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RESEARCH ARTICLE

Photocatalytic and Antibacterial Studies on Poly(Hydroxybutyrate-co-Hydroxyhexanoate) / Titanium Dioxide Composites

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Abstract

Various microorganisms such as bacteria and viruses have been responsible for the rise in food poisoning outbreaks in numerous settings. To prevent this from happening, antimicrobial films can be used as packaging material or coatings on surfaces where food is being processed. However, films like these should not only be effective but also safe. Titanium dioxide (TiO_2) when irradiated with ultraviolet light produces free radicals that can destroy organic contaminants and kill bacteria. TiO_2 is approved by the U.S. Food and Drug Administration and the U.S. Pharmacopeia as a food and pharmaceutical colorant. Polyhydroxybutyrate is a bio-based polymer produced by certain bacteria. Blends of this polymer are being studied for use in implants and drug delivery. Hence, TiO_2 immobilized to blends of this polymer may be suitable for use in food processing. Poly(hydroxybutyrate-co-hydroxyhexanoate) and titanium oxide (PHB-HH/TiO_2) composite films were irradiated under fluorescent and blacklight lamps. The results show that these films can be activated by both lamps. However, the photocatalytic activity is higher when exposed to blacklight. The films were also irradiated in the presence of *Escherichia coli* and *Staphylococcus aureus*. Both bacteria had a 0 log count when a 3% PHB-HH/TiO_2 composite film was exposed to blacklight for 5 h. Exposure to fluorescent light under the same conditions also showed some antibacterial activity. The photocatalytic activity of the films was enough to inhibit bacterial growth even when exposed to fluorescent lamps. PHB-HH/TiO_2 composite films have photocatalytic and antibacterial properties when exposed to both fluorescent and blacklight lamps. The films can therefore be used in the food industry.

Keywords: antibacterial film • bacterial log count • blacklight • fluorescent light • food industry • methylene blue • poly(hydroxybutyrate-co-hydroxyhexanoate) • titanium dioxide

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Introduction

In many cases, food poisoning outbreaks are the result of bacterial contamination. Developing antibacterial films that are suitable for use in the food industry can minimize cases like these. In food processing, these films can be used as coating on surfaces or as packaging material. Antimicrobial films can be made by immobilizing an antibacterial agent into a polymer matrix. For its use in the food industry, both the antibacterial agent and the polymer matrix should be nontoxic.

When light at wavelengths below 415 nm (Fujishima et al. 2000) hits titanium dioxide (TiO_2), positive holes and reactive electrons are formed. These holes and electrons then react with water and oxygen to form hydroxyl and superoxide anion radicals, respectively. The free radicals formed can then wreak havoc (i.e., apoptosis) in the cell by damaging its DNA and other biomolecules.

Being a catalyst, this antibacterial agent is not consumed and can be reused if recovered. Immobilizing TiO_2 in a polymer matrix would greatly improve its ease of recovery. Also, the United States Food and Drug Administration (U.S. FDA) only allows 1% TiO_2 (USFDA 2015) to be mixed with food. If immobilized in some kind of packaging, we can use percentages greater than 1% without violating U.S. FDA regulations. Immobilization of TiO_2 would not only improve its effectiveness but also its economic viability.

The same mechanism is also responsible for its ability to clean organic wastes. TiO_2 is so effective that it has been shown to completely convert organic substances to carbon dioxide (Chen et al. 2004). Currently in Japan, TiO_2-based photocatalytic products are being marketed (e.g., exterior construction materials, interior furnishing materials, road construction materials, purification facilities, and household goods) for its self-cleaning and antibacterial properties (Fujishima and Zhang 2006). On top of its effectiveness, this photocatalyst is also safe for human use, making it an ideal candidate not only for treating wastewater but also drinking water. In fact, the U.S. FDA and U.S. Pharmacopeia allow the use of TiO_2 as an additive in food (USFDA 2015) and pharmaceuticals (USP 2017).

