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RESEARCH ARTICLE

Novel antimicrobial bioplastic based on PLA-chitosan by addition of TiO_2 and ZnO

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Abstract

Purpose The purpose of this study was to develop antimicrobial bioplastics based on Poly Lactic Acid (PLA) with the addition of chitosan-ZnO, and chitosan-TiO_2 to improve antimicrobial properties.

Methods For the preparation of the bioplastics, PLA with chitosan-ZnO or chitosan-TiO_2 were used. The antimicrobial activity, mechanical and thermal properties, and water vapor permeability of bioplastics were evaluated.

Results PLA-chitosan-ZnO indicated a robust antimicrobial activity against bacteria such as *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, yeast such as *Candida albicans*, and fungus *Aspergillus niger*. No formation of new functional groups in PLA-chitosan-ZnO composites. In comparison to other PLA-based bioplastics, this bioplastic has medium tensile strength, tensile modulus, and elongation percentages with low barrier ability to water vapor. Chitosan-ZnO itself has a greater tensile strength compared to chitosan-TiO_2. These two compounds undergo 2 stages of decomposition in a temperature range of 43 °C to 265 °C. The addition of PLA into chitosan-ZnO or chitosan TiO_2 causes the bioplastics decomposed in a single stage. It also increases the decomposition temperature of bioplastic. However, compared to chitosan-ZnO or TiO_2, the PLA-chitosan-ZnO or TiO_2 bioplastics tend to produce a fragile composite indicating by decrease in their tensile strength.

Conclusion In general, the addition of chitosan-ZnO into in PLA-based bioplastic produces better antimicrobial properties compared to TiO_2.

Keywords Antimicrobial properties · Bioplastic · Poly lactic acid (PLA) · Chitosan · TiO_2 · ZnO

Introduction

Recently, petrochemical plastics such as polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyamide (PA) are widely used as packaging materials [1]. Since plastics take longer to degrade completely, their usage needs to be reduced in order to limit environmental pollution. According to the Indonesian Ministry of Environment and Forestry (KLHK), environmental pollution due to waste generation reached 65.2 million tons in 2016, plastic is one kinds of waste that disposed to the sea [2]. The plastic waste might break down into small particles microplastics, which risks to marine biota life since they are indigestible. Besides, plastic packages are often contaminated with food ingredients and biological substances, even though the recycling of contaminated plastics are practical, however it tends to costly and difficult.

Polylactic acid (PLA) is a polymer chemically synthesized from agricultural sources. It is produced from L-lactic acid obtained from the fermentation of corn starch and other sources of polysaccharides [3]. PLA, is biodegradable plastic that has potency to replace petroleum-based fuel plastics due to its high stiffness and strength. However, PLA also has drawbacks such as heat-resistant, easy to break and slow crystallization process. The biodegradable PLA-based plastics were developed with one of the desired characteristics, namely antimicrobial activity. In terms of its nature, PLA has several

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advantages, namely biocompatibility, easy processing, and renewable. PLA itself does not has the ability to inhibit microbial growth. Therefore, this study proposed to add compound that have antimicrobial properties such as chitosan which has antimicrobial properties.

Chitosan is a cationic polysaccharide obtained from the chitin deacetylation process widely used as an active packaging material due to its high biodegradability, non-toxicity, and antimicrobial properties. It is often used as an antimicrobial agent either alone or mixed with other natural polymers. According to Bonilla [4], PLA-chitosan films have antimicrobial activity against mesophilic aerobic bacteria and coliforms. To increase its antimicrobial activity, titanium dioxide (TiO_2) or zinc oxide (ZnO) can be added to the bioplastic. TiO_2 and ZnO, inorganic materials, has been used for energy, medicine, packaging preservatives, and antimicrobial agents [5, 6]. TiO_2 has essential characteristics of excellent chemical stability, being non-toxic, low-cost, and antibacterial properties. TiO_2 has received considerable attention due to its good chemical stability, high photocatalytic activity, and antibacterial properties [7]. The photocatalytic properties of TiO_2 generates highly reactive radical with a highly oxidative and reactive properties under UV-light irradiation [8] can completely decompose the contaminants and also kills the microbes. Synergistic effects of α-Fe_2O_3-TiO_2 and $Na_2S_2O_8$ on the performance of a non-thermal plasma reactor as a novel catalytic oxidation process for dimethyl phthalate degradation These properties have been explored for self-cleaning, disinfectant, and anticancer [9]. These properties have been exploited for self-cleaning, disinfecting, and anticancer.

While ZnO has antibacterial activity against Gram-positive, Gram-negative, and pathogenic bacteria carried by food products [6]. Non-biodegradable plastics are adversely used as food packaging. Utilization of plastic kinds can generate environmental problem due to not degrade in longer time. Therefore, this issue leads to develop degradable plastics with some improvement properties such as antimicrobial properties. Previously Zhang et al. [10] developed antimicrobial paper-based packaging using PLA-ZnO nanocomposite. The thermal properties of PLA-ZnO biocomposite film has been reported by Ghozali et al. [11]. Zhang et al. [12] reported chitosan-TiO_2 composite film that proposed as packaging application. In this present study, we proposed to develop a new method in preparation of antimicrobial PLA based-bioplastic. This bioplastic preparation used modified method from reported studies. PLA-based bioplastic was combined with antimicrobial material such as chitosan, TiO_2 or ZnO.

For observation the antimicrobial properties of bioplastic, the bioplastic was tested into Gram-negative (*Escherichia coli* and *Salmonella typhi*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) *Candida albicans* (yeast), *Aspergillus niger* (fungi). Gram-negative is less resistant that Gram-positive bacteria might be caused by the difference in cell wall constituent [13]. *Escherichia coli* has been selected as target bacterium for comparing the efficacy of many antimicrobial agents since it is an indicator of fecal pollution [14]. *Bacillus subtilis* is a Gram-positive bacterium [13] has been used in many studies [14]. It is a species of aerobic bacteria, and non-pathogenic organism that is widely found in soil, air, decomposed plants, vegetation, the cause of endocarditis disease [15] and highly resistant to disinfection [16]. In addition, this microbe can form spore when the nutrients in the environment is limited, easy to count and identify in laboratory conditions [17].

