Laboratory experiments of lead biosorption by self-immobilized *Rhizopus nigricans* pellets in the batch stirred tank reactor and the packed bed column

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In memoriam Prof. Emeritus Vera Johanides

The biosorption of lead from aqueous solutions was performed in a batch stirred tank reactor and a continuous packed bed column with the purpose of examining the process characteristics on a laboratory scale. As a biosorbent, self-immobilized biomass in the form of spherical pellets of the fungus *Rhizopus nigricans* was used. In the batch stirred tank reactor the influence of the initial lead concentration and the biomass loading were studied. The study of the continuous process in the packed bed column was conducted as a function of the flow rate and the biosorbent bed height. For both types of reactors the metal uptake was compared to the calculated maximum biosorption capacitiy to estimate the efficiency of the process.

Keywords:
Biosorption, batch stirred tank reactor, lead, packed bed column, *Rhizopus nigricans*

Introduction

Biosorption is a promising method for removal of toxic metal ions from waste water. Its advantage is especially in the treatment of large volumes of effluents with low concentration of pollutants. In the past few years a lot of effort has been made on screening of efficient biomass types, its preparation[1–5] and the biosorption mechanism determination[6–10] but only a few patents and large-scale operation plants have been reported so far[4,12,13]. Biosorption is a fast and reversible process which resembles adsorption and in some cases ion exchange. Most often biosorption equilibria are described with adsorption isotherms of the Langmuir or Freundlich type[2]. Furthermore it has been reported that the process does not depend on the viability of the biomass[2,3,14], which is an advantage, because waste biomass can be used for this purpose.

In our work we used biomass of the fungus *Rhizopus nigricans*, which has been recognised for its high metal biosorption capacity. Its capability to bind metal ions is ascribed mainly to high amounts of specific cell wall polymers, chitin and chitosan[9,10,15]. *R. nigricans* can be grown in the form of spherical pellets, considered as a self-immobilized biomass[16]. The pelleted form of the biomass is beneficial for the use in biosorption reactors where it facilitates the solid-liquid separation.

The aim of this work are some preliminary experiments of lead removal from aqueous solutions with respect to reactor design for the process of biosorption on large scale. In this aspect, the experiments in the laboratory scale were performed in a batch and continuous mode, i.e. in a mixing tank reactor and a packed bed column reactor.

Experimental

The biosorbent used in the experiments was a self-immobilized growth form of the fungus *Rhizopus nigricans* (ATCC 6227b), grown in a batch submerged culture. The spherical pellets of average diameter 2.5 ± 0.5 mm were formed in 500 ml Erlenmeyer flasks on a rotary shaker at 225 rpm and the temperature 25 °C. 100 ml of a sterilised nutrient medium, composed of glucose (20 g l^{-1}), soy flour (6 g l^{-1}), yeast extract (5.7 g l^{-1}), NaCl (4 g l^{-1}), K_2HPO_4 (1.98 g l^{-1}), surfactant TWEEN 60 (0.5 g l^{-1}), and the pH set to 5.5 with H_3PO_4, was inoculated with 1×10^5 spores. After 24 h, 10 ml of broth with already formed pellets was used as the inoculum for further cultivation in another 100 ml of fresh medium. After 48 h the formed pellets were harvested, filtered through a gauze, and washed with deionized water five times in order to remove the excess growth medium. The washed biomass was stored frozen at –18 °C. For the experimental use the biomass was defrosted and washed with deionized water again. To ensure equal quality of the biomass during all experiments, several batches were mixed together to represent a uniform biomass.

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The batch biosorption experiments were done in a stirred vessel with a working volume of 100 ml (Fig. 1). A minimal amount of concentrated solution of $Pb(NO_3)_2$ was added into the suspension of fungal pellets in water of various biomass concentrations (25, 50, 100, 150 and 200 g of wet biomass per liter of biomass suspension), to give the desired initial metal concentrations: 10, 20, 50, 100 and 300 mg l^{-1} Pb^{2+}. The diminishing of metal concentration was recorded as a function of the initial metal concentration and the biomass loading.

