WALAILAK JOURNAL
http://wjst.wu.ac.th Agricultural Technology and Biological Sciences

Starch Based Nanocomposites in Active Packaging for Extended Shelf Life of Fresh Fruits

Somruthai TUNMA

Chemistry Program, Faculty of Education, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

(Corresponding author's e-mail: somruthai.tun@crru.ac.th, stunma@hotmail.com)

Received: 27 April 2017, Revised: 15 November 2017, Accepted: 26 December 2017

Abstract

Starch based packaging materials are made from cassava starch with increased antimicrobial activity of titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles. The objectives of this work were to evaluate the quantities of cassava starch, vinegar, glycerin, and water used for 10×10 cm^2 sheet film molding, and to investigate the antimicrobial activity of starch-nanoparticles based films. TiO_2 and ZnO nanoparticles were added into an aqueous starch solution at 0.01, 0.03, and 0.05 %w/w. The antimicrobial activity testing indicated that no inhibition zone against microorganisms was observable for film without the incorporation of nanoparticles. Inhibition areas were yielded by films coated with all concentrations of TiO_2 and ZnO (10 mm for 1×1 cm^2 and 20 mm for 2×2 cm^2 dimensions of films). The results showed that starch films mixed with 0.01 %w/w of TiO_2 were able to extend the shelf life of bananas from 5 - 7 days to 14 days, and tomatoes from 7 - 10 days to 21 days. The biodegradation test of starch film was carried out under the real condition of landfills. The result showed that all starch based films appeared to decompose in the soil within 14 days. The UV-vis spectrum of soil from landfill area containing nanoparticle-starch composites showed no TiO_2 or ZnO absorbance spectra; there was no nanoparticle residue in soil. Starch based nanocomposite films could be used for active packaging applications for post-harvest produce.

Keywords: Starch film, titanium dioxide, zinc oxide, cassava starch, antimicrobial activity

Introduction

Biobased packaging materials are defined as packaging materials derived from renewable sources, such as starch, cellulose, casein, and wheat gluten [1]. Generally, biobased plastics are potentially biodegradable and environment friendly. Food packaging exists to make our lives easier; packaging is needed to contain foods, and to protect foods from the outside environment. Packaging provides protection for food from adulteration by water, gases, microorganisms, and dust. Active packaging is accurately defined as "packaging in which subsidiary constituents have been deliberately included either in or on the packaging material" [2]. Active packaging technologies include some physical, chemical, or biological actions which changes the interactions between a package, product, and/or headspace of the package, in order to get a desired outcome [3,4]. An exciting innovation in active packaging is the potential for the controlled released of antimicrobials from packaging materials. Antimicrobials incorporated in packaging materials could extend shelf life by preventing bacterial growth and spoilage [5]. The concept behind antimicrobial packaging is to enhance the safety and quality measures already used by the food industry. A variety of antimicrobial packaging systems have been reviewed. While some films have incorporated antimicrobial agents into the polymer [6], others have used biopolymer films as effective carriers of antimicrobial agents [6]. Nanoparticles, such as silver, titanium dioxide, and zinc oxide, are highly useful to minimize inhibitory and antimicrobial properties and low toxicity, and receive special attention from the industry [7]. TiO_2 is nontoxic, and the American Food and Drug Administration

(FDA) has approved TiO_2 for use in human food, drugs, cosmetics, and food contact materials [8]. Starch-nanoparticle based films have received attention due to their strong advantages, such as their mechanical and physical properties [9], and their being highly useful for minimizing the growth of contaminants by microorganisms [5]. Among natural polymers, starch is one of the most promising biocompatible and biodegradable materials, due to its strong advantages of low cost and wide availability from many plants, such as corn and potatoes (or cassava) [5,10].

The overall objective of this research has been to develop antimicrobial biodegradable packaging from starch-nanoparticles based film, and to use it to extend the shelf life of fresh fruits.

Materials and methods

Film preparation

The starch based films used in this research were prepared by the solution casting method, using cassava starch (Golden Coins Brand, Thai flour Industry Co. Ltd., Thailand), vinegar (5 % distilled vinegar, aorsorror.com), glycerol or glycerin, a simple polyol compound (1,2,3-propanetriol; C_3H_8O), and distilled water. The aqueous starch solution was heated at 70 - 80 °C until the dissolution of the starch was complete, and the quantities of the component ratio of starch film used for 10×10 cm^2 sheet film molding were studied.

