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Biosorption of aluminum on *Pseudomonas aeruginosa* loaded on Chromosorb 106 prior to its graphite furnace atomic absorption spectrometric determination

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Abstract

A biosorption procedure for separation-enrichment of aluminum in environmental samples has been presented in this work. *Pseudomonas aeruginosa* loaded on Chromosorb 106 has been used as biosorbent for that purpose. *P. aeruginosa* is a gram-negative, aerobic rod. The influences of pH of the aqueous solution, eluent type, eluent volume, sample volume, etc. were examined on the quantitative recovery of aluminum in *P. aeruginosa* loaded on Chromosorb 106. The effects of concomitant ions on the recoveries of aluminum were also investigated. The detection limit based on 3 sigma for aluminum is 30 ng L^{-1}. Three certified reference materials (LGC 6010 Hard Drinking Water, NIST-SRM 1568a Rice Flour and NRCC-DORM-2 Dogfish Muscle) were analyzed for the validation of the presented procedure. The proposed procedure was applied to the determination of aluminum in environmental samples including natural water and food samples. The concentration of aluminum in real samples was found at ppb level.

Keywords: Aluminum; *Pseudomonas aeruginosa*; Biosorption; Preconcentration; Atomic absorption spectrometry

1. Introduction

Aluminum (Al) is a non-essential element to which humans are frequently exposed. Aluminum is widespread throughout nature, air, water, plants and consequently in all the food [1,2]. Aluminum is most commonly used in food technology as cans, packaging materials, kitchen utensils, and vessels [1]. Aluminum is also preferred due to its corrosion resistance and easy processing properties [3]. Aluminum accumulation may increase the risks of neurological and bone diseases, e.g., Alzheimer's disease, Parkinson's disease, encephalopathy/dialysis dementia, and osteomalacia [4,5]. Biologically, aluminum is essentially associated to the development and activity of the brain and to nerve conductivity [1,6]. The determination of very low levels of Al has become increasingly very important in environmental and clinical chemistry since its negative roles in the human life [5,7,8]. Because of aluminum accumulation in the tissues of patients with chronic renal failure, also monitoring of aluminum concentration in dialysis fluids has increasing attentions [5]. The diluted dialysis fluids should not contain aluminum concentrations higher than 10 μg L^{-1} [9,10].

The determination of aluminum and other elements at trace levels by the instrumental techniques including graphite and/or flame atomic absorption spectrometry, inductively coupled plasma mass spectrometry is rather difficult due to interfering effect of matrix and low levels of aluminum in environmental samples. Preconcentration/separation procedures could be used to solve these problems for aluminum and other elements [11–16]. The procedures including solvent extraction, cloud point extraction, coprecipitation, ion-exchange, electrodeposition and solid phase extraction have been used for the preconcentration and separation of trace elements [17–23]. Solid phase extraction of heavy metals on biosorbents is also popular topic at these works [24–26]. The biosorption procedure is based on biosorption of the heavy metals and desorption of these metals from the organisms [27–29]. Microorganisms immobilized on natural and synthetic adsorbents have been used for separa-

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tion and preconcentration of heavy metal including aluminum from various media [30–33].

Pseudomonas aeruginosa is a gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae*. *P. aeruginosa* is pathogens of humans [34,35]. It is often preliminarily identified by its pearlescent appearance and grape-like odor in vitro. It is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon utilizing microorganism, causing microbial corrosion [34,35].

The aim of the presented work is to show possible usage of *P. aeruginosa* loaded on Chromosorb 106 as biosorbent for the separation–preconcentration of aluminum at trace levels.