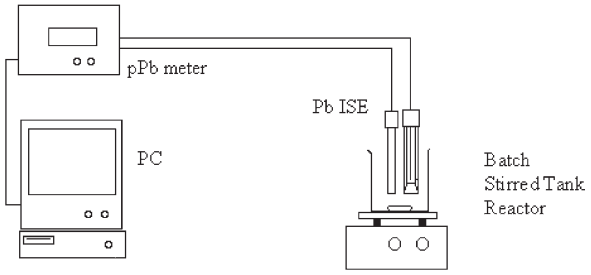


Fig. 1 – *Batch stirred tank reactor*

The continuous experiments were done in a glass column of inner diameter of 5.2 cm with different heights of the packed bed of biomass pellets (20, 40 and 55 cm), set with an adjustable plug (Fig. 2). The solution of $Pb(NO_3)_2$ with initial concentration 100 mg l^{-1} was introduced at the bottom of the column via peristaltic pump with flow rates from 2.5 to 10 ml min^{-1}. An inert bed of glass spheres was placed at the bottom of the column prior the active biomass bed to ensure homogenous distribution of the feeding solution. The remaining metal concentration was measured on-line in the effluent at the top of the column. The breaktrough curves were recorded as a function of the flow rate and the bed height.

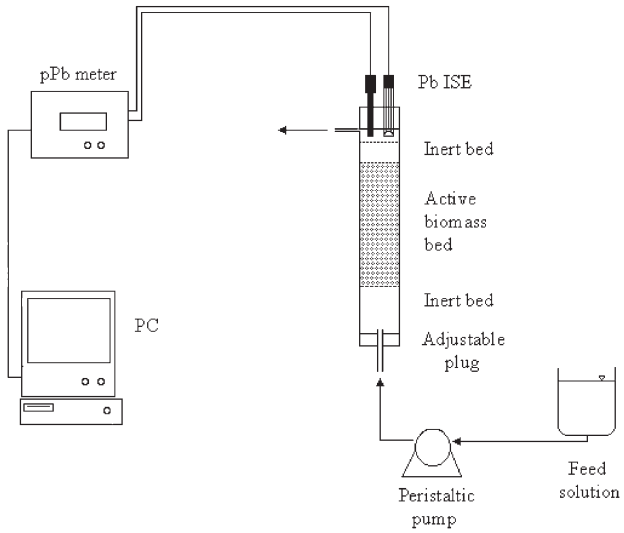


Fig. 2 – *Continuous packed bed column*

The measurements of Pb^{2+} concentration in the solution were performed on-line with a lead ionoselective electrode (WTW, Pb 500). The additional samples were taken to verify the concentration on an atomic absorption spectrometer (Perkin-Elmer 2280 atomic absorption spectrometer).

At the end of each experiment the dry mass of the biomass was determined.

Results and discussion

In the batch stirred tank reactor the experiments were performed in order to investigate the dependence of lead uptake on the concentration of the biomass. As it was expected, the removal of Pb^{2+} was better and faster at higher biomass concentrations (Fig. 3). Increasing of biomass concentration was found to be efficient up to the value of 150 g l^{-1} of wet mass of the biomass. The biomass concentrations above this value still have some positive effect on the biosorption rate but they already hinder the mixing quality in the reactor.

