IMMOBILIZATION OF TRICHOSPORON CUTANEUM R57 ON PVA/TEOS/MPTEOS HYBRID MATRICES FOR REMOVAL OF MANGANESE IONS

Nelly Georgieva, Rayna Bryaskova, Nevena Lazarova, Ralena Racheva
University of Chemical Technology and Metallurgy, Department of Biotechnology, Sofia, Bulgaria
Correspondence to: Nelly Georgieva
E-mail: nelly.georgieva@yahoo.com

ABSTRACT
New hybrid materials based on polyvinyl alcohol (PVA), 3-mercaptopropyltriethoxysilane (MPTEOS) and tetraethoxysilane (TEOS) were prepared, using a sol-gel process. Thermogravimetric analysis (TGA) was performed to determine the thermal stability of the hybrid materials. MPTEOS, as a precursor, ensured the presence of SH-groups, thus providing additional binding places for cell immobilization. Based on good adhesive behavior, the hybrid materials were tested as matrices for immobilization of Trichosporon cutaneum R57 for removal of manganese ions from aqueous solutions. Maximum Mn2+ removal was achieved by cells immobilized onto materials with higher MPTEOS concentration. The synthesized PVA/TEOS/MPTEOS hybrid materials proved to be efficient for use in biosorption applications.

Introduction
Manganese is one of the essential metals for all microorganisms and has been implicated in the regulation of the growth and metabolism in fungi as well as in bacteria, mainly due to its influence on several key enzymes (4, 6). However, excess amounts of the same metal ions are toxic and can cause damage to the function that they serve (18). The main sources of environmental heavy metal pollution are the industry, agricultural use and sewage sludge. The increase in industrial activities intensified environmental pollution problems and the deterioration of ecosystems with the accumulation of toxic metals (24). The search for alternative and innovative treatment techniques has focused on the use of biological materials such as algae, fungi, yeast and bacteria for removal and recovery technologies and has gained importance in recent years because of the better performance and low cost of these biological materials (17, 21, 22). Other added advantages of biological clean-up techniques are the flexibility to handle a range of physicochemical parameters in effluents, the selectivity to remove only the desired metals and the cost-effectiveness (16). Bioremediation is the use of biological systems, almost invariably microorganisms, to clean up a contaminated site (3). Microbes can remove heavy metals from contaminated solutions by bioaccumulation, precipitation, or biosorption (3, 14, 15, 16, 19). Immobilization of biosorbents is a key aspect in the application of biosorption (5, 20). In this regard, the use of sol–gel processes provides a new class of materials which are suitable for immobilization of biomolecules (23). The incorporation of inorganic matrices into organic matrices ensures physical rigidity, photophysical and thermal stability of the obtained hybrid materials (11, 13). Polyvinyl alcohol (PVA) is a good organic matrix due to the large amount of hydroxyl groups which are appropriate for immobilization of different biomolecules. Therefore, the preparation of hybrid materials based on PVA and different silanes with appropriate functional groups is of great importance for successful immobilization (1). MPTEOS (3-mercaptopropyltriethoxysilane) is an organosilane with SH groups that can provide additional adsorption sites and increased efficiency of heavy metal removal (12). The high potential of Trichosporon cutaneum strain R57 for heavy metal (copper, cadmium and chromium) removal from contaminated waters was shown in our previous studies (7, 8, 9, 10). To the best of our knowledge, there are no published reports about preparation of hybrid materials based on PVA, TEOS and MPTEOS in aqueous solutions. The aim of the present work was to investigate the efficacy of removing Mn2+ ions from a liquid medium by strain R57 immobilized on the novel hybrid materials based on PVA/TEOS/MPTEOS.

Keywords: PVA, MPTEOS, sol-gel, Trichosporon cutaneum, manganese
Abbreviations: ICP-MS: inductively coupled plasma mass spectroscopy; PVA: MPTEOS: 3-mercaptopropyltriethoxysilane; polyvinyl alcohol; TEOS: tetraethoxysilane; TGA: thermogravimetric analysis

Materials and Methods
Polyvinyl alcohol (PVA) (Sigma–Aldrich; 87 %–88 % hydrolyzed, Mw = 13000 mol−1–23000 mol−1); HNO3 (Riedel de Haën, Standard solution 2 mol/L); tetraethyl orthosilicate (TEOS) (Fluka); and 3-mercaptopropyltriethoxysilane (MPTEOS), were used as received without further purification.

