In vitro biodegradation evaluation of linear low density polyethylene embedded with TiO$_2$/ZnO couple oxides

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Abstract. In vitro biodegradation of linear low density polyethylene (LLDPE) nanocomposite embedded with TiO$_2$/ZnO (1:3) coupled oxides was studied by Pseudomonas aeruginosa strain in minimal broth (M9) in two different culture systems. A biodegradation effect characterized by low weight loss of LLDPE/TiO$_2$/ZnO nanocomposites thin films (0.99%) with glucose as additional nutrient and (0.65%) weight reduction for nanocomposites thin films being immersed in M9 culture system without glucose was observed within 30 days. Bare LLDPE polymer (without addition of photocatalyst) also having weight reduction for (0.54%). Field emission scanning electron microscopy (FESEM) micrographs of pre-treated LLDPE nanocomposites thin films showed enhancement in surface deformation with formation of holes, wrinkles and rough surfaces after being immersed in M9 solution. Untreated LLDPE nanocomposites thin films had appearance of smooth surface with less defects. This study indicated the influence of TiO$_2$/ZnO couple oxides and glucose to boost the in vitro biodegradation of LLDPE nanocomposites thin films. Further research is needed to study the biodegradation process of LLDPE nanocomposites thin films in prolonged time.

1. Introduction
Since the last decades, synthetic polymers such as polyethylene (PE) have been greatly used for packaging and biomedical industries due to their excellent flexibility, mechanical strength and durability [1].

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It has been specifically designed and synthesized to meet the standard requirements for various types of applications. There have been several efforts aimed to reduce undesirable accumulation of PE since it gave a major threat to environments. Nowadays, the researches on the plastic degradation by the action of naturally occurring microorganism such as bacteria according to the standard protocols (ASTM D 6400-99 1976) have drawn a great attention. PE is one of the most produced synthetic polymers and getting approximately 30% of the plastic demands in Europe [2]. This led to the higher amounts of plastic productions volumes and limited spaces for their accumulations thus attract global demands for an eco-friendly synthetic biodegradable plastic and significantly contribute towards a plastic-free wastes environment mission [3].

Various types of bacteria and fungi such as Arthrobacter sp., Pseudomonas sp., Zalerion maritimum, Brevibacillus borstelensis, Marine Kocuria palustris M16 and Bacillus sp. have been reported for PE biodegradation [4-8]. Previous research has identified the presence of Arthrobacter sp. and Pseudomonas sp. isolated from plastic waste dumped sites caused nearly loss of 12% and 15% weight loss from gravimetric original weight after 30 days [4]. Another study showed a reduction in weight loss up to 1.75% of PE polymer after been incubated for 30 days in the shaken flasks containing marine bacterial isolates [8]. According to Kyaw et al. 2012, the PE films showed a significant degradation up to 20% with the development of Pseudomonas sp. biofilms on each strip [9]. They also point out that the biofilm could enhanced the biodegradation rate by reducing the hydrophobicity of the polymer. In this report, we focus on the degradation of linear low-density polyethylene (LLDPE), a short branch of polyethylene embedded with titanium dioxide (TiO₂) and zinc oxide (ZnO) by Pseudomonas aeruginosa strain. Their biodegradation rate is being analysed through several parameters include bacterial growth (optical density), weight loss reductions of the samples and morphological changes. This study highlighted enhancement of LLDPE degradation with the influence of nanoparticles.

2. Experimental

2.1. LLDPE/TiO₂/ZnO nanocomposites thin films preparation

Material used for synthesizing of LLDPE/TiO₂/ZnO nanocomposites thin films were titanium (IV) isopropoxide (97%, Sigma Aldrich), zinc acetate dehydrate (98%, Sigma Aldrich), ethanol (95%, Biotech Lab Supplies) and 1,2-dichlorobenzene (Merck) and LLDPE pellets (Lotte Chemical Titan (M) Sdn. Bhd.). Both TiO₂/ZnO photocatalyst was prepared by using sol-gel method accordingly to the molar ratio (TiO₂/ZnO = 1:3) and mixed together with LLDPE matrix (5%) as described in previous report [10,11].

2.2. LLDPE/TiO₂/ZnO nanocomposites thin films sterilization

The sterilization of the nanocomposites thin films was performed by using two methods; exposed under ultra-violet (UV) lights for 1 hour and rinsed with ethanol (70%) three times. Then, the nanocomposites thin films were immersed in sterile distilled water for overnight at 37°C and were dried under fume hood prior experiment.

2.3. Bacterial culture

Gram-negative strain, Pseudomonas aeruginosa (P.aeruginosa) was selected for nanocomposites thin films biodegradation studies in three types of culture systems. Subsequently, fresh bacterial inoculum was grown in nutrient LB broth, 18-21 hours at 37°C.

2.4. In vitro degradation test

Sterilized nanocomposites thin films (2.7 x 2.7 cm²) were added to 100 ml of minimal broth (M9) (pH 7.0) filled in 250 ml round conical flasks. Subsequently, 200 µL of P.aeruginosa was added into each flask. The assay was performed in two condition (with and without additional glucose) with respective positive control; (M9 solution, P.aeruginosa and films), negative control ; (M9 solution and films) and
M9 solution and *P. aeruginosa*) without films, respectively. The flasks were incubated at 37 °C with continuous shaking (150 rpm) for 4 weeks. The optical density (OD) at 600 nm were monitored continuously for every 3 days. Samples were collected at the end of experiment to determine any changes occurred.

