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# Chromium(VI) bioaccumulation capacities of adapted mixed cultures isolated from industrial saline wastewaters

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**Abstract**

Enrichment mixed cultures tolerating relatively high concentrations of chromium and salt ions were isolated and their bioaccumulation properties improved by adaptation. Mixed cultures were enriched in Nutrient Broth media containing 25–300 mg $l^{-1}$ Cr(VI) and 0%, 2%, 4%, 6% (w/v) NaCl. Bioaccumulation of Cr(VI) was studied in a batch system as a function of initial pH (7, 8 and 9), Cr(VI) and NaCl concentrations. Increasing NaCl and Cr(VI) concentrations led to significant decreases in percentage uptake and dried weight of mixed cultures but increased maximum specific chromium uptake. The maximum specific chromium uptake value at pH 8 was 58.9 mg $g^{-1}$ for 316.1 mg $l^{-1}$ Cr(VI) in the absence of NaCl, while at pH 9 it was 130.1 mg $g^{-1}$ in media including 194.5 mg $l^{-1}$ Cr(VI) and 2% NaCl concentrations. At 4% NaCl, the maximum Cr(VI) uptake of 127.0 mg $g^{-1}$ for 221.1 mg $l^{-1}$ Cr(VI) occurred at pH 9, while at 6% NaCl the maximum Cr(VI) uptake of 114.9 mg $g^{-1}$ for 278.1 mg $l^{-1}$ Cr(VI) was found at pH 7.


*Keywords:* Bioaccumulation; Chromium(VI); Microorganism; Saline wastewater

## 1. Introduction

Microorganisms have long been known to accumulate heavy metal ions from the environment. This ability offers an attractive option for the removal and recovery of heavy metal ions from wastewater. Microorganisms are much more efficient at removing metal ions than other pollutants. Removal of many heavy metal ions is commonly reported, while chromium is typically removed at low percentages and is considered one of the most difficult metals to remove using biomass (Cervantes et al., 2001; Patterson, 1977).

Chromium is a toxic heavy metal that is widely used in the manufacture of steel, as an electroplated coating for corrosion control, as a mordant in the textile industries and as an anti-corrosive agent in the tanning industry (Patterson, 1977).

Chromium exists in several oxidation states from chromium(II) to chromium(VI). In nature, chromium can be found either as chromium(VI) or as chromium(III). Chromium(III) is rather benign and easily adsorbed in soils, whereas chromium(VI), which is the most toxic form, is not readily adsorbed and most of its salts are soluble (Patterson, 1977).

Some chromium contaminated wastewaters such as tannery wastes contain salt ions. These industrial wastewaters contain both chromium and salt ions which have toxic effects on the microbial consortia of wastewater treatment systems (Stasinakis et al., 2003; Ram et al., 1999; Patterson, 1977).

Different types of microorganisms have been investigated for their ability to take up chromium (Baldrian, 2003; Dursun et al., 2003; Jianlong et al., 2004; Dönmez and Aksu, 2002; Lloyd et al., 2001; Nepple et al., 2000; Dilek et al., 1998; Chirwa and Wang, 1997; Krauter et al., 1996). However, there are no reports on chromium bioaccumulation in saline media. More efficient treatment processes to obtain biomass bioaccumulating both Cr(VI)

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and salt ions are very necessary. The aim of this study was to isolate mixed microbial cultures that can tolerate relatively high concentrations of chromium(VI) and salt ions and to improve the bioaccumulation properties of the biomass by adaptation.

## 2. Methods

### 2.1. Enrichment of mixed microbial cultures

Chromium(VI) bioaccumulating mixed microbial cultures were obtained from industrial saline wastewater (Sepiciler Leather Ind. Inc.) contaminated with chromium(VI). Mixed cultures were prepared by an enrichment procedure. Enrichment was performed by a periodic subculturing of samples from industrial saline wastewater in aqueous Nutrient Broth (NB) medium containing 25 mg $l^{-1}$ chromium(VI) and 0%, 2%, 4% and 6% (w/v) NaCl. One ml samples were incubated in 25 ml of medium in 100 ml Erlenmeyer flasks on a rotary shaker at 100 rpm for 10 days at 20 ± 2 °C. The composition of the NB medium was 5.0 g peptone and 3.0 g beef extract in 1 l. A stock solution of chromium was prepared by dilution of $K_2Cr_2O_7$ to a final concentration of 10 g/l of chromium. Appropriate volumes of the stock solution were added to media. The initial pH was adjusted to 8 with 0.1 M NaOH and 0.1 M HCl. Microorganisms were cultured on four different nutrient agar slants, kept at 4 °C and transferred to fresh media every three months.

