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Immobilization of *Trichosporon cutaneum* R 57 Cells onto Methylcellulose/SiO_2 Hybrids and Biosorption of Cadmium and Copper Ions

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Summary: Methylcellulose/Silica (MC/SiO_2) hybrids were synthesized via poly step sol-gel method. SiO_2 was included into the hybrids from two silica precursors – methyltriethoxysilane (MTES) and ethyltrimethoxysilane (ETMS) with different quantity of organic part-5, 20 and 50 wt. %. The filamentous yeasts *Trichosporon cutaneum* strain R 57 was immobilized onto the synthesized MC/SiO_2 hybrids. After immobilization the hybrid materials were used in the processes of sorption of cadmium and copper ions. The obtained results of protein content analysis indicated that the amount of protein increased with increasing of MC in the hybrids. It was established that the maximal efficiency of copper and cadmium removal were observed for hybrid materials containing MTES and 50 wt.% MC - 66 % and 26 % respectively. For ETMS and 50 wt.% MC a high value of copper removal was 56% and for cadmium – 45 % removal, respectively. FTIR analysis of free and immobilized cells with metal ions was conducted. SEM images showed successful immobilization of the yeasts cells. Second order model was employed in order to investigate the kinetics of copper and cadmium biosorption.

Key words: methylcellulose, silica hybrid materials, sol-gel method, immobilization, metal biosorption

1. INTRODUCTION

If organic polymers and inorganic materials can be combined effectively, then a new class of high performance or highly functional organic-inorganic hybrid materials may be achieved [1, 2]. Sol-gel synthesis, owing to its mild conditions, allows the introduction of organic molecules inside the inorganic network without any damage. In this case, interaction between the organic polymers and the inorganic materials, for example, hydrogen bonding, plays an important role in avoiding phase separation and yielding transparent free standing films [2, 3]. Such hybrids are promising materials for various applications: biocatalysts and as

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materials for further immobilization of different cells and enzymes [3-5] as well as solid-state lasers, optical materials, and other [6-9]. As is known, that a large variety of synthetic or natural polymers soluble in the sol-gel mixture have been used for preparation of hybrid materials [2, 10-17]. Like enzymes, bacteria, antibodies, DNA and fungi, yeast cells can be successfully immobilized in matrices synthesized by the sol-gel method. A living microorganism, *Saccharomyces cerevisiae* [18] has been effectively immobilized in a transparent matrix of porous, gel-derived silica, while preserving the microorganism's bioactivity. The encapsulation of whole yeast cell within sol-gel silica matrices have been reported and describe their enhance stability in various organic solvents [19]. The sol-gel technique has been successfully used for preparation of a heavy metal biosorbent from yeast cells [20].

The objectives of this study were to describe the application of obtained MC/SiO2 hybrids as matrices for immobilization filamentous yeast *Tr.cutaneum* strain *R57* and its Cu^{2+} and Cd^{2+} bioaccumulation capacity.

2. MATERIALS AND METHODS

Hybrid materials

The synthesis and structural characterization of methylcellulose/ silica hybrid materials derived via sol-gel method has been developed within our group previously [21].

Strain and cultivation conditions

Filamentous yeast strain *Trichosporon cutaneum R 57* was used in the investigations. The cultivation conditions and the medium were as described previously [22]. At the 6^{th} hours of the strain cultivation, the SiO_2 hybrid materials were added to the culture medium and the cadmium and copper ions were supplied at the 24^{th} hours of the strain cultivation. The cadmium was applied in the form of $CdSO_4$ in concentration 89.6 mg Cd /l (0.8 mM $CdSO_4$). The copper was applied in the form of $CuSO_4.5H_2O$ in concentration 3.175 mg Cu /l (0.05 mM $CuSO_4.5H_2O$).

Analytical measurements

Determination of copper and cadmium ions uptake by *Tr.cutaneum R57,* were done using an atomic absorption spectrophotometer Perkin-Elmer (England).

Protein assay

Total protein of the immobilized cells was determined by the modified method of Lowry according to Schacterlee et al. [23] using bovine serum albumin (Sigma Co) as a standard protein.

FTIR-analysis

The FTIR spectra of immobilized cells of *Tr.cutaneum R57* before and after biosorption treatment were obtained using FTIR spectrometry (Brucker Tensor 27) in the range 4000-400 cm^{-1} in KBr pellets.

SEM-analysis

Structural details were obtained using a JEOL JSM- 5510 Scanning Electron Microscope (SEM). An JEOL JFC-1200 fine sputter coater was used to sputter the fractured surfaces with gold so as to enhance their conductivity.

