SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 6, N° 6, 1998

COMPARATIVE STUDY OF BIOSORPTION OF METALLIC CATIONS BY DIFFERENT BACTERIA

T. Gavriloaiei*, Raluca Mocanu, Maria Calistru***, R. Olariu****

* University "Al.I.Cuza" Iasi, Faculty of Geography-Geology, 20A Calea Copou, 6600 Iasi, Romania, e-mail: tgavrilo@vela.uaic.ro
** University "Al.I.Cuza" Iasi, Faculty of Chemistry, 71B Bd. Mangeron, 6600 Iasi, Romania
*** Agency of Environmental Protection, 10 Vascauteanu, 6600 Iasi, Romania

ABSTRACT

Certain species of microorganisms have been found to accumulate surprisingly large quantities of important metals, involved in the toxicity provoked by human activities (Cd, Pb, Hg) and metals of economic values (Ag, Au). Microbiological methods are applied to large-scale recovery or removal of metallic ions from aqueous solutions. These apllications involved the removal of heavy metals from sewage sludge, industrial effluents and mine or waste waters. This process has been developed using immobilized extracellular or cellular ligands or more simple chemical models based upon them. The uptake of metal ions on the cell surface and their translocations into the cell are well known natural processes. These adsorption processes could be expressed using Langmuir isotherms. Fe^{2+} and Mn^{2+} appeared to be the most effective cations for adsorption by *Bacillus subtilis*, *Pseudomonas aeruginosa*, even by *Saccharomyces cerevisiae*, while the Zn^{2+} cation, in spite of a great value of maximum adsorption, has a toxic action on microorganisms and the "detoxification" mechanism depends on genetic control.

Key words: bioadsorption, bioaccumulation, bacterial species, Langmuir isotherm

RESUMO

Algumas espécies de microorganismos acumulam quantidades surpreendentes de metais envolvidos na toxicidade provocada por atividades humanas (Cd, Pb, Hg) e de outros importantes em termos de valor econômico (Ag,Au). O presente trabalho trata da remoção de metais pesados do esgoto, efluentes industriais e aguas residuárias provenientes de mineração o outros processos. Foi estudado o processo de bioacumulação de metais usando duas espécies de bactérias e uma levedura. O processo de adsorção foi descrito usando isotermas de Langmuir. A adsorção mais eficiente aconteceu para íons de Fe^{++} e Mn^{++} no caso das tres espécies estudadas, *Bacillus subtilis*, *Pseudomonas aeruginosa* e *Saccharomyces cerevisiae*. Por outro lado, o cátion de Zn^{++} que tem um alto valor de adsorção máxima, exibe um efeito toxico sobre os microorganismos e o seu mecanismo de "detoxificação" depende de controle genético.

DOI: 10.48141/SBJCHEM.v6.n6.1998.35_1998.pdf

INTRODUCTION

The processes of accumulation and uptake of heavy metals by microbial biomass are receiving increasing attention since the microbe-based technologies may provide an alternative to the recovery techniques of heavy metals from different materials (waste waters, low-grade ores etc.). Microbial cells (living or dead) and the products derived from their metabolism may be efficient bioaccumulators, both of the soluble and of the oxidized forms. At the same time, the groups with negative charge from the cell-wall components may ensure an efficient biosorptive system for metallic cations present in high concentrations in solutions.

The use of microorganisms in "leaching" processes for metal recovery from low-grade ores has received much importance in the last decades. The bacterial species involved in the biosolubilization processes are adapted to high concentrations of metallic cations; e.g. a strain of *Thiobacillus ferrooxidans* can be resistant to high concentrations of UO_2^{2+} (0.042 M), Cu (0.87 M), Ni (0.85-1.23 M), Zn (1.7 M) [1] or Al (0.37 M), Co (0.17 M), Mn (0.18 M) and Cr (0.1 M) [2].

The present study describes the bioaccumulation properties of two bacterial species (*Bacillus subtilis, Pseudomonas aeruginosa*) and one yeast (*Saccharomyces cerevisiae*) and the biosolubilization properties of *Bacillus megaterium*. The species (*B. subtilis, P. aeruginosa* and *S. cerevisiae*) have been obtained from the collection of the Department of Microbiology, "Al. I. Cuza" University, Iasi and *B. megaterium* has been isolated from soils by the authors.

