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Research Article

Antimicrobial Activity of TiO_2 Nanoparticle-Coated Film for Potential Food Packaging Applications

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Recent uses of titanium dioxide (TiO_2) have involved various applications which include the food industry. This study aims to develop TiO_2 nanoparticle-coated film for potential food packaging applications due to the photocatalytic antimicrobial property of TiO_2. The TiO_2 nanoparticles with varying concentrations (0–0.11 g/100 mL organic solvent) were coated on food packaging film, particularly low density polyethylene (LDPE) film. The antimicrobial activity of the films was investigated by their capability to inactivate *Escherichia coli* (*E. coli*) in an actual food packaging application test under various conditions, including types of light (fluorescent and ultraviolet (UV)) and the length of time the film was exposed to light (one–three days). The antimicrobial activity of the TiO_2 nanoparticle-coated films exposed under both types of lighting was found to increase with an increase in the TiO_2 nanoparticle concentration and the light exposure time. It was also found that the antimicrobial activity of the films exposed under UV light was higher than that under fluorescent light. The developed film has the potential to be used as a food packaging film that can extend the shelf life, maintain the quality, and assure the safety of food.

1. Introduction

There has been a growing amount of research undertaken into the applications of titanium dioxide (TiO_2) photocatalyst due to the high photocatalytic activity of this material. Currently, there is considerable interest in the antimicrobial property of TiO_2 for applications in the food industry. 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. The photocatalytic reaction of TiO_2 has been used to inactivate a wide spectrum of microorganisms [1–3]. The bactericidal and fungicidal effects of TiO_2 on, for example, *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Pseudomonas putida* have been widely reported [4, 5]. The development of TiO_2-coated or incorporated food packaging has also received attention [6–9].

For food packaging applications, the main purpose of the antimicrobial agent is to act against microorganisms and enhance the functions of conventional food packaging, namely, shelf life extension, maintenance of quality, and safety assurance [10]. The antimicrobial agent inhibits spoilage and reduces pathogenic microorganisms [11]. The antimicrobial agent also helps extend the shelf life of foods by extending the lag period of microorganisms, thereby diminishing their growth and number. Although there are numerous studies of coatings or antimicrobials incorporated into food packaging [6–9], the coating of nanometre sized antimicrobial particles, particularly TiO_2 nanoparticles, onto food packaging film has not been studied extensively.

The advent of nanotechnology has greatly improved the photocatalytic properties of TiO_2. The TiO_2 nanoparticles have attracted considerable attention because they exhibit unique and improved properties compared to their bulk material counterparts [12]. They show quantum size effects in which the physical and chemical properties of materials are strongly dependent on particle size. At the nanoscale level, the particle size decreases and the surface area of the particles

increase dramatically. This is one of the desired features for the nanoparticles to be used and exploited for photocatalytic applications.

Microorganisms can be killed by TiO_2 upon illumination of light due to its photocatalytic properties. Hydroxyl radicals and reactive oxygen species generated on the illuminated TiO_2 surface play a role in inactivating microorganism by oxidising the polyunsaturated phospholipid components of the cell membrane of the microbes [13, 14]. The use of nanometre sized TiO_2 particles has the potential to further enhance the antimicrobial activity of TiO_2. Effective antimicrobial film that can extend the shelf life, maintain the quality, and assure the safety of the food can be developed by coating TiO_2 nanoparticles onto food packaging materials. This creates a large commercial potential for TiO_2 nanoparticles applications in food industry.

The aim of this study is to develop TiO_2 nanoparticle-coated film, particularly low density polyethylene (LDPE) film, by investigating the effect of TiO_2 nanoparticle concentrations (0–0.11 g/100 mL organic solvent), types of light (fluorescent and ultraviolet (UV)), and light exposure time (one–three days) on the antimicrobial activity of the film for potential food packaging applications. The study was undertaken on lettuce packed with uncoated and TiO_2 nanoparticle-coated films against *E. coli*. This research work offers knowledge for developing antimicrobial nanoparticle-coated food packaging film with consideration for advancement in industrial applications.

