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BIOLOGICAL REMOVAL OF Cr(VI) IONS FROM AQUEOUS SOLUTIONS BY *TRICHODERMA VIRIDE*

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Abstract: The paper presents biological removing of Cr(VI) ions from aqueous solution by applying autochtonic fungi seedling of *Trichoderma viride* originated from chromium mud samples. The growth of organism and removing of chromium(VI) was performed in aqueous solution of various chromium(VI) contents and at optimal value of pH. During 14 days of incubation, samples of 5 cm^3 each were collected every day for determination of chromium(VI) in solution and the efficiency of biological removal was specified. Since removal of chromium(VI) from aqueous solution may occur because of reduction, biosorption or bioaccumulation processes, to determine which one occurred, the Cr(III) contents were determined in samples of the medium as well in ooze after mycelium irrigating and in mycelium.

Key words: *bioaccumulation, chromium, Cr(VI), microscopic fungi, Trichoderma viride*

Introduction

Chromium occurs widely in earth's crust as a natural component of rocks, minerals, soils and water. On a worldwide basis, about 80% of the chromium mined goes into metallurgical applications (particularly for manufacture of stainless steel). Only about 15% is used in chromium chemicals manufacture and the remainder is used in refractory applications. In environment, the most often occurred valences of chromium are Cr(III) and Cr(V). The more toxic and harmful, both for environment and human beings, is Cr(VI). Because of its toxic properties and high mobility, effluents and wastes containing this element are treated as highly dangerous. Even relatively small amounts of this element may be a source of danger for ecosystem because of the persistence of its compounds and possibility of multiplying its concentration (Badura, 1993; Barnhart, 1997; Wolak et al., 1997).

The currently applied chemical methods of treatment of effluents containing chromium, which results are not satisfying, require significant financial costs (Kowalski, 2002). The alternative for them may be biotechnological processes (Bai et al., 2003; Chen et al., 1997; Sen et al., 2007). The application of selectively chosen microorgan-

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isms may significantly limit the amount of chromium introduced to the environment. The main advantage of biotechnological methods is the fact that these methods are economic and environmentally friendly. Chromium is removed by a cellular metabolism of microorganisms mainly by bioaccumulation, biosorption and biotransformation.

Previous investigations describe the applications of living and dead microorganisms cells to remove Cr(VI) from aqueos solutions by biosorption (Dönmez and Koçberber, 2005; 2007; Dursan et al., 2003; Ksheminska et al., 2005; Saxena et al., 2000; Srinath et al., 2002) and bioaccumulation (Anjana et al., 2007; Deepa et al., 2006; Donghee Park et al., 2005; Kapoor and Viraraghavan, 1995; Kumar et al. 2008; Ming Zhou et al., 2007; Morales-Barrera and Cristiani-Urbina, 2006; Parka et al., 2005; Srinath et al., 2002; Srivastavaa, Thakurb, 2006; Ziagova et al., 2007). Each of these methods has advantages and disadvantages. The application of dead biomass removes problems of toxic metal concentration in solutions and requirements connected with growth environment – nourishment. Furthermore, the adsorbed metal may be easily removed and the remaining biomass may be applied again. However, the limitation of this method is the fact that no reactions are being continued in the dried cells.

The application of living biomass makes it possible to remove metals during its growth allowing to avoid processes of its reproduction, drying and storage. Unfortunately, in this case the metal concentration in environment is highly important since too high concentration may be toxic for the growing biomass. This problem can be avoided by applying the microorganisms of high tolerance to high concentrations of Cr(VI) or getting it by adaptive processes.

The purpose of the investigation presented in the paper is to optimize the biological process of removing Cr(VI) by application of indigenous microorganisms isolated from chromium mud samples (Hołda et al., 2009).

Materials and methods

The autochthonic fungi seedling *Trichoderma viride*, originating from a chromium mud was selected to research on removing Cr(VI).