B. subtilis, P. aeruginosa and *S. cerevisiae* proved to have a good adsorption capacity for metallic cations from their solutions, a fact which is known in the literature, while *B. megaterium* strain has been tested for the solubilization properties of metallic cations: Fe, Cu, Zn, Pb from copper concentrates, a phenomenon that has not been reported up to the present in the literature. The last aspect has been considered by us recently [3].

MATERIALS AND METHOD

In order to obtain young cultures, *B. subtilis* and *P. aeruginosa* strains have been inoculated on the usual culture media (i.e. broth), while the *S. cerevisiae* one - on the Sobourand medium [4]. After incubation at 37°C (48 h) for bacteria and 28°C (72 h) for yeast, respectively, the cultures have been developed on a special medium, to which the solution of cations subjected to analysis has been added, that means solutions of 0.2 $g.l^{-1}$ Fe^{2+} ($FeSO_4.7H_2O$), Mn^{2+} ($MnSO_4.4H_2O$) and Zn^{2+} ($ZnSO_4.7H_2O$). Subsequently, the samples were incubated at 37°C for bacteria and 28°C for yeast, respectively.

Bioadsorption of metallic cations has been tested in 250 ml Erlenmeyer flasks containing 40 ml culture medium, 10 ml bacterial inoculum and 50 ml solution from each cation subjected to analysis (Fe, Mn, Zn).

Small volumes of solutions have withdrawn every 24 hours, for a period of 6 days. The samples has been filtered and centrifuged, in order to eliminate the bacterial substratum and the supernatant solutions have been analyzed with an atomic absorption spectrophotometer of the Perkin Elmer 3300 type.

The adsorption capacity of metallic cations to the cell-wall surface of microorganisms can be expressed using the Langmuir isotherms in order to obtain the informations on "bioavalability" of active positions for metallic ions sites coordinations. The Langmuir isotherm may be written as follows [5]:

$$\frac{c}{\Gamma} = \frac{c}{\Gamma_{max}} + \frac{1}{k\Gamma_{max}} \qquad (1)$$

where c=is the concentration of adsorbed species at equilibrium, ($mg.l^{-1}$), Γ=is the amount of metallic ions adsorbed per unit of mass of adsorbent ($mg.g^{-1}$), Γ_{max}=is the maximum adsorption ($mg.g^{-1}$), k=the Langmuir constant related with the bonding energy.

The graphical expression of the (c/Γ) function at the equilibrium concentration (1/c) is correlated with the maximum adsorption (Γ_{max}) for each species.

RESULTS AND DISCUSSIONS

Analysing the adsorption capacities of the three cations by the *B. subtilis* species, it may be stressed that the Fe cation is the most adsorbed cation (almost 90% of the initial amount) a maximum being recorded in the 5th day of the process. The Mn cation is well adsorbed too by bacteria, with a maximum values of the adsorption process (82,3%) in the second day, while the Zn cation is "tolerated" by the bacteria. Because the adsorption of Zn^{2+} varied so little from a day to another, is demonstrating that the process take place until chemical equilibrium is attained between adsorption and desoption.

Figure 1 shows a plot of the Langmuir isotherm for the Fe cation adsorption by *B. subtilis*. The nature of the graph obtained for the different values of the equilibrium concentration, indicates that the process is adequate for a good adsorption (Γ_{max}=0.279), even at high concentrations of the analyzed cation. Usually, the solute amount adsorbed on the mass unit of adsorbant, increase in the same time with the metallic ion's amount, but not always in direct proportion. This thing obviously appeared from the graphical plot of the adsorption of Fe cation by *P. aeruginosa* (Fig. 2) (Γ_{max}=0.278), where the appearance of a sigmoidal curves corresponded to unfavourable adsorption at the highest Fe concentration [6].

The Fe adsorption process also occurs in good yields in the case of *S. cerevisiae* strain, as bioaccumulator (Γ_{max}=0.275) (Fig. 3). In the adsorption processes, *S. cerevisiae* is well known for its specificity for UO_2^{2+}: at higher temperatures and at pH values between 3.0 - 4.0, the cells accumulated about 15% of dry weight, but only 32% of cells showed the presence of "uranium dump" [7].