2.5. Variables and analytical techniques associated with LLDPE/TiO$_2$/ZnO nanocomposites thin films

2.5.1. Weight loss

The films after exposure to *P. aeruginosa* bacterial suspensions were taken out and washed thoroughly with sterile distilled water. Then, the films were then dried at 60°C overnight. Weight loss rate was determined by direct measurement using the stated formula:

\[
\text{Weight loss (\%) = } \left( \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right) \times 100
\]

2.5.2. Surface morphology analysis

The surface morphology of the untreated and treated nanocomposites thin films was investigated using field emission scanning electron microscope (FESEM, LEO GEMINI, Carl Zeiss, Oberkochen, Germany) at 5.0 KV and magnification of (5.00 KX). The analysis was carried out to investigate any changes such as biofilm development, micro cracks, erosion and indentation in a surface.

3. Results and Discussion

The growth of *P. aeruginosa* was monitored by measuring the absorbance of cultures (OD 600 nm) for 30 days. Based on the results obtained, it showed *P. aeruginosa* isolated in M9 solution with glucose as additional nutrient exhibited good growth until the end of cultivation (Figure 1). While, another set of samples (without glucose) were at minimal growth without any significant difference between each treated solution (Figure 2). As shown in Figure 1, it proves the influence of glucose been added as additional nutrient into minimal broth with the number of microorganisms. Glucose or sugar-surfactant has been studied for their capability to stimulate the biodegradation assay and as growth booster [12].

Figure 1 showed the rise in number of microorganisms due to the addition of glucose in limited availability of nutrients other than carbon source provided by LLDPE nanocomposite thin films. Untreated *P. aeruginosa* solution showed the least growth, followed with treated solution with LLDPE film only. The present of TiO$_2$/ZnO (5%) did increase the bacterial population. It showed the highest bacterial growth from Day 1 until end of experiment.
Figure 1. Comparative in vitro biodegradation assay of thin film for 4 weeks in the present of glucose as additional nutrient and presence of LLDPE/bare and LLDPE/TiO$_2$/ZnO nanocomposite thin films.

Figure 2. Comparative in vitro biodegradation assay of film for 4 weeks in the absence of glucose and presence of LLDPE and LLDPE/TiO$_2$/ZnO nanocomposite thin films.
After 30 days of incubation period, the percentage of weight reduction was less than 1.0% for all nanocomposites thin films except for LLDPE nanocomposites thin films without glucose in culture system as shown in Figure 3 (with and without glucose as additional nutrient). We observed the weight loss was greater for LLDPE/TiO$_2$/ZnO (0.99%) in glucose culture system and for LLDPE/TiO$_2$/ZnO (0.65%) without glucose in culture system. Whereas, the LLDPE thin films without any additional nanoparticles did having weight reduction for 0.54%. The weight loss of the LLDPE nanocomposites thin films can be attributed from the bacterial enzymatic degradation, which break the carbon backbone [9].

![Figure 3. Percentage reduction in weight of nanocomposites thin films in two different culture systems.](image)

The changes in surface morphology of the nanocomposites thin films were investigated by FESEM before and after be incubated with *P. aeruginosa* bacterial isolation. Figure 4 showed the surface changes on the LLDPE and LLDPE/TiO$_2$/ZnO/5% nanocomposites thin films at magnification (5.00 KX). It was observed that presence of glucose in solution did enhanced the surface deformation after 30 days of incubation. The untreated nanocomposites thin films had appearance of smooth surface with less defects. However, several changes were observed such as formation of holes, wrinkles and rough surfaces formation on both films.

The treated LLDPE nanocomposites thin films in solution without glucose showed a minimal degradation with formation of small wrinkle on the surface, whereas formation of pore was developed on LLDPE/TiO$_2$/ZnO nanocomposites thin films surface.
30 days

|                | Bare LLDPE                          | LLDPE/TiO$_2$/ZnO/5%               |
|----------------|-------------------------------------|------------------------------------|
| No treatment   | ![Image](image1.png)                | ![Image](image2.png)               |
| Presence of glucose in M9 broth | ![Image](image3.png)                | ![Image](image4.png)               |
| Absence of glucose in M9 broth | ![Image](image5.png)                | ![Image](image6.png)               |

**Figure 4.** In vitro biodegradation of nanocomposites thin films in minimal broth (M9) with and without glucose as additional nutrient.

4. Conclusion
This study investigates the biodegradation ability of LLDPE nanocomposites thin films composites embedded with TiO$_2$ and ZnO. The results suggested that the ability of *P.aeruginosa* strain and nanoparticles contribute in biodegradation process of films with additional nutrients (glucose) to boost the bacterial growth.

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**Acknowledgment**
This research was funded by the Ministry of Education (MOE) Malaysia under Transdisciplinary Research Grant Scheme (TRGS) grant no. 6769003.