

Full Length Research Paper

Iron(III) biosorption by *Polyporus squamosus*

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Polyporus squamosus was tested for its ability to absorb Fe(III) ions from solutions. Kinetic and isotherm sorption experiments were conducted to evaluate the effects of contact time, pH, metal concentration, dose of the adsorbent, ionic strength and glucose. The increases in initial concentration of metal and pH of the solutions resulted in an increase in iron uptake. The equilibrium data could be fitted by Langmuir and Freundlich isotherm equation. Both Langmuir and Freundlich sorption models adequately describe the biosorption of Fe(III) by *P. squamosus*. Maximum metal uptake capacities of *P. squamosus* biomass (q_m) were found as 31.2, 18.1 and 12.2 mg/g for 1.5, 3.3 and 6.6 g biomass/l, respectively. With increasing ionic strength, there is a decrease in the metal uptake (q_m) as well as coefficient b in Langmuir equation. It was noticed that lower concentrations of glucose resulted in higher rates and amounts of Fe(III) adsorption, while its concentration above 0.1% (w/v) reduced substantially the ability of the cells to absorb this metal.

Key words: Biosorption, *Polyporus squamosus*, heavy metals, iron, removal.

INTRODUCTION

The production of heavy metals has increased rapidly since the industrial revolution (Ayres, 1992). Heavy metals usually form compounds that can be toxic, carcinogenic or mutagenic, even in very low concentrations (Ruiz-Manriquez et al., 1977). Most the analytical methods that are currently in use for trace metal analysis employ conventional techniques of sample handling. In most cases conventional techniques fail to offer both sample clean up and enrichment (Lopez-Avila, 1999). Conventional techniques aimed at preconcentration of heavy metals usually include chemical precipitation (US EPA, 2000), ion exchange (Riley and Taylor, 1968), some adsorption process (Bhattacharyya and Venkobachar, 1984), membrane process (Kapoor and Viraraghavan, 1995), crystallization, and electrochemical treatment (Schiewer and Volesky, 1995; Wilde and Benemann, 1993). The conventional methods of removing metals from wastewaters are generally expensive and have many limitations (Volesky, 1990). Alternative methods of metal removal and recovery based on biological materials have been considered. Biological process for remo-

val of metal ions from solution can be divided into three general categories: (i) biosorption (adsorption) of metal ions onto the surfaces of a microorganism, (ii) intracellular uptake of metal ions, and (iii) chemical transformation of metal ions by microorganisms. The latter two processes require living organisms (Veglio and Beolchini, 1997; Corder and Reeves, 1994; Darnell et al., 1986).

In the literature, the capability of either living or non-living organisms for fixing metal ions is widely described. The ability of microorganisms to bind heavy metals in aqueous solution has long been of scientific interest. Mapolelo et al. (2005) showed that yeast is a viable trace metal enrichment agent that can be used freely suspended in solution to enrich metal ions at relatively low concentrations. Modak et al. (1996) showed that nonliving *Aspergillus niger* biomass attached to wheat bran was selective for the extraction of copper and zinc. Uranium biosorption by filamentous fungus *Mucor* is well documented (Guibal et al., 1999). Mechanism of aluminium interference on uranium biosorption by *Rhizopus arrhizus* has recently been comprehensively reviewed by Tsezos et al. (1997). Gardea-Torresdey et al. (1996a) performed batch experiments with inactivated cells of *M. rouxii* for Cu(II) binding. The search for alternate and innovative treatment techniques has focussed attention on the metal uptake capacities of various microorganisms and

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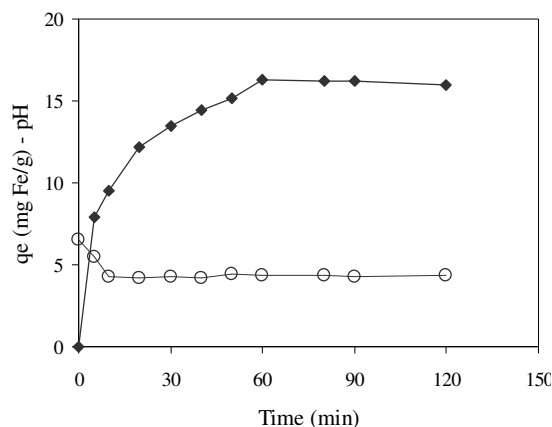


