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PVA-BASED HYBRID MATERIALS FOR IMMOBILIZATION OF *TRICHOSPORON CUTANEUM* R57 EFFICIENT IN REMOVAL OF CHROMIUM IONS

Nelly Georgieva, Rayna Bryaskova, Nevena Lazarova, Dimitar Peshev, Rumiana Tzoneva*

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Abstract

New hybrid materials based on polyvinyl alcohol (PVA) and γ-aminopropyltriethoxyxilane (APTEOS), 3-mercaptopropyltriethoxysilane (MPTEOS) and tetraethoxysilane (TEOS) were prepared. To determine the thermal stability of the hybrid materials, thermogravimetric analysis (TGA) was performed. The new materials were tested for biofilm formation and biosorption studies. They were tested as matrices for immobilization of *Trichosporon cutaneum* R57 capable to remove Cr(VI) from aqueous solutions. The use of APTEOS and MPTEOS as precursors, incorporated new NH– and SH– bonds, which ensured additional binding places for cells immobilization. The presence of SH– and NH– groups in the hybrid matrices resulted in apparently higher Cr(VI) sorption capacity of the immobilized *Tr. cutaneum* R57 cells due to the incorporation of additional adsorption sites in the matrix through the PVA functionalities. The synthesized PVA/APTEOS materials proved efficient for use in biosorption applications.

Key words: polyvinyl alcohol, silica, *Trichosporon cutaneum* R57, chromium

1. Introduction. The chromium emissions and its high toxicity are major concerns in the development of new environmentally safety technologies. The search for new and innovative technology for the remediation of Cr(VI) pollution

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has attracted the attention on the bioremediation potential of microorganisms. The use of microbial biomass for the removal of chromium from industrial waste waters has already been recognized [1–3]. Chromium resistance has been described for bacteria and fungi. The mechanism of chromate resistance is related to limited ion uptake and capability to accumulate Cr ions. The microbial biomass contains chemically active sites that are responsible for sequestering metals from the surrounding solution [4, 5]. To increase the number of chemically active sites and to use this process for industrial scale waste water purification, it is important to immobilize the biomass onto suitable type of matrices [6–7].

In this respect, hybrid organic – inorganic materials attract considerable attention because of their excellent mechanical and thermal properties, chemical reactivity and the presence of specific organic functional groups allowing their modification and application in various fields [8, 9]. Different organic – inorganic hybrid materials have been synthesized and applied as matrices for waste water purification [10–12]. Using polyvinyl alcohol (PVA) as an organic component in the hybrid materials is very attractive because of its excellent chemical and physical properties, easy processing technique and low cytotoxicity. The main PVA disadvantage is its high sensitivity towards moisture thus reducing the film strength which is undesirable for biomedical applications [13–17]. This can be overcome by incorporation of networked silica using precursors such as tetraethyl orthosilicate (TEOS), γ-aminopropyltriethoxysilane (APTEOS) and 3-mercaptopropyl triethoxysilane (MPTEOS) via the sol-gel method into PVA matrix. Amino functional mesoporous silica materials were used as efficient adsorbents of heavy metals such as Cu(II), Cd(II) and Ni(II) in waste water [18]. Recently, we synthesized novel hybrid materials based on PVA and different organosilanes [19].

The aim of the present work was to investigate the newly developed PVA-based hybrid materials for their application in biotechnology. In this respect we studied how the chemical composition and the presence of different functional groups as hydroxyl (–OH), thiol (–SH) and amino ($–NH_2$) of PVA-hybrid materials influence the adhesive behaviour of *Trichosporon cutaneum* strain R57. The efficacy of removing Cr(VI) from medium by strain R57 immobilized on the hybrid materials using kinetic model is also investigated.

2. Experimental. ***2.1. Materials.*** Polyvinyl alcohol (PVA) (Sigma – Aldrich; 87–88% hydrolyzed, Mw = 13000 – 23000 mol^{-1}); HNO_3 (Riedel de Haën, Standard solution 2 mol/L); Tetraethyl orthosilicate (TEOS) (Fluka), γ-aminopropyltriethoxyxilane (APTEOS) and 3-mercaptopropyltriethoxysilane (MPTEOS) were used as received without further purification.