Staphylococcus aureus is a Gram-positive, aerobic bacterium [13] with diameter of 0.5–1.5 μm that is an important cause of infections in humans [18] and cause gastroenteritis disease [19]. Approximately 30% of the healthy human population are carriers of *S. aureus* that can cause life-threatening infections [20]. According to Nataro et al. [21], some examples of anaerobic facultative bacteria are *Eschericia coli* and *Salmonella typii*, types of a Gram-negative [13] and anaerobic facultative bacteria. These bacteria cause diarrhea, urinary tract infections, meningitis and septicemia [22]. *S. typii* bacterium is typhoid-causing pathogenic bacteria [23] and can cause food poisoning [24]. The yeast, *Candida albicans* was also used in this study. This microbe has different cell morphology namely khamir, psedohifa and hyphae [25] and causes infection in the mucous membrane and systemic infections of the blood vessels and the main system [26]. *Aspergillus niger* is a fungus species that causes black fungal disease in plants and fruits as well as lung infections [27]. The fungus grows optimally at temperatures of 24–37 °C and pH 4–6.5 [28].

Materials and methods

Materials

The materials for bioplastic preparation consist of PLA (Ingeo 4060D), chitosan (Degree of deacetylation ≥ 87.5%), TiO_2 (Merck, Darmstadt Germany) that has crystal phase of anatase, ZnO (Wako pure chemical industries, Ltd.), acetic acid (AcOH) 2.5% *v*/v (technical grade), acetone (Merck, KGaA, Darmstadt Germany), and glutardialdehyde (Merck, Schuchardt OHG). The antimicrobial activity test media materials include H_2O, EtOH 70% (technical grade), Nutrient Broth (NB), Nutrient Agar (NA) and Potato Dextrose Agar (PDA) (Himedia Laboratories Pvt.Ltd), Potato Dextrose Broth (GDP) (Le Pont de Claix France), and Bacteriological Agar (LP0011 Oxoid). The observation of antimicrobial activity of bioplastic involved the use of bacteria such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi*, yeast, *Candida albicans,* and fungus, *Aspergillus niger.*

Methods

Chitosan, ZnO, and TiO_2 antimicrobial activity test

The antimicrobial activity test of chitosan, ZnO and TiO_2 was carried according to Zulfiana et al. [13]. The evaluation of the antimicrobial activity uses an agar well diffusion method. *Escherichia coli* and *Samonella typhi* (a type of Gram-negative bacteria), *Staphylococcus aureus* and *Bacillus subtilis* (a type of Gram-positive bacteria), *Candida albicans* (yeast), *Aspergillus niger* (fungi) were used in testing of the antimicrobial activities. One to two loops of microorganism were inoculated into 50 mL of culture medium in 100 mL Erlenmeyer flask. The culture mediums were nutrient broth (NB) and potato dextrose broth (PDB) for bacteria and yeast/fungi, respectively. The flask was incubated in rotary shaker (120 rpm) for 24 h at rooms temperature (30 ± 2 °C). At the end of incubation period, 1% (v/v of inoculum (approximately 10^7 CFU/mL) was inoculated into 200 mL of agar medium and mixed until homogeneous by using magnetic stirrer. As much as 20 mL of the microbial mixtures were poured into a sterilized petri dish. After solidification, hollows of 6.0 mm diameter wells were cut from the agar using a sterile cork borer and 50 μL of each of the tested solutions were poured into the wells. The solutions were tested at samples including chitosan, ZnO, TiO_2 that dissolved at 2.5% v/v AcOH as solvent. This also involved pouring 20 mL of the microbial mixtures into a sterilized petri dish, after solidification, the circular samples of the bioplastic at a diameter of 6.0 mm were placed on the agar surface. The dishes were later incubated at room temperature for 24–48 h after which the diameter of the inhibition zone (mm) was measured. The antimicrobial test was carried out triplicate.

Formulation of chitosan-ZnO and chitosan-TiO_2 bioplastics

The preparation of chitosan-ZnO or chitosan-TiO_2 film was carried out according to Zhang et al. [29] method with the slight modification. In this present study, the cross-linker agent i.e. epichlorohydrin was replaced with glutardialdehyde. Besides that, ZnO was also used in the combination with chitosan in the preparation of bioplastic. Approximately 0.5 g chitosan and 0.05 g ZnO or TiO_2 powder was added into 20 mL AcOH 2.5% v/v to prepare chitosan-ZnO and chitosan-TiO_2 films. Each solution was stirred with a magnetic stirrer to uniformity and sonicated at 120 W for 10 min, followed by the addition of 0.1 mL glutardialdehyde in each solution and stirring for 4 h at room temperature, then placed into a mold and dried it at room temperature [29].

Preparation bioplastics with PLA

PLA-chitosan-ZnO and PLA–chitosan-TiO_2 films were prepared following the Ghozali et al. [11] method with modification. The modification that has been made in this study was by combining PLA with chitosan-ZnO and chitosan-TiO_2, while Ghozali et al. [11] was only prepared PLA-ZnO bioplastic. An amount of 2 g of PLA was dissolved in acetone in separate dishes and carefully stirred at room temperature. This was followed by the addition of chitosan-ZnO and chitosan-TiO_2 in powder forms in each dish and stirred for 2 h. The loading ratio between PLA-chitosan-ZnO/TiO_2 was 40:10:1. Each solution was then placed in the mold and dried at room temperature [11].

Antimicrobial activity test of bioplastics

Bioplastic samples tested for antimicrobial activities include chitosan-ZnO, chitosan-TiO_2, PLA-chitosan-ZnO, and PLA-chitosan-TiO_2. The procedure used was similar to the one employed in the evaluation of the antimicrobial activity of chitosan, ZnO, and TiO_2.

