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Bioaccumulation of Cr(VI) Ions from Aqueous Solutions by *Penicillium citrinum*

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1. Introduction

The growing environmental degradation is consequence of technical development of civilization. Industry and agrochemistry influence on environmental pollution by xenobiotics among which heavy and toxic metals can be found. One of them is chromium occurring in natural environment mainly on two levels of oxidation: III and VI. The more toxic and harmful, both for environment and human beings, is Cr(VI). Chromium(VI) is generally produced by industrial processes. Chromium compounds, mostly in chromium(III) and chromium(VI) forms, produced by the chemical industry are used for chrome plating, the manufacture of dyes and pigments, leather tanning, and wood preserving. Smaller amounts are used in drilling muds, rust and corrosion inhibitors, textiles, and toner for copying machines. Because of toxic properties of chromium(VI) and high mobility, effluents and wastes containing this element are treated as highly dangerous. The additional problem are growing costs of their storage, dump preservation and transport of wastes [2, 15, 17, 30, 31].

Conventional methods for removing dissolved chromium(VI) ions include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment and evaporative recovery. These processes have significant disadvantages like incomplete metal removal, requirements for expensive equipment and monitoring systems, high reagent or energy requirements or generation of toxic sludge or other waste products that require disposal. Besides, the currently applied

chemical methods of treatment of the effluents containing chromium, which results are not satisfying require significant financial costs [17]. The alternative for them may be biotechnological processes [27]. The application of selectively chosen microorganisms may significantly limit the amount of the chromium introduced to environment. The main advantage of biotechnological methods is the fact that these methods are economic and environmentally friendly. The chromium is being removed by the cellular metabolism of microorganisms by mainly bioaccumulation, biosorption and biotransformation.

The previous researches describe the applications of living and dead microorganisms cells to remove Cr(VI) from water solutions by biosorption [1, 3-6, 11, 16, 19-23, 26, 28] and bioaccumulation [4, 7-9, 13, 14, 18, 22, 28, 29, 32]. Each of these methods has advantages and disadvantages. The application of dead biomass removes the problems connected with the toxic metal concentration in researched solution and requirements connected with growth environment – nourishment. Furthermore, the adsorbed metal may be easily removed and the remaining biomass may be applied once again. However, the limitation of this method is the fact that no reactions are being continued in dried cells.

The application of living biomass makes possible to remove metal during its growth what allows to avoid processes of its reproduction, drying and storage. Unfortunately, in this case the metal concentration in environment is highly important – too high may be toxic for growing biomass. This problem can be avoided applying the microorganisms of high tolerance on high concentrations of Cr(VI) [13, 14] 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 clean cultures of sort *Penicillium citrinum.*

2. Methodology of investigation

Clean fungi cultures of *Penicillium citrinum* was selected to research over removing of Cr(VI).

2.1. Investigation of process dependency on pH

95 ml of nourishment and 5 ml of $K_2Cr_2O_7$ solution of concentration 1g of ions Cr^{6+}/l were transferred to Erlenmayer flasks. After deter-

mination of certain pH (acidification of 0,5 M with sulfur acid(VI)) individual species of microscopic fungi were added to prepared medium containing 50 mg of ions Cr^{6+}/l. Every day, the same hour, the 2,5 ml samples of medium were collected from each Erlenmayer flask, which were then transferred to flasks of volume of 25 ml. Then, 0,625 ml of 2M of sulfur acid (VI) and 0,5 ml of 1,5-difenylocarbaside(I) were added to the flasks. After 5 minutes, the flasks were filled up to the line with medium according to Waksman and the determination of chromium(VI) contents by spectrophotometric method.

2.2. Determination of chromium(VI) contents

2.2.1. Agents

Agents being applied to determination of chromium contents, so 1,5-difenylocarbaside(I) and 2M sulfur acid(VI) were prepared according to the norm PN-EN 12441-10 [32].

2.2.2. Determination of chromium(VI) contents

Before starting the measurements, the pattern line was prepared. To this purpose, solutions of sulfur acid(VI), 1,5-difenylocarbaside(I) and certain volumes of pattern solution of chromium(VI) were introduced to 100 ml flasks to get the Cr^{6+} ions concentrations as: 0, 0,1, 0,2, 0,4, 0,6, 0,8, 1 mg/l.

The analytical samples were prepared by mixing solutions of sulfur acid(VI), 1,5-difenylocarbaside(I) and sample solution in 25 ml flask.

2.3. 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 certain fungi grow the best. That was pH = 5,0.

