

## Mathematical Model of Lead Biosorption by *Rhizopus nigricans* Pellets in a Laboratory Batch Stirred Tank

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The aim of this work was to study the  $Pb^{2+}$ -ions biosorption rate with the *Rhizopus nigricans* biomass in a batch stirred tank and to examine the process characteristics with a simple mathematical model. Self immobilized biomass in the form of 2.5–0.5 mm pellets was used in a laboratory scale vessel of 100 ml working volume. Biomass concentrations from 25 to 200 g of wet mass per liter of biomass suspension were used while the initial lead mass concentrations varied from 20 to 300 mg  $l^{-1}$ .

Mathematical model is based on the assumption that the rate of biosorption determines the rate of the process. It combines the differential mass balance equation of the batch reactor and the biosorption rate equation, which is considered as a reversible reaction between metal ions and free binding sites on the biomass. On the basis of lead biosorption rate data and the applied mathematical model, the Langmuir adsorption mechanism was confirmed and the average adsorption rate coefficient  $k_{ads}$   $l\text{mg}^{-1}\text{min}^{-1}$  was determined.

*Key words:*

Biosorption, lead, batch stirred tank, mathematical model, *Rhizopus nigricans*

### Introduction

The guides for the sustainable development challenge the research on the novel, economically acceptable techniques of waste water treatment. Special attention is paid to the exploitation of bio-renewable resources. Having that in mind, the biosorption is considered a novel approach to waste management with a great potential in the future. During the past decade, it has been extensively studied as an alternative method of metal sequestering.<sup>1–4</sup> Some very promising characteristics of this process were found; for example its suitability for the treatment of large volumes of effluents with low concentration of pollutants and also a fact that the process does not depend on the viability of the biomass.<sup>5,6</sup> Therefore, the waste biomass as a low cost material can be used for this purpose. Consequently, no nutrient requirement, simple operating performance, and easy maintenance are of the most important advantages of this process. In the majority of published research work, dried, ground and sieved to an appropriate particle size biosorbents, were used.<sup>7–9</sup> The reports of using fresh, non-modified self-pelleted form of the biomass are scarce, although the waste mycelium after the primary exploitation of industrially important species such as *Aspergillus*, *Penicillium*, *Phanerochaete*, *Rhizopus* and *Streptomyces* is available for this purpose.<sup>10,11</sup>

In general, the performance of the process including economical aspects depends on the reactor type, liquid flow characteristics, as well as the kinetic and mechanical characteristics of the biomass. There is no direct evidence which operating system is most effective for the process of metal uptake using microbial biosorbents.

The open literature deals with the biosorption which involve various metal ions, different forms and states of microorganisms and also different reactor design. However, the kinetics of the biosorption was most commonly studied in the stirred tank. Several mechanisms of metal uptake were proposed, based on the different types of equilibrium reaction kinetics, mass transfer in the stagnant film around particles, through the non-absorbing layers of immobilizing agent or within the pores of the biosorbent, depending on the biomass characteristics. Mathematical models were sometimes developed to describe the process of the biosorption in those particular cases. Often the authors discovered that the biosorption reaction was fast and that the prevailing rate limiting step was the mass transfer, either the external,<sup>12,13</sup> the internal or both.<sup>14–17</sup> Therefore the majority of the models in the open literature propose mass transfer controlled kinetics while for the intrinsic reaction of the biosorption they presume the rapid equilibrium at the biomass surface. The reaction kinetic models seldom appear in the open literature. The approach of the overall

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biosorption reaction kinetics was applied by *Kuyucak* and *Volesky* (1989), who determined the first order overall reaction kinetics. Simple first or second order reaction kinetics and pseudo first or pseudo second order reaction kinetics were also suggested.<sup>19</sup>

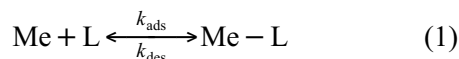
In the present work, a possibility for the use of waste biomass from fermentation industry as an efficient biosorbent for lead ions, has been studied. The lead biosorption from aqueous solutions was performed by non-living, self-immobilized pelleted growth form of fungus *Rhizopus nigricans* in a batch stirred tank. This type of biomass is of particular interest because of its relatively high biosorption capacity and its pelleted form, which is beneficial for the use in biosorption contactors where it facilitates the solid-liquid separation.

