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ScienceDirect
Journal of Hazardous Materials 144 (2007) 549–555
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Cr(VI) and Cr(III) speciation on *Bacillus sphaericus* loaded diaion SP-850 resin

Mustafa Tuzen [a], Ozgur Dogan Uluozlu [a], Mustafa Soylak [b,*]

[a] *Gaziosmanpasa University, Faculty of Science and Arts, Chemistry Department, 60250 Tokat, Turkey*
[b] *Erciyes University, Faculty of Science and Arts, Chemistry Department, 38039 Kayseri, Turkey*

Received 25 September 2006; received in revised form 23 October 2006; accepted 24 October 2006
Available online 28 October 2006

Abstract

A speciation procedure for chromium(III) and chromium(VI) in the environmental samples has been established in the presented work. The procedure presented based on quantitative biosorption of chromium(III) on *Bacillus sphaericus* loaded Diaion SP-850 at pH 5. The Cr(VI) recoveries at pH 5 were below 5% on the biosorbent. After reduction of Cr(VI) by concentrated H_2SO_4 and ethanol, the system was applied to the total chromium. Cr(VI) was calculated as the difference between the total chromium content and the Cr(III) content. Optimal analytical conditions including pH, amounts of biosorbent, etc. for Cr(III) recoveries were investigated. The influences of the some alkaline and earth alkaline ions and some transition metals on the recoveries were also investigated. The capacity of biosorbent for chromium(III) was 6.95 mg g^{-1}. The detection limit (3 sigma) of the reagent blank for chromium(III) was 0.50 μg L^{-1}. The procedure was successfully applied to the speciation of chromium(III) and chromium(VI) in natural water samples (R.S.D. lower than 5%, recoveries greater than 95%).

Keywords: *Bacillus sphaericus*; Diaion SP-850; Biosorption; Speciation; Chromium; Atomic absorption spectrometry

1. Introduction

The determination of metals in environmental samples continues to be a challenge for analytical chemists around the world due to the contribution of metal ions into the effluent by mining, tannery, cement, textile and iron industries [1–4]. Contaminant metals are potentially toxic and may cause health problems to humans via the food chain [5–7]. Metal speciation studies have shown that various oxidation states of metals are toxic to humans and also that they are associated with several types of diseases. The form or species the metal is in is very important, because of the effect it may have on the environment, as some forms of the same metal are more toxic than others. Chromium(VI) is carcinogenic and chromium(III) is found to be involved in some enzyme systems [8–10]. It is therefore important to be able to separate these two species in a water sample and to determine how much of each is present. There is an increasing need to know the concentrations of chromium(III) and (VI) separately rather than only the total chromium content [11–14].

Traces heavy metals quantitatively adsorbed on the higher organisms including mosses, bacteria, algae [15,16]. Biosorption is responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation [15,16]. The biosorption systems for preconcentration-separation and speciation of chromium species are based on biosorption of chromium species on the organisms and desorption of adsorbed chromium from the organisms [17–21]. Bag et al. [22] have been proposed an enrichment and speciation procedure for Cr(III) and Cr(VI) ions on the biosorption on *Saccharomyces cerevisiae* loaded on sepiolite. The possibility of using moss (*Funaria hygrometrica*) immobilized in a polysilicate matrix as substrate for speciation and preconcentration of Cr(III) and Cr(VI) in various water samples has been studied by Krishna et al. [23]. *Saccharomyces cerevisiae* was used to selectively accumulate and preconcentrate Cr(III) from aqueous matrices. The substrate was used in batch and continuous operation immobilized in the latter case on alginate [24]. Biosorption of chromium(VI) was studied by using non-living free and immobilized biomass of *Rhizopus arrhizus*

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[*] Corresponding author. Fax: +90 352 4374933.
E-mail address: soylak@erciyes.edu.tr (M. Soylak).

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doi:10.1016/j.jhazmat.2006.10.069

by Prakasham et al. [25]. The applicability of a fungal biomass *Aspergillus niger* immobilized on a polysilicate matrix as substrate for the separation and determination of Cr(III) and Cr(VI) in natural water samples has been investigated by Rao et al. [26].

Bacillus sphaericus is a naturally occurring bacteria—isolated, cultured, and labeled for mosquito control. *B. sphaericus* has been accepted as one of target specific biological pest control agent. *B. sphaericus* produces an endotoxin that is toxic to some species of mosquito larvae. *B. sphaericus* is found widely in soil substrate available nature and selected because of its effective control of mosquito larvae [27]. *B. sphaericus* has been used at first time by our working group for the preconcentration and separation of copper(II), lead(II), iron(III) and cobalt(II) from the environmental samples. According to our literature survey, it is first time used in the speciation of chromium in the presented work.