Investigation of process dependency on pH

Strains of fungi were grown aerobically at 28 ^{o}C in accumulating medium prepared by mixing Cr(VI) solution autoclaved separately (at 120 ^{o}C for at least 20 min) and sterilized solution according to Waksman. The pH of medium was adjusted to the desired value by using 0.5 M sulfuric acid(VI) solution. Cultures were formed in a 300 cm^3 Erlenmeyer flask with 100 cm^3 of accumulation medium containing 50 mg of ions of Cr^{6+}/dm^3. The 2.5 cm^3 samples of medium were collected from each Erlenmeyer flasks daily, during 14 days, then transferred to flasks of 25 cm^3 volume each. Then, solutions of 2 M sulfur acid(VI) and 1.5-difenylocarbaside(I) were added to the 25 cm^3 flasks. After 5 minutes, the flasks were filled up to the line with medium accord-

ing to Waksman. Residual chromium(VI) ions concentrations in the bioaccumulation medium were determined by measuring the absorption at 540 nm by means of spectrophotometer Cadas 200 type LPG 392 (Gajkowska-Stefańska et al., 2001; Hermanowicz et al., 1999; Marczenko and Balcerzak, 1998; PN-C-04604:1977; PN-EN ISO 18412:2007).

Determination of chromium(VI) contents

Agents used for determination of chromium contents, that is 1.5-difenylocarbaside(I) and 2 M sulfuric acid(VI), were prepared according to the Polish standard (PN-EN 12441-10).

Before starting the measurements, the standard line was prepared. To this purpose, solutions of sulfuric acid(VI), 1.5-difenylocarbaside(I) and certain volumes of standard solution of chromium(VI) were introduced to 100 cm^3 flasks to get the Cr^{6+} ions concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/dm^3.

The analytical samples were prepared by mixing solutions of sulfuric acid(VI), 1.5-difenylocarbaside(I) and sample solution in 25 cm^3 flask.

Dependence of the process of chromium (VI) concentration in nourishment

The investigation of Cr(VI) ions concentration in medium in relation to pH allowed to determine the best reaction by which the fungi grow the best. It was found to be 4.5.

A certain amount of medium and $K_2Cr_2O_7$ (1g of Cr^{6+} ions/dm^3) were transferred to Erlenmeyer flasks to get the required chromium concentration in certain sample in the volume of 100 cm^3 of the bed.

The determination of chromium(VI) contents was conducted every day at the same hour. The samples of 2.5 cm^3 of medium were collected, which were then transferred to 25 cm^3 flasks (10-fold dilution). Next, the solutions of 2M sulfuric acid(VI) and 1.5-difenylocarbaside(I) were added. After 5 minutes, the sample was filled up to the line with the Waksman medium. Chromium(VI) ions concentration in the sample was determined by measuring absorption at 540 nm using spectrophotometer Cadas 200, type LPG 392 (Gajkowska-Stefańska et al., 2001; Hermanowicz et al., 1999; Marczenko, Balcerzak, 1998; PN-C-04604:1977; PN-EN ISO 18412:2007).

In case of the concentration higher than the standard scale range, the samples were adequately diluted.

Determination of biological type of Cr(VI) ions removal

The removal of chromium(VI) from the aqueos solution may occur because of reduction, biosorption or bioaccumulation processes. To determine which of them occurred during the investigation, the Cr(III) contents were determined in samples of medium as well chromium contents in ooze after the mycelium irrigating and in mycelium.

Determination of Cr(III) in medium

The Cr(III) ions contents were determined on the basis on the difference between total chromium and chromium(VI) contents in the medium after 14 days of incubation.

For total chromium contents determination, the samples of the medium with volume assuring the concentration of Cr within the standard scale range, were introduced to a beaker of 200 cm^3 in volume and were filled up to the volume of 50 cm^3. Next, for oxidation of Cr(III) ions to Cr(VI) the solutions of sulfuric acid(VI) and ammonium persulfate were added to the sample and then it was boiled and maintained in this state during 20-25 minutes. After cooling, the samples were transferred to flask of 50 cm^3 in volume and the solution of 1.5-difenylocarbaside(I) was added. After 5 minutes the sample was filled up to the line with the Waksman medium. Chromium(VI) ions concentration in the sample was determined by measuring absorption at 540 nm using spectrophotometer Cadas 200, type LPG 392 (Gajkowska-Stefańska et al., 2001; Hermanowicz et al., 1999; Marczenko, Balcerzak, 1998; PN-C-04604:1977; PN-EN ISO 18412:2007).