2. Experimental

2.1. Preparation of TiO_2 Nanoparticle-Coated Films. Commercial TiO_2 nanoparticles, Aeroxide P25, were obtained from Evonik Industries (average particle size: 25 nm, purity: ≥99.5% trace metals basis, crystalline phase: 80% anatase + 20% rutile). An amount of TiO_2 nanoparticles (0.05, 0.08, and 0.11 g) was mixed with 100 mL organic solvent, particularly ethyl methyl ketone (MEK), to produce TiO_2 nanoparticle concentrations of 0.05 g/100, 0.08 g/100, and 0.11 g/100 mL MEK. The suspensions were ultrasonically irradiated using an ultrasonic probe homogeniser equipped with a temperature controller (Cole-Parmer) for 30 minutes [4]. Based on our previous study related to dispersion and stabilisation of photocatalytic TiO_2 nanoparticles in aqueous suspension, it was expected that the nanoparticles would form agglomerates and that there would be a change in cluster size before and after the ultrasonication [4]. However, note that in this work, changes in cluster size were not measured.

The suspension was then manually coated onto one side of low density polyethylene (LDPE) packaging film (dimensions: 16.5 cm × 17.8 cm, thickness: 0.01 mm) using a K bar coater (RK Print Instruments, UK) at room temperature and dried in air for 10 minutes. A cleanroom was utilised to reduce any possible contamination that could be adsorbed or chemisorbed on to the surface of the coated films. Note that there were no significant changes in the thickness of the film after the coating process, probably due to the nanometre-sized TiO_2 particles suspension being too small to make a measurable difference to the thickness.

2.2. Preparation of E. coli Cells. *E. coli* is a Gram-negative, rod-shaped bacterium that is usually found in the human intestine. Most *E. coli* strains are harmless, but some variations can cause serious food poisoning in their hosts such as *E. coli* O157 : H7. In this work, *E. coli* O157 : H7 was obtained from the Laboratory of Microbiology, Faculty of Food Science and Technology, University Putra Malaysia. The *E. coli* cells were grown in a conical glass flask (Schott Duran) containing 500 mL Luria-Bertani broth (Becton, Dickinson & Co.). The flask was incubated on a rotary shaker (New Brunswick Scientific Co.) at 37°C for 24 hours at 150 rpm. After incubation, the *E. coli* cells were harvested by centrifugation (FinePCR) at 4000 ×g for 20 minutes and washed twice with distilled water. Microbial stock solution was prepared by suspending the final pellets in distilled water. Serial dilution was undertaken to obtain the desired initial concentration of microbial solution. The initial population of *E. coli* (CFU/mL) was determined using a colony count method and was found to be $7.33 \times 10^8 \pm 1.13$ CFU/mL or 8.86 ± 0.12 log CFU/mL (mean value ± standard deviation).

2.3. Actual Antimicrobial Test of Uncoated and TiO_2 Nanoparticle-Coated Films. Fresh lettuce was used in this experiment and cooled overnight at 4°C. The damaged part and outer parts of the lettuce were discarded. Then 25 g lettuce was cut and dipped in 10% sodium hypochlorite solution (PC Laboratory Reagent) for about two minutes for the purpose of cleaning. The cut lettuce was then dip-inoculated with *E. coli* with a concentration of about $7.33 \times 10^8 \pm 1.13$ CFU/mL for two minutes at room temperature and the excess solution was shaken off. The concentration of *E. coli* inoculated on the lettuce was assumed constant. Subsequently, about 25 g of cut lettuce pieces were taken out and packed in the uncoated or the TiO_2 nanoparticle-coated films.

The packages were placed in a dark box complete with an 8W lamp (fluorescent or UV lamp) at room temperature. Then, the packages were exposed to different types of light, namely, fluorescent at a wavelength of 425 nm or UV at a wavelength of 365 nm. An amount of 25 g of lettuce was taken after each light exposure at designated interval times (1, 2, or 3 days) for the determination of the *E. coli* colony whereby the lettuce was placed into 225 mL of distilled water and mixed using a stomacher bag for two minutes. Serial dilution was made in distilled water solution to produce countable *E. coli* colony dilutions and 0.1 mL of the undiluted and diluted solutions were plated onto Luria-Bertani agar (Becton, Dickinson & Co.) in petri dishes using the spread plate technique. A glass rod was utilised to ensure the uniformity of the spread area on the agar plates. The agar plates were then incubated in an incubator at 37°C for 24 hours. Two replicate plates were used for each dilution. After 24 hours, the colonies formed on the agar were calculated using a colony counter machine. The number of viable *E. coli* cells was presented as CFU/g lettuce. The initial concentration of *E. coli* was 9.70 ± 0.10 log CFU/g (mean value ± standard deviation).