The certain amount of medium and amount of $K_2Cr_2O_7$ (of concentration 1g of ions Cr^{6+}/l) were transferred to Erlenmayer flasks to get the required chromium concentration in certain sample in volume of 100 ml of bed.

The determination of chromium(VI) contents was conducted every day at the same hour. The samples of 2,5 ml of medium were collected, which were then transferred to 25 ml flasks (dilution – 10 times). Next,

solutions of 2M sulfur acid(VI) and 1,5-difenylocarbaside(I) were added. After 5 minutes, the sample was filled up to the line with medium according to Waksman. Chromium(VI) ions concentration in the sample was determined by measuring the absorption at 540 nm using spectrophotometer Cadas 200 type LPG 392 [10, 12, 24, 25].

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

2.4. Determination of biological type of Cr(VI) ions removing

The removing of chromium(VI) from water 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 mycelium irrigating and in mycelium.

2.4.1. Determination of Cr(III) in medium contents

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

In purpose of total chromium contents determination, the samples of medium of certain volume assuring the concentration of Cr being inside of pattern scale range were introduced to beaker of 200 ml volume and were filled up to the volume of 50 ml. Next, in purpose of 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 chilling the samples were transfer red to flask of 50 ml volume and the solution of 1,5-difenylocarbaside(I) was added. After 5 minutes the sample was filled up to the line with medium according to Waksman. Chromium(VI) ions concentration in the sample was determined by measuring the absorption at 540 nm using spectrophotometer Cadas 200 type LPG 392 [10, 12, 24, 25].

2.4.2. Determination of chromium contents in ooze after mycelium irrigating

The mycelium was investigated after 14 days of incubation. To determine the presence of chromium adsorbed on the surface mycelium

was irrigated. The given ooze was then analyzed to prove the total chromium presence [10, 12, 24, 25].

2.4.3. Determination of chromium contents in mycelium

After 14 days of incubation dried fungi at 105°C was buried in oven in temperature of 600°C. Next, the chromium compounds were transformed into dilatable nitrates by means of concentrated nitrogen acid (V). The contents of Cr(VI) in mineralized sample was determined by spectrophotometric method [12].

3. Results

3.1. Investigation of process dependency on pH

On the basis of given results the graphs were done presenting the dependency of chromium(VI) ions concentration in medium on pH. Figures shows the change of Cr(VI) concentration in the medium with different initial pH ranging from 4,0–6,5.

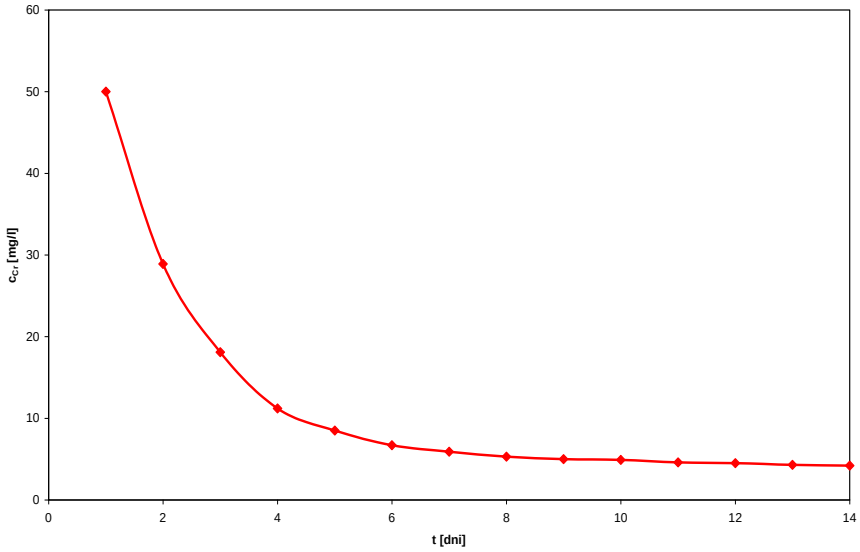

Fig. 1. Dependency $c_{Cr} = f(t)$ for pH = 4,0, initial concentration of Cr(VI) 50 mg/l
Rys. 1. Zależność $c_{Cr} = f(t)$ dla pH = 4,0, stężenie początkowe Cr(VI) 50 mg/l

