



## An Evaluation of Kinetic Parameters of Cadmium and Copper Biosorption by Immobilized Cells

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**Abstract:** Bioremediation is the use of living organisms to reduce or eliminate environmental hazards resulting from the accumulation of toxic chemicals and other hazardous wastes. This technology is based on the utilization of microorganisms to transform organic and inorganic compounds. The filamentous yeast *Trichosporon cutaneum* strain R57, immobilized and free cells was cultivated as batch culture on a liquid medium in the presence of various concentrations of cadmium and copper ions. The simultaneous uptake and accumulation of  $Cd^{2+}$  and  $Cu^{2+}$  ions by *Tr. cutaneum* cells depending on the initial concentration of  $Cd^{2+}$  and  $Cu^{2+}$  in the medium were studied. The potential use of the free and immobilized cells of *Trichosporon cutaneum* to remove cadmium and copper ions, from aqueous solutions was evaluated. Two important physicochemical aspects for the evaluation of the sorption process as a unit operation are the equilibrium of sorption and the kinetics. The  $Cd^{2+}$  and  $Cu^{2+}$  ions biosorption capacities of all tested adsorbent were presented as a function of the initial concentration of metal ions within the aqueous biosorption medium. The individual, as well as bicomponent sorption kinetics of copper and cadmium ions by immobilised cells of *Trichosporon cutaneum* R57 is presented. A second order kinetic model obtains kinetic parameters for the copper and cadmium ions.

**Keywords:** Heavy metals, Biosorption, Bioremediation, Yeasts, *Tr. cutaneum* R57.

### Introduction

In recent years the effect of heavy metals on the environment has attracted the attention of many researchers. The high toxic impact of heavy metals is due to their ability to accumulate in the living organisms and to their transfer through the food chain [21]. The biosorption technologies can be usefully used to extract metal ions by microorganisms from wastewaters where the concentration of metal ions is low. Extreme amounts of metal ions are toxic for the microorganisms, which reduces the efficiency of the process [18]. Every organism has a sensitivity threshold, which has to be determined for each metal ion and taken in consideration. Above this threshold, it is difficult for the microorganisms to utilize carbon sources and thus is ineffective for the bioremediation processes [6]. In our previous investigation of covalently immobilized *Trichosporon cutaneum* R57 we demonstrated the ability and mathematical model of phenol biodegradation [19]. We were interested to go on with heavy metal biosorption by the same strain in the same medium, taking the consideration that this can be used for simultaneous bioremediation of waste waters. Some similar processes were studied by the means of algal-bacterial consortium [13] and dried sewage sludge [17].



A number of researchers have suggested mathematical models to be used to represent the relation between the quantity of metal ions biosorbed by the cells and the quantity of ions remaining in the environment [2].

The aim of the present investigation is the study the kinetics of biosorbption of covalently immobilized *Trichosporon cutaneum* strain R57 and to compare it with free cells and to propose a mathematical model properly describing the process. For these studies the biosorption of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions by free and immobilized cells of *Trichosporon cutaneum* strain R57 was tested under conditions of batch experiments. The cultivation medium contained both ions to be tested under different concentrations.

## Materials and methods

### *Yeast strain and chemicals*

The *Trichosporon cutaneum* R57 strain was obtained from National Bank of Industrial Microbial and Cell Cultures, Bulgaria. The mutant basidiomycete yeast strain of *Trichosporon cutaneum* have been registered by Ivanova et al. [8] under N2414. A copolymer of acrylonitrile with acrylamide, in which the acrylamide units amount to 15% of the total number of units, was obtained at the Department of Biotechnology in UCTM, Sofia. Granules were obtained by dropping the polymer under pressure into water: methanol (7:3) mixture containing  $0.8 \text{ g dm}^{-3}$  NaCl according to reference [22]. Glucose, salts for mineral medium and formaldehyde were obtained from Merck (Germany). Peptone, yeast extract and agar were obtained from Riedel de Haen (Germany).  $\text{CuSO}_4$  and  $\text{CdSO}_4$  were obtained from Merck (Germany). All other chemicals used were of reagent grade or better.

