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Original Research

Bioaccumulation of Cr(VI) Ions from Aqueos Solutions by *Aspergillus niger*

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Abstract

Our paper presents biological Cr(VI) removal from water solution by applying a clean fungi culture, namely *Aspergillus niger*. The growth of the organism and removal of chromium(VI) were done in water solution of various chromium(VI) contents and at optimal pH value. During 14 days of incubation, samples of 5 ml each were collected daily to determinate chromium(VI) contents in solution. Then the efficiency of this biological removal was also specified. The Cr(VI) removal process may occur via reduction, biosorption, or bioaccumulation pathways. To determine which pathway may be used in the study the Cr(III) contents were also determined in the samples as well chromium contents in ooze after mycelium irrigating and in mycelium.

Keywords: bioaccumulation, chromium, Cr(VI), microscopic fungi, *Aspergillus niger*

Introduction

Chromium occurs in the natural environment in various minerals of industrial application. In fact it is a component of industrial sewers and solid wastes. Chromium (VI) is a harmful element for both environments and humans because of its strong toxic properties and high mobility. That is why sewers and wastes containing this element are considered dangerous. Even relatively small amounts of this element may be a source of danger for the ecosystem because of the persistence of its compounds and possibility of multiplying its concentration [1-3].

The currently applied chemical methods for chromium-containing effluent treatments require significant financial costs [4]. The alternative for these methods may be biotechnological processes [5, 7]. The application of selectively chosen microorganisms may significantly limit the amount of the chromium introduced to the environment. The main advantage of biotechnological methods is the fact that these methods are economical and environmentally friendly. The chromium is being removed by the cellular metabolism of microorganisms mainly by bioaccumulation, biosorption, and biotransformation.

Previous research has described the applications of living and dead microorganism cells to remove Cr(VI) from water solutions by biosorption [8, 12, 15-20, 22-24] and bioaccumulation [6, 8-11, 13, 14, 21]. Each of these methods has advantages and disadvantages. The application of dead biomass removes the problems connected with toxic metal concentrations in researched solution and requirements connected with growth environment – nourishment. Furthermore, the adsorbed metal may be easily removed and the remaining biomass and 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 it possible to remove metal during its growth, which allows us to avoid

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processes of reproduction, drying, and storage. Unfortunately, in this case the metal concentration in the environment is highly important – too high may be toxic for growing biomass. This problem can be avoided by applying the microorganisms of high tolerance on high concentrations of Cr(VI) or getting it by adaptive processes.

The purpose of the investigation presented in this paper is to optimize the biological process of Cr(VI) removal by application of clean cultures of *Aspergillus niger*.

Experimental Procedures

The pure mildew fungi culture from *Aspergillus niger* was applied to our investigation. For purposes of identification the diagnostic micro- and macroscopic research was carried out on the basis of mildew fungi determination key [1, 2].

Investigation of Process Dependency on pH

Strains of fungi were grown aerobically at 28ºC in accumulating medium prepared by mixing Cr(VI) solution autoclaved separately (at 120ºC 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 performed in a 300 ml Erlenmayer flask with 100ml of accumulation medium containing 50 mg of ions Cr^{6+}/l. 2.5 ml samples of medium were collected from each Erlenmayer flask daily for 14 days, then transferred to flasks of 25 ml volume each. Then, solutions of 2 M sulfuric acid(VI) and 1,5-difenylocarbaside(I) were added to the 25 ml flasks. After 5 minutes, the flasks were filled to the line with medium according to Waksman. Residual chromium(VI) ion concentrations in the bioaccumulation medium were determined by measuring the absorption at 540 nm by means of a Cadas 200 type LPG 392 spectrophotometer [27-31].

Determination of Chromium(VI) Contents

Agents

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

Determination of Chromium(VI) Contents

Before starting the measurements, the pattern line was prepared. To this purpose, solutions of sulfuric 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 sulfuric acid(VI), 1,5-difenylocarbaside(I) and sample solutions in 25 ml flasks.

Dependence of the Process of Chromium (VI) Concentration on Nourishment

The investigation of Cr(VI) ion concentrations in medium in relation to pH allowed us to determine the best reaction by which certain fungi grow best. That was pH=4.0.

A certain amount of medium and $K_2Cr_2O_7$ (of concentration 1g of ions Cr^{6+}/l) were transferred to Erlenmayer flasks to get the required chromium concentration in certain samples 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 2 M sulfuric acid(VI) and 1,5-difenylocarbaside(I) were added. After 5 minutes, the sample was filled to the line with medium according to Waksman. Chromium(VI) ion concentrations in the sample were determined by measuring the absorption at 540 nm using a Cadas 200 type LPG 392 spectrophotometer [27-31].

In cases of the concentration being higher than the pattern scale range, samples were adequately diluted.