The main structural component of these bacterial walls, involved in bioaccumulation of these cations is peptidoplycan (a repeated dimer of N-acetylglucosamine and N-acetylmuranic acid, linked by a small peptide) [8,9]. This peptide contain rest of glutamic acid, whose carboxylate groups plays an important role in metal-binding. The capacity of *B. subtilis* to bind the metallic cations is

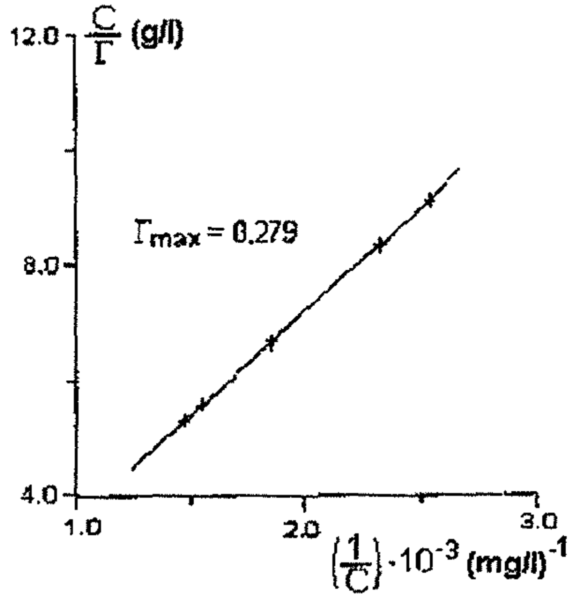

Fig. 1. Langmuir plot of ferrous sorption onto *B. subtilis*

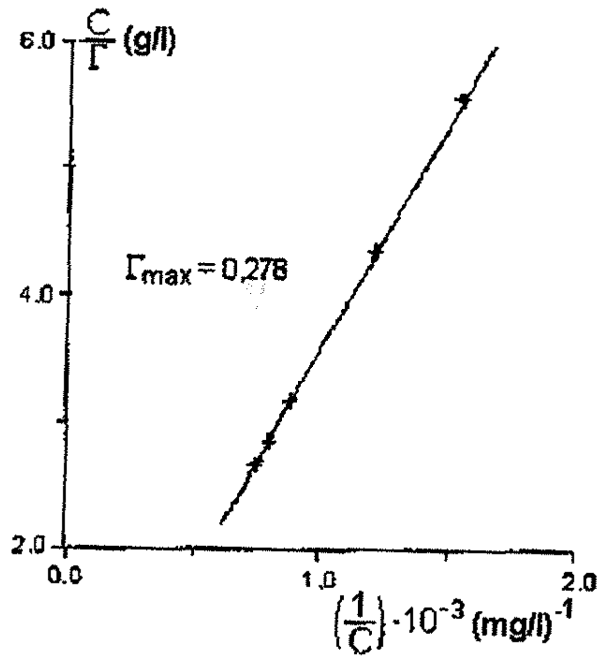

Fig. 2. Langmuir plot of ferrous sorption onto *P. aeruginosa*

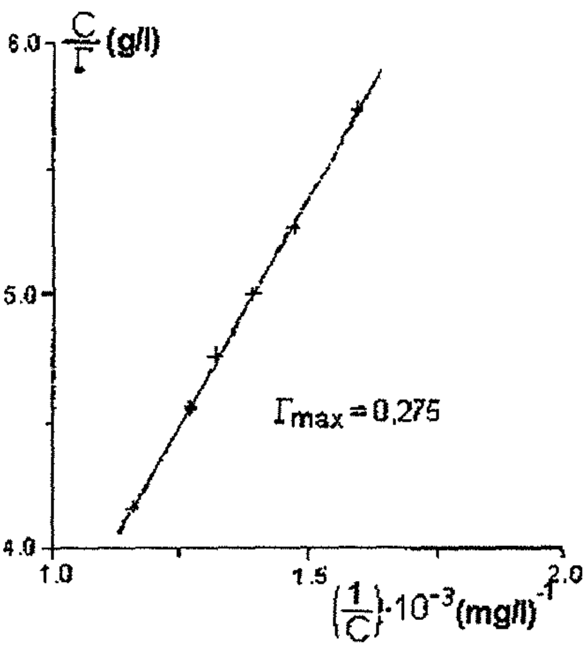

Fig. 3. Langmuir plot of ferrous sorption onto *S. cerevisiae*

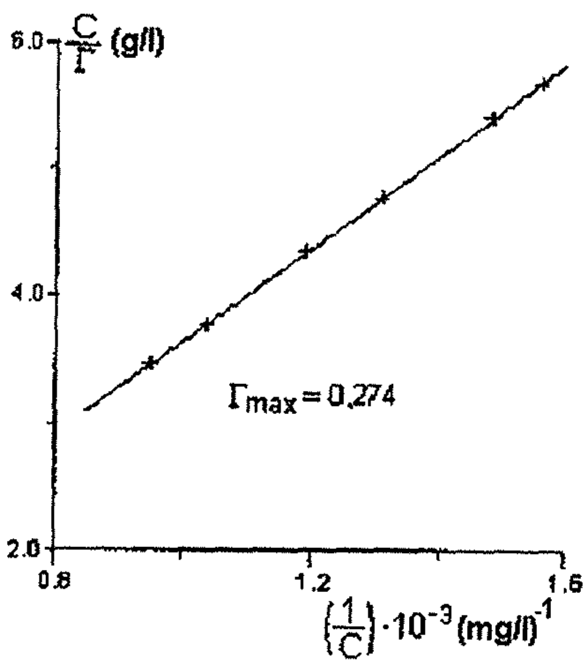

Fig. 4. Langmuir plot of manganese sorption onto *B. subtilis*

dramatically reduced, if the carboxylate group is esterified [10].

The peptidoglycan is followed by a second polymer, teichoic and/or teichuronic acid. These two compounds are linear polymers of glycerol phosphate and their contents in the cell-wall of *B. subtilis* is about 55% of dry weight [7]. Cell-walls of other Gram-positive organisms contain teichuronic acid, a carbohydrate made up of repeating units of a dissaccharide [7,10].

The anionic composition of the cell-walls might be varied function by the metallic cation's reactivity, which can substitute other metals. In this case, a competitivity for the occupancy of "active sites" from cellular walls structure is involved [8,9]. In Table 1 some classes of chemical compounds from the cell-wall composition of *B. subtillis* are given [11].