Figure 1. Time evolution of the biosorption capacity (♦♦) and pH (○) by *P. squamosus* during the experiments. C_0 (Fe(III)) = 100 mg/l, $C_{\text{biomass}} = 3.3 \text{ g}_{\text{dw}}/\text{l}$.

different biomaterials (Mishra et al., 1996; Singleton and Simmons, 1996; Khoshmanesh et al., 1996; Gardea-Torresdey et al., 1996; Gupta et al., 2004).

In metal cleaning, plating and metal processing industries, iron concentrations can approach 11.6-120 mg/l (copper plating) (Patterson, 1977). Fe(III) biosorption has been studied by various investigators using a variety of different biomass types including bacteria and fungi as biosorbent (Selatnia et al., 2004; Sağ and Kutsal, 1998; Chandra Sekhar et al., 1998; Sağ et al., 2001; Aksu and Gülen, 2002). Investigators have reported different biosorption capacities within the range of 19-120 mg/g toward Fe(III) depended of experimental conditions.

Despite the quite extensive literature available on metal biosorption by different biosorbents, little attention seems to have been given to the study of single-metal biosorption by the fungus *Polyporus squamosus* as biosorbent. The use of the white-rot fungus *Polyporus versicolor* in Ni(II) removal as biosorbent was investigated by Dilek et al. (2002). Three different forms of *Polyporus versicolor* were used for the removal of the divalent ions of Group IIB elements from aquatic systems by Satioğlu et al. (2002).

In this work, *Polyporus squamosus* is used to test its ability for iron(III) uptake. The microorganism has been previously used for pectinase production in aqueous two-phase system containing sugar beet extraction waste (Antov et al., 2001). Thus, the present study is directed towards the determining the possibility of using *Polyporus squamosus* biomass originating from an industrial wastewater treatment facility that employs the fungus. The effect of various operational parameters such as biomass and metal concentrations, pH, ionic strength and addition of glucose to the metal-containing solutions on the adsorption of the metals were also studied. Two adsorption models, the Langmuir model and the Freundlich equation were applied to the experimental data obtained for *Polyporus* biomass and correlations were

found for these models.

EXPERIMENTAL

Microorganism and media

Polyporus squamosus MMOL 87, obtained from the Research Institute NPO Biotechnology, Moscow, Russia, was stored on Saboroud maltose agar slants at 4°C. Microorganism was washed from the slant agar surface into 300 ml shake flasks, containing 100 ml of basal medium. The culture was incubated at 28°C for 48 h at 200 rpm. The first vegetative generation obtained in this way was used as inoculum. Basal medium for inoculum was: 5% molasses, 0.15% CSL, 0.1% KH_2PO_4 , 1% NaH_2PO_4 . The pH before sterilisation was adjusted to 6.5. Sterilisation was accomplished at 121°C for 20 min. Erlenmeyer flasks of 300 ml, containing 100 ml of basal media, were inoculated with 5% of inoculum and incubated at 200 rpm and 28°C for a maximum 4 days exposure period.

Sorption experiment

Cells from the stationary growth phase (96 h) were harvested by filtration (through coarse filter paper) at room temperature. This biomass is washed with physiological solution and added to 100 ml distilled water-metal ion solution to a final concentrations of 1.5; 3.3 and 6.6 g (dry weight)/l, respectively, in 250 ml Erlenmeyer flasks. The pH of the suspensions was adjusted to the desired value using 0.1 M NaOH or HCl and samples was agitated for a desired time in a rotary shaker at 200 rpm and 28°C. The slurry after equilibration was filtered through 0.45 μm filters. The clear filtrate was analysed for metal concentration.