Attenuated Total reflectance (ATR)-Fourier transform infra-red (FTIR) analysis

(ATR)-FTIR (Perkin Elmer) was used to characterize the functional groups of bioplastics. Approximately 0.1 mg of bioplastics samples were placed on a plate. IR spectra were recorded with a scan count of 1 per sample in absorption mode and a spectral resolution of 4.0 cm^{-1} in the wave number range of 4000 to 400 cm^{-1} at room temperature using spectrum software (Perkin Elmer, USA).

Tensile strength test of bioplastics

Testing for composite products included tensile strength, which, according to ASTM D882-75b [30], was carried out by cutting the bioplastic into a size of 10 × 1 cm^2. Afterward, the samples were tested using the Shimadzu Universal Testing Machine and pulled at a constant speed until the films were broken. The tensile strength and elongation percentage (% E) can be determined using Eqs. 1 and 2 as follows

$$\sigma = \frac{F}{A} \quad (1)$$

$$\%E = \frac{\Delta L}{L1} \times 100\% \quad (2)$$

Where σ is tensile strength (MPa), F is a force of tensile strength (N), A is field area (mm^2), % E is elongation percentage (%), ΔL is length increase (mm), and L_1 is an initial length (mm).

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA 4000 Perkin Elmer) was carried out based on Bonilla et al. [4] with modification to measure thermal weight loss of each sample formulation. The temperature range was set at 30–900 °C with a heating rate of 10 °C/min under nitrogen flow.

The measurement of water vapor permeability

Water Vapor Transmission Rate (WVTR) and Water Vapor Permeability (WVP) were determined through the cup method, according to ASTM E 95-96 1995 [31]. The WVTR and WVP were measured in triplicates. Water-filled Petri dishes were covered with aluminum foil depending on their sizes. The central part of the aluminum foil paper was perforated by 10% of the area of the petri dish, and the bioplastics were affixed to which the permeability value was to be measured. Petri dishes were weighed and then put into an oven at 37 ± 0.5 °C every 1 h for 7 h. Each sample was measured three times, and the loss of water mass was determined based on time until a steady-state was reached. WVTR and WVP values were calculated using eqs. 3 and 4, respectively.

$$\mathrm{WVTR} = \frac{\mathrm{flux}}{\mathrm{sur\,facearea}} \quad (3)$$

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times \mathrm{d}}{\mathrm{S} \times (\mathrm{R1}-\mathrm{R2})} \times 100\% \quad (4)$$

Where WVTR is water vapor transmission rate ($gs^{-1}m^{-2}$), WVP is water vapor permeability ($gs^{-1}m^{-1}Pa^{-1}$), flux is slope in linear regression, d is film thickness (m), S is saturated air pressure at 37 °C (6266,134 Pa), R_1 is RH in Petri dishes (100%), and R_2 is RH at 37 °C (81%).

Results and discussion

Antimicrobial activity of bioplastics

Antimicrobial activity can be determined based on the formation of inhibition zones on microbial growth media. Figure 1 shows the antimicrobial activity of the initial samples with AcOH 2.5% v/v as a control, which also has antimicrobial activity. Chitosan might inhibit the growth rate of *Escherichia coli, Bacillus subtilis, Salmonella typhi*, and *Staphylococcus aureus*. In general, chitosan and its derivatives are strong antimicrobial agents that inhibit the growth of Gram-positive and Gram-negative bacteria, fungi, and viruses. Under acidic conditions, the C-2 position of the chitosan glucosamine monomer has a positive charge, which interacts easily with the negative charge of microbial cell membranes and causes leakage of proteins and intracellular microbial constituents [32]. Antimicrobial mechanism of chitosan involves penetrating the nucleus, binding to DNA, and inhibiting mRNA synthesis, inhibiting the production of amino acid [33].

According to Wang et al. [34], TiO_2 has antimicrobial ability to mediate photocatalysis under UV light. Reactive oxygen species (ROS), such as OH, H_2O_2, and O_2^- which are essential in destroying microbial cells are produced with enough energy. However, in this study, the results indicate TiO_2 produces antimicrobial activity in the absence of UV light and inhibit the growth of *Escherichia coli, Bacillus subtilis*, and *Candida albicans* since it has significant activity on *Escherichia coli* [35]. Additionally, ZnO inhibits the growth of *Escherichia coli, Bacillus subtilis*, and *Aspergillus niger*. The antimicrobial mechanism of ZnO involves (i) production of ROS, (ii) destabilization of microbial membranes having direct contact with ZnO particles to the cell wall, and (iii) intrinsic antimicrobial Zn^{2+} ion released by ZnO in water media [36].

The antimicrobial activity of the bioplastics refers to the ability of the film to inhibit the growth of microbes, both bacteriostatic and fungistatic. The antimicrobial activity of bioplastic is measured from the diameter of the inhibitor zone formed, as shown in Fig. 2. PLA-chitosan bioplastic does not have antimicrobial activity since chitosan powder is not activated by acetic acid. Chitosan-TiO_2 only inhibits the growth of *Escherichia coli* bacteria, a finding supported by Mihindukulasuriya [35], which established that TiO_2 has significant activity on *Escherichia coli*. In this study, chitosan-ZnO bioplastics have very high antimicrobial activity in which the growth of almost all pathogenic bacteria can be inhibited. This result corresponds to Li et al. [37], which stated that chitosan-ZnO inhibits the growth of bacteria *Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*.

PLA-chitosan-ZnO is among bioplastic composites with the best antimicrobial activity, which can be shown by the inhibition growth activity in all tested microbes. Chitosan-ZnO and PLA-chitosan-ZnO bioplastics have the highest growth inhibition ability in the fungus, *Aspergillus niger*, while PLA-chitosan-TiO_2 only inhibits the growth of *Salmonella typhi*. The antimicrobial activity of *Aspergilus niger* in PLA-chitosan-ZnO is much higher than its activity in coated paper with anionic nanocellulose crosslinked with cationic ion (H^+ and Al^{3+}) [13]. The similar inhibition activity of *Salmonella typhi* also presents in the test of coated paper with anionic nanocellulose crosslinked with cationic ion [13].