In Figures 4 to 6 the Langmuir isotherm for the adsorption processes of Mn by *B. subtilis, P. aeruginosa* and *S. cerevisiae*, respectively are presented. From the calculated values of Γ_{max} it results that both for *B. subtilis* and *P. aeruginosa*, the cation adsorptions by these two species, follow the same trends observed for the adsorption of Fe and Mn. So, the adsorption capacity of these two bacterian species are going on after a favourable mechanism, but there is no specificity for any one of the subjected cations. This thing could be explained based on the basis of the similar chemical's properties of these two cations (the stability in acidic medium, the tendency to form complex or hydratate compounds, close ionic rays, oxidability, medium strength after Pearson's theory), but could not be explained from the chemical composition point of view of cell-wall (Table 2).

Theoretically, it is possible that the metallic ions in high amounts to be intracellular accumulated, in spite of adsorption process to the cell-wall surface. Inside the cell, the cations could be precipitated as an oxalate or polyphosphate [10], but the transmembrane active transport for each cations is still unknown. An interesting example of intracellular accumulation is the metabolism-independent transport for uranium uptake by *P. aeruginosa* [7]. In this example, the uranium is taken up within 10 s and is localized into the cell, but only 44% of the cell, contained visible deposits.

The adsorption process of Mn by *S. cerevisiae* (Fig. 6) has developed with lower yields, Γ_{max}=0,279. In the first two days of determinations the process proceeds through the complexation of "active positions" from the cell-wall, after that the process develop through repeated sorption and desorption, until chemical equilibrium is attained.

