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ANTIMICROBIAL ACTIVITY OF SiO_2/CELLULOSE ETHERS/Ag HYBRID MATERIALS AGAINST *Saccharomyces cerevisiae* 537

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ABSTRACT

The antimicrobial effect against Saccharomyces cerevisiae 537 of silica hybrid materials based on tetraethylorthosilicate, cellulose derivates (hydroxypropyl cellulose and hydroxypropyl methyl cellulose) and silver was investigated. The silver concentration in the hybrid materials varied from 0.5 wt % to 2.5 wt %. The antimicrobial effect was quantified on ground of the inhibition zone measurement and determination of the growth of the synthesized hybrid materials inhibitory effect. The results showed 80 % and 92 % decrease of cell growth by hydroxypropyl cellulose hybrid materials and hydroxypropyl methyl cellulose, respectively, in presence of the highest silver concentration of 2.5 wt %. The lowest silver concentration of 0.5 wt % Ag had no effect on the tested strain viability as the cells observed were not inactivated.

Keywords: antifungal activity, Saccharomyces cerevisiae 537, sol-gel hybrid materials, silver nanoparticles.

INTRODUCTION

Nowadays silver and its compounds are rediscovered by many research groups as antibacterial, antiviral and antifungal agents [1, 2]. The increasing interest and usage of such silver compounds as biocidal factors is due to the well pronounced antibacterial properties against multidrug resistant bacterial strains. Silver ions and silver nanoparticles (AgNps) are among the most commercialized nanoparticles. Different kinds of hybrid materials containing AgNps have an application in many fields such as medicine, food packaging, textiles industry, water purification, etc. [3, 4].

Many authors suggest that silver ions and AgNps can directly damage bacteria cell membranes by causing structural changes, interacting with cytoplasmic components or inhibiting the respiratory chain enzymes [1, 2]. The interaction between microbial membrane structures with silver ions and AgNps leading to increased membrane permeability and cell death is confirmed [5, 6] by scanning microscopic analyses. A possible mode of action of silver and its compounds towards eukaryote can refer to penetration of Nps through the cell membrane and interaction with organelles.

AgNps can also generate silver ions and free radicals due to oxidation processes causing cell death. The usage of silver containing hybrid materials as antimicrobial agents is advantageous because it provides permanent and long time release of silver ions [5, 7].

This study is focused on the antimicrobial effect of two types of silica hybrid materials, SiO_2/HPC/Ag and SiO_2/HPMC/Ag containing different amounts of silver towards *Saccharomyces cerevisiae* 537.

EXPERIMENTAL

Materials

Hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), tetraethylorthosilicate (TEOS) and silver nitrate ($AgNO_3$) were purchased from Aldrich,

while nitric acid was provided by Merck. All chemicals were used as received without any further purification. The strain of *Saccharomyces cerevisiae* 537 was obtained from the Bulgarian National Bank of Industrial Microorganisms and Cell Culture and conserved in our laboratory.

Preparation of SiO_2/HPC/Ag and SiO_2/ HPMC/Ag hybrid materials

The preparation of the hybrid materials was previously described [8, 9]. They contained TEOS and 5 wt. % of HPC as well as TEOS and 5 wt. of % HPMC. Their silver content varied from 0.5 wt. % to 2.5 wt. %.

Antimicrobial experiments

The antimicrobial activity of the two types of hybrid materials described above was studied against *Saccharomyces cerevisiae* 537. The strain was cultured in YEPD medium containing 5.0 g l^{-1} yeast extract, 5.0 g l^{-1} peptone and 10.0 g l^{-1} glucose. Its pH value was equal to 6.0. The cells were cultivated in flasks on a rotary shaker (220 rpm) at 30°C for 24 h.

Agar well diffusion method. This method was previously described [10]. The schema of the experiments is shown in Fig. 1. In each experiment 100 µl cell suspension containing approximately 1×10^5 cell/ml was spread on agar petri dishes. 100 mg of SiO_2/HPC/Ag or SiO_2/HPMC/Ag containing different amounts of Ag (from 0.5 wt. % to 2.5 wt. %) were placed in petri dish wells. Inhibition zones appeared and their size was measured after incubation of the plates for 24 hours at 30°C. Mean values were calculated by performing the experiments in triplicates. The control mixture contained yeast and hybrid materials but no silver.

