Antibacterial Performance in Medium-Density Polyethylene: Effect of type and content of anti-bacterial agents

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Abstract

Anti-bacterial efficacy of medium-density polyethylene (MDPE) with various contents of three different anti-bacterial agents was considered. Halo and Plate-Count-Agar (PCA) methods were used. It was found that the inhibition zones were clearly visible for HPQM agent, the higher the HPQM content the greater the clear zone. ZEOMIC gave no zone of inhibition. The result of PCA suggested that after incorporating HPQM agent in the MDPE matrix, the % reductions of *E. coli* and *S. aureus* bacteria were extremely high (99.9%) for all loadings while those by TROYSAN-S88 were 77.0% for *E. coli* and 96.0% for *S. aureus*.

Keywords: Polyethylene; Anti-bacterial agents; Halo test; Plate count agar method

1. Introduction

Polyethylene is the most widely used among thermoplastics, especially for packaging and constructions applications. In polyethylene packaging, microbial contaminations are of main concern. The application of antimicrobial agents into the polymer products is one of the methods to prevent the products from microbial contaminations **[1]**. There have been many antibacterial agents such as nisin, nano-silver, triclosan and sorbic acid anhydride that could be used by blending with polymers for inhibition of the bacteria growth, using conventional polymer processing methods **[2]**. Some treatment methods for coating the anti-bacterial agents onto the polymer matrices may be required, depending on type, concentration and diffusability of bacteria through the matrices, and testing methods to evaluate the anti-bacterial performance **[3]**. In this present paper, experimental results on anti-bacterial efficacy of medium-density polyethylene added with various contents of three different anti-bacterial agents were reported. Halo and Plate-Count-Agar (PCA) methods were used as qualitative and quantitative measures using *E. coli* and *S. aureus* bacteria as testing bacteria.

2. Experimental

2.1 Materials & Chemicals: Medium-density polyethylene (MDPE, M380RU/RUP, Thai Polyethylene co., ltd., BKK, Thailand) was used as matrix. Carbendazim and Zinc Dimethyl Dithiocarbamate (designated as TROYSAN-S88, supplied by Koventure Co., Ltd, Bangkok, Thailand), 2-Hydroxypropyl-3-Piperazinyl-Quinoline Carboxylic Acid Methacrylate (designated as HPQM, provided by Micro Science Tech Co., Ltd, South Korea) and Silver Substituted Zeolite (designated as ZEOMIC, supplied by V.P. Alliance) were used as the anti-bacterial agents. *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* were used as testing bacteria.

2.2 Preparation of test specimens: The experimental procedure was commenced by mixing MDPE with each anti-bacterial agent for a required dosage using a high speed mixer for 5 min before melt-blended and pelletized by a twin screw extruder whose temperature profiles from feed to die zones were 160, 165, 170 and 170^{o}C until a relatively good dispersion was obtained. After that, the blend of MDPE and anti-bacterial agent was loaded into a compression mould for making a film specimen of 0.2 mm thick for further antimicrobial efficiency analysis **[1]**. The mould pressure, temperature and time used in the compression moulding process were 150 kg.cm^{-3}, 185^{o}C and 5 min, respectively. The obtained film specimens were then cut into disc samples of 6 mm in diameter for halo test, and into square samples of 1x1cm^2 for plate count agar (PCA) test.

2.3 Measurement of antibacterial performance:

Halo test: The test was initiated by pouring the nutrient agar onto sterilized Petri dishes and was allowed to solidify, and then 100 µL of incubated testing bacterial solution (10^6 CFU/ml) was spread uniformly over the plate. The MDPE film samples (6 mm diameter) with and without anti-bacterial agent were gently placed over solidified agar gel in the same Petri dishes which had duplication for obtaining the average. The Petri dishes were then incubated at 37^{o}C ± 0.5^{o}C and examined after 24 h for a zone of inhibition. The diameter of inhibition zone was then measured.

