Bioresource Technology 101 (2010) 7551–7555

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Using of *Rhizopus arrhizus* as a sensor modifying component for determination of Pb(II) in aqueous media by voltammetry

Meral Yüce [a], Hasan Nazır [b], Gönül Dönmez [c,*]

[a] Ankara University, Institute of Biotechnology, 06100 Beşevler-Ankara, Turkey
[b] Ankara University, Faculty of Science, Department of Chemistry, 06100 Beşevler-Ankara, Turkey
[c] Ankara University, Faculty of Science, Department of Biology, 06100 Beşevler-Ankara, Turkey

ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form 25 April 2010
Accepted 29 April 2010
Available online 23 May 2010

Keywords:
Microbial biosensor
Environmental control
Lead
Voltammetry
Rhizopus arrhizus

ABSTRACT

For the sensitive determination of Pb(II) from aqueous solutions, a new voltammetric biosensor based on carbon paste electrode modified with *Rhizopus arrhizus* was developed. The preconcentrated ions at open circuit were reduced by using differential pulse stripping voltammetry technique. The obtained current values were related to the concentration of Pb(II) in the solutions. The best results were achieved at pH 7 with 0.01 M Tris–HCl buffer solution applying a preconcentration time of 12 min. The linear range for the biosensor was found to be within 1.0×10^{-7}–1.25×10^{-5} M, with a detection limit of 0.5×10^{-8} M. The selectivity of the microbial biosensor was explored by adding interfering heavy metals to accumulation medium one by one, and their matrix effects were also investigated in the model metal solutions. Energy dispersive X-ray spectra analysis were applied to show the specific effect of the fungal biomass on the Pb(II) determination.

1. Introduction

In recent years, various types of biosensors have been increasingly used for very different applications from environmental monitoring to disease diagnosis, and biosensor development has become a highly active field of research and development as a fast, practical, economical, and reliable tool in many fields (Chouteau et al., 2004; Lei et al., 2006; Chinalia et al., 2008).

As is well known, the determination of trace levels of heavy metals and their control is important due to their toxic effects on living organisms at high concentrations (Deng et al., 2007). Many techniques have been used for trace level analysis of heavy metals, from atomic spectroscopy and ion chromatography to ICP-MS (Brown and Milton, 2005). Although they are able to detect single elements or species at low concentrations, they are difficult to use for in-situ analysis or low cost screening procedures. Biosensors offer fast, on-site measurements with the same sensitivity and accuracy as most of these routine techniques do, by using low cost and compact portable equipment (Malitesta and Guascito, 2005).

It has been known since the sixties that microorganisms adsorb or bioaccumulate a wide range of chemical substances through their biosorption capacity. Since then, they have been used for the removal of heavy metals from aqueous media (Aksu and Dönmez, 2006; Tuzen et al., 2008). In addition to the use of living microorganisms such as bacteria, algae, yeast, and fungi in bioremediation, they have been used in the construction of biosensors for the determination of many parameters in a wide range of application fields, including environmental ones (Mozaz et al., 2004; Liu et al., 2008). As mentioned above, measurements of trace levels of heavy metal ions are of environmental concern. Biosensors are used in the measurements of several heavy metal species including Cu(II), Cr(VI), and Ni(II) (Verma and Singh, 2005; Michel et al., 2006; Alpat et al., 2008). One of the methods for increasing the performance of biosensors involves the use of microorganisms as a sensor modifying agent, which is known to have some advantages, e.g. it is simple, easy, economical, and suitable for large-scale production (D'Souza, 2001).

As a major pollutant in aquatic and terrestrial ecosystems, discharged through a variety of activities such as mining and metal processing, lead is an environmental concern (Kabata-Pendias, 2004). There are many reports on the toxic effects of Pb(II) in living organisms (Dearth et al., 2002; Malkowski et al., 2002).