Determination of chromium contents in ooze after mycelium irrigating

Mycelium was investigated after 14 days of incubation. To determine the presence of chromium adsorbed on the surface, the mycelium was irrigated. The obtained ooze was then analyzed to find the total chromium content (Gajkowska-Stefańska et al., 2001; Hermanowicz et al., 1999; Marczenko, Balcerzak, 1998; PN-C-04604:1977; PN-EN ISO 18412:2007).

Determination of chromium contents in mycelium

After 14 days of incubation, dried fungi at 105 ^oC were inserted into oven at temperature of 600 ^oC. Next, the chromium compounds were transformed into dilatable nitrates by means of concentrated nitric acid(V). The content of Cr(VI) in the mineralized sample was determined by the spectrophotometric method (Gajkowska-Stefańska et al., 2001).

Results and discution

Investigation of process dependency on pH

On the basis of experimental results graphs were created with dependency of chromium(VI) ions concentration in the medium on pH.

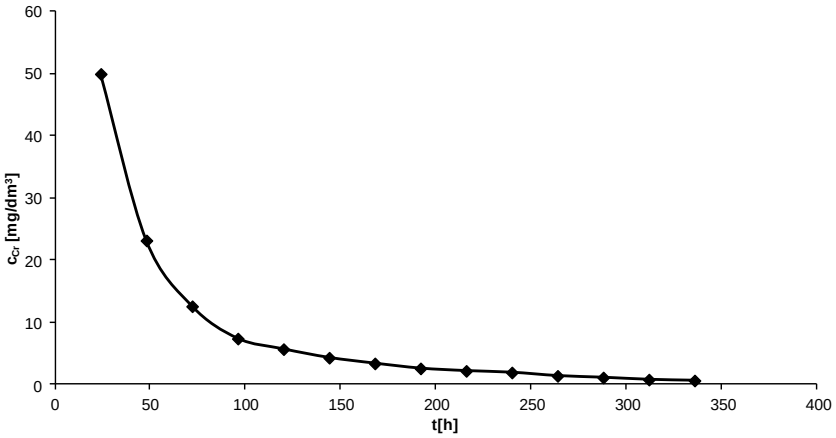

Figure 1. $c_{Cr} = f(t)$ at pH=4.0. Initial concentration of chromium(VI) was 50 mg/dm^3

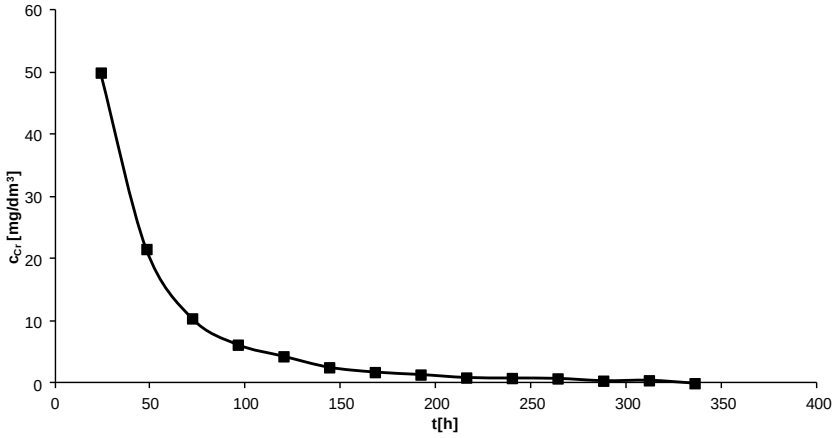

Figure 2. $c_{Cr} = f(t)$ for pH = 4.5. Initial concentration of chromium(VI) was 50 mg/dm^3

Figures 1–7 show the change of Cr(VI) concentration in the medium with different initial pH, ranging from 4.0–6.5. Based on our study, the optimal pH for *Trichoderma viride* was found. At pH = 4.5, chromium(VI) ions were removed the most efficiently and the mildew fungi developed the best.