Antimicrobial activity of hybrids based on the number of surviving cells on agar plates. The microbial inhibiting effect of the hybrid materials was further evaluated by studying the cultures survivale after exposure to the hybrid materials. Aiming this 100 µl strain suspension containing approximately 1×10^5 cell/ml in their logarithmic phase of growth were added to flasks containing 30 ml saline solution and cultivated for 1 h at 30°C. After that 50 mg of each hybrid material of varying silver concentrations were added to the cultivated strain. Samples containing yeast and hybrid materials but no ApNps were used as negative controls. The process was performed on a rotary shaker (220 rpm) at 30°C. Samples were removed from the shaker on the 3rd, 5th and 24th h. They were performed serial dilutions until 10^{-3} and 100 µl of each dilution were transferred drop wise to agar plates. The latter were incubated for 24 hours at 30°C. Then the number of the colonies survived from each dilution was counted.

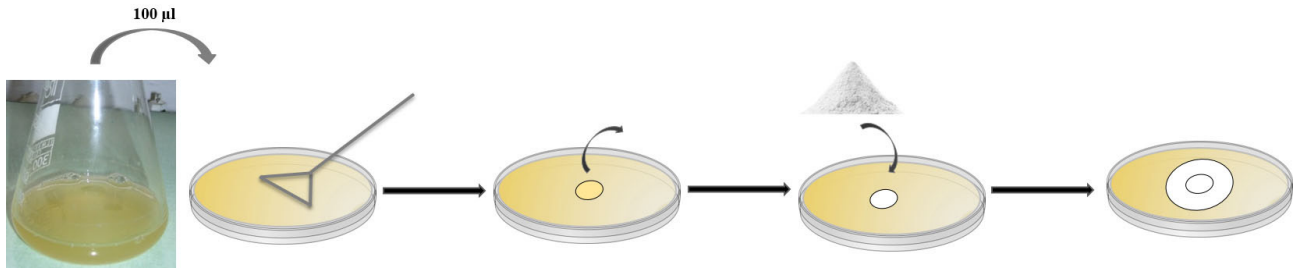

Fig. 1. A schematic presentation of the agar well diffusion method - after cell suspension spreading on the agar petri dish the sample was placed in well.

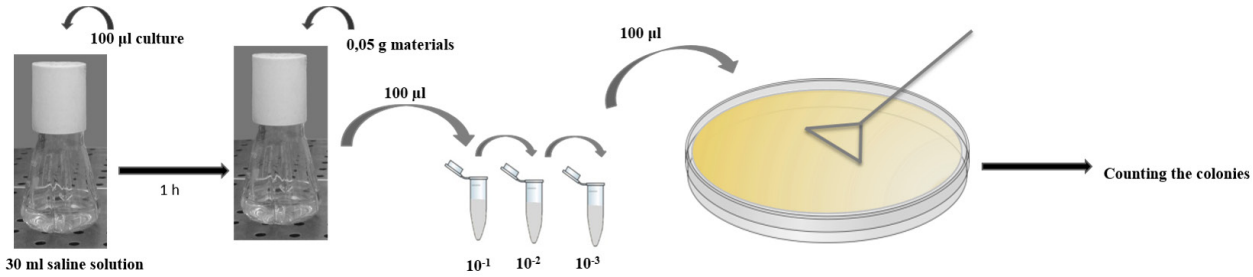

Fig. 2. Test for microbial inhibiting effect of hybrid materials based on YEPD agar plating.

