

Removal of cadmium and copper ions by *Trichosporon cutaneum* R57 cells immobilized onto polyvinyl alcohol/tetraethoxysilane hybrid matrices

Research Article

Rayna Bryaskova^{1*}, Nelly Georgieva², Dimitar Peshev³

¹Department of Polymer Engineering, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria

²Department of Biotechnology, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria

³Department of Chemical Engineering, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria

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Abstract: Polyvinyl alcohol (PVA) and tetraethoxysilane (TEOS) hybrid materials were prepared by sol-gel methods and tested as matrices for immobilization of *Trichosporon cutaneum* R57, capable of removing cadmium and copper ions from aqueous solutions. A kinetic model was applied and the effects of matrix TEOS content on the copper and cadmium uptake equilibria and rate constants were investigated.

Keywords: Cadmium • Copper • Sol-gel • Polyvinyl alcohol • Biosorption

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1. Introduction

Heavy metals are widespread pollutants of great environmental concern, as they are non-degradable and persist in the environment. They are mobilized and carried into the food web by leaching from waste dumps, polluted soils, and water. Even at low concentrations they are usually harmful to human health and other forms of life. Therefore, heavy metal removal is essential prior to disposal of contaminated waste waters [1]. Conventionally, removal is by chemical precipitation, electrochemical treatment, ion-exchange processes, membrane separation, and evaporation [2-4].

Recently, attention has been paid to biosorption

as a promising alternative. Biosorption works through cell surface complexation, ion exchange, and microprecipitation. A large number of microorganisms such as bacteria, fungi, yeasts, and algae have been reported to bind heavy metals [5-7]. Among these, fungi and yeasts were reported as excellent heavy metal biosorbents [8-11]. Microorganisms' potential applications have been well reviewed [1,12].

Biosorption can use either free or immobilized organisms. Especially in column applications, an immobilized form provides advantages such as improved capacity and the ability to regenerate and separate the biomass from the bulk liquid. Facile repetition of biosorption-desorption cycles with immobilized biomass

* E-mail: rbryaskova@uctm.edu

renders the process more economically competitive. The application of immobilization techniques in industry, especially in biological wastewater treatment, offers a high cell concentration in the reactor tank for increased efficiency.

A number of matrices have been used for cell immobilization; the most important are based on alginate, polysulfone, polyacrylamide, polyurethane, and silica [13-14].

The aim of the present study was to synthesize a polyvinyl alcohol (PVA) - tetraethoxysilane (TEOS) hybrid material and to investigate its immobilization of *Trichosporon cutaneum* strain R57. The second objective was to evaluate the efficacy of Cu^{2+} and Cd^{2+} biosorption by the immobilized yeast.

2. Experimental Procedure

2.1. Materials

Polyvinyl alcohol (PVA) (Sigma-Aldrich; 87-88% hydrolyzed, $M_w = 13000 - 23000 \text{ g mol}^{-1}$), HNO_3 (Riedel de Haën, 2 M standard solution), and tetraethyl orthosilicate (TEOS) (Fluka) were used as received.

Filamentous yeast strain *Trichosporon cutaneum* R57, registered by Ivanova *et al.* [15] and maintained in the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures collection (N2414) was used. Details about the growth media can be found elsewhere [16]. Glucose solution (20 g L^{-1}) was sterilized separately and added to the medium.

2.2. Synthesis of PVA/TEOS hybrid materials.

5 g of polyvinyl alcohol was dissolved in 95 mL deionized water by heating for 20 min at 80°C . The silica sol was produced by partial hydrolysis of TEOS in acidified water using HNO_3 as a catalyst (TEOS/ H_2O / HNO_3 volume ratio equal to 1/1/0.1). The mixture was stirred until a clear solution was obtained. Different amounts of TEOS (3 wt%, 5 wt%, and 10 wt% with respect to PVA) were added drop wise to the PVA solution. The final mixtures were stirred for 80 min, cast into films on glass, and dried for 3 days at room temperature. The matrices were denoted as follow: PVA/3 wt% TEOS (M1), PVA/5 wt% TEOS (M2) and PVA/10 wt% TEOS (M3).

