Available online at www.sciencedirect.com
SCIENCE DIRECT®
Enzyme and Microbial Technology 36 (2005) 700–705
ENZYME and MICROBIAL TECHNOLOGY
www.elsevier.com/locate/enzmictec

Isolation of hexavalent chromium resistant bacteria from industrial saline effluents and their ability of bioaccumulation

Gönül Dönmez*, Nur Koçberber

Ankara University, Faculty of Science, Department of Biology, 06100, Beşevler, Ankara, Turkey

Received 7 June 2004; accepted 7 December 2004

Abstract

The mixed cultures has been isolated from industrial saline wastewater contaminated with chromium(VI), using enrichment in the presence of 50 mg l^{-1} chromium(VI) and 4% (w/v) NaCl at pH 8. In this study, the molasses (M) medium was selected a suitable medium for the effective chromium bioaccumulation by the mixed cultures. Eleven pure isolates obtained from mixed cultures and some of them showed high bioaccumulation in the M media containing about 100 mg l^{-1} chromium(VI) and 4% NaCl. The strain 8 (99.3%) and 10 (99.1%) were able to bioaccumulate more efficient than the mixed culture (98.9%) in this media. But the highest specific Cr uptake was obtained by the mixed cultures followed by strain 8 and 10 with 56.71, 33.14 and 21.7 mg g^{-1}, respectively. Bioaccumulation of chromium(VI) ions by the strain 8 growing in the media with chromium(VI) and NaCl was studied in a batch system as a function of initial chromium(VI) (86.6–547.6 mg l^{-1}) and NaCl (0, 2, 4, 6% w/v) concentrations. During all the experiments, the uptake yield of the strain 8 was highly affected from NaCl concentrations in the medium at high initial chromium(VI) concentrations. But at low chromium(VI) concentration, strain 8 was not affected from NaCl concentrations in the medium. The maximum uptake yield were obtained in the M media with 2% NaCl as 98.8% for 110.0 mg l^{-1}, 98.6% for 217.1 mg l^{-1}, 98.6% for 381.7 mg l^{-1} and 98.2% for 547.6 mg l^{-1} initial chromium(VI) concentrations. The strain 8 tolerated a 6% (w/v) NaCl concentration was able to bioaccumulate more than 95% of the applied chromium(VI) at the 97.6–224.4 mg l^{-1} initial chromium(VI) concentrations. The results presented in this paper was shown that these pure and mixed cultures might be of use for the bioaccumulation of chromium(VI) from saline wastewater.

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

1. Introduction

Chromium is a toxic heavy metal that is widely used in industry, including leather tanning, electroplating, paint and pigment manufacturing, textile and fertilizer industries. These industries discharged trivalent and hexavalent chromium with waste effluent to surface water. Chromium(III) is non-toxic and essential to mammals in trace concentrations and relatively immobile in the aquatic system due to its low water solubility. On the other hand, highly soluble chromium(VI) is toxic to many plants, animals and bacteria inhabiting aquatic environments [1,2]. Therefore, it is necessary to remove soluble chromium(VI) from industrial wastewaters.

Many microorganisms are able to remove chromium(VI) from wastewaters. These microorganisms have developed a variety of mechanisms to remove chromium(VI), such as adsorption to cell surfaces, transport into the cell, intracellular accumulation or reduction to non-toxic chromium(III) [3–14]. Chromium(VI) bioaccumulation process might be effectively useful in wastewater treatment systems for detoxification and removal of soluble chromium(VI). However, high concentrations of chromium(VI) are strongly reduced microbial activity of activated sludge [15,16].

Chromium(VI) removal capacity of microorganisms is also effected from some external factors such as salt ions, pH and temperature of wastewater [2,10,17]. Especially high salt concentrations in wastewater treatment systems is the

* Corresponding author. Tel.: +90 312 2126720; fax: +90 312 2232395.
E-mail address: gdonmez@science.ankara.edu.tr (G. Dönmez).

0141-0229/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2004.12.025

most significant factor reduced microbial activity. Therefore, isolation of microbial cultures that was resistant to high chromium(VI) and salt concentrations must be necessary for effectively removal of chromium(VI) from wastewaters.

In this article, we report on the isolation of microorganisms that can resistant high concentration of chromium(VI) and can bioaccumulate chromium(VI) from wastewater contain both chromium(VI) and salt ions. This is the first report of chromium bioaccumulation to saline media with pure cultures.