The adsorption isotherm for the equilibrium distribution of a solute between the adsorbant and Zn solutions is shown in Figures 7 to 9. The values of Γmax are bigger than that obtained for the other two cations: 0.325, 0.324 and 0.329, respectively. This is not surprising because these values didn't show a more favourable adsorption compared with those for Fe and Mn cations. This cation (beside Cu) may be essential for microorganisms at small concentrations, while at high concentrations, he has a potentially action. The larger values for Γ_{max} for Zn adsorption processes are not determined by the ionic speciation, but may be explained by the "detoxification" processes of microorganisms. This process is not an usual one for microorganisms. It is coordinating by plasmides and is a manifestation of their resistance to different external stimuli. Many metallic ions are taken up by bacteria and are biotransformed in different non-toxic products [1,2,12].

Tab. 1. Chemical compiosition of *B. subtilis* cell-wall (11)

Amino-acids (g/100 g wall)	Hydrolysis products of teichoic acids	Monosaccharides present	Lipid contents (%)	Chemical composition
Lysine 6 Histidine 6 Aspartic acid 1 Glycine 5 Tyrosine 1.5	Alanine Glucose Inorg. Phosphate Anhydroribitol Ribitol	Glucose Galactose Manose	0.7 - 3	%N %P $\%S_{red}$ 5.1 5.35 34.0

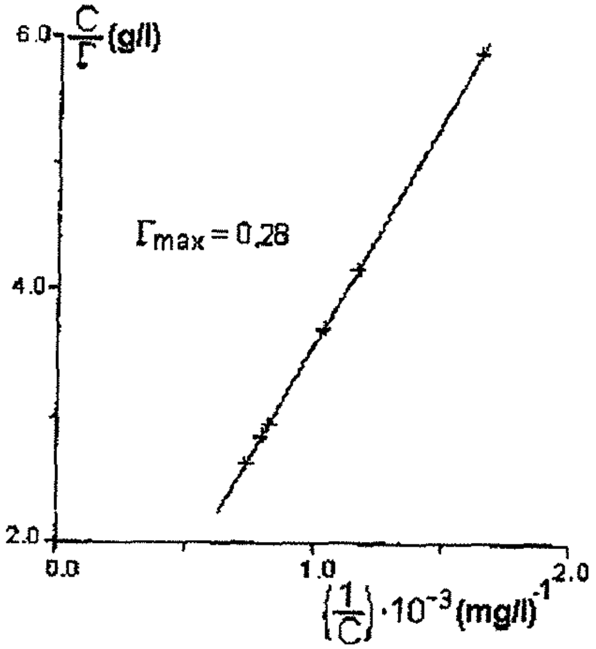

Fig. 5. Langmuir plot of manganese sorption onto *P. aeruginosa*

Tab. 2. Chemical composition of *P. aeruginosa* call-wall [11]

Amino-acids (g/100 g wall)	Monosaccharides present	Major classes of cell-wall constituients	Chemical composition
Alanine 5.1 Arginine 1.3 Aspartic acid 9.3 Phenylalanine 7.3 Glycine 7.1	Glucose Fucose Rafinose	Proteins Polysaccharides Lipids	%N %P $\%S_{red}$ 1.4-10.1 1.6-1.7 8.0-12.1