Visual appearance of bioplastic films

Chitosan-ZnO film in Fig. 3a is more transparent and brighter than chitosan-TiO_2 shown in Fig. 3b. This is because during the dissolution, ZnO dissolves in acetic acid to produce the

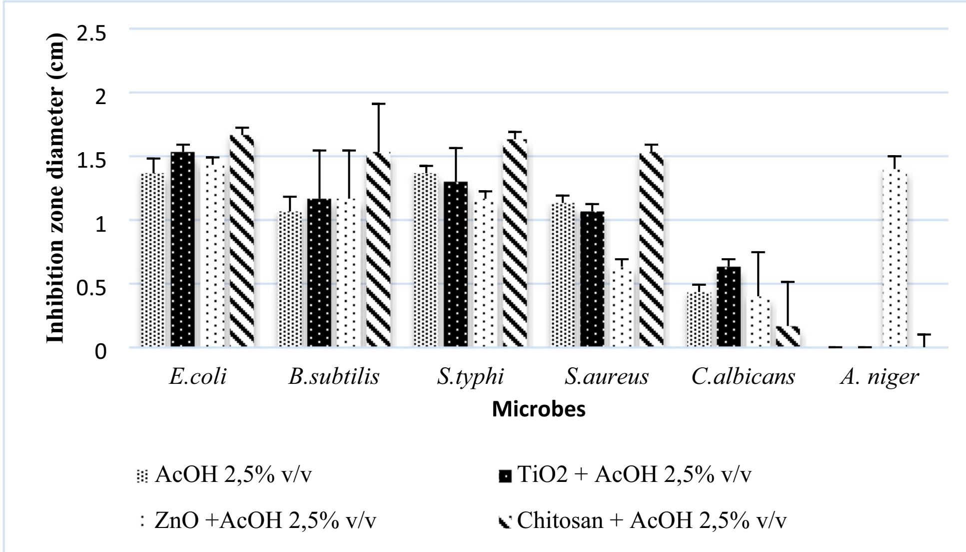


Fig. 1 Antimicrobial activity of initial samples: AcOH 2.5% v/v ( ) AcOH, 2.5% v/v + TiO_2 ( ), AcOH 2.5% v/ v + ZnO ( ) and AcOH 2.5% v/v + chitosan ( )

yellowish color while TiO_2 forms a white solution. This color formation is also caused by the migration of C—H bonds of chitosan in reaction with AcOH. 2.5% *v*/v.

Composite is a microscopic combination of two or more substances having different phases into a new material of better properties. During the preparation of an alloy of bioplastics consisting of PLA, chitosan, ZnO and TiO_2, chitosan-ZnO or chitosan-TiO_2 was prepared before the films were mashed and dissolved in PLA. Figure 3c shows the alloy of PLA-chitosan bioplastic has a flat surface with white color that is fragile and brittle. The bioplastic of PLA-chitosan-ZnO alloy has a slightly wavy and pores surface with orange color as shown in Fig. 3d, while Fig. 3e shows PLA-chitosan-TiO_2 has uneven pores surface with beige color. The formation of pores is attributed to the addition of chitosan-ZnO or chitosan-TiO_2 powder in PLA.

(ATR)-FTIR spectra

Similar to other types of energy absorption, the infrared radiation absorption agitates molecules to a higher energy state. It is a quantized process where molecules only absorb certain frequencies as the energy of infrared radiation. The absorption is related to energy changes at 8 to 40 kJ /mol, and the radiation corresponds to the frequency of vibrational stretching and bonding of most covalent molecules. Usually, the absorption involves infrared radiation frequencies which match the natural vibrational frequencies of the molecules. The energy absorbed increases the amplitude of the vibrational motion of molecule bonds [38]. Figure 4 shows the FTIR spectra of the chitosan, chitosan-ZnO, and chitosan-TiO_2 bioplastics.

The FTIR spectrum of pure chitosan (Fig. 4a) shows the peak at a wavenumber of 2920.44 cm^{-1}, which can be

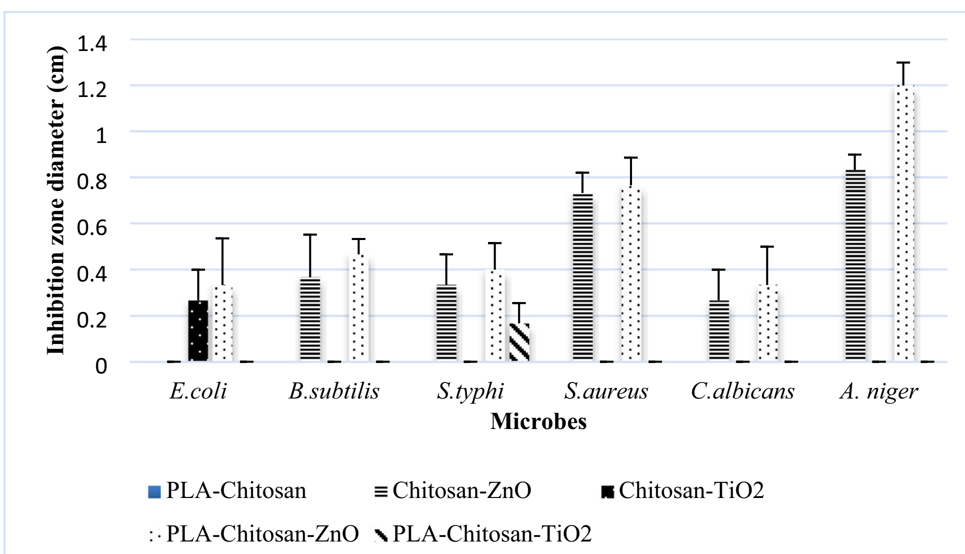


Fig. 2 Antimicrobial activities of bioplastics: PLA-chitosan ( ), chitosan-ZnO ( ), chitosan-TiO_2 ( ), PLA-chitosan-ZnO ( ), and PLA-chitosan-TiO_2 ( )