Polymer-TiO_2 composite films have already been fabricated and proven to be effective in decolorizing certain types of dyes. Furthermore, the films showed antimicrobial property suggesting its application as a disinfectant (Scuderi et al. 2016; Wu et al. 2016). These composites are also being currently studied for its use in wastewater treatment (de Campos et al. 2013; Gadiyar et al. 2013).

In one of the studies, this composite (1 g TiO_2: 2 g cellulose acetate) was used in a fluidized bed reactor to degrade yellow 17 dye (Gadiyar et al. 2013). It was found that a liter solution containing 10 ppm dye was 95% degraded in 7 h (50% in 2 h) with 3 g of TiO_2 or 9 g of the composite. The rate of photocatalysis was almost the same for the immobilized and free TiO_2. This suggests that most of the free radicals produced by TiO_2 attack the dye and not the polymer, which leads us to believe that TiO_2 composites are stable enough to be used as films.

Polyhydroxybutyrate is a nontoxic bio-based polymer produced by certain bacteria (Altaee et al. 2016; Kaewbai-ngam et al. 2016) and is considered to be nontoxic even inside the body. In fact, blends of this polymer are currently being studied for its possible use in drug delivery (Michalak et al. 2013) and biodegradable implants (Meischel et al. 2016; Ni and Wang 2002; Sadat-Shojai et al. 2013). The 3-hydroxyhexanoate is a

conjugate base of a naturally occurring fatty acid (3-hydroxyhexanoic acid) that is normally present in the body especially during fasting (Costa et al. 1998). These facts led us to assume that polyhydroxyhexanoate is safe even when ingested.

This makes poly(hydroxybutyrate-co-hydroxyhexanoate) or PHB-HH an ideal candidate for antibacterial films that can be used in the food industry. This paper reports the photocatalytic and antibacterial properties of TiO_2 immobilized in PHB-HH.

In this paper, we used blacklight and fluorescent light lamps. Blacklight lamps predominantly emit wavelengths in the range of 345 to 400 nm, which is considered safe and yet able to activate TiO_2. Fluorescent lamps predominantly emit wavelengths in the range of 410 to 700 nm but may also emit small amounts (253.7 nm) of ultraviolet or UV radiation. The way fluorescent lamps work is that it initially produces UV radiation, which then hits the fluorescent material in the lamp to produce visible light. Interestingly, our initial studies on free TiO_2 showed that small amounts of UV radiation may have escaped. In a very recent paper, similar results were also obtained (Jawad et al. 2016). Since fluorescent lamps are the usual lamps in many settings, we also explored the possibility of TiO_2 activation under "ordinary lamps."

The objectives of this study are to determine the effect of TiO_2 concentration on the photocatalytic activity of PHB-HH/TiO_2 composite films under fluorescent and blacklight lamps, to determine the changes in photocatalytic activity after repeated use, and to determine if PHB-HH/TiO_2 composite films can kill or at least inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* under fluorescent and blacklight lamps.

Materials and Methods

Time and Place of Study

This study was conducted at the Food Microbiology Laboratory of the Department of Food Science and Chemistry at the University of the Philippines Mindanao from January to July 2016. The overall scheme of the study is shown in Figure 1.

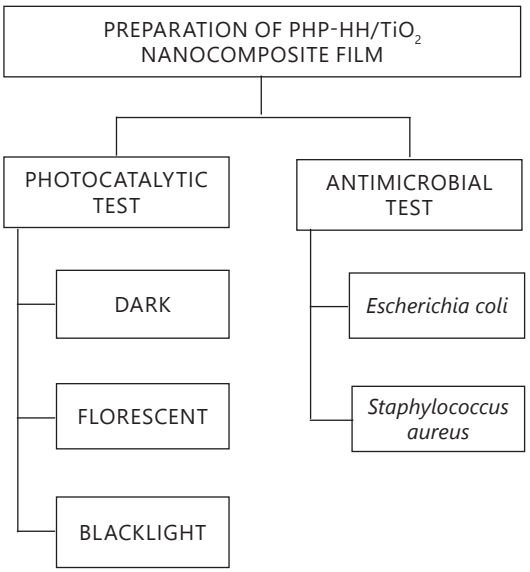

FIGURE 1 Overall process of evaluating the photocatalytic activity and antimicrobial property of poly(hydroxybutyrate-co-hydroxyhexanoate) / titanium oxide (PHB-HH/TiO_2) film