Kinetic model

As to describe the biosorption kinetics of heavy metals by different strains, pseudo first and second order equations were commonly applied as well as intraparticle diffusion equation [24-26]. The applicability of the Ho's second order kinetic model to the biosorption of copper and cadmium ions by the *Trihosporon cutaneum* strain R57 was proved in a previous work [25]. Its integral linearized form can be represented by the following equation [26]:

$$\frac{t}{q} = \frac{1}{k_{ads}\, q_{eq}^2} + \frac{1}{q_{eq}} t \qquad (1)$$

where $q = V(C_0 - C)/m$ is the quantity of the ions adsorbed by a given quantity of biosorbent in time t. The values for the equilibrium heavy metal uptake q_{eq} and the adsorption rate constant k_{ads} can be determined from the slope and the intercept of the plot t/q versus t based on the experimental data. The symbols used in eq. 1 are us follows:

C – concentration of metal ions, [mg·l^{-1}]; C_0 – initial concentration of metal ions, [mg·l^{-1}]; k_{ads} – rate constant, [g·mg^{-1}·min^{-1}]; m – biosorbent dry weight, [g]; q – metal uptake concentration in time t, [mg·g^{-1}]; q_{eq} – metal uptake concentration at equilibrium, [mg·g^{-}]; t – time, min; V – volume of metal solution, [l];

3. RESULTS AND DISCUSSION

The distribution of the immobilized cells on the surface of MC/SiO_2 hybrid materials derived from MTES was visualized by SEM. The images on Fig. 1 indicate successful immobilization of cells to the hybrids surface. During the first 24 hours not only the single cells were attached to the hybrids. The increasing of organic compound content leads to the increase the quantity of functional group on the surface of obtained MC/SiO_2 hybrids, and the quantity of the attached cells onto it. This was proved with increasing of protein amount in the sample with addition of 50% methylcellulose.

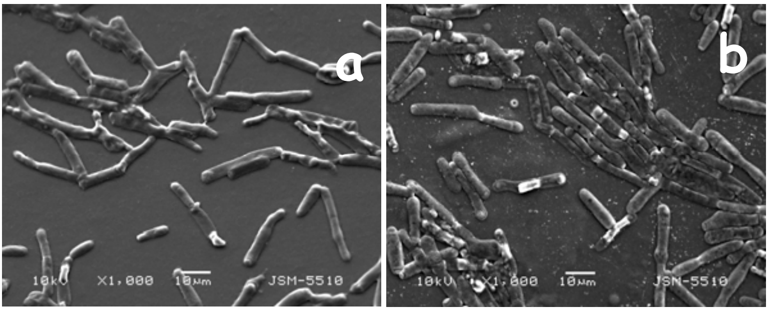

Fig. 1 SEM images of immobilized Tr. cutaneum R57 cells onto MC/SiO_2 hybrid materials derived from MTES containing 5 wt.% methylcellulose (a) and 50 wt. % methylcellulose (b)

The results of protein content analysis for hybrids derived from MTES indicate that the amount of protein is higher in the sample containing 50 wt. % MC 15.18 mg protein/g carrier, while in the samples with 5wt. % and 20 wt. % MC the protein amount is 7.85 and 10.30 mg/g carrier, respectively. The obtained results of protein content analysis for hybrids derived from ETMS are analogous. On the Fig. 2 and Fig. 3 are shown the relationship between protein content and cultivation time. As can be seen from the figures after addition of metals protein content drastically decreased.

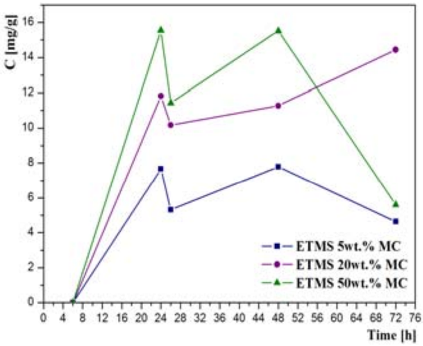

Fig. 2 Protein amount of immobilized yeast cells onto MC/SiO_2 hybrid materials derived from ETMS

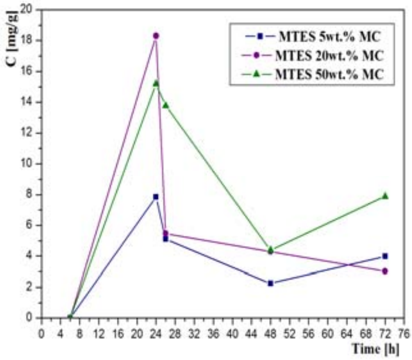

Fig. 3 Protein amount of immobilized yeast cells onto MC/SiO_2 hybrid materials derived from MTES

It was established that the maximal efficiency of copper and cadmium removal were observed for hybrid materials containing MTES and 50 wt.% MC - 66 % and 26 % respectively. For ETMS and 50 wt.% MC a high value of copper removal was 56% and for cadmium – 45 % removal, respectively. Free, not immobilized cells showed copper removal 35% and cadmium removal – 20%.

