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Anti-fungal and Anti-algal Performances of Biocides filled PVC and Wood/PVC Composites

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Abstract. The anti-fungal and anti-algal growth performances of wood poly(vinyl chloride) composite (WPVC) and poly(vinyl chloride) (PVC) containing either fungicides or algaecides at various concentrations were quantitatively evaluated using biological standard tests. The commercial fungicides, namely Carbendazim and IPBC in range of 10,000-50,000 ppm, and algaecides, namely Terbutryn and Isoproturon in range of 250-1,500 ppm, were incorporated into PVC and PVC composites with a fixed wood flour content of 100 pph. Disk diffusion test and dry weight technique, using *Aspergillus niger* as testing fungi, were used for anti-fungal evaluation while inhibition zone test using *Chlorella vulgaris* as testing algae, were utilized for anti-algal evaluation. The results suggested that IPBC exhibited better anti-fungal efficiency than Carbendazim for both PVC and WPVC composites, especially at the suggested IPBC concentrations of 30,000 ppm or higher. Terbutryn showed better anti-algal efficiency than Isoproturon. The recommended loadings of Terbutryn for complete algae killing were 1,000 and 500 ppm for neat PVC and WPVC composites, respectively. The wood particles added in PVC were found to improve the anti-fungal and anti-algal properties in PVC composites, which could be regarded as "anti-microbial promoter" under the commercial biocides used in this work.

Introduction

Wood/poly(vinyl chloride) composite (WPVC) has been increasingly employed for a number of applications, due to high mechanical strength, good dimensional stability, termite free, having a wide range of service temperature and so on. The examples of WPVC products include flooring liner, decking, garden fencing, ceiling and building construction [1-2]. Under terrestrial and aquatic environments, WPVC products may be at risk to contact with microorganisms and formed "biofilm" or "microfouling" on the surfaces. Colonization by fungi and algae have been generally found in all materials and caused not only disfigurement of material surface but also loss in bulk properties [3-6]. Biodeterioration mechanisms by microbial colonization mainly involved biofilm formation, penetration and enzyme activity [7]. Wood particles, cellulosic substances, and some organic compounds, such as, plasticizers and lubricants in WPVC are expected to promote the microbial deterioration due to the carbon source, moisture induction and porous texture [8].

Currently, several commercial fungicides and algaecides have been introduced and availble in agriculture portion, but only a limited number of information has published for wood polymer composites materials. The main objective of this work was to determine the anti-fungal and anti-algal performances for wood poly(vinyl chloride) composites filled with various commercial biocides. Two fungicides: namely; Carbendazim and IPBC, and two algicides: namely; Terbutryn and Isoputoron were introduced in neat PVC and in the wood/PVC composites at different concentrations. Disk diffusion test and dry weight technique (ASTM D1413-76) were used as anti-fungal evaluation methods while inhibition zone test were used as anti-algal evaluation method.

Experimental

Materials and chemicals

PVC powder and necessary additives were supplied by VP Wood Co. Ltd. (Bangkok Thailand) and the compound formulation was the same as used in previous work [9].Wood flour with an average particle size of less than 250 μm (60 mesh size) was obtained from wood particles of the *Xylia Kerrii Craib & Hutch*, supplied by Phongsiri Ltd., Part. (Ratchaburi, Thailand). The chemical treatment on wood surface was carried out by 1% of N-2(aminoethyl)-3-aminopropyltrimethoxysilane (KBM 603, Shin-Etsu Chemical Co. Ltd., Japan) [9]. Biocides which include Carbendazim (Benzimidazol-2-yl-carbamiciacid methyl ester), IPBC (3-Iodopropinyl-N-butylcarbamate), algaecides namely Isoproturon (3-(4-Isopropylphenyl)-1,1 dimethylurea), and Terbutryn (N^2-*tert*-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine) were used and supplied by Troy Asia Co. Ltd. (Bangkok, Thailand). *Aspergillus niger* (*A. niger*), TISTR 3245 and *Chlorella vulgaris* (*C. vulgaris*), TISTR 8580 were selected as the testing fungi and testing algae, respectively.

Specimen preparations

Compression molding technique was used to prepare test specimens. All raw materials were first dry-blended using high speed mixer, and then filled in mould cavity having 1 mm in depth. The processing conditions were carried out under a test temperature of 190^{o}C under a pressure of 150 kg·cm^{-2}, for 5 min, to give PVC and WPVC sheets. For disk diffusion test and inhibition zone test, the sheet was cut in square pieces of 10×10 mm^2 whereas for dry weight technique and chlorophyll-A measurement technique, the sheet was cut in square pieces of 50×50 mm^2.

