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Assessment and Characterization of Antifungal and Antialgal Performances for Biocide-Enhanced Linear Low-Density Polyethylene

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ABSTRACT: In this work, four biocides were used for the purpose of growth inhibition of fungi and algae in linear low-density polyethylene (LLDPE) specimens. Benzimidazol-2-yl-carbamicacid methyl ester [carbendazim (CB)], 5-chloro-2-(2,4-dichlorophenoxy)-phenol [triclosan (TS)], and 3-iodo-2-propynyl *N*-butylcarbamate [iodopropynyl butylcarbamate (IPBC)] were used as antifungal agents, and 2-methylthio-4-ethylamino-6-*tert*-butylamino-triazin-1,3,5 [terbutryn (TT)] was used as an antialgal agent. Antifungal performance was evaluated by disk diffusion and dry weight techniques, and antialgal activities were carried out by disk diffusion and chlorophyll A methods. *Aspergillus niger* TISTR 3245 and *Chlorella vulgaris* TISTR 8580 were used as the testing fungus and alga, respectively. The experimental results suggested that the wettabilities of LLDPE specimens changed with the incorporation of CB, TS, IPBC, and TT biocides without significant changes in chemical structures and mechanical properties of the LLDPE. IPBC with the recommended content of 10,000 ppm was found to give the most satisfactory growth inhibition of *A. niger*. Antifungal performance evaluations were dependent on the testing methods used, whereas those for antialgal activity were not. The optimum concentration of TT agent for effective killing of *C. vulgaris* was 750 ppm; this loading could be reduced from 750 to 250 ppm by the addition of either TS or IPBC agent. TS and IPBC could be used as antialgal promoters in the LLDPE specimens. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Microorganisms are living organisms that can be found everywhere on earth; as most microorganisms are very small in size, they can be easily spread around the world, both by air and water. Under natural and artificial conditions, especially in aqueous environments and high humidity, all surfaces of materials can be covered ubiquitously with microorganisms and hence are susceptible to biofouling and biodeterioration as a result of continuous exposure to physical, chemical, and biological degradation. Generally, biodeterioration is an undesirable degradation of materials, including both metals and polymers, in the presence of microorganisms. An attack on materials by microorganisms can take place either directly or indirectly, depending on the specific microorganisms, chemical and physical properties of the materials, and the environmental conditions.[1–6] Damage to the materials may result in early and unexpected consequences, including system failures and economic losses.

Polyethylene (PE) is a polymer that is widely used in many applications and is preferred among healthcare professionals and the food and agriculture industries. Linear low-density PE (LLDPE) is most suitable for agricultural and food-packaging applications because of its strength and high processability. However, products made from LLDPE are prone to photodegradation and subsequent biodeterioration under natural conditions.[6] It is believed that the necessary additives used in polymers such as starch,

antioxidants, coloring agents, sensitizers, and plasticizers may significantly affect the biodegradability of the parent polymers. LLDPE is one of the potential substrates for phototrophic and heterotrophic microorganisms, including algae, bacteria, and fungi, imbedded in mucilage.[1] Colonization by fungi and algae has also been a primary cause of both disfigurement of the material surfaces and losses in bulk properties.[7–9] To prevent further deterioration of materials, biocides are commonly applied in repairing, cleaning, and maintenance of the targeted polymer products.[2,5,10–12] A series of research studies by Asadinezhad et al.[13–15] revealed the plasma surface treatment on medical-grade PVC films added with antibacterial agents, which included polysaccharides,[13] Irgasan or triclosan (TS),[14] bronopol,[15] benzalkonium chloride,[15] and chlorhexidine.[15] They concluded that the plasma treatment could enhance the roughness on the PVC surfaces, and this led to improvement of antibacterial properties of the PVC.

Carbendazim (CB; methyl-2-benzimidazole carbamate) is one of the commercial biocides in the class of benzimidazole fungicides. The antifungal mechanism of CB involves the inhibition of polymerization of free tubulin molecules by binding an arginine residue of the β-tubulin subunit and by disrupting cell division through linkage to the nuclear spindle.[16] A modified fungicide, chitosan–copper complex (CCC) treated with zinc borate, for wood/polymer composites was studied by Lu et al.[11] Their results suggested that wood/HDPE composites with CCC at a concentration of 3% by weight significantly improved decay resistance against white rot fungus *Trametes versicolor* and brown rot fungus *Gloeophyllum trabeum*. Moreover, CCC-treated wood/HDPE composites performed as well as zinc-borate-treated wood/HDPE composites in terms of fungal decay resistance. Another commercial fungicide is iodopropynyl butylcarbamate (IPBC), which has been used as a preservative in painting, paper, and textile applications as well as adhesives. The decomposition temperature of IPBC is around 100°C, and it is stable to hydrolysis and degradation processes in pH 5 buffered solution.[17] Sørensen et al.[10] found that IPBC encapsulated in microparticles showed a slow release rate and also increased the lifetime of the paint film sample. TS was found to be effective against $\sim$ 100 microorganisms, including nine fungal species, *Clostridium* spp., *Bacillus* spp., *Mycobacterium* spp., *Legionella* spp., and pseudomonads. Jones et al.[18] reviewed the antimicrobial properties of TS, which is commonly used in clinical healthcare settings. However, Merchan et al.[19] clearly suggested that the antimicrobial efficacy was dependent on releasing kinetics of antimicrobial agents, which were also associated with types of testing media, concentration of biocides, and uniformity and distribution of biocides. Some studies[20,21] have indicated that the antialgal mechanism of algaecides involves the inhibition of photosynthesis by preventing oxygen production and blocking photosystem II electron transport. Commercial algaecides, namely isoproturon and terbutryn (TT), were studied by Rioboo et al.[21] who showed that after 96 h of herbicide exposure, TT had a stronger inhibition on the growth of microalgae *Chlorella vulgaris* than isoproturon as the EC_{50} value of TT (EC_{50} of TT = 0.097 μM) for algal growth was two times lower than that of isoproturon (EC_{50} of isoproturon = 0.199 μM).