The aim of this study is to describe a microbial biosensor for determination of Pb(II) ions from aqueous solutions, which is practical to use, and low cost of fabrication. The described microbial sensor is based on voltammetry on a carbon paste electrode (CPE) modified with a dried nonliving fungus, *Rhizopus arrhizus*. Carbon paste electrodes (CPEs) have been extensively used for electro-analytical applications since their introduction in 1958, due to their considerable advantages including low cost of fabrication, low background current and renewable interface (Adams, 1958). As far as we know, the application of a fungal biosensor based on CPE for determination of Pb(II) has not been reported pre-

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

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.04.099

viously. Although there are many studies on the detection and measurement of Pb(II) with electrochemical techniques using different materials as modifying agents, fungal microorganisms have not been used for this purpose up to know.

R. arrhizus was selected, based on its strong biosorption capacity towards Pb(II) (Sağ and Kutsal, 1997). The functional groups and binding cites involved in Pb(II) biosorbtion on the fungal cell were shown by using microscopic and spectroscopic techniques before (Naja et al., 2005). Many other studies confirmed that *R. arrhizus* had an important biosorption capacity for heavy metals (Preetha and Viruthagiri, 2007; Subudhi and Kar, 2008). Based on the findings in these reports, we thought it relevant to test if a new and advantageous method could be developed for voltammetric analysis of Pb(II) on a CPE modified with a dried nonliving fungus, *R. arrhizus*, offering a simple, free of excess preliminary treatment procedure and has low cost of fabrication.

2. Methods

2.1. Chemicals

All solutions were prepared with distilled water. Stock solutions of Co(II), Cu(II), Ni(II), Zn(II), and Pb(II) (1.0×10^{-1} M) were prepared from the corresponding analytical grade metal nitrates (Merck, Darmstadt, Germany). Standard solutions of metal ions were prepared freshly by diluting stock solutions. Electrolyte solution was prepared from analytical grade Tris–HCl (Sigma, Steinheim, Germany). Graphite powder and paraffin liquid (Merck, Darmstadt, Germany) were used for preparing the carbon paste electrode (CPE). Yeast Peptone Glucose (YPG) medium was used for cultivation of the culture. The other chemicals used for the cultivation mediums or buffer solutions were also prepared using analytical grade chemicals. The pH of the supporting electrolyte solution was set using 0.1 M KOH or 0.1 M HCl (Merck, Darmstadt, Germany).

2.2. Microorganism and culture conditions

R. arrhizus was obtained from the United States, Department of Agriculture Collection, and used as a biologic agent to construct the microbial biosensor in this study.

Erlenmeyer flasks filling with 100 ml of the YPG medium were used for cultivation. The microorganism was incubated for 7 days in the stationary phase at room temperature. After the growth period *R. arrhizus* was separated from the medium by using a filter, and washed twice with 100 ml of distilled water in erlenmeyer flasks. The obtained biomass was agitated with 100 ml of 1% formaldehyde for inactivation, and then dried at 110 °C for 24 h in glass plates (Aksu and Gülen, 2002). Finally, heat dried fungal tissue was removed by scratching, and powdered by using a ceramic mortar.

2.3. Operating principle of the developed microbial biosensor

The hand-made working electrode was constructed with using teflon and a short length of copper wire to supply required electrical conductivity.

The modified CPE was prepared by making a homogeneous paste with 10 mg of dried nonliving fungal tissue, 90 mg of graphite powder, and paraffin liquid. The unmodified CPE included only graphite powder (100 mg) and a proper amount of paraffin liquid. The paste mixture was firmly packed into the hole of the electrode body (5 mm diameter), and was compressed with a steel rod.

Then the electrode surface was smoothed on a paper to prevent possible background noises on the voltammograms. The electrode prepared was immersed into the 20 ml of 0.01 M of Tris–HCl for preconcentration/accumulation step. All carbon pastes and electrolyte solutions used for the experiments were prepared freshly.

2.4. Apparatus and experimental method

Electrochemical experiments were performed at room temperature with a CH Instruments 660B model Electrochemical Analyzer (CH Instrument, USA). The instrument was equipped with a conventional three-electrode cell, containing supporting electrolyte solution 0.01 M Tris–HCl, deoxygenated by passing bubbling pure nitrogen through it before the measurements. CPEs modified with *R. arrhizus* were used as working electrodes. The counter electrode was a platinum wire, and Ag/AgCl electrode (saturated KCl) was used as reference electrode. Experiments were done using the accumulation, medium change, and voltammetry steps, respectively (Etienne et al., 2001).

