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Biosorption of Chromium, Copper, Nickel and Zinc Ions onto Fungal Pellets of *Aspergillus niger* 405 from Aqueous Solutions

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Summary

Fungal mycelium pellets of *Aspergillus niger* 405 were used for adsorption of different metal ions. The biosorption of Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} was studied over a range of metal ion concentrations, adsorption time, pH and co-anions. The process of uptake obeyed both the Langmuir and Freundlich isotherms. The results showed better biosorption for the first three metal ions at pH ranging from 4 to 6. On the contrary, pH values from 3 to 7 had no influence on sorption of chromium. Comparison of metal uptake in single and multi-component aqueous solutions of Cu-Zn-Ni-Cr was discussed. Fungus *Aspergillus niger* 405 showed a good affinity for binding of Cu^{2+} , Zn^{2+} and Ni^{2+} ions in single, while in multi-component solution it occurred only for copper and zinc.

Key words: fungus *Aspergillus niger* 405, heavy metals, biosorption, single and multi-component metal aqueous solutions

Introduction

The majority of toxic metal pollutants are waste products of industrial and metallurgical processes. Their concentrations have to be reduced to meet ever increasing legislative standards. According to the World Health Organisation (1), the metals of most immediate concern are cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc. The effluent from metal finishing processes may contain up to 10 mg/L of copper, chromium, nickel and zinc. Usually, when using methods such as chemical precipitation and reverse osmosis for the removal of metal ions from dilute aqueous stream, incomplete metal removal can be obtained. Furthermore, these processes have high reagent or energy requirements and generate toxic sludge that requires careful disposal (2).

The need for cost-effective process and safe method for removing heavy metals from discharging effluents has resulted in search for other unconventional materi-

als such as organic or inorganic sorbents (3). The use of microbial biomass of fungi (4–17), algae (18,19) and bacteria (20–22) for removal of heavy metals from aqueous solutions is gaining increasing attention. It has been found that both living and death microbial cells adsorb metal ions.

The aim of this work was to study the biosorption of metal ions from single and multi-component aqueous metal ion solutions using living mycelium pellets of *Aspergillus niger* 405. The living mycelium in this study means that the fungal cells were not deactivated purposefully after growth and that metal ion adsorption was conducted under non-growth conditions. The influence of initial concentration of heavy metals, pH, and different anions in the aqueous solutions on biosorption of metal ions was studied.

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Materials and Methods

Microorganism and growing conditions

The pure culture of *Aspergillus niger* 405 (Culture Collection of the Faculty of Chemical Engineering and Technology, Zagreb, Croatia) was used in this experiments. It was maintained on potato glucose agar slants at 4 °C. To produce mycelium pellets, 100 mL of potato glucose medium was inoculated in 500 mL conical flasks with spore suspension at final density $5.8 \cdot 10^6$ spore/mL and grown in submerged culture conditions as described earlier (23). When pellets were from 1 to 3 mm in diameter, mycelium pellets were harvested, washed thoroughly with sterile deionised water and stored at 4 °C for adsorption experiments.

Solutions

The aqueous solutions of metal ions chosen for investigations were prepared diluting stock solutions (10^{-2} M) of copper, chromium, nickel and zinc ions. The stock solution of CrO_4^{2-} ion was prepared using Na_2CrO_4 as commercially delivered, while the stock solutions of other metal ions (Cu^{2+} , Ni^{2+} , Zn^{2+}) were prepared from typical noncomplexing perchlorate solutions obtained by precipitation as hydroxides from their chloride solution, dissolved in perchlorate acid and recrystallised using standard laboratory procedures. Several different single-component metal ion (SCM) solutions and multi-component metal ion (MCM) solutions were prepared as follows:

- SCM: corresponding metal ions solution (5, 10, 15 and 20 mg/L) each adjusted to predetermined pH values (2, 3, 4, 5, 6 and 7)
- MCM-1: containing 5.0 mg/L of Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} adjusted at pH=5
- MCM-2: containing 5.0 mg/L of Cu^{2+} and Zn^{2+} and 10.0 mg/L Ni^{2+} and CrO_4^{2-} adjusted to pH=5
- MCM-2 + NaCl: same as MCM-2 plus 50 mg/L of Cl^- in the form of NaCl
- MCM-2 + NaNO_3 : same as MCM-2 plus 50 mg/L of NO_3^- in the form of NaNO_3
- MCM-2 + Na_2SO_4 : same as MCM-2 plus 50 mg/L of SO_4^{2-} in the form of Na_2SO_4

The pH of aqueous solutions were adjusted with 0.1 M HClO_4 or 0.1 M NaOH.