2. Materials and methods

2.1. Isolation of chromium(VI) bioaccumulating mixed microbial cultures

Isolation was performed by culturing of samples from industrial saline wastewater contaminated with chromium(VI) in activated sludge (AS), nutrient broth (NB) and molasses (M) media containing 50 mg l^{-1} chromium(VI) and 4% (w/v) NaCl. The composition of the AS medium is 1.0 g glucose, 0.12 g Na_2SO_4, 0.4 g KH_2PO_4, 0.15 g $MgSO_4{\cdot}7H_2O$, 0.005 g $FeSO_4{\cdot}7H_2O$, 0.042 g NH_4Cl, 0.00021 g $ZnCl_2$, 0.05 g $CaCl_2{\cdot}2H_2O$, 0.00008 g $NiCl{\cdot}6H_2O$, 0.017 g beef extract, 0.0001 g H_3BO_3, 0.4 g CH_3COONa in 1l. The composition of the NB medium is 5.0 g peptone, 3.0 g beef extract in 1l. The composition of the M medium is molasses (beet) solution (approximately equivalent to 10 g l^{-1} sucrose), 1.0 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4 in 1l [18]. 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. The samples (1 ml) were incubated into 20 ml of the media including chromium and NaCl in a 50 ml Erlenmeyer flasks at a room temperature of 20 ± 2 °C on a rotary shaker at 100 rpm for 5–14 days. Mixed cultures growing on the media were activated at the same media for three times before the used to bioaccumulation experiments. The cultures developing on three different media were kept on at 4 °C and was transferred every 3 months.

2.2. Bioaccumulation experiments with mixed microbial cultures

For the experiments, 1 ml mixed cultures was inoculated in 100 ml AS, NB and M media at the pH 8, 4% NaCl and 50 mg l^{-1} chromium(VI) concentration. The cultures were incubated in a 250 ml Erlenmeyer flasks at a room temperature of 20 ± 2 °C on a rotary shaker at 100 rpm for 6 days.

2.3. Selection and isolation of chromium(VI) bioaccumulating pure cultures

Each 0.1 ml aliquot of the growing mixed culture broth was spread on an AS, NB and M agar (1.5%, w/v) plate including 50 mg l^{-1} chromium(VI) and 4% NaCl at pH 8 and incubated at a room temperature of 20 ± 2 °C. After the incubation period (2 days) single colonies on these plates were purified and transferred to agar slants. The pure cultures were kept on at 4 °C and transferred every 3 months. For the selection of high chromium(VI) bioaccumulating cultures, the pure cultures were incubated into 100 ml of the NB and M media including about 100 mg l^{-1} chromium(VI) and 0–4% NaCl in a 250 ml Erlenmeyer flasks at a room temperature of 20 ± 2 °C on a rotary shaker at 100 rpm for 5 days at pH 8.

2.4. Bioaccumulation experiments with pure culture

The experiments were carried out the M media including 86.6–547.6 mg l^{-1} chromium(VI) and 0, 2, 4, 6% NaCl. The pH value of the M media was adjusted to 8. One milliliter of pure culture was grown in 100 ml liquid media at the same chromium(VI) and NaCl concentrations. The cultures were incubated in a 250 ml Erlenmeyer flasks at a room temperature of 20 ± 2 °C on a rotary shaker at 100 rpm for 3–14 days.

In the tests, two control flasks were prepared. First control medium contained M, NB or AS media without any chromium(VI) and NaCl to examine the growth of the microorganisms. Second control medium contained M, NB or AS media, chromium(VI) and NaCl without growing of the microorganisms to observe any reaction of media components with chromium(VI) and NaCl. Each experiment was carried out in triplicate. All determinations were made daily for the incubation period.

2.5. Analytical methods

Three-milliliters sample was taken daily from each flask. Samples were centrifuged 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) [19]. Absorbance measurements were done by 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 using a standard curve of absorbance against dry cell mass.

3. Results

Chromium bioaccumulation properties of mixed and pure cultures were investigated as a function of initial pH and initial chromium concentration. The results are given as bioaccumulated chromium concentration (C_{acc}: mg l^{-1}), bioaccumulated chromium concentration at the end of growth ($C_{acc,m}$: mg l^{-1}), maximum dried cell mass (X_m: g l^{-1}) and maximum specific chromium uptake determined as the maximum amount of chromium per unit of dry weight of microbial cells (q_m: mg g^{-1}). The uptake yield (uptake %) is defined