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Chromium Removal from Tanning Effluent using Biomass of *Aspergillus oryzae*

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Abstract: In tannery industries, Cr^{+3} is used for tanning animal hides. Three valence chromium reacts in specific conditions with existing organic compounds and manganese ions to change to hexavalent chromium which is considered hazardous for human health. *Aspergillus oryzae* grew in different dilutions (10-90%) of tanning house effluent (concentration of Cr^{+3} =120-1080 mg/L). The maximum chromium removal rate, (48.2%), was observed at pH= 3.3, Cr^{+3} concentration=240 mg/L and size of inoculum=0.12% (dry weight). Effects of various factors such as pH, temperature, shaking velocity and nutrients were also investigated. At optimum conditions (pH=5; temperature=30°C, shaking velocity= 150 rpm and nitrogen source of dihydrogen ammonium phosphate concentration=0.3%), biomass growth and chromium removal rate were found to be 0.45% of dry weight and 99.8%, respectively. Statistical studies on factors such as pH, temperature, shaking velocity, type and concentration of nutrients on the "biomass growth" and "residual chromium", showed that all the factors had significant effects.

Key words: *Aspergillus oryzae*, tanning effluent, chromium removal, biomass growth

Introduction

Chromium (+3) is typically treated by raising the pH of the industrial effluent through adding chemicals and coagulants such as lime and Fe compounds in order to recover precipitated chromium hydroxide. Numerous studies have reported 99.5% recovery using this method (Nemerow, 1991; Nancy, 1992).

Fungi, in general, are well known for their metal biosorption (Tobin *et al*., 1998; Venkobachor, 1990; Pillichshammer, 1995). Two species, *Aspergillus oryzae* and *Rhizopous oryzae*, were used for Cu^{+2} removal (Chihpin *et al*., 1998). Another investigation has shown that *Aspergillus niger* can grow in tanning effluent (Sivaswamy, 1988). In another report, the growth of *Aspergillus niger* and *Aspergillus carbonaricus* has been studied (Marakis, 1995).

Regarding the high volume of sludge with high chromium content during chemical treatment of tannery effluents and considering the increasing tanning industries in I.R.Iran, this project was planned and conducted in order to find a more naturally based and feasible treatment method for these effluents.

The objectives of this work were to determine firstly the ability *of A. oryzae* to remove chromium from the tanning effluent at different concentrations of chromium and inoculum size and secondly to specify the removal rate and the effects of optimum conditions on biomass growth and chromium removal rate.

Materials and Methods

The research project was carried out in the Department of Environmental Health Engineering, Tehran University of Medical Sciences during the period from December 2000 to May 2002.

Microorganism: *A. oryzae* was provided from P.T.C.C. (Persian Type Culture Collection) of Biotechnology Division, Iranian Research Organization for Science and Technology. It was maintained at 40°C on Potato Dextrose Agar (PDA) for two months. Potato Dextrose Broth (PDB) was used for mycelium culture of fungi.

Chemicals and reagents: Parameters such as pH, TOC (Total Organic Carbon), TKN (Total Kedjeldal Nitrogen), P (PO_4^{3-}) and Cr^{3+} in tanning effluent were determined and analyzed, based on the standard methods for water and wastewater examination (AWWA, APHA, WEF., 1995). Chemicals such as K_2SO_4, H_2SO_4, $Na_2Br_4O_7$, NaOH (for TKN test), $(NH_4)_6MO_7O_{24}.4H_2O$, NH_4VO_3, KH_2PO_4(for phosphate test), chromium titrisol and concentrated HNO_3 (for Cr^{+3} examination) were used. These chemicals were obtained from Merck Company.

Sources of nitrogen such as NH_4Cl, $(NH_4)_2SO_4$, $H_2PO_4(NH_4)_2$, $NaNO_3$ and $NaNO_2$ were used for the adjustment of carbon to nitrogen ratio in effluent.