2.2. Synthesis of PVA based hybrid materials. 2.2.1. Synthesis of PVA/TEOS hybrid matrix. 5 g of polyvinyl alcohol (PVA) was dissolved in 95 mL deionized water while heating for 20 min at 80 °C. The silica sol was produced by hydrolyzing partially TEOS (0.93 mL) in acidified water (0.93 mL) using HNO_3 as a catalyst to yield $TEOS/H_2O/HNO_3$ volume ration equal to 1/1/0.1.

The mixture was stirred until a clear solution was obtained and subsequently added drop-wise to the PVA solution (25 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. Further thermal annealing was performed for 1 h at 100 °C leading to the formation of PVA/TEOS matrix.

2.2.2. Synthesis of PVA/APTEOS hybrid matrix. 5 g of polyvinyl alcohol (PVA) was dissolved in 95 mL deionized water while heating for 20 min at 80 °C. Then to acidify with HCl until pH = 2, PVA solution (19.6 mL) was added 0.4 mL APTEOS. The mixture was stirred for 10 h and then cast into a film. The films were dried for 3 days at room temperature.

2.2.3. Synthesis of PVA/MPTEOS hybrid matrix. 5 g of polyvinyl alcohol (PVA) was dissolved in 95 mL deionized water while heating for 20 min at 80 °C. The silica sol was produced by hydrolyzing partially MPTEOS (0.93 mL) in acidified water (0.93 mL) using HNO_3 as a catalyst to yield MPTEOS/H_2O/HNO_3 volume ration equal to 1/1/0.1. The mixture was stirred for 40 min added drop-wise to the PVA solution (25 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.

2.3. Microorganism and media. Filamentous yeast strain *Trichosporon cutaneum* R57, maintained in the culture collection of the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N2414 was used in this study. Details about the cultivation media can be found elsewhere [20]. Inoculums were transferred to 300 mL flasks with 100 mL liquid working volume and the cultivation process on the rotary shaker was performed at 30 °C for 48 h.

2.4. Biosorption experiments. The biosorption tests were carried out by using inoculums with cell density of 1.5×10^6 cells mL^{-1}. The hybrid matrices for biosorption experiment were prepared as 10 µL of the solutions cast onto cover glasses (18 × 18) and dried for 48 h. At the 6th h of the strain cultivation (log phase), the hybrid matrix on cover glasses (18/18 mm) was added to 100 mL of the culture medium for cell immobilization. The strain was cultivated in the presence of various concentrations of chromium ions. Potassium dichromate ($K_2Cr_2O_7$) solution was used as a source of hexavalent chromium Cr (VI) in concentrations of 0.2 mM and 1 mM $K_2Cr_2O_7$. The chromium ions were added to the culture broth at 24th h corresponding to the stationary phase of the cultivation process. The heavy metals biosorption experiments were performed under vigorous stirring in order to eliminate the impact of the external diffusion in duration of 120 min. The experimental data for the concentration of residual Cr (VI) in the liquid phase at a given time were represented in terms of the specific heavy metal uptake $q = V(C_0 - C)/m$, where q is the quantity of the ions adsorbed by a given quantity of biosorbent (mg g^{-1}) in time t (min); V is the volume of the liquid phase (L); C is the concentration of metal ions, mg L^{-1}; C_0 is the initial concentration of metal ions, mg L^{-1} and m is the biosorbent dry weight (g). The kinetic data (q versus t) were used to determine the parameters in a pseudo-second order kinetic

model (Eq. (1)) [19] for each type of the immobilization matrix and initial Cr (VI) concentration

$$\frac{t}{q} = \frac{1}{k_{\text{ads}} q_{\text{eq}}^2} + \frac{1}{q_{\text{eq}}} t. \tag{1}$$

The values for the equilibrium Cr (VI) uptake $q_{\text{eq}}^{\text{calc}}$ and the adsorption rate constant k_{ads} (g mg^{-1} min^{-1}) were determined from the slope and the intercept of the plot t/q versus t based on the experimental data. The equilibrium parameter q_{eq}, which is characteristic for the specific uptake capacity (i.e. the efficiency) of the biosorbent and the rate constant, provides tools for the process design and scale up.

