ANNALS of Faculty Engineering Hunedoara
– International Journal of Engineering

Tome XII [2014] – Fascicule 4 [November]
ISSN: 1584-2673 [CD-Rom, online]

a free-access multidisciplinary publication
of the Faculty of Engineering Hunedoara

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DEVELOPMENT OF A QUANTITATIVE METHOD FOR TESTING THE ANTIFUNGAL EFFECT OF FACADE PAINTS WITH BIOCIDES

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Abstract: Biocides are commonly added to facade paints in order to control the growth of algae and fungi, which are usually present on external surfaces of buildings. The antifungal efficiency of biocides are usually determined by producer companies, but those methods are qualitative or semi-quantitative. The aim of this paper is to examine and quantify the antifungal effect of two different biocides and one in-can preservative, usually added to facade paints, as well as the impact of different water quality used during the production of the paints. The conventionelly used fungal nutrient media (PDA-potato dextrose agar) was inoculated with the spores of *Aspergillus niger* ATCC 6275 and the test samples of the facade paints were placed on the inoculated nutrient in Petri dishes. The samples were incubated and the growth was monitored with a camera during the incubation period. In order to quantify the antifungal activity of the commercial facade paints, a specifically designed program code was used. The obtained results showed that the quality of water has no impact on fungal growth. On the other hand, in-can preservative slightly suppresses the growth of fungi. Also, it was determined that one of the tested biocides has a significantly higher antifungal effect than the other one.
Keywords: biocides, facade paints, fungi, program code, antifungal effect

1. INTRODUCTION

Facade paints are used for the protection and decoration of surfaces; both functions may be lost due to the growth of microorganisms which are deposited on the painted surfaces from the surrounding environment. On external surfaces, rain and wind bring small fragments of plant and animal origin, spores and microbial cells, as well as minerals and air pollutants. This kind of contamination usually leads to degradation of facade paints and undesirable aesthetic changes (Gaylarde P. et al., 2011). In order to avoid this kind of contamination, it is important to use antifungal and antialgal biocides.

The primary aim of biocide application is to reduce microbial load on the surface in order to restore or maintain the initial properties of the facade paints. The presence of water, carbon (from resin) and mineral change (inorganic nutrients) makes the facade paint an appropiate place for the development of micoorganisms, evidently the application of biocides is required.

Biocides are usually added to paints for protection during storing (wet-state or in-can biocides) and surface coating preservation (dry-film or in-film biocides). During the storage of the water-based facade paints, the bacteria, which are commonly present in raw materials (e.g. pigments, fillers and water), find an environment favorable to their growth. Contamination may also take place due to inadequate sanitation of the equipment and storage tanks. Inside the can, there are places with oxygen (empty space between the paint and the can lid) that allow aerobic bacteria and yeast growth and places without oxygen (deep within the paint can) where anaerobic bacteria can grow. The resulting biodeterioration leads to changes in viscosity and pH value as well as to production of CO_2, that can cause foam and swelling of the can. Microorganisms which can grow in that kind of environment include *Pseudomonas*, *Aerobacter*, *Flavobacterium*, *Escherichia, Proteus* and *Bacillus species* (Contant et al., 2010).

Nowadays, biocides based on carbendazim, diuron and isothiazolon are widespread due to their broad spectrum against fungi and algae, low solubility in water and good UV stability. It is necessary to point out that the biocides with wide-protection range are forbidden by the law (Biocidal Products Drirective 98/8/EC, 1998) and the paint industry is forced to use substances which can adequately replace them.

The complete mechanism of action of the biocides is not known. Available literature provides some information about the mechanism of the action of isothiazolone biocides. According to literature, the isothiazolones utilize a two-step mechanism involving a rapid inhibition of growth and metabolism, that is followed by irreversibile cell damage resulting in the loss of viability. Cell death results from the destruction of the protein thiols and the production of free radicals (Williams, 2007).