### *Culturing condition*

The *Trichosporon cutaneum* strain R57 cultured on a solid agar medium containing glucose, yeast extract and peptone at  $28^\circ\text{C}$  for 48 hours at pH 6.0. After incubation colonies were picked and suspended in a mineral salt medium with a glucose concentration of  $20 \text{ g}\cdot\text{dm}^{-3}$ . The composition of the nutrient medium was:  $(\text{NH}_4)_2\text{SO}_4 - 4 \text{ g}\cdot\text{dm}^{-3}$ ;  $\text{Na}_2\text{HPO}_4 - 0.75 \text{ g}\cdot\text{dm}^{-3}$ ;  $\text{KH}_2\text{PO}_4 - 1.7 \text{ g}\cdot\text{dm}^{-3}$ ;  $\text{MgSO}_4\cdot 7\text{H}_2\text{O} - 0.02 \text{ g}\cdot\text{dm}^{-3}$ ; thiamine  $- 0.0002 \text{ g}\cdot\text{dm}^{-3}$  and trace mineral medium  $\text{FeSO}_4\cdot 2\text{H}_2\text{O} - 0.001 \text{ g}\cdot\text{dm}^{-3}$ ;  $\text{MnSO}_4\cdot \text{H}_2\text{O} - 0.001 \text{ g}\cdot\text{dm}^{-3}$ ;  $\text{CaCl}_2 - 0.001 \text{ g}\cdot\text{dm}^{-3}$ , according [16]. After 24 h incubation in a bath shaker at  $28^\circ\text{C}$ , pH 6.0, the cells were suspended in the same nutrient medium containing different concentration of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions under the same conditions.

### *Activation of the copolymer*

The immobilization procedure used is based on a method for covalent binding of enzymes to synthetic carriers containing active N- hydroxymethyl groups, which bind to the amino acid residues of proteins [7, 9, 22]. The granulated carrier of copolymer of acrylonitrile with acrylamide was activated according to procedures described in reference [22]. Forty grams of copolymer was activated with 12% (v/v) formaldehyde (dissolved in 0.1 M phosphate buffer, pH 7.8) and stirred 4 hours at  $45^\circ\text{C}$  in a closed vessel. The activated carrier was then washed abundantly with distilled water until no more formaldehyde was observed in the rinsing waters.

### *Covalent binding of Trichosporon cutaneum viable cells to activated carrier*

The activation procedure of the carrier was followed by the immediate treatment with a cell suspension of *Trichosporon cutaneum* (concentration  $80 \text{ mg}\cdot\text{ml}^{-1}$ ). The cells and synthetic

carrier were suspended in a synthetic nutrient medium according to the method described by Andreev [1] containing  $0.1 \text{ g}\cdot\text{dm}^{-3}$  glucose in a  $0.1 \text{ M}$  acetate buffer. The binding was carried out at  $\text{pH } 5.0$  at a temperature  $28^\circ\text{C}$  under continuous stirring for a period of 4 hours.

### *Kinetic experiments*

The free and immobilized cells were grown in batch experiments. This was done in flask cultures at  $\text{pH } 6.0$  at a temperature of  $28^\circ\text{C}$ . The initial concentration of immobilized yeast cells was in same range as for the free ones,  $0.0167 \text{ g}\cdot\text{dm}^{-3}$ . During the experiments the biomass and glucose concentrations was measured every 2 hours. The maximal rate of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  removal from the medium was measured after 24 h of cultivation. Further cultivation did not show changes in the rate of ions removal.

### *Analytical procedures*

The biomass of suspended cells was measured spectrophotometrically at 610 nm. Cell growth of suspended and immobilized cells was also determined as dry cell weight, according to the method described by Mallette [12]. The samples were dried until they reached a constant weight at  $105^\circ\text{C}$ . The analysis was checked by the determination of the protein content using a modified Lowry's method according to Schacterlee et al. [14].