The percentage of cell reduction was calculated as described in ref.[11] in accordance with:

$$Cell\ reduction\ (\%) = \left(1 - \frac{Test\ sample\ \left(\frac{CFU}{ml}\right)}{Control\ \left(\frac{CFU}{ml}\right)}\right).100$$

RESULTS AND DISCUSSION

We have reported the preparation by the sol-gel method of the hybrid materials studied here. Their antimicrobial effect towards *Bacillus subtilis*, *Escherichia coli* K12 [8, 9], *Candida albicans* 74 [10], *Aspergillus niger* [12], and *Penicillium chrysogenum* 2303 [13] was investigated. We have also tested the antimicrobial effect of SiO_2/carboxymethyl cellulose hybrid materials with AgNPs against model yeast *S. cerevisiae* 537 [14]. The results revealed a well pronounced antimicrobial activity depending on the silver content present. The present work is focused on the antimicrobial effect of the materials investigated against strain *S. cerevisiae* 537.

This type of yeast is chosen as a model system for eucaryotic microorganism. Its antimicrobial effect towards prokaryote and eucaryote provokes the interest of many scientists to study the behaviour of fibroblast cells towards this kind of materials.

The agar well diffusion method is one of the experimental methods applied. It is connected with the appearance of inhibition zones with no cell growth. Such zones appear in this study after 24 h of strain cultivation. Fig. 3 illustrates the inhibition zones obtained in presence of *S. cerevisiae* 537 and SiO_2/HPC hybrid material of varying silver content. The silver concentration increase leads to enlargement of the inhibition zones around the materials. Those containing 0.5 wt. % of Ag show the smallest zone of 8.5 mm in diameter and around the control sample was not detected any zone. The highest silver concentration of 2.5 wt. % brings about a clear zone of 16 mm in a diameter, which is in fact 2 fold increase.

The same tendency is observed with the other type of the hybrid materials studied, i.e. SiO_2/HPMC. The smallest