2.5. Crystal violet staining. The filamentous yeast strain *Trichosporon cutaneum* R57 was left to adhere on the materials. After 24-hour incubation the adhered cells were washed once with PBS (phosphate buffered saline), pH 7.4, and were fixed with 3% solution of PFA (paraformaldehyde) for 20 min at room temperature. The cells were washed twice with distilled water, stained with 0.1% solution of crystal violet (St. Louis, Missouri, USA) for 20 min at room temperature, washed again with distilled water and dried for 24 h at 37 °C. The samples were installed on objective glasses by Mowiol. Preparations were analyzed using inverted fluorescent microscope (Leica DMI3000 B, Leica Microsystems GmbH, Germany).

2.6. Analysis. IR spectra of the films were recorded in transmittance mode in the range from 400 to 4000 cm^{-1} using Perkin Elmer FTIR. The IR spectra of immobilized cells of *Tr. cutaneum* R57 before and after biosorption treatment were also obtained in the range 4000–400 cm^{-1} in KBr pellets. TGA–DTA curves were obtained in air conditions from 25 °C to 700 °C at heating rate 10 °C/min using SETERAAM, Labsys 1 EVO 1600 °C DTA–TGA instrument. Determination of chromium ions was done using inductively coupled plasma mass spectroscopy (ICP–MS) Leeman Labs. Prior to analysis all samples were centrifuged by 8000 g for 10 min and the solid and liquid phases were separated.

3. Results and discussion. ***3.1. Thermal behaviour of PVA based hybrid materials.*** The synthesis of hybrid materials on the basis of PVA and organoalkoxysilanes such as TEOS, APTEOS and MPTEOS was carried out via aqueous routes as described elsewhere [19]. The organoalkoxysilanes (TEOS, APTEOS and MPTEOS) were hydrolyzed in the presence of acid catalyst and substitution of alkoxy groups (OR) with hydroxyl groups (–OH) took place, leading to formation of silanol groups. After the casting of the film, a polycondensation process proceeded, in which the subsequent Si-O-Si bonds were formed and alcohol or water was released depending on the degree of hydrolysis.

The thermal behaviour of the matrices used in biotechnology is of notable importance. For this purpose, TGA–DTA analysis was applied by heating the