Determination of cadmium and copper uptake by *Tr. cutaneum*, grown in the presence of  $\text{CdSO}_4$  and  $\text{CuSO}_4$ , was done using atomic absorption spectrometer Perkin-Elmer (Germany).

## **Experimental and mathematical models of the process**

The quantity of adsorbed  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions per unit of sorbent ( $\text{mg}$  metal ions/ $\text{g}$  dry biosorbent) is calculated from the mass balance as follows:

$$q = V(C_0 - C)/M \quad (1)$$

where:  $q$  is the quantity of the ions adsorbed by a given quantity of biosorbent, [ $\text{mg}\cdot\text{g}^{-1}$ ];  $C_0$  – concentrations of the metal ions at the beginning of the cultivation, [ $\text{mg}\cdot\text{l}^{-1}$ ];  $C$  – concentration of the metal ions after the process of biosorption, [ $\text{mg}\cdot\text{l}^{-1}$ ];  $V$  – volume of the liquid phase, [ $\text{l}$ ];  $M$  – quantity of the biosorbent, [ $\text{g}$ ].

There are two important physicochemical aspects for the evaluation of the sorption process as a unit operation, namely the equilibrium of sorption and the kinetics. Biosorption equilibrium is established when the concentration of metal in a bulk solution is in dynamic balance with that of the interface [3, 16]. The  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions biosorption capacities of all tested adsorbents were presented as a function of the initial concentration of metal ions within the aqueous biosorption medium:

$$q_{eq} = f(C) \quad (2)$$

where:  $q_{eq}$  is the amount of adsorbed metal ions on the biosorbent at the equilibrium, [ $\text{mg}\cdot\text{g}^{-1}$ ];  $C$  – initial concentration of metal ions, [ $\text{mg}\cdot\text{l}^{-1}$ ].

In order to examine the controlling mechanism of a biosorption process such as mass transfer and chemical reaction, kinetic models were used to study the experimental data. The kinetic models (the first-order and second-order equations) can be used in this case assuming that



measured concentrations are equal to cell surface concentrations. The experimental data were fitted to the first-order rate equation of Lagergren, which is one of the most widely used equations for the sorption of solute from a liquid solution [10]. It may be represented as follows:

$$\frac{dq}{dt} = k_{1,ads} (q_{eq} - q) \quad (3)$$

where:  $q_{eq}$  is the amount of adsorbed metal ions on the biosorbent at the equilibrium, [ $\text{mg}\cdot\text{g}^{-1}$ ];  $q$  – the amount of adsorbed metal ions on the biosorbent at any time  $t$ , [ $\text{mg}\cdot\text{g}^{-1}$ ];  $k_{1,ads}$  – the Lagergren rate constant of the first-order biosorption, [ $\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ ].

Integrating (3) between the limits,  $t = 0$  to  $t = t$  and  $q = 0$  to  $q = q_{eq}$ , it becomes:

$$\log(q_{eq} - q) = \log q_{eq} - \frac{k_{1,ads}}{2.303} t \quad (4)$$

A plot of  $\log(q_{eq} - q_t)$  against  $t$  should give a straight line confirming the applicability of the kinetic model. In a true first-order process  $\log q_{eq}$  should be equal to the intercept of a plot of  $\log(q_{eq} - q_t)$  against  $t$ .

The pseudo-second order model is based on the assumption that biosorption follows a second order mechanism. So, the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites:

$$\frac{dq}{dt} = k_{2,ads} (q_{eq} - q)^2 \quad (5)$$

where:  $k_{2,ads}$  is the rate constant of second order biosorption, [ $\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ ].