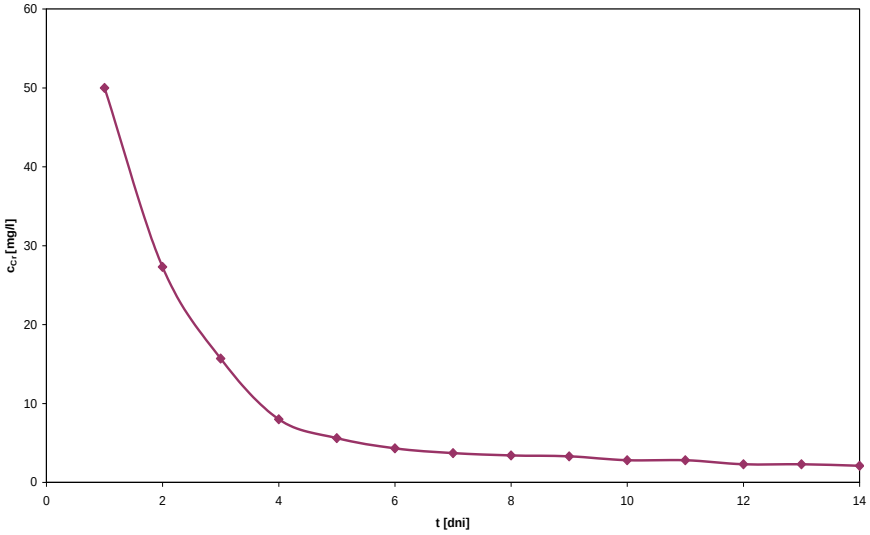

Fig. 2. Dependency $c_{Cr} = f(t)$ for pH = 4,5, initial concentration of Cr(VI) 50 mg/l
Rys. 2. Zależność $c_{Cr} = f(t)$ dla pH = 4,5, stężenie początkowe Cr(VI) 50 mg/l

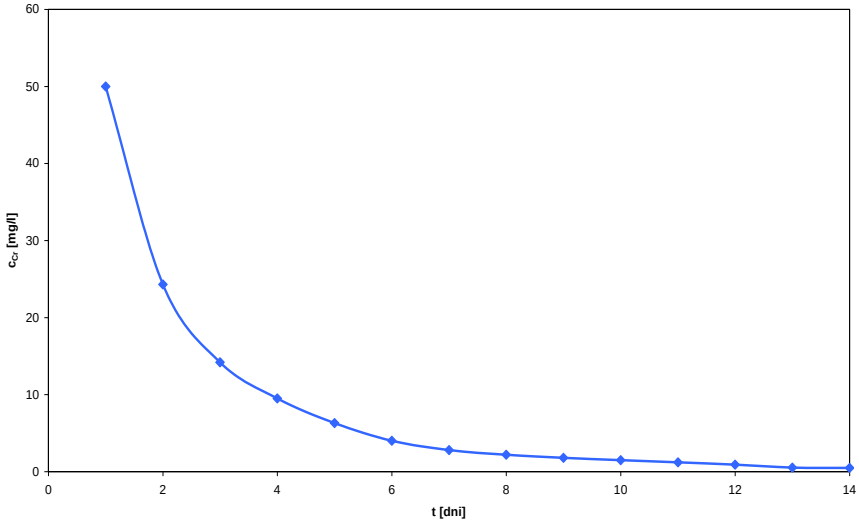

Fig. 3. Dependency $c_{Cr} = f(t)$ for pH = 5,0, initial concentration of Cr(VI) 50 mg/l
Rys. 3. Zależność $c_{Cr} = f(t)$ dla pH = 5, stężenie początkowe Cr(VI) 50 mg/l

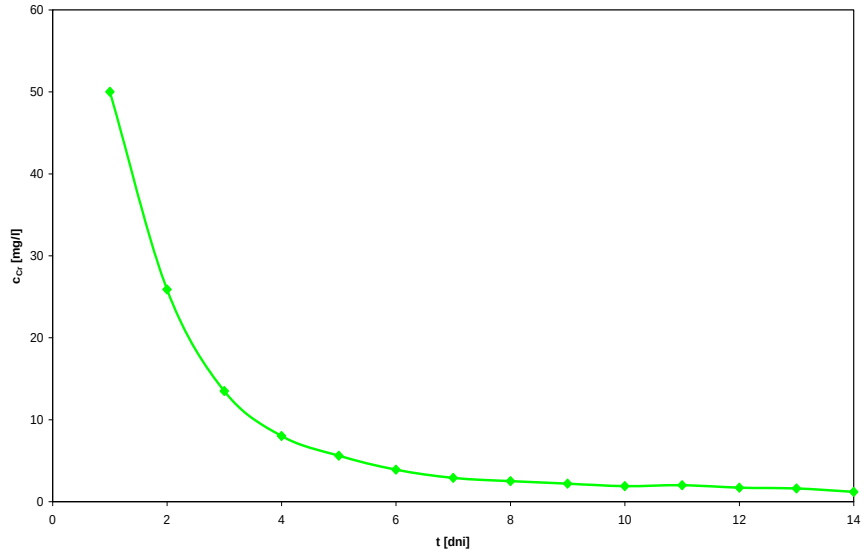

Fig. 4. Dependency $c_{Cr} = f(t)$ for pH = 5,5, initial concentration of Cr(VI) 50 mg/l
Rys. 4. Zależność $c_{Cr} = f(t)$ dla pH = 5,5, stężenie początkowe Cr(VI) 50 mg/l