The optimum initial pH value for *Trichoderma viride* was 4.5. Morrales-Barrera et al. (2006) studied Cr(VI) removal by *T. viride* in a pneumatically agitated bioreactor. In the experiments the initial pH of the culture media was 6.0±0.1 and the pH was not controlled during the experiments. In the case of biosorption by immobilized to Ca-alginate *T. viride* biomass, the optimum pH was 2.5 (Kumara et al., 2011) was determined.

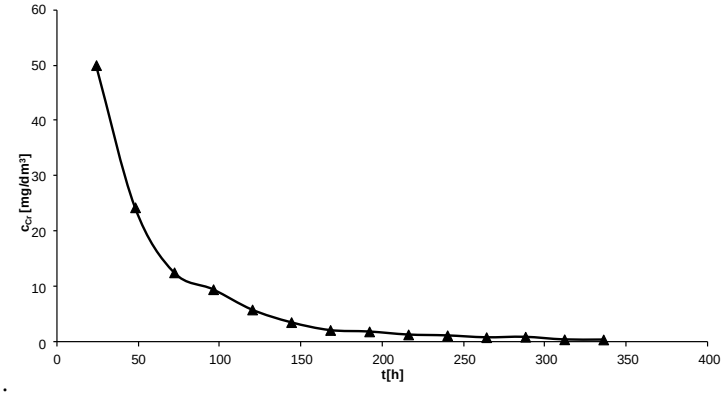

Figure 3. $c_{Cr} = f(t)$ for pH = 5.0. Initial concentration of chromium(VI) was 50mg/dm^3

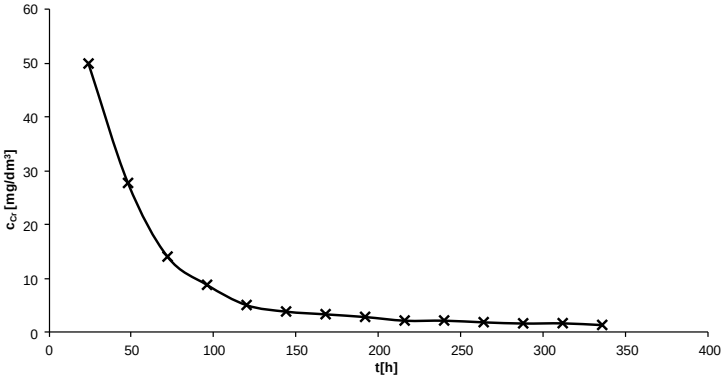

Figure 4. $c_{Cr} = f(t)$ for pH = 5.5. Initial concentration of chromium(VI) was 5 mg/dm^3

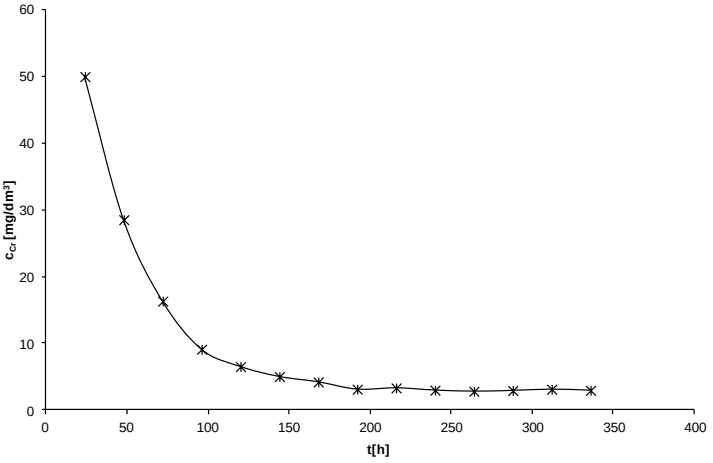

Figure 5. $c_{Cr} = f(t)$ for pH = 6.0. Initial concentration of chromium(VI) was 50 mg/dm^3