The aim of this work was to develop a simple mathematical model of the process which would describe the complex behaviour in a batch stirred tank. The model was based on the basic biosorption reversible reaction kinetics, which was assumed to determine the rate of the process, while the mass transfer resistances were considered to be insignificant in a well-agitated system with highly porous biosorbent. The comparison of the experimental and the model based calculated data of the lead biosorption allowed the examination of the biosorption mechanism and the estimation of the average biosorption rate coefficient, which is necessary for the process scale-up.

## Mathematical model

The mathematical model is based on the assumption of an ideally mixed slurry reactor. A highly porous self-pelleted biomass, which enables the access of liquid to the interior of the particles, was used as the biosorbent. In such a system, the external and internal particle mass transfer resistances are considered to be insignificant so that the rate of the biosorption itself determines the overall rate of the process. The model combines the differential mass balance equation of the batch system with suitable initial conditions and the biosorption rate equation into a single differential equation, which was solved for the unknown parameters numerically.

The biosorption is considered as a reversible reaction between metal ions (Me) and free binding sites (L), which leads to the formation of complexes (Me-L):



The rate of the biosorption is indicated as decreasing of the metal ion concentration in the bulk solution and can be written as:

$$-\frac{d[\text{Me}]}{dt} = k_{\text{ads}} [\text{Me}] [\text{L}] - k_{\text{des}} [\text{Me} - \text{L}] \quad (2)$$

where  $k_{\text{ads}}$  and  $k_{\text{des}}$  are adsorption and desorption rate coefficients. In our model, the metal concentration [Me] is the concentration of free lead ( $\text{Pb}^{2+}$ ) ions in the solution, represented by symbol  $\gamma_{\text{Pb}^{2+}}$ . The mass concentration of free biomass binding sites  $\gamma_{\text{L}}$  can be expressed as the difference between the total ( $q_0'$ ) and the occupied biomass binding sites mass concentration ( $q'$ ) per unit volume of the suspension:

$$\gamma_{\text{L}} = q_0' - q' \quad (3)$$

From the equations 2 and 3, the familiar Langmuir adsorption isotherm can be derived:

$$q_0' = \gamma_{\text{X}_0} \frac{Q_{\text{max}} \gamma_{0, \text{Pb}^{2+}}}{K + \gamma_{0, \text{Pb}^{2+}}} \quad (4)$$

where  $\gamma_{\text{X}_0}$  represents the dry biomass concentration in the suspension,  $Q_{\text{max}}$  is the maximum  $\text{Pb}^{2+}$  biosorption production of the biomass and  $K$  the  $\text{Pb}^{2+}$ -biomass complex dissociation constant:

$$K = \frac{k_{\text{des}}}{k_{\text{ads}}} \quad (5)$$

The differential mass balance and the proper initial conditions can be written for the batch stirred tank:

$$-\frac{d\gamma_{\text{Pb}^{2+}}}{dt} = \frac{dq'}{dt} \quad (6)$$

$$t = 0, \quad \gamma_{\text{Pb}^{2+}} = \gamma_{0, \text{Pb}^{2+}}, \quad q' = 0 \quad (7)$$

The above equations can be rearranged and coupled into a final rate equation:

$$-\frac{d\gamma_{\text{Pb}^{2+}}}{dt} = k_{\text{ads}} \gamma_{\text{Pb}^{2+}} \{q_0' - (\gamma_{0, \text{Pb}^{2+}} - \gamma_{\text{Pb}^{2+}})\} - k_{\text{ads}} K (\gamma_{0, \text{Pb}^{2+}} - \gamma_{\text{Pb}^{2+}}) \quad (8)$$

The only unknown variable in the Equation 8 is the adsorption rate constant  $k_{\text{ads}}$ . The  $\text{Pb}^{2+}$  mass concentration in the biomass, which is in the equilibrium with the initial  $\text{Pb}^{2+}$  mass concentration in the solution ( $q_0'$ ), was calculated from the Langmuir adsorption isotherm (Equation 4). It has already been shown in our previous publication<sup>10</sup> that the Langmuir adsorption isotherm described the bio-

sorption equilibrium of  $\text{Pb}^{2+}$  by mycelial pellets of fungus *R. nigricans* well.

