



# *Pseudomonas aeruginosa* immobilized multiwalled carbon nanotubes as biosorbent for heavy metal ions

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## Abstract

*Pseudomonas aeruginosa* immobilized multiwalled carbon nanotubes has been used as biosorbent for the solid phase extraction of some heavy metal ions in environmental samples. Cobalt(II), cadmium(II), lead(II), manganese(II), chromium(III) and nickel(II) ions have been selected as analytes for the presented study, due to their important negative and positive roles in human life. In order to investigate quantitative biosorption conditions of the analytes, the influences of pH of the aqueous solution, eluent type, eluent volume, samples volume, etc. were examined. The effects of alkaline, earth alkaline and some transitions metals on the biosorption of analyte ions on *P. aeruginosa* immobilized multiwalled carbon nanotubes were also investigated. The presented biosorption procedure was applied to the determination of analytes in tomato leaves, bovine liver, boiled wheat, canned fish, black tea, lichen and natural water samples.

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## 1. Introduction

Heavy metals are extremely persistent in the environment at trace level. They are nonbiodegradable and nonthermally degradable and thus readily accumulate to toxic levels (Sharma et al., 2007). Toxic levels of heavy metals may originate from several sources including air, soil and water (Evans and Miller, 2006; Szentmihályi et al., 2006; Kutlu et al., 2006; Gunsen, 2004). The roles of heavy metal trace amounts in the human body are still under investigation (Gunsen, 2004; Subrahmanyam et al., in press; Praveen et al., in press; Yaman and Ince, 2006). In these studies, atomic absorption spectrometer is one of the main instruments due to its simplicity and its low cost. However there are two big problems for the analytical chemist which are low levels of the metal ions and positive or negative effects

of the matrix components (Dadfarnia et al., 2006; Quinária et al., 2006; Kiran et al., in press). The usage of separation-enrichment procedures could solve these problems, prior to determination of analytes (Lemos et al., in press; Hakim et al., 2007; Ramesh et al., 2007; Ghaedi et al., 2006).

Liquid–liquid extraction, electroanalytical techniques, cloud point extraction, solid phase extraction based on sorption or biosorption, etc. have been used for that purpose (Haji Shabani et al., 2006; Pourreza and Elhami, 2006; Youcef et al., 2006; Seki et al., 2006a,b; Martinez-Garcia et al., 2006; Hosseini and Sarab, 2007). Traces heavy metal ions could be adsorbed on the higher organisms including mosses, bacteria, algae (Seki et al., 2006a,b; Martinez-Garcia et al., 2006; Yan and Viraraghavan, 2001; Barros et al., 2007; Pamukoglu and Kargi, 2007; Karthikeyan et al., 2007). The uptake of metals by biomass can take place actively, by means of a metabolic activity dependent process (bioaccumulation) or by means of a passive and usually rapid (several minutes) metabolism-independent process called biosorption (Godlewska-Zytkiewicz, 2004;

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Godlewska-Żyłkiewicz and Kozłowska, 2005). This point is used by the researchers on the preconcentration–separation of the heavy metals at trace level in the environment. The system is based on biosorption of the heavy metals and desorption of these metals from the organisms. Biosorption of trace metals by microorganisms can be realized in batch and continuous modes (Godlewska-Żyłkiewicz, 2004; Godlewska-Żyłkiewicz and Kozłowska, 2005). An important part of the studies on biosorption is based on the immobilization of the organisms on the natural or synthetic polymeric materials (Godlewska-Żyłkiewicz, 2003, 2004; Godlewska-Żyłkiewicz and Kozłowska, 2005; Baytak and Turker, 2004, 2005a,b; Menegário et al., 2005). Microorganisms immobilized natural and synthetic adsorbents have been used for trace heavy metal separation and preconcentration from various media with successfully results (Godlewska-Żyłkiewicz, 2003, 2004; Godlewska-Żyłkiewicz and Kozłowska, 2005; Baytak and Turker, 2004, 2005a,b; Menegário et al., 2005). *Saccharomyces carlsbergensis*, *Aspergillus niger*, *Agrobacterium tumefaciens*, *Saccharomyces cerevisiae*, etc. were the microorganisms used, while Amberlite XAD resins, silica, sepiolite, Diaion resins, etc. were used as supports (Baytak and Turker, 2004, 2005a,b; Menegário et al., 2005; Godlewska-Żyłkiewicz, 2003). Some applications of microorganisms loaded adsorbent for heavy metal preconcentrations are summarized in Table 1.