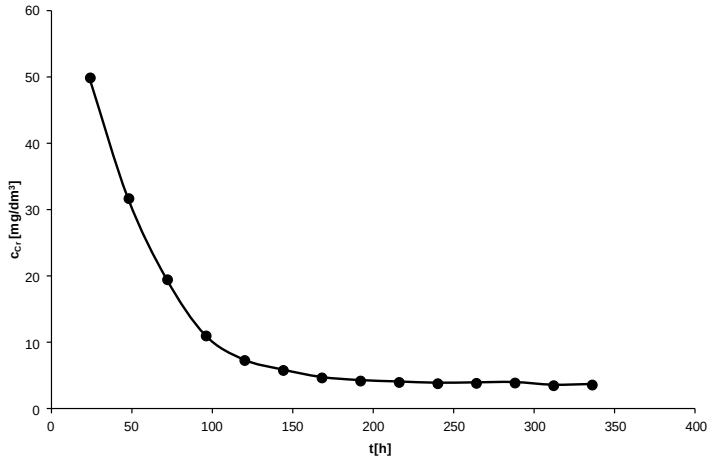

Figure 6. $c_{Cr} = f(t)$ for pH = 6.5. Initial concentration of chromium(VI) was 50mg/dm^3

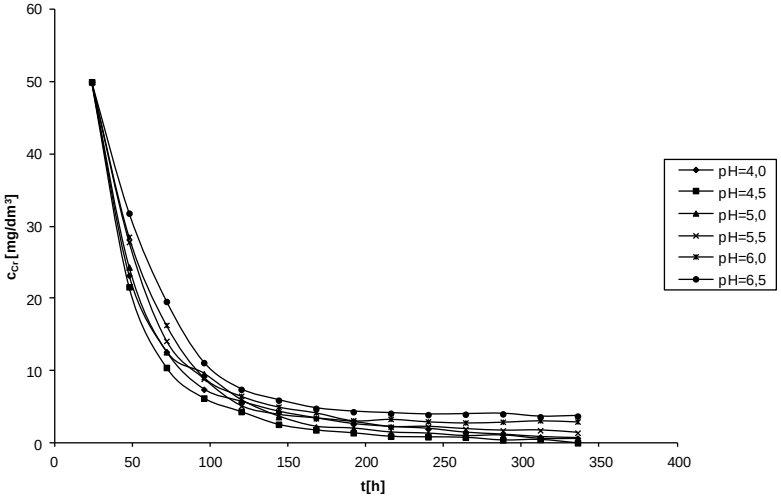

Figure 7. $c_{Cr} = f(t)$ by various values of pH, initial concentration of chromium(VI) 50 mg/dm^3

The determined optimal pH was the basis for further research, where the relation between chromium(VI) ions concentration and initial concentration of chromium(VI)

Dependency on chromium(VI) concentration in the sample

On the basis of measurement results Figures 8–14 present the dependency of chromium(VI) ions concentration in medium on the process time. The effect of initial Cr(VI) concentration was investigated over a range of about 10–125 mg/dm^3.

Obtained results suggested that Cr(VI) removal by *Trichoderma viride* occurred even at the highest concentration of 125 mg/ dm^3, but complete Cr(VI) removal was