Preparation of PHB-HH/TiO_2 Films Using Solvent Casting Method

PHB-HH film with TiO_2 nanoparticles (Sigma-Aldrich, USA) was prepared by first dissolving PHB-HH resin in chloroform. The final solution was placed in six separate 100-mL beakers. Different amounts of TiO_2 corresponding to different ratios of TiO_2/PHB-HH (0%, 1%, 3%, 5%, 10%, 15% w/w) were added to each beaker followed by sonication. All the beakers were covered with perforated foil to allow chloroform to evaporate. Once dried, the polymer forms a network where the TiO_2 nanoparticles become physically entrapped. All composite films used in this experiment had a diameter of 46 mm and a thickness of around 0.119 ± 0.013 mm.

The films were then peeled and tested for photocatalytic and antimicrobial activity. Surface sterilization was done prior to microbial testing by exposing both sides of the film under UV germicidal lamps (253.7 nm) for three minutes.

Photocatalytic Activity Test

Three sets of PHB-HH/TiO_2 composite films at varying ratios (0%, 1%, 3%, 5%, 10%, 15% w/w TiO_2/PHB-HH) were placed in six different transparent plastic cups. In each cup,

30 mL of 0.015 M methylene blue solution was poured. One set designated as control was placed inside a cabinet while the two other sets were exposed under a 36-W fluorescent light (Philips Lifemax Cool Daylight TLD 36W/54-765) and a 40-W blacklight (General Electric / F40BLB), respectively. 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).

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

Photocatalytic Activity Test after Repeated Use

The stability of the films was evaluated based on the photocatalytic activity during its second and third use. All used films were washed before repeat testing using the photocatalytic test described above. The absorbance values of the methylene blue samples were taken after 24 h.

Antibacterial Activity Test

Fresh bacterial cultures of *E. coli* NRRL B-766 and *S. aureus* NRRL B-314 were prepared by inoculating the bacterial cells in nutrient broth and incubating them at 37 °C for 24 h. The bacterial suspension was adjusted to approximately 1 × 108 cfu · mL^{-1} by comparing the suspension with 0.5 M McFarland standard. Further adjustments were made by adding sterile nutrient broth when the bacterial suspension was more turbid or by adding bacterial cells when the inoculum was less turbid. The antibacterial activity of PHB-HH/TiO_2 films with different concentrations of TiO_2 were assessed as described in a previous study (Chawengkijwanich and Hayata 2008) with some modifications. On a sterile petri plate, we aseptically pipetted 4 mL of *E. coli* serially diluted to 10^{-6} and 1 mL of *S. aureus* serially diluted to 10^{-4}. The samples were then exposed under fluorescent and blacklight lamps. Three replicates for each film (three test pieces per PHB-HH/TiO_2 film concentration) were done, and samples from each petri plate were taken after 5 h of exposure to fluorescent and blacklight lamps. For the coliform count, 1 mL of the *E. coli* bacterial suspension was pipetted onto a petri dish. Approximately 15 mL violet red bile agar (VRBA) was then poured. These were mixed with the sample and were allowed to solidify. The solidified agar was then overlaid with approximately 5 mL of melted VRBA. The plates were then incubated at 37 °C overnight followed by the determination of the colony-forming units.