Although the aforementioned studies have clearly documented the antimicrobial effectiveness of biocides available in the market, very few studies have revealed the effectiveness of those biocides when incorporated in polymer products. Our previous reports[22–24] have clearly suggested that the efficacies of many antibacterial agents have worsened when they are added or embedded in polymeric materials. The current work extended our antimicrobial performance evaluation program in polymer systems by studying antifungal and antialgal performances of selected fungicides and algaecides incorporated in LLDPE in both single and mixed biocide systems. Commercial biocides available in the worldwide market were used in this study, including CB, TS, and IPBC (fungicides) and TT (algaecide). Comparisons of the effectiveness of these biocides incorporated in LLDPE at different loadings were made using disk diffusion and dry weight techniques for antifungal testing and using disk diffusion and chlorophyll A methods for antialgal examination. *Aspergillus niger* TISTR 3245 and *Chlorella vulgaris* TISTR 8580 were used as the testing fungus and alga, respectively.

EXPERIMENTAL

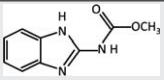
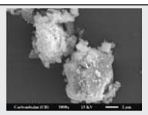
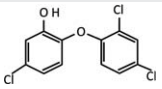
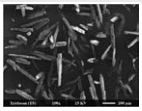
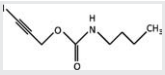
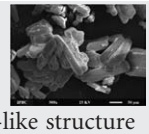
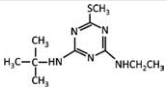
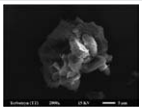
Materials and Chemicals

LLDPE (grade M380RU/RUP), supplied in form of powder by Siam Cement Company (Bangkok, Thailand), was used as a polymer matrix. In this work, four biocides, including antifungal and antialgal agents, were of interest. The three fungicides used were benzimidazol-2-yl-carbamicacid methyl ester (CB), 5-chloro-2-(2,4-dichlorophenoxy)phenol (TS), and 3-iodo-2-propynyl *N*-butylcarbamate (IPBC); 2-methylthio-4-ethylamino-6-*tert*-butylamino-triazin-1,3,5 (TT) was used as an antialgal agent. All biocides were supplied by Troy Asia (Bangkok, Thailand). Table I shows the chemical, physical, thermal, and morphological properties of the biocides used. It can be seen that the main differences were the particle size, morphological properties, and melting temperature. CB appeared to have a relatively high melting temperature greater than the melting temperature of LLDPE (about 120°C). CB and TT had irregular shapes, whereas TS and IPBC were rod-like structures. *Aspergillus niger* TISTR 3245 and *Chlorella vulgaris* TISTR 8580 were obtained from the Thailand Institute of Scientific and Technological Research (Pathum Thani, Thailand).

Preparation of Specimen

Based on the preliminary experiments and information from the biocide suppliers, the recommended concentrations for the fungicides to be introduced into the LLDPE ranged from 1000 to 50,000 ppm, whereas those for the algaecide varied from 250 to 1000 ppm. Test specimens were first prepared by directly mixing LLDPE powder with each fungicide or algaecide at a given concentration using a high-speed mixer. The mixtures were then transformed into film specimens with 1 mm thickness using a hydraulic press. The processing conditions were carried out by three sequential steps as follows: (i) preheating the mixture in a mold at 160°C for 2 min; (ii) introducing pressure of 100 kg/cm^2 into the mold for 10 min; and (iii) cooling the mold with water coolant at 25°C under a pressure of 100 kg/cm^2 for 5 min. To carry out the antifungal and antialgal performance evaluations, the molded film specimens were cut

Table I. Chemical Structure, Morphological, Physical, and Thermal Characteristics of the Biocides Used in This Work

Trade name	Chemical name	Chemical structure	Particle size (μm)	Morphology and physical characteristics	T_m (°C)
Carbendazim (CB)	Benzimidazol-2-yl-carbamicacid methyl ester		0.5–5.0	 Irregular shape	~300
Triclosan (TS)	5-Chloro-2-(2,4-dichlorophenoxy) phenol		10.0–40.0	 Rod-like structure	~57
IPBC	3-Iodo-2-propynyl *N*-butylcarbamate		5.0–50.0	 Rod-like structure	~67
Terbutryn (TT)	2-Methylthio-4-ethylamino-6-*tert*-butylamino-triazin-1,3,5		2.0–20.0	 Irregular shape	~104

into circular shapes with 5 mm in diameter (for a disk diffusion test) and into square shapes 50 × 50 mm^2 (for dry weight and chlorophyll A techniques).

Antifungal and Antialgal Performance Evaluations

Antifungal Test

Disk diffusion test. This was carried out by observing the growth of fungi on an enriched medium (potato dextrose agar) during which a fungal disk, cut from the actual growing edge or hyphal tips of *A. niger* as the test fungus, was located at the center of a Petri dish (90 mm diameter) between two test pieces. The distance from the edge of each specimen to the fungal disk was 15 mm. The conditions for fungal growth were an incubation temperature of 30°C for 7 days. The radius of fungal growth (R_f) was measured to calculate the antifungal efficiency, which was defined as the "inhibition of fungal growth by disk diffusion technique" (IFD). The percentage of IFD was calculated using the following equation:

$$\mathrm{IFD}(\%) = \frac{R_C - R_S}{R_C} \times 100 \quad (1)$$

where R_C is the radius of fungal growth in the control plate (without specimen) (mm), and R_S is the radius of fungal growth in the specimen plate (with specimen) (mm). Photographs of the growth zones of fungi were taken with a digital camera (model 400D; Canon, Tokyo, Japan).

