

REPORT

OF THE RESEARCH ON THE IMPACT OF SELECTED MICROORGANISMS ON ABS COMPOSITES WITH SEANTEX BIOCIDES

/order 514011/

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1 . Materials and Methods

1.1. Materials and methods of testing the anti-bacterial properties of SEANTEX compound.

The tests were effected in two stages. In the first initial stage the impact on selected microorganisms of SEANTEX compound alone was determined. Following strains of bacteria were chosen:

- *Escherichia coli* ATCC 10536;
- *Escherichia coli* D.01 (0157:H7);
- *Staphylococcus aureus* ATCC 43300;
- *Bacillus anthracis* 34F2;

All above strains came from the collection of the Center for Diagnosis of Combating the Biohazards of the Military Institute of Hygiene and Epidemiology in Puławy (Ośrodek Diagnostyki Zwalczenia Zagrożeń Biologicznych Wojskowego Instytutu Higieny i Epidemiologii w Puławach). Bacteria were cultured on TSI /Tryptose Soy Infusion/ substrate for 18 godzin, in temperature of 37°C and next cultured grass-like on TSA /Tryptose Soy Agar/ substrate. After the culture dried the tested preparation in form of a water suspension of 20-0,02 g/ml concentration was placed directly on the substrate, and on the clean tissue circle placed on the substrate, in drops, in quantity of 10 microliters. The reading was made after 24 hours-long incubation in the temperature of 37°C. The biocidal effect of the compound was determined by measuring the diameter of the zone where the growth of given bacteria strain was contained, around the point the compound sample was placed.

1.2. Materials and methods of testing the anti-fungal properties of SEANTEX compound.

For moulds testing, following strains were chosen:

- <i>Aspergillus niger</i> ;	- <i>Penicillium ochro-chloron</i> ;
- <i>Aspergillus terreus</i> ;	- <i>Scopulariopsis brevicaulis</i> ;
- <i>Aureobasidium pullulans</i> ;	- <i>Trichoderma viride</i> ;
- <i>Paecilomyces variotti</i> ;	- <i>Chaetomium globosum</i> .
- <i>Penicillium funiculosum</i> ;	

The fungi strains came from the national collection of the Łódź University of Technology (Politechnika Łódzka).

The contaminated samples were incubated in the temperature of $29\pm 1^{\circ}\text{C}$ for 28 days. The relative humidity in the incubator was over 90%.

It should be noted that SEANTEX compound dissolves in water only to a limited, small degree so its diffusion in the agar substrate is slight, irrespectively of the way it was introduced into the substrate – in form of powder or water suspension.

In the second stage the ABS (Acrylonitrile Butadiene Styrene) composites were tested, containing varying additions of SEANTEX biocide, only on the above enumerated strains of moulds.

The tests were made in compliance with the PN-EN ISO 846 standard, using A and B methods.

The samples were tested on two kinds of substrate:

- incomplete substrate (without glucose) – CD(-S) – deficient
- full substrate (with glucose) – CD(+S)

The deficient substrate is used for determining if the tested material constitutes a source of food for moulds (method A). The full substrate is used for determining if the tested material is subject to the impact of moulds in the presence of additional source of carbon (method B).

The evaluations of the resistance of the tested samples against the above moulds were made visually, using the five-degree scale, and are given in the below table:

Table. 1.

Growth intenseness	Evaluation
0	No microscope visible growth of fungi on the sample
1	Growth not visible with naked eye but clearly visible under the microscope
2	Growth detectable by naked eye; less than 25% of the area covered with fungi (weak fungi growth)
3	Growth detectable by naked eye, covering up to 50% of the tested area
4	Significant growth of fungi, covering over 50% of the tested area
5	Intensive growth covering the entire tested area

Rules of interpretation of the results of testing the resistance of materials against the impact of moulds according to PN-EN ISO 846 standard.

Tab.2.

Method	Growth intenseness	Evaluation of the tested material
A	0	The material is not a nutrient for micro-organisms
	1	The material contains substances being a nutrient or is contaminated to a minor degree enabling slight growth
	from 2 to 5	The material is not resistant to the impact of moulds and contains substances being nutrients for micro-organisms expansion
B	0	Strong fungi-statical effect
	0 + inhibition zone around the sample	Strong fungi-statical effect covering the zone around the sample due to diffusion
	1	The material is not totally fungi-statical
	from 2 to 5	Total lack of fungi-statical effect

2. Effects of testing of biocide properties of SEANTEK compound

2.1. Effects of testing of antibacterial properties

Table.3.

Strain/preparation	Concentration	SEANTEK
Bacteria strains		
Escherichia coli ATCC 10536	20mg/ml	++
	2 mg/ml	++
	0,2 mg/ml	++
	0,02 mg/ml	+
Escherichia coli D.01 (O157:H7)	20mg/ml	++
	2 mg/ml	++
	0,2 mg/ml	++
	0,02 mg/ml	+
Staphylococcus aureus ATCC 43300	20mg/ml	++
	2 mg/ml	++
	0,2 mg/ml	+/-
	0,02 mg/ml	-
Bacillus anthracis 34F2	20mg/ml	++
	2 mg/ml	++
	0,2 mg/ml	++
	0,02 mg/ml	+/-

++ clear lysis zone > 10mm, + clear lysis zone < 10mm, +/- troubled lysis zone, - no lysis zone