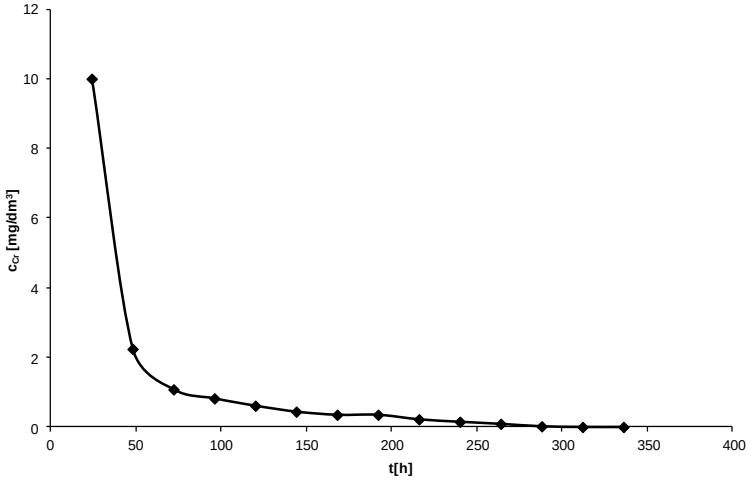

Figure 8. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 10 mg/dm^3; pH = 4.5

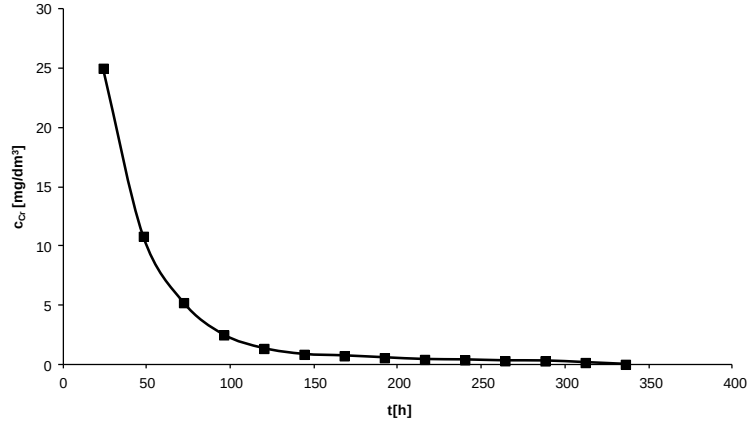

Figure 9. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 25 mg/dm^3; pH = 4.5

observed for 10, 25, 50 and 75 mg/ dm^3 after 9, 11, 12 and 14 days, respectively. However the change of Cr(VI) concentration indicates that at the same incubation time, more Cr(VI) was reduced at higher initial Cr(VI) concentration.

Morrales-Barrera and Cristiani-Urbina (2006) have studied Cr(VI) removal by a microbial culture in a pneumatically agitated bioreactor. In an airlift bioreactor they reported a complete Cr(VI) removal at 1.3 and 1.6 mM initial chromium(VI) concentration after 30 and 80 h of incubation, respectively. Also a very high overall efficiency of Cr(VI) removal was achieved (94.3%) after more than 160 h of incubation at an initial Cr(VI) concentration of 1.94 mM. When *T. viride* was cultivated in unbaffled flasks containing culture medium with initial Cr(VI) concentrations of 1.0, 1.5 and 2.0 mM, the Cr(VI) removal efficiency was from 97 to 100%, respectively.

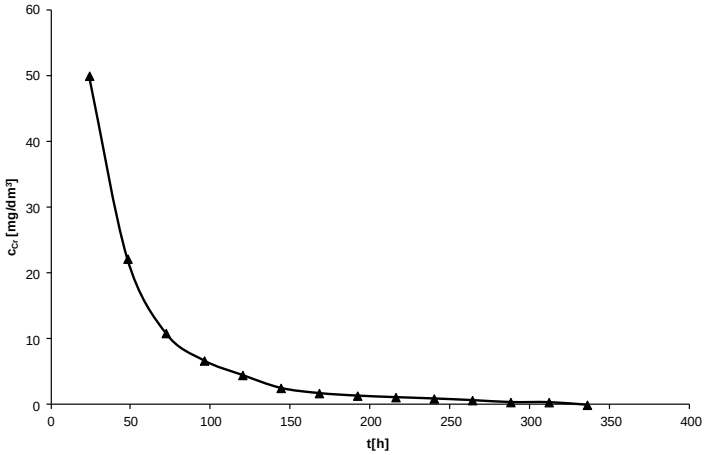

Figure 10. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 50 mg/dm^3; pH = 4.5