The incorporation of metal ions in cells can change significantly their FTIR spectra [27]. Fig. 4 shown that the FTIR spectra of dried unloaded and loaded with copper and cadmium ions cells of *Tr. cutaneum R57*. The FTIR spectrum at 1661, 1456 and 1052 cm^{-1} of unloaded cells assigned C=O stretching [28], -COOH [26] and C-N stretching vibrations [29, 30]. These peak shifts to 1659, 1455 and 1051 cm^{-1} after cadmium and copper biosorption onto immobilized cells of *Tr. cutaneum R57*. These results suggested involvement of these functional groups in cadmium and copper biosorption onto the investigated cells [27]. The stretching vibrations of P=O was observed at 1149 cm^{-1} [27] for FTIR spectrum of unloaded cells and this peak shifts to 1105 cm^{-1} by loaded cells. The absorption bands at 817, 779 and 560 cm^{-1} by unloaded cells were not indicated in the FTIR spectra of loaded cells. The peak at 560 cm^{-1} assigned to deformation of S-O stretch from sulphate anion (SO_4^{2-}) [30]. The most important evidence for the interaction of cadmium and copper ions with the functional groups on the biosorbent was the new peak, which appeared at 988 cm^{-1} after metal biosorption. This peak is due to the connecting of Cd^{2+} and Cu^{2+} to –C-SH groups in accordance with [29]. The formation of new peaks posited at 924 and 869 cm^{-1} of loaded biomass could indicate the metal-sulphur interaction.

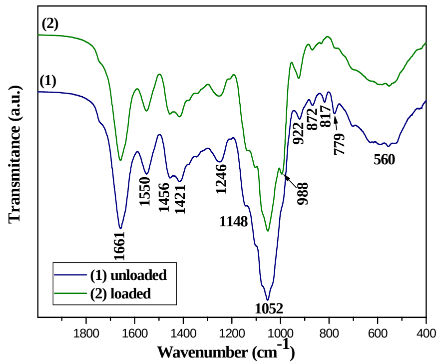

Fig. 4 FTIR spectra of unloaded and Cu^{2+}/Cd^{2+} loaded onto immobilized Tr. cutaneum R57 cells

The experimental data on the kinetics were fitted to the Ho's second order rate equation 1 and the results are summarized in Table 1.

The negative values of the rate constant obtained for some cases correspond to the experimental observation of decreasing with the time specific metal uptake after the initial sharp increase. This may be due to inhibition of the biosorbent in long term process. Representative kinetic curve is shown in Fig. 5 for the case of biosorption of cadmium ions by immobilized strain on hybrid materials containing MTES and 50 wt.% MC when maximum metal uptake concentration was achieved.

Table 1. Data for second order kinetic model parameters

System	Ion	ETMS			MTES		
		q_{eq}, mg g^{-1}	k_{ads}x10^3 g mg^{-1} min^{-1}	R	q_{eq}, mg g^{-1}	k_{ads}x10^3 g mg^{-1} min^{-1}	R
Free cells	Cu^{2+}	11.9617	0.1942	0.3492	25.5754	2.3059	0.9997
5%		16.2866	-1.8599	0.9992	29.3255	0.7116	0.9778
20%		5.8754	-3.4109	0.9670	11.7509	-0.3889	0.9531
50%		8.4962	-1.4283	0.9910	9.0827	-0.4954	0.9586
Free cells	Cd^{2+}	98.0392	-0.2158	0.9999	357.1429	0.0581	0.9901
5%		17.4520	-0.2593	0.8070	384.6154	0.1625	0.9963
20%		16.0514	0.5835	0.9996	285.7143	1.0124	0.9967
50%		9.1491	0.2676	0.7174	400.0000	0.0341	0.9980

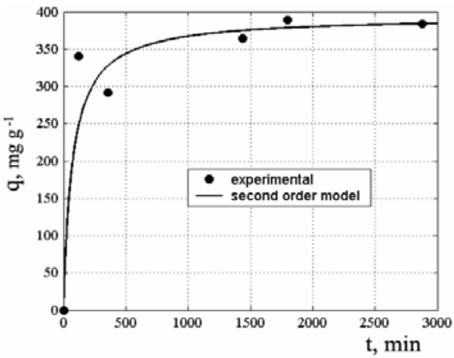

Fig. 5 Plot of the adsorbed amount of cadmium immobilized on MTES type of matrix strain cells versus time – experimental data and equation 1 description

4. CONCLUSIONS

Methylcellulose/Silica hybrid materials were successfully used as matrices for immobilization of filamentous yeasts *Trichosporon cutaneum* strain R 57. After immobilization the hybrid materials were used in the processes of sorption of cadmium and copper ions. It was established that the maximal efficiency of copper and cadmium removal were observed for hybrid materials containing MTES and 50 wt.% MC - 66 % and 26 % respectively. For ETMS and 50 wt.% MC a high value of copper removal was 56% and for cadmium – 45 % removal, respectively. FTIR analysis of free and immobilized cells with metal ions was conducted. SEM images showed successfully immobilization of the yeasts cells. Second order model was employed in order to investigate the kinetics.

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