Dry weight measurement. The test procedure was modified from the dry weight technique used by Melzer et al.[25] and Ikeda et al.[26] The fungal spore suspension was first prepared to obtain the initial testing fungus at a concentration of 10^6 spores·per milliliter in 50 mL of potato dextrose broth. The testing specimen and the fungal spore suspension were then mixed and shaken together in an Erlenmeyer flask (250 mL in volume) at a frequency of 150 rpm and incubation temperature of 30°C for 14 days. At the end of the incubation period, the fungal mycelia were collected by filtration through Whatman filter paper No. 1 and removed from the test specimen. The filter paper with fungal mycelia was dried in an oven at 80°C for 48 h and then held in a desiccator until cooled down for later constant weight determination. The weight of fungi after exposure to the test specimen was obtained by subtracting the weight of the filter paper from the total weight. This was called as the "dry weight value of fungi" (W); the antifungal efficiency was then determined as the "inhibition of fungal growth by dry weight technique" (IFW). The dry weight value (W) and the percentage IFW are described by eqs. ([2]) and ([3]), respectively:

$$W(\mathrm{mg/L}) = \frac{\mathrm{Dryweightoffungi(g)}}{50\mathrm{mL} \times 10^{-3}} \quad (2)$$

$$\mathrm{IFW}(\%) = \frac{W_C - W_S}{W_C} \times 100 \quad (3)$$

where W_C is the dry weight value of fungi from the control flask (without test specimen), and W_S is the dry weight value of fungi from the specimen flask (with test specimen).

Antialgal Test

Disk diffusion test. The procedure commenced by preparing an algal cell suspension with an initial concentration of testing algae of 10^7 cells·per milliliter. The algal cell suspension was then mixed with mineral agar at a mixing ratio of 1 : 1. The mixture was then poured into a Petri dish (9 cm in diameter) to obtain a semisolid agar as an algal testing medium. A test

specimen was gently placed on the testing medium at the center of the dish under aseptic conditions. Incubation was carried out under cyclic dark-light exposure of 12 : 12 h at 28°C for 28 days. The clear zone around the test piece was investigated as the growth inhibition area of algae; however, the results were reported as killing radius (R_a). The antialgal performance of this technique was described in terms of "inhibition of algal growth by disk diffusion technique" (IAD). The calculation of IAD value (mm) is expressed by the following equation:

$$\mathrm{IAD(mm)} = \frac{D_{\mathrm{clearzone}} - D_{\mathrm{specimen}}}{2} \quad (4)$$

where $D_{\mathrm{clear\ zone}}$ is clear-zone diameter (mm) and D_{specimen} is the diameter of the test specimen (mm). Photographs of the algal inhibition zone were digitally taken with a digital camera (model 400D; Canon).

Chlorophyll A measurement. This test followed standard testing method ASTM D-3731-04, entitled "Standard Practices for Measurement of Chlorophyll Content of Algae in Surface Waters." The initial testing density of algae was 10^7 cells·per milliliter, which was first prepared for 100 mL in a flask. The test specimen was then put into the flask, which was then shaken using an orbital shaker at 150 rpm at 28°C for 21 days. After the algal cells were exposed to the test specimen, 1 mL of algal cell suspension was extracted from the flask and put into a test tube containing 9 mL of methanol solvent. The mixture of extracted algal suspension and methanol solvent was kept in the dark for at least 2 h before mechanically separating chlorophyll from broken algal cells using a refrigerated centrifuge at 5000 rpm at 4°C for 5 min. The amount of chlorophyll A, which is a common type of chlorophyll generally found in phytoorganisms (photosynthetic organisms), was determined using a UV spectrophotometer at wavelength numbers of 650 cm^{-1} (A_{650}) and 665 cm^{-1} (A_{665}), as suggested by Lee and Chen.[27] The calculation of chlorophyll A content (C) is demonstrated in eq. (5), whereas the antialgal efficiency is defined by the "inhibition of algal growth by percentage chlorophyll A measurement" (IAM) using eq. ([6]):

$$C(\mathrm{mg/mL}) = [(A_{650} \times 25.5) + (A_{665} \times 4)] \times 10 \times 100 \quad (5)$$

$$\mathrm{IAM}(\%) = \frac{C_C - C_S}{C_C} \times 100 \quad (6)$$

where C_C is chlorophyll A content from the control flask (without test specimen), and C_S is chlorophyll A content from the specimen flask (with test specimen).

Characterization of Materials

Contact Angle Measurement. Changes in the chemistry and surface (wettability) characteristics of LLDPE specimens doped with biocides were investigated using a contact angle goniometer (model 100-00; Ramé-Hart Instrument, Succasunna, NJ) to measure the contact angle of deionized water. The contact angle values of LLDPE doped with each of the fungicides (CB, TS, and IPBC) and the algaecide (TT) were averaged from three independent LLDPE samples.

Image and Morphological Observations. Particle characteristics of biocides, such as size and shape, and surface morphologies of LLDPE doped with the biocides were investigated using a scanning electron microscope (SEM; JSM-6301 F; JEOL, Tokyo, Japan) at 15 kV accelerating voltage. Prior to SEM analysis, the specimens were left to dry for moisture removal and then coated using a gold-sputtering device.[20]

Tensile Properties. Mechanical properties of biocide-filled LLDPE were measured to reveal whether or not the biocide loading had an effect on the material property changes. Tensile testing was selected for this purpose. The testing procedure and specimen preparation used in this work followed the ASTM D-638-09 (2009). A universal testing machine (Model Autograph AG-I; Shimadzu, Tokyo, Japan) was used with 5 kN load using a cross-head speed of 100 mm/min at room temperature.

