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INSTITUTE FOR ENGINEERING OF POLYMER MATERIALS AND DYES**

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Test Report

No. TB/45-46/BIO/2022

Test subject:

Research works on determining:

- 1) the antibacterial properties of a polymer material
- 2) determination of the fungistatic properties of the polymeric material

Test object:

pipes

Sample number:

45/BIO/2022, 46/BIO/2022

Client:

TT PLAST S.A.
Targowisko 476
32-015 Kłaj
NIP: 683-18-62-210

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Date starting of the test: 28.06.2022

Date ending of the test: 29.07.2022

Date of report: 03.08.2022

The test results refer only to the tested sample. Without written permission of the Research Laboratory "Polymer" of the Łukasiewicz Research Network - IMPiB Institute, this report may not be duplicated otherwise than in full.

1. The subject of test

In the study were used samples of the polymer material delivered by the Client:

- sample "control sample"; sample has been renamed **45/BIO/2022**,
- sample "with biocide"; sample has been renamed **46/BIO/2022**.

As indicated by the Client:

- sample 45/BIO/2022 is the control sample.

Test samples were taken by the Client in accordance with the test sample requirements described in the standardized test method(s) given in section 3 of this report.

2. Basis of the study

The tests were carried out on the basis of the order of 29/06/2022 TT PLAST S.A.. Samples for testing were received on June 21, 2021. The order was given the number 150000-61-1.

3. The scope of test

According to the received order, the following tests were performed for each sample:

- determination of the antibacterial properties of the polymer material,
- determination of the fungistatic properties of the polymeric material.

4. Test description

4.1. Determination of the antibacterial properties of the polymer material

Quantitative determination of bactericidal properties of polymeric materials was carried out in accordance with ISO 22196:2011 "Measurement of antibacterial activity on plastic and other non-porous surfaces". Two bacterial reference strains were used in the study: *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538P).

The size, shape of the sample, materials, test methodology and guidelines described in the ISO 22196, 2011 standard were applied to the tests.

Samples characteristics: 50 mm × 50 mm pipe samples with thicknesses of approx. 1.0 mm.

Characteristics of the covering layer: PE film with dimensions of 40 mm × 40 mm and a thickness of 0.06 mm.

Inoculum volume: 0.4 ml.

4.2. Determination of the fungistatic properties of the polymeric material

The fungistatic properties of polymers were determined in accordance with PN-EN ISO 846, 2019 "Plastics. Evaluation of the activity of microorganisms" (Method B).

Five model strains of fungi were used in the study: *Aspergillus niger* (ATCC 6275), *Penicillium pinophilum* (ATCC 36839), *Paecilomyces variotii* (ATCC 18502), *Trichoderma virens* (ATCC 9645) and *Chaetomium globosum* (ATCC 6205).

The size, shape of the sample, materials, test methodology and guidelines described in the PN-EN ISO 846, 2019 standard were applied to the tests.

Samples characteristics: as above

The samples were divided into research batches:

Test series 0: climate control specimens, stored under standard temperature and standard moisture conditions in accordance with PN-EN ISO 291, 2010 (23 °C ± 1 °C, 50% ± 5% RH); 2 specimens.

Test series S: sterile specimens, stored under the same conditions as test series I; 2 specimens.

Test Series I: Test specimens inoculated with microorganisms and incubated; 5 specimens for each sub-test: series Ia (without biocide) and series Ib (with biocide).

Incubation conditions: temperature 29 °C ± 1 °C, 28 days.

The visual assessment of the samples was carried out using photos taken with an automatic SCAN 1200 colony counter (Interscience, France). For microscopic observations, an Olympus SZX 12 stereoscopic microscope (Olympus, USA) was used at $\times 42$ magnification of the sample image, using an Arctam 300 MI camera (Artray, Japan).

5. Results

5.1. Determination of the antibacterial properties of the polymer material

The results of the determinations are presented in the table 1.

Table 1. Test results for the determination of the antibacterial properties of sample 46/BIO/2022 in relation to bacterial strains *E. coli* and *S. aureus*:

Sample symbol	Bacterial strains	The average number of viable bacteria cells cfu/cm ²	Calculated parameter
45/BIO/2022 Control 0h	<i>E. coli</i>	1.7×10^4	$U_0 = 4.2$
	<i>S. aureus</i>	1.9×10^4	$U_0 = 4.3$
45/BIO/2022 Control 24h	<i>E. coli</i>	3.7×10^4	$U_t = 4.6$
	<i>S. aureus</i>	4.5×10^4	$U_t = 4.7$
46/BIO/2022 Sample	<i>E. coli</i>	1.4×10^1	$A_t = 1.2$
	<i>S. aureus</i>	9.4	$A_t = 0.9$

cfu - bacterial colony forming units.

U_0 - mean of the log cfu/cm² number recovered from control samples immediately after inoculation.