Batch forms of kinetic and isotherm sorption experiments were conducted separately to evaluate the effects of initial iron concentration, adsorbent concentration, pH, ionic strength and glucose concentration. Stock iron solution was prepared by dissolving $\text{FeCl}_3 \cdot 2\text{H}_2\text{O}$ (Merck) in double distilled water. The biosorption experiments were carried out in duplicate and the average results are presented in this work. To evaluate the adsorptive capacity of *P. squamosus* in adsorbing Fe(III) from solution, the most commonly used adsorption isotherm models (Freundlich and Langmuir) were used.

Analytical techniques

Dry weight of biomass was obtained by drying at 105°C until constant weight was reached. Metal analyses were determined spectrophotometrically by phenanthroline method with a wavelength at 510 nm (APHA Standard Methods, 1998). Metal uptake (q) was determined as follows:

$$q_e = V (C_0 - C_e) / S$$

Where q_e (metal uptake, mg/g) is amount of metal ions adsorbed on the biosorbent, V (l) is the volume of metal containing solution in contact with the biosorbent, C_0 and C_e (mg/l) are the initial and equilibrium (residual) concentrations of metals in the solution, respectively and S (g) is the amount of added biosorbent on dry basis.

RESULTS AND DISCUSSION

Iron biosorption kinetics

Biosorption kinetics of *P. squamosus* was given in Figure 1. The biosorption of Fe(III) was completed after

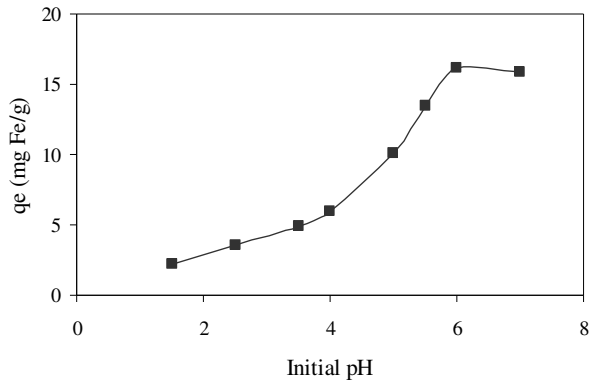


Figure 2. Effect of initial pH on the Fe(III) adsorption capacity by *P. squamosus*. C_0 (Fe(III)) = 100 mg/l, $C_{\text{biomass}} = 3.3 \text{ g}_{\text{dw}}/\text{l}$.

60 min contact with the biosorbent. The short contact time of biosorbent with metal solution for biosorption suggests that adsorption onto the biosorbent surface is the main mechanism of uptake. The metal biosorption depends strongly on the protonation or unprotonation of the functional groups on the cell wall (i.e. carboxylic, hydroxyl and amino groups) (Selatnia et al., 2004; Fourest and Volesky, 1997; Göksungur et al., 2005). The ionic forms of the metal in solution and the electrical charge of the biomass depend on the solution pH. At the very beginning of the experiment, the pH falls from 6.5 to a value of 4.3. These results are in accordance with the data of Selatnia et al. (2004) who suggested that the biosorption mechanism is a ion exchange type between H^+ ions and Fe^{3+} ions, i.e. this can be explained by H^+ liberation of some compounds of the biomass in solution.

Data on the adsorption rates of heavy metal ions by various biosorbent have shown a wide range of adsorption time. For example, the biosorption equilibrium time of Fe(III) on the dead biomass of *Streptomyces rimosus* was 4 h (Selatnia et al., 2004). The Ni(II) biosorption rate of *P. versicolor* reached saturation value with 60 min (Dilek et al., 2002). The Zn(II), Cd(II) and Hg(II) biosorption rate of NaOH treated *P. versicolor* was 60 min (Satiroğlu et al., 2002). Note that there are several parameters which determine the biosorption rate such as stirring rate, structural properties of the support and the biosorbent (e.g. protein and carbohydrate composition and surface charge density, topography and surface area), amount of sorbent, properties of the ions under study, initial concentration of ionic species and the presence of other metal ions which may compete with the ionic species of interest for the active biosorption sites. Therefore, it is difficult to compare the biosorption rates reported (Satiroğlu et al., 2002).