Anti-fungal and anti-algal mearsurements

Anti-fungal evaluation:

Disk diffusion test – Two testing specimens were gently placed onto nutrient agar (potato dextrose agar, PDA) by which a fungal disk of *A. niger* at the center of Petri dish (90 mm diameter) was located between the test specimens. The distance from fungal disk to the edge of the specimens was fixed at 15 mm. The fungi were then incubated at 30^{o}C for 7 days. The fungal growth area was observed and reported in terms of "normalized fungal growth area" which was calculated by the ratio of fungal growth area to Petri dish area.

Dry weight technique – This followed the standard testing method ASTM D1413-76. The fungal testing media was prepared by fungal spore suspension having a concentration of 10^6 spores·ml^{-1} in 100 ml of nutrient broth. Then, a testing specimen and the prepared fungal media were filled together in sterilized erlenmeyer flask. The flasks were circularly shaken at a frequency of 150 rpm at 30^{o}C for 14 days. After the end of incubation period, fungi were filtered using high wet strength Whatman's filter paper, and then dried at 80^{o}C for 48 h to remove moisture before weighing the testing fungi. The results were reported in terms of "Dry weight" value in mg·ml^{-1}.

Anti-algal evaluation:

Clear zone test – The mineral salt soft agar was mixed with culture media of *C. vulgaris* at a concentration of 10^7 cells·ml^{-1} and poured onto agar surface in Petri dish. A testing specimen was then carefully placed at the center of the dish. The incubation temperature and time used were 28^{o}C and 28 days, respectively, in dark and light cycle of 12 h and 12 h. Inhibition area or clear zone was assessed and reported in terms of "Inhibition area of algal growth" in mm^2.

Results and discussion

Fig.1 shows growth of *A. niger* on PDA for PVC (without wood particle) and WPVC containing 100 pph wood content at different concentrations of Carbendazim and IPBC by disk diffusion test. It can be observed that increasing the fungicide content could inhibit the fungal growth. The effect was more pronounced for the IPBC samples than the Carbendazim ones. This could be better

clarified using the normalized fungal growth area given in Fig. 2. The smaller the normalized fungal growth area, the better inhibition of the *A. niger* growth. Therefore, it could be said that IPBC was better anti-fungal performance than Carbendazim. This reason may be because the melting point of IPBC (68-65^{o}C) was much lower than that of Carbendazim (302-307^{o}C). In this respect,the IPBC could perform better dispersion and diffusion around the PVC and WPVC composite matrices. For the effect of wood flour, the results clearly indicated that the PVC with 100pph wood flour exhibited better anti-fungal performance than the neat PVC, the effect being more pronounced at the concentrations of the fungicide of 30,000 ppm or higher. This result suggested that wood particles could enhance the anti-fungal property by acting as anti-fungal promoter for the WPVC composites. The reason behind this was that wood particles can absorb easily moisture due to their high hydrophilicity, and could also generate some voids in the PVC matrix [11], and these two concurrent effects could allow the fungicide to transfer or move from the WPVC test-pieces into nutrient agar and thus resulted in higher inhibition areas (or smaller normalized fungal growth area).

IPBC was selected for further anti-fungal evaluation due to its effectiveness for *A. nige.* Fig. 3 shows anti-fungal performance of PVC and WPVC using dry weight technique. It was found that the results corresponded well to those from the disk diffusion method. That was, the IPBC at 30,000 ppm or higher showed a satisfactory anti-fungal activity than the Carbendazim, and the wood acted as anti-fungal promoter in WPVC system.

