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## Antibacterial properties of TiO<sub>2</sub> nano coating on food packaging surfaces against *Escherichia coli* and *Salmonella typhimurium*

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### ABSTRACT

This work aimed to enhance the surface properties of common food packaging materials (PVC, PS, PET, PVDC) by applying a TiO<sub>2</sub> nano thin film coating. Physical and chemical analyses confirmed a well-defined anatase phase film. PET showed the highest antibacterial activity, followed by PVDC, PS, and PVC. After 60 min of UV-A irradiation, *E. coli* elimination rates were 99.85% (PET), 97.14% (PVDC), 96.5% (PS), and 85.91% (PVC). Similarly, for *S. Typhimurium*, the respective rates were 97.8% (PET), 83.71% (PVDC), 74.79% (PS), and 68.94% (PVC). Complete eradication of both strains occurred within 120 min (*E. coli*) and 180 min (*S. Typhimurium*). Durability testing revealed PET's mass loss of 97 mg/kg after 15 cycles, while PVC had the lowest value of 7 mg/kg. These findings demonstrate that TiO<sub>2</sub> thin film-coated substrates effectively inhibit bacteria growth, extending food product shelf life.

### ARTICLE HISTORY

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### Introduction

In the food production industry, food packaging plays a crucial role as it is responsible for maintaining the quality of food products during storage and transportation until they reach the consumers [1]. In recent years, there has been continuous development in food packaging to incorporate additional functionalities such as moisture barriers [2,3] and antimicrobial properties [4,5]. This is particularly important because bacteria thrive and multiply in environments with high humidity and moisture. The growth of bacteria in food can lead to an undesirable taste and significantly contribute to food spoilage, with meat products being particularly vulnerable to pathogenic and spoilage bacteria [6]. Moreover, the contamination of ready-to-eat products by bacteria poses a severe risk to human health while simultaneously reducing the shelf life of food items and contributing to food waste.

Recent research has focused on utilising nanotechnology to modify antibacterial surfaces. Cheraghcheshm and Javanbakht [7] conducted a study where they applied a coating of ZnO and Ag nanoparticles onto brick surfaces. The coated bricks underwent a calcination process at 350°C to achieve nanoparticle crystallization. Similarly, Zheng et al. [8] aimed to enhance the bioactive glass, a versatile material used

in biomedical applications, by incorporating Ag nanoparticles. A high calcination temperature of 550°C was utilised to stabilise the Ag nanoparticles. While these studies demonstrated effective antibacterial properties, their coating techniques are not suitable for food packaging due to the requirement for high calcination temperatures. In the field of food packaging, plastics and polymers, such as polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) [9], are commonly utilised as core materials. These materials have a melting temperature ranging from approximately 170°C to 280°C. Therefore, developing a simple coating technique at room temperature poses a challenge for producing antibacterial food packaging on a large scale while avoiding the deformation of polymers.

In the literature, the antibacterial properties of food packaging films made from low-density polyethylene (LDPE) material have been evaluated and prepared using a melt blending method [4]. In this method, modified TiO<sub>2</sub> and LDPE granules were blended and subsequently extruded using a twin-screw compounder. The resulting composite LDPE-TiO<sub>2</sub> film, milky whitish in appearance and with a thickness of 30 µm, exhibited a 53% reduction in *Pseudomonas* spp. and a 35% reduction in *Rhodotorula mucilaginosa*. Another approach involved coating oriented-

polypropylene (OPP) plastic film with a TiO<sub>2</sub> suspension using organic solvents and a bar coater [5]. The antibacterial activity of the TiO<sub>2</sub>-coated film against *Escherichia coli* was approximately 38%, and its effectiveness depended on the intensity of UVA light.

Furthermore, active nanocomposite packaging films made from polylactic acid (PLA) reinforced with nano-Ag were prepared using a solvent evaporation method [10]. These nanocomposites exhibited improved physical properties compared to pristine PLA, and the best preservation effect for maintaining the freshness of strawberries was observed in the packaging film containing 5 wt.% nano-Ag. In addition to these approaches, various natural antibacterial agents, such as cinnamon essential oil, lysozyme, and chitosan, have been incorporated into polymeric food packaging to inhibit bacterial growth and extend shelf life [11]. However, the high cost and relatively low activity of these natural agents present significant disadvantages when compared to synthetic antibacterial agents [12].

Various nanomaterials, such as ZnO, Ag, SiO<sub>2</sub>, and TiO<sub>2</sub>, have demonstrated remarkable antibacterial properties. The antibacterial mechanisms of these nanomaterials are associated with photocatalysis and oxidation processes, as elucidated by Sunada et al. [13]. Photocatalysts generate strong reactive oxygen species (h<sup>+</sup>, OH<sup>•</sup>, O<sup>2-</sup>, H<sub>2</sub>O<sub>2</sub>) that partially decompose the outer membrane of bacteria. TiO<sub>2</sub>, in particular, is extensively used as a photocatalyst in wastewater treatment, disinfection, and self-cleaning surfaces [14,15]. Its widespread application is attributed to its chemical stability, abundance, cost-effectiveness, biocompatibility, and non-toxic nature to human health [16]. Wu et al. [17] further clarified that the hydroxyl radical (OH<sup>•</sup>) produced by TiO<sub>2</sub> photocatalysis contributes to the inactivation of microorganisms. Phuinthiang et al. [18] demonstrated the complete eradication of *E. coli* and *S. typhimurium* upon UV-A irradiation of a TiO<sub>2</sub> thin film. Moreover, various microorganisms including fungi, viruses, and other bacteria were effectively eliminated by different concentrations of TiO<sub>2</sub> and increasing irradiation time [19–22].

In this work, we employed four types of plastic food packaging as substrates for developing antibacterial surfaces. These substrates included disposable PVC food containers, styrofoam containers, transparent PET food containers, and food wrap cling film. A TiO<sub>2</sub> thin film was prepared and coated onto these substrates using doctor-blade and UV-curing techniques. The coated substrates underwent characterisation to assess their physical and chemical properties, including surface morphology, chemical composition, bonding, and band gap energy. Furthermore, the viability of *E. coli* and *S. typhimurium* was evaluated in relation to these thin film-coated substrates.

## Materials and methods

### Substrates preparation

Four commonly used plastic food packages, namely disposable PVC food containers (PVC), styrofoam containers (PS), transparent PET food containers (PET), and cling film for food wrapping (PVDC), were procured from local suppliers, as detailed in Table 1. The substrates were then cut into 2 × 2 cm pieces and subjected to a cleaning process using ethanol and DI water in a sonicator for 5 min.