The proposed kinetic model (Equation 8) was fitted to the experimental data,  $\text{Pb}^{2+}$  vs.  $t$ , with the aid of the Madonna computer programme, where Runge-Kutta method was applied to find the best fit.

## Material and methods

### Biosorbent preparation

The biosorbent used in the experiments was a self-pelleted biomass of the fungi *Rhizopus nigricans*, which was grown in a submersed culture as it was described earlier.<sup>10,11</sup> The formed pellets of almost regular spherical shape had an average diameter  $2.5 \pm 0.5 \mu\text{m}$ . For the experimental use the biomass was defrosted and washed with deionized water several times. The detailed procedure of biosorbent preparation is described in our previous publications.<sup>10,11</sup>

### Biosorption experiments

The metal uptake experiments were performed in a well-agitated batch stirred tank with 100 ml working volume (5.6 cm vessel diameter), placed on the magnetic stirrer (1.8 cm stirrer width) and stirred at 300 rpm. A known wet mass of mycelial pellets ( $m_{\text{wet}}$ ) was added into 95 ml of deionized water to set the desired biomass concentrations, from 25 to 200 g of wet biomass per liter of biomass suspension. A minimal amount of concentrated solution of  $\text{Pb}(\text{NO}_3)_2$  with the initial  $\text{Pb}^{2+}$  mass concentration  $10 \text{ g l}^{-1}$  was added into the prepared suspension of fungal pellets to give the desired initial  $\text{Pb}^{2+}$  mass concentrations ( $\text{Pb}^{2+}$ ) from 20 to 300  $\text{mg l}^{-1}$ . The diminishing of  $\text{Pb}^{2+}$  concentration was recorded as a function of the initial  $\text{Pb}^{2+}$  mass concentration and the biomass loading. At the end of each experiment the total volume of the suspension ( $V_0$ ) was measured and the dry mass of the biomass ( $m_{\text{dry}}$ ) was determined by drying the filtered biomass to a constant mass. The experiments were repeated at least twice.

### Concentration determination

The measurements of  $\text{Pb}^{2+}$  mass concentration in the solution were performed on-line with a lead ion selective electrode ( $\text{Pb} \text{ ISE}$  | R502, WTW, Wilhelm). The electrode measurements enabled on-line monitoring of the bulk phase  $\text{Pb}^{2+}$  mass concentration as well as fast and simultaneous data acquisition.

The time constant of the lead ion selective electrode was determined to be in the range of a few seconds (2–8) being considered as a system of the first order (Fig. 1).

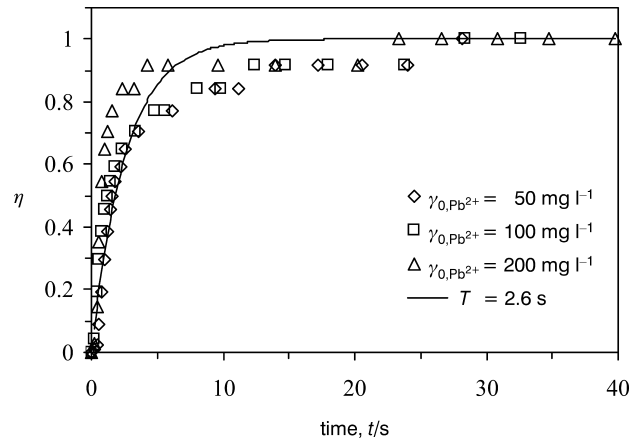


Fig. 1 – The time response of the lead ion selective electrode at different  $\text{Pb}^{2+}$  mass concentrations in the solution and the theoretical response of the first order electrode with time constant 2.6 s

The additional samples of the filtered suspension were taken several times to verify the concentration on an atomic absorption spectrometer (Perkin-Elmer 2280) (Fig. 2).

