli* O157:H7 during washing of lettuce using 200 ppm of chlorine in 10 min (Kondo, Murata, & Isshiki, 2006). Similar to the effect on bacterial inactivation, Feliciano, Li, Lee, and Pascall (2012) observed that the organic content decreased the effect of chlorine on norovirus (MNV-1) as well. Therefore, there is a need for new technologies that can effectively inactivate bacteria and viruses in wash-water.

Photosensitized materials have been used for their antibacterial and antiviral activities in medical and environmental applications (Allison, Mang, Wilson, & Vongtama, 1998; Banfi et al., 2006; Omar, Wilson, & Nair, 2008; Murakami et al., 2015). Interaction of UV-A light with

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endogenous or exogenous photosensitizers can generate reactive oxygen species (ROS) that cause damage to cell membrane, protein, and DNA structures, resulting in cell death. Generation of ROS can also address a critical limitation of UV light alone such as low penetration depth within the medium in the presence of organic load (Ngadi, Smith, & Cayouette, 2003). However, this approach has not been explored significantly in the food industry. The key questions related to applications of photosensitizers in food systems include: (a) the role of organic content in limiting activation of photosensitizers and the resulting microbial decay; (b) the influence of physiological state of microbes in influencing activity of photosensitizers; and (c) the ability of photosensitizers to manage bacterial and viral contaminants in food relevant environment.

ZnO is an inorganic metal oxide, which acts as a photosensitizer in its powder or nanoparticle form under UV-A light (Liu & Yang, 2003; Kairyte, Kadys, & Luksiene, 2013). In addition to its photo-activity, ZnO particles can act as an antimicrobial compound in the dark, possibly due to binding of the particles on the bacterial surface through the electrostatic forces (Zhang, Jiang, Ding, Povey, & York, 2006). ZnO particle at nano and micrometer size itself has been extensively evaluated for its antimicrobial activity (Sawai & Yoshikawa, 2004; Jin, Sun, Su, Zhang & Sue, 2009; Adams, Lyon, & Alvarez, 2006; Huang et al., 2008; Jones, Ray, Ranjit, & Manna, 2008). ZnO particles have been found to have bactericidal property against *Salmonella spp.* (Jin, Sun, Su, Zhang, & Sue, 2009), *Streptococcus spp.*, *Staphylococcus spp.* (Huang et al., 2008), *E. coli* (Zhang et al., 2006; Padmavathy & Vijayaraghavan, 2008; Zhang et al., 2006), *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Rhizopus stolonifer* (Sawai & Yoshikawa, 2004). Moreover, recently ZnO particles were modified by doping with Al, Ta, or Ag to increase their photocatalytic and antimicrobial activities (Guo et al., 2015; Karunakaran, Rajeswari, & Gomathisankar, 2011; Zhang, Hong, Chen, Feng, & Badami, 2014). However, a simultaneous treatment of UV-A and ZnO particles to sanitize wash-water used for produce washing has not been well characterized. ZnO particles including nanoparticles are safe and biocompatible (Zhou, Xu, & Wang, 2006) and FDA has listed ZnO powder as GRAS (generally recognized as safe) (SCOGS,FDA). However, the regulatory status of the nano and micrometer sized ZnO particles is unclear.

Our aim was to investigate the UV-A irradiated ZnO particles based process to inactivate bacteria (*E. coli* BL21) and viruses (T7 phage) in the presence of different organic content to simulate the wash-water used for washing of fresh-produce. To develop further insights into the mechanism of action of photo-irradiated ZnO, membrane damage in bacteria and the generation of reactive oxygen species were also evaluated.

2. Materials and methods

2.1. Materials

Luria–Bertani (LB) broth and agar (LBA) were purchased from Fisher BioReagents (Pittsburgh, PA). NaCl, ZnO, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO). The probes including 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA), and Sytox-Orange were purchased from Life Technologies (Eugene, OR). Hydroxyphenyl fluorescein (HPF) probe was purchased from Cayman Chemical (Ann Arbor, MI). *E. coli* BL21 (ATCC #BAA-1025) and T7 phages (ATCC #BAA-1025-B2) were purchased from ATCC (Manassas, VA). Chemical oxygen demand (COD) standard solution (1000 mg/L) and COD digestion vials (high range) were obtained from Hach Company (Loveland, CO).