The supporting electrolyte for voltammetric determination of copper and zinc was 0.1 M HCl, while for determination of chromium and nickel $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$ buffer solution in equimolar concentrations (0.1 M) was used. All chemicals were of p.a. grade and purchased from Merck (Darmstadt, Germany).

Experimental procedure

Adsorption property of fungal mycelium pellets *Aspergillus niger* 405 for biosorption of different metal ions in single-component aqueous solution were measured with standard batch equilibrium experiments. The mass concentrations of the heavy metal ions ranged from of 5 to 20 mg/L. In the initial experiment the effect of solution pH on biosorption of metal ions was studied. For this purpose aqueous solutions (series SCM) containing

10 mg/L of investigated metal ions, adjusted to the predetermined pH values ranging from 2 to 7, were used. Adsorption was then started by adding 1.0 g of fungal biomass (fresh weight) into 100 mL metal solution in 500 mL Erlenmeyer flasks. The flasks were sealed with Parafilm and agitated on a rotary shaker (Gallenkamp IH 460) at 200 rpm and 25 °C during 10 h. All adsorption tests were overall run in duplicate and relative deviation obtained was less than $\pm 5\%$. Controls without the adsorbent were also run in parallel. Furthermore, the adsorption isotherm experiments were performed under the same conditions but only at pH=5, which was determined as the optimal one.

Uptake of metal ions in experiments with multi-component aqueous solutions containing fungal biomass were carried out similarly as in a single-component metal systems, but only at pH=5. The solution samples were taken at predetermined time intervals during 10 h for evaluation of sorption kinetics.

Measurement procedure

Voltammetric measurements were performed, as described in details elsewhere (24), using the polarographic analyser PAR 264A (EG&G PAR, USA) connected with a XY recorder PL 3 (Lloyd, United Kingdom). A three-electrode electrochemical cell was used. The hanging mercury drop electrode (HMDE) or dropping mercury electrode (DME) were used as working electrode, the saturated calomel electrode (SCE) as reference electrode and Pt wire as an auxiliary electrode. Mass concentration of investigated heavy metals was measured in aqueous solution at the beginning, at the equilibrium and in the residual of biomass. Chromium and nickel content were determined by differential pulse polarography (DPP) on dropping mercury electrode (DME). Voltammograms for chromium(VI) and nickel were observed at approximately -0.34 V and at -0.79 V *vs.* SCE, respectively. Copper and zinc were determined by DPP at hanging mercury drop electrode (HMDE) in 0.1 M HCl as a supporting electrolyte. The voltammograms for copper and zinc were observed at $+0.05$ V and at -0.9 V *vs.* SCE, respectively. To evaluate the metal ion concentration, standard addition procedure was used.

The metal ion concentrations adsorbed onto biomass were calculated from the difference between metal concentration before and after the biosorption process. There was no need for sample digestion (elimination of organic matter) of the solutions separated from biomass. However, as a control, 1.0 g of biomass was wet digested using HNO_3 (67 %) and H_2O_2 (30 %), and the metal ion concentrations were determined in the solution obtained. The differences in the metal ion uptakes measured in solutions and biomass were generally less than 10 %.

Results and Discussion

Effect of pH on metal biosorption

Sorption of heavy metals from aqueous solutions depends on properties of adsorbent and molecules of adsorbate transfer from the solution to the solid phase. It has been also reported that biosorption capacities for

heavy metals are strongly pH sensitive and that adsorption increases as solution pH increases (11,14,25,26).

Initial investigation of biosorption capability of *A. niger* 405 for copper, chromium, nickel and zinc ($\gamma=10$ mg/L) in the single component solution (SCM) at different values of pH (2 to 7) is presented in Fig. 1.