Thermal Stability. Decomposition temperatures (T_d) of the commercial biocides were measured to ensure the thermal stability of the biocides added in the LLDPE matrix during the processing and to reveal whether or not there was any degradation of the added biocides during the processing. This was achieved through thermogravimetric analysis (TGA). The thermogravimetric analyzer (TGA-7HT; Perkin Elmer, Massachusetts, USA) was used under a temperature range from room temperature to 400°C at a heating rate of 10°C/min under a continuous nitrogen stream of 50 mL/min. The onset of decomposition temperature ($T_{d\text{-onset}}$) was reported for thermal stability evaluation.

Chemical Structure Analysis. Fourier transform infrared spectroscopy (ATR-FTIR, Model Spectrum Spotlight 300, Perkin Elmer) was used to monitor the chemical structure changes of LLDPE after being incorporated with the biocides used in this work. The wave number used was in the range of 4000 to 600 cm^{-1}.

RESULTS AND DISCUSSION

Characterization of Materials

Figure 1 shows the effect of biocides on the surface characteristics of LLDPE examined through contact angle and SEM micrographs. It can be seen that the addition of the biocides changed the wettability of the LLDPE, and this was evidenced by contact angle reductions as well as by SEM micrographs, which show apparent physical changes on the LLDPE surfaces. It can be clearly seen that TS, IPBC, and TT had migrated onto the LLDPE surfaces; this effect was less pronounced for TT due to low concentration added, as listed in Table I. The results (Figure 1) confirmed that there was a possibility that all the biocides used could diffuse through the LLDPE matrix and migrated onto the LLDPE surfaces to kill the microbes. However, the migration of the CB additive was hardly seen. This may be because under the melting conditions in which LLDPE was blended with the biocide, the CB was in a solid state and thus had difficulty migrating onto the LLDPE specimen surface. This was also the reason for the unchanged contact angle shown in Figure 1.

Figure 2 shows the TGA curves and the onset of decomposition temperatures ($T_{d\text{-onset}}$) of the biocides used in this study. The $T_{d\text{-onset}}$ values of CB, TS, IPBC, and TT were 243, 237, 171, and

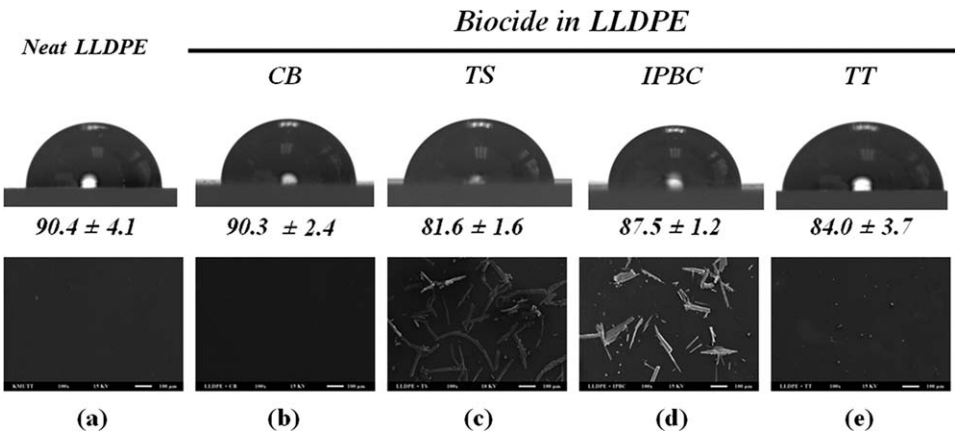


Figure 1. Water contact angle and surface morphology of LLDPE filled with biocides: (a) neat LLDPE, (b) CB/LLDPE, (c) TS/LLDPE, (d) IPBC/LLDPE, and (e) TT/LLDPE.

226°C, respectively. These values were found to be higher than the processing temperature of biocides/LLDPE blends, which was 160°C as indicated in the "Experimental" section. Therefore, there would not be any degradation of the biocides occurring during the processing. Figure 3 shows the FTIR spectra for neat LLDPE and LLDPE blended with all biocides. It can be seen that all FTIR spectra were very similar, and this indicates that the addition of CB, TS, IPBC, or TT at given concentrations did not affect the chemical structure of LLDPE. The mechanical property changes of neat LLDPE and LLDPE filled with CB, TS, IPBC, or TT are given in Table II. It can be seen that the addition of those biocides into LLDPE did not affect the overall tensile properties of the LLDPE, except for ultimate strength and breaking strain of the CB-added LLDPE specimen. This may be expected because, as earlier discussed in Table I, under the melting conditions of the CB/LLDPE blend, the CB was in a solid state. If this was the case, it would have difficulty blending with the LLDPE and thus worsened the mechanical properties, especially at the failure point where the ultimate strength and breaking strain were measured.

