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CHROMIUM BIOREMOVAL FROM TANNERY INDUSTRIES EFFLUENT BY *ASPERGILLUS ORYZAE*

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ABSTRACT

Recently laboratory studies had recognized the capability of alge, fungi, and bacteria in the removal of heavy metals from industrial effluent. In this research, growth of *Aspergillus oryzae* in the tanning house effluent, and its capability in chromium bioremoval were assessed. *Aspergillus oryzae* can grow in different concentration of $Cr^{+,}$ 120-1080 mg/L. Maximum biomass growth and chromium removal rate at pH, 3.3, Cr^{+3} concentration equal to 240 mg/L and inoculum size equal to 0.12% (dry weight) were 0.25% (dry weight) and 94.2%, respectively. Effects of various factors such as pH, temperature, shaking velocity and nutrients were also investigated. At optimum conditions (ie: 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 as 0.45% of dry weight and 99.8%, respectively. Effect of detention time showed that after 30h, biomass growth and chromium removal rate were 0.28% and 97.6%, 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 of the factors had significant effects ($\alpha = 0.05$, $P < 0.001$). Therefore A.niger capable grow in the tannery industries effluent with 240 mg/L chromium and 97.6% chromium removal rate .

Key words: Tannery industries effluent, chromium, bioremoval, *aspergillus oryzae*

INTRODUCTION

Chromium (III) 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, 1991and Nancy, 1992).

Fungi, in general, are well known for their metal biosorption (Tobin and Roux, 1998; Venkobachor, 1990 and Pillichshammer *et al.*, 1995). Biosorption is a process that utilizes biological materials as adsorbent, and this method has been studied by several researchers as an alternative technique to conventional methods for heavy metals removal from waste water (Jean *et al.*, 2001; Sag and Kutsal, 2001). Two species of *Aspergillus oryzae* and *Rhizopous oryzae*, were used for Cu^{+2} removal (Chihpin and Huang, 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). Immobilized *Aspergillus niger* and *Aspergillus oryzae* as biosorbent used for removal of cadmium, lead, nickel and chromium (Prasad and Freitas, 2000).

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

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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 sizes, and secondly to specify the removal rate and the effects of optimum conditions on biomass growth and chromium removal rate.

MATERIALS AND METHODS

Microorgansim preparation

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 4 ˚C on Potato Dextrose Agar (PDA) for two months. Potato Dextrose Broth (PDB) was used for mycellium culture of fungi (Moazami, 1989).

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 titrasol and concentrated HNO_3 (for Cr^{+3} examination) were used. These chemicals were obtained from Merck Company. Sources of nitrogen such as NH_4Cl, $(NH_4)_2$ SO_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 procedure

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

Preculture 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 48h. Pellets of mycelia were formed with a dry weight of 0.8 g/100ml and diameter of 1.1–1.3 mm (Pillichshammer *et al*., 1995).

Sampling and experimental procedure

Serial dilutions of tanning effluent (10 - 90%) with chromium concentration of 1200 mg/L and in each concentration 3 sample were prepared and then sterilized. Different sizes of inoculum (0.04% - 0.24%, 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 environmental factor such as pH, 3 to 8, temperature,10 to 45 ˚C, shaking velocity, 50 to 250 rpm, type and concentration of nitrogen sources (NH_4Cl, $(NH_4)_2SO_4$, $H_2PO_4(NH_4)_2$, $NaNO_3$, $NaNO_2$,upto 0.24%) were also studied in order to find the optimum conditions, dilutions and the best inoculum size. Three samples for each factor prepared. 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 effect of pH on the chromium removal rate was investigated in the pH range of 4-8. The effect of temperature on biomass growth and chromium removal was studied at temperatures ranging from 10 to 45 ˚C. (Fig. 4). Six types of nitrogen sources (ammonium chloride, ammonium sulfate, dihydrogen ammonium phosphate, sodium nitrate and sodium nitrite, were used to adjust the carbon to nitrogen ratio and then compared with each other. Effect of detention time in the range of 0.5 – 36 hours at the best conditions (pH=5,temperature=30˚C, shaking velocity =150rpm and concentration of $H_2PO_4(NH_4)_2$= 0.3%) was studied .