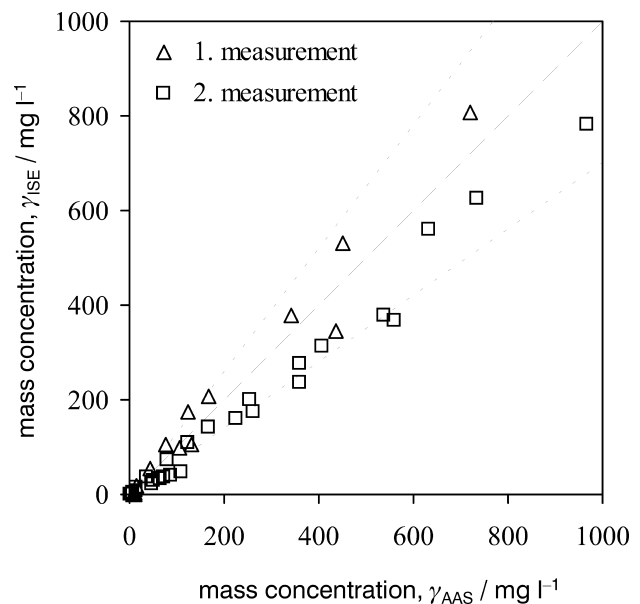


Fig. 2 – Comparison of the  $\text{Pb}^{2+}$  mass concentrations measured by lead ion selective electrode (ISE) and atomic absorption spectrometer (AAS). The dotted lines represent the 30 % ISE experimental error

### Biosorbent characterization

A few representative mycelial pellets were immobilized into a paraffin wax by gradually replacing water phase with series of increasing concentrations of alcohol (ethanol), non polar solvents (xylol), and at last paraffin. The solid preparations were cut into  $20 \mu\text{m}$  thick slices and fixed onto the object glass. Then the paraffin wax was removed from

preparations by desolving in the same solvents in the opposite direction. Microscopic preparations were then photographed under the microscope. From the micrographs the approximate diameter of the mycelial hyphae was determined to be in the range from 5 to 10  $\mu\text{m}$ . The diameter of each slice was calculated from the area of the circle, which area was equal to the outlined area of the slice (equivalent radius). The »greyness« of the outlined area of the slice was determined from the binarized picture (Mean). The porosity of the particle was calculated from the porosity of each slice considering the ratio of slice thickness and hyphae diameter. For the image analysis of the pictures, the computer programme SCION (<http://www.scioncorp.com>) was used.

From the Figure 3 it can be seen, that the estimated pellet porosity does not depend significantly on the slice radius, therefore the pellet porosity was approximated to be the average of the estimated porosity from each slice.

## Results and discussion

In the batch stirred tank it is possible to study the biosorption kinetics at different initial conditions such as biomass and metal ion concentration. However, to directly determine the biosorption kinetics the mass transfer resistance must be avoided by properly setting the process conditions. The satisfactory mixing was confirmed by the short response time of the system in the blank experiment

with no biomass added. The time constant of the blank system was in the range from 5 to 13 s being considered as a system of the first order (Fig. 4). In this well agitated system the external mass transfer resistance was considered to be negligible.

The biosorbent used was in the form of regular spheres and had a highly porous hyphal structure. The pellet porosity was estimated by image analysis to be 0.95 (Fig. 3). The porosity of the fungal pellet represents the volume fraction of the continuous void space between the hyphae, where the fluid can move freely. In a pellet, the internal mass transfer rate could be higher compared to the diffusion rate

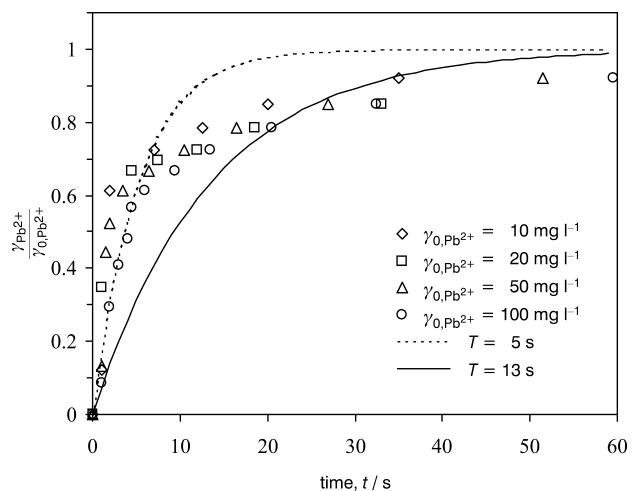


Fig. 4 – The time response of the blank system at different  $\text{Pb}^{2+}$  mass concentrations in the solution and the first order theoretical responses of the system with time constants 5 and 13 s