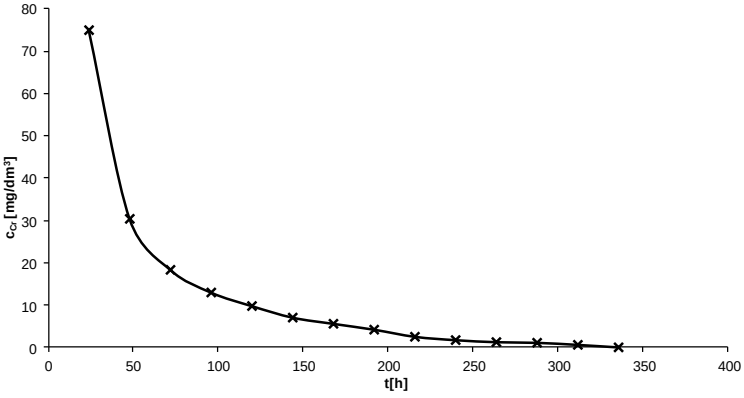

Figure 11. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 75 mg/dm^3; pH = 4.5

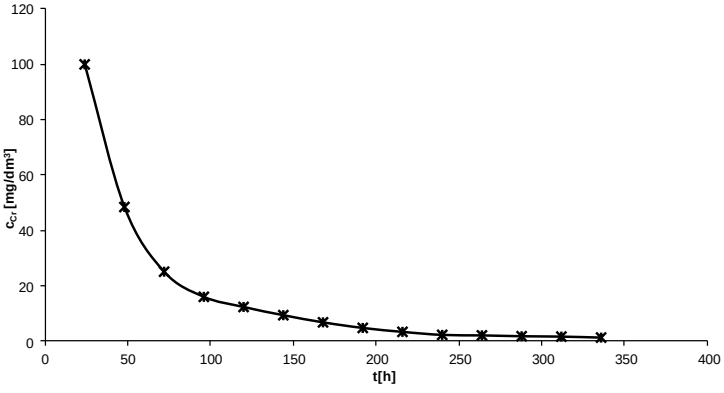

Figure 12. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 100 mg/dm^3; pH= 4.5

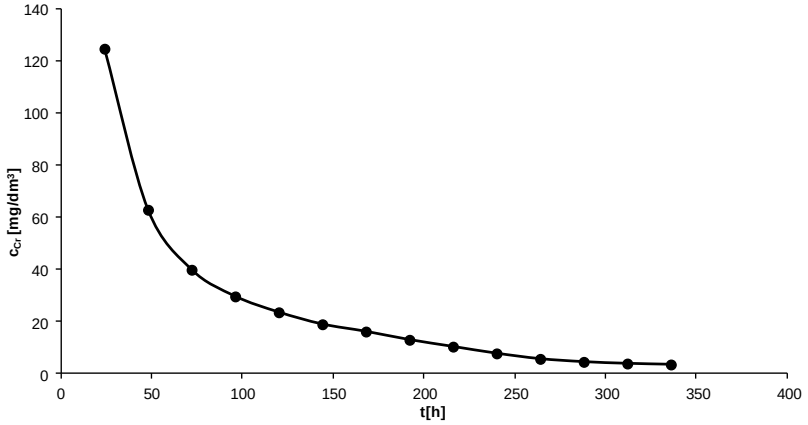

Figure 13. $c_{Cr} = f(t)$. Initial concentrations of chromium(VI) was 125 mg/dm^3; pH = 4.5

