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A preliminary study on antifungal effect of TiO_2-based paints in natural indoor light

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The antifungal activity of four commercial photocatalytic paints (KEIM Ecosil ME, Titanium FA, Photo Silicate and Silicate D) in natural indoor light was investigated. The paints contained TiO_2 in rutile and anatase crystalline forms as evidenced by means of the X-ray diffraction analysis. In most cases the paints inhibited growth of fungi viz. *Trichoderma viride, Aspergillus niger, Coonemeria crustacea, Eurotium herbariorum, and Dactylomyces* sp . The KEIM Ecosil ME paint displayed the highest antifungal effect in the light, which could be explained with the highest anatase content. The paint antifungal activity and the fungal sensitivity to the TiO_2-mediated photocatalytic reaction both decreased in the following orders: KEIM Ecosil ME > Titanium FA > Photo Silicate > Silicate D and *T. viride* > *Dactylomyces sp*. > *A. niger* > *E. herbariorum*.

Keywords: antifungal activity, titanium dioxide, natural indoor light, photocatalysis.

INTRODUCTION

At present, the annual production of TiO_2 exceeds 5,480,000 t [1 – 2]. It is used as a white pigment in paints (51% of the total production), plastics (19%) and paper (17%), which represent the major end-use sectors of TiO_2. Titanium dioxide has received a great deal of attention due to its chemical stability, non-toxicity, low cost and photoinduction phenomena[3]. Since 1985 when Matsunga et al.,[4] reported the photocatalytic inactivation of *E. coli* and *Saccharomyces cerevisiae* in the presence of TiO_2 irradiated with UV light, the photocatalysis has become a promising tool allowing detoxification and disinfection. Principles of the photocatalysis have been described in several reviews[5 – 9]. During the process, titanium dioxide is exposed to sunlight or UV light (λ>400 nm), causing electrons to transfer from the valence band to the conduction band. These highly unstable state have strong oxidation and reduction powers and converts water and oxygen into reactive oxygen species, such as: hydroxyl radicals (·OH), superoxide ion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). These radicals and molecules are termed reactive oxygen species (ROS). The uniqueness of ROS is its strong oxidizing property and antimicrobial effect.

The combination of TiO_2 and light kill bacteria, viruses, prions and cancer cells[10 – 15]. Most of the works has determined the removal rates of pathogenic microorganisms from drinking water and wastewater[6, 8 – 9, 16 – 20], while only some papers have concerned the elimination of fungi by TiO_2-mediated photocatalysis[11, 21 – 25]. Currently, there is considerable interest in the application of TiO_2 photocatalytic reactions in building industry to protection of building materials from biodeteriorating microorganisms, especially from fungi[25, 26].

It has been reported that TiO_2 photocatalysts show little antibacterial efficacy in visible light[27 – 28]. However, there are little data on the antifungal activity of these photocatalysts, especially when added to indoor paints[29]. Therefore, the objective of this study was to evaluate the activity of four commercial TiO_2-based paints under natural indoor light against selected microscopic fungi. A wide variety of fungal isolation sources reflected potential applications of the TiO_2-based photocatalytic reaction for disinfection of plant materials and biosolids and for improvement of hygienic conditions in plant storage and organic waste treatment facilities[18, 23, 25 – 26, 30].

MATERIAL AND METHODS

The photocatalysis paints investigated were commercial: KEIM Ecosil ME (white), Titanium FA (grey), Photo Silicate (white), and Silicate D (white). All paints were manufactured by PIGMENT Building Chemistry Producer, Szczecin, Poland. Paint samples were delivered to the laboratory by the producer, thoroughly mixed under aseptic conditions, and instantly used in the experiment.

The fungi were isolated from dried herbs, sunflower seed, wood chips and from flotation waste. The fungal species and isolates were as follows: *Trichoderma viride* (KW8; from flotation waste), *Aspergillus niger* (AN1; from dried herbs), *Coonemeria crustacea* (A50; from sunflower seed), *Eurotium herbariorum* (R19; from dried herbs), and *Dactylomyces* sp. (T1; from wood chips). The isolates are deposited at the West Pomeranian University of Technology fungi collection, Szczecin, Poland.