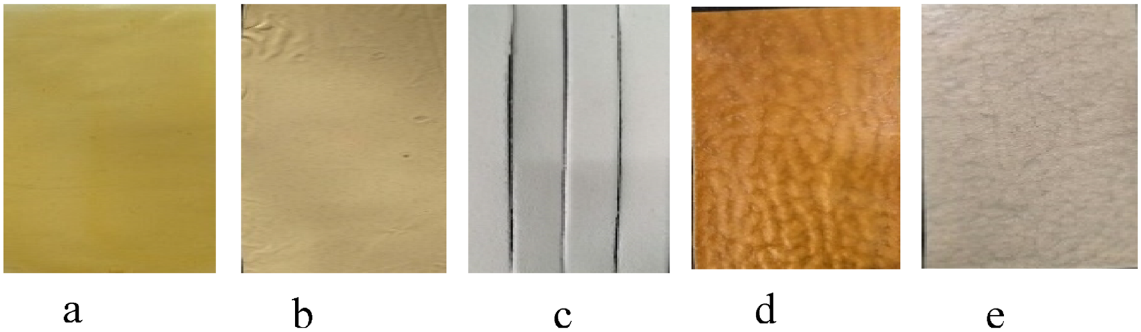
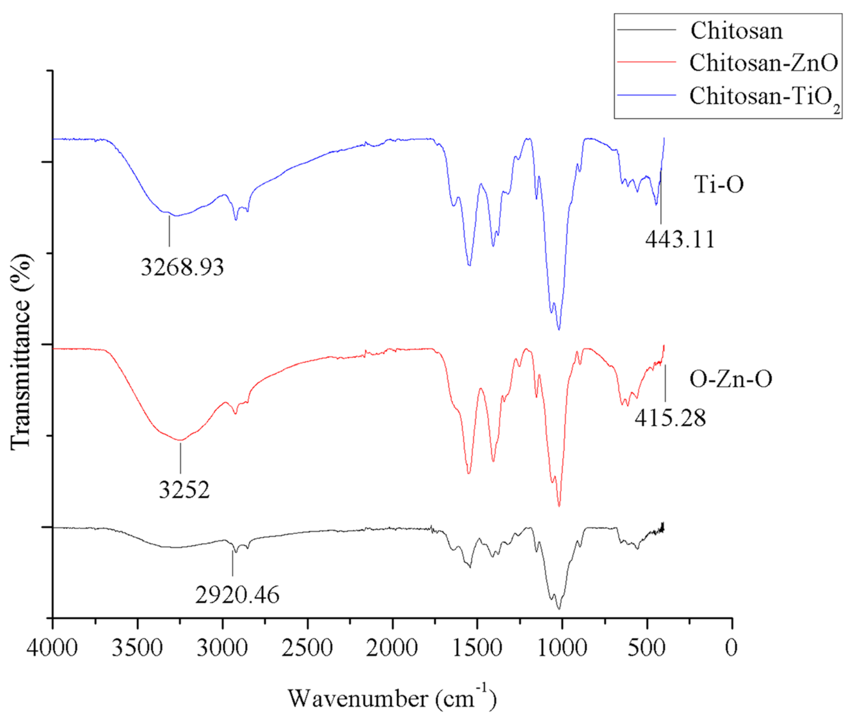


Fig. 3 Bioplastics: Chitosan-ZnO (**a**), Chitosan-TiO_2 (**b**), PLA-chitosan (**c**), PLA-chitosan-ZnO (**d**), and PLA-chitosan-TiO_2 (**e**)

identified as absorption —OH. According to Badi et al. [39] and Zulfiana et al. [13], and many similar studies, the broad peak in FTIR spectrum between around 3300 cm^{-1} may be attributed to the adsorbed water molecules (OH). Spectrum of 2900 cm^{-1} is the stretching vibration symmetry of the -CH group. Moreover, the wavenumbers of 1735 cm^{-1} is the vibration of the ester group while spectrum of 1050–1025 cm^{-1} presents the primary and secondary alcohol groups [13]. The addition of ZnO and TiO_2 facilitates each Zn^{2+} and Ti^{4+} ions to immediately form a coordination bond with —OH and —NH from chitosan [40]. There is a shift in the absorption peak of —OH in the chitosan-ZnO (Fig. 4b) and chitosan-TiO_2 (Fig. 4c) bioplastics towards the 3252 cm^{-1} and 3269.02 cm^{-1} wavenumbers, respectively. The absorption peak in the range of wavenumber of 580–400 cm^{-1} originates from O—Zn—O vibrations [38]. In the chitosan-ZnO bioplastic, the peak absorption of O—Zn—O is at the wavenumber of 415.28 cm^{-1}, as shown in Fig. 4b. The absorption peaks in the range fingerprint of wavenumbers of 700–400 cm^{-1} are derived from Ti—O absorption peaks [40]. A peak of absorption of Ti—O at the wavenumber of 443.11 cm^{-1} appears in the chitosan-TiO_2. The FTIR spectra confirm that chitosan-ZnO and chitosan-TiO_2 interact chemically with the proposed reaction, as shown in Fig. 5.

