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Synthesis and characterization of hybrid materials with embedded silver nanoparticles and their application as antimicrobial matrices for waste water purification

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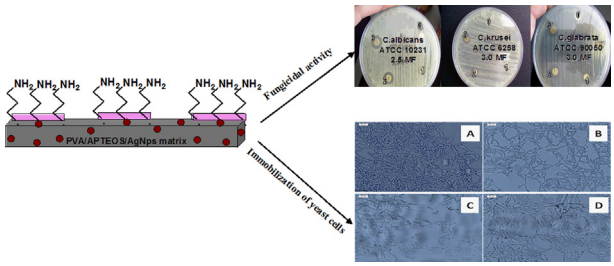
HIGHLIGHTS

- Novel PVA/APTEOS materials with embedded silver nanoparticles were prepared via sol–gel method.
- The fungicidal activity of PVA/APTEOS/AgNps against control and clinical strains was tested.
- The efficacy for removing Mn^{2+} ions from the medium by strain *Trichosporon cutaneum* R57 immobilized onto matrices was studied.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyvinyl alcohol/aminopropyltriethoxysilane hybrid materials with embedded silver nanoparticles (PVA/APTEOS/AgNps) have been synthesized for the first time using sol–gel method. The successful formation of silver nanoparticles embedded into PVA/APTEOS matrices has been proven by UV–vis and TEM analyses. The presence of fungicidal activity of thus prepared PVA/APTEOS/AgNps materials against different control and clinical strains has been established applying disk diffusion method (DDM). The hybrid materials were tested as matrices for immobilization of Trichosporon cutaneum R57 capable to remove Mn^{2+} from aqueous solutions. This strain showed considerable ability to remove Mn^{2+} ions from aqueous solutions at low silver nanoparticles content into the PVA/APTEOS matrices.

1. Introduction

The pollution with heavy metals represents a serious environmental problem and increasing attention to overcome this problem is paid nowadays. The presence of heavy metals in the environment even in a low concentration could cause different medical problems such as dermal damages, respiratory problems, and etc. [1,2]. There are several techniques employed for purification of waste waters such as precipitation [3], ionic exchange [4–6] ultra-filtration [7], and reverse osmosis [8]. One innovative and interesting approach is the use of biosorption potential of microbial biomass [9,10]. The presence of chemically active sites onto microbial biomass is responsible for sequestering metals from the surrounding solution [11]. The biosorption process offers several advantages over the traditional techniques as low cost, high efficiency of metal removal, withoutadditional nutrient requirements, and regeneration of biosorbent [12]. The use of appropriate matrices leads to good removal efficiency of different toxic heavy metals [13,14].

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Therefore, organic–inorganic hybrid materials are considered as suitable materials for the adsorption of heavy metals from aqueous solutions. Hybrid materials for immobilization of microorganisms based on polyvinyl alcohol and different silanes with proven removal efficiency of heavy metals have already been applied [15,16]. However, there are no data in the literature reporting the use of hybrid materials with embedding silver nanoparticles as matrices for immobilization of microorganisms for treatment of waste water. It is well known that silver nanoparticles (AgNps) demonstrated high antimicrobial activity against bacteria, viruses, and fungi [17–20]. Some studies have shown that the biofilms used for biologic waste water treatment processes are more tolerant to antimicrobial agents such as silver than to the planctonic bacteria [21,22]. The high tolerance to AgNps is due to physical protections in the biofilms, microbial community interactions in the biofilms, and the slow growth rate of certain microorganisms in the biofilms which plays important role in controlling the antimicrobial effects of AgNPs.

The aim of the present work is to prepare hybrid materials based on PVA/APTEOS with embedded silver nanoparticles and to investigate the presence of fungicidal activity as well as their efficacy for removing Mn^{2+} ions from the medium by strain *Trichosporon cutaneum* R57 immobilized onto matrices.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA) (Sigma–Aldrich; 87–88% hydrolyzed, Mw = 13,000–23,000 mol^{-1}); HNO_3 (Riedel de Haën, Standard solution 2 mol/L); silver nitrate (Acros Organics); and aminopropyltriethoxysilane (APTEOS) (Fluka) were used as received without further purification.

