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Antimicrobial Property of Sodium Alginate/TiO_2 Nanocomposite Film

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Food poisoning outbreaks are commonly caused by bacterial contamination. These incidents can be minimized by using antimicrobial films that are suitable for use as packaging material. These films can be made by immobilizing an antibacterial agent to a non-toxic polymer matrix. Titanium dioxide (TiO_2), when irradiated with ultraviolet light, produces free radicals capable of killing bacteria. Sodium alginate (SA) is an edible polymer taken from brown algae. Both TiO_2 and SA are approved by the U.S. Food and Drug Administration as an additive in food. Therefore, composites made from SA and TiO_2 are considered safe. SA/TiO_2 nanocomposite films can be activated by both fluorescent and black light lamps. As evidenced by the percent color removal of methylene blue, the photocatalytic activity appeared to be higher when exposed to black light. SA/TiO_2 composite films were exposed to fluorescent and black light lamps for 5 h in the presence of *Escherichia coli* and *Staphylococcus aureus*. Under fluorescent lamps, the photocatalytic activity of the SA/TiO_2 composite films was enough to at least inhibit the proliferation of both bacteria. However, exposure of the 5% SA/TiO_2 composite film to black light resulted to a 0 log count for both bacteria. These results showed that SA/TiO_2 composite films can therefore be used in the food industry as an antibacterial film.

Key words: antibacterial film, food packaging, methylene blue, sodium alginate, titanium dioxide

INTRODUCTION

Bacterial contamination is often the culprit in several food poisoning outbreaks. In the case of the durian candy that poisoned 2,000 people in the Philippines on Jul 2015, *Staphylococcus aureus* bacteria was reported as the etiologic agent (FDA 2015). Developing antibacterial films that are suitable for use in food packaging can minimize occurrence of food poisoning. This can be done by immobilizing an antibacterial agent to a polymer matrix. For use in packaging, both the polymer matrix and the antibacterial agent should be proven to be safe even when ingested.

Titanium dioxide (TiO_2) immobilized to various polymer films are being studied for its use in waste water treatment (Gadiyar et al. 2013). The same property that gives TiO_2 its ability to act as a cleaning agent also enables it to act as an antimicrobial agent (Wu et al. 2016). TiO_2 is a photocatalyst. Once activated by light, it reacts with water and oxygen to form hydroxyl and superoxide radicals (Lan et al. 2013). These radicals can then react with the cells

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biomolecules and wreak havoc from within. There are also studies (Chawengkijwanich & Hayata 2008; El-Wakil et al. 2015) on light assisted control of microorganisms using TiO_2 coatings or TiO_2/polymer composite films. Though already gaining attention, application of this technique in the food industry is still minimal (Ramesh et al. 2016).

TiO_2 has been successfully immobilized to edible polymer films like TiO_2/wheat gluten/nanocellulose (El-Wakil et al. 2015), TiO_2/graphine oxide /chitosan (Liu et al. 2017), and Ag/TiO_2/chitosan (Xiao et al. 2015). All these polymer nanocomposites showed photocatalytic activity.

The U.S. Food and Drug Administration (USFDA) allows TiO_2 to be mixed with food only up to a maximum of 1% (USFDA 2015). If immobilized in some kind of packaging, concentrations greater than this can be used. At present, majority of packaging materials are petroleum-based plastics. Since the disposal of this type of plastic poses an environmental concern, the use of biodegradable films as a packaging material is gaining attention.

Polysaccharide-based films have the advantage of not only being biodegradable but also edible (Cazón et al. 2017). Sodium alginate (SA) is a bio-based polymer that has been used to develop films for food packaging (Hamedi et al. 2017; Şen et al. 2017). Since SA films are edible, these films may be used as immobilization matrices for TiO_2.