### TiO<sub>2</sub> sol-gel preparation and substrates coating

Titanium isopropoxide (TTIP, 97%) was combined with acetic acid (≥99.5%) in a 1:2 w/w ratio. After thorough mixing, 80 mL of deionised water was added to the solution, which was continuously stirred at 80°C for 15 h. This process resulted in the formation of a transparent TiO<sub>2</sub> sol-gel, which was subsequently used for coating the food packaging substrates. The doctor-blade technique was employed to coat the substrates with the TiO<sub>2</sub> thin film. The TiO<sub>2</sub> sol-gel was positioned in front of the blade, which was then moved across the surface of the substrate to create a wet film. The TiO<sub>2</sub> wet film-coated substrates were exposed to UV-C radiation (TUV 36W, Philips) at room temperature for a duration of 3 h. More detailed information regarding the coating method can be found in previous work [15]. To confirm the presence of TiO<sub>2</sub> and assess its properties, the TiO<sub>2</sub> thin film-coated substrates, including PVC, PS, PET, and PVDC, underwent characterisation.

### Nano layer characterisation

The surface morphology of both coated and uncoated substrates was characterised using a Field Emission Scanning Electron Microscope (FESEM, JSM-IT800) and an Atomic Force Microscope (AFM, Park Systems AFM XE-120). The crystallographic orientation, chemical composition, and bonding of the TiO<sub>2</sub> thin films were investigated through grazing incidence X-ray diffraction (GIXRD, Bruker D8 Advance), X-ray photoelectron spectroscopy (XPS, Axis Ultra DLD, Kratos Analytical), and Raman spectroscopy (HORIBA, Scientific LabRAM HR Evolution), respectively.

**Table 1.** Summary of food packages used in this study.

Sample name	Substrate	Polymer type
PVC	Disposable PVC food container	Polyvinyl chloride
PS	Styrofoam containers	Polystyrene
PET	Transparent PET food container	Polyethylene terephthalate
PVDC	Food wrap cling film	Poly (vinylidene chloride)

The hydrophilicity of the thin film surface was measured using a contact angle analyzer (Dataphysics OCA-20). Additionally, the band gap energy of the TiO<sub>2</sub> thin films was measured using a UV-Vis spectrophotometer (Mapada, UV-6100) and calculated using Tauc's relation.

### Antibacterial activity test of coated substrates

Two pathogenic bacteria, *Escherichia coli* (*E. coli*, ATCC 25922) and *Salmonella typhimurium* (*S. typhimurium*, TISTR 1469), were utilised in this study. A single colony of *E. coli* and *S. typhimurium* were incubated at 37°C for 18 h for cultivation, and the bacterial suspension was diluted to an initial concentration of  $1 \times 10^9$  colony-forming units (CFU) per millilitre for the antibacterial test. The procedure for the antibacterial activity test was as follows: the coated substrates were disinfected with 75% ethanol, and the dried substrates were immersed in the bacterial suspension for a brief duration. It should be noted that the uncoated substrates (without TiO<sub>2</sub>) were used as controls for the corresponding type of substrate in this study. The uncoated substrates (PVC, PS, PET, and PVDC) were subjected to the same conditions as the coated substrates by being immersed in the bacterial suspension for a short period. Subsequently, both the coated substrates and uncoated samples were exposed to UV-A light (with a light intensity of 2.5 W/cm<sup>2</sup>) for 0, 30, 60, 150, and 180 min. After irradiation, the surface of the coated substrates was rinsed with 10 mL of nutrient broth, and the nutrient broth was incubated at 37°C for 18 h. The bacterial growth was quantified

by measuring the cell density at 600 nm (OD600) and employing the plate count technique.

### Robustness test

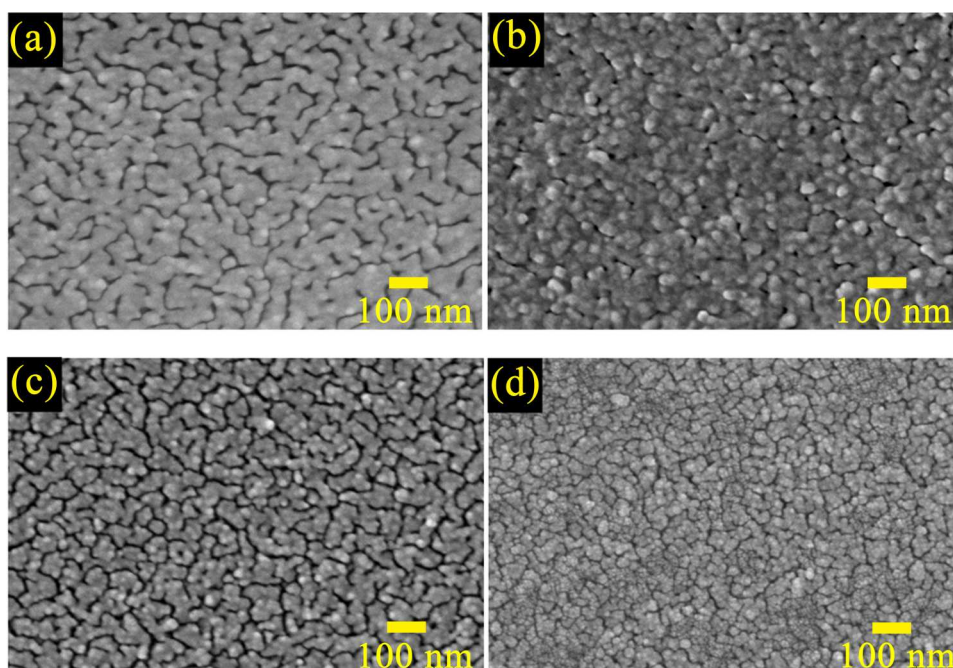
The durability of the TiO<sub>2</sub> thin film coated on the food packages is a significant concern for commercialisation. To assess their durability, a robustness test was conducted [23]. The procedure for the robustness test was as follows: the coated substrates were placed in a centrifuge tube containing 25 mL of deionised water and centrifuged at 10,000 rpm for 20 min (ScanSpeed, 1248). The solution was then separated, and the absorbance value was measured using a UV-vis spectrophotometer (Mapada, UV-6100). The TiO<sub>2</sub> concentration in the solution was determined by utilising the standard curve of P25 concentration and its absorbance. To ensure precision, five replicates of each coated substrate were performed, and the uncoated substrate was used as the blank in the test.