Integrating (5) for the boundary conditions  $t = 0$  to  $t = t$  and  $q = 0$  to  $q = q_{eq}$ , and then linearizing it, determines an equation (6) such as:

$$\frac{t}{q} = \frac{1}{k_{2,ads} \cdot q_{eq}^2} + \frac{1}{q_{eq}} t \quad (6)$$

where:  $q_{eq}$  and  $k_{2,ads}$  can be calculated from the slope and the intercept of the plot  $t/q$  versus  $t$ . It is important to notice that it is not necessary to estimate the experimental value of  $q_{eq}$  for the application of such a model.

## Results and discussion

The amount of immobilized cells on synthetic polymer was  $38.4 \text{ mg g}^{-1}$  dry carriers. Heavy metals biosorption ability of the covalently bound cells after immobilization treatment was studied. Cells were grown in defined medium first with 10% glucose without presence of  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ , and then subsequently in defined medium with presence of both investigated heavy metals' ions. Biomass growth and glucose consumption on a defined medium are shown in Fig. 1 and Fig. 2 respectively.

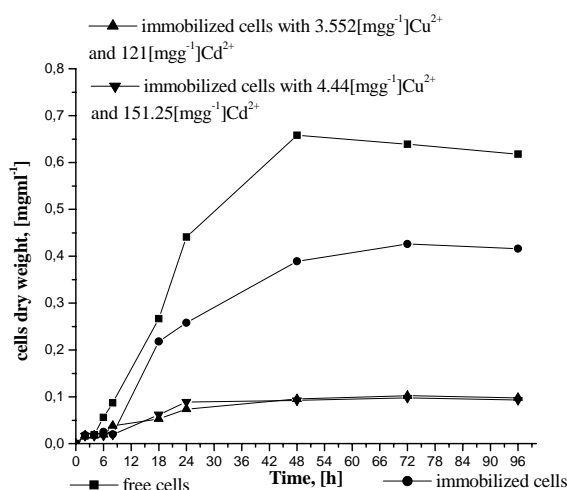


Fig. 1 Growth of free and immobilized yeast cells without presence and with presence of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions

As can be seen from the figure free and covalently immobilized cells show clearly determined growth phases, such as the lag phase of covalently immobilized cells are much longer than the free cells. In the presence of heavy metals during the incubation time has not clearly determined growth phases of the strain.

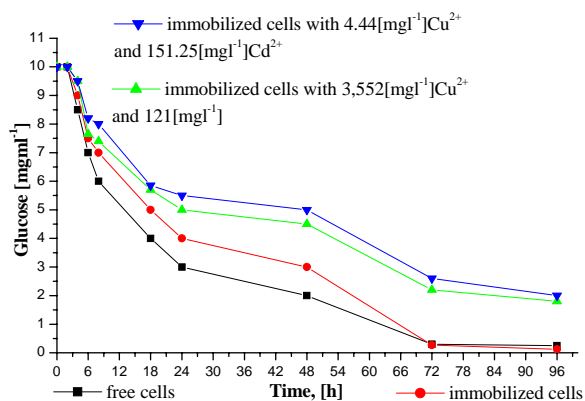


Fig. 2 Glucose consumption by free and immobilized yeast cells without presence and with presence of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions

The results show that the cells treated with heavy metals consume in the lower range glucose than the free and immobilized cells without presence of the  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  ions. It is probably due to of toxic effect of the heavy metals in the nutrient medium and the less growth of the biomass.

### Biosorption rate

The biosorption rates of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  on the free and immobilized cells were obtained by following the decrease of the concentration of  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  within the adsorption medium with time.

Fig. 3 shows the biosorption of copper ions by immobilised and free cells in a one-component system. The adsorption rate is higher at the beginning of the process and the copper ion saturation level can be observed at the 48<sup>th</sup> hour of the incubation time for both initial concentrations (3.552 mg·l<sup>-1</sup>, 4.44 mg·l<sup>-1</sup>) under study.