For staphylococci count, 0.1 mL of the bacterial suspension with *S. aureus* NRRL B-314 was spread plated onto Mannitol salt agar (MSA). The plates were then incubated for 48 h at 37 °C and the colony-forming units (cfu) was manually counted after incubation. The cfu · mL^{-1} was computed using Equation 2:

$$\frac{\text{cfu}}{\text{mL}} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{mL plated}} \qquad (2)$$

Statistical Analysis

Experimental data were statistically analyzed with Minitab® 17 software. The result of each test was subjected to one-way analysis of variance (ANOVA) at $p < 0.05$ level of significance. Tukey's honest significant difference (HSD) test was also used to determine the significant differences between treatments. All data points were done in triplicates.

Results and Discussion

Photocatalytic Activity Test

Positive holes and reactive electrons are formed when TiO_2 is irradiated by light below 415 nm (Jawad et al. 2016). These holes and electrons then react with water (H_2O) and oxygen to form hydroxyl radicals and superoxide anions, respectively. The color of methylene blue is dependent on its molecular structure. Destruction of its molecular structure through free radical attack would therefore result in decolorization.

The photocatalytic activity of different TiO_2 concentrations (0%, 1%, 3%, 5%, 10%, 15% w/w) in PHB-HH/TiO_2 films were assessed under dark, fluorescent, and blacklight lamps exposed for 24 h under various light conditions (Table 1). There was an observed initial color reduction of methylene blue (at 5 h) even under dark

TABLE 1 Percent color removal of methylene blue by poly(hydroxybutyrate-co-hydroxyhexanoate) polymer films containing various concentrations of titanium oxide after 24 h of exposure under various light conditions (ANOVA, HSD: $p < 0.05$)

Concentration	Dark	Fluorescent	Blacklight
Control	6.04 ± 2.70[a]	27.67 ± 1.07[a]	9.05 ± 2.10[a]
1%	6.78 ± 1.22[a]	35.64 ± 7.44[b]	51.12 ± 5.28[b]
3%	6.94 ± 2.86[a]	42.80 ± 5.90[c]	69.04 ± 0.64[c]
5%	7.25 ± 0.77[ab]	51.48 ± 1.03[d]	72.43 ± 3.18[c]
10%	10.50 ± 2.80[b]	54.22 ± 0.96[d]	71.39 ± 2.25[c]
15%	1.65 ± 3.33[c]	55.59 ± 0.37[d]	71.85 ± 4.67[c]

NOTE: Data are means of three replicates. Values within a column with similar letters are not significantly different at $p < 0.05$.

conditions. This is due to the adsorption of the dye on the film, which could be attributable to a combination of Van der Waals, hydrophobic, and ionic interactions. The same results was also observed by another group (Thakur et al. 2016).

Exposure of the PHB-HH/TiO_2 composite films to fluorescent and blacklight lamps showed an increase in % color removal of methylene blue with increasing concentration of TiO_2. However, the % color removal was less for the fluorescent light. TiO_2 once activated by UV light (418 nm or lower) can act as a catalyst for the oxidation of organic compounds (Fujishima et al. 2000). Blacklight produces UV (315–400 nm) radiation and fluorescent light produces visible light. However, UV (253.7 nm) radiation is also an intermediate product of fluorescent lamps, and a small portion of that radiation may escape. Therefore, fluorescent lamps also have the ability to activate TiO_2 but to a lesser extent than blacklight lamps.

There was a consistent increase in % color removal with increasing % TiO_2 under both lamps (Table 1). However, the rate of increase in % color removal (Δ % color removal / Δ % TiO_2) slows down at high concentrations of catalyst. There was no statistically significant difference in terms of % color removal using (5% to 15%) and (3% to 15%) TiO_2 under fluorescent and blacklight lamps, respectively. The data is in agreement with the results of another group (Jawad et al. 2016) where the rate of increase in the first order rate constant (Δ rate constant / Δ % TiO_2) decreases with increasing catalyst concentration. This property is indicative of the decrease in the average photocatalytic activity per unit weight of our catalyst. The decrease in photocatalytic activity per unit weight of catalyst can be due to partial shielding from UV light because of overcrowding and/or aggregation (Sridewi et al. 2011).

