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Research of the bacterial and fungistatic effect on specimens of steel and plastic materials

According to ”PN-LN ISO 846:2002” standard.

“Plastics-Evaluation of the Action of Microorganisms" under certain environmental conditions the microorganisms may settle and colonize the surface of the materials or plastic products. Their presence and/or their metabolic products may not only damage the plastic itself, but also affect the serviceability of the materials.

Basing on “PN-EN ISO 846:2002” standard, the deterioration and extend of the deterioration of materials exposed to selected microorganisms was determined and also, the bacterial and fungistatic effect was examined on the specimens delivered by the customer, namely:

- powder-coated steel (colour of the paint: white and black)
- plastic material with the admixture of the substance inhibiting the development of microorganism

by a visual examination and changes in their physical properties.

This research made use of the following definition of the bacterial and fungistatic effect:

The bacterial and fungistatic effect is according to point 3 of the above-mentioned standard the effect is the result of an antimicrobial treatment which prevents a given material from being overgrown by fungi under moist conditions.

The examination involved exposing the test specimens to the action of selected strains of bacteria and fungi for a specified period of time (here 28 days) under specified conditions of humidity and temperature (according to point 8.1 of the standard the temperature was 23±1 °C); and relative humidity of > 90 % - such humidity is achieved by agar – according to table 3 of the above-mentioned standard). The cultures of strains were treated on the agar slants in line with point 5.2.2 of the above-mentioned standard.

1. Determination of the fungistatic effect (including, in this case, Aspergillus niger) was carried out according to point 4.2.1.1. – *method B* – determination of the fungistatic effect. In line with this point the specimens are exposed to the action of suspension (conidia cells of mould) in the presence of a complete nutrient medium, namely, containing carbon. Each inhibition of growth, both on the examined material and the nutrient (inhibitor zone) indicates the fungistatic action of the material or the presence of the agent inhibiting the growth of microorganisms. It was decided that *A. niger* strain of mould shall be chosen for the test as it is indicated in point 5.2.1 which states that the selection of strains shall depend on the agreement concluded by the interested parties.

   The specimens for the tests were prepared in line with point 6.1. of the standard and they were in the form of 30mm x 30 mm squares.

   B variant of the standard was used in the research (according to 4.2.1.1), namely the evaluation of the fungistatic effect. The specimens were exposed to the action of the mixture of conidia *A. niger* in the presence of complete nutrient agent, namely containing the source of carbon, which was prepared in line with point 5.2.3.5. of the above-mentioned standard (source of carbon: glucose solution of the 30g/L concentration).

   The viability of the tested mould was checked in line with point 8.2.1.4 of the above-mentioned standard.

   For this reason, the sterile Petri dishes were filled with complete nutrient (5.2.3.5) proceeding in line with point 8.2.1.1 and were inoculated with one drop of each cell suspension. They were incubated at the temperature of 24±1 °C for 3-4 days. After the required test period the abundant growth of the microorganism was observed which confirmed that the cells had been properly prepared and had achieved the required viability.

   According to the standard, after the end of the exposure of the specimens, the visual evaluation was carried out before and after the cleaning. The tests results concerning the cells exposed to the biological activity were comparable to the results obtained on the basis of the evaluation of specimens which had not been exposed to the action of microorganisms.

   The evaluation of the modification of examined specimens was performed in line with point 9.1. of the standard – the visual assessment of the growth of the fungi and in line with point 9.2.1 – the evaluation of the cleaned specimens. After the 28-day-long incubation, the specimens were subject to the visual and microscopic evaluation.