After that, the quantities of cassava starch, vinegar, glycerol, and water were defined, according to the above. Nanoparticles, such as TiO_2 and ZnO (TiO_2, ZnO from King Mongkut's Institute of Technology, Thailand), were added into the aqueous starch solution at 0.01, 0.03, and 0.05 %w/w., mixed, and left stirring for 10 min. Then, all of the mixtures were poured into aluminum plates (11×11 cm^2). All solutions were dried at room temperature for 24 h and then placed in an oven at 35 °C, 5 h. All films were stored in a desiccator at room temperature for 24 h before analysis.

Characterization

Antimicrobial evaluation - disk diffusion method

Bacillus cereus (*B. cereus*) and *Escherichia coli* (*E. coli*) are common in the environment, and can be found in soil, dust, air, water, and on decaying matter such as food, causing food poisoning [10]. Inoculums (1 mL) were spread on the surface of Petri dishes prepared with nutrient agar [11]. Starch based films were cut into squares of 1×1 cm^2 and 2×2 cm^2 and placed on the surface of prepared Petri dishes. A 12.5 mg/ml of ceftriaxone (sold under the trade name Rocephin, an antibiotic useful for the treatment of a number of bacterial infections) antibiotic solution was used as a positive control. Petri dishes were then incubated at 37 °C for 2 days, and the inhibitory zone determined using measurement of its diameter. Antimicrobial agent efficiency was evaluated by the formation of an inhibition zone around the film samples, which was characterized by surrounding clear areas. All tests were performed in triplicate.

Shelf-life determination of banana and tomato

Pisang Mas (*Musa sapientum L.*), also known as "Kluai Khai", and tomato (*Solanum lycoperisicum L.*) are widely cultivated in Thailand. Fruits were collected from Ban Du fresh market, Mueang Chiang Rai District, Thailand. They were washed thoroughly with tap water to remove dirt and other undesirable particles from the surface. After washing, fruits were dried at room temperature for 2 - 3 h. Thereafter, the fruits were packed in different packaging films (10×10 cm^2); 1) unpacked, 2) petroleum plastic, 3) starch based film, 4) 0.01 % TiO_2 starch based film, 5) 0.03 % TiO_2 starch based film, 6) 0.05 % TiO_2 starch based film, 7) 0.01 % ZnO starch based film, 8) 0.03 % ZnO starch based film, and 9) 0.05 % ZnO starch based film. Each condition was replicated 4 times. Packed fruits were kept at ambient conditions, and data were recorded at 2 day intervals.

Biodegradation of starch based nanocomposite films

Soil degradation

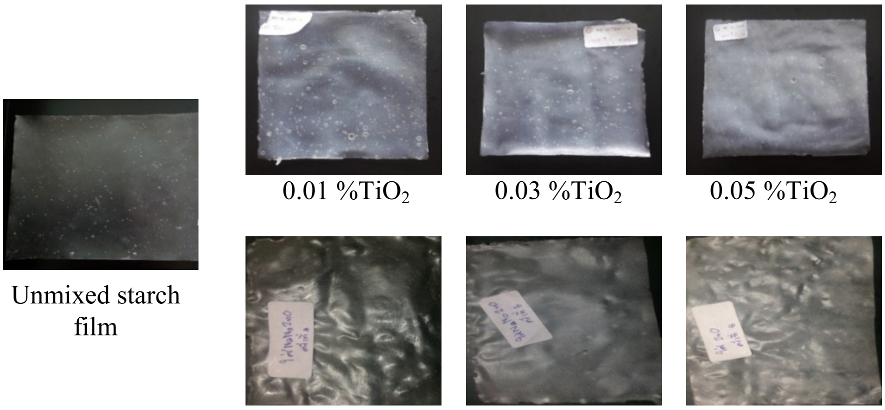
All packaging films were cut into squares of 5×5 cm^2 and buried in soil on a landfill site (10×10×10 cm^3 in dimension). Soil before and after film composting was collected every 7 days to confirm the biodegradability of all packaging films in the soil environment (adapted from the “soil degradation determination” of Borghei *et al.* [15].

Evaluating the residues of nanoparticle in soil

To determine the nanoparticle residues in soil at the landfill site, UV-vis absorbance measurement of TiO_2, ZnO, and compost soil of starch based films dispersed in water was monitored using Genesys 10S UV-vis, Thermo Fisher Scientific, USA.