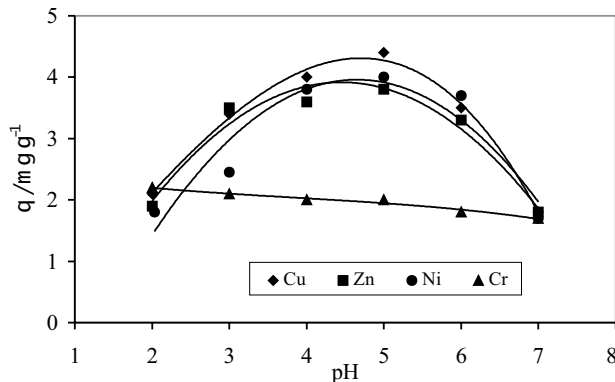


Fig. 1. The effect of solution pH on biosorption of Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} by *A. niger* 405 at 25 °C and $\gamma_{\text{Me}}=10$ mg/L

It has been found that the fungal biomass possessed maximum sorption capacity for the cationic metal ions at pH values between 4 and 6. At pH below 3, uptakes of copper, nickel and zinc were negligible, probably due to the cation competition effects with oxonium (hydronium) ion H_3O^+ . Furthermore, at pH=6 biosorption of cationic metals decreased probably because of chemical precipitation. Sorption studies were meaningless above pH=7, due to the formation of insoluble products in investigated solutions, what is in accordance with the solubility products of metal hydroxides as follows: $K_{\text{sp}}(\text{Cu}(\text{OH})_2)=10^{-19}$ M^3 ; $K_{\text{sp}}(\text{Zn}(\text{OH})_2)=10^{-17}$ M^3 ; $K_{\text{sp}}(\text{Ni}(\text{OH})_2)=10^{-14}$ M^3 (26). On the contrary, insignificant uptake of CrO_4^{2-} by *A. niger* 405 has been found in the whole investigated pH range, though increased removal of CrO_4^{2-} by complexation on bacteria, algae, fungi and yeast at pH=2 was reported (15,18,28). According to the results of this initial experiment, the further biosorption investigations were performed at pH=5 as an optimal value.

Adsorption isotherms

Sorption isotherms represent the concentration dependent equilibrium distribution of metal ions between aqueous and solid phases. Fig. 2 shows the isothermal adsorption equilibrium of Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} at 25 °C and pH=5 on fungal mycelium of *Aspergillus niger* 405. Each point represents a mean value obtained from six independent solutions. The biosorption rate was fast within the first 2 h of contact time with primary rapid uptake, and then slowly reached the equilibrium. The biomass became saturated within 10 h.

These isotherms follow the typical Langmuir adsorption pattern as shown by the linear transformation. The Langmuir model is based on the assumption of a single layer adsorption on a complete homogenous surface. In spite of this, it has been often used as a model for metal adsorption on various types of biosorbents,

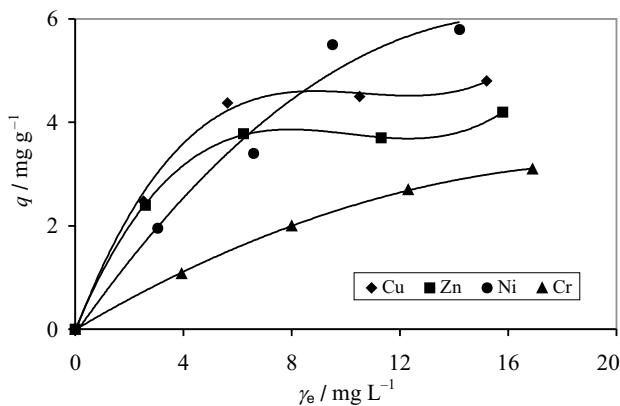


Fig. 2. Equilibrium sorption isotherms of Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} by *A. niger* 405 at 25 °C and pH=5 during 10 h

even though the metal uptake may not exactly follow the single layer adsorption mechanism (14). The linearised form of Langmuir equation is represented by the following expression (29):

$$\frac{\gamma_e}{q} = \frac{1}{q_{\text{max}}b} + \frac{\gamma_e}{q_{\text{max}}} \quad /1/$$

where γ_e is the equilibrium solution concentration (mg/L), q_{max} is the amount adsorbed at equilibrium (mg/g), the Langmuir constants q_{max} and b are related to adsorption capacity and energy of adsorption, respectively. The linear plot of γ/q vs. γ_e shows that investigated metal ions adsorption by *A. niger* 405 obey the Langmuir plots. The isotherm constants obtained by linear regression are listed in Table 1.