Interaction of chitosan and metal ions forms complex compounds (Fig. 5). Chitosan is an efficient chelating agent that bind metals to remove toxic produced by microorganisms. A regular absorption of each type of bond, such as N—H, C—H, O—H, C—X, C═O, C—O, C—C, C═C, C≡C, and C≡N, is only found in certain parts of the infrared vibration region. A small absorption range can be determined for each type of bond, though other types of bonds form absorption outside this range. Absorption in the range of 3000 ± 150 cm^{-1} is often attributed to the presence of C—H bond in the molecules. However, the absorption in the range of 1715 ± 100 cm^{-1} is caused by the presence of the C═O bond (carbonyl groups) in

Fig. 4 FTIR spectra of chitosan (**a**), chitosan-ZnO (**b**), and chitosan-TiO_2 (**c**) bioplastics



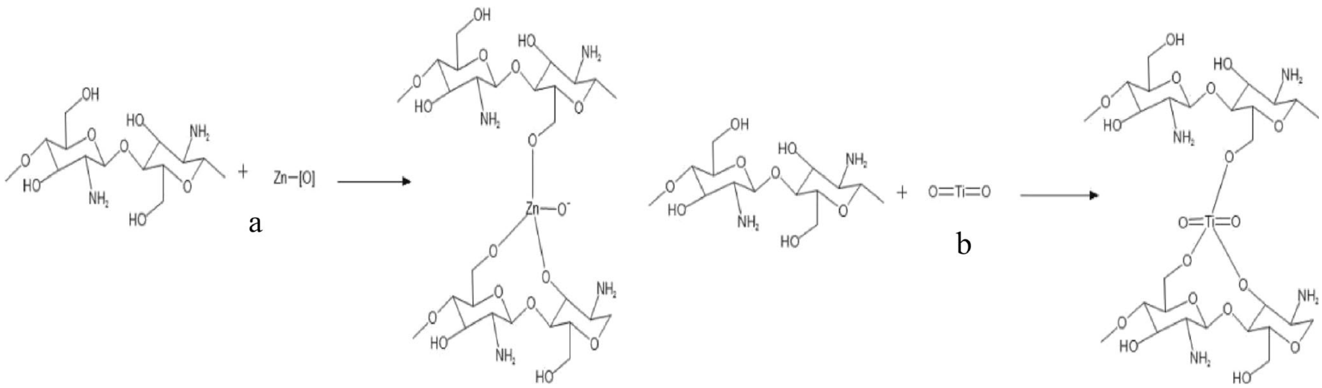


Fig. 5 Interactions in the chitosan-ZnO (**a**) and chitosan-TiO_2 (**b**) films

the molecules. The same range applies to each type of bond [38].

The spectra of bioplastics in the presence of additional PLA is shown in Fig. 6. The regions of interest for PLA and the composites are 1780, and 1680 cm^{-1} for the C=O and 3600–3000 cm^{-1} for the O-H stretch. The peaks of about 1750 and 1180 cm^{-1} belong to the C=O and the C-O-C stretching of PLA [41]. In general, all PLA bioplastics contain these two absorption peaks, and therefore the interactions between PLA and chitosan, chitosan-ZnO, and chitosan-TiO_2 do not occur chemically. Their occurred interactions are only physically in the alloy form.

Mechanical properties of bioplastics

Mechanical properties, Tensile Strength (TS), percentage elongation (% E), and Young's Modulus according to the bioplastic composition and constituent component features. By measuring these properties, the limitation of allowed external pressure in the bioplastic can be determined. TS is the maximum value of the bioplastic holding a given load before breaking. The elongation increases to the maximum length after the bioplastics are pulled up to break. The results of the tensile strength of bioplastics are summarized in Table 1.

The addition of ZnO and TiO_2 to chitosan increases the TS and the % E, respectively. TS of chitosan-ZnO bioplastics is higher than chitosan-TiO_2. Based on the result, the chitosan-ZnO bioplastics is hard and brittle, while the chitosan-TiO_2 film is hard and tough. This is in line with Cazon et al. [42], which stated that the TS of polysaccharide-based films, such as chitosan and synthetic polymers are similar. The TS of chitosan mixed with different molecular weights and solvents ranged from 6.7 to 150.2 N/mm^2, the same range for Chitosan-ZnO and chitosan-TiO_2 bioplastics fall in this range [42],

Pure PLA has a higher TS compared to % E. The addition of chitosan, ZnO, and TiO_2 decreases TS but increases the % E. The mixing of PLA and chitosan leads to a low TS of PLA-chitosan while the addition of ZnO and TiO_2 increases it to 193.64% and 102.91%, respectively. Moreover, the TS of PLA-chitosan-ZnO and PLA-chitosan-TiO_2 bioplastics are lower compared to chitosan-ZnO and chitosan-TiO_2 due to

Fig. 6 FTIR spectra: PLA (**a**), PLA-chitosan (**b**), PLA-chitosan-ZnO (**c**), dan PLA-chitosan-TiO_2 (**d**) bioplastics

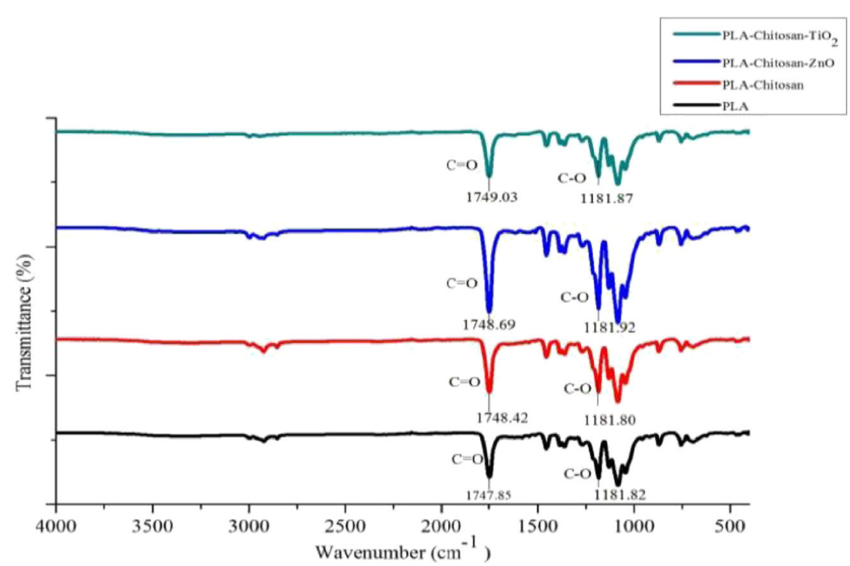


Table 1 Mechanical properties of bioplastics

Bioplastics	Young's Modulus (N/mm^2)	Tensile strength (N/mm^2)	Elongation at break (%)
Chitosan [22]	0.54–1.71	22.2–39.6	13–73.6
Chitosan-ZnO	10.496	31.403	2.992
Chitosan-TiO_2	0.714	27.030	37.876
PLA [23]	36.241	30.08	0.83
PLA-Chitosan	0.980	1.716	1.750
PLA-Chitosan-ZnO	2.861	5.039	1.761
PLA-Chitosan-TiO_2	0.745	3.482	4.673