## Results and discussion

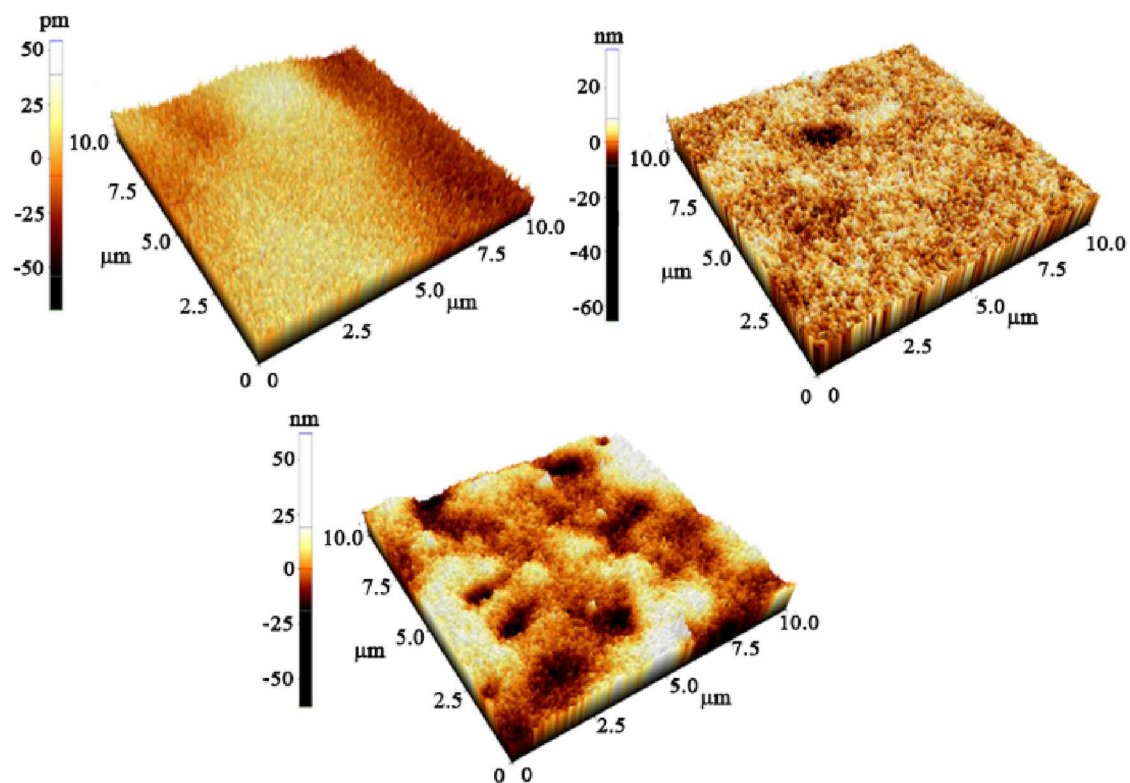
### Coated substrates characterisation

#### Surface morphology

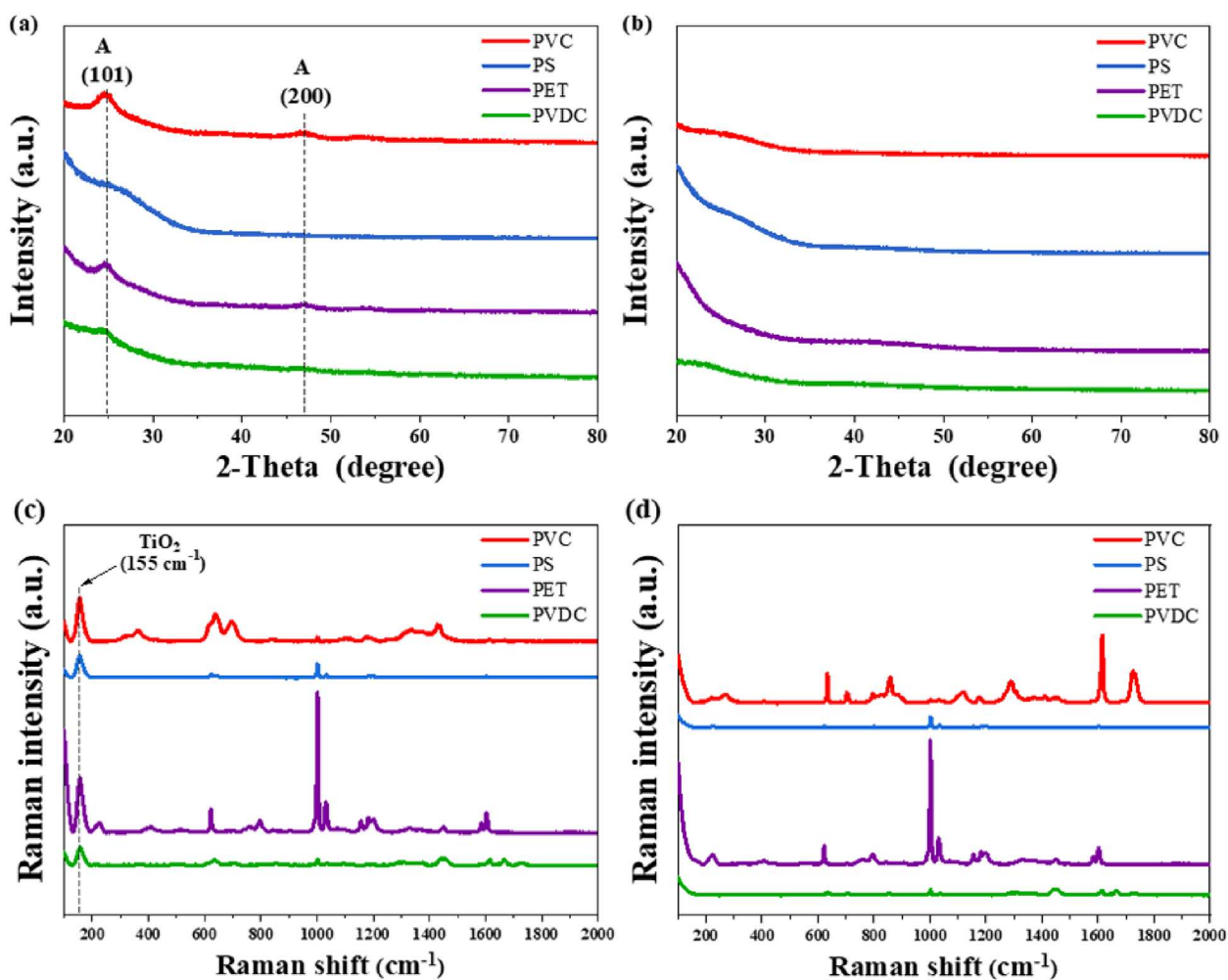
The coated and uncoated substrates exhibited high transparency, and no differences in appearance were observed. The TiO<sub>2</sub> thin film was extremely thin and smooth, displaying no cracks or pinholes, as depicted in Figure 1. Moreover, at a magnification of 100,000, the crystal TiO<sub>2</sub> was found to be densely structured. The surface roughness of the coated substrates is presented in Figure 2. The root mean square (RMS)



**Figure 1.** FESEM image of TiO<sub>2</sub> thin film coated on (a) PVC, (b) PS, (c) PET, and (d) PVDC (100,000 magnification).