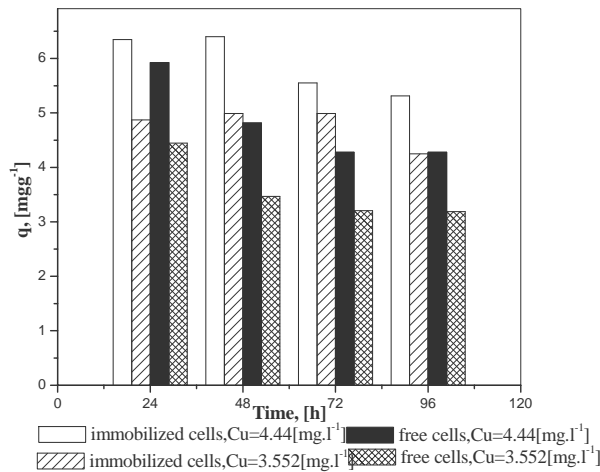


Fig. 3 Biosorption of copper ions by free and immobilized cells in a one-component system

Fig. 4 shows the biosorption of cadmium ions by immobilised and free cells in a one-component system. As can be seen from the figure the adsorption rate is higher at the beginning of the process and the cadmium ion saturation level is reached at the 96<sup>th</sup> hour of the incubation process for both initial concentrations (151.25 mg·l<sup>-1</sup>, 121 mg·l<sup>-1</sup>).

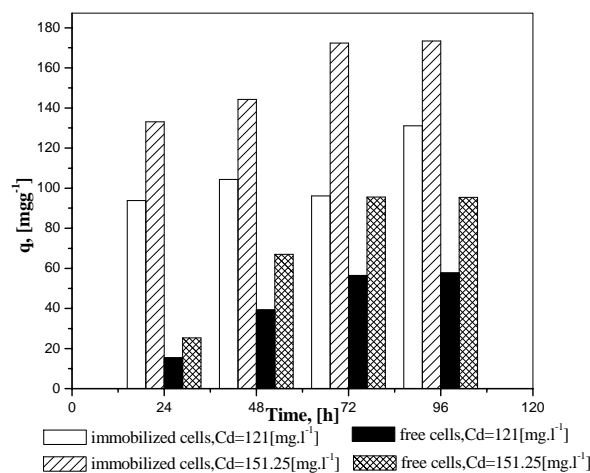


Fig. 4 Biosorption of cadmium ions by free and immobilized cells in a one-component system

The experimental results show that the biosorption ability of the immobilised cells is better compared to the free cells.

The biosorption of metal ions by immobilised and free cells was also tested in two-component solutions:

Solution-1:  $4.44 \text{ mg}\cdot\text{l}^{-1} \text{ CuSO}_4$  and  $151.25 \text{ mg}\cdot\text{l}^{-1} \text{ CdSO}_4$  and solution-2:  $3.552 \text{ mg}\cdot\text{l}^{-1} \text{ CuSO}_4$  and  $121 \text{ mg}\cdot\text{l}^{-1} \text{ CdSO}_4$ .

As can be seen from the following two figures (Fig. 5 and Fig. 6), the biosorption rate is higher at the beginning of the process and the metal ion saturation levels are completely reached at the 72<sup>th</sup> and 96<sup>th</sup> hour of the incubation time, for both systems respectively. Again the immobilised cells showed better biosorption capacity than the free cells.

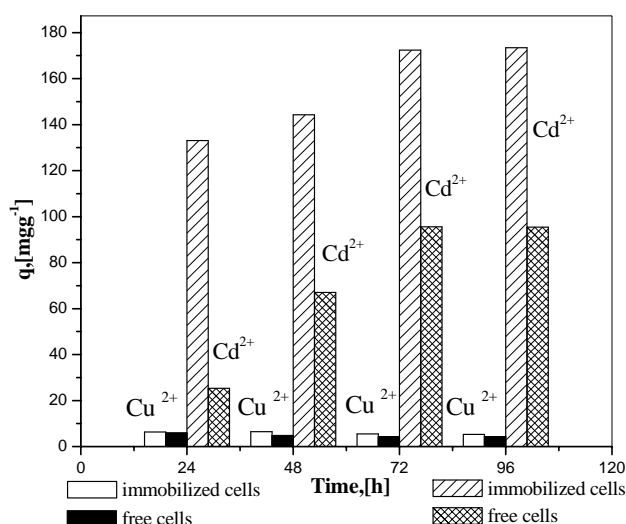


Fig. 5 Biosorption of copper and cadmium ions by immobilized and free cells in solution 1