Effect of initial pH on Fe(III) biosorption

Many researchers have demonstrated that the binding of heavy metal ions on biomaterials is pH dependent. In this

work the effect of pH on biosorption was examined in the pH range of 1.5-7.5. At pH values higher than 6.5, biosorption studies could not be performed due to the precipitation of iron ions. *P. squamosus* ($3.3 \text{ g}_{\text{dw}}/\text{l}$ medium) was used as the biosorbent and a medium containing 100 mg Fe(III)/l was used as the biosorption medium.

Figure 2 shows that the Fe(III) adsorbed quantity increases with increasing initial solution pH. That fact can be explained by strong relation between the biosorption and the number of negative charges at the biomass surface which is itself related with the functional groups. The maximum of the Fe(III) adsorbed quantity at the surface of the biomass is 16 mg Fe(III)/g biomass. This maximum occurs at pH 6.5. There is a sharp increase of the adsorbed quantity from pH 4.3 which corresponded to the dissociation of functional group or their deprotonation. For low pH values, one may consider an adsorption competition between H^+ protons and metallic ions on the active sites on the cell wall of the biomass, as it has been suggested by many other authors (Selatnia et al., 2004; Fourest and Volesky, 1997).

Several researchers have also investigated the effect of pH on biosorption of heavy metals by using different microbial biomass and found similar results with this study. Aksu and Gülen (2002) investigated the biosorption of iron(III) and iron(III)-cyanide complex ions on *Rhizopus arrhizus*. The working pH value was determined as 2.0. Also, Sağ and Kutsal (1998, 1996) found the optimum pH for Fe^{3+} uptake as 2.0 for *R. arrhizus* cells. Yin et al. (1999) determined that biosorption capacity of *R. arrhizus* biomass increased with increasing pH of the solution and gave a peak at pH 5.5. Chandra Sekhar et al. (1998) used industrial biomass for iron removal and found that metal uptake increased with increase in medium pH and had a maximum value at pH 4-5. The biosorption of Fe(III) ions on *Zoogloea ramigera* and *R. arrhizus* was studied in systems at optimum pH value 2.0 (38, 47). Selatnia et al. (2004) reported that maximum of the Fe^{3+} adsorbed quantity occurs at pH 10. The effect of pH was also investigated by Satiroğlu et al. (2002) and maximum biosorption of metal ions on the three different forms of *P. versicolor* were observed at pH 6.0. Ni(II) biosorption by *P. versicolor* was investigated at pH 5 (Dilek et al., 2002).

Effect of biomass and iron concentrations

The investigate the effects of the Fe(III) and biomass concentrations on the uptake of this metal, the process was carried out with initial Fe(III) concentrations between 0.4 and 150 mg/l and various biomass concentrations in the range of 1.5-6.6 $\text{g}_{\text{dw}}/\text{l}$. Initial pH was 6.5. At equilibrium (60 min contact time), the Fe(III) concentration in each system was measured and isotherms, displayed in Figure 3, were taken as a function of Fe(III) and biomass concentration.

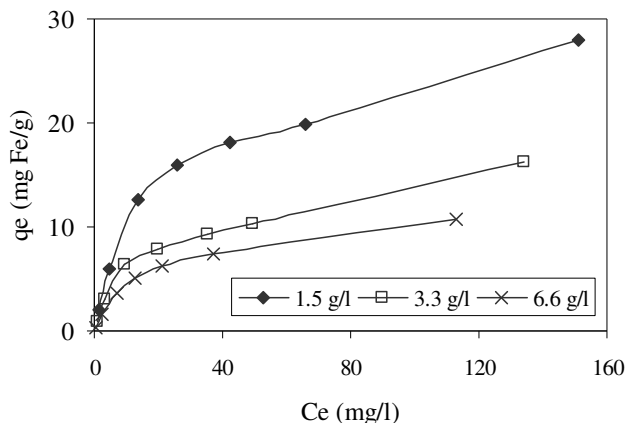


Figure 3. Adsorption isotherms of Fe(III) by different *P. squamosus* concentrations.