**Figure 2.** AFM micrographs of coated substrates; (a) PVC, (b) PET, and (c) PVDC.



**Figure 3.** (a) GIXRD patterns of coated substrates, (b) GIXRD patterns of uncoated substrates, (c) Raman spectra of coated substrates and (d) Raman spectra of uncoated substrates.

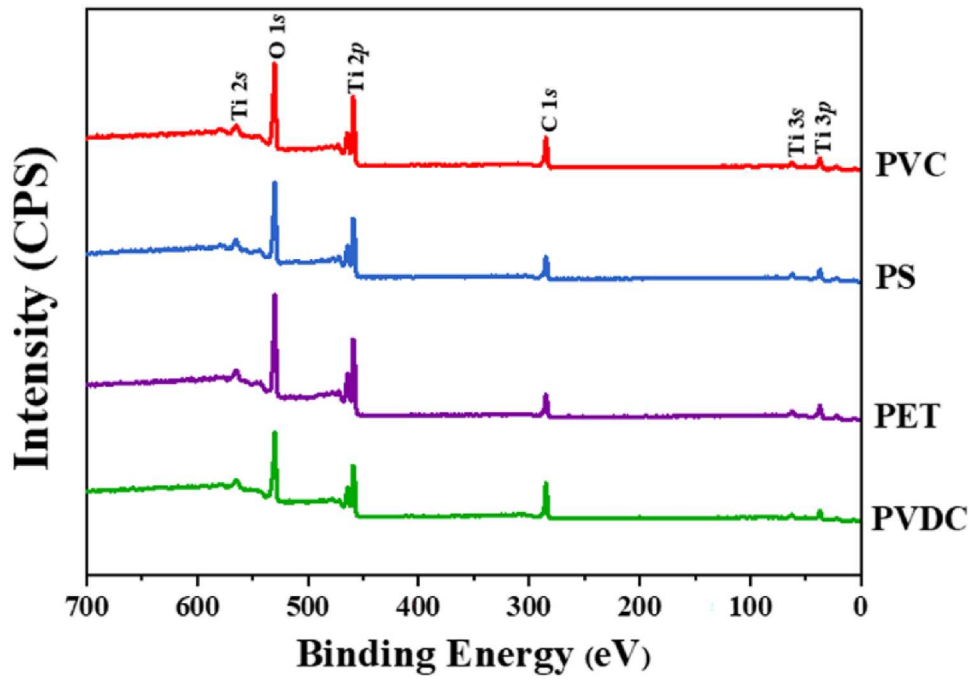


Figure 4. XPS survey spectra of coated substrates.

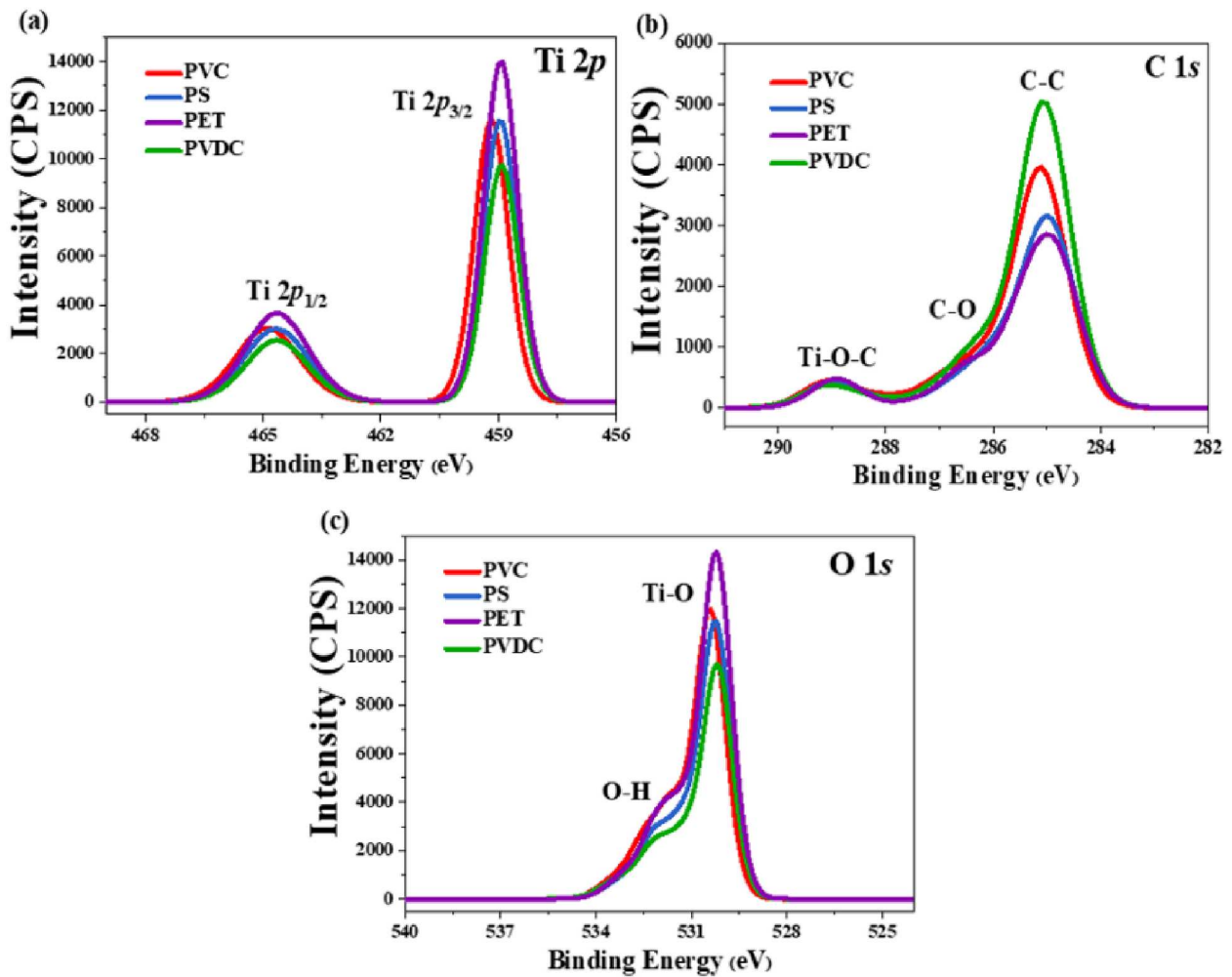


Figure 5. XPS spectra of (a) Ti 2p, (b) C 1s, and (c) O 1s of coated substrates.

roughness values for PVC, PET, and PVDC were 3.89, 4.47, and 9.71 nm, respectively. The increased roughness of PVDC in comparison to the others can be

attributed to the original surface characteristics of the substrate. It is worth noting that the AFM image of the PS sample was not included in this study due

to the unique surface properties of PS, which featured numerous shallow depths that the instrument could not accurately measure.

