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An advanced investigation on a new algal sensor determining Pb(II) ions from aqueous media

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ABSTRACT

It has been well documented that heavy metal accumulation in environment is harmful for living organisms at even trace levels. A new voltammetric algal sensor based on *Phormidium* sp. modification for Pb(II) determination from aqueous solutions was developed, and selectivity of the biomass to Pb(II) was investigated comprehensively. Many important experimental parameters were performed by using electrochemical techniques, including cyclic voltammetry and differential pulse stripping voltammetry. The preconcentrated ions at open circuit were reduced by scanning the potential from −1.5 to 1.5 V and current values obtained were related to the concentration of Pb(II) in the solutions. The best peak values belonging to Pb(II) were achieved at pH 8.0 with 0.05 M Tris–HCl solution. Preconcentration time was selected as 10 min, and the sensor was found in a linear range from 5.0×10^{-8} M to 2.0×10^{-5} M Pb(II) (0.01–4.0 mg L^{-1}) with a detection limit of 2.5×10^{-8} M. Other analytical properties of the developed microbial biosensor were also investigated.

According to the Fourier transform infrared attenuated total reflectance (FTIR-ATR) analyses, the possible functional groups involved in Pb(II) accumulation in the *Phormidium* sp. were defined as carboxyl, sulphoxide and alcoholic groups. A simple chemical modification by formaldehyde both enhanced Pb(II) determination and content of functional groups involving Pb(II) binding. The proposed usage form of *Phormidium* sp. does not need complicated immobilization procedures and expensive preliminary preparations.

1. Introduction

During the last two decades, extensive attention has been paid on the management of environmental pollution caused by hazardous material, such as heavy metals. Presence of heavy metals even in trace levels is toxic and detrimental to both flora and fauna. Wastewaters containing large amounts of heavy metals are being discharged into the environment, by either directly or indirectly, resulting from development of the some industries and installations, including mining, leather, metallurgy, electrolysis, and metal surface treating.

Among these heavy metals, lead is extremely toxic even at low concentrations, and can damage the nervous system, reproductive system, and kidneys particularly in children (Nriagu, 1988). Lead concentration must be brought to 0.1 mg/L as per instruction of EPA before discharging into the surface water (Friberg, 1977).

Researches on heavy metal detection and removal from wastes have been a crucial current issue for the cost effective procedures, in terms of the harmful impacts of these metals on living organisms. To overcome present environmental problems, conventional methods have some restrictions, such as expensiveness, insufficiency, and particularly having too low permissible limit.

Many biosensors involving different species of microorganisms have been constructed in order to detect pollutants in the environment (Ivask et al., 2009; Lin and Chung, 2009; Liu et al., 2009; Wu et al., 2009). The use of microalgae as sensor modifying agents has recently become very interesting topic in biotechnology.

Usage of algal biomass as recognizer component in the biosensor construction has considerable advantages, including being able to live under high pH conditions preventing contamination by other microorganisms, and to give high productiveness with both low expenses and low nutrient requirements (Aksu et al., 2009). In addition, they generally do not produce toxic substances unlike other microorganisms, and being autotrophic, they produce a large amount of biomass (Alpat et al., 2007; Campanella et al., 2001; Chouteau et al., 2005; Shitanda et al., 2005).

However, the cell wall components of the algae, such as alginate and fucoidan, have made them suitable sorbents for heavy metal detection or removal heavy metals from aqueous solutions effectively. The affinity of some divalent metal ions to alginates

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containing large amounts of carboxylate groups has been demonstrated on early reports (Fourest and Volesky, 1996; Haug, 1961). However, these considerable properties of the algal cells have not been studied comprehensively for biosensor applications, except a few reports about the usage of algal biomass as sensor component to detect toxic substances (Alpat et al., 2007; Chouteau et al., 2005; Frense et al., 1998; Lojou and Bianco, 2000; Podola et al., 2004; Shitanda et al., 2005).

In the present study, a strain of filamentous *Cyanobacterium*, *Phormidium* sp. has been used as a biological material for the construction of Pb(II) ion selective algal sensor. The usage of *Phormidium* sp. has not been reported yet in the biosensor applications to perform heavy metal detection although, some species of *Phormidium* have been previously figured out in heavy metal biosorption studies (Aksu et al., 2009; Blanco et al., 1999; De Philippis et al., 2003; Wang et al., 1998). Besides, the relation between *Phormidium* species and some heavy metals have not been clearly analyzed either.