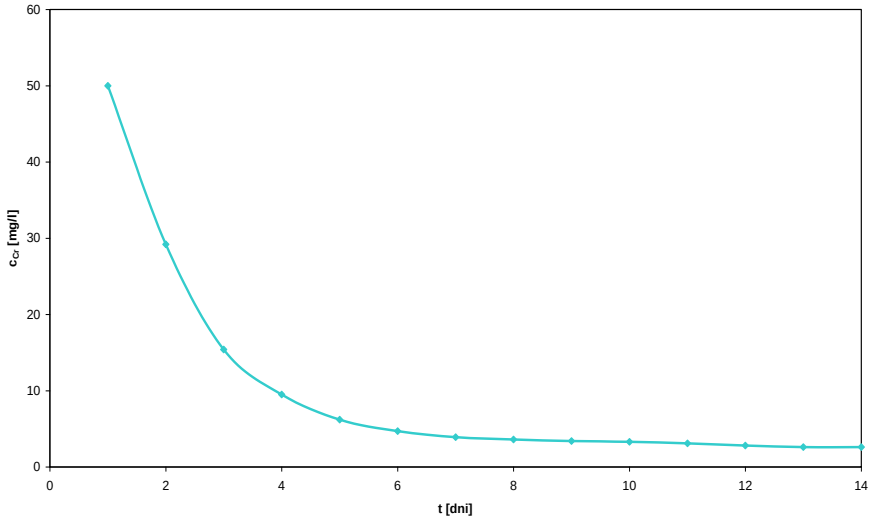

Fig. 5. Dependency $c_{Cr} = f(t)$ for pH = 6,0, initial concentration of Cr(VI) 50 mg/l
Rys. 5. Zależność $c_{Cr} = f(t)$ dla pH = 6,0, stężenie początkowe Cr(VI) 50 mg/l

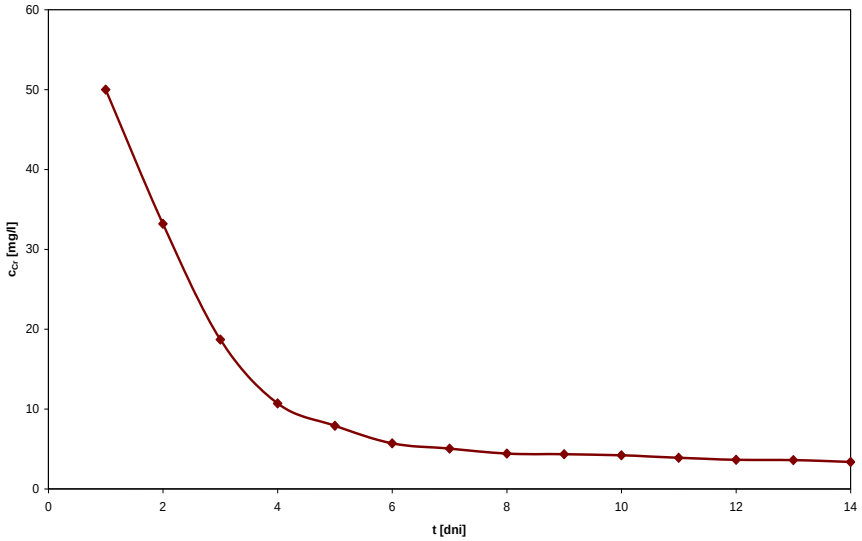

Fig. 6. Dependency $c_{Cr} = f(t)$ for pH = 6,5, initial concentration of Cr(VI) 50 mg/l
Rys. 6. Zależność $c_{Cr} = f(t)$ dla pH = 6,5, stężenie początkowe Cr(VI) 50 mg/l