### Structural property

The GIXRD patterns of the four coated substrates exhibited two prominent peaks at  $24.9^\circ$  and  $47.1^\circ$ , which corresponded to the (101) and (200) planes of anatase  $\text{TiO}_2$  [24,25]. In contrast, these two peaks were not detected in the uncoated substrates (Figure 3(a and b)). Similarly, the Raman spectra displayed distinct peaks at  $155\text{ cm}^{-1}$  for anatase  $\text{TiO}_2$  (Figure 3 (c and d)). Although anatase  $\text{TiO}_2$  is typically observed at  $144\text{ cm}^{-1}$  [26,27], the broadened and shifted peak indicated the presence of other contaminants (such as carbon atoms) that disrupted the  $\text{TiO}_2$  bonding. Furthermore, the GIXRD and Raman spectra confirmed that the coated layer exclusively consisted of the anatase phase of  $\text{TiO}_2$ , without any traces of rutile or brookite phases.

### Chemical states and compositions

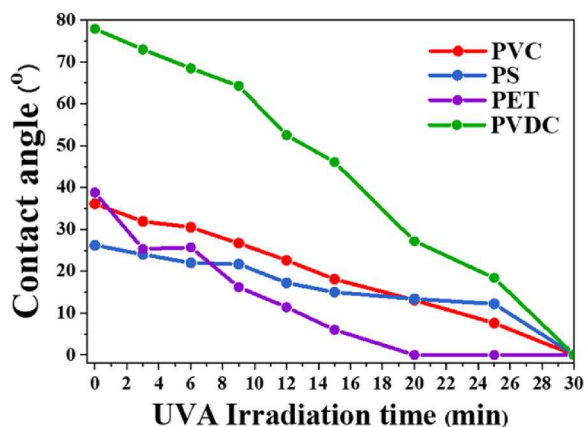
The presence of contaminants in the  $\text{TiO}_2$  thin film was examined in this section. The XPS survey spectra of the coated substrates displayed Ti2p and O1s peaks corresponding to  $\text{TiO}_2$ , as shown in Figure 4. However, the significant intensity of the C1s peak at a binding energy of 285 eV indicated a substantial amount of

carbon, which could have originated from the substrate during the coating process or surface contamination from the air [28].

From Figure 5(a), high-resolution Ti 2p<sub>3/2</sub> and Ti 2p<sub>1/2</sub> peaks were detected at a binding energy of 458.8 and 464.7 eV in all coated substrates, indicating the presence of  $\text{Ti}^{4+}$  species in the anatase phase of  $\text{TiO}_2$  [29]. In the C 1s spectra (Figure 5(b)), peaks were observed at binding energies of 285.0, 286.3, and 289.0 eV in all coated substrates, corresponding to C–C, C–O, and Ti–O–C bonding, respectively [30]. The presence of Ti–O–C bonding aligns with the explanation above that the  $\text{TiO}_2$  thin film was disrupted by carbon atoms. Additionally, two peaks of O 1s were found in the coated substrates: a binding energy of  $530.2 \pm 0.2\text{ eV}$  was associated with Ti–O bonding, and that of  $532.2 \pm 0.3\text{ eV}$  (as shown in Figure 5(c)) indicates the presence of hydroxyl groups [31,32].

### Hydrophilicity and wettability of surface

The surface wettability of the coated substrates was analysed by measuring the contact angle. The substrates were exposed to UV-A light for different periods (ranging from 0 to 30 min), and the results are presented in Figure 6. The PVDC-coated substrate showed the highest contact angle of  $78^\circ$ , followed by PET ( $40^\circ$ ), PVC ( $36^\circ$ ), and PS ( $26^\circ$ ). As the irradiation time increased, the contact angle continuously decreased for all coated substrates. This decrease indicated an increase in the hydrophilicity of the  $\text{TiO}_2$  thin film, leading to an enhancement of the photocatalytic process. According to Lee and Park [33], water molecules close to the  $\text{TiO}_2$  molecules on the thin film surface were dissociated into –OH and H atoms. The –OH group later bonded with Ti to form a hydroxyl group (Ti–OH), resulting in the formation of a hydrophilic surface. Table 2 shows that PET exhibited the highest O1s value of 48.91% (indicating Ti–OH bonding), making it the most hydrophilic surface. On the other hand, PVDC had the most hydrophobic surface among the coated substrates, with a relatively low O 1s value of 36.46%.



**Figure 6.** Water contact angle of coated substrates at various irradiation times.

**Table 2.** Correlation of %atomic of O 1s and water contact angle of coated substrates.

	PVC	PS	PET	PVDC
Atomic% of O1s	41.87	43.55	48.91	36.46
Contact angle	~36°	~26°	~40°	~78°
25 min of irradiation time	~7°	~12°	<1°	~18°

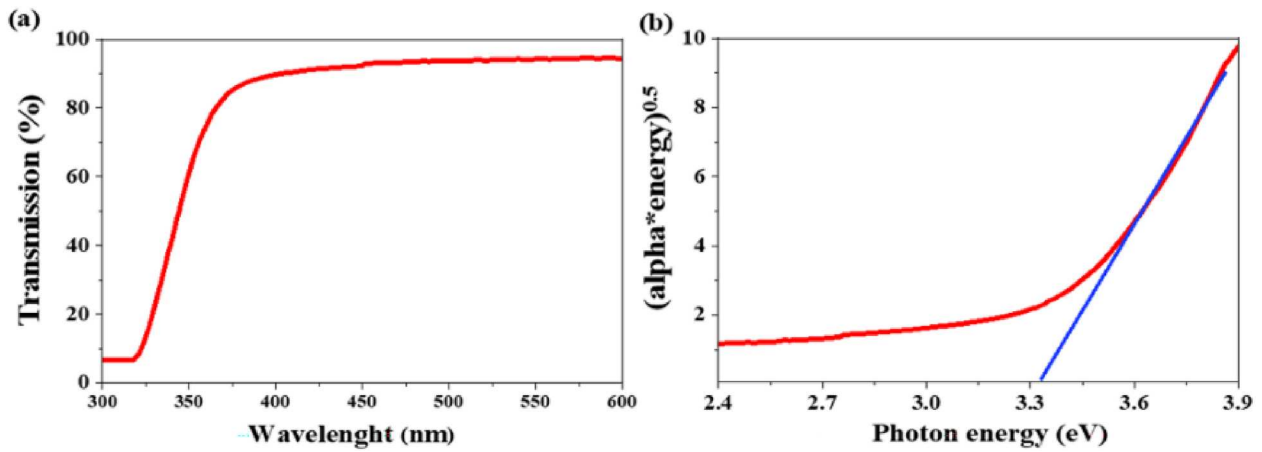


Figure 7. (a) Transmission spectra and (b) band gap energy of coated PVC.