The main goals of the current search were (i) to reveal the usage potential of the *Phormidium* sp. for Pb(II) qualification and quantification at trace levels from aqueous solution, (ii) to optimize the analyzing conditions deeply by using electrochemical techniques, and (iii) to expose simply some important functional groups involved in Pb(II) binding to the algal biomass. The proposed algal sensor based on carbon paste has been found accessible to be used because of requiring low cost for reproducibility, and having a broad detection range for Pb(II). Moreover, the modification of electrode surface by formaldehyde has increased the electrochemical response and has served to find binding sites involving Pb(II) sorption on the algal cell.

2. Experimental

2.1. Chemicals

2.1.1. Metal solutions

All solutions used 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). Working solutions of metal ions were prepared freshly from stock solutions after appropriate dilutions with distilled water.

2.1.2. Supporting electrolyte solutions

Tris–HCl buffer solution was used as main electrolyte solution in all experiments, and was prepared from the analytical grade of trizma base and hydrochloric acid (Sigma). Sodium acetate solution was prepared by mixing the certain amounts of glacial acetic acid (Carlo Elba) and sodium acetate (Merck). Sodium phosphate buffer solution was prepared by mixing the appropriate amounts of monobasic potassium phosphate and dibasic potassium phosphate. The pH of all solutions was adjusted with 0.1 M stock solution of HCl, KOH, NaOH or HNO_3 (Merck).

1% formaldehyde solution diluted from 37% solution (Merck) was used for chemical modification of the biomass. Graphite powder (Sigma) and paraffin liquid (Merck) were used for preparing the carbon paste electrode.

2.2. Procedures

2.2.1. Microorganism and culture conditions

A strain of filamentous *Cyanobacterium*, *Phormidium* was isolated from a hot spring located in Ayaş, Ankara, Turkey, as described in the previous report (Sadettin and Dönmez, 2006).

The microorganism was cultivated in BG11 medium using shake flask method. The medium pH was adjusted to 8.5 by 0.1 M H_2SO_4 and 0.1 M NaOH, before autoclaving. Once inoculated, unshaken flasks containing 100 ml of BG11 medium were incubated under continuous illumination at 2400 lx light intensity provided by cool white fluorescent lamps for 35 days at 35 °C in a plant growth chamber (Lab-line, Biotronette).

After the incubation period, *Phormidium* sp. was harvested by centrifugation (M.E.D. Instruments, 351R) at 10,000 rpm for 15 min, the supernatant was discarded, and pellet was rinsed twice with distilled water. Obtained biomass was dried at 80 °C for 24 h in glass plates. Finally, heat dried biomass was removed from the plates by scratching, and was powdered by using a ceramic mortar. All the experiments were conducted by heat-dead biomass, which provides easy handling and storage without nutrient supply requirement. Additionally, it has been reported that dead algal cells sorb metal ions more than alive ones (Mehta and Gaur, 2005).

2.2.2. Electrode preparation

The modified carbon paste electrode (MCPE) was prepared by making a homogeneous paste with 5.0 mg of heat dried algal biomass, 95 mg of graphite powder, and a proper amount of paraffin liquid. The unmodified carbon paste electrode (UMCPE) was used as a control sample, included only graphite powder and paraffin liquid. The paste mixture was firmly packed into the hole of the handmade electrode body (1 mm in diameter), and was compressed with a steel rod. Then the electrode surface was smoothed on a paper for making the surface available for further electrochemical steps. After each determination, electrode was treated with HCl (0.1 M) and distilled water to eliminate Pb(II) adsorbed on the electrode.

2.2.3. Apparatus and operating principle of the developed algal sensor

All electrochemical experiments were performed at room temperature with a CH Instruments 660B model Electrochemical Workstation (CH Instrument, USA). The instrument was equipped with a conventional three-electrode cell, containing 20 ml of supporting electrolyte solution, 0.05 M of Tris–HCl, deoxygenated by passing bubbling pure nitrogen through it before the measurements. The counter electrode was a platinum wire, and Ag/AgCl electrode (saturated KCl) was used as reference electrode. Experiments have been done using the preconcentration, accumulation, medium change, and voltammetry steps, respectively (Yüce et al., 2010). All experiments were done twice, and results obtained were presented as mean of the measurements ± standard deviation in the graphics and/or tables.