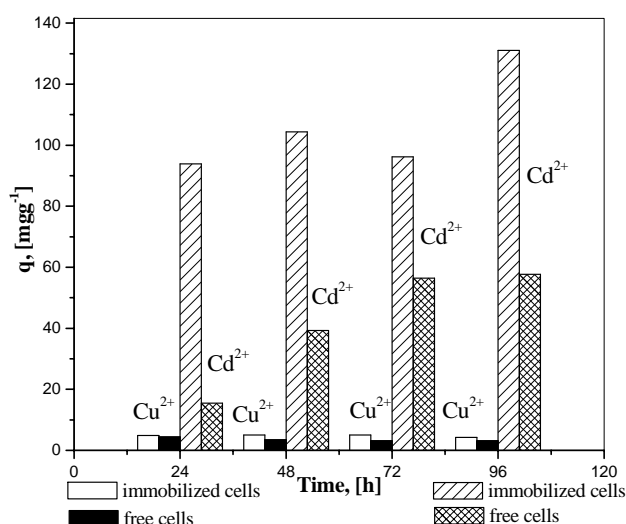


Fig. 6 Biosorption of copper and cadmium ions by immobilized and free cells in solution 2

### Determination of the kinetic parameters of a one-component system

Chemical reaction kinetic models were used to analyse the experimental data in order to study the controlling mechanism of the biosorption process of copper and cadmium ions by immobilised cells of *Trichosporon cutaneum* R57, as well as the effect of the mass transfer. The applicability of the two models to the experimental data was compared.

The values of  $q_{eq}$ ,  $q_{exp}$ ,  $k_{2,ads}$  and  $R^2$  (correlation coefficient) obtained through the second order kinetic model are presented in the Table 1.

Table 1. Parameters values

Initial metal-concentration [mg·l <sup>-1</sup> ]	Second-order kinetic model			
<b>Cd<sup>2+</sup></b>	$k_{2,ads}$ [g·mg <sup>-1</sup> ·min <sup>-1</sup> ]	$q_e$ [mg·g <sup>-1</sup> ]	$q_{exp}$ [mg·g <sup>-1</sup> ]	$R^2$
C <sub>0</sub> = 30.25	0.0021290	33.445	36.74	0.8145
C <sub>0</sub> = 121.00	0.0007809	166.667	157.33	0.9730
<b>Cu<sup>2+</sup></b>				
C <sub>0</sub> = 3.552	0.3232000	4.060089	4.2840	0.9992
C <sub>0</sub> = 4.440	0.4362500	4.413063	4.5994	0.9973

The theoretical values ( $q_{eq}$ ), calculated using the first order kinetic model (4), significantly differ from the experimentally obtained values and the computed correlation coefficients are low. The negative values obtained for the rate constant correspond to the experimental observation of decreasing kinetic curve in the case of the highest 151.25 mg·l<sup>-1</sup> initial concentration.

The correlation coefficients for the graphics from  $t/q$  versus t for the second order model are higher. The theoretical values for  $q_{eq}$  for the investigated single-component systems of copper and cadmium ions biosorption by immobilised cells are very close to the experimental  $q_{exp}$  values when a second order kinetic model is used.