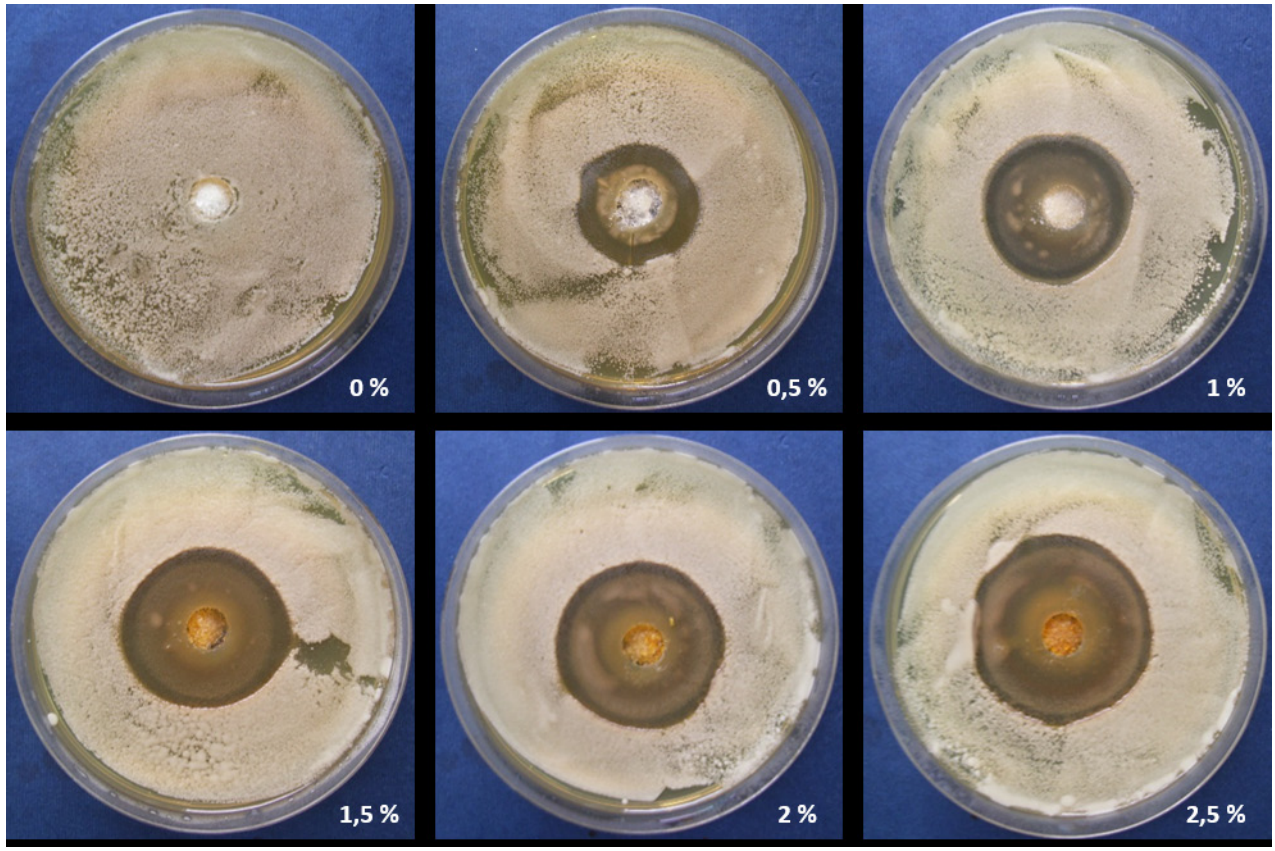

Fig. 3. Inhibition zones provided by SiO_2/HPC hybrid materials of a varying silver concentration against *S. cerevisiae* 537 after 24 hours incubation at 30°C.