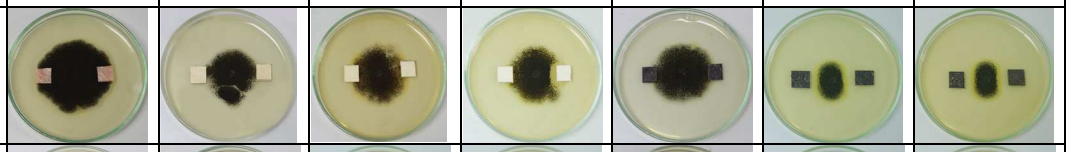
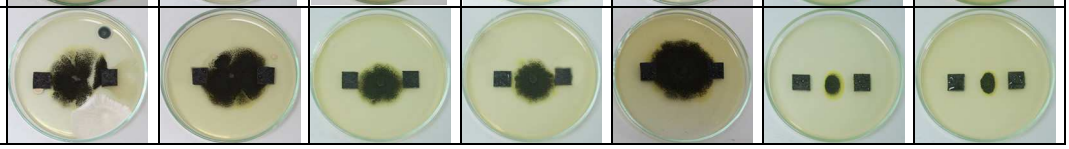
Wood content (pph)	Fungicide content (ppm)						
	-	Carbendazim			IPBC		
	0	10000	30000	50000	10000	30000	50000
0							
100							

Figure 1 Visual growth of *A. niger* on nutrient agar for different types and contents of fungicides in PVC and WPVC.

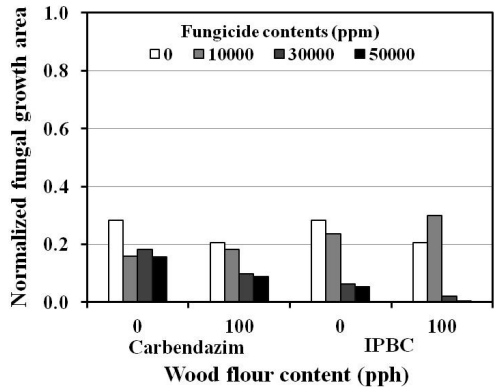


Figure 2 Normalized fungal growth areas of PVC and WPVC samples at different fungicide contents

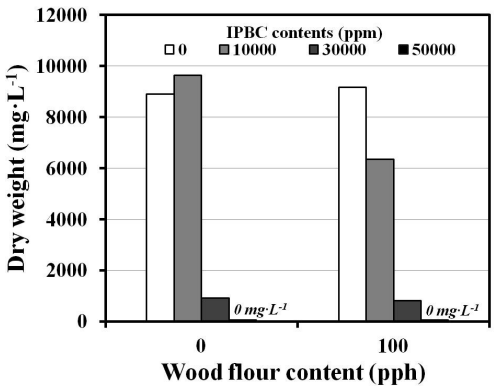


Figure 3 Dry weight of *A. niger* after exposure to PVC and WPVC samples at different IPBC contents

Fig. 4 shows inhibition zone of *C. vulgaris* growth on mineral-salts agar for neat PVC and WPVC composites with different types and loadings of Terbutryn and Isoproturon algaecides. It was found that the greater the algaecide concentration the greater inhibition area. The results also showed the anti-algal performance by Terbutryn was apparently higher than that by Isoproturon for neat PVC and WPVC composites. Only 250 ppm of Terbutryn could initiate the *C. vulgaris* killing, but in

order to completely killing of the *C. vulgaris*, 1,000 ppm of Terbutryn may be required. It was interesting to mention that the presence of 100pph wood fibers accelerated or promoted the *C. vulgaris* killing at which the loading of Terbutryn to obtain the complete algae termination reduced to 500 ppm. Thus, this could be said that that the wood acted as the anti-algae improver for WPVC system, likely found in case of the IPBC filled WPVC.

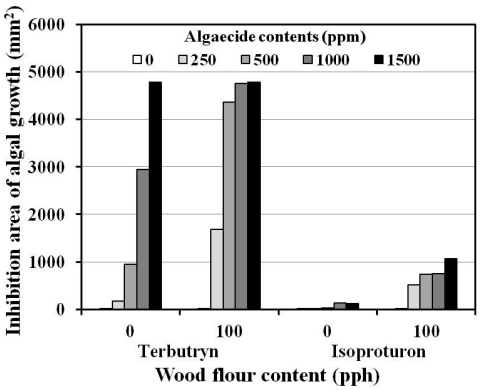


Figure 4 Inhibition areas of *C. vulgaris* on nutrient agar for PVC and WPVC samples at different algaecide contents

Conclusion

The results suggested that IPBC and Terbutryn were recommended to be effective antifungal and anti-algal agents, respectively for neat PVC and WPVC composites. The recommended dosages for IPBC and Terbutryn in PVC were 30,000 ppm and 1,000 ppm, for PVC and WPVC composites. The wood particles added in PVC could act as anti-microbial promoters under the test conditions and commercial biocides used in this work.

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