Standard glass plates (9-cm diam) with MEA medium (Malt Extract Agar, Merck) were used in the experiment. In each plate the medium layer was ca. 7 mm thick. In one part of the plates their agar layer tops were coated with a ca. 2-mm paint layer, while another part with no paint layer were used as controls. The paint coating on the top of MEA plates was prepared manually with a sterilized small soft brush.

Ten-day old fungal cultures on MEA slants at 25°C were used for preparing fungal spore suspensions. 5 ml of sterile physiological saline was added to each slant. The slants

were then vigorously shaken with a Vortex for three minutes. Each plate was centrally inoculated with 5 μl of spore suspension.

The plates inoculated separately with *T. viride*, *E. herbariorum* and *A. niger* and with *C. crustacea* and *Dactylomyces* sp. were incubated in the non-reversed position at 25°C and 37°C, respectively. The incubation temperature 37°C was chosen due to the thermophilic nature of the two last species. The incubation was carried out for 10 days. One part of the plates was incubated in the dark, while another part was everyday exposed to natural indoor light (on the windowsill and without window opening) for six hours (without plate opening). Colony diameters were measured after 2, 4, 6, 8 and 10 days of incubation with a ruler. The daily growth rates ($mm*day^{-1}$) were calculated from the linear regression equation, $r = a*t + b$, in which: r – colony radius (mm); t – incubation time (day); a – daily growth rate; and b – growth retardation time (lag phase; λ). The calculations were performed in the Excel program. According to Dantigny et al.[31], the lag time has no biological significance, because its calculation results from macroscopic observations of the mycelium. It was the reason why the analysis of b values were abandoned in this study. The experiment was verified three times. The statistical significance of the differences in daily growth rates was evaluated with one-way ANOVA test at $p \leq 0.05$.

Based on the mean daily growth rates two antifungal activity indices of the paints were calculated: (1) the paint antifungal activity in the dark (AAD) = daily growth rate on paint-coated MEA plates / daily growth rate on uncoated MEA plates (both plates were incubated in the dark throughout the incubation period); and (2) the paint antifungal activity in the light (AAL) = daily growth rate on paint-coated MEA plates incubated in the dark / daily growth rate on paint-coated MEA plates everyday exposed to natural indoor light for six hours. When the paint coat stimulates the fungi to grow the AAD and AAL values are >1, whereas the values <1 indicates the inhibition of fungal growth. When the total growth inhibition is observed the AAD and AAL values are zero.

The X-ray diffraction analysis was used to determine the contents of rutile and anatase in the paints. The samples of paints was dried at the temperature 70°C for 4 h. X-ray diffraction patterns of the sample were obtained with a X'Pert PRO Philips (The Netherlands) diffractometer in the diffraction angle range $2\theta = 10 - 80^{o}$ using CuK_{α} radiation. Obtained XRD patterns were compared with Join Committee on Powder Diffraction Standards (JCPDS) cards.

In all paints the X-ray diffraction analysis revealed rutile (Table 1). Three paints (Ecosil ME, Titanium FA, Photo Silicate) were enriched with TiO_2 powder in the anatase crystalline form. The Silicate D contained only rutile. The highest amount of anatase the relative to dry mass (4.5 wt.%) was in paint Ecosil Me. The rutile contents were comparable in all paints.

The light intensity was measured by Radiation Intensity Meter LB 901/WCM3 & PD 204AB *cos*. Sensor (The Netherlands). The intensity of light ranged from 3.1 to 24.6 $W\cdot\times m^{-2}$. The UV intensity range was 0.1 – 0.3 $W\cdot\times m^{-2}$.