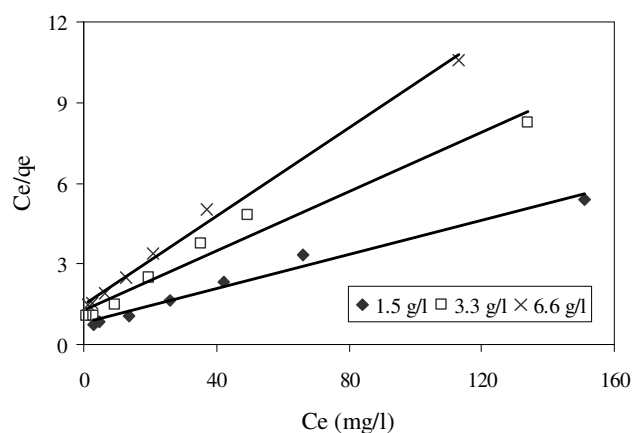


Figure 4. Application of the Langmuir equation to the adsorption data of Fe(III) onto different *P. squamosus* concentrations.

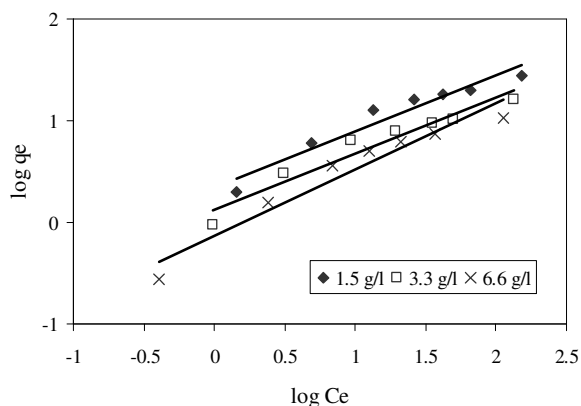


Figure 5. Application of the Freundlich equation to the adsorption data of Fe(III) onto different *P. squamosus* concentrations.

In order to optimize the biosorption process parameters, we have modelised the equilibrium curve (Figure

3). Two equations of isotherms of Langmuir and Freundlich were tested (Figures 4 and 5). Figures 4 and 5 are the transformed forms of these models which permit to calculate Langmuir's constants (q_m and b) and Freundlich's constants (K and n).