In the Pb(II) accumulation step, the modified CPE was immersed in 20 ml of stirred 0.01 M Tris–HCl ion solution, including Pb(II) at a definite concentration for a selected time at open circuit. After this step, the biosensor was taken out from the accumulation solution, and its surface was washed carefully with 5 ml of distilled water to remove any solution component. In the second stage called medium change, the biosensor was placed in the conventional three-electrode cell filled with only 20 ml of 0.01 M Tris–HCl solutions, and connected to the electrochemical workstation carefully. Voltammetric measurements were performed in the final stage. All steps were repeated for each new CPE. Cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPSV) assays were performed with both modified and unmodified CPEs, which were preconcentrated in Pb(II) solutions as control samples. The voltammograms were scanned from −1.3 to 1.3 V potential ranges, and were swept back for CV assays versus the Ag/AgCl reference electrode. The background current was also obtained after the accumulation step in the absence of Pb(II) blank solution.

After the optimum operating conditions for Pb(II) determination was reached, model solutions were prepared in order to reveal the interference effects of some important heavy metals that could influence the electrochemical response. For this aim, 20 ml of 0.01 M Tris–HCl buffer solutions, containing certain concentration of Ni(II), Co(II), Zn(II), and Cu(II) heavy metals were used as accumulation medium, and the measurements were conducted in turn as mentioned above.

2.5. Elemental analysis of the modified electrode surface

Elemental composition analysis for the surfaces before and after loading of Pb(II) was performed by using an Energy Dispersive X-ray Spectra (JEOL JSM-7000F Field Emission Analytical SEM-EDAX). Analyses were performed for both the surface of blank and Pb(II) loaded microbial biosensors. The elemental analysis of the Pb(II) loaded microbial biosensor was carried out after the accumulation stage mentioned above.

3. Results and discussion

3.1. Electrochemical behaviors of the developed biosensor

Cyclic voltammograms of the unmodified and modified CPEs were obtained in 0.01 M Tris–HCl for 1.0×10^{-4} M Pb(II). The electrochemical behaviors of the biosensors were shown in Fig. 1. As seen in Fig. 1(b), although voltammetric peaks were not traceable when the unmodified electrode was used, *R. arrhizus* modification increased the electrode response to a level sufficient for the determination of Pb(II) (Fig. 1a). A well defined cathodic peak corresponding to reduction of surface bonded Pb(II) was obtained in a

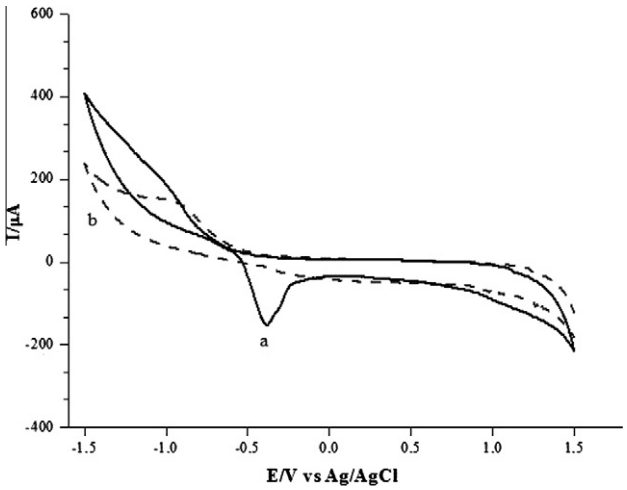

Fig. 1. Cyclic voltammograms of *R. arrhizus* modified CPE (a) and unmodified CPE (b) (concentration of Pb(II): 1.0×10^{-4} M, detection: cyclic voltammetry in 0.01 M Tris–HCl with 50 mV/s scan rate, preconcentration time: 15 min).

potential range of −0.6 to −0.4 V with modified CPEs. Woolever and Dewald (2001) also reported that Pb(II) had a potential range of similar magnitude.

3.2. Elemental analysis of the developed microbial biosensor's surface

Elemental analysis of the developed microbial biosensor surface after loading of Pb(II) (5.0×10^{-5} M in the accumulation solution), and unmodified electrode surface before loading of Pb(II) were obtained to show the differences between them. The modified carbon paste electrode was preconcetrated for 15 min at open circuit, under stirring conditions before EDAX analysis.