2.2.4. FTIR-ATR studies

FTIR-ATR spectra of *Phormidium* sp. before and after loading of Pb(II), were taken in FTIR-ATR Spectroscopy Affinity model equipped with ATR (Shimadzu) in the region of 700–4000 cm^{-1} at a resolution of 4 cm^{-1}. FTIR-ATR spectra of *Phormidium* sp. were taken after chemical modification of algal cell, as well. Peaks appearing in the spectrum of native *Phormidium* sp. were assigned to various groups and bands in accordance with their respective wave numbers (cm^{-1}) as reported in literature.

2.2.5. Chemical modification of the algal biomass and the electrode surface

Modification method was adapted from a biosorbtion study (Chen and Yang, 2006). 0.2 g of the heat-dried algal biomass was added into the 150 ml Erlenmeyer flask containing 50 ml of 1% formaldehyde solution, and was agitated at 30 °C in a rotary shaker at 120 rpm for 4 h. Obtained biomass was used for experiments after centrifugation and drying steps.

CPE including 5.0 mg of heat-dried algal biomass was immersed into 20 ml of 1% formaldehyde solution and was agitated on a mag-

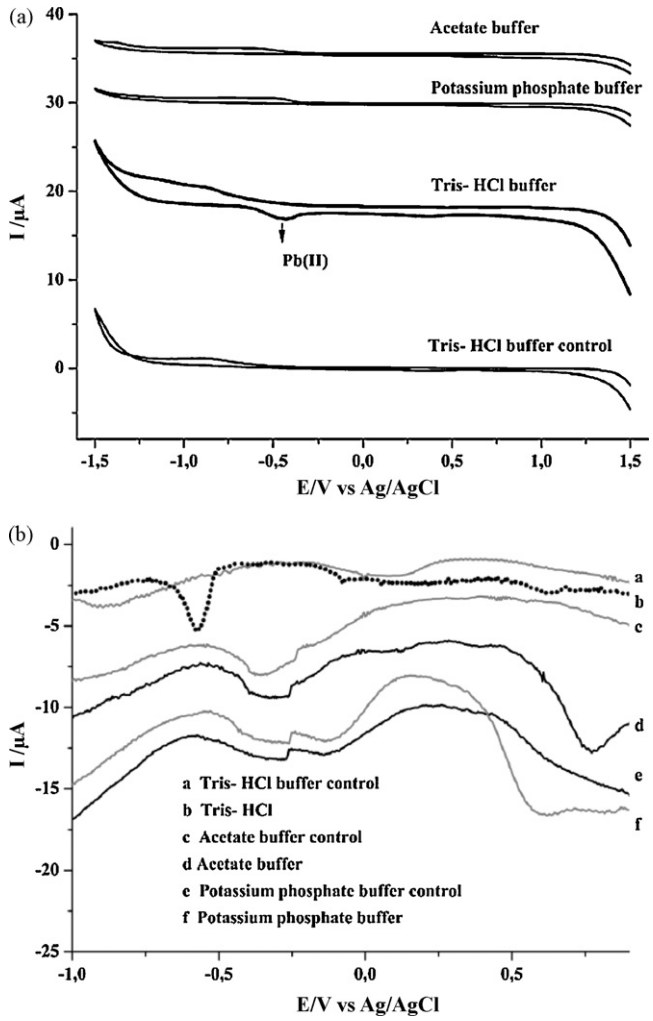

Fig. 1. CV (a) and DPSV (b) results of the electrodes, obtained at different electrolyte solutions (concentration of Pb(II): 5.0×10^{-5} M, preconcentration time: 10 min at open circuit, electrolyte solution's concentration: 0.1 M, the solution's pH: 6.0).

netic stirrer for 90 min. After stirring step, the electrode was taken from the solution, and was washed with plenty of water, carefully. Chemically modified carbon paste electrode (CMCPE) was immersed into 20 ml of electrolyte solution including a certain amount of Pb(II) and was measured electrochemically after accumulation step.