Table 1. Content of polymorphic phases of TiO_2 in the paints

No	Kind of paint	Content, wt.%	
		anatase	rutile
1.	KEIM Ecosil ME	4.5	27.7
2.	Titanium FA	1.9	28.7
3.	Photo Silicate	2.3	26.9
4.	Silicate D	–	29.7

Results and Discussion

Except for *C. crustacea*, the other fungi grew on paint coatings in MEA plates; indicating that the nutrients from MEA penetrated the coatings and were available to the fungi.

The daily growth rates are presented in Table 2. Statistically significant differences at $p \leq 0.05$ were observed for the growth of *A. niger*, *C. crustacea*, and *Dactylomyces* sp. on MEA without paint coating under dark and light exposure. The *A. niger* exposure to light considerably decreased the daily growth rate compared to the control in the dark. Less significant decrease was observed in *Dactylomyces* sp. On the contrary, the light exposure slightly increased the *C. crustacea* daily growth rate on MEA. Thus, the influence of natural indoor light on fungal growth was found to be diverse.

The inhibitory or stimulatory effect of paint coatings on fungal growth under dark and natural indoor light exposure can be well observed while comparing AAD and AAL indices. The AAD values are illustrated in Fig. 1. In most cases the coatings exerted inhibitory effect against the fungi in the dark (AAD values <1). The antifungal activity of the paints was dependent on the specified sample and the fungal species. However, *A. niger* was found to be the most resistant to the paints. The KEIM Ecosil ME even slightly stimulated the growth of this fungus (AAD value >1). The growth of *T. viride* was stimulated by Silicate D.T. *viride* is a well known biocontrol agent against different fungi. Hence, the stimulation of its growth may control the growth of some fungal forms .

The AAL values were illustrated in Fig. 2. The Photo Silicate and Titanium FA paint coatings everyday exposed to light exerted little or no inhibitory effect against the fungi examined (AAL values close to 1). Subsequently, the KEIM Ecosil ME showed the strongest antifungal activity; causing the total growth inhibition of *E. herbariorum*, *T. viride* and *Dactylomyces* sp. (AAL=0). The *A. niger* growth on this paint was found to be highly restricted (AAL <0.5). Since the KEIM Ecosil ME had the highest anatase content, and since the anatase shows much higher photocatalytic activity compared to the rutile, the results appear to be explainable[4, 20, 32 – 33].

The Silicate D antifungal activity against *A. niger*, *T. viride* and *Dactylomyces* sp. was also high (AAL close to or <0.5). However, this paint stimulated the growth of *E. herbariorum* to a high degree (AAL >3).

In general, the fungi displayed diverse reactions to the paints in the dark. The paints were found to usually inhibit the growth of the fungi. Due to the paint compositions were only known to the producer, it was impossible to specify inhibition or stimulation agents in the paints. In comparison with controls in the dark the exposure of paints and fungi to natural indoor light resulted in the

Table 2. Effect of light exposition on fungal daily radial growth rate (mean ± standard deviation) on MEA coated with photocatalytic paints and on MEA without coating

Strain	MEA Dark	MEA Light	KEIM Ecosil ME Dark	KEIM Ecosil ME Light	Photo Silicate Dark	Photo Silicate Light	Silicate D Dark	**Silicate D Light**	Titanium F A Dark	Titanium FA Light
Trichoderma viride	11.3±0	10.9 ±0.3	4.6±1.3*	0±0	5.8±0.1*	4.1±0.4	13.7±2.2*	3.4±0.9	6.5±0.4*	4.4±0.1
Aspergilus niger	10.8±1.1*	4.6±0.3	11.7±0.3*	4.9±0.2	8±0.1	8.3±0.6	10±0.5*	5.5±0.6	8.1±0	8.8±0.2
Coonemeria crustacea	6.5±0.2*	8.3±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Eurotium herbariorum	3.3±0.8	3.3±0.8	0±0	0±0	0.4±0.2	0.4±0.2	0.5±0.1*	1.6±0.4	0.6±0	0.5±0
Dactylomyces sp.	9.9±1.2*	8.3±0	1.8±0.4*	0±0	3.9±0.6*	4.9±0.3	4.2±0.2*	1.8±0	4.9±0.2	4.7±0.2