2. Experimental

2.1. Apparatus

A Perkin-Elmer AAnalyst 700 atomic absorption spectrometer equipped with HGA graphite furnace and with deuterium background corrector was used. For graphite furnace measurements, argon was used as inert gas. The operating parameters for working elements were set as recommended by the manufacturer given in Table 1. Pyrolytic-coated graphite tubes (Perkin-Elmer part no. B3 001264) with a platform were used. The samples of 20 μL plus 5 μL of 10,000 mg L^{-1} $Mg(NO_3)_2$ as matrix modifier during the study were injected into the furnace using Perkin-Elmer AS-800 autosampler. The signals were measured as peak areas.

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. Ethos D (Milestone S.r.l., Sorisole, BG, Italy) closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used.

2.2. Reagents and solution

All chemicals used were of analytical reagent grade and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1}) 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. Aluminum standard solution used for calibration was produced by diluting a stock solution of 1000 mg L^{-1} (Sigma Chem. Co. St. Louis, MO, USA). Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Table 1
Instrument settings and analytical conditions for GFAAS determination of aluminum

Wavelength (nm)	309.3
Slit width (nm)	0.7
Instrumental conditions	
Argon flow (mL min^{-1})	250
Heating program temperature °C (ramp time (s), hold time (s))	
Drying 1	100 (5, 20)
Drying 2	140 (15, 15)
Ashing	1700 (10, 20)
Atomization	2500 (0, 5)
Cleaning	2600 (1, 3)

Phosphate buffer solutions ($H_2PO_4^-$/H_3PO_4) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} sodium dihydrogen phosphate and phosphoric acid solutions for pH 2 and 3. Acetate buffer solutions (CH_3COO^-/CH_3COOH) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} acetic acid and 0.1 mol L^{-1} sodium acetate solutions for pH 4. Phosphate buffer solutions ($H_2PO_4^-$/HPO_4^{2-}) were prepared by mixing of appropriate volumes of 0.1 mol L^{-1} sodium dihydrogenphosphate and 0.1 mol L^{-1} sodium hydrogen phosphate for pH 5, 6 and 7. Ammonium buffer solutions were prepared by mixing of appropriate amounts of 0.1 mol L^{-1} ammonia and 0.1 mol L^{-1} ammonium chloride solutions for pH 8–9.

2.3. Preparation of biomass

The liquid medium was prepared by mixing 2 g of peptone, 2 g meat extract and 1 g mineral medium (10 g $CaCl_2 \cdot 2H_2O$, 20 g $MgCl_2 \cdot 6H_2O$ and 1 g $MnCl_2 \cdot 4H_2O$) and was dissolved in the 200 mL distilled water, and sterilized at 120 °C for 20 min. To prepare a starter culture, the bacterial strain, *P. aeruginosa* was grown in solid stock medium. It was inoculated into a 10 mL liquid nutrient medium. It was incubated at 30 °C for 24 h. The previously prepared 200 mL sterile liquid mediums were inoculated with 2 mL of the starter culture, and incubated in 10 vials at pH 7.3. The bacterial cultures were kept in continuous shaking at 30 °C. The stationary phases of each 200 mL liquid bacterial cultures were detected by microscopic observations. After reaching stationary phases, 16–24 h of incubation periods, *P. aeruginosa* cell density was 4.0–4.6 at 600 nm, and at this time the bacterial cells were harvested and separated from the media using centrifugation at 7000 rpm for 15 min. The isolated biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried.

Hundred and fifty milligrams of dry and dead *P. aeruginosa* was mixed with 500 mg of Chromosorb 106 [24]. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *P. aeruginosa* and Chromosorb 106, thereby improving the immobilization efficiency. Then, the product obtained was used as biosorbent for the present work.

2.4. Column preparation

A 10 cm in length and 1 cm in diameter column, with a small plug of glass wool, placed on the bottom of the column was used. The column was filled with 650 mg of biosorbent. The bed depth of biosorbent in the column was approximately 2.5 cm. The resin column was prepared by aspirating water slurry of *P. aeruginosa*-loaded on Chromosorb 106 into the glass column. It was conditioned by passing phosphate buffer solution then it was used for separation–preconcentration study. After each