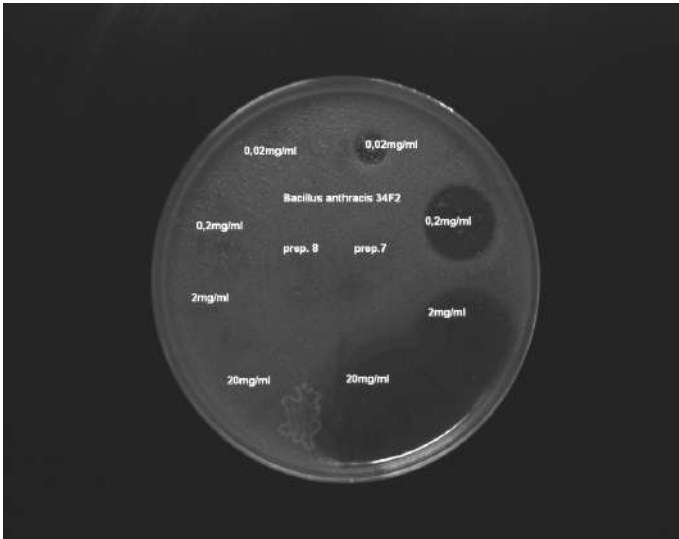


Photo.1. A sample photograph showing the germicidal activity of SEANTEX compound (marked as 'prep.7')

Germicidal properties of SEANTEX compound were tested against the strains of *Escherichia coli* ATCC 10536, *Escherichia coli* D.01 (O157:H7), *Staphylococcus aureus* ATCC 43300, *Bacillus anthracis* 34F2. All tested concentrations of the compound proved to have strong germicidal effect, as shown by a sample photo above. On the photograph the SEANTEX compound is marked as 'preparat 7'. The dark round zones are the places where drops of particular concentrates were inserted; they show the lysis of the bacteria cells in these locations.

2.2. The results of testing the anti-fungal properties.

Tab.4. Resistance of particular strains of moulds against SEANTEX compound.

Mould string	SEANTEX
<i>Aspergillus niger</i>	-
<i>Aspergillus terreus</i>	-
<i>Aureobasidium pullulans</i>	-
<i>Paecilomyces variotti</i>	+
<i>Penicilium funiculosum</i>	+
<i>Penicilium ochro-chloron</i>	+
<i>Scopulariopsis brevicaulis</i>	+
<i>Trichoderma viride</i>	+
<i>Chaetomium globosum</i>	+

- + growth inhibition zone present,
- no growth inhibition zone.

The strains *Aspergillus niger*, *Aspergillus terreus*, *Aureobasidium pullulans* are resistant against the concentration of SEANTEK compound used in the tests.

Photographs were taken after 28 days of incubation in temperature of $29 \pm 1^{\circ}\text{C}$.



Photo.2. A sample photograph showing fungicidal activity of SEANTEK compound

Testing of properties of sample of Acrylonitrile Butadiene Styrene (ABS) with addition of SEANTEK compound

items 1 and 3 – samples of product
item 2 - composite

Table.5. Results of testing the antifungal properties of ABS samples with varying content of SEANTEK

	ABS samples with different SEANTEK content	Resistance results on SD(+S) substrate
1	ABS+SEANTEK 3%/1000g	0
2	ABS+10%SEANTEK	0
3	ABS+SEANTEK 1.5%/1000g	0

Photographic documentation of samples after finishing the tests of resistance against fungi (after 28 days of incubation)

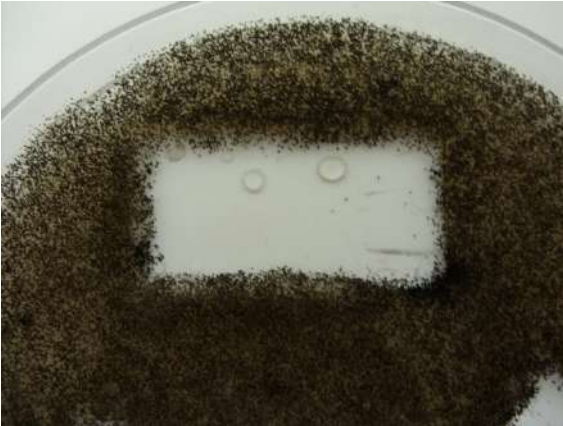


Photo.1. ABS+SEANTEK (3%/1000g) sample on CD(+S) substrate



Photo.2. ABS+10% SEANTEK sample on CD(+S) substrate

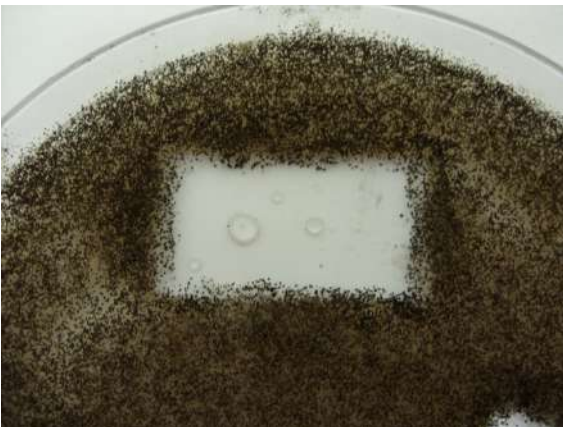
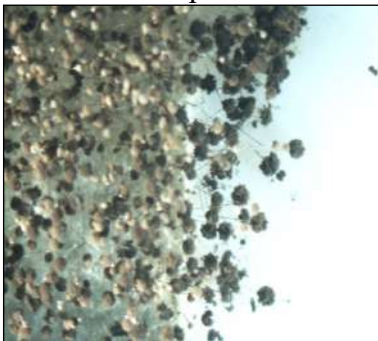


Photo.3. ABS+SEANTEK (1.5%/1000g) sample on CD(+S) substrate

Camera-taken photo under stereo microscope, ~50x magnification



The photograph shows the sample is not infested by fungi. The mould hypha cover the verge of the sample only because they are much higher than the surface of the sample.