In case of the visual evaluation on the basis of table 4 of the above-mentioned standard, the following grades of the evaluation were applied:

0 – no growth apparent under the microscope;

1 – no growth visible to the naked eye, but clearly visible under the microscope;

2 – growth visible to the naked eye, covering up to 25% of the test surface;

3 - growth visible to the naked eye, covering up to 50% of the test surface;

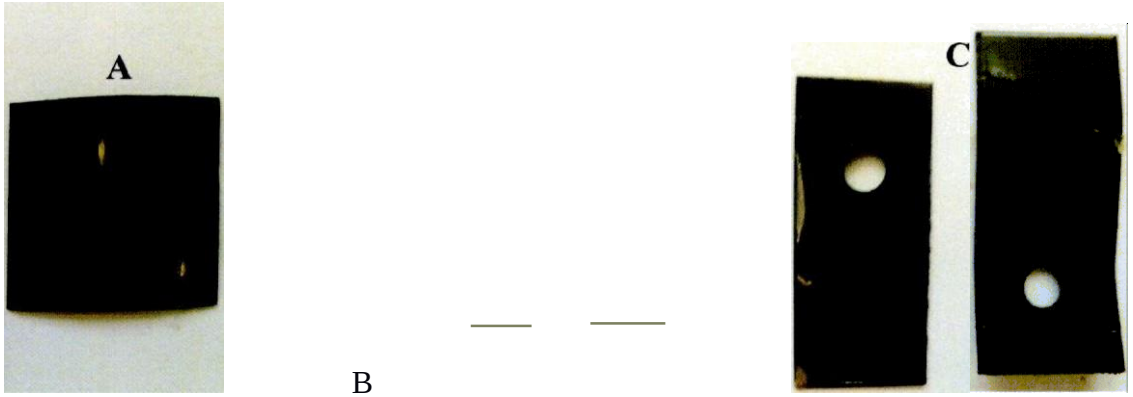
4 – significant growth of the mould on the specimen covering more than 50% of the examined surface;

5 – heavy growth covering the entire test surface.

On the basis of the experiments carried out in accordance with point 9.1 of the above-mentioned standard, the tests were viewed with a naked eye and then in each case it was confirmed under a stereomicroscope (enlarged x 50). The mould growth was evaluated according to table 4 of the above-mentioned standard -"the evaluation of the growth of fungi". An additional assessment of the specimens was carried out after their cleaning in accordance with section 9. 1 (cleaning of the specimens was carried out in accordance with section 9.2.1. , namely, the specimens were removed from agar, immersed in a mixture of ethanol and water prepared according to 5.1.11.1, and rinsed with running water, dried with paper filter and left for the night to dry in a room temperature).

The visual evaluation of each specimen was expressed as the degree of the cell growth in a way described in table 4.

- The appearance of the specimens which were delivered for tests after 28 days of incubation in the presence of microorganisms is shown in picture 1A-C. As shown, none of the specimens during the contact with the microorganisms shows the apparent change.

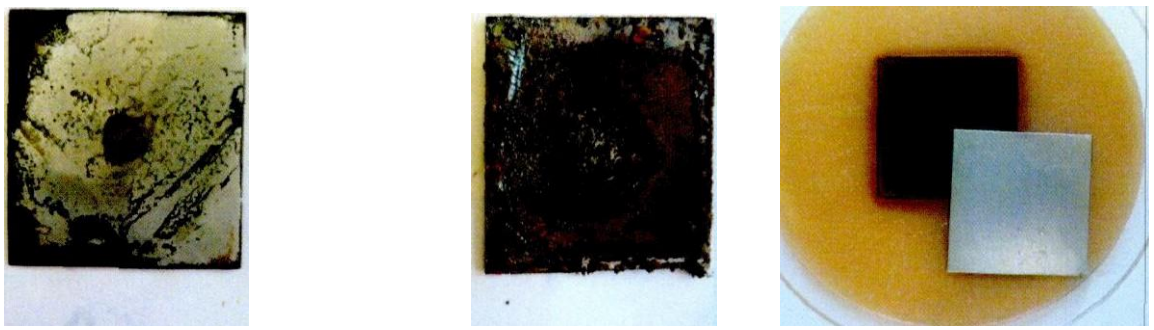


Drawing 1: The image of the specimen surface after 28 days of incubation in the presence of A. niger.

A - The plastic material with the mixture of the antimicrobial agent; B – powder-coated steel (white); C - powder-coated steel (black).

- The appearance of the control specimens (steel) which were exposed to the action of mould, after 28 days of incubation is shown in drawings 2 D-E. As it is shown, the specimens which were delivered for testing of steel during the testing showed apparent changes and a strong corrosion of the tested material took place. On the nutrient, in the contact place of the specimens and surface no inhibition of growth of the tested strain was observed on *A – niger* which proves the absence of the inhibition agent which could inhibit the growth of the tested microorganism.