Cultivation: Pure culture of *A. oryzae* was transferred to sterile PDA slant and maintained at 30°C. The spores were collected by washing with distilled water and counted by the hemocytometric methods (Becker *et al*., 1990). The number of spores in suspension were 10^7 to 10^8 conidia/ml. The suspension was stored at 4°C for 3 months in sterile conditions.

Pre-culture preparation: The spores of the suspension were inoculated to potato dextrose liquid culture and maintained in a shaker incubator at 30°C and 150 rpm for 48 h. Pellets of mycellia were formed with a dry weight of 0.8 g/100 ml and diameter of 1.1–1.3 mm.

Experimental procedure: Serial dilutions of tanning effluent (10 - 90%) with chromium concentration of 1200 mg/L were prepared and then sterilized. Different sizes of inoculum (0.4 - 2.4%, dry weight) were inoculated to sterile samples and maintained in shaker incubator at 30°C and 150 rpm for 2 days. In this stage, the effects of inoculum size and effluent dilutions on the biomass growth and chromium removal rate were investigated. Effects of pH (3-8), temperature (10-45°C), shaking velocity (50-250 rpm) and type and concentration of nitrogen sources (NH_4Cl, $(NH_4)_2SO_4$, $H_2PO_4(NH_4)_2$, $NaNO_3$, $NaNO_2$, up to 0.24%) were also studied in order to find the optimum conditions, optimum dilutions and the best inoculum size.

Results and Discussion

Tanning effluent quality: The composition of tanning wastewater (Table 1) showed that pH of the effluent was 3-3.5, since stabilization of chromium on the hide must be carried out in acidic environment. The carbohydrates and organic acids (formic acid) were the sources of total organic carbon (TOC). TOC in the effluent varied from 27000-41000 mg/L. Enzymes (such as tripsin and protease) and proteins of hides were the sources of nitrogen compounds. The concentration of nitrogen compounds varied between 160 and 245 mg/L. The C/N ratio was observed to be 139-169, which was very high for fungal growth (the general

Table 1: Composition of tanning house effluent

Parameters	Amount
pH	3–3.5
TOC, mg/L	27000–41000
TKN, mg/L	160–245
$P(PO_4^{3-})$, mg/L	65–118
Cr^{+3}, mg/L	1000–1300

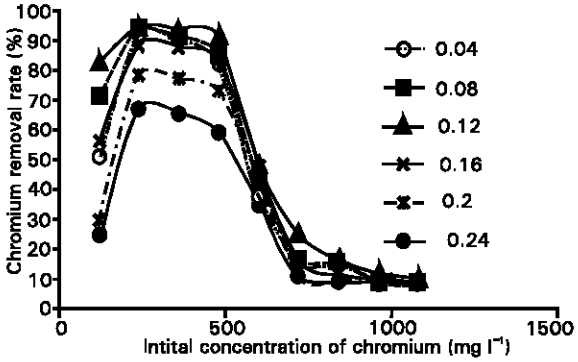

Fig. 1: Effect of different concentrations of chromium in the effluent on chromium removal rate by different inoculum size of *A. oryzae* (0.04 - 0.24% dry weight of biomass)

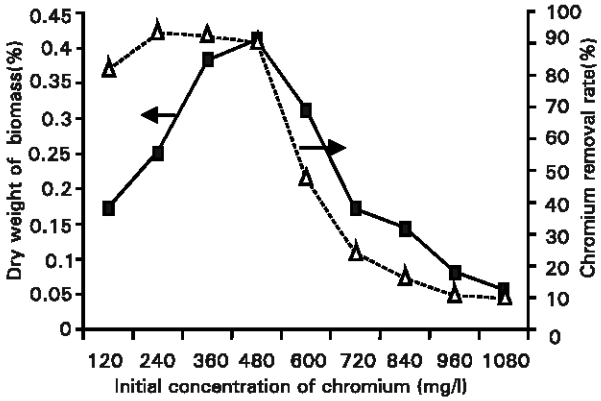

Fig. 2: Effect of different concentrations of chromium in the effluent on biomass growth and chromium removal rate by optimum inoculum size of *A. oryzae* (Size of inoculum = 0.12% dry weight)