The procedures were repeated for different light exposure times (1, 2, and 3 days). The actual antimicrobial test was

Table 1: The *E. coli* colony values (CFU/g) of the uncoated and coated films for various TiO_2 concentrations (0.05, 0.08, and 0.11 g/100 mL MEK) at designated interval times (1, 2, 3 days) exposed under fluorescent and UV light.

Light	Sample film	Day	Log CFU/g
Fluorescent	Uncoated	1	10.06 ± 0.11
		2	10.18 ± 0.18
		3	10.31 ± 0.13
Fluorescent	Coated—0.05 g TiO_2/100 mL MEK	1	9.08 ± 0.15
		2	8.38 ± 0.13
		3	8.67 ± 0.20
Fluorescent	Coated—0.08 g TiO_2/100 mL MEK	1	7.96 ± 0.10
		2	7.72 ± 0.11
		3	7.47 ± 0.15
Fluorescent	Coated—0.11 g TiO_2/100 mL MEK	1	7.44 ± 0.14
		2	7.30 ± 0.17
		3	7.07 ± 0.09
UV	Uncoated	1	9.36 ± 0.18
		2	9.94 ± 0.12
		3	10.04 ± 0.12
UV	Coated—0.05 g TiO_2/100 mL MEK	1	7.16 ± 0.11
		2	7.02 ± 0.20
		3	6.86 ± 0.16
UV	Coated—0.08 g TiO_2/100 mL MEK	1	6.96 ± 0.07
		2	6.94 ± 0.22
		3	6.44 ± 0.15
UV	Coated—0.11 g TiO_2/100 mL MEK	1	6.82 ± 0.10
		2	6.68 ± 0.09
		3	6.29 ± 0.17

repeated at least twice for all the conditions (different concentrations of TiO_2, different light exposure times, and different types of light). Note that all the procedures were undertaken inside a cleanroom to minimise any possible contamination.

3. Results and Discussion

The effect of various TiO_2 concentrations (0–0.11 g/100 mL MEK), types of light (fluorescent and UV), and the time of exposure to light of the films (one–three days) on the antimicrobial activity of the films was determined and plotted in Figure 1. For purposes of clarity, the *E. coli* colony values (log CFU/g) obtained are also tabulated in Table 1. Note that the initial *E. coli* colony value determined using the colony count method was 9.70 ± 0.10 log CFU/g.

As expected, Figure 1 and Table 1 show that the *E. coli* colony for lettuce packed with TiO_2 nanoparticle-coated films decreased over time after being exposed to both fluorescent and UV light. This is attributed to the antimicrobial property of the TiO_2 nanoparticles. For instance, the *E. coli* colony for film coated with 0.05 g TiO_2/100 mL MEK reduced from an initial value of 9.70 ± 0.10 log CFU/g to 8.67 ± 0.20 and 6.86 ± 0.16 log CFU/g after being exposed for three days to fluorescent and UV light, respectively. Moreover, although not significant, it seems that the *E. coli* colony values decreased with an increase in the light exposure time due to the higher chances for TiO_2 to photocatalytically react at longer light exposure time. For example, the *E. coli* colony for film coated with 0.08 g TiO_2/100 mL MEK reduced from an initial value of 9.70 ± 0.10 log CFU/g to 7.96 ± 0.10,

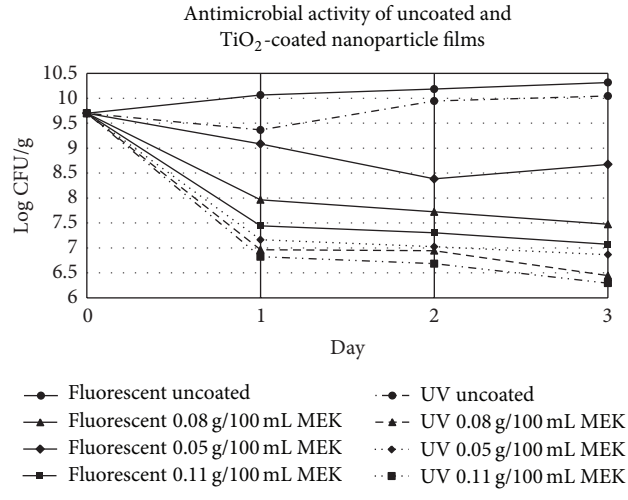


Figure 1: Antimicrobial activity of uncoated and coated films for various TiO_2 concentrations (0.05, 0.08, and 0.11 g/100 mL MEK) at designated interval times (1, 2, and 3 days) exposed to fluorescent and UV light.