After the application of facade paint on a surface, the formed film may be in contact with fungi and algae from the air. Fungi are particularly dangerous to paint films because they can be developed on both, external and internal walls. The literature has pointed to fungi as to major microorganism responsible for microbiological spoilage of painted surfaces. Examples of fungi that are frequently found on the facade paints are *Aspergillus niger*, *Cladosporium* and *Alternaria species* (Gaylarde et al., 2011; Contant et al., 2010). The growth of phototrophic microorganisms, algae and cyanobacteria, on the external surfaces of buildings occasionally cause discoloration and physico-chemical deterioration. The main consequences of such growth are disfigurement, retention of water, encouragement of colonisation by fungi and macroorganisms and, in some cases, corrosion caused by organic acids. Algae which can be developed on painted surfaces belong to *Chlorophyceae* and *Cyanophyceae* families (Gaylarde P. and Gaylarde C., 2000; Barberousse et al., 2007).

Available literature is mainly based on leaching biocides from surfaces (Schoknecht et al., 2009); statistical analysis of fungicide activity (Gaylarde P., 2004); and the isolation of microorganisms (Gaylarde C. and Gaylarde P., 2005). On the other hand, there are no literature or standardized methods for quantifying the antifungal efficiency of biocides. The antifungal efficiency of biocides are usually determined by producer companies, but those methods are qualitative or semi-quantitative.

The aim of this paper is to examine and quantify the antifungal effect of two different biocides and one in-can preservative that are commonly added to the facade paints, as well as the impact of different water quality used during the production of the paints. In order to achieve the goal, specifically designed program code was used.

2. MATERIALS and METHODS

2.1. Materials

In order to examine the antifungal effect of the commonly used biocides, three groups of experiments were set up. In these experiments, six different facade paint systems were used. Details about the experiments and the used facade systems are shown in Table 1. The composition of the biocide 1 is the following: carbendazim, isoproturone, propium iodide, butylcarbamate and terbutryn. On the other hand, the composition of the biocide 2 is: diuron, carbendazim, octylisothiazolinon, while the composition of the in-can preservative is: ethylenedioxydimethanol, 5-chloro-2-methyl-4-isothiazolin and 2-methyl-2H-isothiazon.

All facade paint samples had a round form (Φ50mm). Before the beginning of the experiment, the facade samples were rinsed with tap water for 2 hours and then they were dried at room temperature. The fungal media, Potato Dextrose Agar (PDA), was inoculated with the test microorganism- *Aspergillus niger* ATCC 6275.

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Table 1. Groups of experiments and tested facade paint systems

Group	Experiment	Facade paint system
I	Determination of the antifungal effect of the biocide 1 and biocide 2	facade paint produced with tap water + biocide 1 + in-can preservative
		facade paint produced with tap water + biocide 2 + in-can preservative
II	Determination of the antifungal effect of the in-can preservative	facade paint produced with tap water without biocides + in-can preservative
		facade paint produced with tap water without biocides and in-can preservative
III	Determination of the water quality impact on antifungal effect of the biocide 1	facade paint produced with tap water + biocide 1 + in-can preservative
		facade paint produced with tap water without biocides + in-can preservative
		facade paint produced with distilled water + biocide 1 + in-can preservative
		facade paint produced with distilled water without biocides + in-can preservative

2.2.Methods

In order to determine the antifungal effect of the biocides, the round test samples were placed with the painted surface on the PDA media. The prepared samples were incubated at 23°C during 8 days.