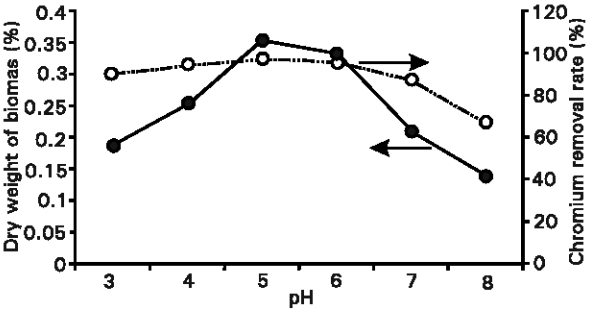

Fig. 3: Effect of pH on *A. oryzae* biomass growth and chromium removal rate in the optimum inoculum size (Size of inoculum = 0.12% dry weight , Cr^{+3} = 240 mg L^{-1})

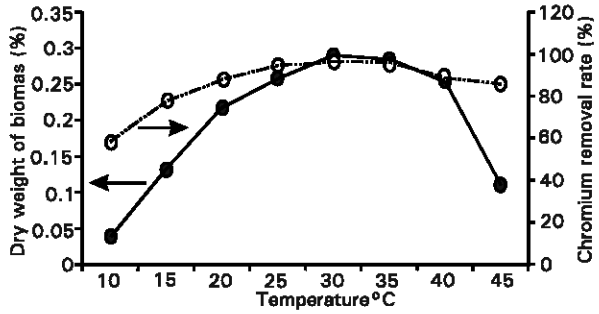

Fig. 4: Effect of temperature on *A. oryzae* biomass growth and chromium removal rate in the optimum inoculum size (Size of inoculum = 0.12% dry weight , Cr^{+3} = 240 mg L^{-1})

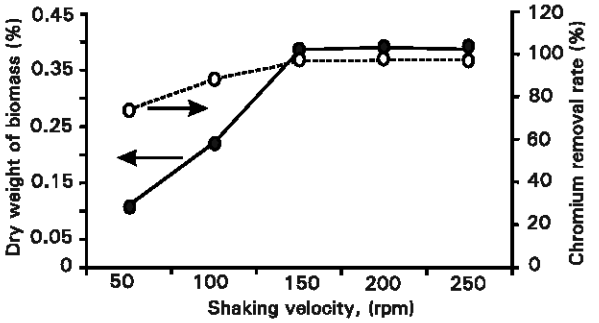

Fig. 5: Effect of Shaking velocity on *A. oryzae* biomass growth and Chromium removal rate in the optimum inoculum size (Size of inoculum = 0.12% dry weight , Cr^{+3} = 240 mg L^{-1})

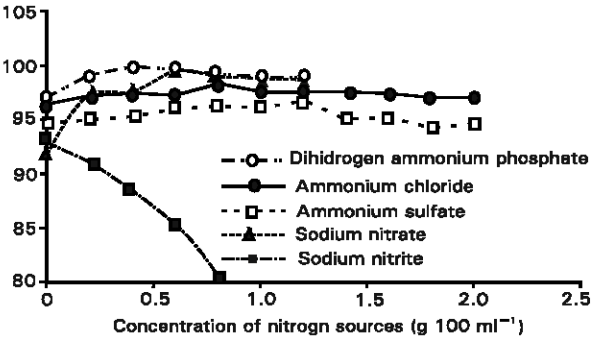

Fig. 6: Effect of type and quality of nitrogen sources on Chromium removal rate by *A. oryzae* biomass and the optimum inoculum size (Size of inoculum = 0.12% dry weight , Cr^{+3} = 240 mg L^{-1})

formula for fungi is $C_{10}H_{17}O_6N$ (Griffin, 1994) and the C/N ratio is 10). Phosphate concentration range in the effluent was 65-118 mg L^{-1}. The sources of phosphate in the effluent were the enzymatic compounds, antiseptic and detergent materials. Phosphorous was the important element in cellular respiration and metabolism of fungi.