TiO_2 is activated only at wavelengths below 385 nm (Huang et al. 2000). Black light lamps predominantly emit wavelengths in the range of 345–400 nm, which is considered safe and yet able to activate TiO_2. Fluorescent lamps predominantly emit wavelengths in the range of around 410–700 nm. The way fluorescent lamps work is that it initially produces UV radiation (253.7 nm), which then hit the fluorescent material in the lamp to produce visible light. Therefore, it is possible that small amounts of UV radiation may be emitted in the process.

To the surprise of the authors, antibacterial activity was observed when polycaprolactone/TiO (Muñoz-Bonilla et al. 2013) and PVDF/TiO_2 (Muñoz-Bonilla et al. 2015) composites were irradiated at 500 nm inside a UV-VIS spectrometer. However, these instruments are well-known to allow small amounts of light outside the wavelength of interest to pass (stray light). This is actually the major reason behind the limited linear range of UV-VIS absorbance spectrometers. We think that the above observation is due to a small amount of UV light that was not effectively filtered by the spectrometer. This suggests that TiO_2 can be activated by small amounts of UV radiation.

Recent studies suggests that small amounts of UV radiation can escape from fluorescent lamps (Fundador et al. 2017; Jawad et al. 2016). Therefore, the researchers also explored the possibility of using fluorescent lamps for activating our SA/TiO_2 composite films.

This paper reports the photocatalytic and antibacterial properties of TiO_2 immobilized in SA films when exposed to black light and fluorescent light lamps. The objectives of this study were: (a) to determine the effect of TiO_2 concentration on the photocatalytic activity of SA/TiO_2 composite films under fluorescent and black light lamps and (b) to determine if photocatalytic activity is sufficient enough to kill or at least inhibit the growth of food pathogens *Escherichia coli* and *S. aureus.*

MATERIALS AND METHODS

Preparation of SA/TiO_2 Nanocomposite Films Using Solvent Casting Method

SA films (Sigma Aldrich, USA) were prepared based on the method described in previous study (Rhim 2004) with some modifications. SA, glycerol, and water were mixed at a ratio of 0.2 g, 0.1 g, and 5 ml, respectively. The mixture was then heated with constant stirring until the mixture became clear. TiO_2 nanoparticles (Sigma Aldrich, USA; <25 nm, anatase) were then added to make SA/TiO_2 films at concentrations of 0, 1, 3, 5, 10, and 15% and then sonicated (Elma Ultrasonic LC30H). The solution was poured on a plastic petri plate and oven dried at 50 °C for 24 h. The resulting films were soaked in 2.5% w/v calcium chloride ($CaCl_2$) solution for 5 min. After which, the $CaCl_2$ solution was discarded and the films were dried at ambient condition. The films prepared had a diameter of 60 mm and thickness of 0.080 mm.

Photocatalytic Activity Test

SA/TiO_2 composite films at varying concentrations of 0, 1, 3, 5, 10, and 15% TiO_2 were placed in separate plastic cups. Thirty milliliters (30 mL) of 0.015 M methylene blue solution at pH 2.0 was then poured in each cup. Each set was then exposed under a 36-W fluorescent light (Philips Lifemax Cool Daylight TLD 36W/54-765), a 40-W black light (General Electric /F40BLB), and inside the cabinet. Sampling and testing was done after 5 h and 24 h intervals for 72 h. Absorbance values at 668 nm were determined using a spectrophotometer (Shimadzu UV-1601, Japan). The % color removal on the dye was then calculated using Equation 1 (Sridewi et al. 2011).

$$\text{Color removal}(\%) = \frac{\text{Abs}_{\text{init}} - \text{Abs}_{\text{final}}}{\text{Abs}_{\text{init}}} \times 100 \qquad (1)$$