*– statistically significant differences in the means for plates incubated in the dark and daily exposed to light at $p \leq 0.05$

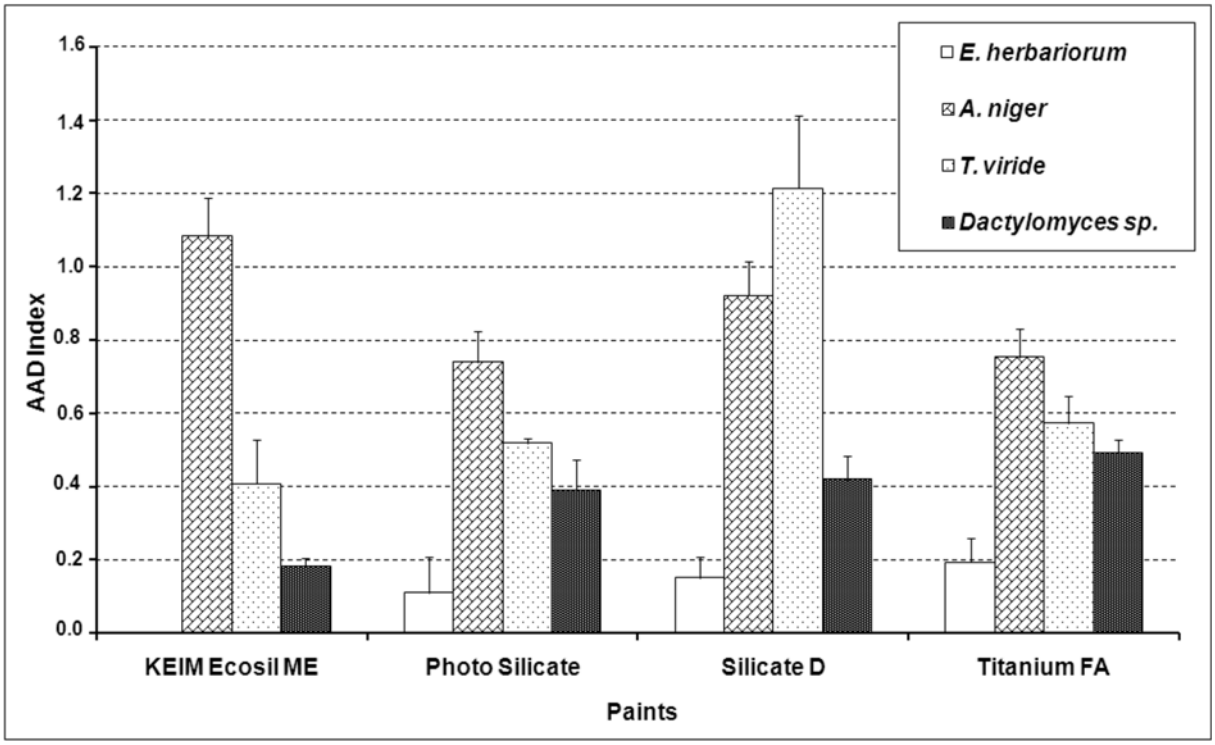

Figure 1. The antifungal activity of paint coatings on MEA in the dark (AAD index values)

considerable changes of fungal growth rates. The KEIM Ecosil ME paint displayed the highest antifungal effect, which could be explained with the highest anatase content. The fungi sensitivity to photocatalytic paints decreased in the following order: *T. viride* > *Dactylomyces* sp. > *A. niger* > *E. herbariorum*. Due to *C. crustacea* did not grow on paint-coated MEA under both dark and light exposure conditions, the species was excluded from this list. The results have confirmed the findings by Maneerat and Hayata[21] and Mitoraj et al.[34]; concerning the inhibition effect of TiO_2-mediated photocatalysis on fungal growth. Chen et al.[25] also found that photocatalytic disinfection processes were effective in the *A. niger* growth inhibition only under UVA irradiation.