Statistical analysis

Analysis of variance on data was carried out. The effect of effluent dilution, inoculum size and type of fungi with interaction of them was tested. In addition, environmental factors effect on biomass growth and chromium removal rate were analyzed (Bernard, 2000).

RESULTS

Tanning effluent quality

The composition of tanning wastewater is shown in Table 1. The pH of the effluent was 3 to 3.5 which was very acidic condition. TOC in the effluent varied from 27000 to 41000 mg/L.

The concentration of nitrogen compounds varied between 160 and 245 mg/L. The C/N ratio was observed to be 139 to169, which was very high for fungal growth. Phosphate concentration range in the effluent was 65 to 118 mg/L. Chromium concentration in the effluent was 1000-1300 mg/L. The source of chromium was chromium sulfate that was used for tanning of hides in all countries.

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

Effect of chromium concentration and inoculum size

The Study of the trend of biomass growth and chromium removal rate (Fig. 1 and 2), shows their increase with chromium concentration in the range of 120 to 480 mg/L. Results showed that the maximum chromium removal rate were 0.25% (Dry weight) and 94.2% in the biomass growth equal to 0.25%, at the best inoculum size (0.12%, dry weight), and initial Cr^{+3} concentration of 240 mg/L (dilution 20%).

Effect of pH

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

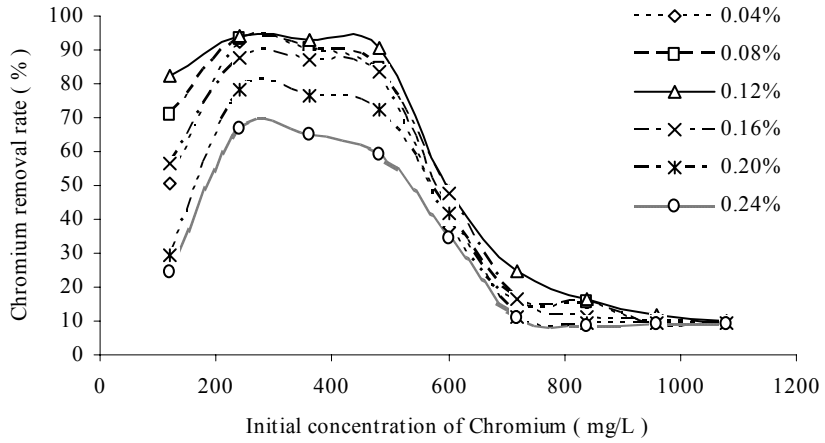

Fig.1: Effect of initial concentrations of Chromium in the effluent on chromium removal rate by different inoculum size of *A. oryzae* (0.94%-0.24% dry weight)

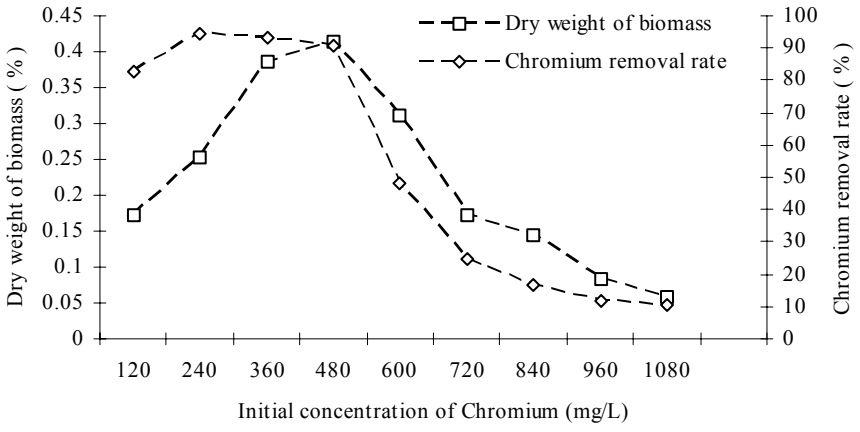

Fig. 2: Effect of initial concentrations of Chromium in the effluent on Chromium removal rate by optimum inoculum size of *A.oryzae* 0.12 % (dry weight)

chromium removal rate occurred at pH, 5, and were 0.35% (Dry weight) and 96.6%, respectively. Enzymatic activities of *A.oryzae* at pH 5 is very suitable in which the living cell of fungi are able to grow significantly (Fig. 3).