7.72 ± 0.11, and 7.47 ± 0.15 log CFU/g after being exposed for, respectively, 1, 2, and 3 days to fluorescent light.

In contrast, the *E. coli* colony for the lettuce packed with uncoated films increased from 9.70 ± 0.10 log CFU/g to 10.31 ± 0.13 and 10.04 ± 0.12 log CFU/g after being exposed for three days to fluorescent and UV light. The result occurred due to the absence of the TiO_2 antimicrobial agent, thus proving that the uncoated films did not exhibit any antimicrobial effect. This finding is consistent with the work of Chawengkijwanich and Hayata [9] who found that after two days, the number of cells of *E. coli* from cut lettuce which packed in uncoated film was higher than the initial concentration of *E. coli*, whereas the number of cells from TiO_2-coated polypropylene film was lower than the initial concentration. This result implies that the TiO_2 nanoparticle-coated film has the ability to decrease the microbial contamination on food products as well as decrease the risk of microbial growth in food packaging.

Note that for the uncoated film under UV light illumination, the *E. coli* colony decreased slightly from an initial value of 9.70 ± 0.10 log CFU/g to 9.36 ± 0.18 log CFU/g (day 1) possibly due to the sterile property of the UV light whereby UV light impairs microorganism cells by means of oxidative stress caused by oxygen radicals inside the cells [15]. However, UV light alone, without the presence of an antimicrobial agent is not capable of adequately inactivating the *E. coli*. The *E. coli* colony for the uncoated film under UV light illumination increased from 9.36 ± 0.18 log CFU/g to 10.04 ± 0.12 log CFU/g from day 1 to day 3.

The mechanism for the events occurring on the TiO_2 nanoparticle coating can be explained as follows. When TiO_2 nanoparticles are irradiated with light suitable to their bandgap energy of 3.2 eV or higher (wavelengths below 385 nm), they have a tendency to experience all of the physical phenomena that include absorption, reflection, and scattering of light. Apart from that, TiO_2 nanoparticles will also be involved in photophysical and photochemical processes. In a photophysical process, the absorbed photons of light will excite the electrons (e^-) from the valence band to the conduction band leaving holes (h^+) in the valence band which generate electron and hole pairs (1). These energised electron and hole pairs can either recombine and dissipate the energy as heat (2) or dissociate because of charge trapping thus producing charge carriers available for the redox reactions ((3) and (4)) in the photochemical processes [16].

$$TiO_2 + h\nu \longrightarrow e^- + h^+ \quad \text{(Photogeneration of electron and hole pairs)} \tag{1}$$

$$e^- + h^+ \longrightarrow \text{light or phonon emission} - \text{heat} \quad \text{(Electron and hole recombination)} \tag{2}$$

$$D + h^+ \longrightarrow D^{\bullet +} \quad \text{(Oxidation)} \tag{3}$$

$$A + e^- \longrightarrow A^{\bullet -} \quad \text{(Reduction)} \tag{4}$$

A portion of the photoexcited electron and hole pairs will diffuse to the surface of the TiO_2 nanoparticles and take part in the chemical reaction with the adsorbed electron donors (D) or adsorbed electron acceptors (A). The holes can oxidise adsorbed electron donors (3), whereas the electrons can reduce appropriately adsorbed electron acceptors (4) [17].

The water in the air acts as an electron donor to react with the holes to produce the highly reactive hydroxyl radical ($OH^\bullet$) (5). Oxygen that is omnipresent on the surface of the particles acts as an electron acceptor by forming the superoxide ion (O_2^-) (6). The holes, the hydroxyl radicals, and superoxide ion are very powerful oxidants that can be used to oxidise and naturally decompose common organic matters such as odour molecules, bacteria, and viruses to water and carbon dioxide (7). Among them, hydroxyl radicals play the most important role in inactivating microorganism by oxidising the polyunsaturated phospholipid component of the cell membrane of microbes