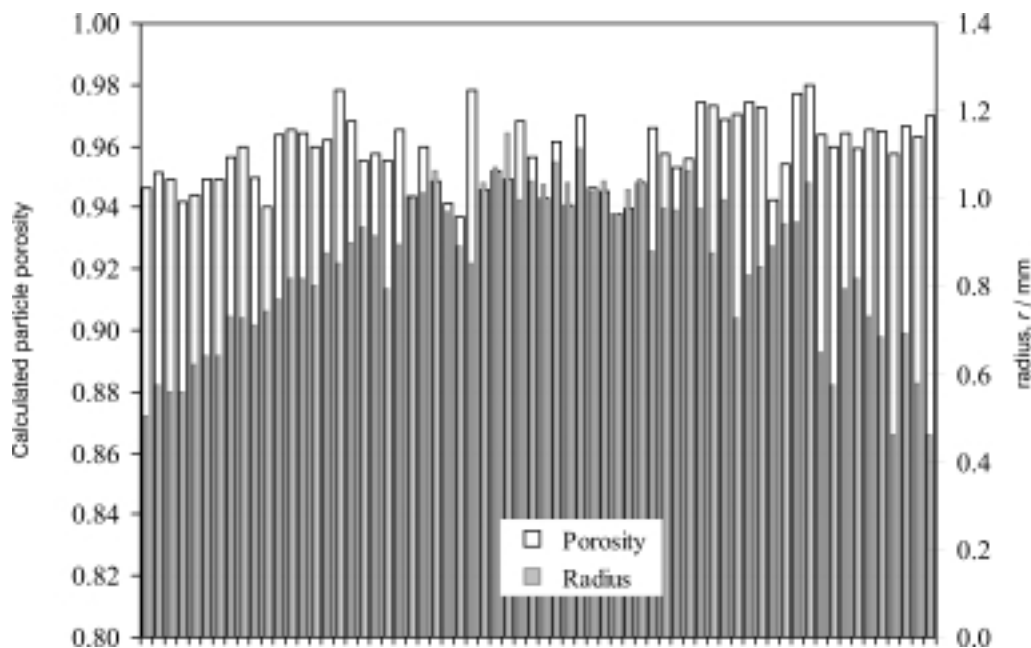


Fig. 3 – The radius of several successive slices from solid preparation of mycelial pellet determined by image analysis and the calculated porosity of the particle

in a solid particle with porous structure. According to this, we considered the internal mass transfer resistance to be insignificant and was therefore omitted from the mathematical model.

The shape of the bulk  $\text{Pb}^{2+}$  mass concentration curves obtained in the presented experimental system (Figures 5 and 6) implies that the kinetic equation should describe the two characteristics of the batch system response: the steep initial drop of the  $\gamma_{\text{Pb}^{2+}}$  mass concentration in the solution and the final establishment of the equilibrium. By fitting the model (Equation 8) to the experimental data we tried to confirm the selected mechanism of the biosorption and to determine the biosorption rate constant.