Plate Count Agar (PCA) method: Plate Count Agar (PCA) was suitable for quantitative assessment of bacteria reduction, which follows the test standard of ASTM E-2149 (2001). 25 pieces of MDPE film sample of 1x1 cm^2 were used. Nutrient broth (NB) was used as a growing medium for *E. coli* and *S. aureus bacteria* and peptone solution (prepared by 1 g / L peptone, pH 6.8 – 7.2) was chosen as a testing medium. Bacteria were

cultivated in 5ml of NB at 37°C for 24 h. The anti-bacterial efficacy of the film samples was measured by the following testing method. Samples were placed into a 250 ml flask with peptone solution and the bacteria cell suspensions were diluted with distilled water to the required initial bacteria density. In this work, the dilution factor either 10^5 or 10^6 CFU/ml was considered depending on the preferred number of initial bacteria colonies (ranging from 30 to 300 colonies). The flask was shaken on a reciprocal shaker at a speed of 100 rpm at 37°C ± 0.5°C for a contact time of 2h. 100 µL of bacterial solution after shaking were placed over the agar into sterilized Petri dishes. The inoculated plates were cultivated at 37°C ± 0.5°C for 24 h before counting the active bacteria and evaluating the anti-bacterial effect using Equation 1 **[4]**.

$$R = \frac{A - B}{A} \times 100 \quad (1)$$

where: R is percentage reduction of bacteria (%)
A is average number of bacteria from MDPE without antibacterial agent (CFU/ml)
B is average number of bacteria from MDPE with antibacterial agent (CFU/ml)

3. Results and discussion

Table 1 shows the clear zone results for MDPE film samples with different loadings of TROYSAN S88, HPQM and ZEOMIC agents for *E. coli* and *S. aureus* bacteria. It was found that TROYSAN-S88, and HPQM agents could generate zones of inhibition of 13.5 and 20.5 mm for *E. coli* and of 14.5 and 24.0 for *S. aureus*, respectively, on the Petri dishes. This also indicates that these two anti-bacteria agents were diffusible and could perform an inhibition of the bacteria growth. The clear zone was clearly visible only for HPQM, the higher the HPQM content the greater the clear zone. There was no zone of inhibition for ZEOMIC agent in all cases.

Table 2 gives the percentage reductions of *E. coli* and *S. aureus* for MDPE samples for different loadings of TROYSAN-S88, HPQM and ZEOMIC agents by PCA method. It was found that the incorporations of TROYSAN-S88 and HPQM gave the positive reductions of bacteria whereas those of ZEOMIC did not show any indications of bacteria reduction in the MDPE sample under the experimental conditions used in this work. Comparing the anti-bacterial efficacies of TROYSAN-S88 and HPQM, the pure TROYSAN-S88 and materbatch HPQM gave similar percentage reductions of bacterial up to 93.0% and 99.9% for *E. coli* and 98.0 and 99.9% for *S. aureus*, respectively. However, after incorporating with MDPE matrix, the % reductions of *E. coli* and *S. aureus* bacteria by HPQM was still high (99.9%) for all loadings while those by TROYSAN-S88 decreased to 77.0% for *E. coli* and 96.0% for *S. aureus*.

Table 1. Effect of type and loading of antibacterial agents on zone of inhibition

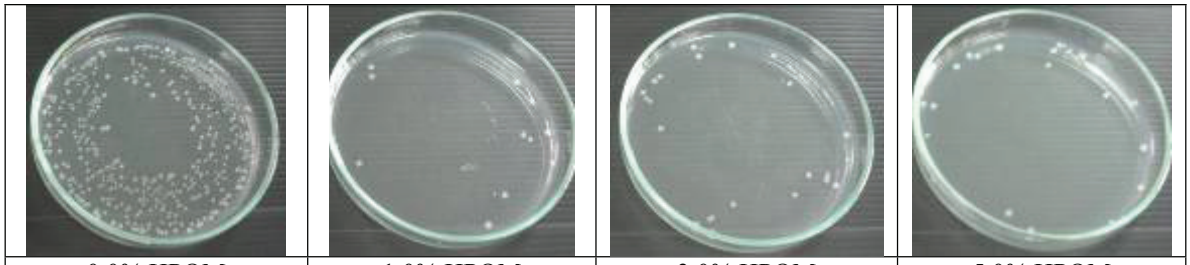
Anti-bacterial agents	Diameter of clear zone (mm)							
	Anti-bacterial agent content (%wt)							
	0.0	1.0	3.0	5.0	7.0	10.0	15.0	Pure or masterbatch
E. coli								
• TROYSAN-S88	0.0	0.5	0.5	0.9	-	-	-	13.5
• HPQM	0.0	12.0	14.5	17.5	-	-	-	20.5
• ZEOMIC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. aureus								
• TROYSAN-S88	0.0	0.5	0.8	1.1	-	-	-	14.5
• HPQM	0.0	15.5	22.5	23.5	-	-	-	24.0
• ZEOMIC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Effect of type and loading of antibacterial agents on % reduction of bacteria