Antifungal and Antialgal Performance Evaluations

Figures 4 and 5 show the qualitative and quantitative results of the disk diffusion test for LLDPE incorporated with CB, TS, and IPBC ranging from 0 to 50,000 ppm. It can be seen that IPBC exhibited the most satisfactory inhibition of *A. niger* growth, the recommended content being 10,000 ppm. CB was not found to act as an antifungal agent in this case. Previous studies have indicated that the chemical functional groups involved in biocidal activity are the azole groups in CB,[16] phenol and chlorine groups in TS,[18] and iodine groups in IPBC.[10,17] The killing mechanisms for CB, TS, and IPBC in neat form have been discussed in a number of reports.[10,16–18] The inhibition differences between IPBC and CB could be explained by their diffusibility through the LLDPE matrix, as already discussed (Figure 1). The comparison of IPBC with TS revealed that IPBC was more effective at inhibiting *A. niger*. As shown in Figure 5, increasing the concentrations of TS and IPBC increased the inhibition of *A. niger* growth, the effect being more pronounced for IPBC. This corresponded to the disk

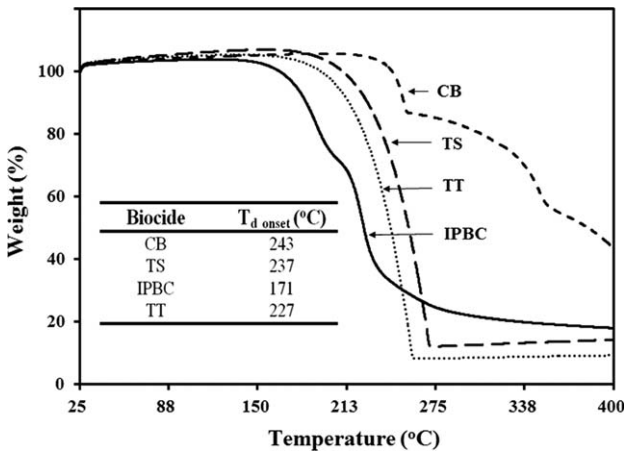


Figure 2. TGA curves and onset of decomposition temperatures for CB, TS, IPBC, and TT.

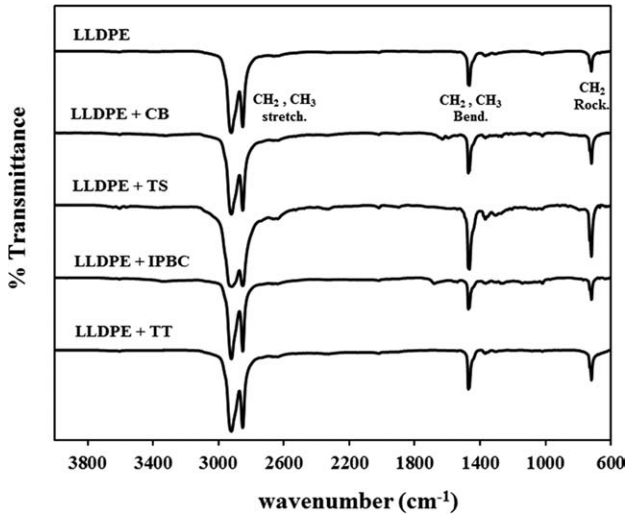


Figure 3. FTIR spectra of neat LLDPE and LLDPE with CB, TS, or IPBC at 10,000 ppm and LLDPE with TT at 500 ppm.

Table II. Tensile Properties of Neat LLDPE and LLDPE Filled with CB, TS, IPBC, and TT at Various Concentrations

Materials/biocide content (ppm)		Elastic modulus (MPa)	Yield stress (MPa)	Ultimate stress (MPa)	Breaking strain (%)
Neat LLDPE	-	33.84 ± 4.96	18.77 ± 0.97	28.39 ± 0.19	1856.84 ± 24.50
CB	10,000	33.85 ± 3.46	18.82 ± 0.43	11.86 ± 3.15	185.37 ± 32.43
	30,000	35.61 ± 6.61	18.20 ± 0.44	13.33 ± 2.56	194.20 ± 28.97
	50,000	28.15 ± 2.05	17.40 ± 0.23	12.97 ± 0.63	289.65 ± 54.12
TS	10,000	28.73 ± 4.52	17.86 ± 0.27	26.03 ± 1.06	1765.97 ± 96.69
	30,000	29.44 ± 3.04	16.92 ± 0.30	27.58 ± 0.94	1995.79 ± 68.09
	50,000	30.76 ± 2.32	16.81 ± 0.44	27.10 ± 0.92	1931.79 ± 78.51
IPBC	10,000	30.43 ± 1.97	18.27 ± 0.30	26.22 ± 0.82	1849.61 ± 70.78
	30,000	29.55 ± 1.30	17.34 ± 0.25	27.67 ± 0.92	2020.53 ± 79.22
	50,000	35.00 ± 1.77	17.75 ± 0.31	27.98 ± 0.65	2009.92 ± 42.44
TT	250	33.81 ± 4.84	18.62 ± 0.40	28.27 ± 0.94	1805.85 ± 40.93
	500	36.64 ± 5.61	18.14 ± 0.74	28.27 ± 1.03	1923.83 ± 70.04
	750	33.08 ± 2.98	18.55 ± 0.51	29.11 ± 1.06	1866.34 ± 77.22
	1000	33.08 ± 2.98	18.55 ± 0.51	29.11 ± 1.06	1866.34 ± 77.22

diffusion results (Figure 4). The differences in the *A. niger* growth inhibition by IPBC and TS could be explained indirectly using minimum inhibition concentration (MIC), which is defined as the amount of a chemical in neat form to inhibit the microorganism growth. In this case, IPBC had a MIC of 0.6–5.0 mg/L, whereas TS had a MIC of 3.0–30.0 mg/L.[28] This was the reason why the IPBC required lower dosage for a complete killing of the *A. niger*. Taking the results in Figures 4 and 5 into account, the recommended dosages for TS and IPBC for satisfactory killing of the *A. niger* in this work were 30,000 and 10,000 ppm, respectively.

