P-052	**Yeast cells immobilization on HPC/SiO_2 sol-gel based materials for removal of Cr (VI) ions**

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INTRODUCTION AND OBJECTIVES

Immobilization of biomolecules and cells within sol-gel matrices has gained considerable importance in large-scale biotechnological processes. There are many reports on immobilization of yeast cells on organic-inorganic supports having different applications (Podrazky and Kuncova 2005, Meena 2006, and Guan 2008). Biosorption of heavy metals from aqueous solutions is a relatively new process that has been proved very promising process in the removal of heavy metal contaminants. The major advantages of biosorption are its high efficiency in reducing the heavy metal ions and the use of inexpensive biosorbents. Microbial removal of metal ions from wastewaters has been shown to be highly effective (Cojocaru 2009). The high potential of *Trihosporon cutaneum* strain R57 for heavy metals removal from contaminated waste waters has been revealed in previous investigation (Georgieva 2008). The immobilization by attachment onto hybrid matrices has been shown to retain the high strain biosorption capacity (Rangelova 2009). In the present work, Cr (VI) removal in aqueous solutions has been studied using free and immobilized *Trichosporon cutaneum* R57 cells. The experimental data were used to derive the parameters in a pseudo second order kinetic model.

MATERIALS AND METODS

Hybrid materials preparation. Silica hybrids with different hydroxypropyl cellulose (HPC) content- 5, 20 and 50 wt % were obtained by acid-catalyzed sol-gel method. The molar ratio SiO_2:H_2O:HCl= 1:3.3:0.01. Obtained sol-gel materials were used as matrices for cells immobilization by attachment of the filamentous yeast cells.

Yeast strain. The filamentous yeast strain *Trichosporon cutaneum R 57* was used in this investigation.

Cultivation conditions, immobilization and biosorption. The cultivation conditions and the medium were described previously (Rangelova 2009). After 6 hours of the strain cultivation, the HPC/SiO_2 materials were added to the culture medium and the Cr^{6+} ions were supplied after 24 hours of the strain cultivation. $K_2Cr_2O_7$ in concentrations 0.2 and 1.0 mM was added to the medium.

Kinetic model. The heavy metals biosorption experiments were performed under vigorous stirring in order to eliminate the impact of the external diffusion. The experimental data for the concentration of residual Cr^{6+} in the liquid phase at given time were represented in terms of the specific heavy metal uptake $q = V\left(C_0 - C\right)/m$. 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 were used to determine the parameters in a pseudo-second order kinetic model according to Rangelova 2009. For microscope observations the samples were analyzed by using bright field microscope Olympus BX53, Camera SC30 (Japan). Residual Chromium concentrations was determinated by inductively coupled plasma mass spectroscopy (ICP) Leeman Labs.

RESULTS AND DISCUSSION

The filamentous yeast *Tr.cutaneum R57* was immobilized onto HPC/SiO_2 hybrid materials with different HPC content. The Immobilization procedure was carried out at early stationary phase of the culture.

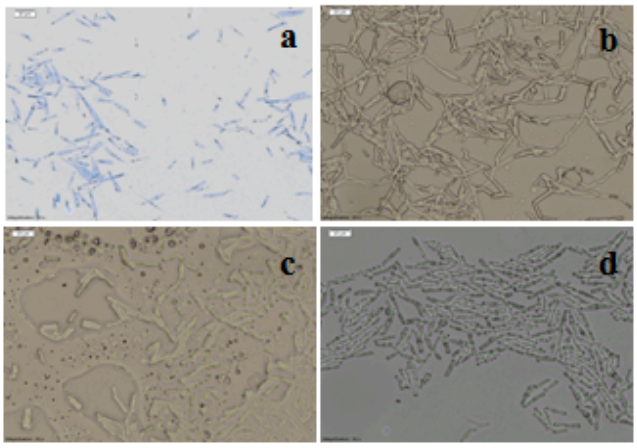

Figure 1 : Microscopic photographs of free *Tr. cutaneum* cells (a) and attached onto ETMS 5 wt% HPC (b), ETMS 20 wt% HPC (c) and ETMS 50 wt% HPC (d) at 0.2 mM $K_2Cr_2O_7$. Magnification 20×

The added concentration of 0.2 mM $K_2Cr_2O_7$ can be considered as an inhibitory threshold value since it changed the duration of stages of growth compared to controls (Georgieva 2011). The microscopic study showed filamentous nature of the cells (Fig.1). More cells can be seen on the hybrids containing 50 % HPC compared to the control free cells (Fig.1a and d). During the treatments with 1.0 mM $K_2Cr_2O_7$, the cell culture grew very slowly, showing no typical stage of growth (Fig.2a). As can be seen the more cells were attached to the hybrids with maximum HPC content (Fig. 2d). Cr(VI) removal was monitored with the time. On Fig. 3 is shown comparison between representative kinetic data for free and immobilized cells. With immobilized cells, the specific uptake concentration increases continuously with the time and approaches a plateau.