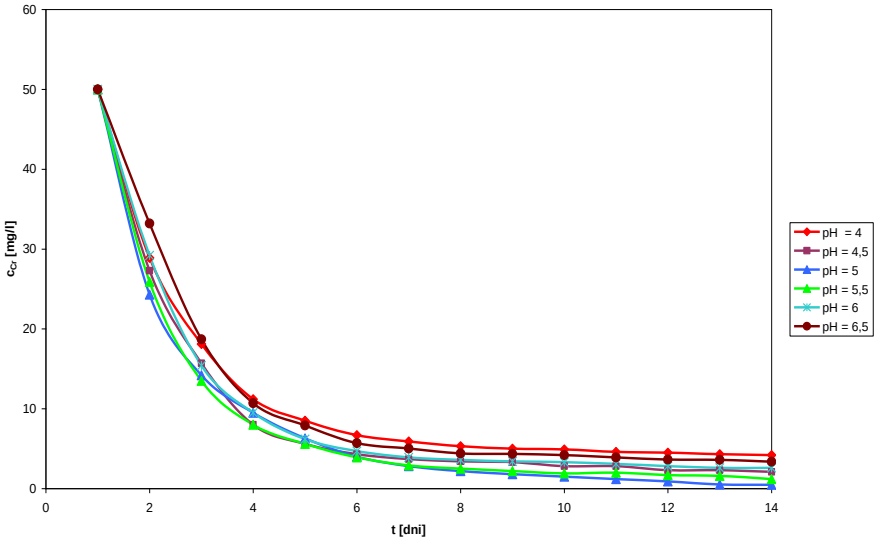

Fig. 7. Dependency $c_{Cr} = f(t)$by various values of pH, initial concentration of chromium(VI) 50 mg/l
Rys. 7. Zależność $c_{Cr} = f(t)$ dla różnych wartości pH, stężenie początkowe chromu 50 mg/l.

From this figures optimal pH was determined for *Penicilium citrinum* – pH = 5,0. This was the one were the chromium(VI) ions were removed the most efficiently and the certain mildew fungi developed the best.

3.2. Dependency of process on chromium(VI) concentration in sample

On the basis of measurement results the figures presenting relations of chromium(VI) ions concentration in medium on this process time were prepared.

Obtained results suggested that Cr(VI) removal for the *Penicillium citrinum* occurred even at the highest concentration of 125 mg/l, but complete Cr(VI) removal was observed for 10 and 25 mg/l at 14 days in both concentrations. However the change of Cr(VI) concentration indicates that in the same incubation time, more amount of Cr(VI) was reduced at higher initial Cr(VI) concentration.

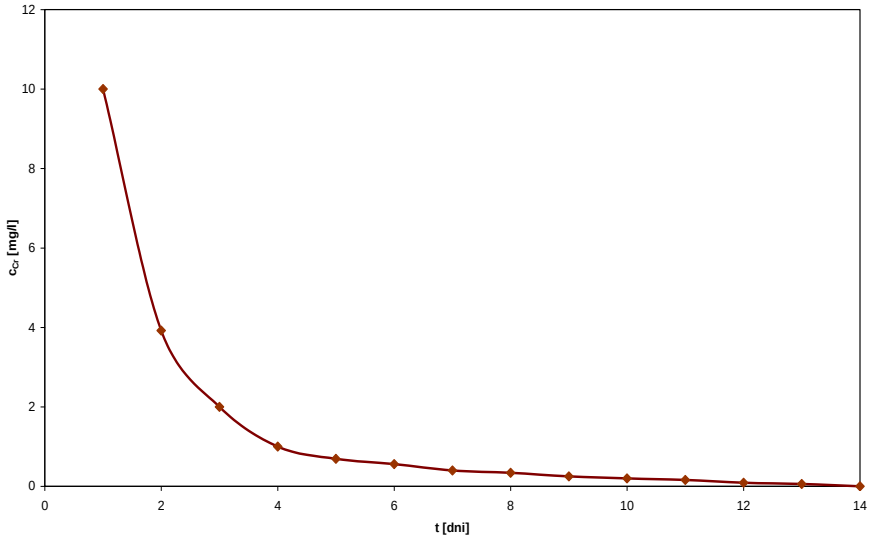

Fig. 8. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 10 mg/l; pH = 5
Rys. 8. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 10 mg/l