Effect of temperature

It can be observed that the maximum biomass growth and chromium removal rate was achieved at 30ºC and were 0.29% (Dry weight) and 96.9%, respectively. Decreasing temperature below 24ºC decreased fungal growth and enzymatic activity. Furthermore, increasing the temperature up to about 40°C, decreased the fungal growth and consequently the chromium removal extent.

Effect of shaking velocity

In order to study the effect of shaking velocity on chromium removal rate, this parameter was varied in the range of 50 and 250 rpm. The maximum amount of biomass growth and chromium removal rate occurred at 150 rpm Maximum biomass growth and chromium removal rate were 0.38% (Dry weight) and 98.31% at 150 rpm, respectively (Fig. 5). With increasing shaking velocity, biomass growth was stationary.

Effect of nitrogen sources

The biomass growth and chromium removal rate were determined at the nitrogen source concentration of 0-1.2% (weight %). The maximum amount of biomass growth and chromium removal rate were 0.45% (Dry weight) and 99.8% in 0.3% of dihydrogen ammonium phosphate, respectively (Fig. 6).

Effect of detention time

The maximum amount of *A.oryzae* biomass and chromium removal rate were estimated as 0.28% and 97.6%, respectively (Fig.7). After 24h, fungi growth was stopped.

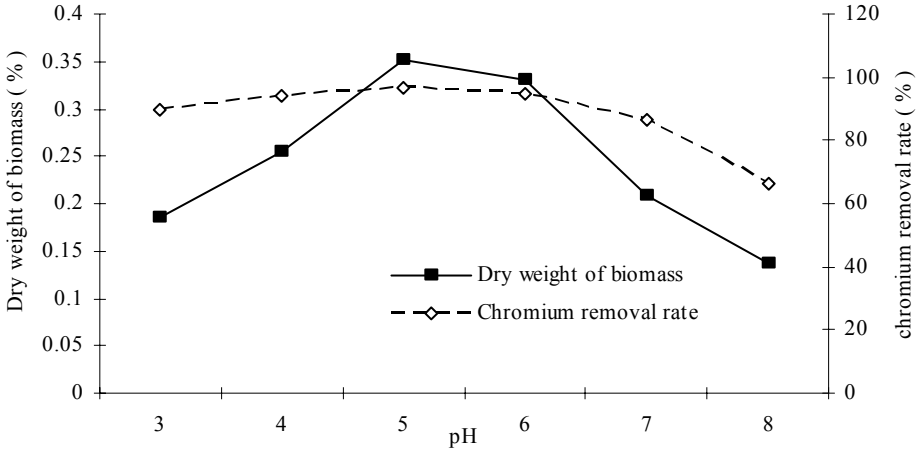

Fig.3:Effect of pH on *A.oryzae* biomass growth and Chromium removal rate in the optimum inoculum size 0.12% (dry weight) and initial concentration of Chromium 240 mg/L

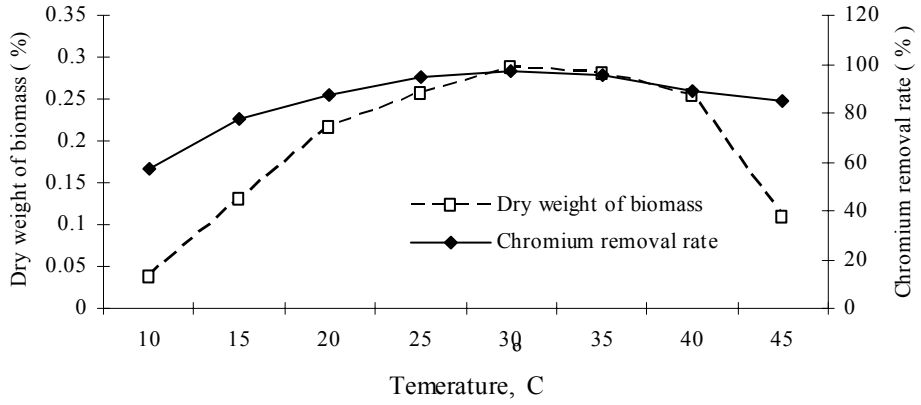

Fig.4: Effect of temperature on *A.oryzae* biomass growth and Chromium removal rate in the optimum inoculum size 0.12% (Dry weight) and initial concentration o Chromium 240mg/L