Determination of Biological Type of Cr(VI) Ion Removal

The removal 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 as chromium contents in ooze after mycelium irrigation and in mycelium.

Determination of Cr(III) in Medium Contents

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

For the purpose of total chromium content determination, the samples of medium of certain volume assuring the concentration of Cr being inside the pattern scale range were introduced to a beaker of 200 ml volume and were filled to a volume of 50 ml. Next, for purposes of oxidation of Cr(III) ions to Cr(VI), the solutions of sulfuric acid (VI) and ammonium persulfate were added to the sample. Then it was boiled and maintained in this state for 20-25 minutes. After chilling the samples were transferred to a flask of 50 ml volume and the solution of 1,5-difenylocarbaside(I) was added. After 5 minutes the sample was filled to the line with medium according to Waksman. Chromium(VI) ion concentrations in the sample were determined by measuring the absorption at 540 nm using a Cadas 200 type LPG 392 spectrophotometer [27-31].

Determination of Chromium Contents in Ooze after Mycelium Irrigation

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 total chromium presence [27-31].

Determination of Chromium Contents in Mycelium

After 14 days of incubation dried fungi at 105ºC was buried in an oven at 600ºC. Next, the chromium compounds were transformed into dilatable nitrates by means of concentrated nitrogen acid (V). The contents of Cr(VI) in a mineralized sample was determined by spectrophotometric method [27].

Results

Investigation of Process Dependency on pH

On the basis of given results a graph presents the dependency of chromium(VI) ion concentration in medium on pH. Fig. 1 shows the change of Cr(VI) concentration in the medium with different initial pH ranging from 4.0-6.5. The results suggest that the optimum initial pH value was 4. This was the one where the chromium(VI) ions were removed most efficiently and the certain mildew fungi developed the best. Similar results were obtained by Dursun et al. [9].

In the case of the biosorption process Mungasavalli et al. [12] reported that the optimum pH for the sorption of chromium(VI) by the pretreated *A. niger* was 3.0. Other authors [7, 18] showed that the complete removal of Cr(VI) by the pure colony of *A. niger* was observed only at highly acidic pH such as 2.0 because of insufficient contact time (i.e. below 24 h) at higher pH values.

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

Dependency of Process on Chromium(VI) Concentration in Sample

On the basis of measurement results, the figure presenting relations of chromium(VI) ion concentrations in medium on this process time were prepared. The effect of initial Cr(VI) concentration was investigated over a range of about 10-125 mg/l. As shown in Fig. 2, Cr(VI) removal occurred even at the highest concentration of 125 mg/l, but complete Cr(VI) removal was observed only for 10, 25, and 50 mg/l at 9, 11, and 12 days, respectively. However, the change of Cr(VI) concentration indicates that in the same incubation time, more amounts of Cr(VI) were reduced at higher initial Cr(VI) concentrations.

In the previous study Dursun et al. [9] have reported that maximum uptake capacity (defined as the ratio of bioaccumulated concentration of metal ion at the end of growth to the initial metal ion concentration) was determined at 50 mg/l initial chromium(VI) concentration and no significant microbial activity and chromium(VI) uptake were observed above this chromium(VI) concentration. Authors applied the strain of *A. niger* obtained from the United States Department of Agriculture culture collection. Removal of hexavalent chromium by *Aspergillus niger* isolated from soil of leather tanning effluent was reported by Srivastava et al. [14]. This strain removed more than 70% chromium in soil contaminated by 250 and 500 ppm of chromate (*A. niger* was introduced in soil microcosm, 40% moisture content, with different concentration of chromate). The difference in obtained results shows how colony source affects its tolerance of high concentrations of toxic chromium.

In the case of biosorption, an effect of initial Cr(VI) concentration on Cr(VI) removal by the dead fungal biomass of *A. niger* was reported by D. Park et al. [18]. The concentration of Cr(VI) versus time was examined at initial Cr(VI) concentrations from 25 to 200 mg/l. According to authors, Cr(VI) was completely removed from the solution in 30 hours for Cr(VI) concentration of 25 mg/l, whereas the complete removal of 200 mg/l of Cr(VI) required about 400 h of contact time.

Determination of Type of Biologia Cr(VI) Ion Removal Process

Determination of Cr(III) Contents in Medium

The results of analysis of total chromium presence in medium and initial chromium(VI) contents was presented in Table 1. The Cr(III) ion contents were determined on the

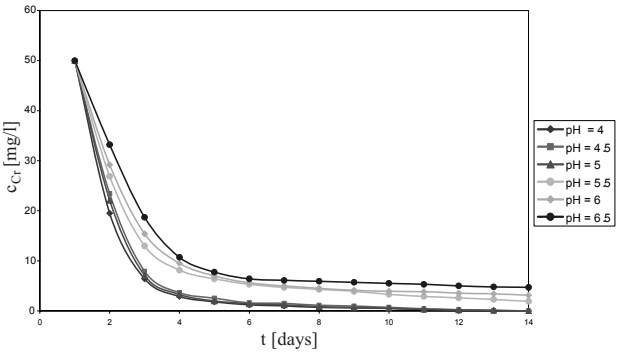

Fig. 1. Dependency $c_{Cr} = f(t)$ by various values of pH, initial concentration of chromium(VI) 50 mg/l.