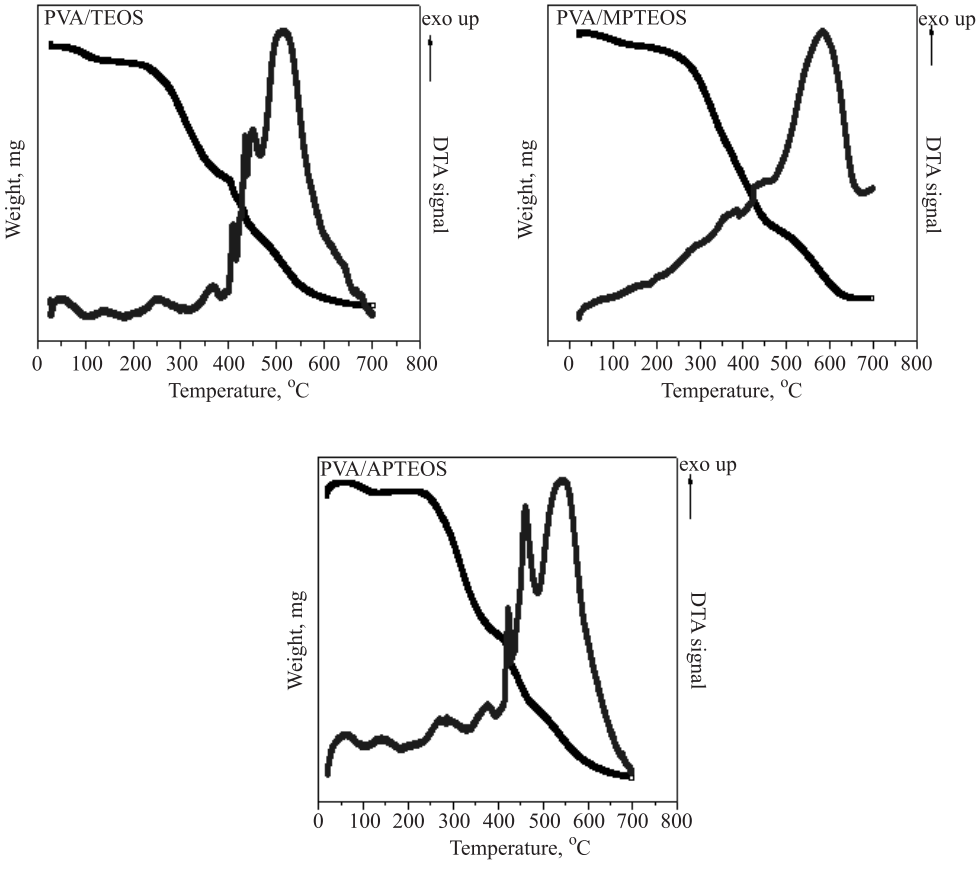

Fig. 1. TGA–DTA curves for PVA/TEOS, PVA/APTEOS and PVA/MPTEOS

hybrid material in air until 700 °C. The obtained thermal curves are presented in Fig. 1.

The TGA–DTA curves of all tested hybrid materials showed the presence of few peaks. The small peaks at 100–150 °C were associated with the elimination of water, which was a result of polycondesation process. The thermal degradation of the organic fraction took place in the range 300–600 °C where two main degradation peaks were observed. The peak at 360 °C was due to the PVA side chain elimination or to the loss of mercaptopropyl or aminopropyl groups at PVA/MPTEOS and PVA/APTEOS hybrid materials. The peaks at 400–460 °C originated from degradation of the main PVA chain. The strong exothermic peak at 530–570 °C was associated with some crosslinking reactions occurring in PVA chains.

3.2. Immobilization of yeast strain and biosorption of Cr (VI). The synthesized PVA-based hybrid materials were applied as matrices for immobi-

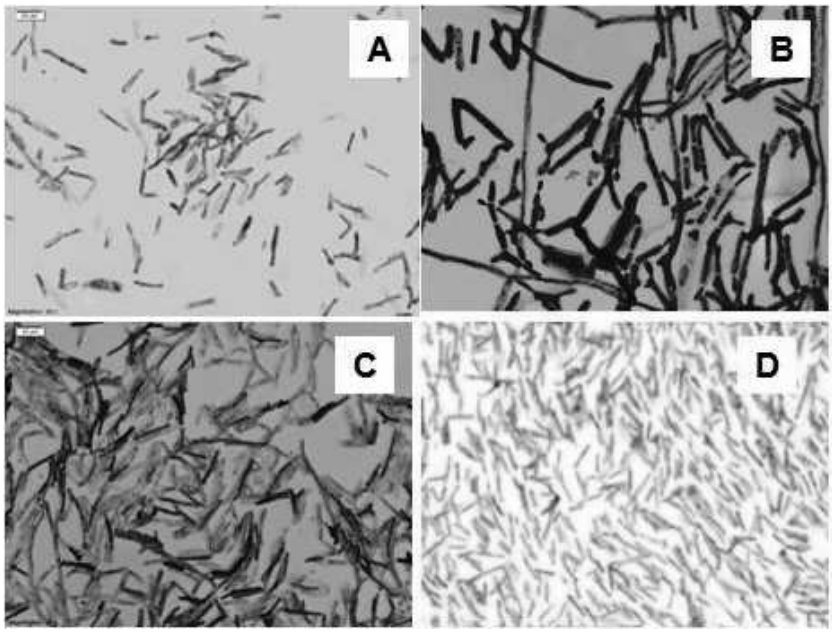

Fig. 2. *Tr. cutaneum* R57 immobilized on: glass (A), on PVA/TEOS (B), on PVA/APTEOS (C), PVA/MPTEOS (D), Bar is 20 µm

lization of filamentous yeast *Trichosporon cutaneum* strain R57. The microscope observation showed that the yeast cells are uniformly distributed onto carriers (Fig. 2).

The added concentration of 0.2 mM $K_2Cr_2O_7$ could be considered as an inhibitory threshold value since it changed the duration of growth stages compared to controls, and concentration of 1 mM $K_2Cr_2O_7$ was a moderate inhibitory value, since an abnormal morphology with short, thickened hyphae was observed (Fig. 3).