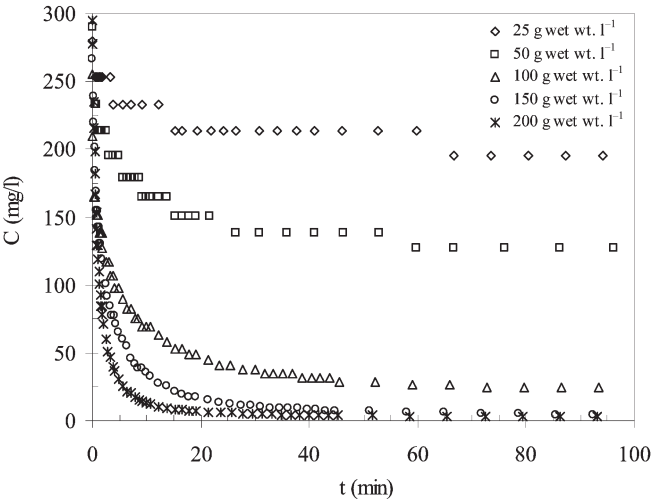
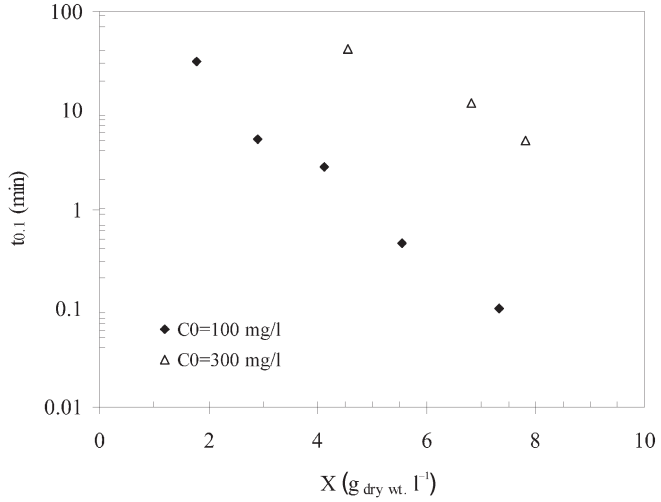
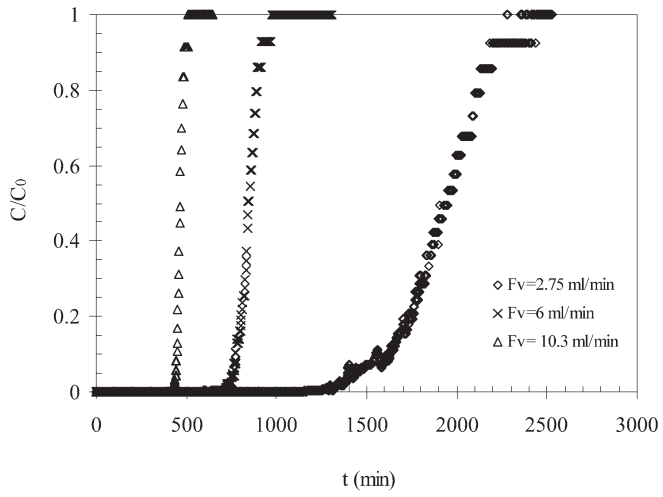


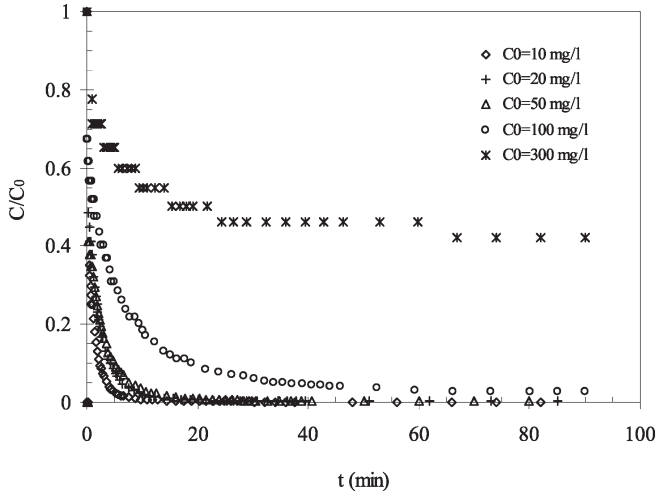
Fig. 3 – *Biosorption in the batch stirred tank reactor as a function of wet biomass concentration X, at initial Pb^{2+} concentration C_0 = 300 mg l^{-1}*