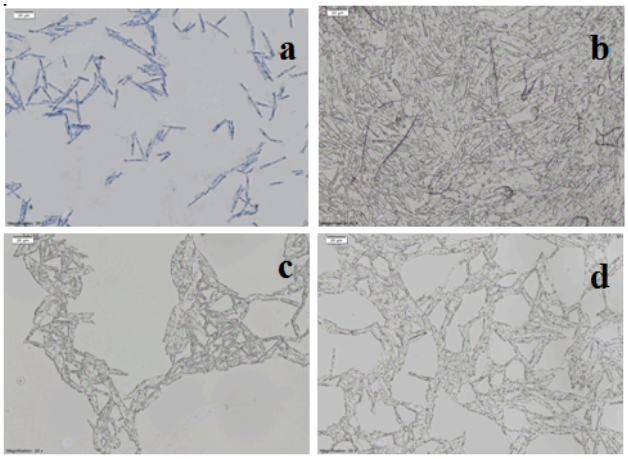

Figure 2 : Microscopic photographs of free *Tr. cutaneum* cells (a) and attached onto ETMS 5 wt% HPC (b), ETMS 20 wt% HPC (c) and ETMS 50 wt% HPC (d) at 1.0 mM $K_2Cr_2O_7$. Magnification 20×

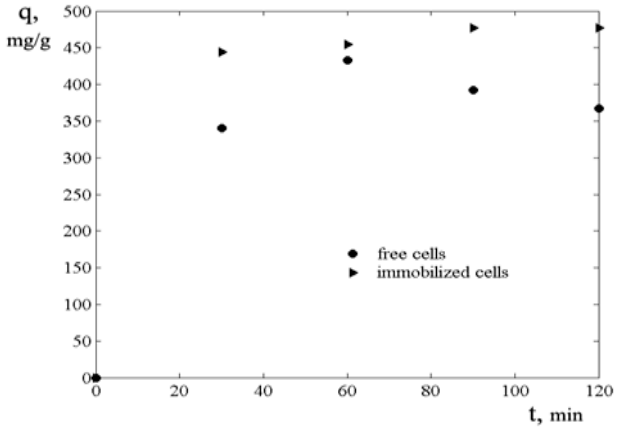

Figure 3 : Kinetic data for biosorption of Cr(VI) by free and immobilized on hybrids with 20 wt% HPC cells at initial concentration in the medium 104 mg/L

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 the heavy metal ions. Table 1 summarizes the values for the calculated k_{ads} and q_{eq}^{calc} in case of immobilized cells and the measured $q_{\max}^{\exp}$ for free cells as biosorbent. The results showed that the content of HPC in the hybrids does not influence the sorption capacity of the filamentous cells as far as no correlation between its content and q_{eq}^{calc} can be derived. The calculated based on the experimental data and the pseudo second order kinetic model values for the equilibrium Cr(VI) uptake concentration, q_{eq}^{calc}, for immobilized cells are close to the maximum values measured in the case of free cells. However, there is a trend of increasing the biosorption capacity of the immobilized compared to the fee cells when increasing the initial concentration of Cr (VI).

Table 1: Data for second order kinetic model parameters

C_0, mg/L	ETMS 5%HPC		ETMS 20%HPC		ETMS 50%HPC	
	$k_{ads}\times10^3$	q_{eq}^{calc}	$k_{ads}\times10^3$	q_{eq}^{calc}	$k_{ads}\times10^3$	q_{eq}^{calc}
20.8	1.5546	57.013	1.7505	60.827	1.1323	78.370
104	0.23243	520.83	0.52406	493.097	0.68873	520.56

This phenomena indicate additional benefits from the biomass immobilization on the synthesized matrices considering its application for Cr (VI) contaminated waste waters recovery.

CONCLUSIONS

The obtained results indicated the potential of this functional material that combines the biological activity of yeast cells with the adsorbent capacity of sol–gel materials. The immobilization by attachment to the synthesized hybrids ceases the inhibition of the cells by heavy metal ions thus increasing their potential for application in Cr^{6+} contaminated waste waters recovery. This effect is even more prominent at higher Cr^{6+} concentrations in the medium.

Acknowledgements

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