Table 1. Regression parameters for Langmuir isotherm for biomass of *A. niger* 405 (equilibrium pH=5) Regression line: $(\gamma / q) / ((\text{mg}/\text{kg}) / (\text{mg}/\text{g})) = (a(1) \pm \text{std.err.}) + (a(2) \pm \text{std.err.}) \gamma / (\text{mg}/\text{kg})$; R = correlation coefficient; b and q_{max} are parameters in Eq. / 1 /

Metal	a(1) ± std.err.	a(2) ± std.err.	R	q_{max}	b
Cu	0.45 ± 0.21	0.177 ± 0.015	0.993	5.66	0.39
Zn	0.47 ± 0.26	0.213 ± 0.018	0.993	4.70	0.45
Ni	1.33 ± 0.35	0.071 ± 0.029	0.867	14.1	0.05
Cr	2.99 ± 0.26	0.139 ± 0.017	0.985	7.2	0.04

Greater values of b mean higher affinity of biosorbent to investigated metals. Consequently, the preference of investigated biosorbent for metal ions is as follows: $\text{Zn} > \text{Cu} > \text{Ni} > \text{Cr}$.

Furthermore, the Langmuir model has been used in this study to enable comparison of the biosorbent characteristics of fungal mycelium of *A. niger* 405 with other types of biomasses described in the literature (5,8,12,26). Table 2 provides a comparison on heavy metal capacities of some fungal organisms (based on measurement of mg of metal ion adsorbed per g of biomass). Adsorption capacity of *A. niger* 405 for copper and nickel was comparable to other fungal adsorbents. The values indicate that the living *A. niger* 405 is a quite good biosorbent. Its relatively high affinity to copper and nickel,

Table 2. The metal adsorption capacities of some fungal organisms

Metal type	Biomass type	Metal adsorption capacity (mg/g) ^{a,b,c}	Reference
Copper	<i>A. niger</i> 405	4.4	This study
	<i>A. niger</i>	2.9 ^c	Kapoor and Viraraghavan, 1998
	<i>Mucor rouxii</i>	2.6	Mullen <i>et al.</i> , 1992
	<i>P. chrysosporium</i>	3.9	Sing and Yu, 1998
Nickel	<i>A. niger</i> 405	2.0	This study
	<i>A. niger</i>	1.1 ^c	Kapoor and Viraraghavan, 1998
	<i>R. nigricans</i>	1.0	Holan and Volesky, 1995

^{a,b}Represents the values obtained at equilibrium metal concentration of 10 mg/L and 5 mg/L, respectively

^c The metal adsorption capacity in term of biomass where the matrix contains approximately two-third biomass

compared with the other biosorbents, might be attractive in treatment of industrial waste water (13) because of its capability of uptaking investigated metal ions from a diluted solution.

The Freundlich equation is another model which has been commonly used to describe adsorption isotherms. Its linearised form is represented by the equation (25):

$$\log q = \log K + \frac{1}{n} \log \gamma_e \quad /2/$$

where q is the amount adsorbed per unit mass of adsorbent and γ_e is the equilibrium concentration (mg/L). The plot of $\log q$ vs. $\log \gamma$ is linear and constants K and n can be evaluated from the slopes and intercepts. The Freundlich constants are shown in Table 3.

Table 3. Regression parameters for Freundlich isotherm for biomass of *A. niger* 405 (equilibrium pH=5) Regression line: $(\log \gamma / \log q) / ((\text{mg/kg}) / (\text{mg/g})) = (a(1) \pm \text{std.err.}) + (a(2) \pm \text{std.err.}) \gamma / (\text{mg/kg})$; R = correlation coefficient; K and n are parameters in Eq. /2/

Metal	$a(1) \pm \text{std.err.}$	$a(2) \pm \text{std.err.}$	R	n	K
Cu	0.30 ± 0.12	0.353 ± 0.116	0.907	2.83	1.98
Zn	0.29 ± 0.09	0.289 ± 0.085	0.924	3.46	1.94
Ni	-0.07 ± 0.12	0.758 ± 0.119	0.976	1.32	0.85
Cr	-0.39 ± 0.07	0.740 ± 0.063	0.993	1.35	0.41