Elemental analysis results confirmed that a significant amount of Pb(II) (30.564% of the total concentration) was accumulated by the modified electrode's surface through the strong biosorption ability of the fungal biomass. The decrease in the oxygen level during analysis from 27.397% to 12.319% indicated the oxidation of surface bonded Pb(II) ions during analysis. All these data verified that the *R. arrhizus* bound the Pb(II) ions, and modified carbon paste electrodes with this microorganism could be used for determination of Pb(II) ions from aqueous solutions in short times.

3.3. The effect of preconcentration time

Preconcentration time is an important factor to define the optimum conditions for the determination of elements. The effects of varying preconcentration times for 1.0×10^{-5} M Pb(II) at the modified CPE were examined in the range from 3 to 15 min. Fig. 2(a) shows the influence of the preconcentration time on the microbial biosensor response. The preconcentration time increases the microbial biosensor response up to 12 min, because of the increases in the metal ion concentration at the sensor surface. This maximum concentration was then followed by a stationary phase at the surface when the accumulation time was 15 min. This relation can be explained by saturation of the binding sites at the electrode surface with longer preconcentration periods. The voltammograms showed that the optimum accumulation time for the sensor was 12 min.

3.4. The effect of electrolyte solution on the microbial biosensor response

It is important to choose an appropriate electrolyte solution, because of its effects on the electrochemical response of the electrode, including elimination of electro-migration, providing a constant ionic strength, and decreasing the resistance of solution for the best analysis conditions (Wang, 2000).

Five different supporting electrolyte solutions (0.05 M of NH_4Cl/NH_3 buffer, HNO_3 solution, CH_3COONa/ CH_3COOH, $NaNO_3$ solution, and Tris–HCl buffer) were tested to obtain the best defined catho-

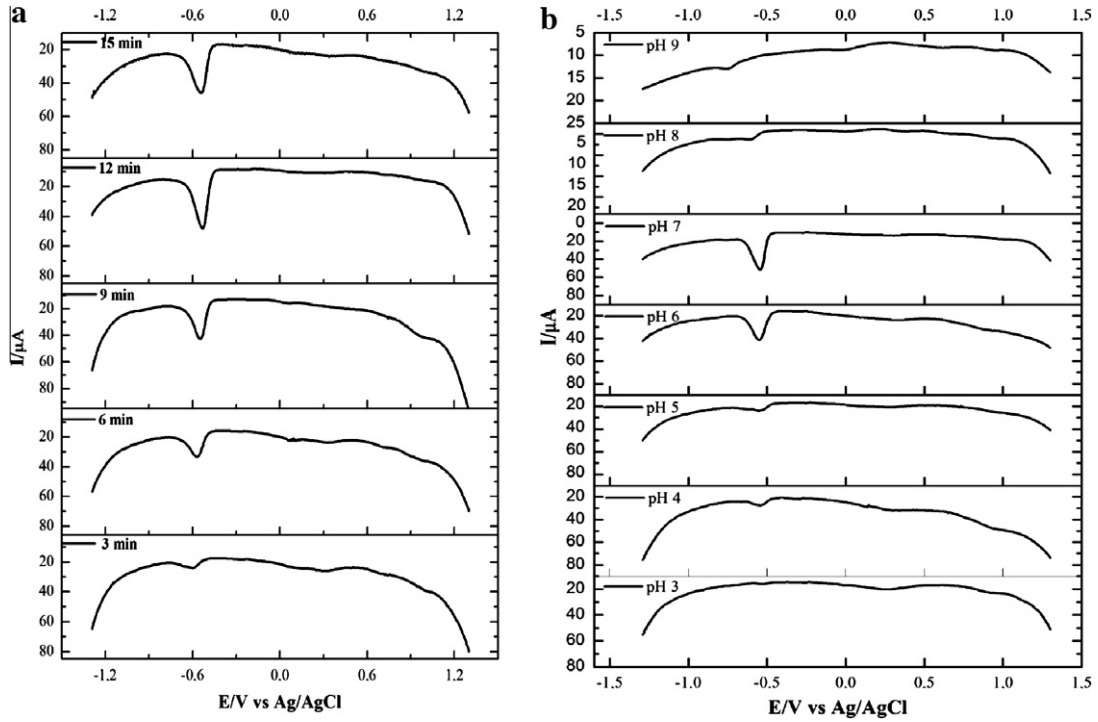

Fig. 2. Effect of preconcentration time (a) and preconcentration solution pH (b) on the microbial biosensor response (concentration of Pb(II): 1.0×10^{-5} M, detection: DPSV in 0.01 M Tris–HCl with a deposition potential of −0.6 V).