2.2. Simulated wash-water

LB broth was used to simulate organic matter usually present in fresh produce wash-water. The concentrations of LB broth used were 200, 600, and 1000 mg/L to mimic varying organic load. The sample without organic content was prepared with 0.9% NaCl to provide osmotic balance for the microorganisms. The chemical oxygen demand (COD) of this simulated wash-water was measured using a US-EPA reactor digestion method (Standard Method 5220 D, USEPA). Two milliliters each of simulated wash-water samples and the COD standard solutions were digested in high range COD digestion vials at 150 °C in DRB200 Reactor (Hach Company, Loveland, CO) for 2 h. Then, the digested samples and standards were cooled down to room temperature and the COD values were determined by a colorimetric method at 620 nm using a colorimeter (Hach Company, Loveland, CO). The COD values of the samples were calculated from the interpolation on the standard curve obtained from the absorbance values of the standards.

2.3. Microbiological cultivation

E. coli BL21 was grown in 10 mL of LB broth for 3 h and 24 h at 37 °C to obtain the bacterial population in logarithmic (absorbance of 0.4 at 600 nm) and stationary phases (absorbance of 1.5 at 600 nm), respectively. The culture of *E. coli* BL21 grown in LB broth was washed twice with 0.9% NaCl to remove the adsorbed culture medium components, followed by a centrifugal separation for 2 min at 10,000 × *g* and 4 °C. After the washing step, the pellet was re-suspended in simulated wash-water of varying organic loads (0–1000 mg/L LB). The initial population of *E. coli* BL21 was adjusted to 1×10^6 CFU/mL for both stationary and logarithmic phase cultures.

T7 phages were used as a model for non-enveloped, double-strand DNA viruses. The stock phage sample was prepared by incubating the phage and the mid-log phase culture of *E. coli* BL21 in LB broth in a shaking incubator at 37 °C and 250 rpm for 4 h. Then, 20% *v/v* chloroform was added to lyse the remaining bacteria and the lysate was incubated at 37 °C for 20 min with 150 rpm shaking. The solution was centrifuged at 5652 × *g* for 10 min, the supernatant was recovered and vacuum filtered with a 0.45 μm filter. The phage activity of stock phage sample was approximately 1×10^{11} PFU/mL.

2.4. ZnO and UV- A treatment

A bench-top, batch type equipment was used for the UV-A light treatment. The apparatus consisted of four UV-A lamps (320–400 nm, 18 W, Actinic BL, Philips N.V.) mounted on the ceiling of a closed box (Fig. 1). The predominant wavelength range emitted by the lamp was 340 to 400 nm with a peak at 360 nm. Two milliliters of the test sample was placed in an individual well of a 12-well plate and the entire plate was placed under the UV- A lamps enclosed within the box. The depth of the sample was 1 cm. The distance between the lamps and the surface of the solution within the wells was 8.0 cm. The average incident UV-A intensity measured at the location where the well-plates were kept was 2646 ± 212 μW/cm^2. Test solution consisted of ZnO (0, 0.1, 1, 5 mM) sonicated for 1 min (100 W, 42 kHz) to disintegrate the aggregates, mixed with the simulated wash water of varying organic load containing 1×10^6 CFU/mL of bacteria or 1×10^7 PFU/mL of T-7 phage. The

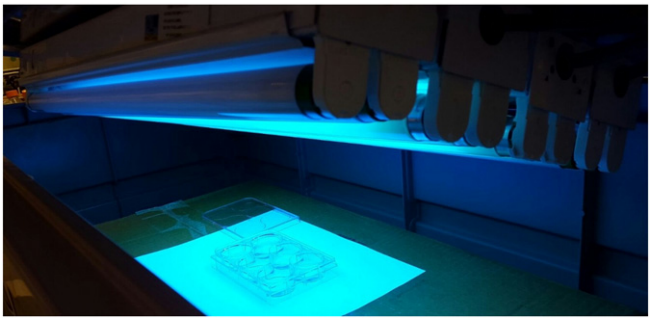

Fig. 1. A photograph of the bench-top photo-reactor.