Infrared spectra of biomass without chromium and biomass loaded with 1 mM Cr and 1.5 mM Cr are presented in Fig. 4. It is well known that the cell wall consists of different functional groups which can provide binding sites for the metal ions. These functional groups are carboxyl (–COOH), phosphate (PO_4^{3-}), amine (–NH_2) and hydroxyl (–OH). The presented FTIR spectra confirm their existence. The presence of a broad absorption at around 3400 cm^{-1}, which is characteristic for –OH group and amino groups stretching vibrations, was observed in all spectra. The peak characteristic for amino group at 1640–1650 cm^{-1} was also detected. Slight shifting of the peak from 1646 cm^{-1} to 1656 cm^{-1} was observed with increasing the concentration of Cr ions. Similar observation was detected for the peak typical for the hydroxyl group from 3415 to 3400 cm^{-1}. The phosphate groups show some characteristic adsorption peaks at 1150, 1070 and 1035 cm^{-1} representing P=O, P–O–C and P–OH stretching vibrations. The

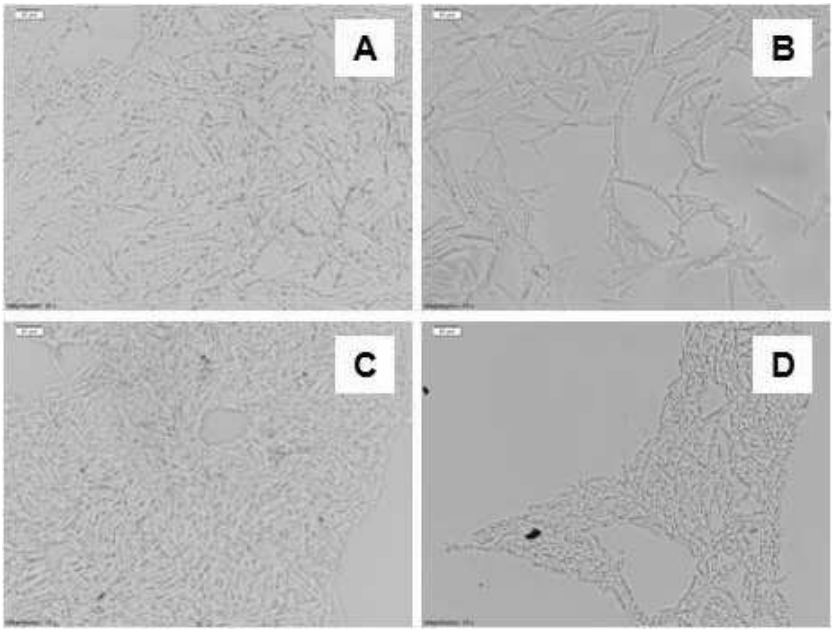

Fig. 3. *Tr. cutaneum* R57 immobilized on: PVA/TEOS in the presence of 0.2 mM $K_2Cr_2O_7$ (A) and 1 mM $K_2Cr_2O_7$ (B), PVA/APTEOS in the presence of 0.2 mM $K_2Cr_2O_7$ (C) and 1 mM $K_2Cr_2O_7$ (D), Bar is 20 µm

strong peaks observed at 1410 and 1240 cm^{-1} are characteristic for the C=O stretching band of carbonyl groups. These spectra are indicative for the role of –NH and –OH groups as binding sites for metal ions complexation. As we have described previously in [20], the immobilization could be due to OH– bond as well as SH– and NH– bond of PVA-matrices with carboxyl or hydrogen groups in the yeast cell wall. Using APTEOS and MPTEOS as a precursor for new hybrid matrices, the appearance of new bond NH– and SH– can afford additional binding places for cell immobilization.

In Figure 5 a comparison between representative kinetic data for free and immobilized cells is shown. With immobilized cells, the specific uptake concentration increases continuously with time and approaches a plateau. The adsorption capacity of the free cells reached a maximum followed by steep decrease. This phenomenon may be due to inhibition of the strain cells by heavy metal ions.

Table 1 summarizes the values for the calculated k_{ads} and q_{eq}^{calc} in case of immobilized strain cells and the measured q_{max}^{exp} for free cells as biosorbent.