2.3. Cultivation conditions

Starter cultures were prepared by loop-inoculating 100 mL liquid medium and incubating for 18 hours in an intensely stirred environment (rotary shaker, 180 rpm at 30°C). For experimental cultures, 100 mL of medium was inoculated with 5 mL of the starter culture (with an

initial biomass concentration of approximately 0.1 mg dry wt per mL) and incubated on the shaker for 48 hours at 30°C . At the 6th hour of cultivation, 0.01 g PVA/TEOS matrix was added to 100 mL of culture. The cadmium and copper ions were added at the 24th hour. Cadmium was added as 0.8 mM CdSO_4 (89.6 mg Cd per L). Copper was added as 0.05 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3.175 mg Cu per L). To eliminate diffusion limitation and ensure kinetic control of the overall biosorption process the mixing was intense (rotary shaker at 180 rpm). Liquid phase samples were taken periodically for heavy metal determination. Prior to atomic absorption analysis all samples were centrifuged to remove the biosorbent.

2.4. Analysis

SEM observation was carried out on a JEOL JSM- 5510 after coating in a JEOL JFC- 1200 fine coater.

Transmittance IR spectra of the films were recorded from 400 to 4000 cm^{-1} using a Perkin Elmer FTIR.

Thermogravimetric analysis (TGA) was performed under nitrogen from 25°C to 600°C at $20^\circ\text{C min}^{-1}$ using a TA TGA Q500.

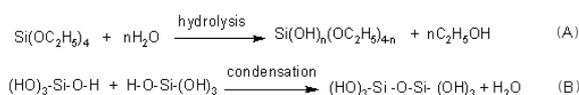
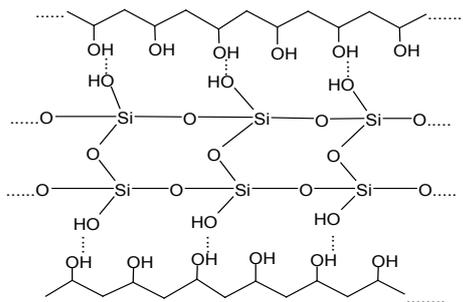
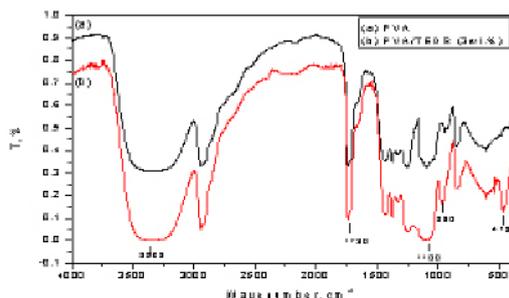
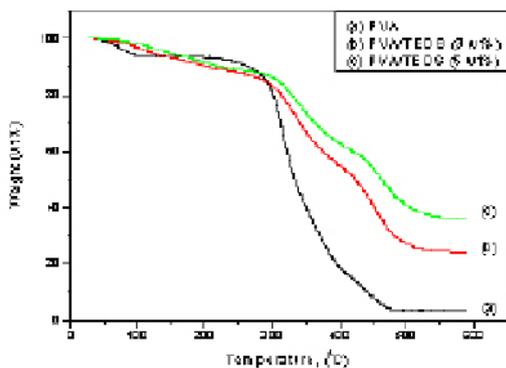
Determinations of copper and cadmium were done using a Perkin-Elmer (England) atomic absorption spectrophotometer.

3. Results and Discussion

Hybrid materials based on polyvinyl alcohol and tetraethoxysilane were used as an immobilization matrix for *Trichosporon cutaneum* strain R57 yeast. Partially hydrolyzed TEOS (alcoxy groups replaced with hydroxyl; Scheme 1A) was added to PVA solution. Upon film casting, polycondensation forms Si-O-Si bonds and alcohol or water is released (Scheme 1B).

The hydroxyl groups in PVA form strong hydrogen bonds with the silanol groups on the TEOS (Scheme 2) forming a PVA/TEOS matrix.

The hybrid matrices were characterized by FTIR spectroscopy. Fig. 1 presents the FTIR spectra of pure PVA and the PVA/3 wt% TEOS matrix. A broad O-H stretch centered at 3300 cm^{-1} is observed in both, due to hydrogen bonding [17]. In all spectra there is a strong carbonyl peak at 1740 cm^{-1} from acetate in the partially hydrolyzed PVA. The peaks at 1440 cm^{-1} and 1330 cm^{-1} are from O-H bending and C-H deformation respectively. The peak at $1000-1100 \text{ cm}^{-1}$ can be attributed to C-O stretching and O-H bending vibrations from the PVA chain. The asymmetric Si-O-Si stretch appears at $1120-1080 \text{ cm}^{-1}$. The PVA/TEOS absorption at 470 cm^{-1} is due to Si-O-Si deformation. The peak at 960 cm^{-1} is

**Scheme 1.** Hydrolysis and condensation reactions of TEOS.**Scheme 2.** PVA/TEOS matrix.**Figure 1.** FTIR spectrum of PVA/3 wt% TEOS matrix.**Figure 2.** Thermogravimetric analysis of pure PVA and PVA/TEOS matrices (3 wt% and 5 wt%).

characteristic of Si-OH stretching. No Si-O-C peaks were observed, which indicates that hydrogen bonding between PVA -OH and TEOS -OH holds the material together.