Anti-bacterial agents	Reduction of bacteria (%)							
	Anti-bacterial agent content (%wt)							
	0.0	1.0	3.0	5.0	7.0	10.0	15.0	Pure or masterbatch
E. coli								
• TROYSAN S88	0.0	77.0	77.0	75.0	-	-	-	93.0
• HPQM	0.0	99.9	99.9	99.9	-	-	-	99.9
• ZEOMIC	0.0	neg	neg	neg	neg	neg	neg	neg
S. aureus								
• TROYSAN S88	0.0	96.0	96.0	97.0	-	-	-	98.0
• HPQM	0.0	99.9	99.9	99.9	-	-	-	99.9
• ZEOMIC	0.0	neg	neg	neg	neg	neg	neg	neg

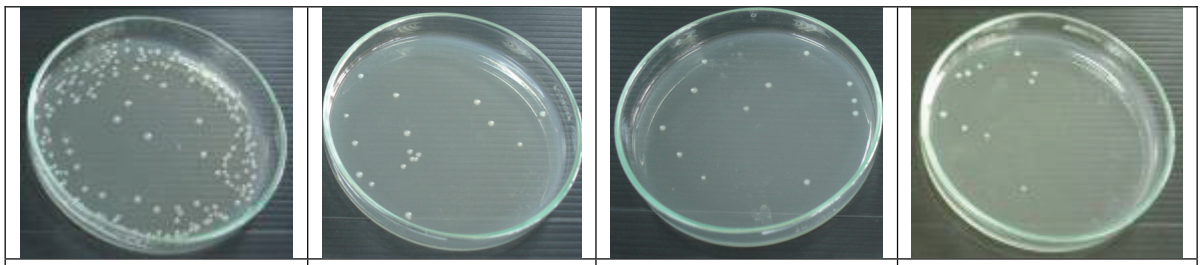
Note "neg" = bacteria growth rate > bacteria reduction rate

This clearly indicates that HPQM was more diffusible from MDPE matrix to the surroundings where the bacteria were present. Examples of number of living bacteria left in the Petri dishes of MDPE matrix for different loadings of HPQM agent for *E. coli* and *S. aureus* are given in **Figures 1 and 2**. The results from halo and PCA methods clearly indicate that HPQM agent was the most effective among the three anti-bacterial agents used in this work.



0.0% HPQM	1.0% HPQM	3.0% HPQM	5.0% HPQM

Figure 1. Living bacteria for MDPE matrix filled with HPQM using *E. coli* as a testing bacterium by PCA method



0.0% HPQM	1.0% HPQM	3.0% HPQM	5.0% HPQM

Figure 2. Living bacteria for MDPE matrix filled with HPQM using *S. aureus* as a testing bacterium by PCA method

4. Conclusions

The results indicated that HPQM could give the most inhibition zones, the effect being more pronounced with increasing HPQM content. ZEOMIC gave no zone of inhibition. After incorporating HPQM agent in the MDPE matrix, the % reductions of *E. coli* and *S. aureus* bacteria were 99.9% for all loadings while those by TROYSAN-S88 were 77.0% for *E. coli* and 96.0% for *S. aureus*.

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