The data show that the presence of –SH and $–NH_2$ groups in PVA–hybrid matrices used for immobilization resulted in apparently higher sorption capacity of the immobilized *Trichosporon cutaneum* R57 cells. Practical explanation of this phenomenon could be the incorporation of additional adsorption sites in the matrix through PVA functionalities. The calculated values for the equilibrium

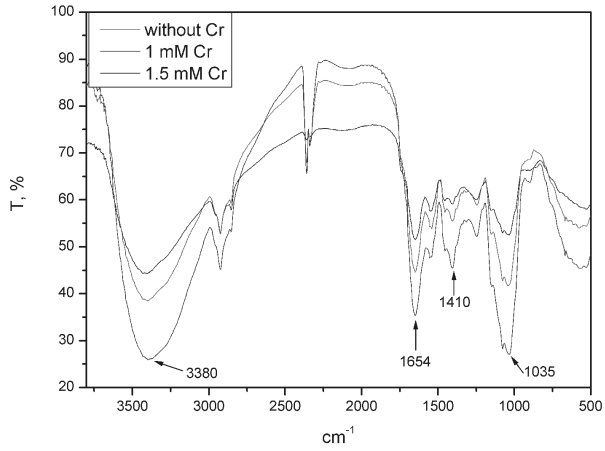

Fig. 4. IR spectra of biomass without Cr and biomass loaded with 1 mM Cr and 1.5 mMCr

Cr(VI) uptake based on the experimental data and the pseudo second order kinetic model concentration, $q_{\mathrm{eq}}^{\mathrm{calc}}$, for immobilized cells vary from about 1.2 to 1.5 times the maximum values measured in the case of free cells. There is also a trend of increasing the biosorption capacity of the immobilized cells compared to the free cells when increasing the initial concentration of Cr(VI) ions. This trend indicates additional benefits from the biomass immobilization on the synthesized

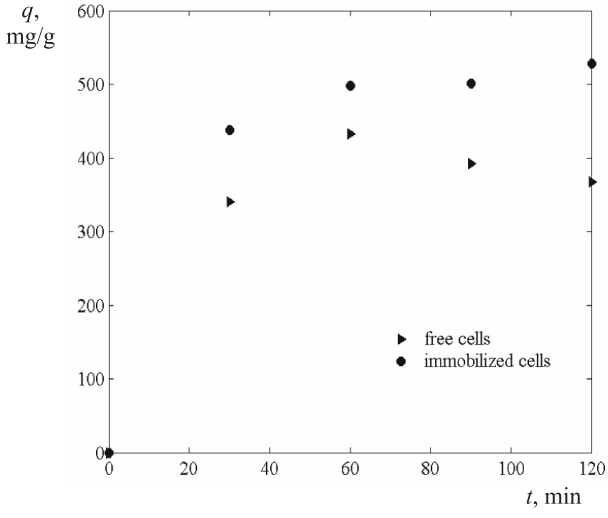

Fig. 5. Kinetic data for biosorption of Cr(VI) ions by free and immobilized on PVA/APTEOS matrix *Tr. cutaneum* R57 cells at initial concentration in the medium $C_0 = 104$ mg L^{-1}

Table 1

Data for second order kinetic model parameters

C_0,	PVA/TEOS		PVA/APTEOS		PVA/MPTEOS		Free cells
mg/L	$k_{ads} \times 10^3$	q_{eq}^{calc}	$k_{ads} \times 10^3$	q_{eq}^{calc}	$k_{ads} \times 10^3$	q_{eq}^{calc}	q_{max}^{exp}
20.8	1.2653	122.055	1.19	119.73	0.51662	142.45	97.08
104	0.46086	633.312	0.21063	560.54	0.28391	658.33	432.82

matrices considering its application for recovery of waste waters contaminated with Cr(VI) ions.

The immobilization by attachment to the surface of the synthesized hybrid materials ceases the inhibition of the cells by the heavy metal ions thus increasing their potential for application in Cr(VI) contaminated waste waters recovery. This effect is even more prominent pronounced at higher Cr(VI) concentrations in the medium. The presence of –SH– and $–NH_2$ groups in PVA–hybrid matrices used for immobilization resulted in apparently higher Cr(VI) sorption capacity of the immobilized *Tr. cutaneum* R57 cells, which can be explained by the incorporation of additional adsorption sites in the matrix through the PVA functionalities and some affinity of Cr(VI) ions to these functional groups. A similar observation was also reported by M. Irani [11], where the Cd^{2+} and Ni^{2+} biosorption was studied by using PVA/TEOS/TMPTMS hybrid membrane.