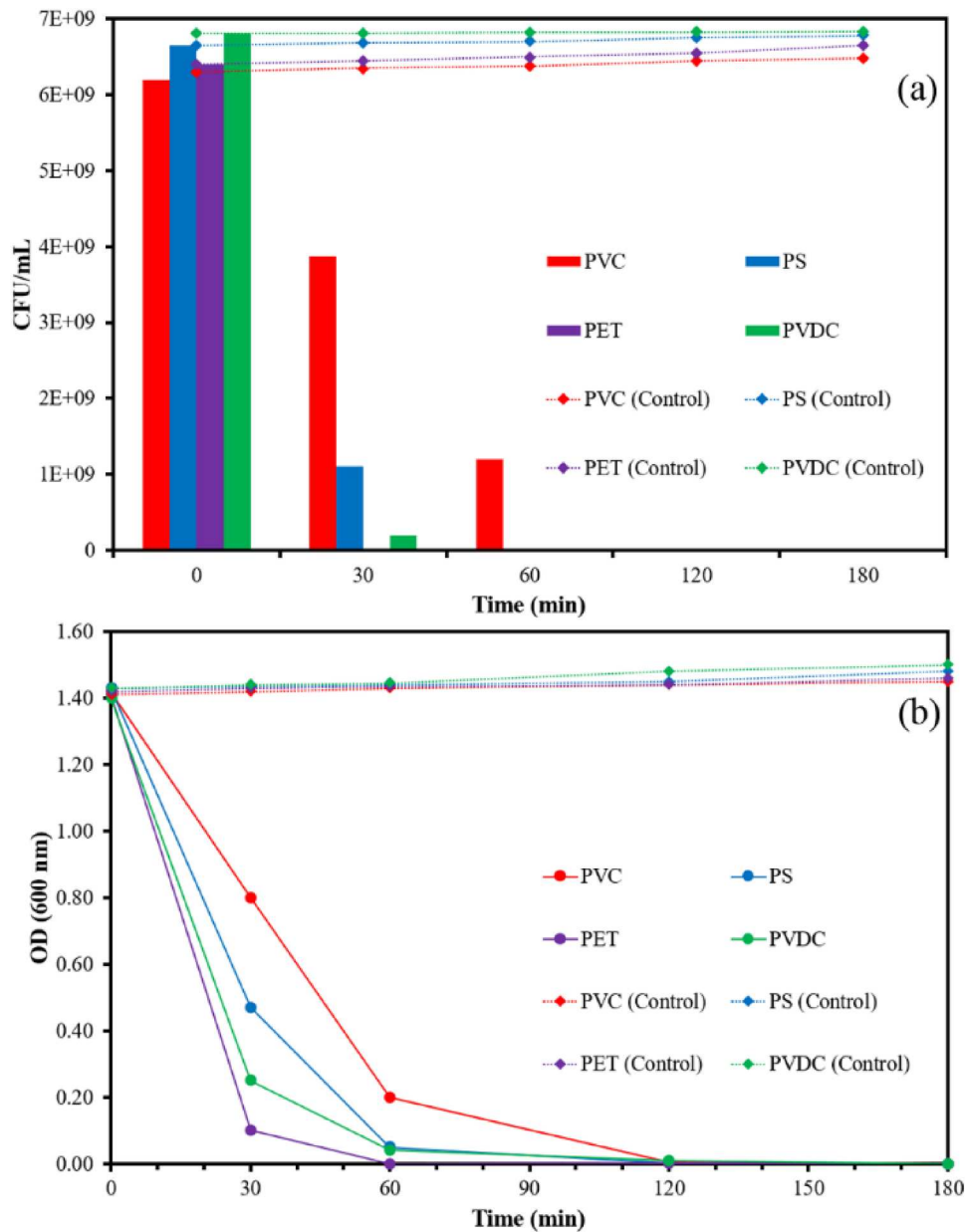


Figure 8. Reduction of *E.coli* number on coated substrates under UV-A irradiation: (a) CFU number and (b) OD absorption.

#### Optical property

The coated PVC substrate was used as a representative of TiO<sub>2</sub> thin film-coated substrates to investigate the