Results and discussions

Starch base films were produced by casting technique, using the methodology and optimum contents of cassava starch, vinegar, glycerol, and water at a ratio of 20:5:5:80 g. The total weight of the mixture was 110 g. The mold used was a plastic plate with dimensions of 10×10 cm^2. After that, TiO_2 and ZnO (0.01, 0.03, 0.05 %w/w) were mixed with the starch solution using a hotplate (60 - 80 °C) and stirred for 10 min. The solution was used for film molding by aluminum plate of 11×11 cm^2, then left to cool, as shown in **Figure 1**.



0.01 %TiO_2 0.03 %TiO_2 0.05 %TiO_2

Unmixed starch film

0.01 %ZnO 0.03 %ZnO 0.05 %ZnO

Figure 1 Starch based nanocomposite films.

Starch based films blended with TiO_2 and ZnO are more opaque than unmixed starch based film. The TiO_2 blended film showed more white opaqueness and was more hard-brittle than the ZnO blended film.

Antimicrobial activity of biodegradable starch based nanocomposites

The inhibition areas yielded by Ceftriaxone biotic (control) and starch based films with different contents of nanoparticles (TiO_2, ZnO) against each studied microorganism (*E. coli* and *B. cereus*) are shown in **Figure 2**. The results indicated that no inhibition zone against the microorganisms was observable for film without the incorporation of nanoparticles. Inhibition areas were yielded by all concentrations of TiO_2 and ZnO coated films (10 mm for 1×1 cm^2 and 20 mm for 2×2 cm^2 films). The mechanism of antimicrobial activity of metal oxide nanoparticles and their selective toxicity to biological systems can be explained as follows. The microorganisms carry a negative charge of cellular membrane due to the presence of carboxyl (-COOH), phosphate (PO_4^{3-}), and amino ($-NH_2$) groups [12] while metal oxides carry a positive charge, which creates an electromagnetic attraction between the microbe and nanoparticle coated surface; the microbe is oxidized and dead instantly [8,13,14]. As shown in **Figure 3**, no microorganism growth was observed in the film contact area for all nanoparticle concentrations.

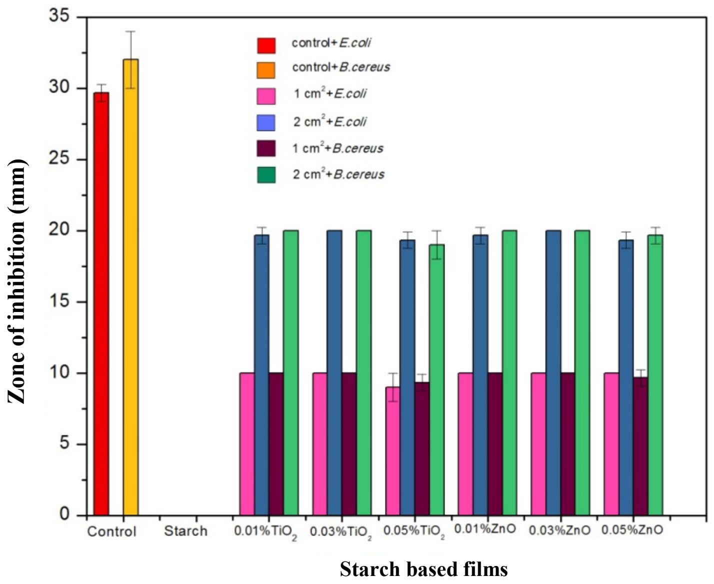


Figure 2 Inhibition zone of TiO_2 and ZnO blended starch based films against *E. coli* and *B. cereus*.