Therefore, the second order kinetic model describes the experimental data better than the first order kinetic model. This is presented on the figures below:

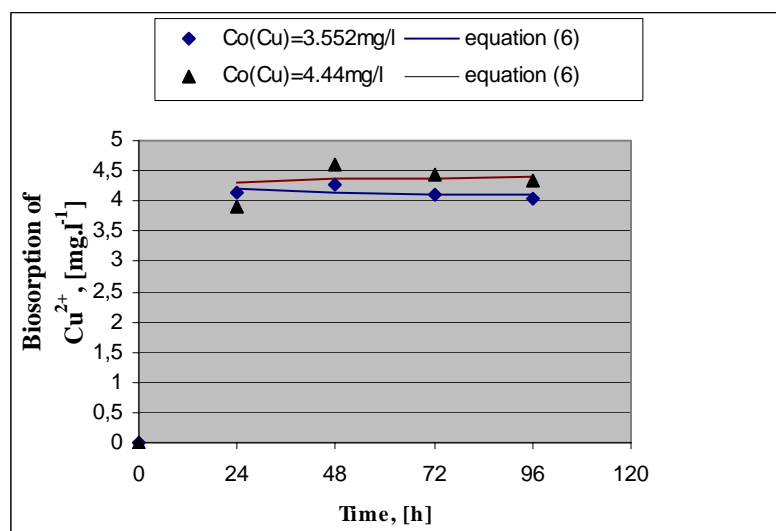


Fig. 7 Kinetics of the copper ions biosorption by immobilised cells *Trichosporon cutaneum* R57 relative to two initial concentrations



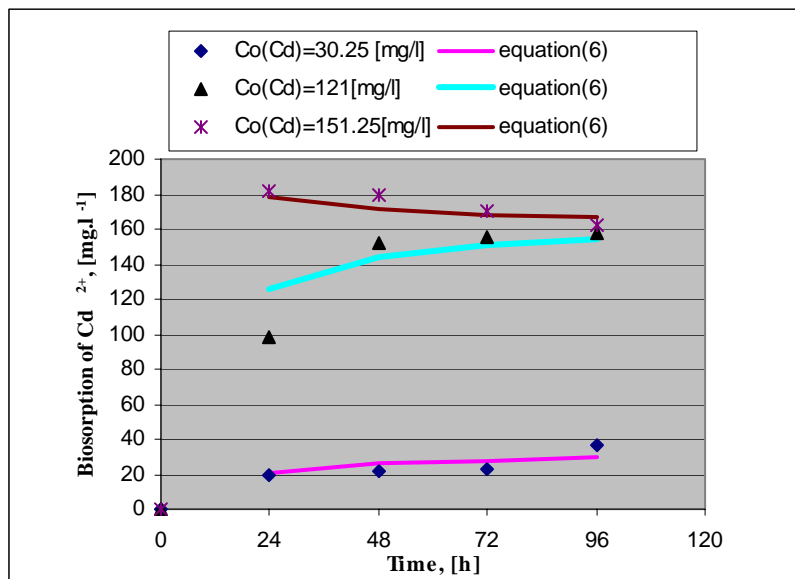


Fig. 8 Kinetics of the cadmium ion biosorption by immobilised cells *Trichosporon cutaneum* R57 relative to three initial concentrations

The second model has been successfully applied to biosorption of various metals by different microorganisms [4, 5, 11]. Other authors [17] have also found that there is a tendency the values of  $k_{2,ads}$  [ $\text{g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ ] to decrease with the increase of the cadmium ions in the medium. Investigations of the kinetic parameters of biosorption of copper ions by brown alga *Sargassum* [4] also produced high correlation coefficients when the second order kinetic model was applied.

#### Comparison of heavy metal ions biosorption depending on the number of components of the system

The following two figures (Fig. 9 and Fig. 10) show the biosorption of cadmium and copper ions by immobilised cells of *Trichosporon cutaneum* R57 depending on the number of the components of the system.