It can be observed that there is some initial color removal (at 5 h) even under dark conditions for all the films (Figure 2a). However, the color removal does not represent the photocatalytic activity of TiO_2. It appears that the PHB-HH/TiO_2 composite absorbs the dye since the film was observed to turn blue. There is an increase in % color removal with time under fluorescent light (390 to 700 nm) even in the absence of TiO_2 (Figure 2b). Based on its absorbance spectra (Ho et al. 2004), methylene blue absorbs fluorescent light better than blacklight. Absorption of light is a prerequisite for photodegradation. Therefore, fluorescent light by itself can degrade methylene blue.

In the presence of TiO_2, there was an increase in % color removal with time under both fluorescent and blacklight lamps. The % color removal is lower for fluorescent lamps in comparison to blacklight lamps. The increase in % color removal with increasing % TiO_2 with fluorescent lamps indicates the presence of photocatalytic activity. The color removal by blacklight lamps is due to the fact that TiO_2 can be activated at wavelengths below 415 nm (Fujishima et al. 2000) to produce free radicals that degrade methylene blue. On the other hand, fluorescent lamps emit "small" quantities of UV radiation in addition to visible light and, therefore, have the ability to activate TiO_2 to a limited extent.

Photocatalytic Test after Repeated Use

TiO_2 in the PHB-HH composite films may leach after repeated use and result in decreased

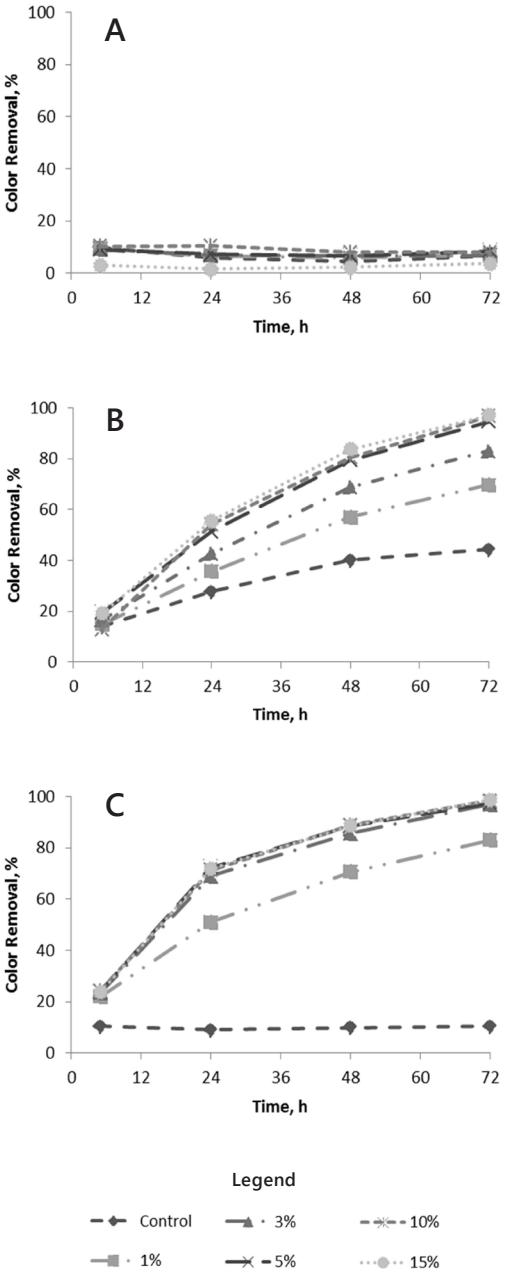

FIGURE 2 Percent color removal of methylene blue using different concentrations of TiO_2 in PHB-HH films after 5 h and 24 h intervals for 72 h exposure under (A) dark, (B) fluorescent light, and (C) blacklight

photocatalytic activity. For films containing higher concentrations of TiO_2 (i.e., 3% and 5%), it was generally observed that the % color removal for the first use is actually lower than the second use (Table 2). This could be due to swelling of the polymer after the first use.