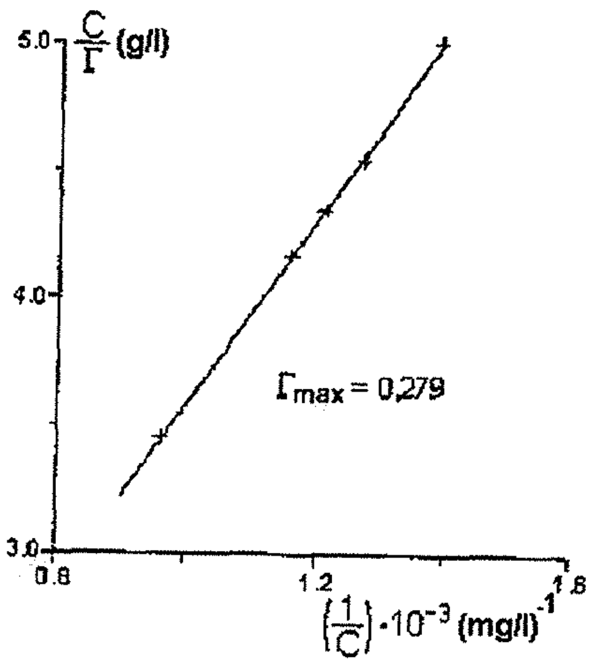

Fig. 6. Langmuir plot of manganese sorption onto *S. cerevisiae*

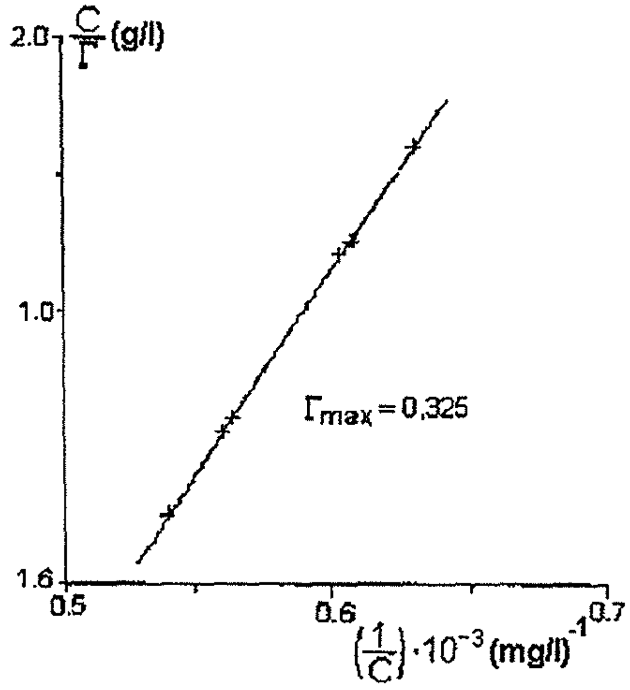

Fig. 7. Langmuir plot of zinc sorption onto *B. subtilis*

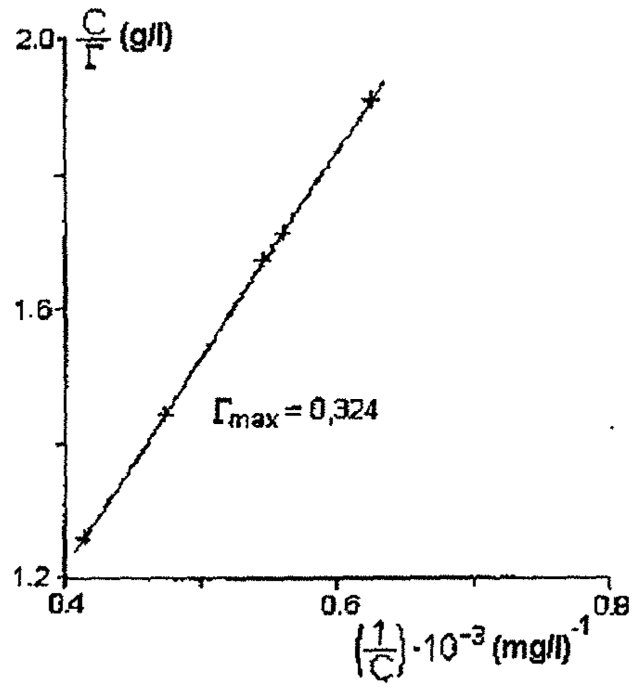

Fig. 8. Langmuir plot of zinc sorption onto *P. aeruginosa*

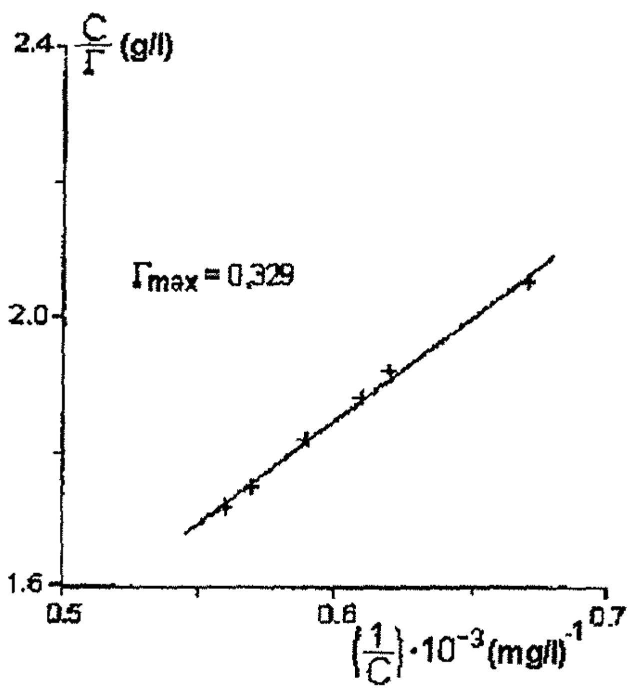

Fig. 9. Langmuir plot of zinc sorption onto *S. cerevisiae*

Most microorganisms are adopting different strategies for decreasing metal ion toxicity, strategies which are almost completely unknown at molecular levels. The metal toxicity can be decreased either through extracellular deposition as a insoluble form (like sulphide, oxalates, citrates etc.) or by through the intracellular deposition in the presence of varied proteins.

CONCLUSIONS

The positive adsorption of these three cations (Fe, Mn, Zn) by the microbian species (*B. subtilis, P. aeruginosa, S. cerevisiae*) followed the Langmuir isotherm, indicating a single layer adsorption to the cell-wall surface [6]. The process is develop by cations substitution from the initial solutions and their concentration to the cell-wall surface (bioadsorption) or inside the cell (bioaccumulation) [7], until the chemical equilibrium between adsorption and desorption is attained.

The adsorption isotherm may characterize the accumulation processes of metallic cations from solutions. Thus, *B. subtilis* and *P. aeruginosa* seems to be nonspecific for the adsorption of Fe and Mn cations. For Zn adsorption the values of Γ_{max} are bigger as a result of the detoxification process at high concentrations. A transmembrane-transport process with precipitation as a metal-protein may occur for Zn.

At high concentration of metallic ions the adsorptions process for *P. aeruginosa* presents a sigmoidal type curve, which show that the kinetics process is more complex and the graphical representation followed a different Langmuir curve [6].