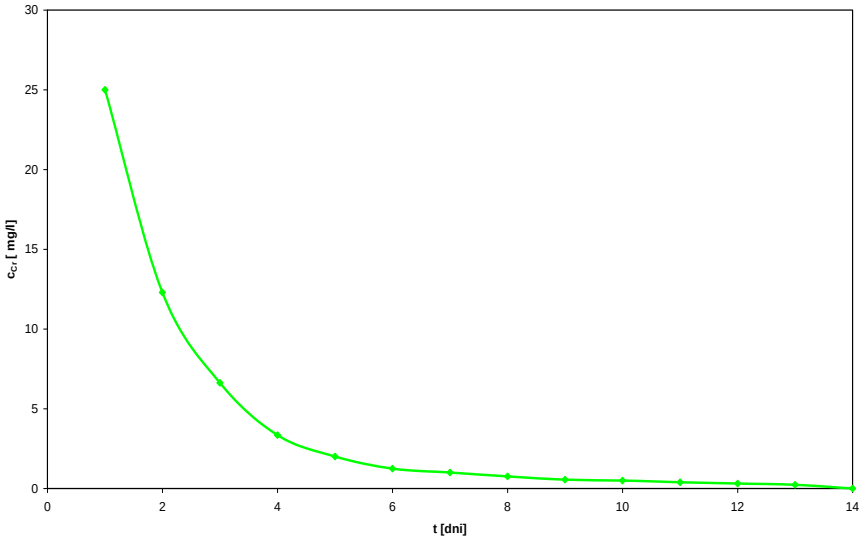

Fig. 9. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 25 mg/l; pH = 5
Rys. 9. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 25 mg/l

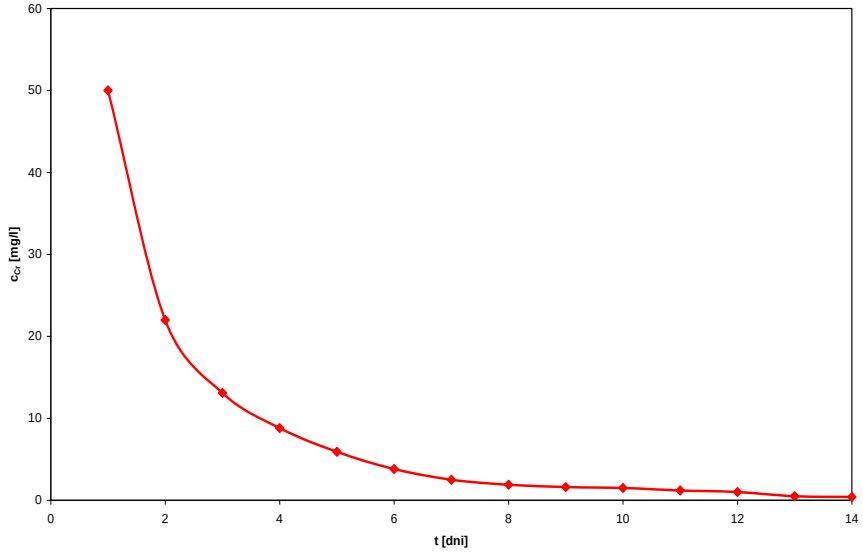

Fig. 10. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 50 mg/l; pH = 5
Rys. 10. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 50 mg/l

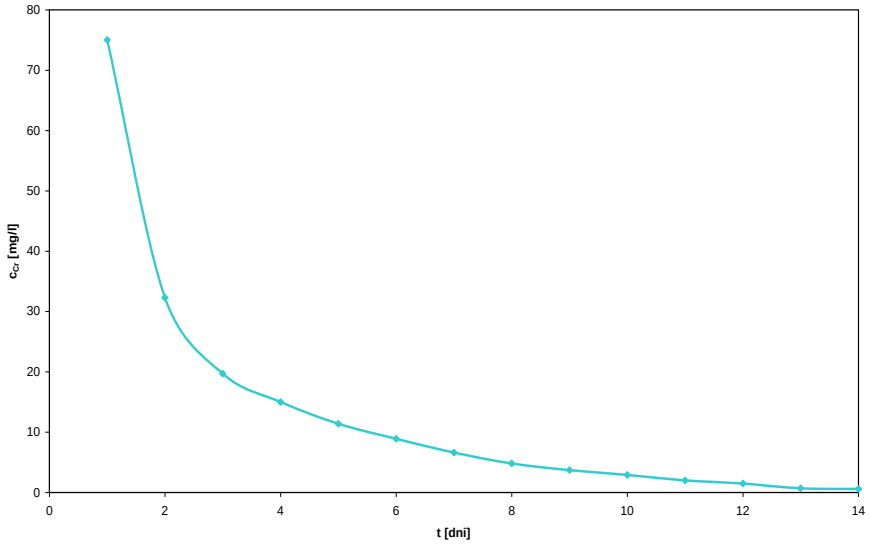

Fig. 11. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 75 mg/l; pH = 5
Rys. 11. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 75 mg/l