It was found that the adsorption equilibrium data were better fitted by the Freundlich isotherm, although they can also be modelled by the Langmuir isotherm, in the concentration range studied. Using the Freundlich isotherm model, the agreement of biosorption of metal ions with the Irving-Williams series ($\text{Cu} > \text{Zn} > \text{Ni} > \text{Cr}$) of stability (30) is better and better linear correlation estimated by correlation coefficients was obtained. The values of correlation coefficients generated by linear regressions performed on isotherm data is in accordance with

literature (3). The biosorption of the CrO_4^{2-} , as a chromate ion was low probably because of the lack of positively charged functional groups.

Biosorption kinetics of metals on the biomass of *A. niger*

The kinetics of the selected metal ions biosorption by fungal mycelium pellets in multi-component systems (MCM-1 and MCM-2) was investigated. The results are presented in Figs. 3A and 3B for MCM-1 and MCM-2, respectively. The sorption processes were found to be slower than in the single-component metal solutions, and equilibrium was reached after 5 h of the experiments. Reaching the equilibrium point, copper and zinc were bound 46 %, nickel 30 % and chromium 20 %. Moreover, during the next 5 hours there was no evidence in further uptake of metal ions. Concerning copper, chromium and zinc no significant removal efficiency could be noticed between single (SCM) and multi-component (MCM-1) systems. On the contrary, removal efficiency of nickel diminishes in the MCM-2 multi-component system. Generally, it can be concluded that the kinetics of biosorption appears to be faster in the single-component systems in the comparison with the multi-component one. It is probably due to the absence of competitive processes between metals and biomass. Their interactions take place at the active adsorption sites where solid-liquid phase equilibrium occurs. Therefore, de-

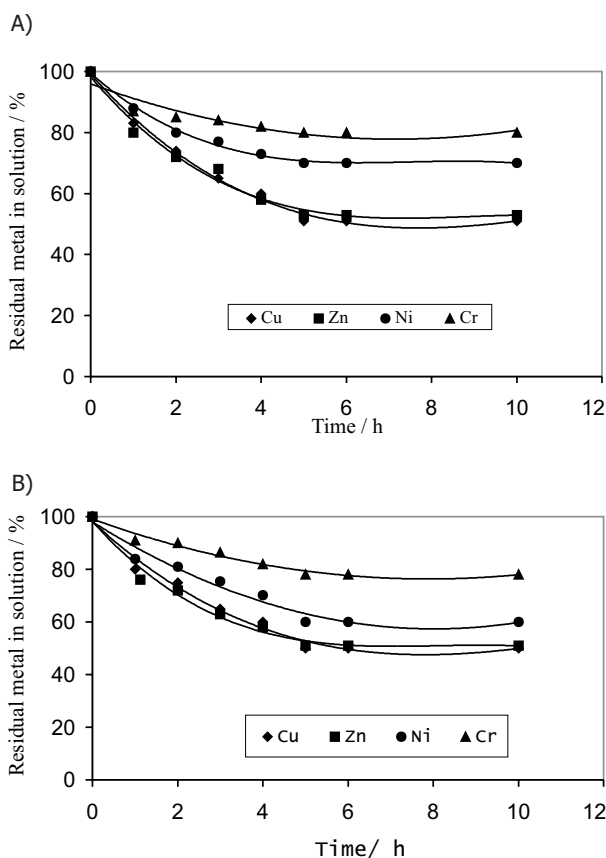


Fig. 3A and B. Metal concentrations in MCM-1 solution (A) and in MCM-2 solution (B) during Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} biosorption by *A. niger* 405 at 25 °C and pH=5 during 10 h

creased biosorption of Ni²⁺ in MCM-2 multi-component system can be explained by possible simultaneous competition between metal ions and biomass in different competitive equilibria.