TGA was performed to determine the thermal stability of the hybrid material (Fig. 2). Pure PVA and

PVA/TEOS hybrids (3 wt% and 5 wt% TEOS) showed two main decomposition peaks. The first peak at 315 °C is due to PVA side chain elimination and the second at 450 °C is from degradation of the main PVA chain.

For PVA/TEOS hybrid materials the decompositions shifted to higher temperatures; TEOS incorporation leads to increased thermal stability. Increasing the TEOS content also increased the residue. The residue for pure PVA was negligible (3 wt%) in comparison with PVA/TEOS hybrids where the residue increased to 30 wt% and 40 wt% for materials containing 3 wt% and 5 wt% TEOS respectively.

These hybrid materials were used as matrices for immobilization of yeast *Trihosporon cutaneum* R57. SEM (Fig. 3) showed the presence of yeast cells uniformly distributed on the surface. This immobilization could be due hydrogen bonding of PVA hydroxyl groups with carboxyl or hydroxyl groups in the yeast cell wall.

The high potential of *Trihosporon cutaneum* R57 as an adsorbent for heavy metals is due to the presence of polar groups on the cell surface which can bind metal cations. These can be -SH, -OH, -CN, -OCH₃, -COO⁻, and other negatively charged hydrophilic groups. These are usually part of polysaccharide moieties such as glucans and manans incorporated in the yeast cell wall. Protein can also contribute to such binding.

Process design and scale-up usually require well-defined kinetics and equilibrium at the operating conditions selected. To describe heavy metal biosorption kinetics, pseudo first and second order equations as well as an intraparticle diffusion equation have been commonly applied [18-21]. However in many studies of bivalent metal ion sorption a second order kinetic model adequately described the experimental data [18-20]. Its applicability to the biosorption of copper and cadmium ions by *Trihosporon cutaneum* strain R57 was shown in previous work [19]. Therefore we selected the pseudo second order reaction rate model [20] to evaluate copper and cadmium adsorption kinetics. This assumes that the rate of removal of metal ions from the liquid is proportional to the square of the number of unoccupied matrix adsorption sites. Its linearized integral form can be represented by [20]:

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

where $q = V(C_0 - C)/m$ is the quantity of the ions adsorbed by a given quantity of biosorbent (mg mg⁻¹) in time t (min); V is the volume of metal solution (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

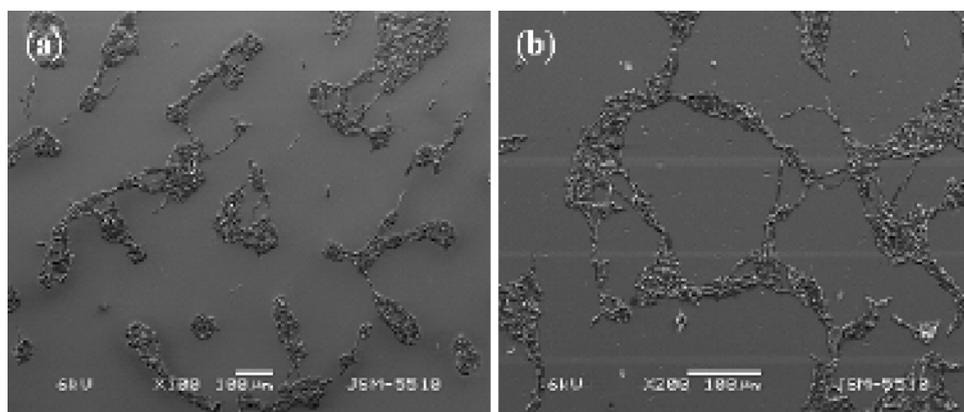


Figure 3. SEM images of *Tr. cutaneum* R57 immobilized onto PVA/TEOS matrices: (a) 3 wt% TEOS, and (b) 10 wt% TEOS.

