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Solid Phase Extraction Based on the Use of Thermophilic Bacteria *Bacillus aerius* VO-8 Immobilized XAD-16 for The Preconcentrations of Co and Ni

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Abstract—Bacillus aerius VO-8 (Accession:KJ842091.1), a thermophilic bacteria, was immobilised on Amberlite XAD-16 and used as solid phase biosorbent for preconcentrations of Co(II) and Ni(II) before their determination by inductively coupled plasma optical emission spectrometry (ICP-OES). Effect of various parameters such as pH, flow rate of the sample solution, amount of the biosorbent, type of eluent and reusability of column were investigated. For Co and Ni the optimum pH values, flow rate and type of eluent were found pH 6.0 and 5.0, 2 ml/ min, 1.0 mol/L HCl, respectively. Maximum biosorption capacity was observed 13,8 mg/g for Co and 10.5 mg/g for Ni. The accuracy of the proposed method was verified through the analysis of certified reference samples of tea (NCS ZC-73014) and applicability of the method was validated through the concentrations of Co and Ni were determined in some edible vegetables.

Index Terms— Preconcentration, Bacillus aerius, XAD16, biosorption,

I. Introduction

Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters [1]. There are several physicochemical tratment technologies used in the removal of heavy metals. These conventional methods include solid phase extraction, precipitation, ion exchange, reverse osmosis,, electrochemical processes, cloud point extraction and membrane processes [2]. Among them, SPE has some advantages compared with other methods, such as reduced solvent usage, no use of toxic organic solvents, low disposal costs and short experimental times [3].

The use of biological materials for removing and recovering heavy metals from contaminated industrial effluents has emerged as a potential alternative method to conventional techniques [4]. In recent times, the method of removal of metallic ions from liquid effluents with the help of microorganisms has received greater attention over the conventional physical/chemical methods [5].

In the present work, we focused on the development of a sensitive and selective preconcentration method. Bacillus aerius was used as the bacterial biomass for the preconcentration of Co and Ni prior to its determination by ICP-OES. Critical parameters in SPE experiments were optimized.

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II. Material and Methods

A. Instrumentation

The concentrations of metal ions were determined by ICP-OES (Perkin-Elmer Optima 2100 DV; Shelton CT). SPE experiments were performed on polypropylene column (1.0 cm x 10.0 cm).

B. Reagents and Solutions

Standard solutions of 1000 mg/L Co and Ni were diluted to prepare working standards. Certified reference tea sample was obtained from China National Analysis Center for Iron and Steel Amberlite XAD-16 (Sigma Chemical, St. Louis, MO, USA) was used as a support material for the immobilization of Bacillus aerius. pH was adjusted by the addition of diluted HCl or NaOH to sample solution.

C. Preparation of SPE Column

Thermophilic bacteria was isolated from hot-spring water of Norsin, Bitlis in Turkey, was used as biosorbent in this study. It was cultivated in 500-ml Erlenmeyer flasks containing 100ml Nutrient Broth (NB) medium by shaking at 50 °C for 36h (150 rpm). The cultures were centrifuged at 8,000 rpm for 7 min, and then the pellets were washed with 0.9% NaCl and dried in an oven at 80 °C 24 h. To obtain a fine powder of the dried cells, they were ground in a porcelain mortar and then were autoclaved at 121 °C for 15 min to assess complete death of the dried cells. Finally, the cells were inoculated to liquid medium, and the absence of any growth indicated positive results (complete death of the bacteria). Dry biomass powder (200 mg) was mixed with 800 mg of Amberlite XAD-16 and 5 ml of distilled water and then thoroughly mixed [6,7]. Samples were collected along the cultivated banks of the Botan River (Siirt, Turkey) and stored in polyethylene bags according to their type and then brought to the laboratory for preparation and treatment. They were first washed with tap water to remove the pollutions from soil and then rinsed by distilled water. Then, they were dried at 80 °C for 24 h in oven. The samples were ashed at 480 °C in a furnace for 3h, and the ashed samples were dissolved with a mixture of nitric acid–hydrogen peroxide (2:1, v/v). 3 ml of the acid mixture was then added; the process was repeated two times with occasional shaking on a hot plate. After drying, 5 ml of 1 mol/L nitric acid was added to dissolve the remaining residue. It was then diluted up to 50 ml with distilled water and SPE procedure was applied. The same procedure was also applied to the certified reference tea sample, NCS ZC73014.