*Pseudomonas aeruginosa* is a gram-negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae*. *P. aeruginosa* is pathogens of humans (Menegário et al., 2006). *P. aeruginosa* is often preliminarily identified by its pearlescent appearance and grape-like odor in vitro. Definitive clinical identification of *Pseudomonadaceae aeruginosa* often includes identifying the production of pyocyanin and fluorescein as well as its ability to grow at 42 °C. *Pseudomonadaceae aeruginosa* is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon utilizing microorganism, causing microbial corrosion (Gelmi et al., 1994).

Carbon nanotubes (CNTs) are one of the most commonly used building blocks of nanotechnology. With one hundred times the tensile strength of steel, thermal conductivity better than all but the purest diamond, and electrical conductivity similar to copper, but with the ability to carry much higher currents, they seem to be a very interesting material (Seki et al., 2006a,b). Carbon nanotubes (CNTs) have been proposed as a novel solid phase extractor for various inorganic and organic materials at trace levels (Wikipedia.org.; Zhou et al., 2006; Iijima, 1991; Liang et al., 2004, 2005).

According to our literature survey, *P. aeruginosa* and multiwalled carbon nanotubes combination is not used on the biosorption of traces heavy metal ions. Possible usage of the *P. aeruginosa* immobilized multiwalled carbon nanotubes for biosorption of metals was investigated. The analytical conditions for the quantitative recoveries of the analytes including pH of solutions, sample volume, etc. were investigated.

## 2. Experimental

### 2.1. Instrument

A Perkin Elmer AAnalyst 700 atomic absorption spectrometer with deuterium background corrector was used. All measurements were carried out in an air/acetylene flame. A 10 cm long slot-burner head, a lamp and an air–acetylene flame were used. The operating parameters for working elements were set as recommended by the manufacturer. SEM image was obtained on a LEO 440 scanning electron microscope (SEM).

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for

Table 1  
Comparative data from some recent studies on biosorption of heavy metals on microorganism immobilized on adsorbents

Elements	Media	Adsorption capacity (mg g <sup>-1</sup> )	PF	DL (μg l <sup>-1</sup> )	RSD (%)	Reference
Fe <sup>3+</sup> , Co <sup>2+</sup> , Mn <sup>2+</sup> , Cr <sup>3+</sup>	<i>Agrobacterium tumefaciens</i> immobilized on Amberlite XAD-4	1.21–1.71	25	2.8–3.6	<10	Baytak and Turker (2005)
Fe <sup>3+</sup> , Co <sup>2+</sup> , Cr <sup>3+</sup>	<i>Saccharomyces carlsbergensis</i> immobilized on Amberlite XAD-4	1.41	–	2.8–7.4	<5	Baytak and Turker (2005)
Mn <sup>2+</sup>	<i>Saccharomyces carlsbergensis</i> immobilized on Amberlite XAD-4	–	–	60	<5	Baytak and Turker (2004)
Cr <sup>3+</sup> , Cr <sup>6+</sup>	<i>Saccharomyces cerevisiae</i> immobilized on controlled pore glass	–	12	0.45–1.5	–	Menegário et al. (2005)
Pt <sup>2+</sup> , Pd <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> and <i>Chlorella vulgaris</i> immobilized on silica gel	–	–	0.4–0.8	<5	Godlewska-Żyłkiewicz (2003)
Cu <sup>2+</sup> , Pb <sup>2+</sup> , Fe <sup>3+</sup> , Co <sup>2+</sup>	<i>Bacillus sphaericus</i> loaded Diaion SP 850	4.3–9.2	50	0.20–0.75	<5	Tuzen et al. (2007)
Cu <sup>2+</sup> , Pb <sup>2+</sup> , Zn <sup>2+</sup> , Fe <sup>3+</sup> , Ni <sup>2+</sup> , Co <sup>2+</sup>	<i>Aspergillus fumigatus</i> immobilized on Diaion HP-2MG	4.4–8.5	50	0.30–0.72	<7	Soylak et al. (2006)
Co <sup>2+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> , Mn <sup>2+</sup> , Cr <sup>3+</sup> , Ni <sup>2+</sup>	<i>Pseudomonas aeruginosa</i> immobilized on multiwalled carbon nanotubes	5.25–6.23	50	0.24–2.60	<10	This study

PF, preconcentration factor.

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