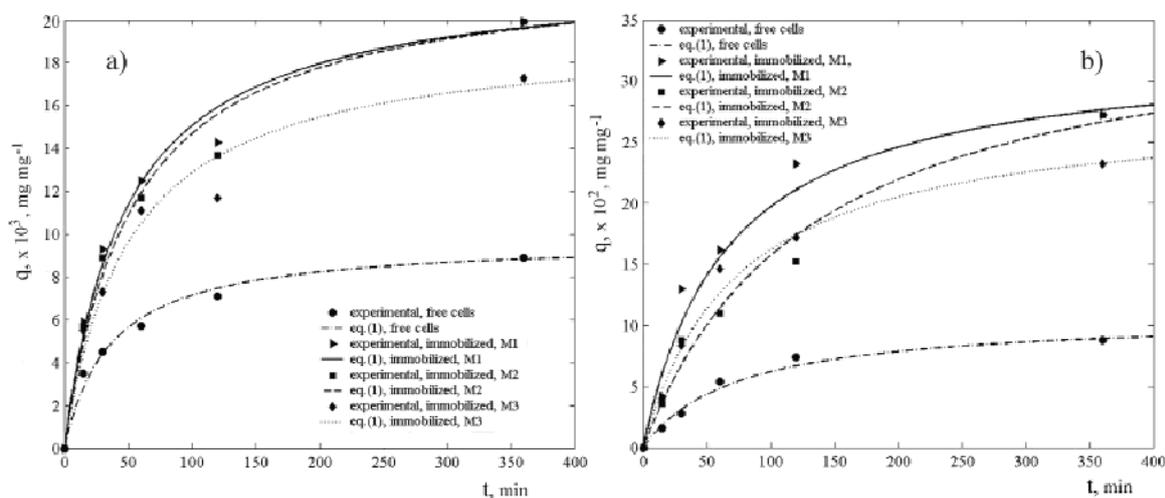


Figure 4. Metal ion adsorption by free and immobilized *Tr. cutaneum* R57 cells; (a) copper, (b) cadmium.

dry weight (mg). The equilibrium heavy metal uptake q_{eq}^{calc} ($mg\ mg^{-1}$) and the adsorption rate constant k_{ads} (min^{-1}) can be determined from the slope and intercept of the t/q vs. t plot. The equilibrium parameter q_{eq} , a measure of the biosorbent specific uptake capacity, and the rate constant provide tools for process design as well as for laboratory comparisons of different techniques and operating conditions.

Kinetics experiments were performed in an intensely stirred environment to eliminate mass transfer limitations on the rate and to ensure that measured bulk concentrations were equal to cell surface concentration. The experimental data were fitted to the second order rate Eq. 1. Results are summarized in Table 1 and illustrated in Fig. 4. As can be seen from Table 1, R^2 values for the t/q vs. t plots are sufficiently high in all cases ($R > 0.96$) to demonstrate the applicability of the second order kinetic model.

The specific equilibrium uptake concentration (q_{eq}^{exp})

was also directly measured by monitoring the medium's residual heavy metal concentration during long-term experiments (Table 1). For immobilized cells, Cu and Cd removal efficiency (q) reached a maximum at the 6th hour of cultivation. For free cells equilibration was considerably slower (24 hours). The measured values of q_{eq}^{exp} for both ions by free cells and those immobilized on different matrices are also given in Table 1. There is no significant difference (average deviation 13.2%) from the values estimated based on the kinetic experiments and the pseudo second order reaction rate model (q_{eq}^{calc}).

Cell immobilization resulted in approximately three times lower rate constants for both ions in all matrix types. This may be due to increased steric hindrance of the adsorption sites after immobilization. Despite the lower rate constant, immobilization led to a higher equilibrium metal uptake. Due to this increased driving force, the effective rate of heavy metal recovery from

Table 1. Second order kinetic model parameters

System	Ion	R ²	k _{ads} ' min ⁻¹	q _{eq} ^{calc.} , mg mg ⁻¹	q _{eq} ^{exp.} , mg mg ⁻¹	$\frac{ q_{eq}^{calc} - q_{eq}^{exp} }{q_{eq}^{calc}} \cdot 100, \%$
Free strain R57 cells	Cu ²⁺	0.9975	2.842	0.00972	0.0105	8.0
Immobilized cells onto M1		0.9943	0.934	0.0223	0.0199	10.8
Immobilized cells onto M2		0.9908	0.834	0.0225	0.0199	11.6
Immobilized cells onto M3	Cd ²⁺	0.9872	1.013	0.0194	0.0173	10.8
Free strain R57 cells		0.9890	0.128	0.108	0.114	5.6
Immobilized cells onto M1		0.9750	0.0463	0.327	0.272	16.8
Immobilized cells onto M2		0.9657	0.0210	0.364	0.272	25.3
Immobilized cells onto M3		0.9922	0.0487	0.280	0.232	17.1

the liquid is higher for immobilized biomass than for free cells.