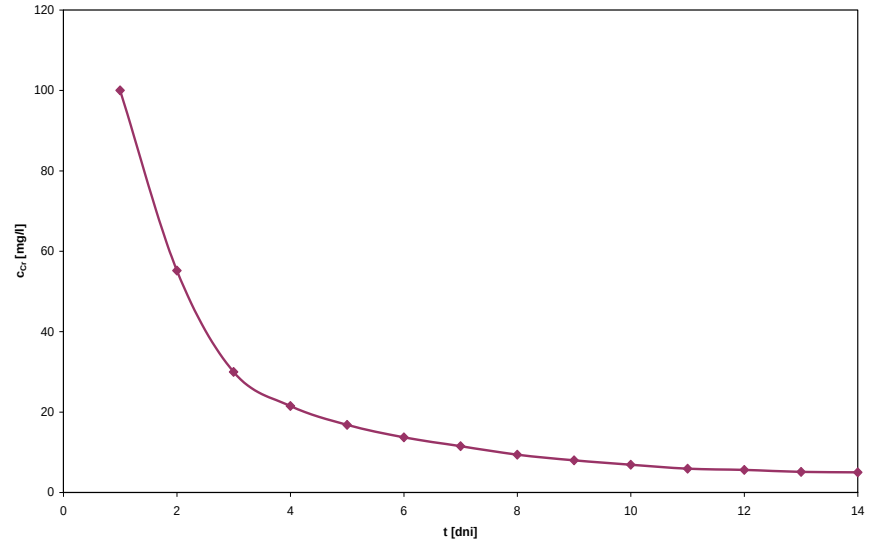

Fig. 12. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 100 mg/l; pH = 5
Rys. 12. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 100 mg/l

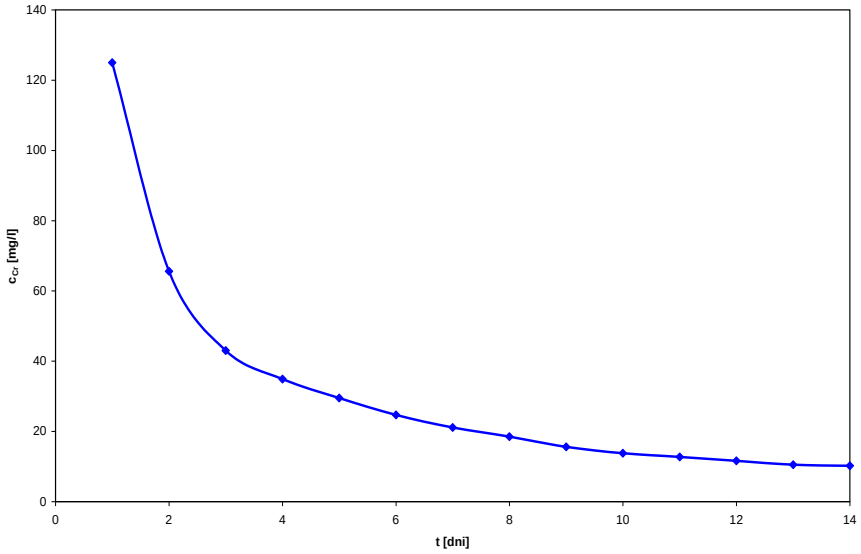

Fig. 13. Dependency $c_{Cr} = f(t)$, initial concentrations of Cr(VI) 125 mg/l; pH = 5
Rys. 13. Zależność $c_{Cr} = f(t)$, pH = 5, stężenie początkowe chromu(VI) 125 mg/l

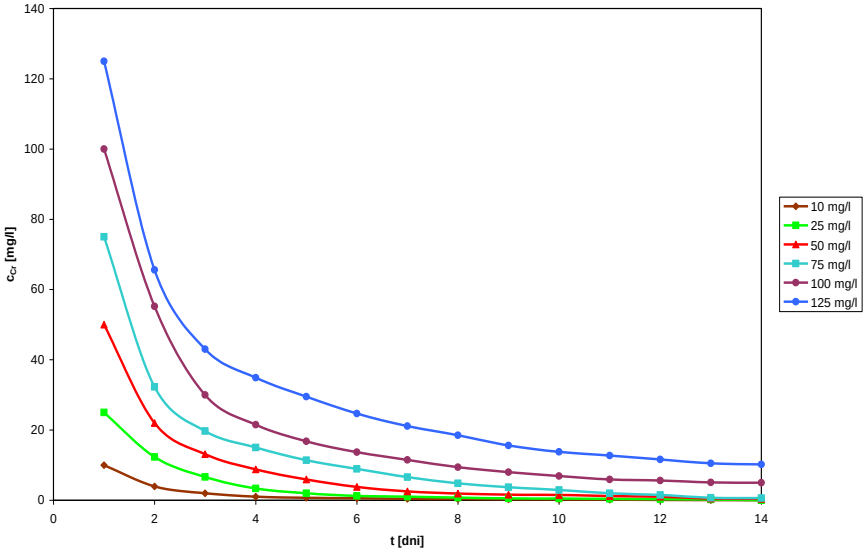

Fig. 14. Dependency $c_{Cr}=f(t)$ for various initial concentrations of Cr(VI); pH = 5
Rys. 14. Zależność $c_{Cr}= f(t)$ dla różnych stężeń początkowych Cr(VI), pH = 5