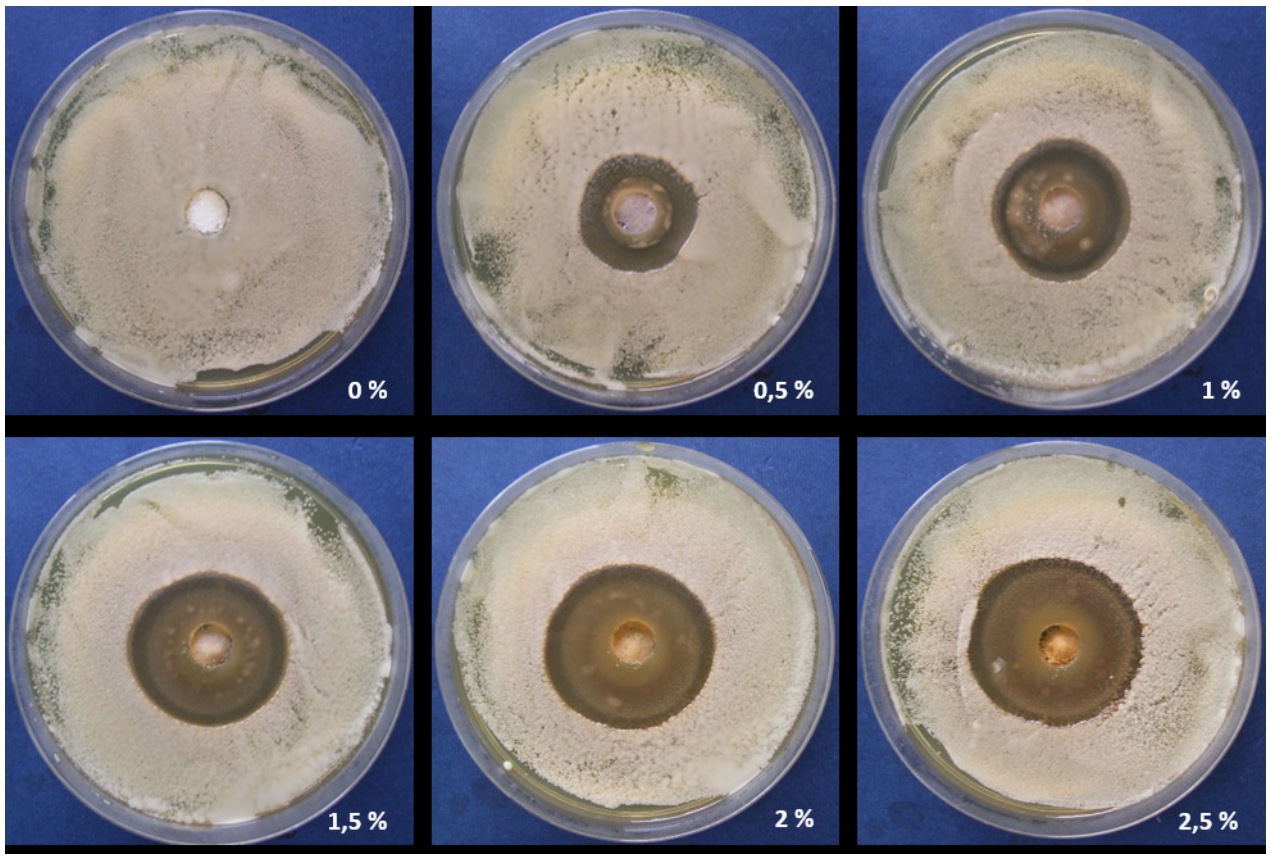

Fig. 4. Inhibition zones provided by SiO_2/HPMC hybrid materials of a varying silver concentration against *S. cerevisiae* 537 after 24 hours incubation at 30°C.

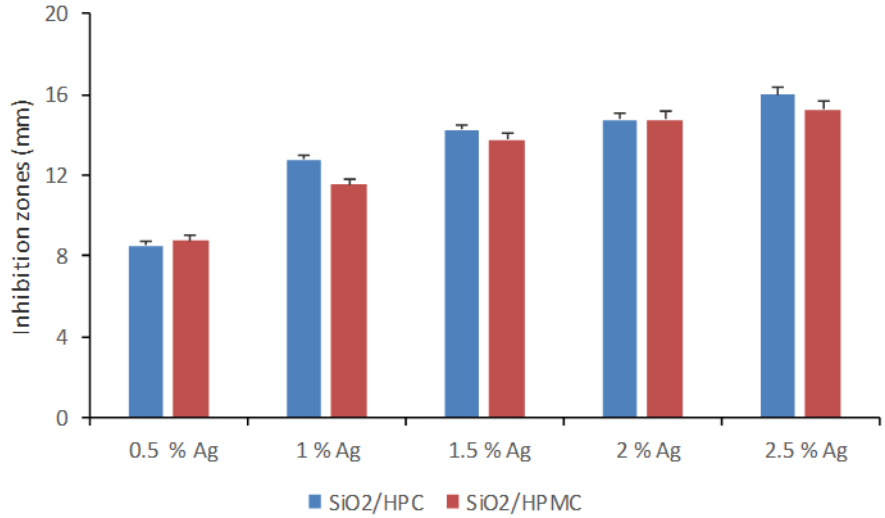

Fig. 5. A comparison between the inhibition zones formed around the hybrid materials of different silver concentrations in case of *S. cerevisiae* 537.

inhibitory zone of 8.75 mm diameter is detected in presence of 0.5 wt % of Ag, while that obtained in case of 2.5 wt % of Ag content has a diameter of 15.25 mm (Fig. 4).

Both hybrid materials show similar antimicrobial behavior towards the tested strain *S. cerevisiae* 537. Fig. 5 illustrates the comparison between the inhibition zones formed around the hybrid materials of different silver concentrations.

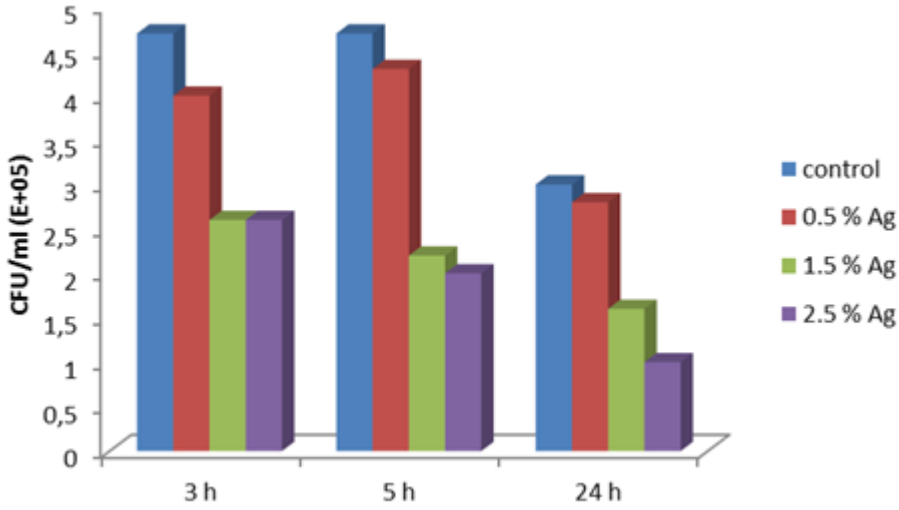

Fig. 6. Growth inhibiting effect of SiO_2/HPC hybrid materials of different silver concentrations in case of *S. cerevisiae* 537 after 24 h incubation.