Antibacterial Activity Test

An overnight culture of the test organisms *E. coli* NRRL B-766 and *S. aureus* NRRL B-314 were prepared in nutrient broth. The bacterial suspension was adjusted

to approximately 1×10^8 cfu·mL^{-1} by comparing the suspension with 0.5 M McFarland standard. The antibacterial activity of SA/TiO_2 films with different concentrations of TiO_2 were assessed as described in a previous study (Chawengkijwanich & Hayata 2008) with some modifications. Four milliliters (4 mL) of *E. coli* serially diluted to 10^{-6} and 1 mL of *S. aureus* serially diluted to 10^{-4} were aseptically pipetted onto each test piece placed on a sterile petri plate. The samples were then exposed under fluorescent and black light lamps. Three replicates for each film (three test pieces per SA/TiO_2 film concentration) were done, and samples from each petri plate were taken after 5 h of exposure to fluorescent and black light lamps. For the coliform count, 1 mL of the *E. coli* suspension was plated on violet red bile agar (VRBA) using the overlayered pour plating method and incubated overnight at 37 °C. For staphylococci count, 0.1 mL of *S. aureus* suspension was spread plated on mannitol salt agar (MSA) followed by incubation at 37 °C for 48 h. Colonies were counted and reported as log CF/mL.

Statistical Analysis

The result of each test was subjected to one-way analysis of variance (ANOVA) at $p<0.05$ level of significance. Significant difference between treatments was also determined using Tukey's HSD test. The tests were done using the Minitab ® 17 software. All data points were done in triplicates.

RESULTS AND DISCUSSION

Photocatalytic Activity Test

Titanium dioxide (TiO_2) can be activated by light below 385 nm (Huang et al. 2000). Once activated, TiO_2 can then react with water and oxygen to form hydroxyl and superoxide radicals, respectively. The radicals formed can then attack methylene blue and result to a decrease in color of our solution.

The photocatalytic activity of different TiO_2 concentrations (0, 1, 3, 5, 10, 15% w/w) in SA nanocomposite films were assessed under dark, fluorescent, and black light lamps exposed for 24 h under various light conditions (Table 1). Exposure of the SA/TiO_2 composite films to fluorescent and black light lamps showed an increase in % color removal of methylene blue with increasing concentration of TiO_2. It is known that TiO_2 can act as a catalyst for the oxidation of organic compounds by UV (385 nm or lower) light (Huang et al. 2000). Black light produces UV (345–400 nm) radiation and fluorescent light produces visible light. However, UV (253.7 nm) radiation is also an intermediate product of fluorescent lamps. Most of this UV radiation is absorbed by phosphor and converted to visible light while a small portion of that radiation may escape. This explains why fluorescent lamps have the ability to activate TiO_2 but to a lesser extent than black light lamps. As seen in Table 1, the % color removal was less when exposed to fluorescent light. The same results were observed in a previous paper (Fundador et al. 2017) and from another research group (Jawad et al. 2016).

Color removal was also observed under dark conditions. However, the researchers did not observe a consistent increase in % color removal with increasing TiO_2 concentration. This is because color removal was just the result of dye adsorbing to the polymer. The same results was also observed in a previous study (Fundador et al. 2017). In this case, adsorption could be primarily due to the ionic interaction of the negatively-charged carboxylate groups of the alginate and the positively-charged methylene blue. To minimize ionization of the carboxylate groups, photocatalytic studies have to be performed at pH 2.0. In fact, hydrogels made from SA and TiO_2 act as a good adsorbent for methylene blue at pH>3 (Thakur et al. 2016).

Table 1. Percent color removal of methylene blue by SA polymer films containing various concentrations of titanium oxide after 24 h of exposure under various light conditions (ANOVA, HSD: $p<0.05$).

TiO_2 Concentration	Dark	Fluorescent	Blacklight
Control	27.69 ± 1.88a1	32.88 ± 2.27a12	31.27 ± 1.86a2
1%	23.89 ± 0.50b	34.90 ± 1.14ab	65.10 ± 4.18b
3%	19.38 ± 2.08bc	37.13 ± 1.39bc	68.02 ± 4.98b
5%	21.98 ± 0.13c	38.67 ± 1.33cd	70.32 ± 3.71bc
10%	31.47 ± 1.09d	41.36 ± 2.59d	75.02 ± 2.50cd
15%	27.97 ± 2.76a	45.19 ± 1.07e	76.21 ± 0.97d

Note: values with similar superscripts are not significantly different at $p<0.05$.
a–b: compares significant differences between different TiO_2 concentrations
1–2: compares significant differences between light sources

As seen in Table 1, color removal was also observed even under dark conditions. However, the researchers did not observe an increase in % color removal with time. It appears that color removal was just the result of the dye adsorbing to the polymer. In the presence of TiO_2, there was a consistent increase in % color removal with time under both fluorescent and black light lamps. As expected, the % color removal is lower for fluorescent lamps in comparison to black light lamps.