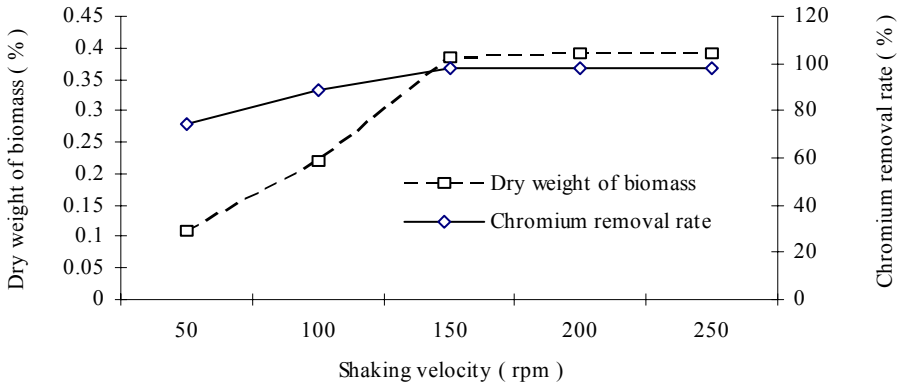

Fig.5: Effect of shaking velocity on *A. oryzae* biomass growth and Chromium removal rate in the optimum inoculum size 0.12% (Dry weight) and initial Chromium Concentration 240mg/L

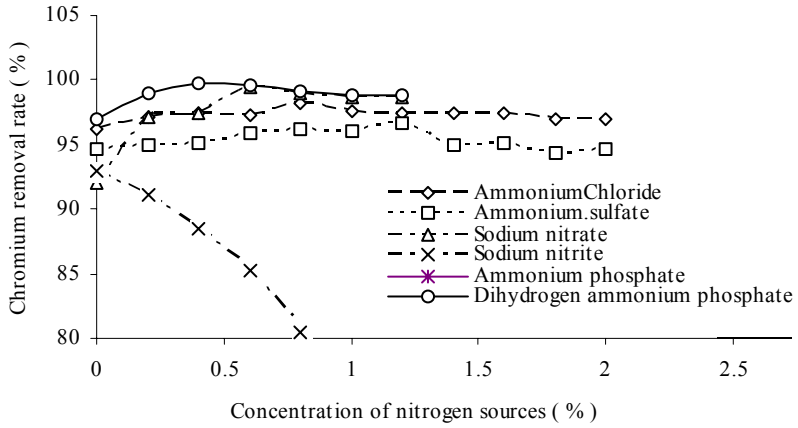

Fig. 6: Effect of type and quantity of nitrogen sources on Chromium removal rate by the *A.oryzae* biomass and optimum inoculum size 0.12% (Dry weight) and initial Chromium concentration 240 mg/L

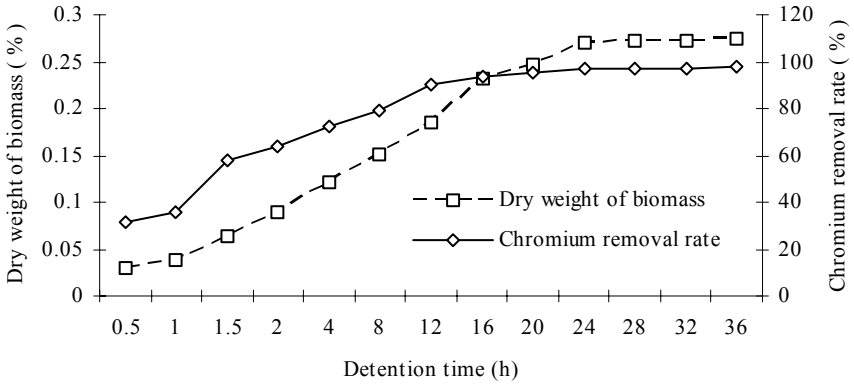

Fig.7: Effect of detention time on *A. oryzae* biomass growth and chromium removal rate in the optimum inoculum size 0.12% and initial Chromium concentration 240 mg/L