dic peaks for 1.0×10^{-5} M Pb(II) concentration, and the results were presented at Table 1. There were no detectable peaks when 0.05 M NH_4Cl/NH_3 buffer was used as electrolyte solution. On the other hand, may be over complexation reactions occurred between Pb(II) and acetate ions when the CH_3COOH buffer solution was used, and caused the precipitation of Pb(II) ions in the solution. Although well defined peaks were also obtained with 0.05 M $NaNO_3$, Tris–HCl was selected due to the lower background noises.

3.5. The effect of preconcentration solution pH and ionic strength

The pH of preconcentration solution affects the solubility of metal ions and the ionization state of the functional groups of the fungal cell wall. Gardea-Torresdey et al. (1999) reported that as a member of the first class of metal ions Pb(II) bonded tightly and rapidly at pH 5 or higher pH values, and could be stripped at pH 2 and lower values. They described the biosorption of metal ions in this class in terms of ion exchange, where the cations were competing with protons for negatively charged binding cites on the cell wall.

The effect of different pH values of the preconcentration solution on the microbial biosensor response was investigated in the present study, within the pH range from 3.0 to 9.0 for 1.0×10^{-5} M Pb(II). Peak current increased up to pH 7.0 slowly. Below this pH level, the sensitivity of the developed biosensor for Pb(II) decreased as reflected by the lower peak current values measured, which were due to the increased competitive binding of Pb(II) ions with permanently existing ligand hydrogen ions (H^+). The fluctuations over pH 7 could be explained by the formation of the insoluble hydroxylic Pb(II) complexes in the solution that inhibited lead accumulation on the electrode surface.

The results presented in Fig. 2(b) showed that the maximum peak current was obtained when the pH of the electrolyte solution was 7.0. This pH value was also reported in similar studies (Elvina and Mojica, 2005).

The ionic strength was also investigated for Tris–HCl in the range of 0.01–0.5 M. According to the results obtained, peak currents decreased when ionic strength was raised up to 0.5 M. Because of high ionic concentration effects solubility of ions and adsorption process, the peak current showed an important decrease when the ionic strength was raised up to 0.5 M. Subsequently, the Tris–HCl buffer solution with a 0.01 M concentration was selected due to providing best electrochemical response.

3.6. Analytical data for developed microbial biosensor

The linear ranges of DPSV responses for Pb(II) detection were determined in 0.01 M Tris–HCl solution adjusted to pH 7.0; the preconcentration time was 12 min at open circuit. At the obtained optimum experimental conditions, the microbial biosensor showed a linear range between 1.0×10^{-7} and 1.25×10^{-5} M Pb(II) (0.02 and 2.5 mg L^{-1}) (R^2: 0.982). At higher concentrations of Pb(II) (>1.25×10^{-5} M) a deviation from linearity was experienced, which may have been due to saturation of the binding regions on the electrode surface. Limit of detection (LOD) value of the developed microbial biosensor was also calculated as 0.5×10^{-8} by using $3S_b/m$ criteria (Akyılmaz et al., 2010). Mojica et al. (2007) reported that a modified carbon paste electrode with banana tissues exhibited a limit of detection of 0.1 mg L^{-1}. They also found the linear range to be between 1 and 20 mg L^{-1}.

A biosensor was also reported that was based on bovine serum albumin (BSA) for the detection of Pb(II) with a linear range from 1.0×10^{-7} to 3.0×10^{-9} M using Piezo electric quartz crystals (Yin et al., 2007). Although the limit of detection found in that study was lower than that from our microbial biosensor, there were some drawbacks when compared with our procedure, including the long preparation time (over 2 h), difficulty of application, and high cost.

Table 1
Effect of different supporting electrolyte solutions.