3. Results and discussion

3.1. Cyclic voltammetry assays and effect of different supporting electrolyte solutions

The cyclic behaviors of the MCPEs and UMCPEs (as controls) were investigated in 0.1 M of acetate buffer, potassium phosphate buffer and Tris–HCl buffer for 5.0×10^{-5} M Pb(II) concentration. The pH of all buffer solutions was adjusted to 6.0 to obtain equal conditions. As shown in Fig. 1(a), the cyclic behavior of MCPE in Tris–HCl buffer solution made a difference unlike the other solutions tested, and a peak belonging to Pb(II) was observed at potentials between −0.6 and −0.4 V. The relevant potential range, in which Pb(II) peak occurred on the voltammogram was in agreement with previous results (Kirowa-Eisner et al., 1999; Woolever and Dewald, 2001).

The same solutions were tested by using DPSV to show different solution's effects in detail. As shown in Fig. 1(b) there were no traceable peaks indicating the existence of Pb(II) on the electrode surface, when the acetate and potassium phosphate buffers were used. These results could be explained by the competition between Pb(II) ions and other protons for binding cites in the phosphate buffer solution, or formation of weak lead acetate complexes in the acetate buffer. However, a well-defined cathodic peak depicting the capability of the algal biomass to adsorb Pb(II) has been observed when Tris–HCl buffer was used as electrolyte solution. The strong acidic nature of HCl may have resulted from releasing of the light metal ions (Na(I), K(I), Ca(II), Mg(II)) from the algal binding cites (Davis et al., 2003), and facilitated the sorption of Pb(II) to algal biomass. Consequently, Tris–HCl solution was used for further experiments.

3.2. Effects of pH and ionic strength of electrolyte solution

pH of the electrolyte solution greatly influences metal sorption process. Depending on nature of the biomass, different optimum pH values for biosorbtion have been reported for different metals (Chang et al., 1997; Panda et al., 2007). Electrochemical responses have been tested in a pH range from 5.0 to 9.0, and the best sensitivity has been found at pH 8.0 for 5.0×10^{-6} M Pb(II) as presented in Fig. 2(a). A gradual increase on the peak current was observed up to pH 8.0, and the relevant peak shape improved clearly. The reason for this increment could be explained by the increasing competitive binding of Pb(II) ions with permanently existing ligand hydrogen ions. At more acidic pH values, many of the binding cites could be protonated and this situation may prevent the possible Pb(II) binding. However, the peak current decreased sharply above pH 8.0, probably due to the formation of the insoluble hydroxylic Pb(II) complexes in the solution that inhibited lead accumulation on the algal biomass. The pH value obtained was either in the optimum working range of Tris–HCl buffer (pH 7.0–9.0), or very close to growth conditions of the *Phormidium* sp. (pH 8.5).

Effect of ionic strength on the electrochemical response has been investigated by using five concentrations of Tris–HCl buffer for 5.0×10^{-6} M Pb(II) (0.01, 0.05, 0.1, 0.2 and 0.3 M), owing to the fact that the ionic concentration can affect solubility of metal ions and adsorption process. As presented in Fig. 2(b), increase in the ionic power concluded a decrease considerably in the electrochemical response. This may reflect either the electrostatic component of the complexation reactions or the formation of stable metal complexes in solution, or both (Davis et al., 2003). Previous studies also showed that adsorption decreases with the increase in ionic strength (Bennouna et al., 1997; Dönmez, 2002). Subsequently, the Tris–HCl buffer solution with a 0.05 M concentration was selected due to the best electrochemical response.

3.3. Effect of preconcentration time

Preconcentration or accumulation time has a substantial impact on the determination of target analytes. Preconcentration time may vary from minutes to hours according to the properties of functional binding cites on the biomass and target analytes (Ouangpipat et al., 2003; Yin et al., 2007). In the light of the above information, a time range from 5.0 to 25 min was operated for 5.0×10^{-6} M Pb(II) at open circuit conditions (Fig. 3).