III. RESULTS AND DISCUSSION

A. Effects of pH

The pH is one of the most significant empirical parameters in heavy metal biosorption studies that affects the protonation of the active sites on the biomass [8]. All bacteria shows diverse biosorption characteristics at a given pH owing to differences in the cell wall structure. The effect of pH on SPE of Co and Ni by immobilized thermophilic Bacillus aerius is showed in Fig. 1. To study the influence of pH, a series of experiments were performed using 50.0 ml of 10 µg/L Co and Ni, with different pH from 2.0 to 8.0. The optimum pH was found to be 6.0 for Co and 5.0 for Ni. After this result, all the subsequent biosorption studies were performed at pH 6.0 and 5.0 for Co and Ni, respectively.

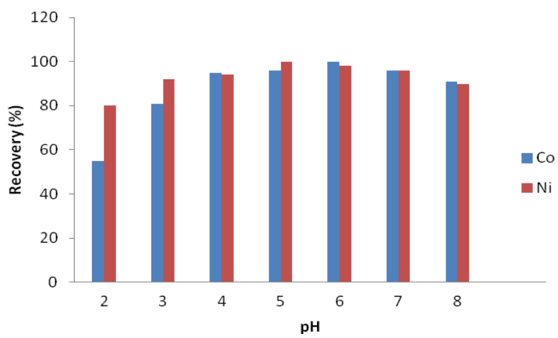

Fig. 1. Effect of pH on solid phase extraction of Co and Ni

B. Effects of flow rate

The effect of flow rate on recoveries of Co and Ni, from aqueous solution by immobilized thermophilic *Bacillus aerius* onto XAD16 was investigated. 50.0 ml of 10 µg/L Co and Ni was adjusted to the optimum pH value. Then, these solutions were passed from the bacteria immobilized column with flow rates between 1.0 and 6.0 mL/min. The quantitative recoveries were obtained at the flow rates 2.0 mL/min for both metals (Fig. 2). The flow rate of the sample solution is major parameter affecting both the uptake of the analyte metal ions and controlling the time of analysis [9].

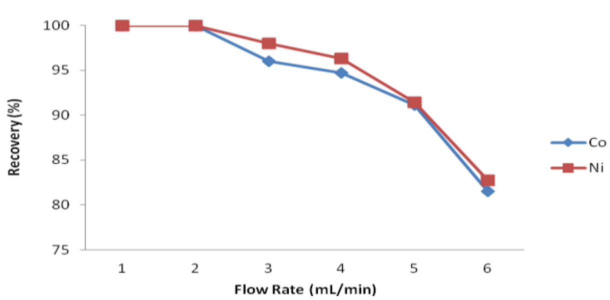

Fig.2. Effect of flow rate on recovery of Co and Ni

C. Effects of amount biosorbent and resin

The preconcentration of Co and Ni depending on the amount of biosorbent and resin XAD16 were analyzed in the range of 50–300 mg and 500-1000 mg for *Bacillus aerius* and resin, respectively.. Maximum uptake of Co and Ni ions was monitored when the amount of biosorbent was 200 mg and resin was 800 mg (Fig. 3,4). Thus, future experiments were carried out using 200 mg of biosorbent and 800 mg of resin XAD16.

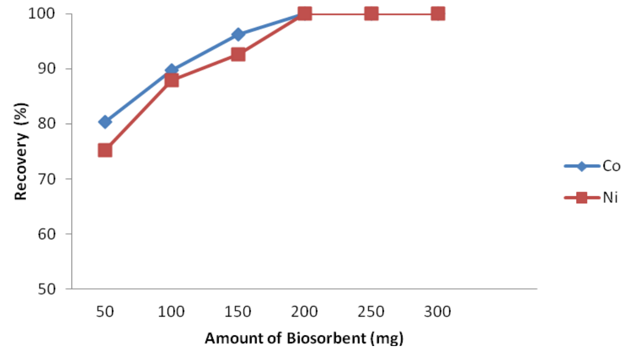

Fig. 3. Effect of amount of biosorbent on recovery of Co and Ni

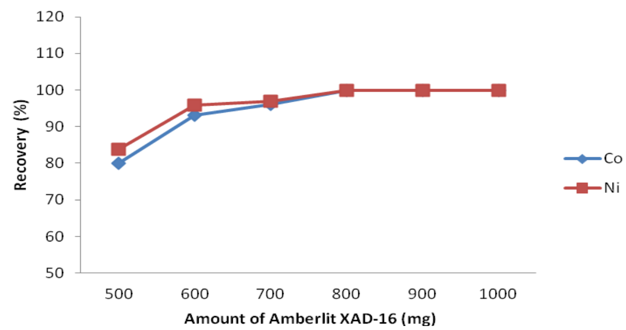

Fig. 4. Effect of amount of resin XAD16 on recovery of Co and Ni

D. Reusability of solid phase column

In SPE process, the potential reusability of the column is a very important parameter to evaluate the biosorbent which can guaranty its common application in industry [10]. The stability of the SPE column exhibits that the uptake of Co and Ni ions were stable at least 20 biosorption-elution cycles with the recovery for the Co and Ni ions greater than 96.1% and 94.5%, respectively (Fig. 5).