The etalon strains *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* B 6030413, and *Candida glabrata* ATCC 90050 were received from the collection of "laboratory for control of *in vitro* diagnostic medical devices" at Bul Bio-NCIPD, Bulgaria. The perti dishes with MHA agar and disks from chromatographic paper "Munktell" were produced by Bul Bio-NCIPD. The fungicidal activity was also studied with clinical strains isolated from patients with urinary track infections. (*C. glabrata* 8–122, *C. albicans* 8–127, *C. albicans* 8–137, *C. krusei* 8–126). The used clinical strains were kindly obtained from the reference microbiologic laboratory "Mycosis" to NCIPD, Bulgaria. Their sensitivity was tested with the trade kit ATB Fungus 3 "Bio Merieux" with five included antimycotics such as flucytosin (5FC), amphotericin B (AMB), fluconazol (FCA), itraconazol (ITR), voriconazol (VRC).

Filamentous yeast strain *Trichosporon cutaneum* R 57 maintained in the culture collection of Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N2414 was used in this study. The cultivation was carried out in a medium under conditions described elsewhere [23].

2.2. Methods

Transmission electron microscopy (TEM) images were recorded on a FEG-TEM (Phillips CM 200 field emission gun TEM). Samples were prepared by placing a drop of the precursor solutions on carbon-coated grids and dried under air at room temperature. TEM observations of the films were performed after annealing of the coated grids at 100 °C for 1 h.

UV–vis absorption spectra of the hybrid films were recorded at room temperature in the wavelength range from 200 to 800 nm using PerkinElmer spectrophotometer. IR spectra of the films were recorded in transmittance mode in the range from 400 to 4000 cm^{-1} using PerkinElmer FTIR.

Manganese ions concentration in the medium was measured by inductively coupled plasma mass spectroscopy (ICP-MS) of Leeman Labs. After supplying manganese ions to the cultivated strain, samples for ICP analysis were taken at every 30, 60, and 120 min. Prior to analysis, all samples were centrifuged by 8000 g for 10 min, and the solid and liquid phases were separated.

For microscope observations, the cells were washed twice with distilled water, stained with 2% solution of methylene blue for 20 min at room temperature, washed again with distilled water, and dried for 24 h at 37 °C. Further, the samples were analyzed by using bright field microscope Olympus BX53, Camera SC30 (Japan).

2.3. Synthesis of PVA/APTEOS/AgNps hybrid materials via thermal annealing of the films.

5 g of polyvinyl alcohol (PVA) was dissolved in 95 mL deionized water while heating for 20 min at 80 °C. Different amounts of silver nitrate (0.01, 0.025, and 0.05 g dissolved in 0.5 mL (water) were added to 18 mL PVA (5%). The silica sol was produced by hydrolyzing APTEOS (0.93 mL) in acidified water (0.93 mL) using HNO_3 as a catalyst to yield APTEOS/H_2O/HNO_3 volume ration equal to 1/1/0.1. The mixture was stirred until a clear solution is obtained and subsequently added drop-wise to the PVA/$AgNO_3$ solution thus achieving final concentration of silver nitrate in the solution equal to: 0.5, 1.2, and 2.5 mg/mL. The final mixture was stirred for 80 min and then cast into a film. The films were dried for 3 days at room temperature in dark place. Furthermore, thermal annealing was performed for 1 h at 100 °C leading to formation of silver nanoparticles (AgNps) in PVA/APTEOS matrix. The color of the annealed films depending on the Ag concentration varied from dark yellow to brown.

2.4. Measuring of the fungicidal activity of the films by disk diffusion method (DDM) [24,25]

The disks from chromatographic paper (d = 6 mm) were impregnated with 5 μL of the solutions prepared following the procedures mentioned above and allowing them to dry for three days at room temperature. Further, disks were annealed at 100 °C for 1 h. These disks were tested against *Candida* etalon and clinical *Candida* strains using DDM.

The Mueller-Hinton agar (MHA) was chosen for routine susceptibility testing of yeasts, standardized to 3MF density of the suspension. A control sample of impregnated disks with PVA/APETOS solution without silver nanoparticles under the same conditions was also prepared. Each plate was examined after 20–24 h of incubation. Then, monitoring of the inhibition zones was performed and determined. When the growth was insufficient after 24 h incubation, the results were collected after 48 h incubation according to CLSI M44-A. The clinical strains used were proven with resistance towards at least two routine tested in the clinical practice antimycotics.

2.5. Cells immobilization and biosorption experiments

The biosorption of manganese ions was performed in a batch system as described previously [15,16]. The hybrid matrices for biosorption experiment were prepared as 10 μL of the PVA/APTEOS/AgNps solutions were cast onto cover glasses (18 × 18) and dried for 48 h. The immobilization of cells by attachment was carried out at the 6th hour (log phase) of the strain cultivation when the hybrid materials were added to 100 mL of culture medium. In order to study the biosorption by free and immobilized cells, the manganese ions were supplied to the cultivated strain at the 24th hour of cultivation in concentrations of 5