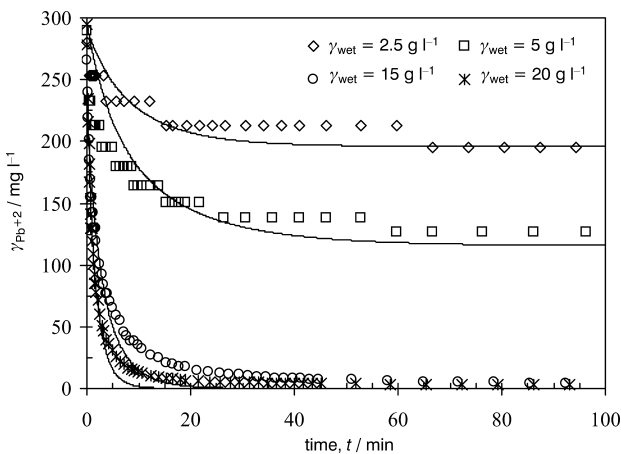


Fig. 5 – The comparison of the experimental data at different wet biomass concentrations,  $\gamma_{\text{wet}}$  and the model best fit (—) at the initial  $\text{Pb}^{2+}$  mass concentration  $\gamma_{0,\text{Pb}^{2+}} = 300 \text{ mg l}^{-1}$

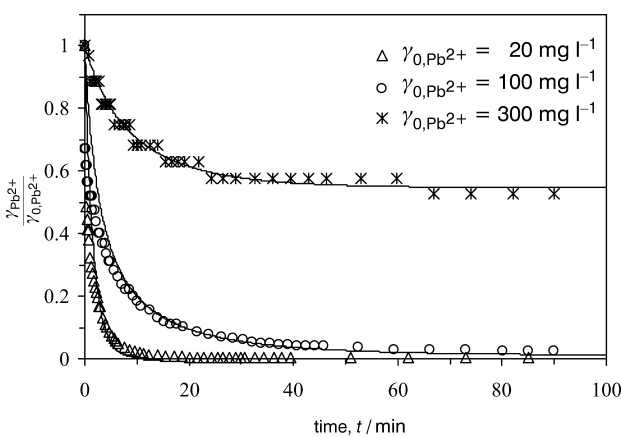


Fig. 6 – The comparison of the experimental data at different initial  $\text{Pb}^{2+}$  concentrations and the model best fit (—), at the wet biomass concentration  $\gamma_{\text{wet}} = 5 \text{ g l}^{-1}$

In the Table 1 the model parameters are summarized. The nominal  $\text{Pb}^{2+}$  mass concentrations,  $\text{Pb}^{2+}$  – nominal, were calculated from the known volume of the concentrated  $\text{Pb}^{2+}$  solution added and

Table 1 – Estimated parameters of the biosorption kinetics model. Experimentally determined values set as a model constants and numerically determined parameters of the best model fit

$\gamma_{0,\text{Pb}^{2+}}$ [mg l <sup>-1</sup> ] – nominal	20	50	100	300
* $Q_{\text{max}}$ [mg g <sup>-1</sup> ]	83.5	83.5	83.5	83.5
* $K$ [mg l <sup>-1</sup> ]	8.06	8.06	8.06	8.06
$m$ [g]	0.207	0.140	0.120	0.132
$V_0$ [l]	0.096	0.107	0.10	0.098
$\gamma_{0,\text{Pb}^{2+}}$ [mg l <sup>-1</sup> ]	16	40	80	240
$k_{\text{ads}}$ [l mg <sup>-1</sup> min <sup>-1</sup> ]	$6.8 \cdot 10^{-4}$	$9.3 \cdot 10^{-4}$	$4.6 \cdot 10^{-4}$	$6.7 \cdot 10^{-5}$

$\gamma_{\text{wet}}$ [g l <sup>-1</sup> ] – nominal	2.5	5	15	20
* $Q_{\text{max}}$ [mg g <sup>-1</sup> ]	83.5	83.5	83.5	83.5
* $K$ [mg l <sup>-1</sup> ]	8.06	8.06	8.06	8.06
$m_{\text{dry}}$ [g]	0.130	0.245	0.688	0.780
$V_0$ [l]	0.11	0.11	0.11	0.11
$\gamma_{0,\text{Pb}^{2+}}$ [mg l <sup>-1</sup> ]	292	298	245	285
$k_{\text{ads}}$ [l mg <sup>-1</sup> min <sup>-1</sup> ]	$5.7 \cdot 10^{-5}$	$5.9 \cdot 10^{-5}$	$1.0 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$