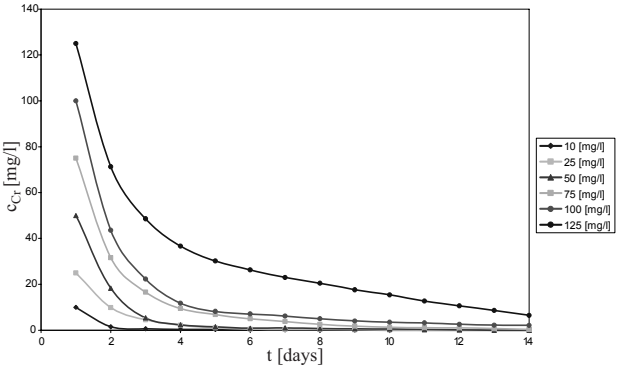

Fig. 2. Dependency $c_{Cr} = f(t)$ for various initial concentrations of chromium(VI); pH=4.

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

Initial concentration of Cr(VI) [mg/l]	Total chromium concentration [mg/l]	Cr(III) concentration [mg/l]
10	0	0
20	0.032	0.032
50	0.159	0.159
75	0.56	0.15
100	2.325	0.194
125	7.03	0.529

basis of the difference between total chromium contents and chromium(VI) contents in medium after 14 days of incubation.

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

Determination of Overall Chromium Content in Ooze after Mycelium Irrigating

The results of analysis of total chromium presence in ooze on initial chromium(VI) contents was 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.

Determination of Chromium Contents in Mycelium

Table 3 presents the results of total chromium contents in mycelium dependably on initial Cr(VI) ion concentrations in medium. The results indicate that the growth of Cr(VI) ions in mycelium occurred in comparison with these ions concentrations in the surrounding environment.

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

Initial concentration of Cr(VI) [mg/l]	Total chromium concentration in ooze [mg/l]
10	0
20	0
50	0.056
75	0.11
100	0.195
125	0.23

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

Initial concentration of Cr(VI) [mg/l]	Total chromium concentration in mycelium [mg/l]
10	9.89
20	19.25
50	49.29
75	74.09
100	97.12
125	117.05

This may prove that the removal of Cr(VI) from water solutions by macroscopic fungi *Aspergillus niger* occurs by intracellular bioaccumulation.

Conclusions

A wide variety of growing, living or non-living microbial biomasses has been used for chromium(VI) removal. Many species of yeasts, bacteria, and fungi have been investigated to asses their suitability for Cr(VI) removal. Chromium(VI) tolerance and bioaccumulation in 51 strain of yeast was reported by Ksheminska et al. [13] while bioaccumulation in fungi was studied by Sen et al., Dursun et al., and Dönmez and Koçberber and Srivastavaa et al. [6, 9-11, 14]. Use of dead fungal biomass for the detoxification of hexavalent chromium has been investigated by many authors [7, 12, 15-18, 22]. Biosorption of Cr(VI) by biomass of different strains of bacteria isolated from various environments was also often studied [8, 19, 20, 23, 24]. In general, authors examined the influence of pH, initial chromium concentration, and biomass concentration on metal uptake.

The purpose of the investigation presented in this paper was to optimize the biological process of Cr(VI) removal by *Aspergillus niger*. On the basis of results the following conclusions were made:

- Removal of Cr(VI) from water solutions by application of microscopic fungi *Aspergillus niger* occur by intracellular bioaccumulation.
- The process of intracellular chromium absorption with alimentary substances is greatest during the first 5 days of mycelium growth.
- Bioaccumulation of chromium(VI) is dependent on environmental pH and is the most efficient by pH 4.0.
- The greater the chromium(VI) concentration, the smaller the accumulation of this element from the environment, and the growth of mycelium is slower.

The culture of *Aspergillus niger* investigated in this study could grow at 10-125 ppm chromium concentration, which indicates that it possessed a high tolerance to various concentrations of chromium. At 125 ppm chromium, this organism could successfully accumulate about 90% of chromium. High tolerance of this culture make it a potential candidate as a heavy metal scavenger with respect to chromium.

The application of mildew fungi to biological removal of chromium(VI) may be a perfect alternative for expensive chemical methods. Its disadvantages are a longer time for bioaccumulation and lack of possibility of metal recovery without destruction of the mycelium – this causes the application of these microorganisms to be possible only once.

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