The second microbiological test method used in this study reveals the hybrids antimicrobial activity based on the number of the surviving cells on the agar plates. Microbial inhibiting effect of the hybrid materials was further evaluated by studying the ability of the cells to survive after exposure to the tested hybrid materials of different silver concentrations. Fig. 6 presents the antimicrobial effect of SiO_2/HPC hybrid materials of a varying silver concentration on the number of surviving cells.

It is seen that the control sample and that of the lowest silver concentration (0.5 wt % of Ag) show a constant level in survived cells at the 3rd and 5th hour of cells incubation, i.e. this silver containing material has no effect on the viability of the strain tested as the cells were not inactivated. The samples containing 1.5 wt. % and 2.5 wt. % of Ag show the most pronounced level of inhibition after 24 h of cells incubation, namely 68% and 80%, respectively (Table 1).

Fig. 7 illustrates the antimicrobial effect of SiO_2/HPMC hybrid materials of a varying silver concentration on the tested cells viability.

These hybrid materials, similar to the SiO_2/HPC, containing 0.5 wt % of Ag show a constant level of the cells survived which coincides with that of the control sample. The most significant decrease of the cells number is observed by materials containing 1.5 wt % and

Table 1. Antimicrobial test results referring to *S. cerevisiae* 537 after 24 h cultivation in the presence of hybrid materials of different silver concentrations.

	SiO2/HPC		SiO2/HPMC	
Ag [wt %]	CFU/ml [E+05]	Reduction of cells [%]	CFU/ml [E+05]	Reduction of cells [%]
control	5	-	5	-
0.5	2.8	44	2.3	54
1.5	1.6	68	1.2	76
2.5	1	80	0.4	92

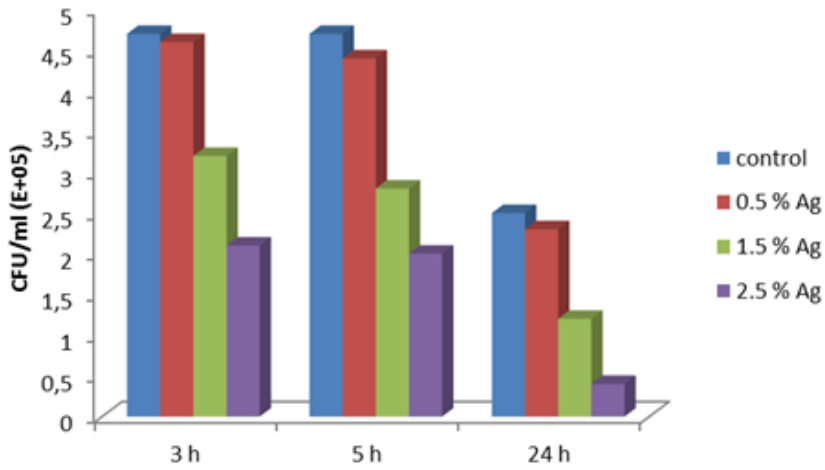

Fig. 7. Growth inhibiting effect of SiO_2/HPMC hybrid materials of different silver concentrations in case of *S. cerevisiae* 537 after 24 h incubation.