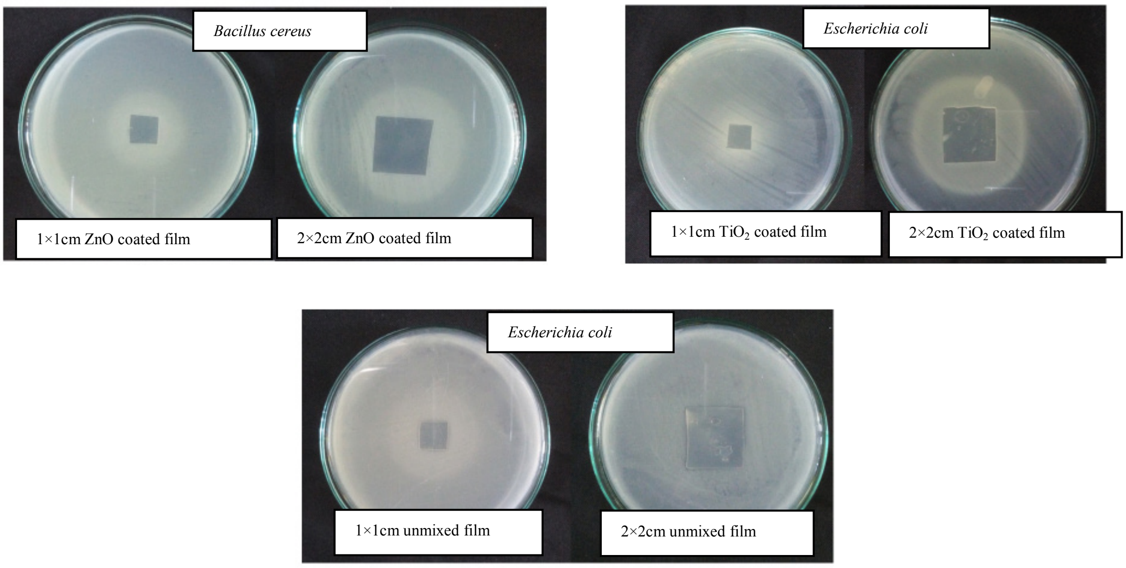


Figure 3 Petri dishes with squares of 1×1 cm^2 and 2×2 cm^2 starch based films, showing inhibition zones against *E. coli* and *B. cereus* due to effects of nanoparticles mixed films; no inhibition zone was detected for unmixed film.

Degradation of starch based nanocomposite film was investigated in landfill. Starch based films and petroleum film were buried in soil. All starch based films, including with and without nanoparticles, decomposed absolutely within 14 days, thus indicating soil biodegradation of starch based films whereas the petroleum film showed no evidence of degradation, as shown in **Figure 4**. Soil microorganisms were attracted to the cassava starch content of blended films. Microorganisms consumed starch and caused bioplastics to fracture [15].

(a) Unmixed starch film

(b) ZnO mixed starch film

(c) TiO_2 mixed starch film

(d) Petroleum film

Figure 4 Degradation of starch based nanocomposite films (a, b, and c) and petroleum film (d) in landfill.

Although nanoparticles have many beneficial applications, there is concern about what might happen if they were to be released into the environment, especially in soil around landfill. This study reviewed the residue of TiO_2 and ZnO in soil by using a UV-vis spectrophotometer. TiO_2 and ZnO dispersed in water and gave a near-UV absorbance peak at 336 nm for TiO_2 [17], and one of 258 nm for ZnO [18], as shown in **Figures 5(a)** and **5(b)**, respectively. The UV-vis spectrum of soil at the nanoparticle-bioplastic composite landfill area showed no TiO_2 and ZnO absorbance spectra, as shown in **Figures 5(c)** and **5(d)**, leading to the preliminary assumption that there was no nanoparticle residue in soil.

The use of nanoparticle-bioplastic films for fresh fruit packaging will be highlighted in a discussion of 2 products, bananas and tomatoes, concerning extended shelf life. Bananas and tomatoes were packed in starch based films (both with and without TiO_2 and ZnO) and petroleum film. Each contained one piece of fruit, and was stored in ambient conditions. All samples were evaluated in triplicate. The results showed that the starch based films mixed with 0.01 %w/w of TiO_2 resulted in an extended shelf life of banana, from 5 - 7 days to 14 days, as shown in **Figure 6**. For tomato, the starch film mixed with 0.01 % of TiO_2 extended shelf life from 7 - 10 days to 21 days, as shown in **Figure 7**.