Supporting electrolyte solutions	U (V)	I_p (μA)
0.05 M NH_4Cl/NH_3	–	No peak detected
0.05 M HNO_3	−0.42	0.46 ± 0.08
0.05 M CH_3COONa/CH_3COOH	−0.56	1.28 ± 0.03
0.05 M $NaNO_3$	−0.71	4.73 ± 0.20
0.05 M Tris–HCl	−0.56	6.10 ± 0.06

3.7. Single effects of interfering metal ions on the microbial biosensor developed

The presence of interfering metal ions either enhances or decreases the peak current of a certain metal in analyte. The response of the microbial biosensor was evaluated with the interfering metal ions Ni(II), Co(II), Zn(II), and Cu(II). The ions present in natural water were used as possible sources of interferences in the present

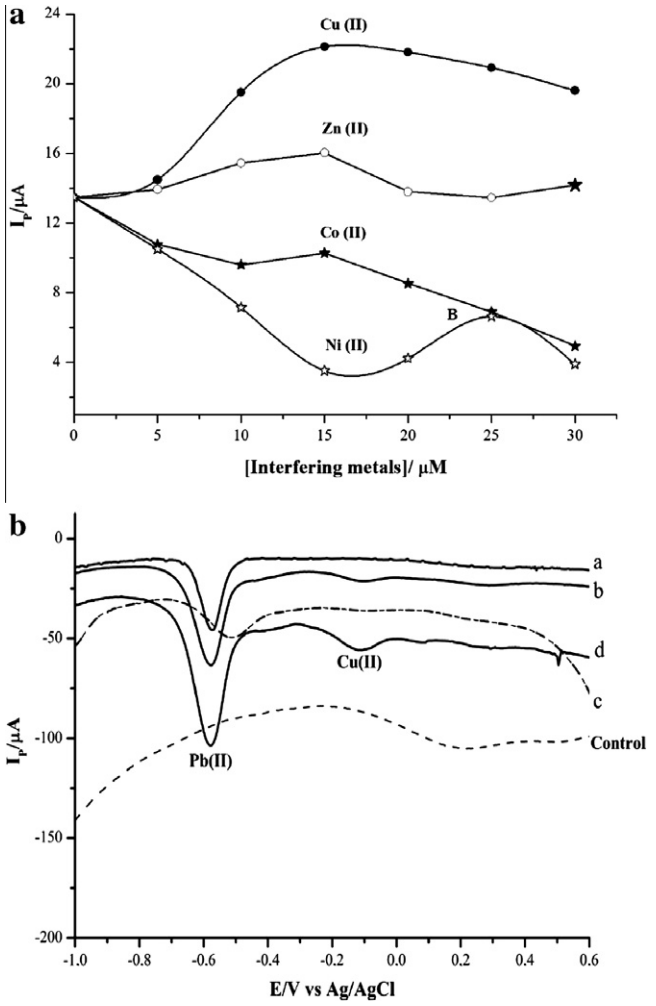

Fig. 3. Single (a) and matrix (b) effects of the interfering metal ions ((a) concentration of Pb(II): 2.5×10^{-6} M and the other heavy metal ions, Ni(II), Zn(II), Cu(II) and Co(II) at concentrations of 2.5×10^{-6}–3.0×10^{-5} M, (b) a; including of 5.0×10^{-6} M Pb(II), b; including of 5.0×10^{-6} M of all interfering metals and Pb(II), c; including of 1.0×10^{-5} M of all interfering metals and 5.0×10^{-6} M of Pb(II), d; including of 2.0×10^{-5} M of all interfering metals and 5.0×10^{-6} M of Pb(II), detection: DPSV in 0.01 M Tris–HCl with a deposition potential of −0.6 V, preconcentration time of 12 min).

study. A fixed concentration of 2.5×10^{-6} M of Pb(II) solution was used, with increasing concentrations of the interfering metal ions from 2.5×10^{-6} to 3.0×10^{-5} M. The results were shown in Fig. 3(a). According to the results obtained, peak current toward Pb(II) was not influenced negatively by the presence of Cu(II) and Zn(II). Moreover, the current increased with increasing Cu(II) concentrations and a second peak was observed at about −0.2 V belonging to Cu(II) at higher concentrations of it. Although Co(II) and Ni(II) ions affected the peak current in a negative way, the peak currents belonging to Pb(II) obtained were clear at all the tried concentrations of these two interfering metal ions. As a result of these, the high concentration of Cu(II) could also be detectable as qualitative with current form of the developed biosensor.