D E



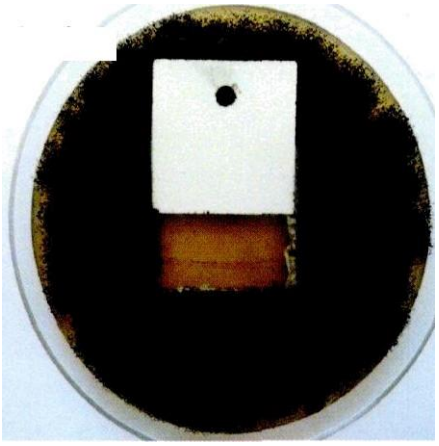
Drawing 2: The image of the specimen surface after 28 days of incubation in the presence of A-niger. D and E – steel – control; on the right it is possible to see the changes in colour of the nutrient as a result of corrosion and lack of inhibition of the growth of microorganisms.

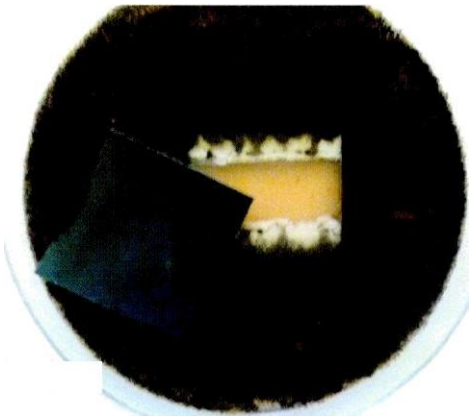
Fungistatic action of the tested specimens

The below drawings present the inhibition of the mould growth *A. niger* in the presence of tested specimens, namely, the powder-coated steel and the plastic with the admixture of the antimicrobial material. As it is shown in the below drawings, the specimens provided for testing show no apparent changes. On the nutrient, in the contact place of the specimens and surface the inhibition of growth of the tested strain was observed on *A – niger* which proves the presence of the inhibition agent which inhibits the growth of the tested group of microorganisms.

A. niger

A.niger





A. niger

Drawing 2. The image of the tested specimen after 28 days of incubation of *A. niger* in the presence of the tested sample; at the top, to the left, there is a powder-coated steel (black) and powder-coated steel (white); at the bottom, there is a plastic with the admixture of antimicrobial material – images after removing the specimens .

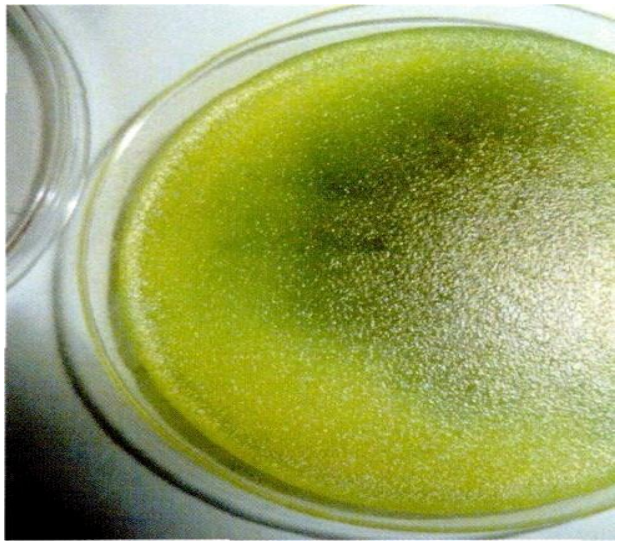
2. The tests with the participation of bacteria were carried out in line with point 8.2.3 (method C).

   The method of cleaning the specimens was carried out in line with point 8.2.3.1. The tests were performed on the agar slants prepared according to point 8.2.3.2. The bacterial cultures developed on agar nutrient were flushed with the enriched bullion (5.3.2.2.) and was incubated for 24 hours at the temperature of 29°C ± 1°C and proceeded according to the description.