3.3. Determination of type of biologia Cr(VI) ions removing process

3.3.1. Determination of Cr(III) contents in medium

Results of Cr(III) contents investigation in medium were presented in Table 1.

Table 1. Results of analysis of Cr(III) presence in medium
Tabela 1. Wyniki analizy na obecność Cr(III) w pożywce

Initial Cr(VI) concentration [mg/l]	Total chromium concentration in medium [mg/l]	Cr(III) concentration in medium [mg/l]
10	0	0
20	0	0
50	0,279	0,121
75	0,735	0,113
100	6,03	1,029
125	11,62	1,38

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

3.3.2. Determination of overall chromium contents in ooze after mycelium irrigating

The results of investigation were presented in Table 2.

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

Table 2. Results of analysis of overall chromium presence in the ooze
Tabela 2. Wyniki analizy na obecność chromu ogólnego w przesączu

Initial Cr(VI) concentration [mg/l]	Total chromium concentration in ooze [mg/l]
10	0
20	0
50	0,092
75	0,33
100	0,25
125	0,411

3.3.3. Determination of chromium contents in mycelium

The results of investigation were presented in Table 3.

Table 3. Results of analysis of overall chromium presence in mycelium
Tabela 3. Wyniki analizy na obecność chromu ogólnego w grzybni

Initial Cr(VI) concentration [mg/l]	Total chromium concentration in mycelium [mg/l]
10	9,90
20	19,76
50	49,11
75	73,69
100	93,12
125	112,82

The results indicate that the growth 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 water solutions by macroscopic fungi occurs by intracellular bioaccumulation.

4. Conclusions

On the basis of given results the following conclusions were made:

- Removing of Cr(VI) from water solutions by application of microscopic fungi *Penicillium citrinum* occur by intracellular bioaccumulation;
- Process of intracellular chromium absorption with alimentary substances is the biggest during first 5 days of mycelium growth;
- Bioaccumulation of chromium(VI) is dependent on environmental pH and is the most efficient by pH 5,0 for *Penicillium citrinum*;
- The bigger is chromium(VI) concentration the smaller is accumulation of this element from the environment and the growth of mycelium is slower;
- The degree of removal of Cr(VI) ions from water solutions by application of microscopic fungi *Penicillium citrinum* was: 100% for $c_{Cr(VI)}$ = 10,20,50 mg/l; 98% for $c_{Cr(VI)}$ = 75 mg/l; 93% for $c_{Cr(VI)}$ = 100 mg/l; 90% for $c_{Cr(VI)}$ = 125 mg/l.

Application of mildew fungi to biological removing of chromium(VI) may be a perfect alternative for 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.

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Bioakumulacja jonów Cr(VI) z roztworów wodnych przy wykorzystaniu grzyba *Penicillium citrinum*

Abstrakt

W artykule przedstawiono biologiczne usuwanie jonów Cr(VI) z roztworu wodnego przy użyciu czystej kultury grzyba *Penicillium citrinum*. Wzrost organizmu oraz usuwanie chromu(VI) przeprowadzono w roztworze wodnym o różnej zawartości chromu(VI), oraz przy określonym optymalnym pH. W czasie 14 dni inkubacji, codziennie, pobierano 5ml próbki roztworu w celu oznaczenia zawartości chromu(VI) w roztworze i na tej podstawie określono efektywność biologicznego usuwania tego pierwiastka. Ponieważ usuwanie chromu(VI) z roztworu wodnego może zachodzić wskutek procesów redukcji, biosorpcji lub bioakumulacji to aby określić, który z tych procesów miał miejsce w trakcie badań wykonano oznaczenia zawartości Cr(III) w próbkach pożywki oraz zawartości chromu ogólnego w przesączu po płukaniu grzybni oraz w grzybni. Badania wykazały wysoką skuteczność grzyba mikroskopowego z gatunku *Penicillium citrinum* w eliminacji jonów chromu(VI) z roztworu – w przypadku najwyższego stężenia Cr(VI) równego 125 mg/l stopień usunięcia wynosił 90%.