### 2.2. Adaptation experiments

After the enrichment period, mixed cultures were grown on NB media (pH 8) containing 25, 50, 100, 200 and 300 mg $l^{-1}$ chromium at 0%, 2%, 4% and 6% (w/v) NaCl concentrations on a rotary shaker at 100 rpm for 7–14 days at 20 ± 2 °C. In the adaptation experiment, cultures adapted to low chromium(VI) and NaCl concentrations were used to inoculate high chromium(VI) and NaCl supplemented media. The experiments were repeated at pH 7 and 9.

### 2.3. Bioaccumulation experiments

In order to examine the bioaccumulation capacity of mixed cultures, two sets of experiments were carried out. Firstly, to find a suitable pH level for more efficient bioaccumulation, pH was adjusted to 7, 8 and 9 in NB media including 25–50 mg $l^{-1}$ Cr(VI) and 0%, 2%, 4% and 6% (w/v) NaCl concentrations. For these experiments, 1 ml of adapted mixed culture was used to inoculate 100 ml of the same NB media used for adaptation experiments. Secondly, bioaccumulation capacities of mixed cultures were investigated at different chromium concentrations. One milliliter of mixed culture adapted in each of the NB media containing 25–300 mg $l^{-1}$ chromium was grown in 100 ml liquid medium at the same chromium concentration. The effect of chromium concentration on the bioaccumulation was studied at different pH (pH 7, 8 and 9) and NaCl concentrations (0%, 2%, 4% and 6% w/v). The cultures were incubated in 100 ml of the NB medium including chromium and NaCl in 250 ml Erlenmeyer flasks on a rotary shaker at 100 rpm for 10 days at 20 ± 2 °C.

In the tests, control cultures containing no chromium were prepared. Each experiment was carried out in triplicate. All determinations were made daily for the incubation period.

### 2.4. Analytical methods

A 3-ml sample was taken daily from each of the flasks. Samples were centrifuged at 3.421g for 5 min to remove suspended biomass and the concentration of chromium in the supernatant was determined spectrophotometrically at 540 nm using diphenyl carbazide reagent in acid solution as the complexing agent for chromium(VI) (Snell and Snell, 1959). Absorbance measurements were done using a Shimadzu UV 2001 model spectrometer.

For the measurement of microbial growth, the biomass concentration was determined by measuring the turbidity of the diluted sample at 540 nm and using a standard curve of absorbance against dry cell mass.

### 2.5. Statistical analysis

The experiments were set up in a completely randomized design with three replicate. Analysis of variance was performed by using Minitab 14 on the data and significant differences among treatment means were compared by descriptive statistics (±SE).

## 3. Results

Chromium bioaccumulation properties of mixed culture were investigated as a function of initial pH and initial chromium concentrations. The percentage uptake of Cr(VI) was calculated from

$$\text{uptake } \% = ((C_0 - C_f)/C_0) \times 100$$

The bioaccumulation capacity Cr(VI) is the concentration of Cr(VI) on the biomass and can be calculated based on the mass balance principle where

$$q_m = (C_0 - C_f)/X_m$$

In these two equations, $q_m$ (the maximum specific chromium uptake) represents the maximum amount of chromium per unit dry weight of microbial cells (mg $g^{-1}$), $X_m$ maximum dried cell mass (g $l^{-1}$), and $C_0$ and $C_f$ the initial and final concentrations (mg $l^{-1}$), respectively.

### 3.1. Microbial adaptation in media including chromium(VI) and NaCl

To examine the tolerance of chromium and salt by mixed culture, cells were cultivated in NB media supplemented