In the presented work, a biosorption procedure on *B. sphaericus* loaded Diaion SP-850 for speciation of chromium(III) and chromium(VI) in natural water samples has been established.

2. Experimental

2.1. Instrument

A Perkin-Elmer Analyst 700 atomic absorption spectrometer with deuterium background corrector, equipped with a chromium hollow cathode lamp operated at 25 mA, was used throughout this work. The wavelength and spectral band pass were set at 357.9 nm and 0.7 nm, respectively. A 10 cm long slot-burner head and an air-acetylene flame were used. The nebulizer uptake rate was adjusted to give the optimum response for conventional sample introduction, the resulting rate being 6.0 mL min^{-1}. An acetylene flow rate of 2.5 L min^{-1} was used with an air flow rate of 17.0 L min^{-1}. A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase.

2.2. Reagents and solution

All chemicals used in this work, were of analytical reagent grade and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistivity) was used for all dilutions. All the plastic and glassware were cleaned by soaking in dilute HNO_3 (1 + 9) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} of the given element supplied by Sigma and Aldrich. Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Sodium phosphate buffer (0.1 M) was prepared by adding an appropriate amount of phosphoric acid to sodium dihydrogen phosphate solution to result in a solution of pH 2 and 3. Ammonium acetate buffers (0.1 M) were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions to result in solutions of pH 4–6. Ammonium chloride buffer solutions (0.1 M) were prepared by adding an appropriate amount of ammonia to ammonium chloride solutions to result in solutions of pH 8–9.

Diaion SP-850 is an aromatic type adsorbent is the standard grade and is based on crosslinked polystyrenic matrix. Its surface area is 1000 m^2 g^{-1}. Diaion SP-850 was purchased from Sigma Chem. Co., St. Louis, USA. It (20–60 mesh) was washed successively with methanol, water, 1 mol L^{-1} HNO_3 in acetone, water, 1 mol L^{-1} NaOH, and water, sequentially.

2.3. Model studies

B. sphaericus Loaded Diaion SP-850 was prepared according procedure given by Tuzen et al. [28]. The *B. sphaericus* loaded Diaion SP-850 column was 10 cm long, and 0.5 cm in diameter. A small plug of glass wool was placed on the bottom of the column. The column contained about 500 mg resin. The *B. sphaericus* loaded Diaion SP-850 column was prepared by aspirating water slurry of the *B. sphaericus* loaded Diaion SP-850 into the glass column. It was conditioned by passing 10–15 mL of buffer solution then it was used in the presented work.

The biosorption procedure on *B. sphaericus* loaded Diaion SP-850 for chromium speciation was tested with model solutions. For chromium(III) determinations, to 40–50 mL of solution containing 20 μg of chromium(III) ion was added 10 mL of buffer solution (to give the desired pH between 2 and 9). The *B. sphaericus* loaded Diaion SP-850 column was preconditioned by passing buffer solution. The solution was passed through the column at a flow rate of 5 mL min^{-1}. The sample solution was permitted to flow through the column under gravity. After passing of this solution, the column was rinsed twice with 5–8 mL of water. The adsorbed chromium on the biosorbent column was eluted with 10 mL portion of 1.0 mol L^{-1} HCl. The eluent was analyzed for the determination of chromium(III) concentrations by an atomic absorption spectrometer.

Total chromium was determined as Cr(III) by the method described above after reducing Cr(VI) to Cr(III). The reduction of Cr(VI) to Cr(III) was performed by the addition of 0.5 mL of concentrated H_2SO_4 and 0.5 mL of ethanol to the test solution [22]. The level of Cr(VI) is calculated by difference of total chromium and Cr(III) concentrations. The optimum conditions for separation of Cr(III) from Cr(VI) and for preconcentration of Cr(III) have been determined by using the test procedure given above.

2.4. Application to natural waters

Water samples analyzed were filtered through a cellulose membrane filter (Millipore) of 0.45 μm pore size. The pH of the samples was adjusted to 5.0 with buffer solution. The sample was passed through the *B. sphaericus* loaded Diaion SP-850 column. Chromium(III) adsorbed on column was eluted with 1 mol L^{-1} HCl. The levels of chromium in the samples were determined by flame atomic absorption spectrometry.

Total chromium in natural water samples was determined as chromium(III) after reducing chromium(VI) to chromium(III)