Antibacterial Activity

For the antibacterial studies, *E. coli* and *S. aureus* were chosen as representative Gram-negative and Gram-

positive bacteria, respectively. As shown in Table 2, the log count of the bacterial suspensions on the control films after 5 h exposure to fluorescent lamp increased from 5.64 to 6.45 for *S. aureus* and from 7.68 to 8.48 for *E. coli.* Under the black light lamp, the log count increased from 5.64 to 6.14 for *S. aureus*, while an increase from 7.68 to 8.19 was observed for *E. coli*. Thus, bacterial growth is significantly lower in black light lamps than in fluorescent lamps. However, it appears that these lamps do not have the capability to kill or prevent bacterial growth on their own. It is worth noting that the UV spectra emitted by black light is in the UVA region and not the UVC region emitted by germicidal lamps. This is the reason why the bacterial count still increases after 5 h in the presence of black light.

Table 2. Log count of the test microorganisms after 5 h of exposure under fluorescent and blacklight on different TiO_2/SA nanocomposite films.

	E. coli		***S. aureus***	
Initial count	7.68 ±0.08		5.64 ±0.11	
TiO_2 conc.	Fluorescent	Blacklight	Fluorescent	Blacklight
Control	8.48 ± 0.05a	8.19 ± 0.06a	6.45 ± 0.02a	6.14 ± 0.05a
1%	8.08 ± 0.04b	1.00 ± 2.45b	5.66 ± 0.06ab	4.37 ± 0.16b
3%	7.91 ± 0.02c	1.00 ± 2.45b	4.68 ± 0.30b	1.38 ± 2.14c
5%	7.68 ± 0.07d	0.00 ± 0.00b	2.67 ± 2.06c	0.00 ± 0.00c

Note:
1. There was a decrease in bacterial log count with increasing TiO_2 concentration. Values within a column with similar letters are not statistically significant at $p<0.05$;
2. Without TiO_2, the log count for *E. coli* and *S. aureus* significantly increased under both lamps at $p<0.05$; and
3. In the presence of TiO_2, the bacterial log count was always lower after exposure to black light in comparison to fluorescent light; all differences were statistically significant at $p<0.05$ except for *S. aureus* at 3% and 5% TiO_2.

TiO_2 can be activated by UV light. As shown in Table 2, there was an observed increase in antibacterial activity with increasing TiO_2 concentration. Black light lamps were able to sufficiently activate the films and decrease the bacterial log count of both bacteria. Black light lamps predominantly emit UV light while fluorescent lamps only emit small amounts of UV light as a by-product. Exposure to black light lamps resulted in films having more antimicrobial activity in comparison to films exposed to fluorescent lamps. Both films exhibited higher antimicrobial activity with increasing TiO_2 concentration under both lamps. However, exposure to black light lamps resulted in higher antimicrobial activity because of better TiO_2 activation. The bacterial log count using 5% SA/TiO_2 films was already 0 for both *E. coli* and *S. aureus* after 5 h exposure to black light lamps. Similar results were observed in two other studies – increased antibacterial activity was observed when *E. coli* was exposed to black light in the presence of free TiO_2 (Wu et al. 2016) and TiO_2 films (Scuderi et al. 2016). Exposure to fluorescent lamps for 5 h using 5% SA/TiO_2 films resulted in no bacterial growth for *E. coli*. It was observed that the bacterial log count remained at 7.68. Fluorescent lamps were able to sufficiently activate the films and at least inhibit the proliferation of both bacteria. In the case of *S. aureus*, a 0.96 and a 2.97 reduction in log count was observed using 3% and 5% TiO_2, respectively. This is probably due to the small amounts of UV light emitted by fluorescent lamps. In another study, significantly smaller amounts of antibacterial activity was also observed when apatite-coated TiO_2 was exposed to fluorescent light as opposed to black light (Kangwansupamonkon et al. 2009). This was done against *E. coli*, *S. aureus, Micrococcus luteus*, and methicillin-resistant *S. aureus.*