the porous and slightly bumpy surface. As a result, the PLA-Chitosan, PLA-Chitosan-ZnO, and PLA-Chitosan-TiO_2 bioplastics are soft and weak.

Young's Modulus measures the stiffness of solid material and defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material in the linear elasticity regime of uniaxial deformation. It indicated chitosan-ZnO of 10.496 N/mm^2, and this means the film is more resistant to tensile pressure compared to others. As stated before, the hard and brittle structure of chitosan-film affects this value.

Thermal properties of bioplastics

The thermogravimetry (TGA) test determines the changes in sample weight-loss as a function of temperature variation. It relies on a high degree of precision in three measurements, including weight, temperature, and temperature changes. The principle of TGA helps to measure the average velocity of mass changes of a material/sample as a function of temperature or time in a controlled atmosphere condition.

This method characterizes a sample through its mass loss or the decomposition, oxidation, or dehydration. The mechanism of mass changes in the TGA in the material indicates a loss or gain in mass. Generally, mass loss occurs since the decomposition process involves the breaking of chemical bonds, evaporation (loss of volatility as temperature increase), reduction of the interaction of the material with reducing agents, and desorption. The increase in mass is due to the oxidation process, which involves the interaction of the material with the oxidizing agent and absorption [43]. Table 2 shows the thermal stability of chitosan, chitosan-ZnO, and chitosan-TiO_2.

Table 2 Temperature decomposition of bioplastics

Bioplastics	Temperature decomposition °C	
	Stage 1	Stage 2
Chitosan	24.91	258.73
Chitosan-ZnO	47.24	249.49
Chitosan-TiO_2	43.6	261.47
PLA	355.53	
PLA-Chitosan	287.87	
PLA-Chitosan-ZnO	275.83	
PLA-Chitosan-TiO_2	304.58	

The addition of ZnO and TiO_2 in chitosan reduces and increases the decomposition temperature in stage 2, respectively. Furthermore, addition of ZnO into PLA also decreases decomposition temperature. This finding is similar with previous observation of Ghozali et al. [11]. The chitosan-based bioplastics have a similar decomposition model in which they are decomposed in two stages, as shown in Table 2. The first mass loss below 100 °C is associated with water loss and shows very small shape as presented in the TGA curve. There is a lot of mass loss between temperatures of 200 °C and 300 °C, as shown in Fig. 7a. This is due to dehydration of the saccharide ring complex, depolymerization, and decomposition of the acetate polymer unit and its deacetylation [40]. The addition of PLA to the chitosan-ZnO bioplastics and chitosan-TiO_2 causes an increase in decomposition temperature, as shown in Table 2. PLA-chitosan, PLA-chitosan-ZnO, and PLA-chitosan-TiO_2 decompose in one stage in the temperature range of 270 to 310 °C, as shown in Fig. 7b.

Water vapor permeability

WVTR describes the ability of a material to hold water vapor into the plastic. This transmission value affects the WVP, which is the ability of a material to pass water vapor in an area unit. This value is affected by the pore size and hydrophilicity of the bioplastic constituent component. Figure 8 shows the WVP of bioplastics.

The WVP values of chitosan-ZnO and chitosan-TiO_2 bioplastics are 4146×10^{-9} ($gs^{-1}m^{-1}Pa^{-1}$) and 4263×10^{-9} ($gs^{-1}m^{-1}Pa^{-1}$). Chitosan-ZnO has a lower WVP value than chitosan-TiO_2. A hard and brittle property of chitosan-ZnO allows water vapor to pass through it easily. In contrast, the chitosan-TiO_2 film is hard and tough, and therefore water vapor hardly passes through it. WVP values of chitosan-ZnO and chitosan-TiO_2 is greater than WVP of pure chitosan, which is 1.05×10^{-14} ($gs^{-1}m^{-1}Pa^{-1}$)[41].

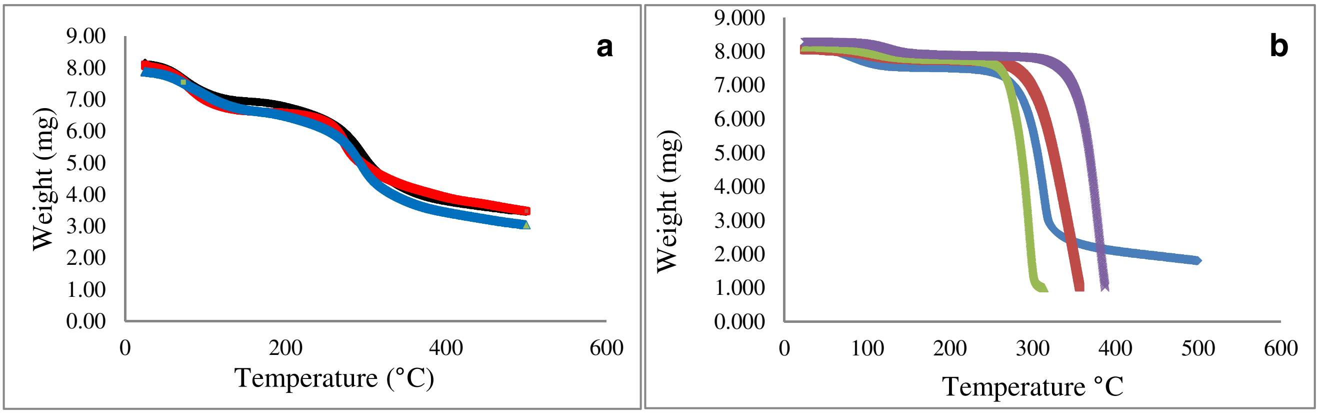


Fig. 7 **a**. TGA curves of chitosan (blue square), chitosan-ZnO (red square), chitosan TiO_2 (black square); **b**. PLA (violet square), PLA-chitosan (blue square), PLA-chitosan-ZnO (green square), PLA-chitosan-TiO_2 (red square)