\* published in ref. 11

represent the individual experiments. Likewise the nominal biomasses,  $m_{\text{wet}}$  – nominal, represent the individual experiments with different starting wet biomass concentrations. To find the best fit, some of the model parameters were set to constants.  $Q_{\text{max}}$  and  $K$  were determined previously in the equilibrium experiments<sup>10</sup> to be  $83.5 \text{ mg g}^{-1}$  of biomass and  $8.06 \text{ mg l}^{-1}$  and were set as constants in this experiment to minimize the number of unknown parameters. The dry mass of the biomass,  $m_{\text{dry}}$ , and the volume of the suspension,  $V_0$ , were set as a constant values for each run and were determined as described in *Material and methods* section. Although the electrode and the system as a whole had relatively small time constants, it was practically impossible to measure the initial  $\text{Pb}^{2+}$  concentration and  $\text{Pb}^{2+}$  mass concentrations at the very beginning of the process. During the experiments it was noticed, that the determined  $\text{Pb}^{2+}$  mass concentrations at the beginning of the reaction were lower than the nominal or set initial  $\text{Pb}^{2+}$  mass concentrations. The lower response of the electrode was probably due to the »non ideal« solution of pellet suspension, which differs from standard  $\text{Pb}^{2+}$  solutions in which calibration was performed. Because of the difficulties

with the determination of the initial  $\text{Pb}^{2+}$  mass concentration, the model quantity  $\gamma_{0,\text{Pb}^{2+}}$  was allowed to deviate from nominal values up to  $\pm 20\%$ , which was in the limits of the determined experimental error of the electrode (Fig. 2).

By fixing or limiting most of the model parameters, the only unknown parameter of the model remained the adsorption rate coefficient,  $k_{\text{ads}}$ . It was determined from the best fit of the model to the experimental data for each experiment separately. In the Table 1 only one of the repeated experiments from each series with most suitable determined model parameters is presented. The adsorption rate constants determined from other experiments within the same series agree approximately within  $\pm 40\%$ .

The proposed reversible kinetic model describes well the experimental concentration curves. The agreement between the experimental data and the model is better at lower biomass concentrations ( $\gamma_{\text{wet}} = 2.5 \text{ g}$  and  $\gamma_{\text{wet}} = 5 \text{ gY}^{-1}$ ), where the final equilibrium of  $\text{Pb}^{2+}$  mass concentrations in the solution have substantial values (Fig. 3). The higher biomass concentration represent a great initial biosorption potential. In such case the initial rate of the biosorption can no longer be assumed to be the rate limiting step of the process and the mass transfer resistances can not be neglected any more. Accordingly, some discrepancies can be observed between the experimental and modelled concentrations in the cases with high biomass concentrations ( $\gamma_{\text{wet}} = 15 \text{ g}$  and  $\gamma_{\text{wet}} = 20 \text{ gY}^{-1}$ ) (Fig. 3). At the beginning of the process, the predicted  $\text{Pb}^{2+}$  concentrations are lower than measured because the model does not account for the mass transfer rate limitations. A good agreement between experimental data and the model can be observed also at different initial  $\text{Pb}^{2+}$  mass concentrations in the solution at low initial biomass concentration  $\gamma_{\text{wet}} = 5 \text{ gY}^{-1}$ . This again confirms the predicted biosorption kinetics at the conditions of no mass transfer limitations (Fig. 3).

The presumption of insignificant internal mass transfer resistance was checked by calculation of the respective Thiele moduli and estimation of the effectiveness factor<sup>20</sup>. At given experimental conditions Thiele moduli were in the range from 0.5 to 2, which corresponds to effectiveness factors from 0.9 to 0.4. Higher values of Thiele modulus were obtained at higher initial lead mass concentrations and higher biomass concentrations. From the estimated values of the effectiveness factors it can be seen that the internal mass transfer resistance can not be totally neglected in all the experiments. This explains the observed deviations between the mathematical model and the experimental data in particular cases described above.