3.8. Matrix effects of interfering metal ions on the microbial biosensor developed

We also investigated the matrix effect of interfering metal ions by preparing model solutions, including different concentrations of related heavy metals (Fig. 3(b)). The first solution (a) included only 5.0×10^{-6} M Pb(II) in 20 ml of Tris–HCl buffer solution. All of the remaining solutions prepared included equal concentrations of 5.0×10^{-6}, 10×10^{-6}, and 20×10^{-6} M of the interfering metal ions, and these solutions were indicated by b, c, and d, respectively in Fig. 3(b). All of these solutions (b, c, and d) included 5.0×10^{-6} M of Pb(II) as well. As seen in Fig. 3(b), the peak height increased up to 10×10^{-6} M of interfering metal ions, but showed an important decrease above this concentration. At higher concentrations of interfering metal ions, peak current belonging to Pb(II) was affected unfavorably due to the competition between metal ions for binding to functional groups at the electrode surface.

4. Conclusion

The usage of heat-dried *R. arrhizus* cells on carbon paste modification to determine Pb(II) at trace levels was investigated for the first time. This procedure has the advantages of low cost for instrumentation, simplicity of use, and not requiring an extensive sample treatment procedure. On the other side, the selectivity of the developed microbial sensor can be enhanced by applying different electrochemical conditions, including deposition time, deposition potential, and scan rates. Moreover, the biological material can be modified with different chemicals, which interact with the functional metal binding sites on the biomass. Apart from these, the manuscript presents a different aspect that about ability of the microorganisms, which have been used for the biosorption of heavy metals, to determine specific heavy metals in short times by using electrochemical techniques.

Acknowledgement

Financial support was provided by the Scientific and Technical Research Council of Turkey (TÜBİTAK-BİDEB).