Figure 6 shows the inhibition of *A. niger* growth by dry weight technique for a LLDPE matrix loaded with CB, TS, or IPBC biocides ranging from 0 to 50,000 ppm. It was interesting to observe that although the results by dry weight technique had similar trends with those from the disk diffusion method, the values and the optimal loadings for all biocides were found to be different. This implied that the antifungal performance evaluations were dependent on the testing method. This has practical implications for the selection of the testing method used. In other words, one should consider that the environmental conditions of the testing methods must be similar to those of actual use. Based on the results in Figure 6, the recommended dosages for TS and IPBC to achieve a complete killing of the *A. niger* were 5000 and 1000 ppm. The differences in the recommended dosages for TS and IPBC were associated with differences in incubation time and state or form of the nutrition used. The incubation time for the disk diffusion test was 7 days, whereas that for the dry weight method was 14 days. In addition, the disk diffusion test used a solid medium that did not promote the diffusion of TS and IPBC biocides, whereas the dry weight method used a liquid medium that facilitated the dynamic diffusion of the biocides.

In general, TT Isoproturon and Diuron are used as antialgal agents; however, when considering the MIC value, it is found that TT and Diuron have relatively low MIC, suggesting high antialgal performance when compared with isoproturon. However, when

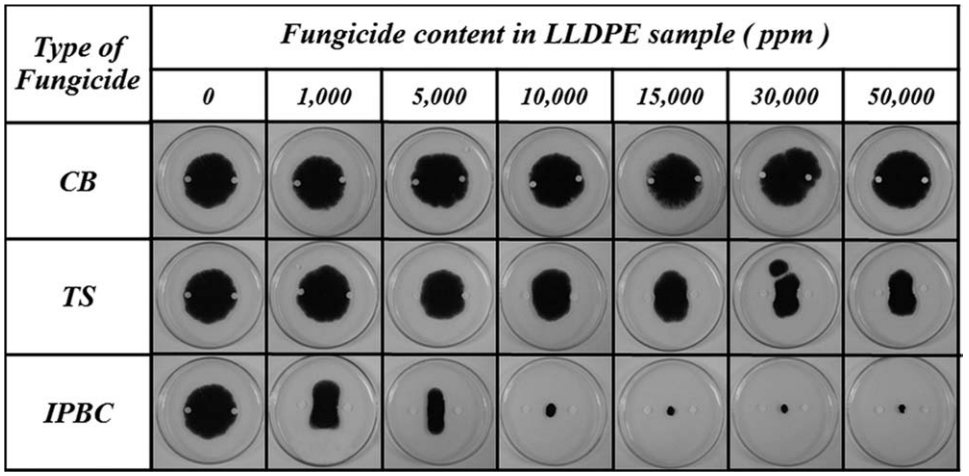


Figure 4. Digital images of *A. niger* growth on potato dextrose agar for LLDPE filled with CB, TS, or IPBC at different concentrations.

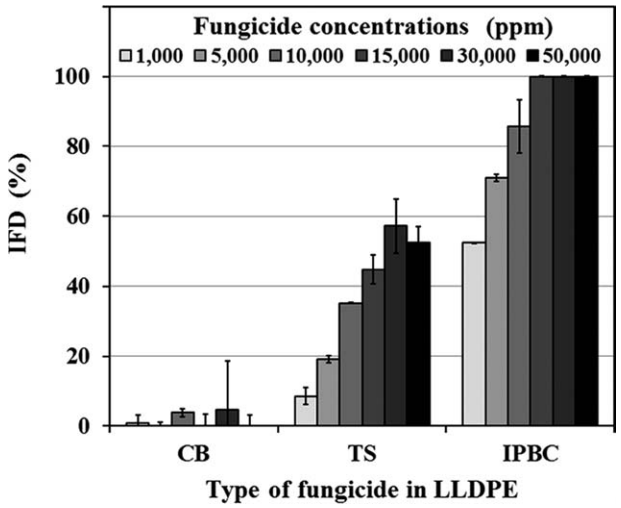


Figure 5. Inhibition of fungal growth by disk diffusion technique (IFD) for LLDPE filled with CB, TS, or IPBC at different concentrations.

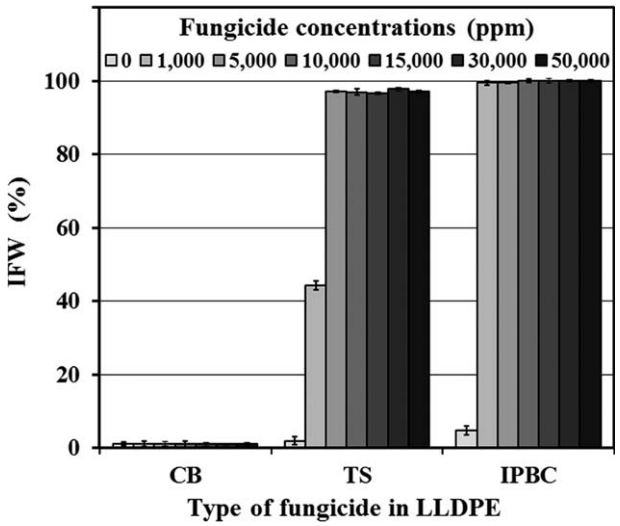


Figure 6. Inhibition of fungal growth by dry weight technique (IFW) for LLDPE filled with CB, TS, or IPBC at different concentrations.