DISCUSSION

Tanning effluent quality

The color 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. The tanning house effluent was very acidic, since stabilization of chromium on the hide must be carried out in acidic environment. The carbohydrates and organic acids (formic acid) are the sources of total organic carbon (TOC). Enzymes (such as tripsin and protease) and proteins of hides are the sources of nitrogen compounds. The general formula for fungi is $C_{10}H_{17}O_6N$ (Griffin, 1994) and the C/N ratio is 10 (weight ratio). Therefore, C/N ratio in the effluent is very high and must be adjusted. The sources of phosphate in the effluent were the enzymatic compounds, antiseptic and detergent materials. Phosphorous is the important element in the cellular respiration and metabolism of fungi.

Effect of chromium concentration and inoculum size

While the chromium concentration exceeded 480 mg/l (Figs. 1 and 2), the aforementioned parameters decreased significantly, because of the inhibitor role of high concentration of chromium for fungal growth (Griffin, 1994). Smooth chromium removal rate obtained with 0.04 %-0.12% of inoculum size related to the 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 ($P< 0.001$).

Effect of pH

With increasing pH beyond 5, the chromium removal rate decreased, which might be due to osmotic changes and hydrolyzing effect (Fig. 3). Analysis of variance showed that the interaction of *A.oryzae* with pH was significant ($P < 0.001$, $\alpha = 0.05$). Multiple comparisons has shown that for *A.oryzae* and pH 5 to 6, variations in the biomass growth and chromium removal rate were not significant and that the significant level was 1.0.

Effect of temperature

Temperature affects on biosorption and bioaccumulation process in fungi cells by influencing enzymatic systems. In addition, the solubility of metals in the effluent is affected by temperature and its adsorption rate. The number of samples for each temperature was 3. Analysis of variance showed that the interaction of *A.oryzae* with temperature was significant and P value was less than 0.001 ($\alpha = 0.05$). Post hoc tests and multiple comparison (Bernard, 2000) showed that for *A.oryzae* with temperatures between 25, 30, 35 and 40°C, variations in biomass growth and chromium residual were not significant (significance level was 0.997).

Effect of shaking velocity

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 decreased. The reason is that when shaking velocity and agitation is very high, fungal cells are not capable to use the nutrient and transfer oxygen. Analysis of variance was showed shaking velocity was significant on biomass growth and chromium residual ($P<0.001$). Multiple comparisons have shown that for *A.oryzae* in shaking velocities between 150 and 200 rpm, variation in biomass growth and chromium residual were not significant, and significance level was 1.0.

Effect of nitrogen sources

All of the nitrogen sources except nitrite were effective 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 is toxic for almost all species of fungi, as well as for *A.oryzae* (Griffin, 1996). 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. Significance level was less than 0.001 ($\alpha = 0.05$). Post hoc test and multiple comparisons showed that for *A.oryzae* no significant difference in the biomass growth and chromium residual were observed, if we used dihydrogen ammonium phosphate at 0.4% or sodium nitrate at 0.6% (significance level was 0.08). However, significant difference was detected when ammonium chloride was used.

Effect of detention time

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 cells and dead cells were fixed. Detention time for *A.oryzae* growth was determined as 24 h (Fig. 7). In this condition chromium uptake rate was 83.7 mg/g.

Conclusion of this research indicates that for *A. oryzae* the maximum chromium removal efficiency of 97.6 % and biomass growth of 0.28 % (dry weight) could be achieved in pH =5, T= 30 ^{0}C, 150 rpm and residence time of 24 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 with 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 and Roux, 1998), uptake rate of 21.4 mg/g obtained with *Mucor hiemalis* (Pillichshammer *et al*., 1995), and Cu^{2+} removal efficiency of 80 % with *A. oryzae* (Chihipin and Haung, 1996).

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