Chromium concentration in the effluent was 1000-1300 mg/L. The source of chromium was chromium sulfate that, used for tanning the hides in all countries. The colour of tanning house effluent was dark blue, due to Cr^{+3} cation. Chromium cation has an important role in transforming the hide into leather. In fact, the collagen proteins of hides can complex with chromium and change to leather.

Effect of chromium concentration and inoculum size: The effect of chromium concentration in the range of 120 to 1080 mg/L (dilutions = 10 - 40%) in tanning house effluent, as well as different inoculum sizes of *A. oryzae* were examined. The C/N ratio of the effluent was adjusted at about 10 by ammonium chloride. The range of inoculum size was 0.04 -0.24%.

Study of the trend of biomass growth and chromium removal rate (Figs. 1 and 2), showed their increase with chromium concentration in the range of 120 to 480 mg/L; while the chromium concentration exceeded 480 mg/L. The up mentioned parameters decreased significantly, because of the inhibiting role of high concentration of chromium for fungal growth (Griffin, 1994). The maximum chromium removal was accomplished at the

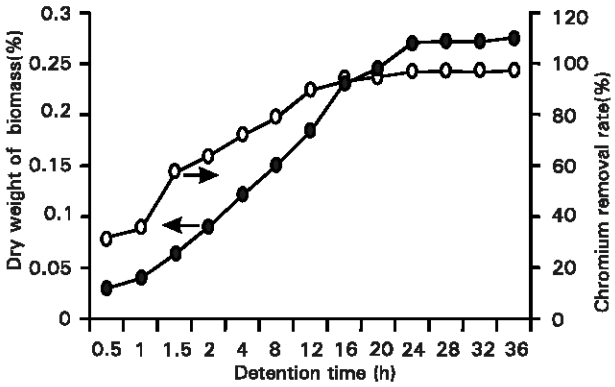

Fig. 7: Effect of Detention time on *A. oryzae* biomass growth and Chromium removal rate in the optimum inoculum size (Size of inoculum = 0.12% dry weight , Cr^{+3} = 240 mg L^{-1})

best inoculum size (0.12%, dry weight) and Cr^{+3} concentration of 240 mg/L (dilution 20%). Smooth chromium removal rate was obtained with 0-0.12% of inoculum size related with availability of nutrients and the effect of population density on the hydrodynamic characteristics of the medium, which consequently influenced the final chromium removal rate. Analysis of variance showed that the chromium concentration and inoculum size were both effective on the biomass growth and chromium residual ($P < 0.001$) ($\alpha = 0.05$).

Effect of pH: The effect of pH on the chromium removal rate was investigated in pH range of 4-8. Phosphoric acid was used to adjust the pH, since it is a valuable buffering agent for fungal growth. The maximum amount of biomass growth and chromium removal occurred at pH 5, where chromium removal rate was 96.6%.

Enzymatic activities of *A. oryzae* at pH 5 was very suitable in which the living cells of fungi were able to grow significantly. With increasing pH beyond 5, the chromium removal rate decreased, which might be due to the osmotic changes and hydrolyzing effect (Fig. 3).

For each pH, five samples were examined and analysis of variance showed that the interaction of *A. oryzae* with pH was significant [$P < 0.001$ ($\alpha = 0.05$)]. Multiple comparisions showed that for *A. oryzae* and pH = 5-6, variations in the biomass growth and chromium removal rate were not significant and that the significant level was 1.0.

Effect of temperature: The effect of temperature on biomass growth and chromium removal was studied at temperatures ranging from 10 to 45°C. (Fig. 4). The maximum biomass growth and chromium removal rate was achieved at 30°C. Decreasing temperature below 24°C, decreased the fungal growth and enzymatic activity. Furthermore, increasing the temperature up to about 40°C, decreased the fungal growth and consequently the chromium removal extent. Temperature affected the biosorption and bioaccumulation process in fungi cells by influencing the enzymatic systems. Also, the solubility of metals in effluent was affected by temperature and its adsorption rate. Anaylsis of variance showed that the interaction of *A. oryzae* with temperature was significant ($P < 0.001$) ($\alpha = 0.05$). Post hoc tests and multiple comparison (Bernard, 2000; Sheskin, 1997) showed that for *A. oryzae* with temperatures between 25, 30, 35 and 40°C, variation in biomass growth and chromium residual was non significant (significance level was 0.997).