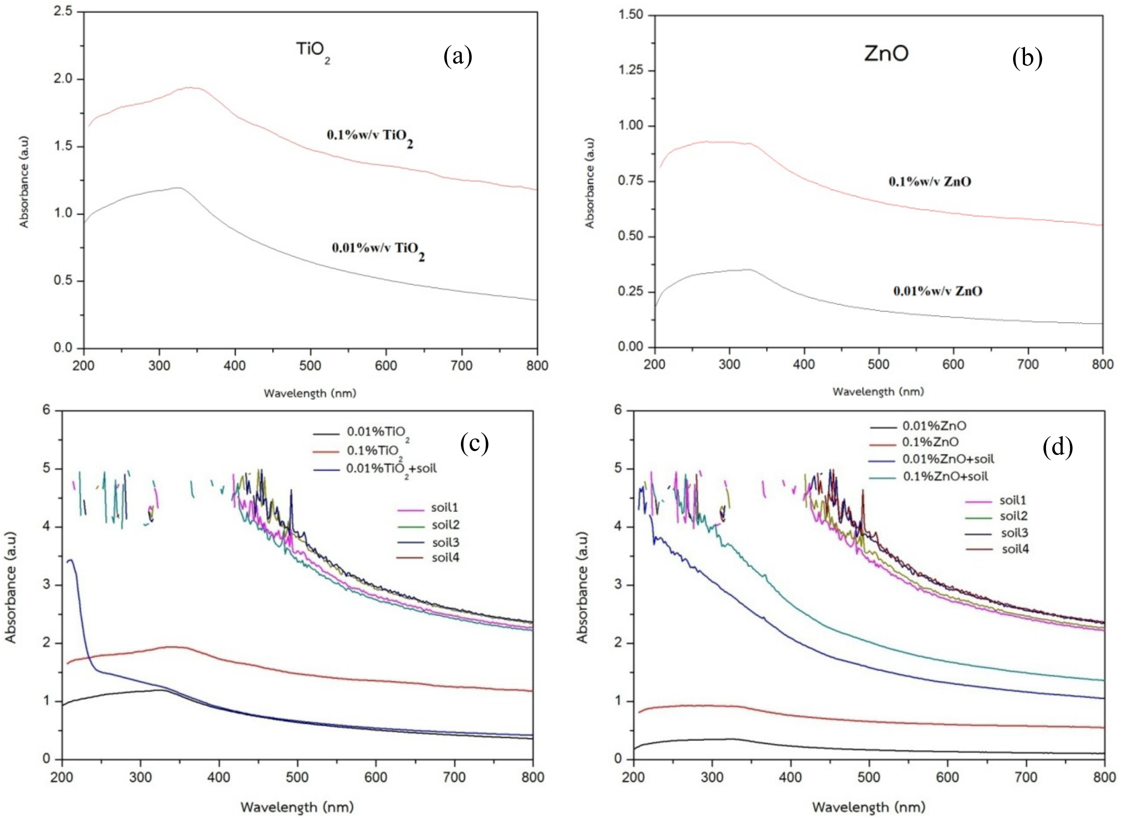


Figure 5 UV-vis spectra of TiO_2 (a) and ZnO (b) dispersed in water, TiO_2+soil and soil at landfill area (c), and ZnO+soil and soil at landfill area (d).

Day 1 **Day 7** **Day 14** **Day 14**

Unwrapped.

Day 1 **Day 7** **Day 14** **Day 14**

Petroleum film

Unmixed nanoparticle starch film.

0.01 %TiO_2 mixed starch film.

Figure 6 Use of starch based films for banana packaging.

Unwrapped.

Petroleum film.

0.03 %ZnO mixed starch film.

Day 1 **Day 14** **Day 21**

0.01 %TiO_2 mixed film.

Figure 7 use of starch based films for tomato packaging.

Antimicrobial activity testing indicated that all concentrations (0.01, 0.03, and 0.05 %w/w) of TiO_2 and ZnO nanoparticles, mixed in starch based films, yielded inhibition areas against *E. coli* and *B. cereus*. However, the results of using nanoparticle-starch based films for banana and tomato wrapping showed that only 0.01 % of TiO_2 was effective for extending shelf life. It could be explained that a high percentage of TiO_2 and ZnO caused brittleness in films and led to the fracture of fruit packaging during storage. In the case of ZnO mixed in cassava starch, the result showed fungal infection on the film surface over time, whereas no observed fungal infection was found in the TiO_2 mixed films. It could be implied that TiO_2 starch film was more effective in extending the shelf life of fresh fruits than ZnO starch film. TiO_2 and ZnO nanoparticles are important photocatalysts, and have been extensively studied for the removal of organic compounds from contaminated air and water and for microbial disinfection. However, some research has reported that TiO_2 nanoparticles generated more reactive oxygen species (ROS) than ZnO nanoparticles, as they showed higher levels of photocatalytic inactivation on the 4 species of bacteria examined, *S. aureus* and *B. subtilis* (gram-positive) and *E. coli* and *P. aeruginosa* (gram-negative) [16].