CONCLUSION

SA/TiO_2 nanocomposite films were irradiated under fluorescent and black light lamps. The composite films exhibited stronger photocatalytic activity when exposed to black light lamps. Under both lamps, the photocatalytic activity increases with increasing concentration of TiO_2.

Under both lamps, the photocatalytic activity was strong enough to exhibit at least some antibacterial activity. Antibacterial activity was very much stronger in the presence of black light lamps. The bacterial log count using 5% SA/TiO_2 films was already 0 for both *E. coli* and *S. aureus* after 5 h exposure to black light lamps.

The ability of SA/TiO_2 nanocomposite films to exhibit photocatalytic/antibacterial activity even under ordinary lamps (e.g., fluorescent lamps) gives rise to its many possible uses. Based on the results, these films can potentially be used as a packaging material. Depending on the exact industry specifications, it is possible that the fluorescent lamps used in food shelves may already provide sufficient antimicrobial activation.

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REFERENCES

CAZÓN P, VELAZQUEZ G, RAMÍREZ JA, VÁZQUEZ M. 2017. Polysaccharide-based films and coatings for food packaging: A review. Food Hydrocolloids 68: 136–148.

CHAWENGKIJWANICH C, HAYATA Y. 2008. Development of TiO_2 powder-coated food packaging film and its ability to inactivate *Escherichia coli in vitro* and in actual tests. International Journal of Food Microbiology 123: 288–292.

EL-WAKIL NA, HASSAN EA, ABOU-ZEID RE, DUFRESNE A. 2015. Development of wheat gluten/nanocellulose/titanium dioxide nanocomposites for active food packaging. Carbohydrate Polymers 124: 337–346.

[FDA] Food and Drug Administration (Philippines). 2015. Public Health Advisory on the Alleged Durian Candy "Wendy's" Brand Poisoning in CARAGA Region. Retrieved from http://www.fda.gov.ph/advisories-2/food-2/253125-fda-advisoryno-2015-043-public-health-advisory-on-the-alleged-durian-candy-wendy-s-brandpoisoning-in-caraga-region on 10 Dec 2015.

FUNDADOR EO, LAGMAY G, DE CADIZ A, FUNDADOR NG. 2017. Photocatalytic and Antibacterial Studies on Poly(Hydroxybutyrate-co-Hydroxyhexanoate) / Titanium Dioxide Composites. Banwa 12: 1–10.

GADIYAR C, BORUAH B, MASCARENHAS C, VIDYA S. 2013. Immobilized Nano TiO_2 for Photocatalysis of Acid Yellow-17 Dye in Fluidized Bed Reactor. International Journal of Current Engineering and Technology 4(S1): 84–87.

HAMEDI H, KARGOZARI M, SHOTORBANI PM, MOGADAM NB, FAHIMDANESH M. 2017. A novel bioactive edible coating based on sodium alginate and galbanum gum incorporated with essential oil of *Ziziphora persica*: The antioxidant and antimicrobial activity, and application in food model. Food Hydrocolloids 72: 35–46.

HUANG Z, MANESS P-C, BLAKE DM, WOLFRUM EJ, SMOLINSKI SL, JACOBY WA. 2000. Bactericidal mode of titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology A: Chemistry 130: 163–170.

JAWAD AH, MUBARAK NSA, ISHAK MAM, ISMAIL K, NAWAWI WI. 2016. Kinetics of photocatalytic decolourization of cationic dye using porous TiO_2 film. Journal of Taibah University for Science 10: 352–362.