The lowest WVP is found in PLA-chitosan, which has a great barrier against water vapor. However, the PLA-Chitosan-ZnO has the highest WVP, which means it has a low ability to resist water vapor passage. PLA-Chitosan-ZnO and PLA-Chitosan-TiO_2 have a high WVP due to the formation of pores on the film surface, which allows easy passage of water vapor. The WVP value of bioplastic produced is higher than that of commercial plastic and pure PLA. A low-density polyethylene (PE) has a WVP value of $(7.3–9.7) \times 10^{-13}(gs^{-1}m^{-1}Pa^{-1})$ [39], while PLA has WVP value 2.47×10^{-11} $(gs^{-1}m^{-1}pa^{-1})$ [44].

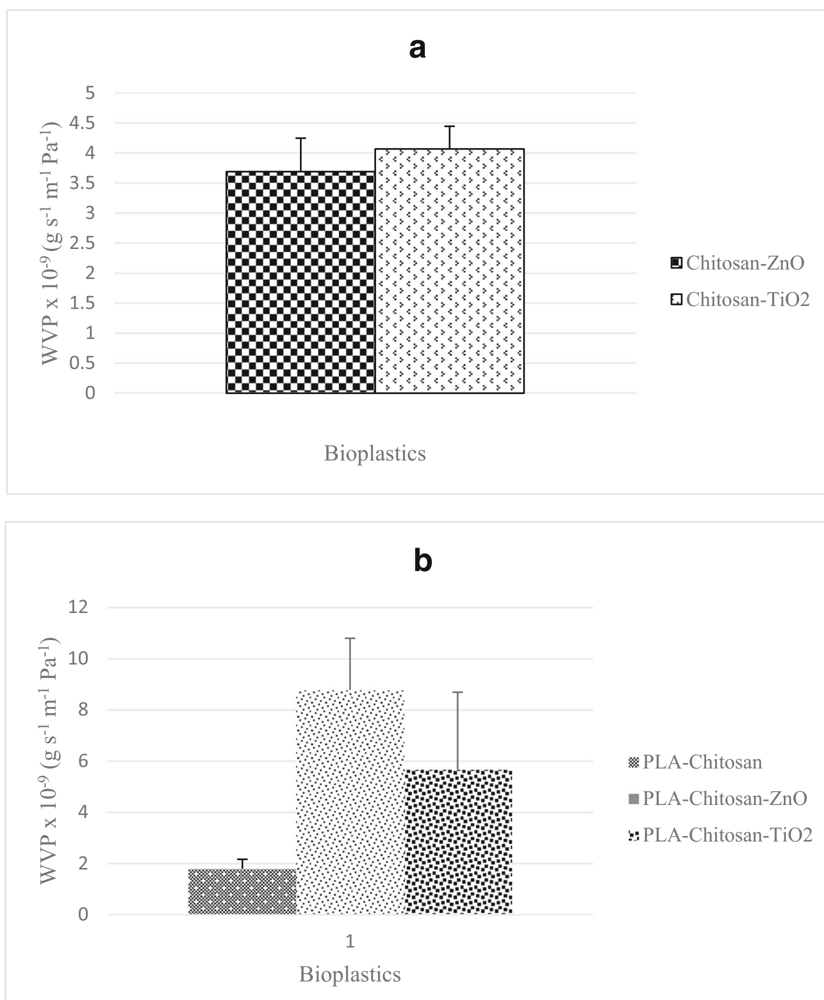


Fig. 8 A.Water vapor permeability of chitosan-ZnO ( ), chitosan-TiO_2 ( ), bioplastics; B. PLA-chitosan ( ), PLA-chitosan-ZnO ( ), and PLA-chitosan-TiO_2 ( )

Conclusions

The present study was to prepare PLA-based bioplastics that has antimicrobial activity by addition of chitosan-ZnO or chitosan-TiO_2. The interactions between chitosan-ZnO or TiO_2 form chelate compound structure. Meanwhile, the interaction between PLA with chitosan-ZnO or TiO_2 composites only form physical interaction indicated by no new functional groups. These interactions result in different mechanical properties of the bioplastic. The PLA-chitosan-ZnO and PLA-chitosan-TiO_2 bioplastics have a much lower tensile strength than pure chitosan and PLA. Meanwhile, tensile strength of chitosan-ZnO and chitosan-TiO_2 bioplastics was higher compared to chitosan alone. The PLA addition in chitosan-ZnO or TiO_2 increases the decomposition temperature and causes the change of decomposition stage of bioplastics into one stage. Water vapor permeability of chitosan-ZnO is lower than chitosan-TiO_2, however PLA addition into bioplastic increases this value. It might be affected by the mixing method of PLA into chitosan-TiO_2 or ZnO that need to be improved. The PLA-chitosan-ZnO bioplastic shows antimicrobial activity against to Gram-negative (*Escherichia coli* and *Salmonella typhi*), Gram-positive bacterium (*Staphylococcus aureus* and *Bacillus subtilis*) *Candida albicans* (yeast), *Aspergillus niger* (fungi). Generally, addition of chitosan- ZnO in PLA-based bioplastic has better antimicrobial activity than TiO_2.

Compliance with ethical standards

Conflict of interest The authors of this article declare that they have no conflict of interest.

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