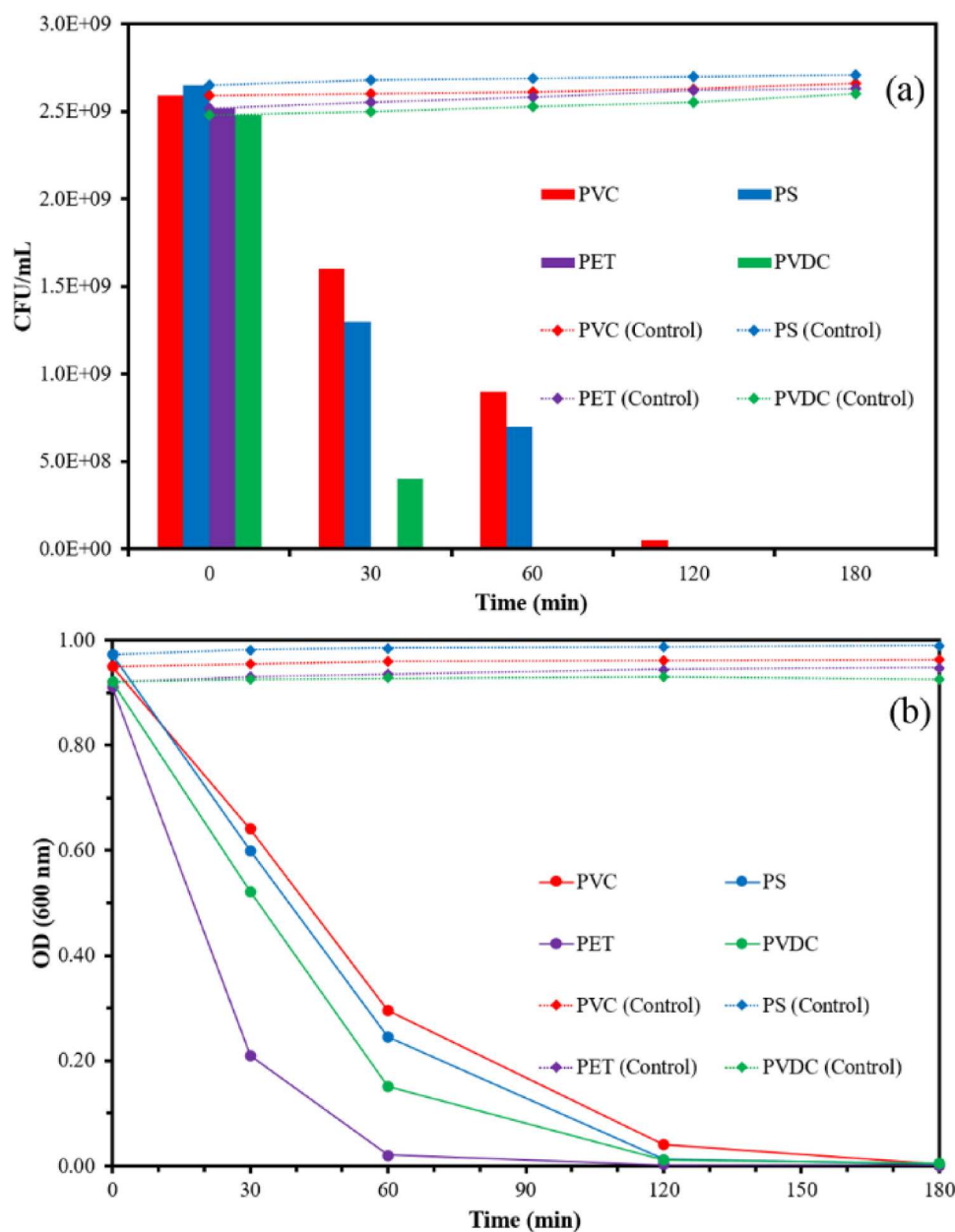
band gap energy of the thin film, which is further related to the light energy required to combat bacteria through the photocatalysis process. The optical



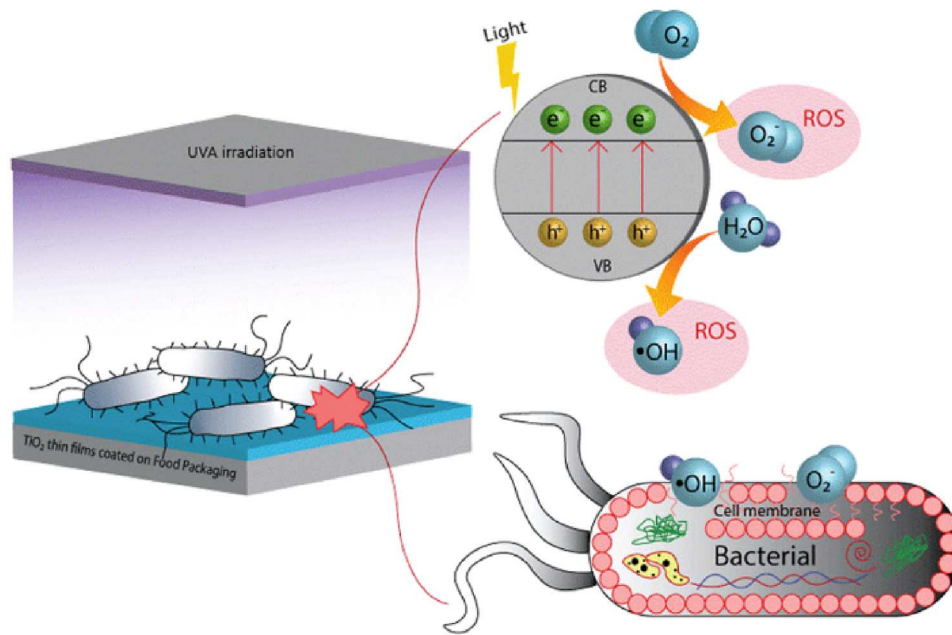
transmittance and band energy of the coated PVC substrate are shown in Figure 7(a and b), respectively. The transmittance of 85%–90% was observed in the spectral region spanning wavelengths from 375 to 600 nm. According to Tauc's relation, plotting  $(\alpha \times \text{energy})^{0.5}$  versus the photon energy gives a straight line in a certain region. The x-axis interception of this straight line gives the indirect optical energy gap, which was approximately 3.32 eV in this study. The band gap energy of the coated PVC substrate was slightly higher than that of  $\text{TiO}_2$  in the literature (3.20 eV) [34]. This could be due to the phase composition [35] resulting from the presence of carbon atoms in the  $\text{TiO}_2$  thin film, as indicated by the above results of Raman and XPS analysis.

### Antibacterial property

Since the band gap energy of the coated  $\text{TiO}_2$  thin film on the substrate was approximately 3.32 eV, UV-A light was required to activate the photocatalysis process for antibacterial activity on the coated substrates. The reduction in bacterial numbers was established using both CFU and OD600. Figure 8 (a and b) shows that the number of *E. coli* rapidly decreased on PET, with the initial concentration of  $6.5 \times 10^9$  CFU/mL being reduced to  $2.5 \times 10^5$  CFU/mL at 30 min (92.9%) and reaching zero at 60 min (100%). On the other hand, PVC showed the poorest antibacterial property against *E. coli*. The same results were also observed in the OD600 measure-



**Figure 9.** Reduction of *S. typhimurium* number on coated substrates under UV-A irradiation: (a) CFU number and (b) OD absorption.