TT and Diuron are used and compared, the water solubility of Diuron is higher,[28] and this makes it less suitable for use in antialgal applications. Therefore, this work intentionally selected TT as the only antialgal agent of interest. Figures 7 and 8 show the qualitative and quantitative assessments for inhibition of growth from a disk diffusion test of LLDPE incorporated with TT agent ranging from 0 to 1000 ppm. It was clearly found that TT antialgal agent was effective at inhibiting the growth of *C. vulgaris*, with an optimal loading of 750 ppm. The main chemical functional group responsible for the biocidal properties was suggested to be triazines.[25] It is interesting to note that although the optimum TT dosage was 750 ppm, a TT loading of 250 ppm seemed to result in the most effective biocidal activity against *C. vulgaris*, as evidenced by a sharp decrease in the growth rate of the algae. The inhibition of *C. vulgaris* appeared to decrease for TT loadings of greater than 250 ppm. This may be due to the relatively low residual content of *C. vulgaris* in contact with the TT agent. The chlorophyll A content of LLDPE specimens loaded with different TT concentrations (Figure 9) confirmed the optimum loading of TT required for complete killing of *C. vulgaris*, as suggested by Figures 7 and 8.

Effect of Mixed Biocides on Antifungal and Antialgal Performance Evaluations

During the experiments, an interesting question was raised as to whether the antifungal agents (CB, TS, and IPBC) could inhibit the growth of *C. vulgaris* and also whether the TT antialgal agent could inhibit the growth of *A. niger*. To answer these questions, separate experiments on disk diffusion measurements for *C. vulgaris* by CB, TS, or IPBC and for *A. niger* by TT were carried out. The results are given in Table III. It can be seen that the antifungal agents TS and IPBC were able to inhibit *C. vulgaris*, whereas CB did not. TT agent was also found to not inhibit the growth of *A. niger*. It could thus be concluded that antifungal agents like TS and IPBC could function as biocides for both *C. vulgaris* and *A. niger*. Therefore, it would be very interesting to examine the changes in inhibition of growth of *C. vulgaris* and *A. niger* under a mixture of antifungal and antialgal agents. Another interesting aspect to consider was whether or not the optimal dosages of the antifungal and antialgal agents would change if they both were added to the LLDPE at the same time. Figures 10 and 11 show the effect of antifungal and antialgal ratio, by using a fixed concentration of antifungal agents, on the growth inhibition of *A. niger* and *C. vulgaris*, respectively. It was observed in Figure 10, in comparison with the results in Figure 5, that an increase in the TT loading in LLDPE containing 10,000 ppm of CB, TS, or IPBC did not change the inhibition performance of *A. niger*. This suggested that the TT agent could neither kill nor inhibit the growth of *A. niger*. On

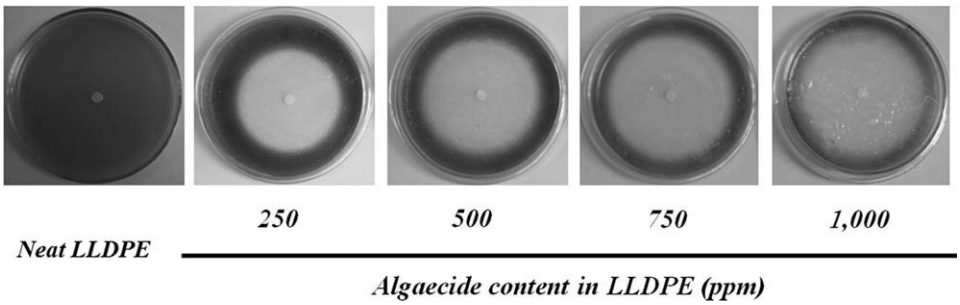


Figure 7. Digital images of clear zone for *C. vulgaris* on mineral agar for LLDPE filled with TT at different concentrations.

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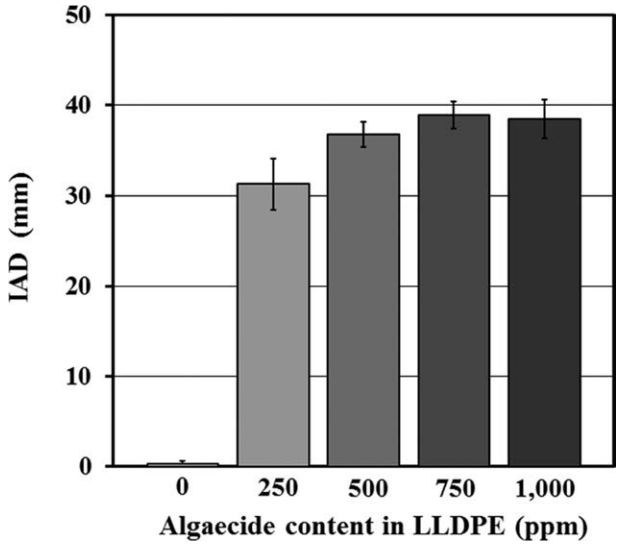

Figure 8. Inhibition of algal growth by disk diffusion technique (IAD) for LLDPE with various concentrations of algaecide.