Microorganisms and growth conditions
Filamentous yeast strain Trichosporon cutaneum R 57 maintained in the culture collection of the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N2414 was used in this study. The cultivation medium and conditions were as previously described (7).
Hybrid materials preparation

PVA (5 g) was dissolved in 95 mL of deionized water while heating for 20 min at 80 °C. The silica sol was produced by partially hydrolyzing TEOS (0.37 mL) in acidified water (0.37 mL), using HNO₃, as a catalyst to yield a TEOS/H₂O/HNO₃ volume ratio of 1:1.0:1. Then, different amounts of MPTEOS were added (0.2 mL, 0.4 mL, and 0.6 mL) and stirred for additional 4 h. The obtained solutions were added drop-wise to the PVA solution (19.2 mL). The final mixtures were stirred for 80 min and then cast into films which were dried for 3 days at room temperature. The PVA/TEOS and PVA/TEOS/MPTEOS films were thermally annealed for 1 h at 100 °C for the formation of PVA/TEOS and PVA/TEOS/MPTEOS matrices which were denoted as PVA/TEOS/MPTEOS 1; 2 and 3.

Cell immobilization and biosorption experiments

The biosorption of manganese ions was performed in a batch system as described previously (7). The hybrid matrices for the biosorption experiment were prepared by casting 10 µL of the solutions onto cover glasses (18×18) and dried for 48 h followed by annealing at 100 °C for 1 h. The immobilization of cells by attachment was carried out at the 6th h (log phase) of the cultivation process, 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 h of the cultivation process, i.e. the stationary phase, at concentrations of 5 mmol/L and 10 mmol/L MnSO₄·H₂O. The cultivation experiments were carried out at 30 °C.

Analytical measurements

The structural groups of obtained hybrid materials were determined by Fourier Transform infrared (FT-IR) spectroscopy with a MATSON 7000 Fourier Transforming Infra-Red spectrometer using KBr tablets. TGA-DTA curves were obtained in air from 25 °C to 700 °C at a heating rate of 10 °C/min, using SETERAAM, Labsys 1 EVO 1600°C DTA-TGA equipment.

The manganese ion concentration in the medium was measured by inductively coupled plasma mass spectroscopy (ICP-MS) Leeman Labs. After supplying manganese ions to the cultivated strain, samples for ICP analysis were taken every 30 min, 60 min and 120 min. Prior to analysis, all samples were centrifuged at 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 with a bright-field microscope Olympus BX53, Camera SC30 (Japan).

Results and Discussion

The synthesis of hybrid materials on the basis of PVA, TEOS and MPTEOS was performed using a sol-gel process in aqueous solutions. The organoalkoxysilanes (TEOS and MPTEOS) were hydrolyzed in the presence of an acid catalyst. During the hydrolysis process, the alkoxy groups (OR) were substituted with hydroxyl groups (-OH), followed by a polycondensation process. After the casting of the film, polycondensation proceeds, in which the subsequent Si-O-Si bonds are formed and alcohol or water is released depending on the degree of hydrolysis. The hydroxyl and thiol groups in the silanols can form hydrogen or covalent bonds with the hydroxyl groups arising from the PVA chains during the polycondensation process and subsequent annealing of the films.

Thus prepared PVA/TEOS and PVA/TEOS/MPTEOS hybrid materials were characterized by FT-IR spectroscopy (Fig. 1). In all cases, a broad absorption peak centered at around 3300 cm⁻¹ was observed and it is attributed to the O-H stretching vibration of hydrogen bonded hydroxyl groups arising from the main PVA chain as a result of intramolecular and intermolecular hydrogen bondings. A strong absorption peak observed at 1730 cm⁻¹ is characteristic for carbonyl groups arising from the acetate groups in the partially hydrolyzed PVA. The peaks at 1430 cm⁻¹ and 1326 cm⁻¹ are typical for the O-H groups and C-H deformation vibration respectively in PVA and are present in all spectra. The absorption peak at (1090–1020) cm⁻¹ can be assigned to the C-O stretching and O-H bending vibrations arising from the PVA chain. In the same region, the asymmetric stretching vibration due to the Si-O-Si linkage as a result of the condensation reaction between hydrolyzed silanol Si-OH groups is also presented. The Si-O-C bonds, which can be formed as a result of chemical crosslinking of silanol groups (Si-OH) and -OH groups arising from PVA during the thermal annealing of the films, appear with a peak in the range of (1100–1000) cm⁻¹ and it is overlapped with the absorption interval of Si-O-Si bonds. At PVA/TEOS/MPTEOS film among several peaks, a peak at 2560 cm⁻¹, which is characteristic for thiol (-SH) groups arising from MPTEOS, is observed (Fig. 1) (2).