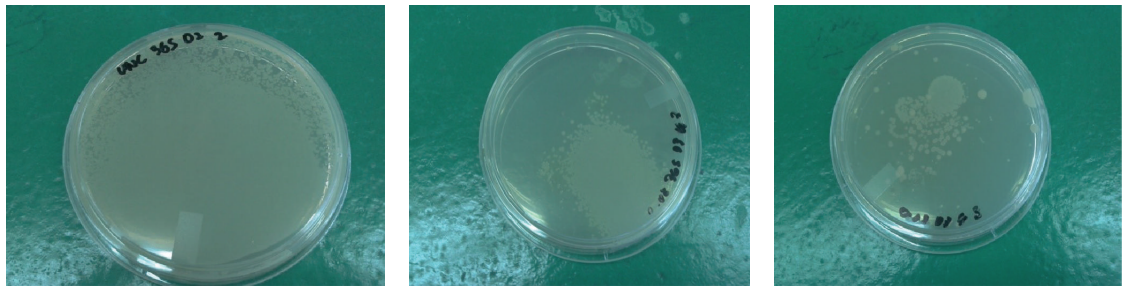
$$H_2O + h^+ \longrightarrow OH^\bullet + H^+ \quad \text{(Formation of hydroxyl radicals)} \tag{5}$$

$$O_2 + e^- \longrightarrow O_2^- \quad \text{(Formation of superoxide ion)} \tag{6}$$

$$OH^\bullet + \text{organic contaminants} \longrightarrow xCO_2 + yH_2O \quad \text{(Radical oxidation of organic compound)} \tag{7}$$

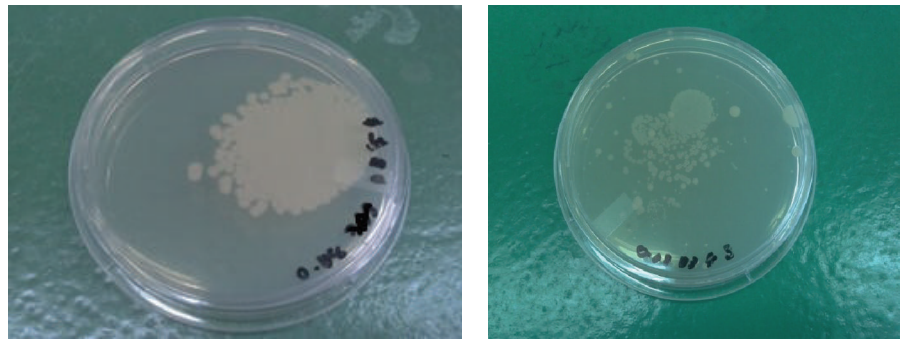
Furthermore, Figure 1 also shows that the trend of the antimicrobial activity of the coated films is the same despite different concentrations of TiO_2 nanoparticle and different types of light being used whereby the *E. coli* colony decreased over time after being exposed to both types of lighting. However, it can be clearly observed from Figure 1 that the antimicrobial effect becomes more pronounced as the TiO_2 concentration was increased from 0.05 to 0.11 g/100 mL MEK. After three days of fluorescent light exposure, the *E. coli* colony for the film coated with 0.05 g TiO_2/100 mL MEK was 8.67 ± 0.20 log CFU/g compared to 7.07 ± 0.09 log CFU/g for the film coated with 0.11 g TiO_2/100 mL MEK. Meanwhile, after three days of exposure to UV light, the *E. coli* colony for film coated with 0.05 g TiO_2/100 mL MEK was 6.86 ± 0.16 log CFU/g compared to 6.29 ± 0.17 log CFU/g for film coated with 0.11 g TiO_2/100 mL MEK, respectively. This finding is consistent with the fact that the higher the concentration of TiO_2 used as a coating, the higher the chances of photocatalytic reaction to occur, thus more *E. coli* can be inactivated.

This finding can also be evidenced from Figures 2(a)–2(c) which compares the *E. coli* colony on agar plates for lettuce packed inside uncoated and coated films of 0.05 and 0.08 g TiO_2/100 mL MEK. Note that Figure 2 is for visualisation purposes only to differentiate the effect of uncoated and coated films on the number cells in the *E. coli* colonies. Further serial dilution was undertaken in order to count the colony forming unit. From Figures 2(a)–2(c), it can be obviously seen that the *E. coli* colony area for lettuce packed with TiO_2 nanoparticle-coated films is much smaller compared to that packed with uncoated film, which demonstrates the antimicrobial activity of the coated films. Moreover, the colony area for 0.08 g TiO_2/100 mL MEK coated film (Figure 2(c))



(a) (b) (c)

Figure 2: *E. coli* colony on agar plate for lettuce packed inside (a) uncoated (b) 0.05 g TiO_2/100 mL MEK and (c) 0.08 g TiO_2/100 mL MEK coated films after three days of UV light illumination.