U_t - mean of the logarithm of cfu/cm² recovered from control samples after 24 h.

A_t - the mean of the logarithm of cfu/cm² that was recovered from the samples tested after 24 hours.

Calculation of antibacterial activity according to the table:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Sample 46/BIO/2022:

relative to *E. coli*: $R = 3.4$

relative to *S. aureus*: $R = 3.7$

Validation conditions for samples 46/BIO/2022:

I. The logarithm value of the number of bacteria recovered immediately after inoculation from the untreated test specimens shall the following requirement:

$$(L_{\max} - L_{\min}) / (L_{\text{mean}}) \leq 0.2, \text{ where}$$

L_{\max} is the common logarithm (i. e. base 10 logarithm) of the maximum number of viable bacteria found on a specimen;

L_{\min} is the common logarithm of the minimum number of viable bacteria found on a specimens;

L_{mean} is the common logarithm of the mean of viable bacteria found on the specimens.

Obtained results:

for *E. coli*: 0.02 (fulfills the condition)

for *S. aureus*: 0.02 (fulfills the condition)

II. The average number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall be within the range 6.2×10^3 cfu/cm² to 2.5×10^4 cfu/cm².

Obtained results:

for *E. coli*: 1.7×10^4 cfu/cm² (fulfills the condition)

for *S. aureus*: 1.9×10^4 cfu/cm² (fulfills the condition)

III. The number of viable bacteria recovered from each untreated test specimen after incubation for 24h shall not be less than 6.2×10^1 cfu/cm².

Obtained results:

for *E. coli*: 3.7×10^4 cfu/cm² (fulfills the condition)

for *S. aureus*: 4.5×10^4 cfu/cm² (fulfills the condition)

5.2. Determination of the fungistatic properties of the polymeric material

The results of the determinations are presented in the table 2 and figure 1.

Table 2. Test results for the determination of the fungistatic properties of polymeric material (sample 45/BIO/2022 and 46/BIO/2022). Assessment made on the basis of the scale adopted in the standard *:

Test series		45/BIO/2022 Control	46/BIO/2022 Sample
I _a	visual assessment	3	-
0	visual assessment	-	0
S	visual assessment	-	0
I _b	visual assessment	-	0 or 1
	microscopic assessment	does not require	1c

*Assessment of fungal growth on the samples according to the scale: 0 - no visible growth under the microscope; 1 - growth invisible to the unaided eye, but clearly visible under the microscope, including: 1a - covering up to 25%, 1b - up to 50%, 1c - over 50% of the sample surface; 2 - growth noticeable with the naked eye, covering up to 25% of the tested area; 3 - growth noticeable with the naked eye, covering up to 50% of the tested area; 4 - significant increase, covering more than 50% of the tested area; 5 - intensive growth covering the entire test area.

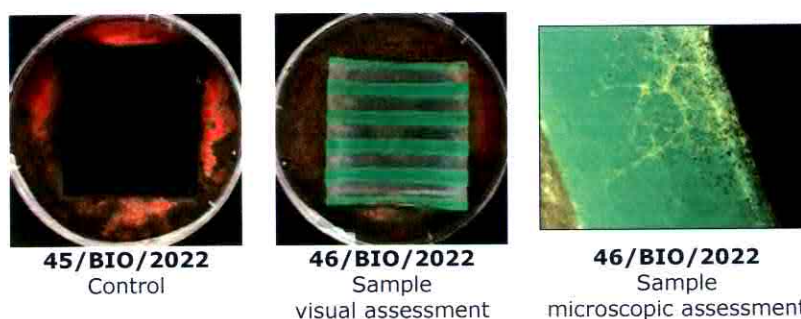


Figure 1. Illustrative photos of the visual and microscopic evaluation of batch Ia / Ib samples after incubation

6. Research evaluation

6.1. Determination of the antibacterial properties of the polymer material

For the tested sample **46/BIO/2022**, the reduction value of the decimal logarithm cfu/cm^2 ($\text{Red}_{\log_{10}}$) relative to the control sample (**45/BIO/2022**) was 3.4 ($R = 3.4$) for *E. coli* and at a level of 3.7 ($R = 3.7$) for *S. aureus*.

The higher the antimicrobial activity, the higher the $\log \text{cfu/cm}^2$ reduction ($\text{Red}_{\log_{10}}$) relative to the control.

6.2. Determination of the fungistatic properties of the polymeric material

For the test sample **46/BIO/2022**, no visible growth of fungi was observed under the microscope, while for the control sample **45/BIO/2022**, growth was visible with the naked eye, covering up to 50% of the test area (see Figure 1).

Any inhibition of growth in both the sample and the medium (zone of inhibition) relative to the control sample (without biocide) indicates a fungistatic activity of the material.

Test prepared by:

Test report authorized by:

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- end of report -

C/O:

- Client,
- TB.