Although the adsorption process by *S. cerevisiae* occurs with good yields for the subjected cations, the results obtained by the authors, did not confirm those from literature (they present lower values). The literature present *S. cerevisiae* as a good and rapid adsorbant of metallic cations, even at high concentrations.

The results obtained allow us to conclude that the method may be applied to real systems, which involve the adsorption of metallic cations from mine waters, waste waters etc. The originality of the work lies in the comparative biosorption study of the microorganisms considered for analysis.

ACKNOWLEDGEMENTS

The authors are grateful to Miss S. Dunca, Department of Microbiology, University "Al. I. Cuza" Iasi, for her technical assistance.

REFERENCES

1. A. E. Torma and K. Bosecker, *Prog. Ind. Microbiol.*, **16**, 77-118, (1982).
2. A. G. Lundgren and M. Silver, *Ann. Rev. Microbiol.*, **34**, 263-283, (1980).
3. R. Mocanu, I. Miu, T. Gavriloaiei, *Euroanalysis IX, Eur. Conf. on Anal. Chemistry*, Bologna, 1-7 sept, FECS, P45, (1996).
4. O. Ailisei, E. Nimiţan, St. Comănescu, *Lucrări practice de microbiologie generală*, Universitatea "Al.I.Cuza" Iaşi, (1980).
5. V. Isac, A. Onu, C. Tudoreanu, Gh. Nemtoi, *Chimie fizica, lucrari practice*, Ed. Stiinta, Chisinau, (1995), pp. 575-605.
6. G. M. Gadd and L. de Rome, *Apll. Microbiol. and Biotechnol.*, **29**, 610-617, (1988).
7. G. W. Strandberg, S.E. Shumate II, J.R. Parrett, Jr., *Appl. Environ. Microbiol.*, **41**,237-245, (1981).
8. T. G. Beveridge and R.G.E. Murray, *J. Bacteriol.*, **127**, 1502-18, (1976).
9. T. G. Beveridge and R.G.E. Murray, *J. Bacteriol.*, **141**, 876-87, (1980).
10. M. N. Hughes and R. K. Poole, *Metals and Microorganisms*, Chapman and Hall, London, New York, (1989), pp. 257-358.
11. M. R. J. Salton, *The Bacterial Cell Wall*, Elsevier Publishing Co., Amsterdam, London, New York, (1964).
12. N. Kuyucak and B. Volesky, *Biorecovery*, **1**, 189-204, (1988).