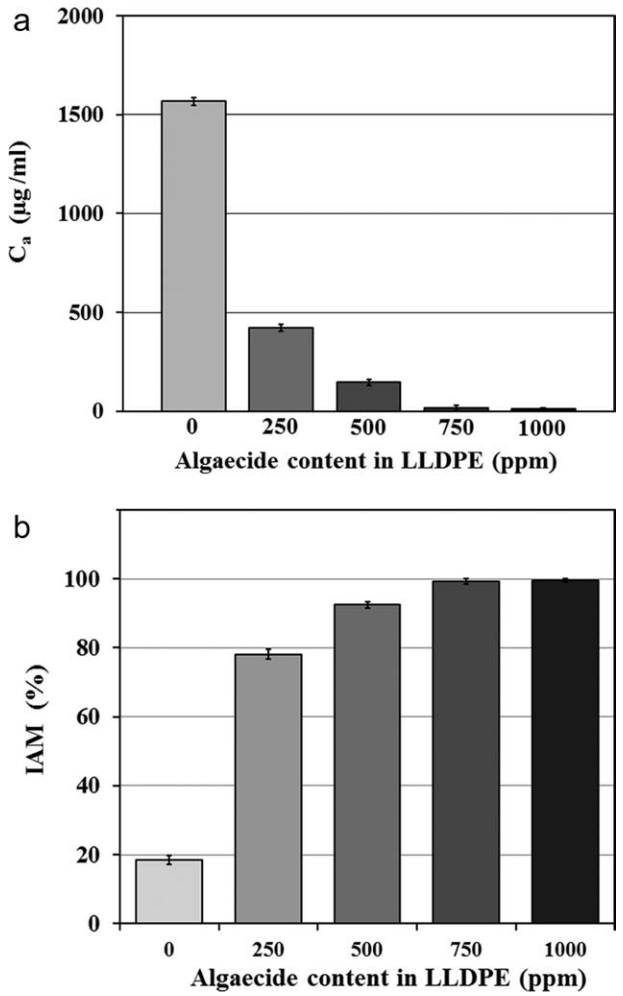

Figure 9. Antialgal properties of LLDPE with different concentrations of algaecide: (a) *C* value from chlorophyll A measurement and (b) IAM value.

Table III. Clear-Zone Radius of *C. vulgaris* for LLDPE Filled with CB, TS, or IPBC at 10,000 ppm and TT at 1000 ppm

Clear-zone radius of fungicide-filled LLDPE (mm)			
CB	TS	IPBC	TT
0.0	14.0	19.3	38.5

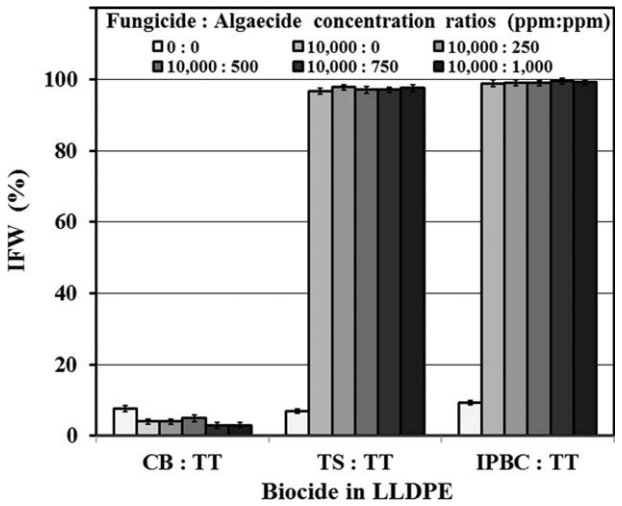

Figure 10. IFW values of LLDPE filled with mixed fungicide and algaecide at different mixing ratios.

the other hand, Figure 11 shows that the incorporation of CB, TS, or IPBC into LLDPE together with various contents of TT agent could improve the growth inhibition of *C. vulgaris*; this suggests, in comparison with the results in Figure 6, that TS and IPBC antifungal agents could be regarded as antialgal promoters in the LLDPE specimens. This was clearly demonstrated by a reduction in the optimum dosage of TT agent from 750 to 250 ppm.

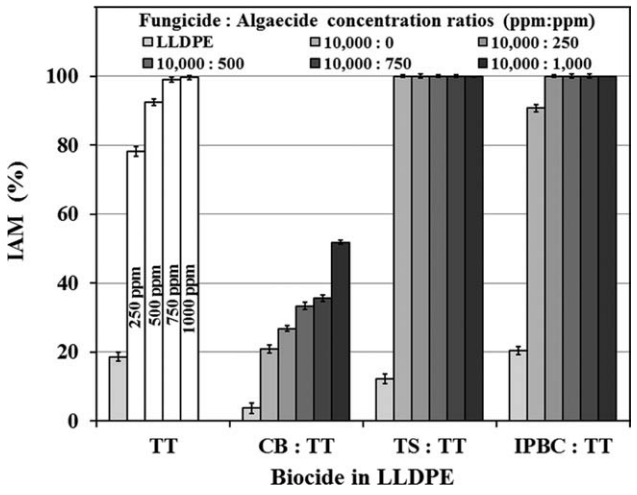

Figure 11. IAM values of LLDPE filled with mixed fungicide and algaecide at different mixing ratios.

CONCLUSION

The addition of CB, TS, IPBC, and TT biocides changed the wettability of LLDPE specimens; this in turn affected the diffusibility of the biocides and their ability to kill the fungi and algae on the specimen surfaces. There were no changes in chemical structure and mechanical properties of LLDPE by the incorporation of all biocides used in this work, except for the CB. IPBC exhibited the most satisfactory inhibition of *A. niger* at the recommended content of 10,000 ppm. The differences in effectiveness of the studied antifungal agents could be explained in relation to their particle size, melting temperature, and 50% lethal dose value. The results of antifungal performance were dependent on the testing methods used, whereas those for antialgal activity were not. TT with an optimal loading of 750 ppm could be used to effectively inhibit the growth of *C. vulgaris*. Mixing the antifungal and antialgal agents could improve the inhibition efficiency of *C. vulgaris*. TS and IPBC could be used as antialgal promoters in the LLDPE specimens. The dosage of TT antialgal agent could be reduced from 750 to 250 ppm by the addition of either TS or IPBC agent.

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