KANGWANSUPAMONKON W, LAURUENGTANA V, SURASSMO S, RUKTANONCHAI U. 2009. Antibacterial effect of apatite-coated titanium dioxide for textiles applications. Nanomedicine: Nanotechnology, Biology and Medicine 5: 240–249.

LAN Y, LU Y, REN Z. 2013. Mini review on photocatalysis of titanium dioxide nanoparticles and their solar applications. Nano Energy 2: 1031–45.

LIU L-P, YANG X-N, YE L, XUE D-D, LIU M, JIA S-R, HOU Y, CHU L-Q, ZHONG C. 2017. Preparation and characterization of a photocatalytic antibacterial material: Graphene oxide/TiO_2/bacterial cellulose nanocomposite. Carbohydrate Polymers 174: 1078–86.

MUÑOZ-BONILLA A, CERRADA M, FERNÁNDEZ-GARCÍA M, KUBACKA A, FERRER M, FERNÁNDEZ-GARCÍA M. 2013. Biodegradable Polycaprolactone-Titania Nanocomposites: Preparation, Characterization and Antimicrobial Properties. International Journal of Molecular Sciences 14: 9249.

MUÑOZ-BONILLA A, KUBACKA A, FERNÁNDEZ-GARCÍA M, FERRER M, FERNÁNDEZ-GARCÍA M, CERRADA ML. 2015. Visible and ultraviolet antibacterial behavior in PVDF-TiO_2 nanocomposite films. European Polymer Journal 71: 412–422.

RAMESH T, NAYAK B, AMIRBAHMAN A, TRIPP CP, MUKHOPADHYAY S. 2016. Application of ultraviolet light assisted titanium dioxide photocatalysis for food safety: A review. Innovative Food Science & Emerging Technologies 38, Part A: 105–115.

RHIM J-W. 2004. Physical and mechanical properties of water resistant sodium alginate films. LWT – Food Science and Technology 37: 323–330.

SCUDERI V, BUCCHERI MA, IMPELLIZZERI G, DI MAURO A, RAPPAZZO G, BERGUM K, SVENSSON BG, PRIVITERA V. 2016. Photocatalytic and antibacterial properties of titanium dioxide flat film. Materials Science in Semiconductor Processing 42, Part 1: 32–35.

ŞEN F, UZUNSOY İ, BAŞTÜRK E, KAHRAMAN MV. 2017. Antimicrobial agent-free hybrid cationic starch/sodium alginate polyelectrolyte films for food packaging materials. Carbohydrate Polymers 170: 264–270.

SRIDEWI N, TAN L-T, SUDESH K. 2011. Solar Photocatalytic Decolorization and Detoxification of Industrial Batik Dye Wastewater Using P(3HB)-TiO_2 Nanocomposite Films. CLEAN – Soil, Air, Water 39: 265–273.

THAKUR S, PANDEY S, AROTIBA OA. 2016.

Development of a sodium alginate-based organic/inorganic superabsorbent composite hydrogel for adsorption of methylene blue. Carbohydrate Polymers 153: 34–46.

[USFDA] United States Food and Drug Administration. 2015. Code of Federal Regulations, Title 21. Retrieved from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFR on 21 May 2015.

WU X, HUANG Y-Y, KUSHIDA Y, BHAYANA B, HAMBLIN MR. 2016. Broad-spectrum antimicrobial photocatalysis mediated by titanium dioxide and UVA is potentiated by addition of bromide ion via formation of hypobromite. Free Radical Biology and Medicine 95: 74–81.

XIAO G, ZHANG X, ZHANG W, ZHANG S, SU H, TAN T. 2015. Visible-light-mediated synergistic photocatalytic antimicrobial effects and mechanism of Ag-nanoparticles@chitosan–TiO2 organic–inorganic composites for water disinfection. Applied Catalysis B: Environmental 170–171: 255–262.