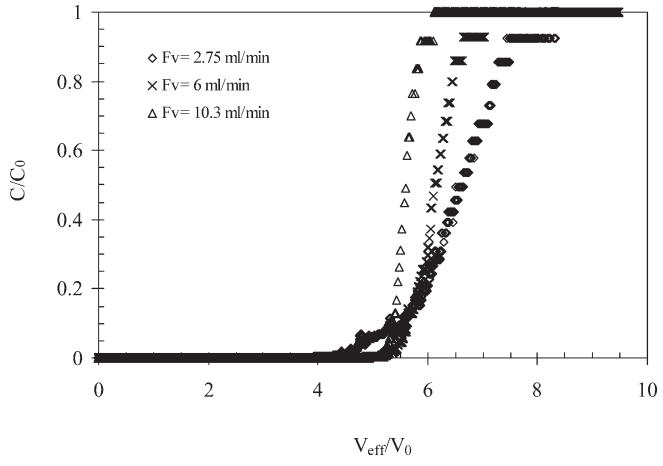
Fig. 4 shows the characteristic time, $t_{0.1}$, when the residual concentration in the reactor falls to 10 % of the initial metal concentration, as a function of biomass concentration. As expected, the characteristic time is reciprocal to the biomass loading and positively related to the initial metal concentration. At the same time it is conditional on the biosorption equilibrium.

The time course of Pb^{2+} concentration in the batch stirred tank reactor, as a function of the initial


100
10
1
0.1
0.01
$t_{0.1}$ (min)
C0=100 mg/l
C0=300 mg/l
0
2
4
6
8
10
X ($g_{dry\ wt.}\ l^{-1}$)


1
0.8
0.6
0.4
0.2
0
C/C0
Fv=2.75 ml/min
Fv=6 ml/min
Fv= 10.3 ml/min
0
500
1000
1500
2000
2500
3000
t (min)


1
0.8
0.6
0.4
0.2
0
C/C0
C0=10 mg/l
C0=20 mg/l
C0=50 mg/l
C0=100 mg/l
C0=300 mg/l
0
20
40
60
80
100
t (min)


1
0.8
0.6
0.4
0.2
0
C/C0
Fv= 2.75 ml/min
Fv= 6 ml/min
Fv= 10.3 ml/min
0
2
4
6
8
10
V_{eff}/V_0

The efficiency of the biosorption process depends on the reactor type and the operating conditions. In the performed batch and continuous experiments, the final metal uptake was compared to the maximum uptake, calculated from the Langmuir adsorption isotherm on the presumption of complete saturation of the biomass, i.e. maximum biosorption capacity. The maximum biosorption capacity of *R. nigricans* for the Pb^{2+} ion determined in the previous batch equilibrium experiments[17] was 83.5 mg $g^{-1}_{\text{dry wt.}}$. The metal uptake (Q, mg $g^{-1}_{\text{dry wt.}}$) on the basis of dry biomass (m, $g_{\text{dry wt.}}$) was calculated from the initial Pb^{2+} concentration (C_0, mg l^{-1}) and the determined final concentration (C_f, mg l^{-1}), where V is the volume of the suspension (l) and X ($g_{\text{dry wt}}$ l^{-1}) the concentration of the biomass, calculated per dry weight:

$$Q = \frac{(C_0 - C_f)\cdot V}{m} = \frac{(C_0 - C_f)}{X} \qquad (1)$$

The ratio of the final metal uptake to the maximum biosorption capacity Q/Q_{max} was considered as the efficiency of the metal uptake. At the conditions of low initial Pb^{2+} concentration (100 mg l^{-1}) or high biomass concentration (150 and 200 $g_{\text{wet wt.}}$ l^{-1}) the biomass capacity appears to be higher than the available amount of free Pb^{2+} in the reactor, which leads to the final Pb^{2+} concentrations approaching the zero value. In this case the metal uptake and its efficiency become reciprocal to the biomass concentration, which is clearly indicated in Fig. 8.