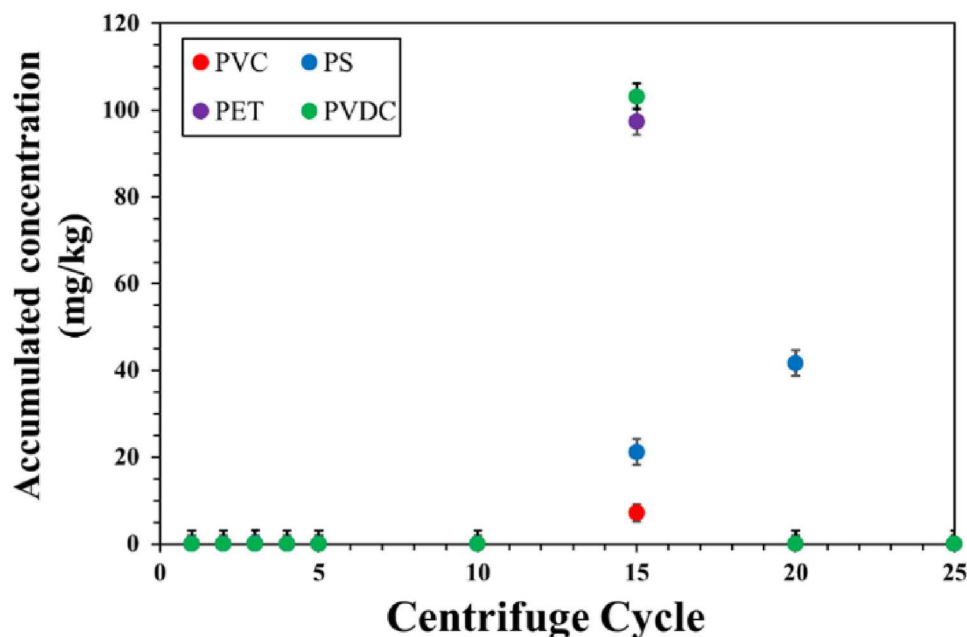


**Figure 10.** Schematic diagram of bactericidal mechanism of  $\text{TiO}_2$  thin films coated substrates through photocatalysis process.

ment. Due to the reduction kinetics, the antibacterial property of coated substrates followed the order  $\text{PET} > \text{PVDC} > \text{PS}$  and  $\text{PVC}$ , with first-order reaction constants of 0.109 1/s for PET, 0.059 1/s for PVDC, 0.053 1/s for PS, and 0.050 1/s for PVC. However, complete *E. coli* reduction occurred in 120 min for all coated substrates. On the other hand, steady growth of *E. coli* was observed over the incubation time in the uncoated substrates. It indicates that UV-A irradiation did not cause any *E. coli* death.

Figure 9(a and b) presents the *S. typhimurium* test, with the number of bacteria decreasing rapidly from

$2.5 \times 10^9$  CFU/mL to  $4.2 \times 10^6$  CFU/mL at 30 min (76.95%) and reaching  $3 \times 10^3$  CFU/mL at 60 min (97.8%) on PET. Meanwhile, the other substrates exhibited a lower reduction rate, ranging from 32% to 43% at 30 min and 68% to 83% at 60 min. Among the four substrates, PET still exhibited the best antibacterial property with the highest constant rate of 0.052 1/s, followed by PVDC, PS, and PVC, respectively. Conversely, *S. typhimurium* increased steadily over the incubation time in the case of uncoated substrates, indicating that UV-A irradiation did not harm *S. typhimurium*. In addition, PET had the most hydrophilic surface and a lower Ti–O–C bonding in the thin



**Figure 11.** Amount of  $\text{TiO}_2$  peeled off at increasing centrifuge cycles.

film compared to the others, enabling the effective photocatalysis process of TiO<sub>2</sub> to deactivate bacterial cells. Although *E. coli* and *S. typhimurium* are both Gram-negative bacteria, the coated substrates were more effective against *E. coli* than *S. typhimurium*. In the literature, TiO<sub>2</sub>-incorporated PE film exhibited antibacterial activity against *E. coli* of around 89.3% under 60 min of UV irradiation [36]. Nano TiO<sub>2</sub> demonstrated intense disinfecting activity against both Gram-negative and Gram-positive pathogens under UV light, with no *S. typhimurium* observed after 180 min [37].

The bactericidal mechanisms of TiO<sub>2</sub> thin film-coated substrates through the photocatalysis process are suggested in Figure 10. When the TiO<sub>2</sub> thin film on the coated substrates was irradiated, electrons on the surface were transferred from the valence band (VB) to the conduction band (CB), generating reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anions. The ROS then attached to the cell membrane of bacteria, namely *E. coli* and *S. typhimurium*, promoting the peroxidation of polyunsaturated phospholipid components of bacterial lipids. Eventually, the bacterial cells lost their components, leading to their death [36].

### Coated substrates robustness

This section investigates the leaching of TiO<sub>2</sub> from the coated substrates to identify the durability of the antibacterial property of food packaging. As shown in Figure 11, there was no leaching of TiO<sub>2</sub> from the coated substrates during the first 10 centrifuge cycles. However, suspended TiO<sub>2</sub> was observed during the 15th centrifuge cycle, with approximately 7, 21, 97, and 103 mg/kg for the coated substrates PVC, PS, PET, and PVDC, respectively. PET and PVDC showed a high amount of leached TiO<sub>2</sub>, but the values remained consistent in the later cycles of 20 and 25. In contrast, PS showed a continuous increase in leached TiO<sub>2</sub>, reaching a maximum value of 62 mg/kg. On the other hand, only 0 mg/kg of TiO<sub>2</sub> was leached from the coated PVC after 25 centrifuge cycles, indicating the high durability and resistance of the thin film surface. Thus, the materials used for food packaging significantly impacted the durability of the coated TiO<sub>2</sub> thin film on the substrates. However, leaching of TiO<sub>2</sub> did not occur easily and required high turbulence and contact time.

### Conclusions

The main points of emphasis in this work are:

- This work demonstrated a simple coating technique on PVC, PS, PET, and PVDC food packaging substrates for antibacterial surfaces.

- The TiO<sub>2</sub> thin film was smoothly coated on the substrates without cracks or pinholes and was in the anatase phase with a band gap energy of 3.32 eV.
- The antibacterial properties of the coated substrates depended on the polymer types and their physico-chemical properties.
- PET had the highest antibacterial activity of 97% at 60 min, but peeling TiO<sub>2</sub> of 97 mg/kg was observed after 15 cycles of 20 min and 10,000 rpm centrifuge.
- All coated substrates achieved disinfection of *E. coli* and *S. Typhimurium* after 120 and 180 min of UV-A irradiation, respectively.
- This work indicated that various food packages coated with TiO<sub>2</sub> thin films obtained excellent antibacterial properties and could potentially extend the shelf life of food products.

In conclusion, this study successfully demonstrated a simple coating technique on common food packaging substrates for antibacterial surfaces using TiO<sub>2</sub> thin films. The coated substrates exhibited excellent antibacterial properties that varied depending on the polymer types and their physico-chemical properties. PET had the highest antibacterial activity of 97% at 60 min, but it also showed the highest amount of peeling TiO<sub>2</sub>. All coated substrates achieved disinfection of *E. coli* and *S. Typhimurium* after 120 and 180 min of UV-A irradiation, respectively. The findings suggest that TiO<sub>2</sub> thin films could potentially extend the shelf life of food products. Further studies are recommended to explore this possibility.

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### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### Data availability statement

Derived data supporting the findings of this study are available from the corresponding authors (A. Nakaruk and W. Khanitchaidecha) on request.

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