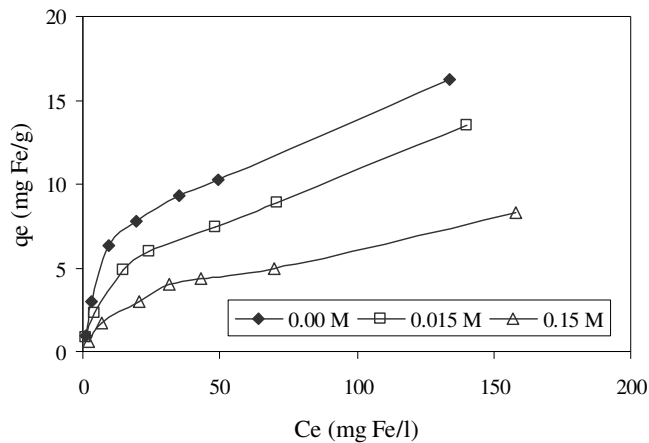
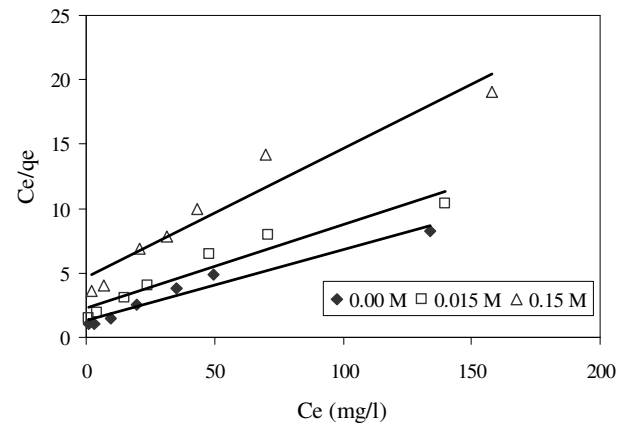
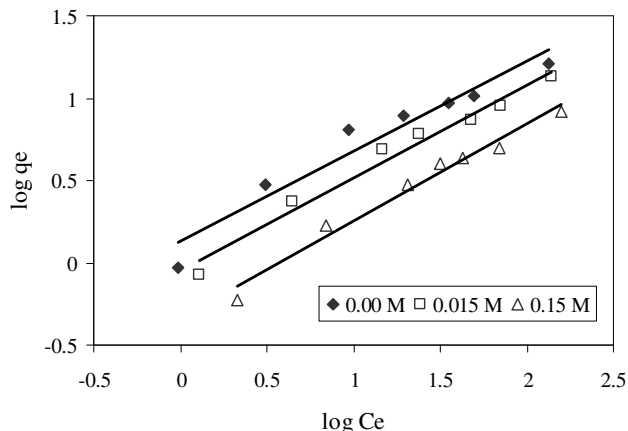
Table 1 shows the values of the computed adsorption constants. It can be noted that the Fe(III) uptake per unit weight of the sorbent increased with a decrease in the biomass concentration, while the total amount of the metal removed increased with an increase in the sorbent concentration. The results in Figures 3, 4 and 5 also indicate that the increase in the Fe(III) uptake was associated with an increase in its concentration. Central to the development of the adsorption plus membrane transport model is the basic assumption that a simple linear relationship exists between the concentration of the metal ions adsorbed on the surface of the cell and the metal ion concentrations in solution (Satiroğlu et al., 2002). Also, the isotherm curve shows a limiting biosorption capacity attained at Fe(III) equilibrium concentration of about 150 mg/l, 130 mg/l and 110 mg/l for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively. As seen in Table 1, regression correlation coefficients for Fe(III) - *P. squamosus* systems from the Langmuir model were 0.9821, 0.9632 and 0.9942 for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively. For Fe(III) ions, the correlation coefficients for Freundlich equation were 0.9387, 0.9395 and 0.9395 for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively. The Langmuir equation was more in correlation with the experimental data. The Langmuir model makes several assumptions, such as monolayer adsorption and constant adsorption energy, while the Freundlich equation deals with heterogeneous surface adsorption (Zhou and Kiff, 1991). The agreement of the experimental data with the Langmuir model implied that monolayer adsorption existed for the experimental conditions used.

The values of $1/n$ and K are Freundlich constants which are indicators of adsorption intensity and adsorption capacity. The value of $1/n$ obtained in this study were 0.553, 0.548 and 0.652 for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively, and K values were 2.19, 1.34 and 0.75 l/g for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively.

The coefficient b in Langmuir equation is a measure of the stability of the complex formed between metal ions and biomass under specified experimental conditions (Say et al., 2001). The small b values (0.0408, 0.0427 and 0.0546 l/mg for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively) obtained in this research implied strong binding of metal ions to *P. squamosus* biomass. Maximum metal uptake capacities of *P. squamosus* biomass (q_{max}) were found as 31.2, 18.1 and 12.2 mg/g for 1.5, 3.3 and 6.6 g_{dw} biomass/l, respectively. Selatnia et al. (2004) found the maximum metal uptake of about 122 mg_{Fe/g} biomass for biosorption of Fe³⁺ by a bacterial dead *Streptomyces rimosus* biomass. Maximum biosorption capacities three different forms of *P. versicolor* was