TiO_2 upon exposure to UV light catalyzes the production of oxygen free radicals that are capable of killing bacteria and/or destroying organic pollutants. However, these free radicals can also attack the polymer and functionalize it with hydroxyl and carboxylate groups. Introduction of these functional groups will increase the hydrophilic characteristic of the polymer and result to increased swelling and/or solubility. Swelling may initially increase the photocatalytic activity of the films by decreasing the shielding between the TiO_2 particles. Shielding is more likely at higher concentrations of TiO_2 (i.e., 5%). In a similar study (Ren et al. 2015), it was shown that the TiO_2/polyvinyl alcohol composite increases its photocatalytic activity after repeated use even with the loss of some TiO_2. However, it is expected that an increase in pore size due to swelling would also lead to increased leaching of TiO_2. Eventually, there is going to be a decrease in photocatalytic activity. In this study, the decrease in photocatalytic activity can be seen by the lower % color removal at the third use in comparison to the second use. Therefore, it is expected that these films will have a finite lifespan. The actual lifespan of these films would depend on its specific use.

Antibacterial Activity

The log count of the bacterial suspensions on the control films after 5 h under the fluorescent lamp increased from 6.89 to 7.12 for *S. aureus* and from 7.46 to 8.31 for *E. coli* (Table 3). Under the blacklight lamp, the log count did not change for *S. aureus* while an increase from 7.46 to 7.93 was observed for *E. coli*. Bacterial growth is lower in blacklight lamps than in fluorescent lamps. However, it appears that these lamps do not have the capability to kill bacteria on their own. It is worth noting that the UV spectra emitted by

TABLE 2 Percent color removal of methylene blue by poly(hydroxybutyrate-co-hydroxyhexanoate) polymer films containing various concentrations of titanium oxide after first, second, and third use under fluorescent light and blacklight

Concentration	First use*	Second use*†	Third use†
Florescent light			
Control	27.67 ± 1.07[a]	27.16 ± 2.10[a1]	22.46 ± 1.56[1]
1%	35.64 ± 7.44[b]	34.15 ± 0.82[b]	27.47 ± 2.00
3%	42.80 ± 5.90[c]	45.49 ± 5.16[c3]	39.47 ± 3.59[3]
5%	51.48 ± 1.03	62.96 ± 2.12	55.00 ± 3.11
Blacklight			
Control	9.05 ± 2.10	1.46 ± 0.51[1]	3.79 ± 3.92[1]
1%	51.12 ± 5.28[b]	48.57 ± 3.33[b]	28.91 ± 7.69
3%	69.04 ± 0.64	80.57 ± 0.93	63.60 ± 6.36
5%	72.43 ± 3.18	84.40 ± 3.10[4]	81.02 ± 4.34[4]

NOTES: Data are means of three replicates.
* For comparison of photocatalytic activity during first and second use, values within a row with similar letters (a–d) are not significantly different at $p < 0.05$. Values with no superscript are statistically significant.
† For comparison of photocatalytic activity during second and third use, values within a row with similar number (1–4) are not significantly different at $p < 0.05$. Values with no superscript are statistically significant.

TABLE 3 Initial log count and log count of the bacterial suspensions on poly(hydroxybutyrate-co-hydroxyhexanoate) films after exposure to fluorescent light and blacklight for 5 h

Bacteria	Initial log count	Log count after 5 h	
		Fluorescent	Blacklight
Staphylococcus aureus	6.89[X1]	7.12[aX]	6.89[b1]
Escherichia coli	7.46[X1]	8.31[aY]	7.93[b2]

NOTES: Values within a row with similar superscripts are not significantly different at $p < 0.05$
a–b: compares significant differences between different light sources
X–Y: compares significant differences between initial and after 5 h log count under fluorescent light
1–2: compares significant differences between initial and after 5 h log count under blacklight

blacklight is in the UVA region and not the UVC region emitted by germicidal lamps.