The specimens for testing were prepared in line with point 6.2.1. Their quantity was compliant with point 6.2.2 of the above-mentioned standard. The method applied was compliant with point 8.2, table 3 of the above-mentioned standard.

The strain *Pseudomonas aeruginosa* was selected for tests in line with point 5.3.1 ATCC 13388, which was grown on the substrate defined in point 5.3.2.1. of the above-mentioned standard.

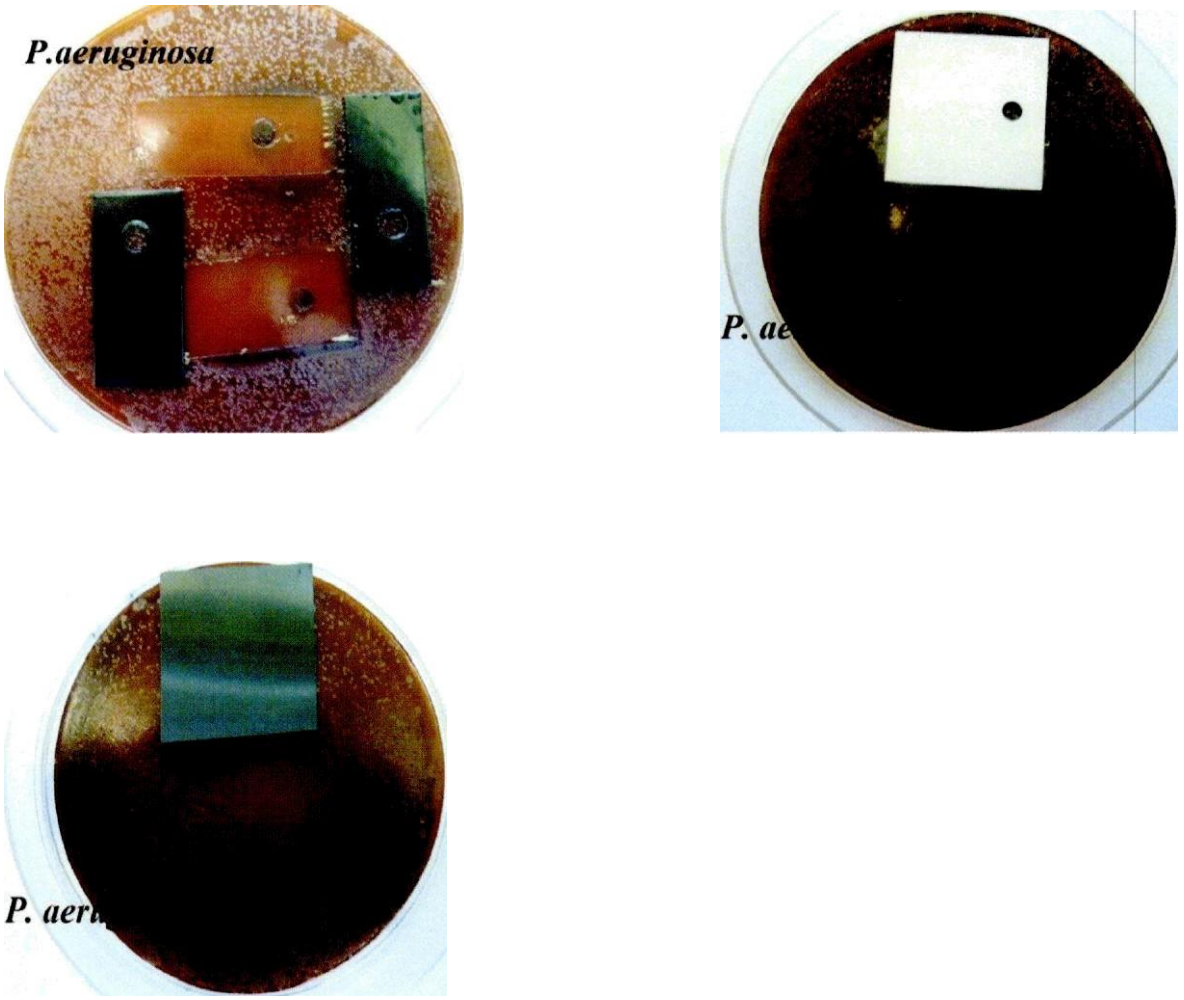
The strain viability was checked in line with point 8.2.3.4. The agar nutrient used for the test was inoculated in line with point 8.2.3.5. The exemplary image of the viability status of P. *aeruginosa* is shown in drawing 3.