A significant difference was not observed on the electrochemical response after 5 min preconcentration time, probably indicating the saturation of the binding cites on the algal biomass. The reaching to equilibrium phase in a short time could be explained by being narrow of the electrode surface, and using only a small amount of biomass for carbon paste preparation that implies presence of fewer functional groups on the electrode surface. These properties have provided important advantages to algal sensor in economic and practical senses.

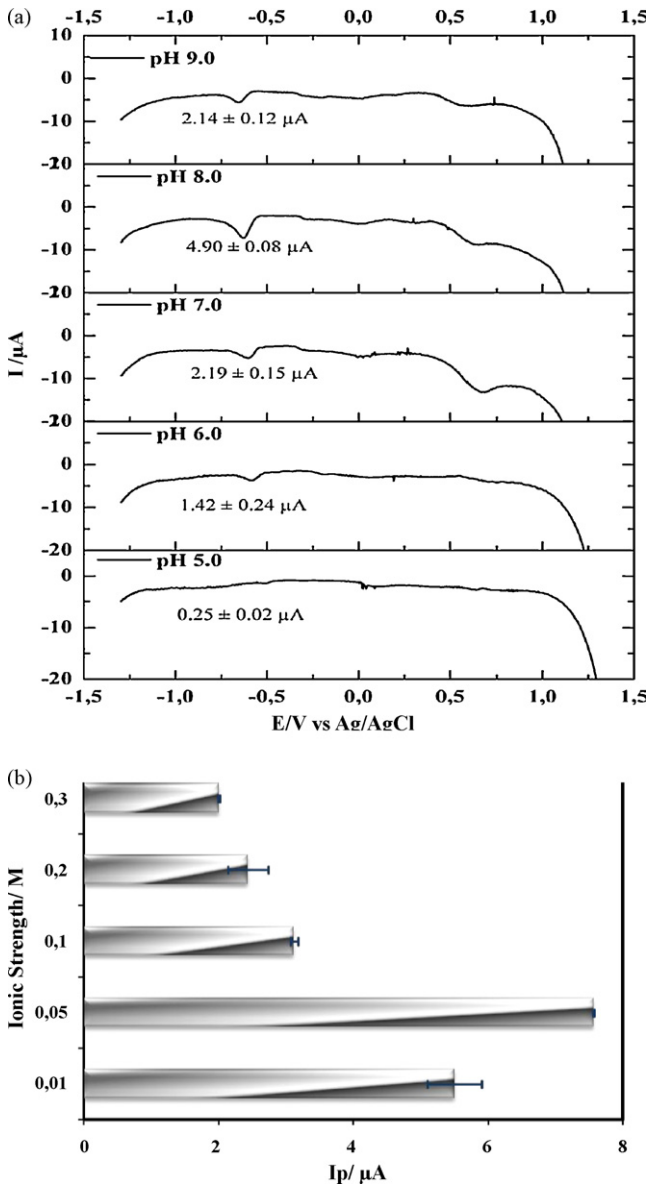

Fig. 2. Effect of electrolyte solution's pH (a) and ionic strength (b) on electrochemical response (concentration of Pb(II): 5.0×10^{-6} M for both assays, concentration of electrolyte solution: 0.1 M for pH assays, preconcentration time: 10 min at open circuit, detection: DPSV).

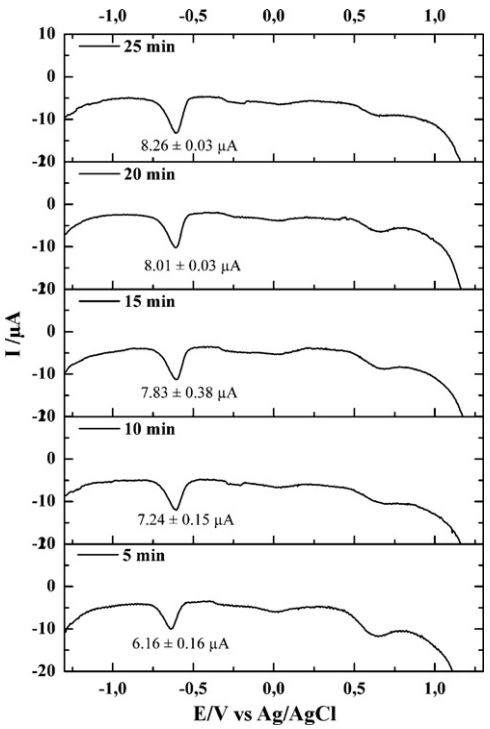

Fig. 3. Effect of preconcentration time on electrochemical response (concentration of Pb(II): 5.0×10^{-6} M, electrolyte solution: 0.05 M Tris–HCl, electrolyte solution's pH: 8.0, detection: DPSV).