Table 1. Langmuir and Freundlich isotherm parameters for Fe(III) uptake by *P. squamosus* at different biomass concentration.

| Biomass conc. (g _{dw} /l) | Langmuir | | | Freundlich | | |
|---------------------------------------|-----------------------|----------|----------------|------------|-------|----------------|
| | q _m (mg/g) | B (l/mg) | R ² | K (l/g) | 1/n | R ² |
| 1.5 | 31.2 | 0.0408 | 0.9821 | 2.19 | 0.553 | 0.9387 |
| 3.3 | 18.1 | 0.0427 | 0.9632 | 1.34 | 0.548 | 0.9395 |
| 6.6 | 12.2 | 0.0546 | 0.9942 | 0.75 | 0.652 | 0.9395 |

**Figure 6.** Adsorption isotherms of Fe(III) by *P. squamosus* at different ionic strength. $C_{\text{biomass}} = 3.3 \text{ g}_{\text{dw}}/\text{l}$.**Figure 7.** Application of the Langmuir equation to the adsorption data of Fe(III) onto *P. squamosus* at different ionic strength. $C_{\text{biomass}} = 3.3 \text{ g}_{\text{dw}}/\text{l}$.**Figure 8.** Application of the Freundlich equation to the adsorption data of Fe(III) onto *P. squamosus* at different ionic strength. $C_{\text{biomass}} = 3.3 \text{ g}_{\text{dw}}/\text{l}$.

found as 139.3, 70.8 and 54.1 mg/g for Zn(II); 232.2, 118.2 and 90.0 mg/g for Cd(II) and 290.3, 168.9 and 131.4 mg/g for Hg(II), respectively (Satiroğlu et al., 2002). The biosorption capacity of Ni(II) was 57 mg/g for *P. versicolor* (Dilek et al., 2002). The comparison of the biosorption capacities of biomass used in this study with those reported in the literature shows that *P. squamosus* biomass is suitable for biosorption processes.

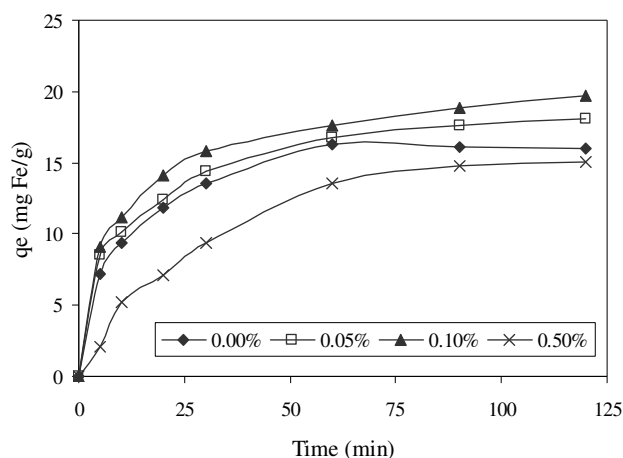
Effect of ionic strength

The effect of ionic strength on the adsorption of iron is very interesting. The alkali and alkali earth metal ions such as Na^+ , K^+ , Mg^{2+} and Ca^{2+} , have been known to interact with fungal cells through weak electrostatic bonding and play an important role in many biological functions, such as in metal transport channels or pores in the membrane of fungal cells. They may also be used in cationic exchange with metals such as Co^{2+} , Cd^{2+} , Cu^{2+} and Zn^{2+} resulting in their uptake. This cationic exchange can occur either in the cell wall, cell membrane or cytoplasm. Thus, the knowledge of the effect of these metal ions, is important for efficient application of the biomass. The effect of ionic strength was tested by the addition of sodium chloride to the iron(III) solution. Two different ionic strength values 0.015 M and 0.15 M NaCl were used and the results were compared with those obtained for control samples. The effect of NaCl can be evaluated using the results (Figures 6, 7 and 8) obtained from modelling the data with the Langmuir and Freundlich equation (Table 2).