Effect of shaking velocity: This parameter varied in the range of 50 and 250 rpm. The maximum amount of biomass growth and chromium removal rate occurred at 150 rpm. Decreasing shaking velocity below 150 rpm, caused the fungal growth and chromium removal to decrease (because aeration rate was insufficient for fungal growth) and when exceeding 150 rpm, fungal growth and chromium removal rate was decreased. The reason might be that when shaking velocity and agitation was very high, fungal cells were not capable to use the nutrient and transfer the oxygen. Maximum chromium removal was 98.31% at 150 rpm (Fig. 5). For each shaking velocity, 3 samples were taken and analyzed and the biomass growth and chromium residual were significant [$P < 0.001$ ($\alpha = 0.05$)]. Multiple comparisons showed that for *A. oryzae* in shaking velocities between 150 and 200 rpm, variation in biomass growth and chromium residual were non significant and level of significance was 1.0.

Effect of nitorgen sources: The biomass growth and chromium removal rate were determined at the nitrogen source concentration of 0-1.2% (weight %). All of the nitrogen sources except nitrite were effecitve for the growth of *A. oryzae* and removing chromium, because fungi use nitrogen in the form of ammonium and nitrate nitrogen as a nitrogen source. Nitrite was toxic for almost all species of fungi, as well as for *A. oryzae*. The maximum amount of chromium removal was 99.8% and occurred in 0.3% of dihydrogen ammonium phosphate (Fig. 6). Three samples were examined for each amount of nutrients. Analysis of variance showed that the type and concentration of nitrogen source had a significant effect on the biomass growth and chromium removal rate. Level of significance was less than 0.001 ($\alpha = 0.05$). Post hoc test and multiple comparisons showed non significant difference in the biomass growth and chromium residual of *A. oryzae* if dihydrogen ammonium phosphate was used at 0.4% or sodium nitrate at 0.6% (significance level was 0.08). But, significant difference was detected when ammonium chloride was used.

Effect of detention time: Effect of detention time in the range of 0.5–36 h at the best conditions (pH = 5, temperature = 30°C, shaking velocity = 150 rpm and concentration of $H_2PO_4(NH_4)_2$ = 0.3%) was studied and the maximum amount of *A. oryzae* biomass and chromium removal rate were estimated to be 0.28 g/100 ml and 97.6%, respectively. The growth curve for *A. oryzae* had three stages: lag phase, logarithmic phase and stationary phase. The growth rate during the lag phase was very low, because *A. oryzae* was adapting with the environment. After this stage, *A. oryzae* grew in logarithmic form using the nutrients. In the third stage, the number of living and dead cells were fixed. Detention time for *A. oryzae* growth was determined as 36 h (Fig. 7).

The maximum chromium removal efficiency of 97.6% and biomass growth of 0.28% (dry weight) for *A. oryzae* could be achived at pH 5, 30°C, 150 rpm and residence time of 36 h. Hence the uptake rate of Chromium was determined as 83.7 mg/g. It should be mentioned that this project was based on studying the living cells of *A. oryzae* with tanning industry effluent and not on synthetic wastewater and dead cells of fungi like other recent researches, such as the chromium uptake rate of 31–59 mg/g obtained with dead cells of *Rhizopus arrhizus* (Tobin, 1998), uptake rate of 21.4 mg/g obtained with *Mucor hiemalis* (Pillichshammer, 1995) and Cu^{2+} removal efficiency of 80% with *A. oryzae* (Chihipin, 1996).

Hence, *A. oryzae* may be applied for the chromium removal from tanning effluent without chemical process.

It is to be noted that the same research project is planned to be carried out in unsterile conditions and with other types of fungi in pilot scale.

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