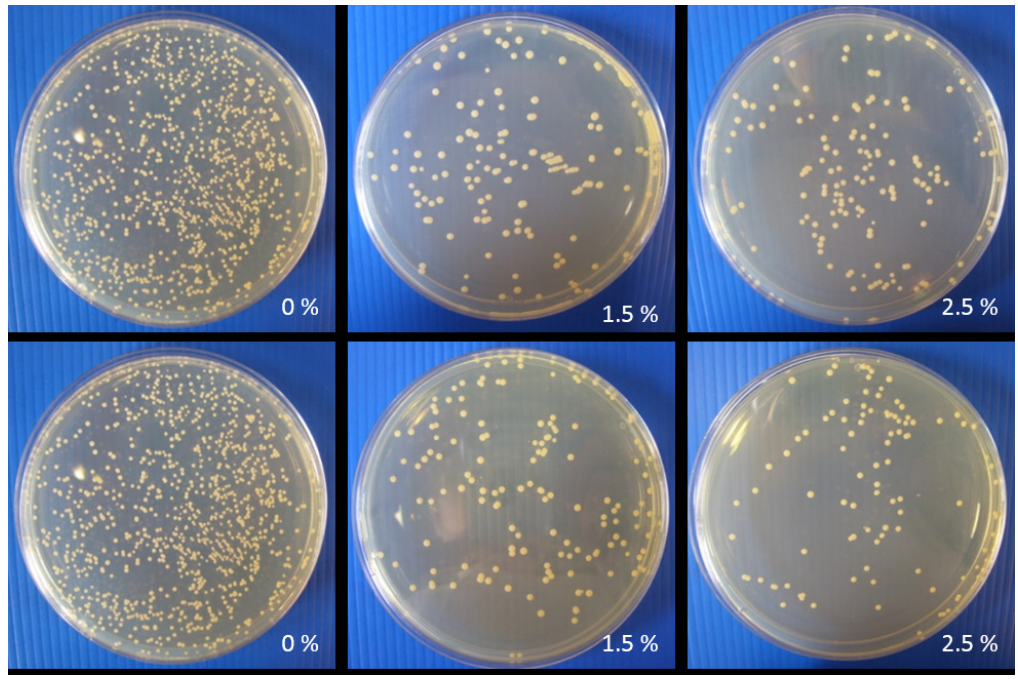

Fig. 8. Test results referring to *S. cerevisiae* 537 cultivated on solid YEPD after 24 h with SiO_2/HPC/Ag (on the upper panel) and SiO_2/HPMC/Ag (on the lower panel).

2.5 wt % Ag, where the decrease amounts to 76 % and 92 %, respectively (see Table 1).

Fig. 8 presents the test results referring to 24 h cultivation of *S. cerevisiae* 537 on solid YEPD in presence of hybrid materials SiO_2/HPC/Ag and SiO_2/HPMC/Ag of a varying silver concentration. It is worth noting that the decrease observed is close to that found in the study of the antimicrobial behavior of these hybrid materials towards *Candida albicans* 74 [10]. The cells number decrease by SiO_2/HPC/Ag containing 2.5 wt.% of Ag amounts to 88 %, while that found in presence of SiO_2/HPMC/Ag with an identical silver concentration is equal to 62.5 %. The lowest silver concentration of 0.5 wt. % Ag does not affect the tested strain cell viability. We have found that these hybrid materials affect more significantly the growth of *B. subtilis* and *E. coli* K12 strains [15]. In that case the decrease of the cell number of *B. subtilis* reached 100 % in presence of 1.5 wt %

of Ag, whereas the cell growth decrease by *E. coli* K12 was 95 %. The lowest silver concentration of 0.5 wt. % affected also bacterial growth and the decrease reached 75 % by *B. subtilis* and 53 % by *E. coli* K12. The results confirmed that the bacteria exhibited higher sensitivity to the hybrid materials than to the yeast. Silver (in its lowest concentration of 0.5 wt %) does not strongly affect the viability of yeast cells unlike the case with the prokaryotes cells. Many authors suggest that the yeast cells in the presence of silver ions and AgNps possess some kind of a defense mechanism protecting the growth processes [1].

CONCLUSIONS

Antimicrobial SiO_2/HPC and SiO_2/HPMC silver-doped hybrid materials were prepared by the sol-gel method. They showed high antimicrobial activities towards *Saccharomyces cerevisiae* 537 depending on the silver concentration present. The lowest silver content of 0.5 wt % does not affect yeast cells viability.

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