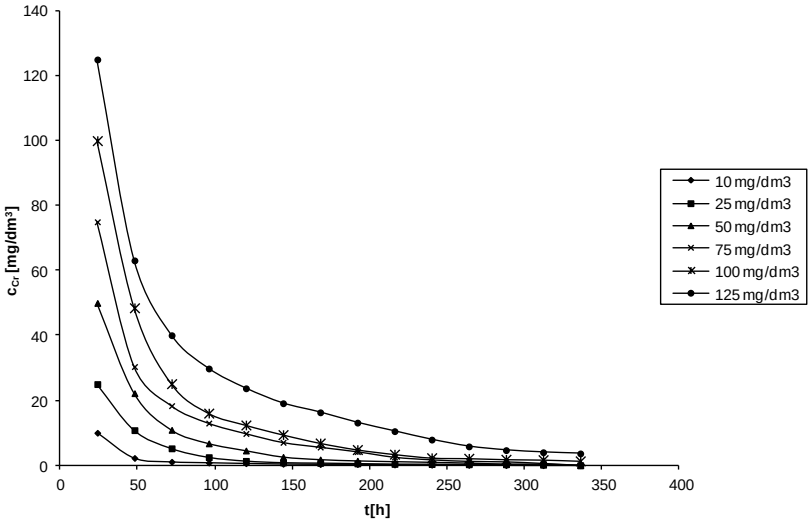

Figure 14. $c_{Cr} = f(t)$ for various initial concentrations of chromium(VI); pH = 4.5

Determination of type of biology Cr(VI) ions removing process

Determination of Cr(III) contents in medium

The results of analysis of total chromium presence in medium and initial chromium(VI) contents were presented in Table 1. The Cr(III) ions contents were determined on the basis on differences between the total chromium content and chromium(VI) content in the medium after 14 days of incubation.

Table 1. Results of analysis of Cr(III) presence in medium

Initial concentration Cr(VI) [mg/dm^3]	Total chromium concentration in medium [mg/dm^3]	Cr(III) concentration in medium [mg/dm^3]
10	0	0
20	0.15	0.15
50	0.408	0.408
75	0.625	0.605
100	2.15	0.806
125	4.012	0.376

Modest amounts of Cr(III) in the medium might occur because of acidification of environment by products of fungi metabolism in the final stage of 14-days period of culture. Such a small chromium(III) concentration proves also that the reduction process is not a cause of biological removal of Cr(VI) ions by application of the mildew fungi.

Determination of overall chromium contents in ooze after mycelium irrigating

The results of the analysis of total chromium presence in ooze, which depends on the initial chromium(VI) contents, were presented in Table 2.

Table 2. Results of analysis of overall chromium presence in the ooze

Initial concentration Cr(VI) [mg/ dm^3]	Total chromium concentration in ooze [mg/ dm^3]
10	0
20	0
50	0.02
75	0.092
100	0.131
125	0.3

Trace amounts of total chromium in ooze rather eliminates ion adsorption of this element on the surface of mycelium. This process could occur at the initial phase of the mycelium growth and was the first stage of the intracellular accumulation.

Determination of chromium contents in mycelium

Table 3 presents the results of total chromium contents in mycelium as a function of initial Cr(VI) ions concentration in the medium.

Table 3. Results of analysis of overall chromium presence in mycelium

Initial concentration Cr(VI) [mg/dm^3]	Total chromium concentration in mycelium [mg/ dm^3]
10	9.92
20	19.52
50	49.18
75	74.01
100	97.61
125	120.31

The results indicate that the increase of Cr(VI) ions in mycelium occurred in comparison with these ions concentration in surrounding environment. This may prove that the removal of Cr(VI) from aqueous solutions by macroscopic fungi occurs by intracellular bioaccumulation.

Conclusions

On the basis of presented results the following conclusions can be made:

- removal of Cr(VI) from water solutions by application of microscopic fungi *Trichoderma viride* occurs by intracellular bioaccumulation
- process of intracellular chromium absorption with alimentary substances is the largest during the first 5 days of mycelium growth
- bioaccumulation of chromium(VI) is dependent on environmental pH and is the most efficient at pH 4.5 for *Trichoderma viride*
- the greater is chromium(VI) concentration the smaller is accumulation of this element from the environment and the growth of mycelium is slower.

Application of mildew fungi to biological removing of chromium(VI) may be a suitable alternative to expensive chemical methods. Its disadvantages are longer time of bioaccumulation and lack of possibility of metal recovery without destruction of the mycelium. This causes that the application of these microorganisms is possible only once.

Acknowledgements

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