Drawing 3. The evaluation of the viability of the tested strain of *P. aeruginosa*

As it is shown in above drawing, the microorganism showed the characteristic (abundant) growth on the nutrient which is confirmed by its good viability.

The appearance of the tested specimens exposed to the action of the bacteria strain after 28 days of incubation is presented in drawing 4.



Drawing 4. The image of the surface of the plate after 28 days of incubation of P. aeruginosa in the presence of the tested sample; at the top, to the left, there is a powder-coated steel (black) and powder-coated steel (white); at the bottom, there is a plastic with the admixture of antimicrobial material – images after removing the specimens .

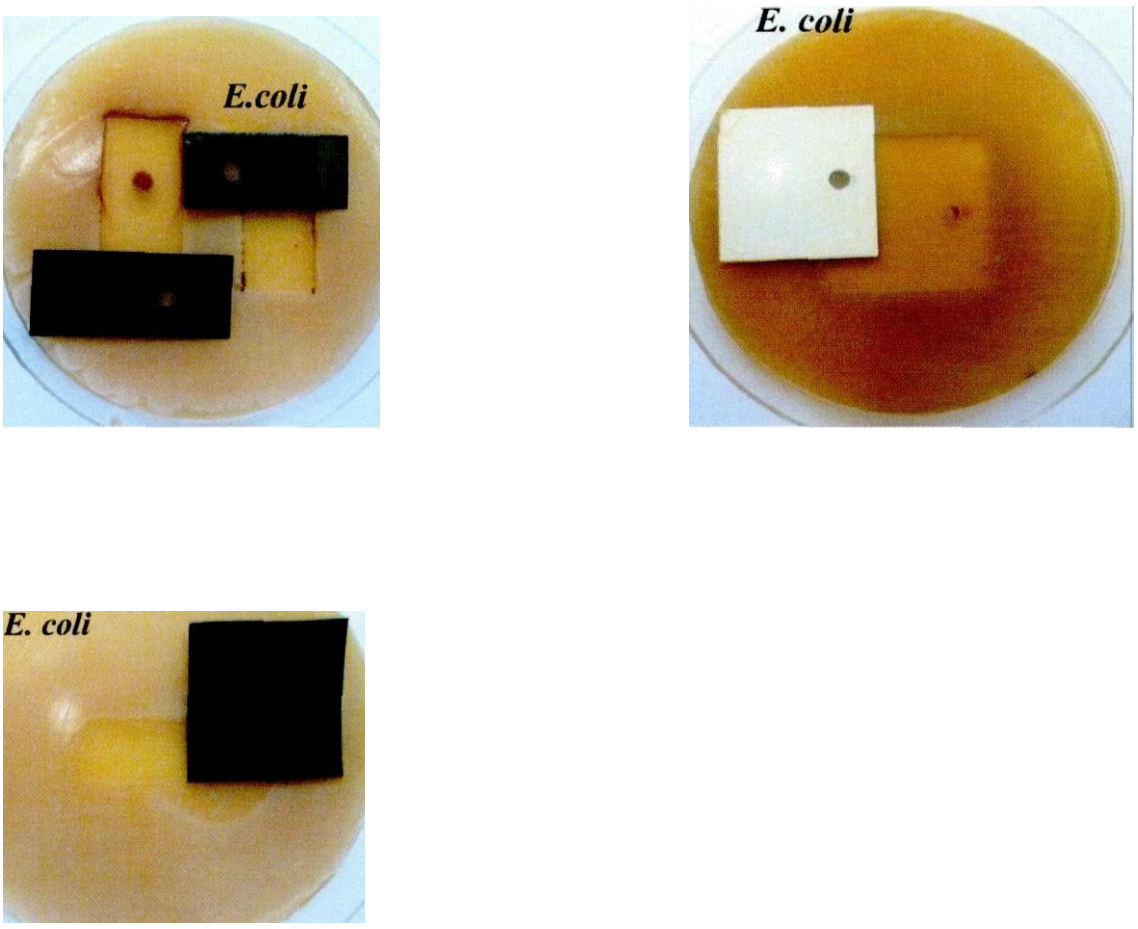
As it is shown, the specimens delivered for testing during the 28 day contact with the bacteria did not show any apparent changes. On the nutrient, in the contact place of the profile with the surface, a strong inhibition of the growth of the tested strain of Pseudomonas aeruginosa was observed, which confirms the presence of the growth agent inhibiting the development of the tested group of microorganisms.