As already mentioned, TiO_2 photocatalysts are considered to display little antimicrobial activity in visible light[27, 29]. However, the results of the present study have demonstrated that the TiO_2-supplemented paints inhibit the growth of some fungal species under natural indoor light conditions. The antifungal potential of the paints examined was found to be diverse and decrease in the following order: KEIM Ecosil ME > Titanium FA > Photo Silcate > Silicate D. Further studies are needed to find out the magnitude and mechanisms of fungi killing by paint coatings containing TiO_2 and exposed to natural indoor light. Due to the biodeterioration of walls increases bioaerosol levels, the findings would be essential for improvement of hygienic conditions in the indoor environment. Interestingly, the purification of volatile organic compounds (VOCs) in indoor air has succeeded[35].

The fungi biodeteriorating the walls also contaminate the food commodities stored in such rooms. The findings of the present study would be also helpful in providing hygienic atmosphere for safe storage of different commodities within the wall coated with antifungal paints.

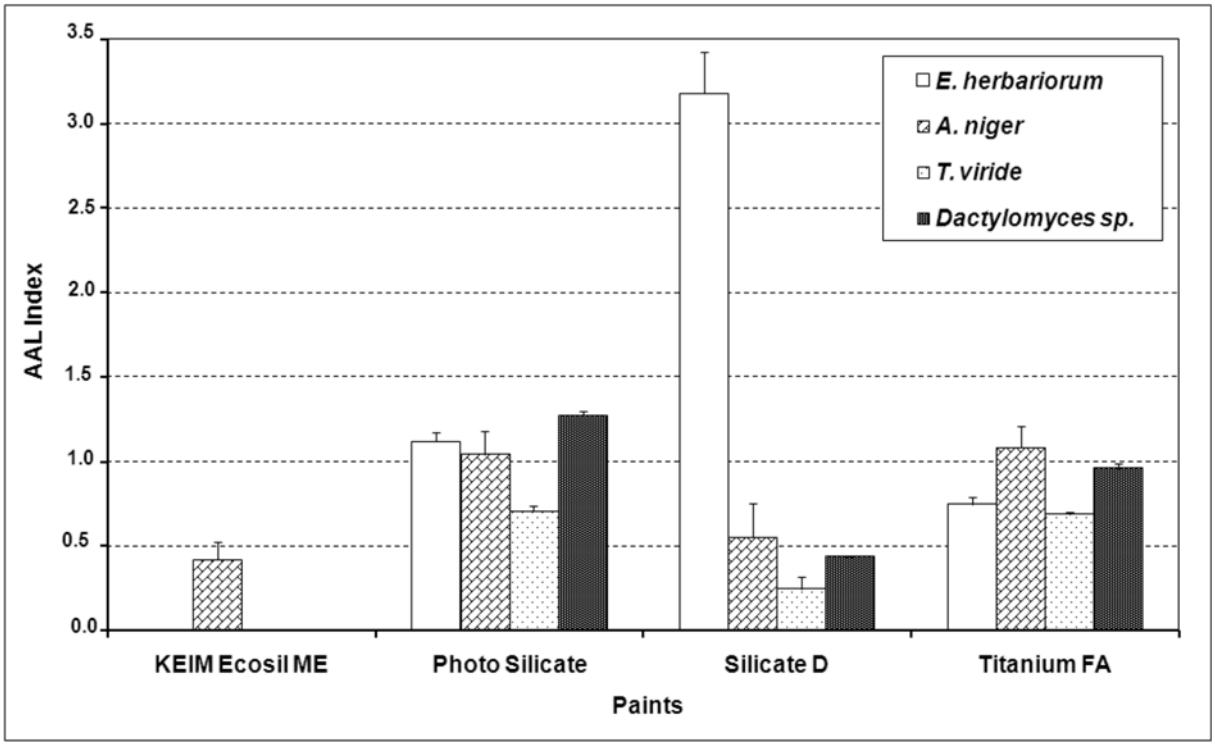

Figure 2. The antifungal activity of paint coatings on MEA everyday exposed to natural indoor light (AAL index values)

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