4. Conclusion. New hybrid materials on the basis of PVA and organosilanes as TEOS, APTEOS and MPTEOS were characterized by TGA analysis and successfully applied as matrices for yeasts immobilization. The results drawn from that initial study present NH_2-bearing groups polymer (PVA/APTEOS) as the most suitable polymer matrices for *Trichosporon cutaneum* R57 strain immobilization. Further experiments are needed to test that most compatible material for biosorption application.

REFERENCES

[1] Cardenas-Gonzalez J., I. Acosta-Rodriguez. Bioinorg. Chem. Appl., DOI: 10.1155/2010/676243.

[2] Francisco R., M. Alpoim, P. Morales. J. Appl. Microbiol., **92**, 2002, No 5, 837–843.

[3] Ksheminska H., D. Fedorovych, T. Honchar, M. Ivash, M. Gonchar. Food Technol. Biotechnol., **46**, 2008, No 4, 419–426.

[4] Parameswari E., A. Lakshmanan, T. Thilagavathi. Austr. J. Basic. Appl. Sci., **3**, 2009, No 2, 1363–1368.

[5] Arief V., K. Trilestari, J. Sunarso, N. Indraswati, S. Ismadji. CLEAN – Soil Air Water, **36**, 2008, No 12, 937–962.

[6] Ting Y., G. Sun. J. Chem. Technol. Biotechnol., **75**, 2000, No 7, 541–546.

[7] Georgieva N., N. Rangelova, D. Peshev, S. Nenkova. Compt. rend. Acad. bulg. Sci., **64**, 2011, No 10, 1421–1428.
[8] Zou H., S. Wu, J. Shen. Chem. Rev., **108**, 2008, No 9, 3893–3957.
[9] Binsu V., R. Nagarale, V. Shahi. J. Mater. Chem., **15**, 2005, No 45, 4823–4831.
[10] Prakash S., M. Kumar, B. Tripathi, V. Shahi. Chem. Eng. J., **162**, 2010, No 1, 28–36.
[11] Irani M., A. Keshtkar, M. Mousavian. Chem. Eng. J., **175**, 2011, No 1, 251–259.
[12] Jin X., Y. Li, C. Yu, Y. Ma, L. Yang, H. Hu. J. Hazar. Mater., **198**, 2011, No 1, 247–256.
[13] Bandyopadhyay A., A. De Sarkar, A. Bhowmick. J. Mater. Sci., **40**, 2005, No 19, 5233–5241.
[14] Uragami T., K. Okazaki, H. Matsugi, T. Miyata. Macromol., **35**, 2002, No 24, 9156–9163.
[15] Zhang Q., Q. Liu, Zh. Jiang, Y. Chen. J. Membr. Sci., **287**, 2007, No 2, 237–245.
[16] Andrade G., E. Barbosa-Stancioli, A. Piscitelli-Mansur, W. Vasconcelos, H. Mansur. Biomed. Mater., **1**, 2006, No 4, 221–228.
[17] Guo R., X. Ma, Ch. Hu, Zh. Jiang. Polymer, **48**, 2007, No 10, 2939–2945.
[18] Aguado J., J. Arsuaga, A. Arencibia, M. Lindo, V. Gascon. J. Hazard. Mater., **163**, 2009, No 1, 213–221.
[19] Bryaskova R., D. Pencheva, G. M. Kale, U. T. Kantardjiev. J. of Colloid and Interface Sci., **349**, 2010, No 1, 77–85.
[20] Bryaskova R., N. Georgieva, D. Peshev. Cent. Eur. J. Chem., **8**, 2010, No 5, 1053–1058.

University of Chemical Technology
and Metallurgy
8, St. Kl. Ohridski Str.
1756 Sofia, Bulgaria
e-mail: nelly.georgieva@yahoo.com

**Institute of Biophysics*
and Biomedical Engineering
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21
1113 Sofia, Bulgaria