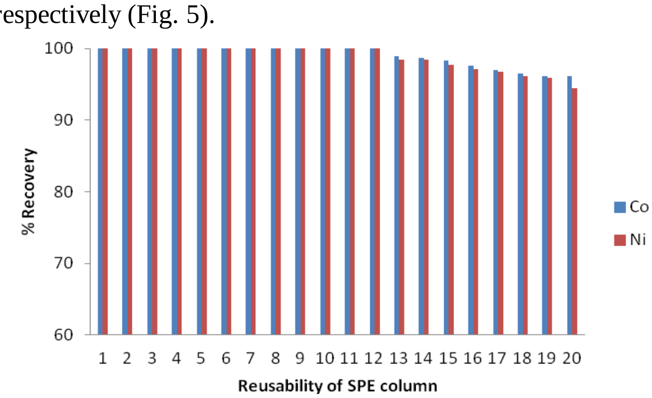

Fig. 5 Reusability of the column for further preconcentration of Co and Ni

E. Effect of the type and volume of elution solutions

HCl and HNO3 were investigated as eluent. Variations in recovery values of metal ions with different concentrations and volumes of acids were investigated (Table 1). When concentration of HCl was increased from 0.5 to 1.0 mol/L by a volume of 5.0 ml, recovery values of Co and Ni increased from 94.1% to 99.5% and from 95.6% to 99.2%, respectively. After this result, all the subsequent experiment were performed 5 ml of 1.0 mol/L HCl was selected as the optimum volume HCI for elution of both metal ions.

TABLE I
EFFECT OF THE TYPE AND VOLUME OF ELUTION SOLUTIONS ON THE RECOVERY OF CO AND NI

Type of elution	Volume (mL)	Concentration (mol/L)	Recovery (%)	
			Co^{2+}	Ni^{2+}
HCl	3.0	0.5	86.7±0.5	84.6±0.4
	5.0	0.5	91.2±0.8	91.7±0.3
	3.0	1.0	94.1±0.7	95.6±0.5
	5.0	1.0	99.5±0.4	99.2±0.8
HNO_3	3.0	0.5	81.2±0.9	83.9±0.8
	5.0	0.5	91.9±0.6	90.0±0.7
	3.0	1.0	92.7±0.9	86.1±0.8
	5.0	1.0	94.1±0.5	93.3±0.9

The accuracy of the method was validated by applying the method to the certified reference tea sample (Table 2). The obtained values were found to agree with the certified values. 200 and 400 ng/g of Co and 1 and 3 µg/g of Ni were also added to the sample. The results exhibited that spiked amounts of Co and Ni were quantitatively recovered. As a result, it can be said that the method can be applied to real samples without the loss of analytes.

TABLE II
THE RESULTS FOR CO AND NI IN CERTIFIED REFERENCE SAMPLES OF TEA LEAVES (NCS ZC-73014), N=3

Metal	Added	Certified	Found
Co (ng/g)	-	220±20	218.3±19
	200	-	409.5±12
	400	-	632.4±26
Ni (µg/g)	-	3.4±0.3	3.3±0.4
	1.0	-	4.6±0.3
	3.0	-	6.2±0.4

The developed method was applied to real vegetable samples collected along the cultivated banks of the Botan River to determine the concentrations of Co and Ni. The results obtained are shown in Table 3. Concentrations of Co were in the range of 21.52- 45.34 ng/g. The highest Co and Ni concentration were determined in the purslane and onion, respectively. The lowest Co and Ni concentration was determined in the aubergine.

TABLE III
THE LEVEL OF TOTAL CO AND NI IN VEGETABLES (N=3)

Vegetable	Metals	
	Co(ng/g)	Ni(µg/g)
Aubergine	21.52±0.55	1.77±0.22
Okra	39.43±0.92	1.82±0.31
Onion	34.55±0.87	6.56±0.54
Purslane	45.34 ± 0.75	3.45 0.25

IV. CONCLUSION

An alternative method was developed for the preconcentration of Co and Ni by solid phase extraction (SPE). *Bacillus aerius* was used as a bacterial biosorbent for the preconcentration of Co and Ni prior to its determination by inductively coupled plasma optical emission spectrometry (ICP-OES). The important experimental parameters in the developed SPE method were optimized. The applicability of the method was validated through the analysis of certified reference tea samples. Results showed that founded concentrations for Co and Ni agreed with certified values. Edible vegetables were collected along the cultivated banks of the Botan River. It was also found that the same SPE column can be used 20 times without loss of efficiency.

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