References

Adams, R.N., 1958. Carbon paste electrodes. Anal. Chem. 30, 1576.
Aksu, Z., Dönmez, G., 2006. Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vulgaris*: Co-ion effect on mono-component isotherm parameters. Process Biochem. 41, 860–868.
Aksu, Z., Gülen, H., 2002. Binary biosorption of iron(III) and iron(III)–cyanide complex ions on *Rhizopus arrhizus*: modelling of synergistic interaction. Process Biochem. 38, 161–173.
Akyılmaz, A., Yorgancı, E., Asav, E., 2010. Do copper ions activate tyrosinase enzyme? A biosensor model for the solution. Bioelectrochem. 78, 155–160.
Alpat, Ş., Alpat, S.K., Çadırcı, B.H., Yaşa, I., Telefoncu, A., 2008. A novel microbial biosensor based on *Circinella* sp. modified carbon paste electrode and its voltammetric application. Sens. Actuators, B 134, 175–181.
Brown, R.J.C., Milton, M.J.T., 2005. Analytical techniques for trace element analysis: an overview. TrAC Trends Anal. Chem. 24, 266–274.
Chinalia, F.A., Patron, G.I., Killham, K.S., 2008. Physiological and toxicological characterization of an engineered whole cell biosensor. Bioresour. Technol. 99, 714–721.
Chouteau, C., Dzyadevych, S., Chovelon, J.M., Durrieu, C., 2004. Development of novel conductometric biosensors based on immobilised whole cell *Chlorella vulgaris* microalgae. Biosens. Bioelectron. 19, 1089–1096.
Dearth, R.K., Hiney, J.K., Srivastava, V., Burdick, S.B., Bratton, G.R., Dees, W.L., 2002. Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. Reprod. Toxicol. 16, 343–352.
Deng, X., Yi, X.E., Liu, G., 2007. Cadmium removal from aqueous solution by gene-modified *Escherichia coli* JM109. J. Hazard. Mater. 139, 340–344.
D'Souza, S.F., 2001. Microbial biosensors. Biosens. Bioelectron. 16, 337–353.
Elvina Jr., R.O., Mojica, E.R.E., 2005. Orange peel essential oil as component of a metal sensor lead(II) ion determination in aqueous solutions. J. Appl. Sci. Environ. Manage. 9, 23–27.
Etienne, M., Bessiere, J., Walcarius, A., 2001. Voltammetric detection of Cu(II) at a carbon paste electrode containing on organically modified silica. Sens. Actuators, B 76, 528–531.
Gardea-Torresdey, J.L., Tiemann, K.J., Gamez, G., Dokken, K., 1999. Effects of chemical competition for multi-metal binding by Medicago sativa (alfalfa). J. Hazard. Mater. 69, 41–51.
Kabata-Pendias, A., 2004. Soil-plant transfer of trace elements – an environmental issue. Geoderma 122, 143–149.
Lei, Y., Chen, W., Mulchandani, A., 2006. Microbial biosensors. Anal. Chim. Acta 568, 200–210.
Liu, J.X., Zhou, W.J., Gong, J.L., Tang, L., Zhang, Y., Yu, H.Y., Wang, B., Xu, X.M., Zeng, G.M., 2008. An electrochemical sensor for detection of laccase activities from *Penicillium simplicissimum* in compost based on carbon nanotubes modified glassy carbon electrode. Bioresour. Technol. 99, 8748–8751.
Malitesta, C., Guascito, M.R., 2005. Heavy metal determination by biosensors based on enzyme immobilised by electropolymerisation. Biosens. Bioelectron. 20, 1643–1647.
Malkowski, E., Kita, A., Galas, W., Karcz, W., Kuperberg, J.M., 2002. Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. J. Plant Growth Regul. 37, 69–76.
Michel, C., Ouerd, A., Battaglia-Brunet, F., Guigues, N., Grasa, J.P., Bruschi, M., Ignatiadis, I., 2006. Cr(VI) quantification using an amperometric enzyme-based sensor: interference and physical and chemical factors controlling the biosensor response in ground waters. Biosens. Bioelectron. 22, 285–290.
Mojica, E.R.E., Vidal, J.M., Pelegrina, A.B., Micor, J.R.L., 2007. Voltammetric detection of lead(II) ions at carbon paste electrode modified with banana tissues. J. Appl. Sci. 7, 1286–1292.
Mozaz, R.S., Marco, M.P., Lopez de Alda, M.J., Barcelo, D., 2004. Biosensor for environmental applications: future development trends. Pure Appl. Chem. 76, 723–752.
Naja, G., Mustin, C., Berthelin, J., Volesky, B., 2005. Lead biosorption study with *Rhizopus arrhizus* using a metal-based titration technique. J. Colloid Interface Sci. 292, 537–543.
Preetha, B., Viruthagiri, T., 2007. Bioaccumulation of chromium(VI), copper(II) and nickel(II) ions by growing *Rhizopus arrhizus*. Biochem. Eng. J. 34, 131–135.
Sağ, Y., Kutsal, T., 1997. The simultaneous biosorption process of lead(II) and nickel(II) on *Rhizopus arrhizus*. Process Biochem. 32, 591–597.
Subudhi, E., Kar, R.N., 2008. *Rhizopus arrhizus* – an efficient fungus for Cu(II) effluent treatment. Int. J. Integr. Biol. 2, 166.
Tuzen, M., Saygı, K.Ö., Usta, C., Soylak, M., 2008. Pseudomonas aeruginosa immobilized multiwalled carbon nanotubes as biosorbents for heavy metal ions. Bioresour. Technol. 99, 1563–1570.
Verma, B., Singh, M., 2005. Biosensors for heavy metals. Biometals 18, 121–129.
Wang, J., 2000. Analytical Electrochemistry, second ed. Analytical Electrochemistry, New York.
Woolever, C.A., Dewald, H.D., 2001. Differential pulse anodic stripping voltammetry of barium and lead in gunshot residues. Forensic Sci. Int. 117, 185–190.
Yin, J., Wei, W., Liu, X., Kong, B., Wu, L., Gong, S., 2007. Immobilization of bovine serum albumin as a sensitive biosensor for the detection of trace lead ion in solution by piezoelectric quartz crystal impedance. Anal. Biochem. 360, 99–104.