The quantification of the antifungal effect of the tested facade paint systems was performed using a specifically designed program code. Every day the fungal growth was monitored with camera. The created images were loaded in the designed application and cropped in order to eliminate the influence of the image background. Every image was cropped three times in order to get the exact areas of the Petri dish, the facade paint samples and of the inhibition zones (Fig.1). The percentage of the surface coverage was calculated by applying a specifically written program code, which determines the ratio of the black and white pixels. The black pixels correspond to the surface covered with hyphae and mycelia of the fungi. The following equation, Eq. 1, was used in order to obtain the percentage of the surface without a visible fungal growth:

$$x_f = \frac{A_f}{A_p - A_s} \cdot 100\% \quad (1)$$

where x_f is the percentage of the surface without a visible fungal growth, A_f, A_p, A_s are areas (in pixels) of the inhibition zones, Petri dishes and the facade painted samples, respectively. A_p and A_s were calculated from the cropped image as an ellipse surface area. The ellipse shape was chosen in order to minimize the errors which could appear during the preparation of the images (caused by the different angles of the photography shots).

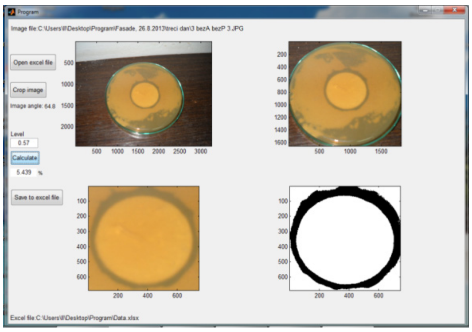

Figure 1. Working steps in the designed program code

3. RESULTS and DISCUSSION

In real terms, raining leads to the leaching of the biocides form facade paints, which results in the loss of their concentration (Wittma et al., 2011). At the start of the analysis, rinsing of all samples was performed in order to simulate real conditions.

After setting the experiment, an inhibition zone (zone without fungal growth) is formed around the facade samples, which is the consequence of the biocides properties. The biocidal components are soluble in water and immediately after the placing of the samples in the inoculated nutrient, as well as during the incubation. These components "migrated" from the sample surface into the inoculated media, with effect similar to the biocides leaching from the facade paints (Coutu et al., 2012).

By using the designed program code, two different biocides were tested on the antifungal efficiency (Fig.2), as well as one in-can preservative (Fig.3).

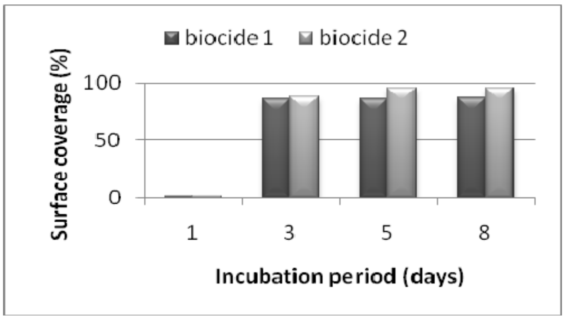

Figure 2. Antifungal effect of the tested biocides

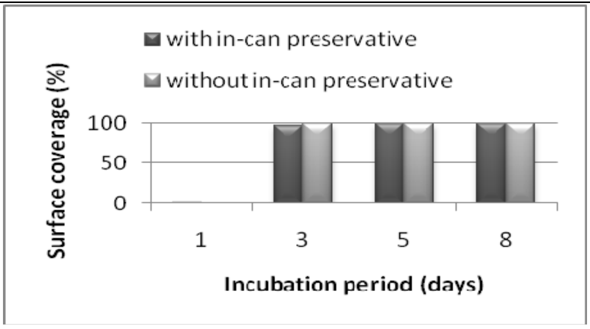

Figure 3. Antifungal effect of the in-can preservative

The obtained results show that biocide 1 has higher antifungal effect than the biocide 2 (Fig.2). During the first 3 days of the incubation period, there were almost no differences between two tested biocides. Major differences were observed on the fifth day when it was noted that the surface coverage of the sample with the biocide 2 is about 10% greater than the surface coverage of the sample with biocide 1. The minimum surface coverage (about 86%) and the maximum inhibition zone (about 14%) were obtained for the biocide 1 after 8 days. Also, it should be noted that the percent of the surface coverage for the biocide 1 was approximately the same after the third day. On the other hand, the percent of the surface coverage for the biocide 2 was increasing until the last day of the incubation period. This dissimilarity may be related to their different composition.