Effect of anions on metal biosorption

Domestic and industrial waste waters usually contain respectable amounts of different anions, what may influence the biosorption processes investigated. Thus, experiments were carried out with multi-component system (MCM-2) containing different anions. The efficiency of biosorption of investigated metals in the presence of Cl⁻, NO₃⁻ and SO₄²⁻ anions, beside the presence of perchlorate, is shown in Fig. 4. Evidently, concerning copper, chromium and zinc, no significant removal efficiency could be noticed between single (SCM) and multi-component (MCM-1) systems. On the contrary, presence of chloride ions significantly decreased efficiency of Cu²⁺, Zn²⁺, Ni²⁺ and CrO₄²⁻ biosorption. Thus, observed efficiency decreased more than 50 % in the presence of chloride ion in respect of its absence.

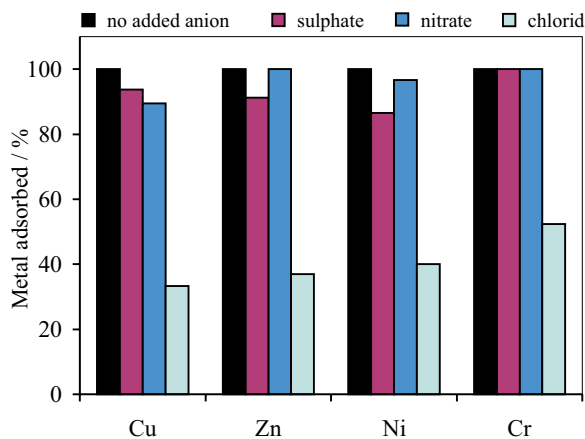


Fig. 4. Effect of anions on Cu²⁺, Zn²⁺, Ni²⁺ and CrO₄²⁻ biosorption by *A. niger* 405 at 25 °C and pH=5 during 10 h; the adsorption capacity at zero added anions is taken as 100 %

It is well known that among the investigated anions, chloride ions form the most stable complexes with the investigated metal ions (31). So, one could expect that this difference in behaviour of chloride ions compared to nitrate and sulfate ions is due to different chloride complex species formed in aqueous solutions.

Conclusion

It has been found that the fungal biomass possessed maximum sorption capacity for Cu²⁺, Ni²⁺ and Zn²⁺ at pH ranging from 4 and 6, while insignificant uptake of chromium (CrO₄²⁻) was noticed. The biosorption equilibrium data obeyed Langmuir and Freundlich model in the concentration ranges studied.

The preference of investigated biosorbent for metals is as follows Cu>Zn>Ni>Cr.

The sorption processes were found to be slower in multi-component than those in the single-component

metal solutions, though no significant differences in uptake efficiencies among them were observed.

Concerning copper, chromium and zinc, no significant removal efficiency could be noticed between single and multi-component aqueous metal solutions in the presence of NO₃⁻ and SO₄²⁻ anions. On the contrary, presence of chloride ions significantly decreased efficiency of metal biosorption.

This study showed that *A. niger* used in industrial production of citric acid is present as mycelial waste in great quantities, and that it can be used for metal uptake in waste water treatment of metal finishing industry.

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Biosorpcija kroma, bakra, nikla i cinka iz vodenih otopina na micelijska zrna *Aspergillus niger* 405

Sažetak

Ispitana je mogućnost uklanjanja metalnih iona iz vodenih otopina s pomoću plijesni *Aspergillus niger* 405. Biosorpcija Cu^{2+} , Zn^{2+} , Ni^{2+} i CrO_4^{2-} praćena je u različitom rasponu koncentracije iona, ovisno o vremenu adsorpcije, pH otopine i prisutnosti dodanih aniona. Provedeni postupci uklanjanja mogu se uspješno modelirati Freundlichovom i Langmuirovom izotermom. Rezultati pokazuju da je biosorpcija za prva tri metalna iona bolja pri pH od 4 do 6, dok u pH području od 3 do 7 ne postoji utjecaj na sorpciju kroma. Rezultati uklanjanja metalnih iona iz vodenih otopina s pojedinim ionom uspoređeni su s rezultatima dobivenim iz otopina smjese iona. Plijesan *A. niger* 405 pokazala je dobar afinitet za vezivanje Cu^{2+} , Zn^{2+} i Ni^{2+} u jednostavnom sustavu, a u smjesi metalnih iona to su svojstvo zadržali samo bakar i cink.