Considering the increased recovery, *Trihosporon cutaneum* R57 immobilization improves its biosorbent performance. As illustrated in Fig. 4 and Table 1 for copper, the best results were achieved by immobilization matrices M1 and M2. For cadmium, immobilization on M1 should be preferred due to the much higher rate constant, with the equilibrium adsorption almost equal to that with M2.

4. Conclusions

Novel hybrid materials based on PVA and TEOS were synthesized and successfully used as matrices for the immobilization of yeast cells *Trihosporon cutaneum* strain R57. The experimental data on biosorption of Cu and Cd by free cells as well as those immobilized on different matrices were used to evaluate adsorption

rate constants and equilibrium uptake concentrations as parameters of a second order kinetic model. For all matrices studied, immobilized cells showed higher equilibrium Cu and Cd uptake than free cells. PVA/3 wt% TEOS (M1) matrix was found most suitable for immobilization for both ions in terms of process rate and degree of metal recovery.

Immobilization of strain R57 on the surface of these matrices can be used to improve its bioremediation capacity. These results will guide further investigations into the use of microorganisms for cadmium and copper polluted wastewater bioremediation.

Acknowledgements

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References

- [1] P. Baldrian, *Enzyme and Microb. Technol.* 32, 78 (2003)
- [2] B. Volesky, *Hydrometallurgy* 59, 203 (2001).
- [3] F. Veglio, F. Beolchini, *Hydrometallurgy* 44, 301(1997)
- [4] F.R. Espinoza-Quiñones, M.A. Rizzutto, N. Added, M.H. Tabacniks, A.N. Módenes, S.M. Palácio, E.A. Silva, F.L.Rossi, N. Martin, N. Szymanski, *Nucl. Instrum. Methods Phys. Res. B* 267, 1153 (2009)
- [5] F.R Espinoza-Quinones, E.A da Silva, M de Almeida Rizzutto, S.M Palacio, A.N. Modenes, N. Szymanski, N. Martin, A. Kroumov, *World J. Microbiol. Biotechnol.* 24, 3063 (2008)
- [6] A. Jarosz-Wilkolazka, E. Malarczyk, J. Pirszel, T. Skowronski, A. Leonowicz, *Cell Biol. Inetnat.* 26, 605 (2002)
- [7] S. Kahraman, D. Asma, S. Erdemoglu, O. Yesilad, *Eng. Life Sci.* 5, 72 (2005)
- [8] M.A. Castro-Silva, A. Oliveira, A. Gerchenski, D. Jaques, A. Rodrigues, P. Souza, L. Rorigues, *Braz. J. Microbiol.* 34, 45 (2003)
- [9] P. Kujan, A. Prell, H. Safar, M. Sobotka, T. Rezanka, P. Holler, *Folia Microbiol.* 51(4), 257 (2006)
- [10] P. Vasudevan, V. Padmavathy, S. Dhingra, *Biores. Technol.* 89, 281 (2003)
- [11] C.L. Wang, P.D. Maratukulam, A.M. Lum, D.S. Clark, J.D. Keasling, *Appl. Environ. Microbiol.* 66, 4497 (2000)
- [12] E. Breierova, I. Vajezikova, V. Sasinkova, E. Stratilova, M. Fisera, M. Gregor, J. Sajbidor, *Z. Naturforsch.* 57c, 634 (2002)
- [13] J. Wang, C. Chen, *Biotechnol. Adv.* 27, 195 (2009)
- [14] K. Vijayaraghavan, Y.S. Yun, *Biotechnol. Adv.* 26, 266 (2008)
- [15] N. Ivanova, Z. Alexieva, *Bulg. J. Agric. Sci.* 2, 761 (1996)

- [16] N. Georgieva. Intern. J. Agr. Biol. 10, 325 (2008)
- [17] R.L. Guo, C.L. Hu, F.S. Pan, H. Wu, Z.J. Jiang, Membr. Sci. 281, 454 (2006)
- [18] Y.A Yahaya, M.D.Mashitah, B. Subhash, J. Hazard. Materials 161, 189 (2009)
- [19] D. Marinkova, I. Tsibranska, L. Yotova, N. Georgieva, Bioautomation 7, 46 (2007)
- [20] Y.-S. Ho, Bioresource Technology 96, 1292 (2005)
- [21] P. Mondal, B. Mohanty, C.B. Majumder, N. Bhandari, AIChE Journal 55(7), 1860 (2009)