The presented results in Fig.3 show that the in-can preservative slightly suppressed the fungal growth on the third day of the incubation period when the surface coverage for this sample was about 97%. By the end of the experiment, the surface coverage of the sample with the in-can preservative reached 100%. It should be pointed out that the in-can preservative was added to the facade paints during packaging, and had manifested the biocide effect inside the can, not after the application of the paint. The surface coverage was 100% for the samples without the in-can preservative which was expected, because there are no limited agents in the paint for the fungal growth.

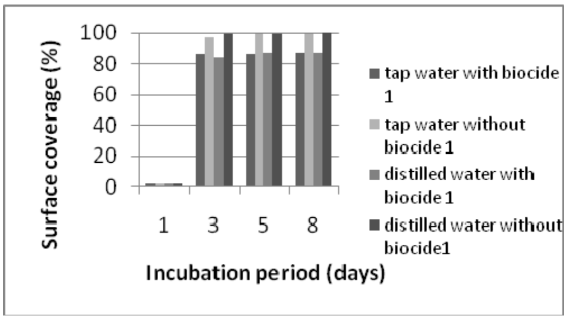

Figure 4. Impact of the water quality on antifungal activity of biocide 1

The same program code was used in order to determine the impact of water quality on the antifungal effect of the biocides. It was assumed that the samples prepared with tap water would have higher percent of surface coverage than the samples prepared with distilled water, due to the higher content of organic matter. In order to achieve the goal, the percent of the surface coverage of the facade paints with and without biocide 1 prepared with tap and distilled water were compared (Fig.4). It can be seen from Fig. 4 that the quality of water does not have an important role in the efficiency of the biocides. Both samples without biocide 1, prepared with tap and distilled water, reached almost 100% of the surface coverage within 3 days. Also, for the samples with biocide 1, water quality has no influence on the antifungal activity of the biocide 1 (Fig.2 and Fig.4).

4. CONCLUSIONS

The biocide 1 (carbendazim, isoproturone, propium iodide, butylcarbamate and terbutryn) showed the highest antifungal effect, while the biocide 2 (diuron, carbendazim, octylisothiazolinon) possessed 10% less antifungal activity. On the other hand, the in-can preservative (ethylenedioxydimethanol, 5-chloro-2-methyl-4-isothiazolin and 2-methyl-2H-isothiazon) had the ability to slightly suppress the fungal growth. It can be concluded, from the obtained results, that water quality had no impact on the antifungal effect of the biocides. The producers of the facade paints may use tap water in order to reduce costs, maintaining in the same time the requested quality of the products. After the data collection presented in this paper, the next step will be the testing of the physical, structural and surface properties of the facade systems in order to study the action of mechanism of the examined biocides.

ACKNOWLEDGEMENT

The financial support from the Serbian Ministry of Education, Science and Technological Development (Contract No. III45008) and from the Provincial Secretariat for Science and Technological Development of Vojvodina Region (Contract No 114-451-3426/2013-02) are gratefully acknowledged.

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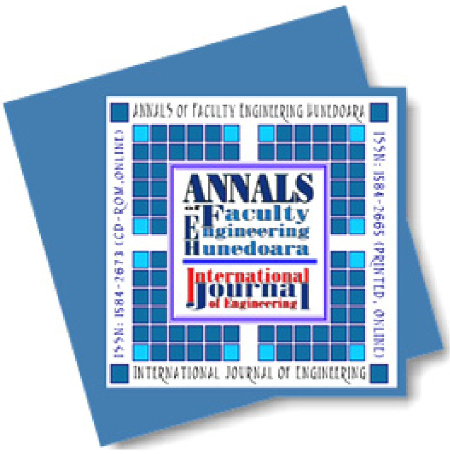

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