By a mutual agreement of both interested parties, the tests involved the use of additional strains, namely: E. coli PCM 2209; S. aureus PCM 458 and Bacillus cereus PCM 2018;

The specimens for tests were prepared in line with point 6. 1. Of the standard and were in a form of 30mmx30mm squares.

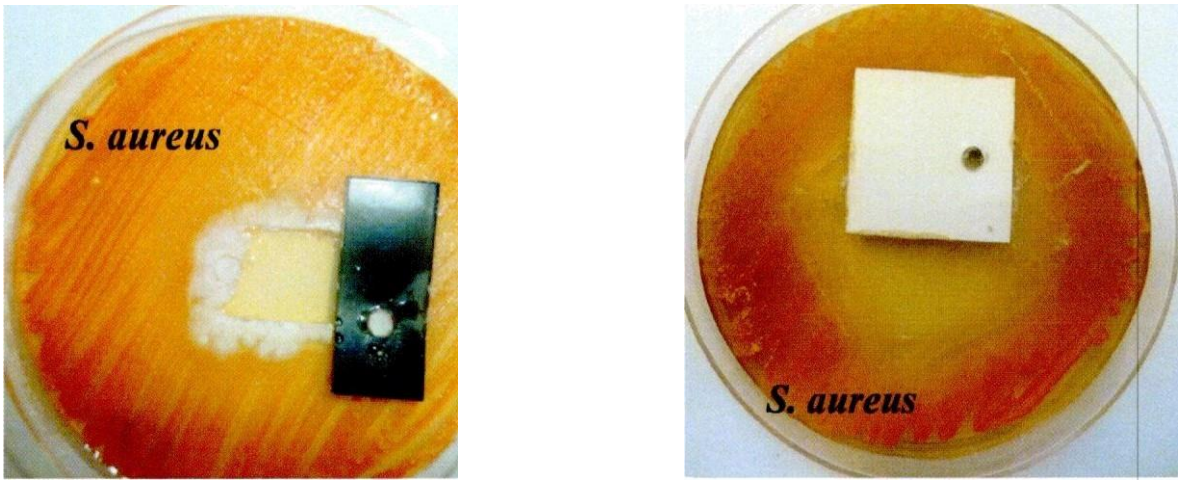
The appearance of the specimens exposed to the action of the test strains of bacteria after 28 days of incubation are shown in drawings 5, 6 and 7. As it is shown, specimens delivered for tests during the incubation period did not show substantial changes. On the nutrient in the contact place of the specimens with the surface a heavy inhibition of the growth of tested strains of *E. coli* PCM 2209; *S. aureus* PCM 458 and *B. cereus* PCM 2018 was observed, which proves the presence of the growth agent inhibiting the development of the tested group of microorganisms.

The below pictures (drawing 5) presents the appearance of the agar plates after 28 days of incubation of selected bacteria (E. coli) in the presence of test specimens.