With increasing ionic strength, there is a decrease in the metal uptake (q_m) as well as the coefficient b in Langmuir equation. The most logical reason for the antagonistic action is claimed to be competition for adsorption sites on the microbial cells and or the screening effect by the second metal ion (Sağ and Kutsal, 1996). On the

Table 2. Langmuir and Freundlich isotherm parameters for Fe(III) uptake by *P. squamosus* at different ionic strength.

| Ionic strength (M NaCl) | Langmuir | | | Freundlich | | |
|----------------------------|--------------|----------|--------|------------|-------|--------|
| | q_m (mg/g) | B (l/mg) | R^2 | K (l/g) | 1/n | R^2 |
| 0 | 18.1 | 0.0427 | 0.9632 | 1.336 | 0.548 | 0.9395 |
| 0.015 | 15.3 | 0.0295 | 0.9309 | 0.894 | 0.563 | 0.9761 |
| 0.15 | 10.0 | 0.0216 | 0.9321 | 0.461 | 0.589 | 0.9739 |

**Figure 9.** Effect of glucose concentration on Fe(III) uptake by *P. squamosus*. C_0 (Fe(III)) = 100 mg/l, $C_{biomass}$ = 3.3 g dw/l .

basis of this results it can be concluded that the effect of ionic strength of NaCl on biosorption of Fe(III) depends of experimental conditions for metal-biomass system. From inspection of Table 2, it is also noticed that the value 1/n changed between 0.548 and 0.589. Since there was no significant change in the value of 1/n on 0.015 M and 0.15 M NaCl, it can be concluded that the sorption mechanisms for these ionic strength were similar. Values of coefficients of correlation R^2 shows that the Langmuir's and Freundlich's models, fits fairly well our experimental data.

Glucose effect

The effect of glucose on the metal adsorption by microbial cells has been investigated by various workers (Norris and Kelly, 1977; Al-Asheh and Duvnjak, 1995). In our study it was also noticed that glucose affected the amount and the rate of Fe(III) uptake by the cells of *P. squamosus* (Figure 9).

It was noticed that lower concentrations of glucose resulted in higher rates and amounts of Fe(III) adsorption, while its concentration above 0.1 % (w/v) reduced substantially the ability of the cells to absorb this metal. The increase in the adsorption can be the result of an increase of cells' activities, including the metal adsorption activity, caused by the increase in the energy available to

the cells in the presence of glucose. At higher glucose concentrations, it may happen that this compound interferes with the cells' metal active sites, thus preventing their interactions with metal ions. Therefore, it is necessary to find the optimum glucose concentration for the adsorption of metals by microbial biomass. In this study, the highest Fe(III) adsorption was noticed when the glucose concentration was 0.1% (w/v). This is in agreement with already published data for *Aspergillus carbonarius* (Al-Asheh and Duvnjak, 1995).

Conclusions

In this study, the ability of the fungal biomass *P. squamosus* to bind Fe(III) in solution was investigated and the results were compared with other published data. Adsorption is moreover influenced by various parameters such as initial pH, initial Fe(III) concentration, biomass concentration, ionic strength, glucose concentration. An increase in pH significantly increased the iron uptake per unit weight of *P. squamosus*. Decreasing biomass concentration and increasing Fe(III) ions concentration resulted in an increase in Fe(III) uptake per unit weight of biomass. The obtained results showed that *P. squamosus* has a high affinity to Fe(III) when compared with other microbial species. Up to 31.2 mg of iron can be fixed by each gramme of *P. squamosus* biomass. Both Freundlich and Langmuir sorption models adequately describe the biosorption of Fe(III) by *P. Squamosus*. Adsorption of Fe(III) ions in aqueous solution by *P. squamosus* was characterized as function of ionic strength. Addition of glucose enhances iron adsorption; the optimum glucose concentration for this adsorption is 0.1 %. Still, these results are far from ideal, being necessary to make more detailed studies to clarify the iron biosorption mechanism by *P.squamosus*.

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