(a) (b)

Figure 3: *E. coli* colony on agar plate for lettuce packed inside 0.08 g TiO_2/100 mL MEK films after three days of (a) fluorescent and (b) UV light illumination.

is smaller than for the 0.05 g TiO_2/100 mL MEK coated film (Figure 2(b)) revealing improved antimicrobial activity of the 0.08 g TiO_2/100 mL MEK coated film. This supports the previous findings whereby antimicrobial activity becomes more pronounced with the increase in TiO_2 concentration. Thus, it can be deduced that it is vital to determine the right concentration of TiO_2 nanoparticles in order to ensure the effectiveness of the packaging application.

Moreover, from Figure 1 and Table 1 a comparison of the antimicrobial activity under UV and fluorescent light showed that the UV light was more effective at inactivating the *E. coli* than the fluorescent light. It was found that film coated with 0.05 g TiO_2/100 mL MEK managed to reduce the *E. coli* colony up to 8.67 ± 0.20 log CFU/g after three days of fluorescent illumination compared to 6.86 ± 0.16 log CFU/g after three days of UV illumination. Similarly, film coated with 0.08 g TiO_2/100 mL MEK managed to reduce the *E. coli* colony up to 7.47 ± 0.15 log CFU/g after three days of fluorescent illumination compared to 6.44 ± 0.15 log CFU/g after three days of UV illumination. Lastly, film coated with 0.11 g TiO_2/100 mL MEK managed to reduce the *E. coli* colony up to 7.07 ± 0.09 log CFU/g after three days of fluorescent illumination compared to 6.29 ± 0.17 log CFU/g after three days of UV illumination. This outcome is most likely related to the bandgap energy of TiO_2 nanoparticles (3.2 eV ≈ 385 nm) which is more suitable and closer to the wavelength of the UV light (365 nm) than fluorescent light (420 nm). The bandgap energy can be converted to wavelength by applying the following equation [18]:

$$\lambda\,(\text{nm}) = \frac{1240}{\text{bandgap}\,(\text{eV})}. \tag{8}$$

Apart from that, Horie et al. [19] who compared the photocatalytic sterilisation rates of *E. coli* cells in TiO_2 slurry irradiated with various light sources found that the inactivation rate of *E. coli* was dependent on the intensity of UVA light. Since the UVA light intensity of the UV light was much higher than the fluorescent light, more OH radicals formed on the surface of the TiO_2 coated films under UV light illumination, resulting in an increase in antimicrobial activity of the coated film under UV rather than fluorescent light. Cho et al. [14] in their study reported the linear correlation between inactivation of *E. coli* and hydroxyl radical concentration in TiO_2 photocatalytic disinfection. Photocatalytic disinfection was significant for higher hydroxyl radical concentration.

This result can also be evidently seen from Figures 3(a) and 3(b) in which the figures show the *E. coli* colony on agar plate for lettuce packed inside 0.08 TiO_2 g/100 mL MEK coated films exposed to both fluorescent and UV light for three days. Note that Figure 3 is for visualisation purposes only to differentiate the effect of the type of light on the number of cells in the *E. coli* colonies. Further serial dilution was done in order to count the colony forming unit. It can

be clearly seen from Figure 3 that the *E. coli* colony area for lettuce packed with coated film exposed to UV light is much smaller compared to that exposed to fluorescent light. This finding implies that selecting a suitable light in terms of wavelength and UVA intensity is important in order for the TiO_2 to work efficiently as a photocatalyst or antimicrobial agent.

4. Conclusion

This study demonstrated that the produced TiO_2 nanoparticle-coated films exhibited potential for antimicrobial applications in food packaging. The actual test revealed that the antimicrobial activity of the films exposed to both fluorescent and UV light increased with an increase in the TiO_2 nanoparticle concentration. The UV light was found to be more effective in expediting the antimicrobial activity of TiO_2 compared to fluorescent light due to the suitable bandgap energy of UV light and the higher hydroxyl radical concentration on the surface of the coated films. It is believed that the films can not only be used for food packaging but also other packaging applications that demand a hygienic environment. The use of nanometre sized TiO_2 has the prospect for further enhancing the antimicrobial activity of TiO_2 especially for applications in the food industry for which the antimicrobial agent is important in order to ensure food safety.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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