The metal uptake 80.8 mg $g^{-1}_{\text{dry wt.}}$, determined with the minimal tested biomass concentration 25 $g_{\text{wet wt}}$ l^{-1} at the initial Pb^{2+} concentration 300 mg l^{-1}, approaches the maximum biosorption capacity 83.5 mg $g^{-1}_{\text{dry wt.}}$ and consequently the corresponding efficiency is 0.98. This experiment setting represents the optimal process parameters for the biosorption of Pb^{2+} in the batch stirred tank reactor.

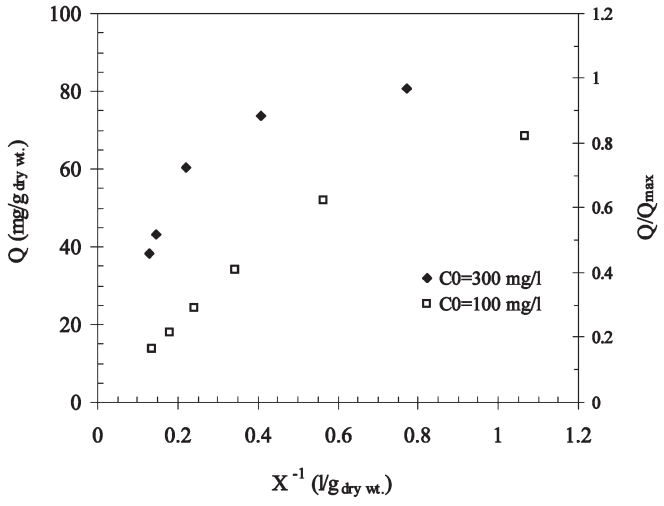


Fig. 8 – *Metal uptake in the batch stirred tank reactor as a function of reciprocal biomass concentration.*

In the continuous experiments the metal uptake or the total capacity of the bed was calculated from the breakthrough curves for each experiment and again was the efficiency of metal uptake represented as the Q/Q_{max} ratio.

Because in a continuous process there is no equilibrium limit to the final concentration the metal uptake should theoretically reach the maximum biosorption capacity. The experiments however showed varying and much lower metal uptake 56 mg $g^{-1}_{\text{dry wt.}}$ ± 22%. We belive, that corresponding lower metal uptake efficiency 0.67 ± 0.15 is a consequence of the packed bed non-uniformity. It turned out that it is very difficult to ensure equal quality of the porous bed packing, because the fungal mycelial pellets are soft structures of intertwining hyphae which easily adhere and form aglomerates or are compressed by the gravity or due to the flow pressure. Therefore, the formation of channels and zones of unexploited biomass within the bed was inevitable.

Conclusions

The choice of an optimal reactor for the removal of lead ions from solutions by biomass depends predominately on the biomass characteristics. It was shown that a self-pelleted form of *R. nigricans* could be efficiently used in the batch stirred tank reactor, while for the use in the packed bed some adjustments should be taken to consideration. In our experiments it was demonstrated that the naturally formed fungal pellets were too soft to withstand even the small pressure drops or flow rates which caused the compressing of the bed. Besides this disadvantage, channelling is another inconvenience that commonly occurs because of clogging of the biomass. In such case the strengthening of the biomass consistency is necessary. From the same point of view the batch stirred tank reactor operation is much simpler, but the biomass exploitation may be inferior, and the final metal concentration in the solution is not optional because of establishment of the equilibrium. Therefore, for the operation of batch reactors optimization of operation conditions such as initial metal concentration, biomass loading and residence time has to be made. On the basis of the performed laboratory experiments it can be concluded that for the examined type of biomass, i.e. the spherical pellets of the fungal mycelia of *R. nigricans* and similar types, a continuous reactor with good mixing and high biomass loading, for example the continuos stirring tank reactor or the fluidized bed reactor, would be preferable.

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