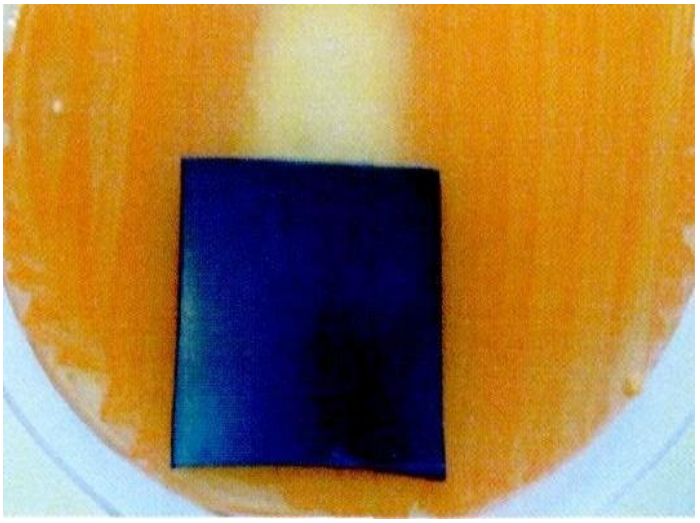


Drawing 5. The image of the plate after 28 days of incubation of E. coli in the presence of the test specimen; at the top, to the left there is a powder-coated steel (black) and powder –coated steel (white); at the bottom there is a plastic with the admixture of antimicrobial agent – images after removing the specimens.

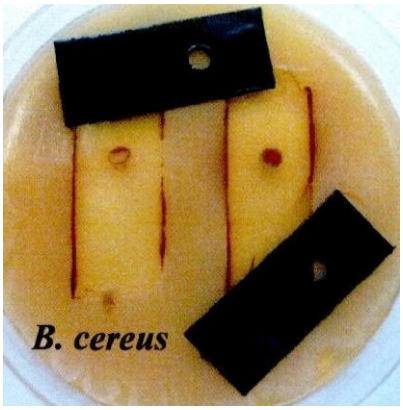
The below pictures (drawing 7) presents the appearance of the surface of agar plates after 28 days of incubation of selected bacteria (B. cereus) in the presence of test specimens.

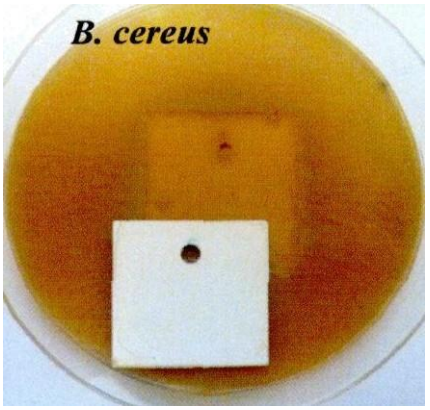


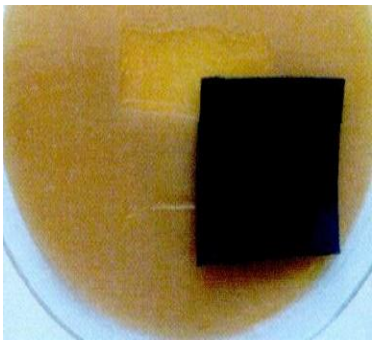
S. aureus



Drawing 6. The image of the test plate after 28 days of incubation of *S. aureus* the presence of the test specimen; at the top, to the left, there is a powder-coated steel (black) and powder-coated steel (white); at the bottom there is a plastic with the admixture of antimicrobial material – images after removing the specimens.







Drawing 7. The image of the test plate after 28 days of incubation of *B. cereus* the presence of the test specimen; at the top, to the left, there is a powder-coated steel (black) and powder-coated steel (white); at the bottom there is a plastic with the admixture of antimicrobial material – images after removing the specimens.

Conclusions

1. The specimens delivered for tests (powder-coated steel (white and black)) are covered with a permanent layer of the agent strongly inhabiting the growth of tested microorganisms, namely: *A. niger, P. aeruginosa, E. coli, S. aureus* and *B. cereus.*
2. The layer prevents the growth of microorganisms in the direct contact.
3. The steel which is not coated with an antimicrobial layer does not inhibit the growth of microorganisms and is exposed to corrosion in contact with the nutrient.
4. The plastic which does not have any antimicrobial properties is not exposed to biodeterioration but does not inhibit the growth of microorganisms.
5. The plastic with the admixture of antimicrobial agent inhibits the growth of: *A. niger,*

P. aeruginosa, E. coli, S. aureus and *B. cereus.*

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