An increase in antibacterial activity with increasing TiO_2 concentration is observed (Table 4). It appears that both fluorescent and blacklight lamps were able to sufficiently activate the films and cause bacterial inhibition. It is the UV light that activates TiO_2. Blacklight lamps predominantly emit UV light while fluorescent lamps only emit small amounts of UV light as a by-product. Exposure to blacklight lamps resulted in films having more antimicrobial activity in comparison to films exposed to fluorescent lamps. The films exhibited higher antimicrobial activity with increasing TiO_2 concentration under both lamps. However, exposure to blacklight lamps resulted in higher antimicrobial activity. The bacterial log count using 3% PHB/TiO_2 films was already 0 for both *E. coli* and *S. aureus* after 5 h exposure to blacklight lamps (Table 4). Similar results were observed in two other studies: increased antibacterial activity was observed when bacteria (*E. coli*) was exposed to blacklight in the presence of free TiO_2 (Wu et al. 2016) and TiO_2 films (Scuderi et al. 2016). Exposure to fluorescent lamps using 3% PHB/TiO_2 films also resulted in a decrease in log count. However, the decrease in log count though statistically significant is still very small. At 3% TiO_2, the log count was 7.86 for *E. coli* and 6.34 for *S. aureus*. The control values (0% TiO_2) were 8.31 for *E. coli* and 7.12 for *S. aureus*. 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 blacklight. This was done against *E. coli*, *S. aureus*, *Micrococcus luteus*, and methicillin-resistant *S. aureus* (Kangwansupamonkon et al. 2009).

TABLE 5 Log count of the test microorganisms after 5 h of exposure under fluorescent and blacklight on different titanium oxide concentrations in poly(hydroxybutyrate-co-hydroxyhexanoate) nanocomposite films

Concentration	*Escherichia coli*		*Staphylococcus aureus*	
	Fluorescent	Blacklight	Fluorescent	Blacklight
Control	8.31 ± 0.02[a]	7.93 ± 0.05[a]	7.12 ± 0.07[a]	6.90 ± 0.05[a]
1%	8.08 ± 0.03[ab]	4.00 ± 3.00[b]	6.76 ± 0.07[b]	2.31 ± 2.74[b]
3%	7.86 ± 0.10[b]	0.00 ± 0.00[c]	6.34 ± 0.21[c]	0.00 ± 0.00[c]
5%	7.37 ± 0.34[c]	0.00 ± 0.00[c]	5.80 ± 0.44[d]	0.00 ± 0.00[c]

NOTE: Data are means of three replicates. Values within a column with similar letters are not significantly different at $p < 0.05$.

Conclusion

PHB-HH/TiO_2 composite films were irradiated under fluorescent and blacklight lamps. The composite films exhibited stronger photocatalytic activity when exposed to blacklight lamps. Under both lamps, the photocatalytic activity increases with increasing concentration of TiO_2. However, the rate of increase (Δ photocatalytic activity / Δ % TiO_2) decreases with increasing concentration of the photocatalyst. There was no statistically significant increase in photocatalytic activity when the TiO_2 concentration was increased from 5% to 15% and 3% to 15% when exposed to fluorescent and blacklight lamps, respectively. Therefore, there is no need to further increase the % TiO_2 above a certain limit under our experimental conditions.

Under both lamps, the photocatalytic activity was strong enough to exhibit at least some antibacterial activity. Antibacterial activity was much stronger in the presence of blacklight lamps. Exposure of 3% PHB-HH/TiO_2 composite films to blacklight lamps resulted to 0 log count for *E. coli* and *S. aureus* after 5 h.

A decrease in photocatalytic activity of the films was observed when used for the third time. Therefore, these films have a finite lifespan. The actual lifespan of the films would depend on its specific application.

The ability of PHB-HH/TiO_2 composite films to exhibit photocatalytic/antibacterial activity even under ordinary lamps (e.g., fluorescent lamps) gives rise to its many possible uses. These films can be applied to surfaces where its photocatalytic/antibacterial characteristics would be beneficial. In the food industry, examples of these surfaces would be packaging materials or any surface where food preparation is done.

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