The adsorption rate coefficient determined from the best fit of the model vary in a broad range among experiments (Table 1). Significant deviations can be ascribed mainly to the poor repeatability of the experiments in a biological system and partly to the simplification of negligible mass transfer resistances in the applied mathematical model. The average adsorption rate coefficient was estimated to be  $k_{\text{ads}} = 8 \times 10^{-4} \text{ Yng}^{-1} \text{ min}^{-1}$ . From Equation 5 also the desorption rate coefficient could be easily calculated,  $k_{\text{des}} = 2 \times 10^{-3} \text{ Ymin}^{-1}$ . The estimated order of magnitude of the rate coefficients is valuable for the process design and scale-up.

In general, good agreement of the model and the experimental data at the conditions, where the presumptions of neglected mass transfer resistances can be accepted, confirms the proposed reversible mechanism of the biosorption (Eq. 1).

## Conclusions

The presented work shows the possibility of the use of biosorption as a perspective method for lead removal from waste water. In addition, biomass is a cheap natural source of biosorbent. If it is available as a waste from other fermentation industries this represents further advantage to the process, regarding to the cumulative costs and simplicity of the technology. The non-living biomass of fungus *Rhizopus nigricans* was shown to be an efficient biosorbent for lead ions with a promising biosorption capacity. Its self-pelleted growth form facilitates manipulation in a standard adsorption process equipment especially the final solid-liquid separation. Solutions with a broad range of lead ion concentrations can be efficiently treated in a simple mixing tank. The on-line measurements of  $\text{Pb}^{2+}$  mass concentration in the solution has been successfully applied with a fast lead ion selective electrode.

It was shown that in the system under consideration the external mass transfer resistances can be neglected because of the satisfactorily mixed suspension of highly porous pellets. The classical engineering calculation of internal effectiveness factor showed that in some experiments mass transfer resistances were important. However, with respect to the highly porous biosorbent structure, enhanced internal mass transfer and, therefore, higher effectiveness factors could be expected.

The specific characteristics of the described biological system require careful application of classical chemical engineering principles. Having this in mind, the developed reversible kinetic mathematical model describes the experimental concentration curves reasonably well and confirms the proposed

reversible biosorption reaction. The reported values of adsorption and desorption rate coefficients should be taken as rough estimates, which are applicable to process design and scale up.

### ACKNOWLEDGEMENTS

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### Symbols

- $k_{\text{ads}}$  – adsorption rate coefficients,  $\text{lYng}^{-1}\text{Ymin}^{-1}$   
 $k_{\text{des}}$  – desorption rate coefficients,  $\text{min}^{-1}$   
 $K$  – dissociation constant,  $\text{mgY}^{-1}$   
 $m_{\text{dry}}$  – dry mass of the biomass, g  
 $m_{\text{wet}}$  – wet mass of the biomass, g  
 $q'$  – metal uptake or  $\text{Pb}^{2+}$  mass concentration in the biomass, calculated per volume of the suspension,  $\text{mgY}^{-1}$   
 $q'_0$  – maximal metal uptake or  $\gamma_{\text{pb}^{2+}}$  mass concentration in the biomass in equilibrium with initial  $\text{Pb}^{2+}$  mass concentration in solution, calculated per volume of the suspension,  $\text{mgY}^{-1}$   
 $Q_{\text{max}}$  – maximum biosorption capacity of the biomass,  $\text{mgY}^{-1}$   
 $t$  – time, min, s  
 $T$  – time constant, s  
 $V_0$  – total volume of the suspension, l  
 $\gamma_{\text{x}_0}$  – (dry) biomass concentration,  $\text{g l}^{-1}$   
 $[\text{L}]$  – concentration of free biomass binding sites,  $\text{mol l}^{-1}$   
 $[\text{Me}]$  – concentration of free metal ions in the solution,  $\text{mol l}^{-1}$   
 $[\text{Me-L}]$  – concentration of biomass-metal ion complexes,  $\text{mol l}^{-1}$

- $\gamma_{\text{pb}^{2+}}$  –  $\text{Pb}^{2+}$  mass concentration in the solution,  $\text{mgY}^{-1}$   
 $\gamma_{0,\text{pb}^{2+}}$  – initial  $\text{Pb}^{2+}$  mass concentration in the solution,  $\text{mgY}^{-1}$   
 $\eta$  – efficiency

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