Conclusions

This study demonstrated that the produced TiO_2 and ZnO nanoparticle-coated cassava starch films exhibited potential usage for active packaging and application for extending the shelf life of fresh fruits. The results of used starch based nanocomposite films for fresh fruit packaging revealed that 0.01 % of TiO_2 extended the shelf life of bananas and tomatoes for longer than fruits unwrapped or wrapped with petroleum films. The use of nano-starch based plastic packaging materials to extend the shelf life of fresh fruits may be considered as an environmentally healthy alternative method of fresh fruit storage at ambient conditions.

Acknowledgements

We thank the Research and Development Institute, Chiang Rai Rajabhat University, for financial support, and the College of Nanotechnology, King Mongkut's Institute of Technology Ladkrabang, for nanoparticle support.

References

[1] NL Garcia, L Fama, NBD Accorso and S Goyanes. Biodegradable starch nanocomposites. *Eco-Friendly Polym. Nanocompos. Adv. Struct. Mater.* 2015; **75**, 17-77.
[2] GL Robertson. A*ctive and Intelligent Packaging*. 2nd ed. CRC Press, Boca Raton, 2006.
[3] KL Yam. Intelligent packaging for the future smart kitchen. *Packag. Tech. Sci.* 2000; **13**, 83-5.
[4] KL Yam, PT Takhistov and J Miltz. Intelligent packaging: concepts and applications. *J. Food Sci.* 2005; **70**, R1-R10.
[5] AS Abreu, M Oliveira, A de Sa, RM Rodrigues, MA Cerqueira, AA Vicente and AV Machado. Antimicrobial nanostructured starch based films for packaging. *Carbohyd. Polym.* 2015; **129**, 127-34.

[6] K Huff. 2016, Active and Intelligent Packaging: Innovations for the Future. Graduate Student. Department of Food Science and Technology, Virginia Polytechnic Institute and State Univerisity, Blacksburg, USA.

[7] SM Amini, M Gilaki and M Karchani. Safety of nanotechnology in food industries. *Electron. Phys.* 2014; **6**, 962-7.

[8] SH Othman, NR Abd Salam, N Zainal, RK Basha and RA Talib. Antimicrobial activity of TiO_2 nanoparticle-coated film for potential food packaging applications. *Int. J. Photoenerg*. 2014; 2014, 945930.

[9] CC Okpala. The benefits and applications of nanocomposites. *Int. J. Adv. Eng. Tech*. 2014, **4**, 12-8.

[10] L Liu. 2006, Bioplastics in Food Packaging: Innovative Technology for Biodegradable Packaging. San Jose State University, USA.

[11] AC Souza, GEO Goto, JA Mainardi, ACV Coelho and CC Tadini. Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *Food Sci. Tech*. 2013, **54**, 346-52.

[12] A Bbaszadegan, Y Ghahramani, A Gholami, B Hemmateenejad, S Dorostkar, M Nabavizadeh and H Sharghi. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study. *J. Nanomat*. 2015; **2015**, 720654.

[13] A Jesline, NP John, PM Narayanan, C Vani and S Murugan. Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant *Staphylococcus aureus*. *Appl Nanosci*. 2015, **5**, 157-62.

[14] A Kubacka, MS Diez, D Rojo, R Bargiela, S Ciordia, I Zapico, JP Albar, C Barbas, VAPM dos Santos, M Fernandez-Garcia and M Ferrer. Understanding the antimicrobial mechanism of TiO_2-based nanocomposite films in a pathogenic bacterium. *Sci. Rep.* 2014, **4**, 4134.

[15] M Borghei, A Karbassi, S Khoramnejadian, A Oromiehie and AH Javid. Microbial biodegradable potato starch based low density polyethylene. *Afr. J. Biotechnol*. 2010, **9**, 4075-80.

[16] RJ Barnes, R Molina, J Xu, PJ Dobson and IP Thompson. Comparison of TiO_2 and ZnO nanoparticles for photocatalytic degradation of methylene blue and the correlated inactivation of gram-positive and gram-negative bacteria. *J. Nanopart. Res*. 2013, **15**, 1432.