![Fig. 1. FT-IR spectra of PVA/TEOS films; PVA/TEOS/MPTEOS films at the highest MPTEOS concentration.](image)
The TGA curves of all tested hybrid materials show the presence of few peaks. The small peaks at (100–150) °C are associated with the elimination of water, which is a result of a polycondensation process. Thermal degradation of the organic fraction was observed between 300 °C and 600 °C, with two main degradation peaks. The peak at 360 °C is a result of PV A side-chain elimination or of the loss of mercaptopropyl groups at PV A/TEOS/MPTEOS hybrid materials. The peaks at (400–460) °C are originated from degradation of the main PV A chain. The increased content of MPTEOS into the hybrid materials lead to higher solid content.

For industrial scale applications, it is very important to immobilize the microbial cells on an inert carrier while maintaining their activity. Further, the synthesized PV A/TEOS/MPTEOS hybrid materials were applied as matrices for immobilization of the filamentous yeast Trichosporon cutaneum R57. The high potential of this strain to remove copper, cadmium, manganese and chromium ions from contaminated waters was demonstrated in previous studies (7, 8, 9, 10). In our preliminary experiments, it was established that a concentration of 5 mmol/L MnSO4 can be considered as an inhibitory threshold value and a concentration of 10 mmol/L MnSO4 as a moderately inhibitory value, since an abnormal morphology was observed. In this study, both concentrations of MnSO4 = 5 mmol/L and 10 mmol/L, were used. The microscopic observations of immobilized cells are shown in Fig. 3. In the presence of manganese ions, the cells were well distributed onto the hybrid materials with morphology typical for the strain. The microscopic observations also showed that the attached cells are more tightly distributed in the PV A/TEOS/MPTEOS-3 matrices (Fig. 3D) than in the control matrices PV A/TEOS (Fig. 3A). This indicates that the presence of SH-groups in the PV A/TEOS/MPTEOS hybrid matrices leads to higher cell immobilization which can influence the Mn2+ sorption capacity of the immobilized cells. Fig. 4 shows the removal efficiency of strain R57 immobilized onto hybrid materials in the presence of manganese ions. The removal effect was more prominent with higher MPTEOS content, where the strain showed 18 % removal capacity compared to 16 % for the control PV A/TEOS matrices. This could be explained with the incorporation of MPTEOS into the hybrid matrices, which posses -SH functional groups, thus providing additional adsorption sites in the matrix through the PVA functionalities and some affinity of Mn2+ ions to these functional groups. The introduction of additional -SH groups into the system through modification of the PVA surface in order to increase the sorption capacity of the used hybrid materials has also been reported for adsorbents of Hg2+ and Ni2+ from industrial sewage (12).

We demonstrated increasing of biosorption capacity of the investigated strain immobilized onto PV A/TEOS/MPTEOS hybrid materials, due to incorporation of additional adsorption sites in the matrix. This trend indicates additional benefits from the immobilization of biomass on the synthesized matrices, considering its application for recovery of waste waters contaminated with Mn2+ ions. The immobilization by attachment to the surface of the synthesized hybrid materials reduces the inhibition of the cells by the heavy metal ions, thus increasing their potential for application in Mn2+ contaminated waste water recovery. The biosorption process includes physicochemical interactions between metal ions and several anionic ligands present on the biomass such as carboxyl, phosphoryl, carbonyl and sulphydryl groups. Our results
clearly supported the benefit of incorporating strain R57 cells into the studied matrices for removing manganese ions from waste waters. Thus, this study may be regarded as an initial step for the development of technologies for bioremediation of waste water polluted with heavy metals.

**Conclusions**

New PVA-hybrid materials functionalized with -SH groups were successfully used as matrices for immobilization of *Tr. cutaneum* R57. Maximum Mn²⁺ removal efficiency was obtained with immobilized cells onto polymer matrices with higher MPTEOS content. The obtained encouraging results on enhancing the biosorption capacity of the investigated cells, the relatively high specific surface, physicochemical properties and mechanical stability of the synthesized hybrid materials highlight the potential benefits from their industrial-scale application for bioremediation of Mn²⁺ contaminated waste waters.

**Acknowledgements**

This work was supported by the National Science Fund (Ministry of Education, Youth and Science of the Republic of Bulgaria) grant DDVU 02-96/2010.

**REFERENCES**