Evaluation of the antimicrobial efficacy of industrial substrata

M. A. Calvo1, J.M. Vaquero2, G Girmé1, E.L. Arosemena1, S. Manso2, I. Segura2 and A. Aguado2

1 Grup de recerca en Microbiologia Aplicada i Mediambiental. Universidad Autònoma de Barcelona. Campus de Bellaterra 08193 Bellaterra (Barcelona), Spain
2 Departament d’Enginyeria de la Construcció. Universitat Politècnica de Catalunya, 08028 Barcelona, Spain

In this chapter we provide different methodologies to assess the antimicrobial activity of various substrates for industrial application. The capacity of microorganisms to colonize or remain viable on a variety of media: paintings, all kinds of plastic, wood, stone, concrete, etc., can produce a deterioration and even the possible role of these substrates as infection vectors processes in humans and animals. In this sense and based on Standards of International control we have developed a simple and reliable method to verify, if the ability of individual carriers treated with natural products with inhibitory capacity of microorganisms to prevent, delay and/or minimize its colonization and biodeterioration.

Keywords: Antimicrobial activity; industrial substrata; biodeterioration

1. General remarks

The capacity of microorganisms to colonize or remain viable on a variety of media: paintings, all kinds of plastic, wood, stone, concrete, etc., can produce a deterioration and even the possible role of these substrates as infection vectors processes in humans and animals. In this regard and based on Standards of International control we have developed a simple and reliable method to verify, if the ability of individual carriers treated with natural products with inhibitory capacity of organisms to prevent, delay and/or minimize its colonization and biodeterioration. [1,2].

2. Microorganisms assayed

We propose to evaluate different microorganisms on the basis of industrial substrates whose antibiotic activity will be evaluated. The main microorganisms are: Thiobacillus thiooxidans, Escherichia coli, Staphylococcus aureus, Salmonella spp., Bacillus subtilis, Proteus mirabilis, Klebsiella pneumoniae, and Candida albicans, among others.

3. Methodologies

Among the methodologies for the evaluation of the antimicrobial efficacy of different industrial substrata we can mention:

3.1 Impregnation Assay

3.1.1 General

We proceed to impregnate samples of the substrates under study with a suspension of different microorganisms and immediately after impregnation, and time sequenced in function of applications of substrats we proceeds to recover the inoculated microorganisms by sterile swab. These samples were inoculated and incubated on Petri dishes with specific media.

Concentrations of 2x10^5 CFU/mL were prepared from cultures from 24-48h, and impregnation by using sterile swabs on the surface to be evaluated was prepared. Prepare as many samples as desired sampling. Samples were kept in controlled conditions of light, temperature and humidity. 24h after your impregnation the deposited suspensions are recovered and made the inoculation of the same in order to establish the number of viable microorganisms present in the sample freshly inoculated (Time0) and at 24h (Time24) the study started and so on every 24 hours until the end of it or in the intervals you want to set for each study. All assays were performed in triplicate and the results as percent recovery of CFU or viable cells per cm of analyzed sample, depending on the inoculated suspension expressed. Some of the samples were stain with vital dyes and their preparation to be observed under microscope.

3.1.2 Particular case of Thiobacillus thiooxidans

The test consists in embedding by impregnating the concrete surface, treated and untreated, with slurry containing Thiobacillus thiooxidans. This operation is performed in duplicate using two suspensions with two different concentrations of microorganisms.
a. Preparation of suspensions. Preparing two suspensions of microorganisms: 1x10^9 CFU/ml (high concentration) and 1x10^6 CFU/ml (low concentration).

b. Impregnation of the concrete surface. Once the solutions obtained are impregnated with each of these surfaces of the concrete samples, both the samples treated and non-treated. This process is carried out in duplicate 16 samples thus obtained.

c. Recovery solution. The samples were recovered from each of the test pieces using a sterile swab and plated on specific medium for *Thiobacillus thiooxidans*. This process is evaluated: time zero (initial time), day 1, day 4, day 6 and day 9, in order to track the development of the organism over time.

d. Incubation and reading the plate. Petri plates are incubated sown 10 days at 37 °C and then the colonies developed are quantified.

The assay results extracted by impregnation are expressed as the number of colonies formed in each Petri dish.

![Fig. 1](image1.png) Development *Thiobacillus thiooxidans* at high concentration in the Petri dish.

![Fig. 2](image2.png) Development *Thiobacillus thiooxidans* at low concentration in the Petri dish.
3.2 Immersion Assay

In order to know if a product intended for the construction has a bactericidal active which will determine possible impairment may cause *Thiobacillus thiooxidans*, is proposed to immersion Assay.

The methodology for performing the dipping test is based on the procedure performed by Vincke (Vincke et al. 1999) was carried out in duplicate using two means of crops: specific culture medium for the growth of *Thiobacillus thiooxidans*, and isotonic culture medium. The duration of this test was 51 days, divided into 3 cycles of 17 days each, as shown in Figure 1a, and each cycle, in turn, comprises the following 4 stages:

a. Incubation in airlock. The concrete samples were incubated in an atmosphere of hydrogen sulfide at a concentration of 250 ppm for 3 days

b. Incubation in solution with inoculated with *Thiobacillus thiooxidans*. Each concrete specimen were immersed in 150 ml of culture medium, to which were added 1.5 ml of the suspension of microorganisms to a concentration of the order of 1x10^7 CFU (Colony Forming Units) / mL, are covered and incubated for 10 days. The containers were kept in rotation at a speed of 90 rpm. During incubation 4 samples of culture medium on days 1, 4, 6 and 9 of each cycle are removed to determine the amount of SO_4^{2-} and kept refrigerated until analysis.

c. Washing. The concrete samples were washed in Milli- Q water for 2 days by rotating agitators.

d. Drying. The duration of the drying process of the samples was 2 days.

The viability of microorganisms is established according to: 1 - Evolution of the concentration of SO_4, expressed in ppm and 2 - Mass loss. values weight concrete specimens obtained after each of the 3 cycles consisting of the assay, expressed as% weight loss.

3.3 Diffusion Assay

In this type of assay used a modification of the technique of Kirby-Bauer test [3], known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what treatment of antibiotics should be used when treating an infection. In our study, instead of using antibiotic disks inhibitory activity, we were evaluated aliquots of the samples under study. This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic, is placed on the medium. The organism will grow on the agar plate while the antibiotic “works” to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a “zone of inhibition” can be observed and measured to determine the susceptibility to an antibiotic for that particular microorganism.

The culture used in this test has to be the Mueller-Hinton agar because it is an agar that is thoroughly tested for its composition and its pH level. Also, using this agar ensures that zones of inhibitions can be reproduced from the same organism, and this agar does not inhibit sulfonamides. The agar itself must also be only 4mm deep. This further ensures standardization and reproducibility.

The size of the inoculated organism must also be standardized (using barium sulfate standards, McFarland standards). The reasons are because if the size of the inoculum is too small, the zone of inhibition will be larger than what it is supposed to be and if the inoculum is too large, the zone of inhibition will be smaller.

After the plates have been incubated, there should be a “clearing” zone around each of the substrata discs. The diameter of each zone should be measured and recorded in millimeters (mm).

![Fig. 3 Assay with *Aspergillus flavus* in front of treated wood](image1.png)

![Fig. 4 Assay with *Aspergillus brasiliensis* in front of treated textile](image2.png)
4. Conclusion

These methods allow the selection of simple and efficient way, industrial substrates and products that delay or prevent biodeterioration of microbial origin and so can facilitate the control of the disease in the environment of these materials

References