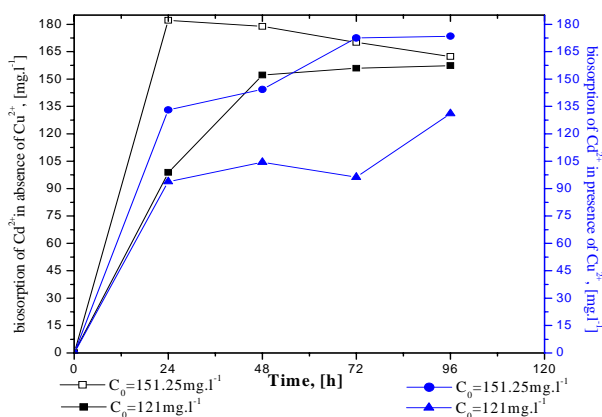


Fig. 9 Biosorption of cadmium ions by immobilised cells relative to the number of the components of the system

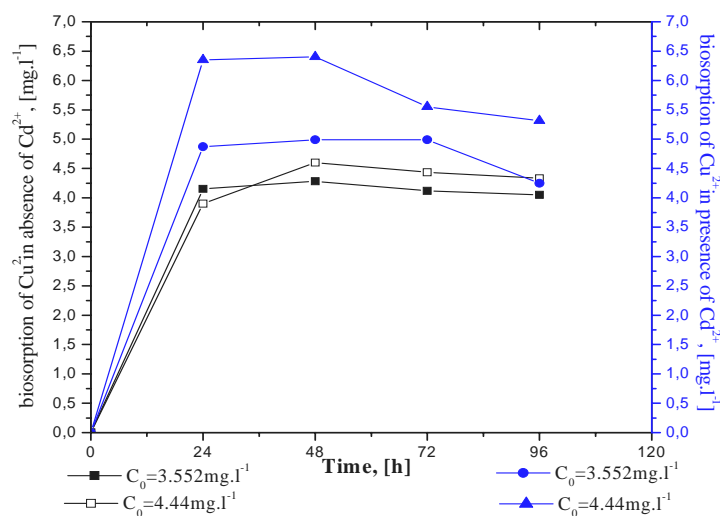


Fig. 10 Biosorption of copper ions by immobilised cells relative to the number of the components of the system

It can be seen from the figures above that the biosorption rate of each ion in the two-component system is affected by the presence of the second one. In the two-component system the adsorption of  $\text{Cu}^{2+}$  is stimulated while the adsorption of  $\text{Cd}^{2+}$  is suppressed by the presence of the second component – copper ions. Fig. 9 clearly shows that the adsorption of  $\text{Cd}^{2+}$  increases after 48 hours in the presence of copper ions so competitive adsorption takes place. Most probably, the competitive adsorption of the copper ions on the account of the cadmium ions is due to the passive mechanisms of sorption of the ions by the cells of the studied strain. In this type of biosorption the ions accumulate in the cells without cell energy expenditure. The future investigations intend to reveal the exact mechanism of this adsorption.

## Conclusions

The filamentous yeast strain *Trichosporon cutaneum* R57 showed moderate tolerance of growth and bioaccumulation ability to cadmium and copper ions, which can be a useful tool for further investigations to elucidate the mechanisms of bioremediation of metal ions from wastewaters.

This work indicates that investigated yeasts cells (free and covalently immobilized biomass to synthetic carrier) can be used for the removal of cadmium and copper ions from wastewaters with a biosorption capacity comparable or higher than other commercial materials.

The experimental results show that the biosorption ability of the immobilized cells is better compared to the free cells, as well as in bicomponent solutions. The kinetics of the individual biosorption of copper and cadmium ions by immobilised cells of *Trichosporon cutaneum* R57 is successfully described by a second order kinetic model. From the analysis of the experimental kinetic curves for the bicomponent system, it can be seen that the adsorption of  $\text{Cu}^{2+}$  is stimulated while the adsorption of  $\text{Cd}^{2+}$  is suppressed by the presence of the second component.

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