3.4. Some analytical and technique specifications of the developed algal sensor

The calibration graph, obtained by using the optimum experimental conditions, was defined by the equation $y = 1.1878x$ according to the calibration graphic (supplementary data). The peak current belongs to Pb(II) (y) was correlated with Pb(II) concentration (x). It was linear from 5.0×10^{-8} M to 2.0×10^{-5} M Pb(II) (0.01–4.0 mg L^{-1}) ($R^2 = 0.9901$). At higher concentrations, standard curve showed a deviation from the linearity. Limit of detection was calculated as 2.5×10^{-8} M by using $3S_b/m$ formula (Akyılmaz et al., 2010). The LOD value obtained is both lower than many other reports based on carbon paste detecting Pb(II) ions (Friberg, 1977; Ganjali et al., 2010; Mojica et al., 2007; Yao and Ramelow, 1998), and the Pb(II) limit determined by EPA. Analytical and technique properties of some Pb(II) sensors and present developed microbial biosensor can be found as a table in supplementary data.

The reproducibility of the algal sensor has also been tested for 1.0×10^{-5} M Pb(II) ($n = 8$) under optimized experimental conditions. The mean of the replicates, percent relative standard deviation (RSD%), standard error and percent coefficient of variation (CV %) were found to be 10.09 ± 1.32 μA, 13.08, 0.47, and 1.63%, respectively. Ouangpipat et al. (2003) reported a RSD value of 5.39 with a detection limit of 0.01 mg L^{-1} for Pb(II) determination based on bovine serum albumin modification. Although this value was better than that of the present study, preparation of the working electrode was more difficult, expensive, as well as time consuming. However, current RSD value is lower than the one found by Demetriades et al. (2004). A CV % obtained from carbon paste electrode modified with banana tissue was defined 6.7% by Mojica et al. (2007).

3.5. Effect of interference ions

The electrochemical behavior of developed algal sensor for some heavy metals has been tested in order to estimate its performance in more realistic conditions, and results obtained were presented in Table 1.

3.6. FTIR-ATR studies and effect of formaldehyde modification

FTIR spectroscopy was conducted on native, Pb(II) loaded and chemical modified *Phormidium* sp. in order to obtain information about the nature of the possible cell–metal ion interactions. FTIR spectrum pattern of native *Phormidium* sp. (unloaded, unmodified) showed five distinct groups including hydroxyl, amino, phosphate, carboxyl, and sulphoxide, as shown in Fig. 4(a).

Broad bands at 3284 cm^{-1}, represented bonded –OH and –NH groups (Gnanasambandam and Proctor, 2000; Sheng et al., 2004). The bands observed at about 2879 cm^{-1} could be assigned to the –CH stretch vibrations, and the peak at about 1631 cm^{-1} represented a chelated form of carbonyl on the carboxyl group or

Table 1
Effect of interfering metal ions on electrochemical response (electrolyte solution: 0.05 M Tris–HCl, electrolyte solution's pH: 8.0, preconcentration time: 10 min at open circuit, detection: DPSV).

Interferences	Metals (μM)	I_p (μA)	%Activity
Pb(II)	5	7.15 ± 0.05	100
Pb(II)	10	10.84 ± 0.18	100
Co(II)	5	0	0
Pb(II) + Co(II)	5 + 5	3.38 ± 0.01	47
Cu(II)	5	0	0
Pb(II) + Cu(II)	5 + 5	8.67 ± 0.04	121
Ni(II)	5	0	0
Pb(II) + Ni(II)	5 + 5	3.25 ± 0.30	45
Zn(II)	5	0	0
Pb(II) + Zn(II)	5 + 5	4.01 ± 0.06	56
Pb(II) + Zn(II) + Cu(II) + Co(II) + Ni(II)	5 + 5 + 5 + 5 + 5	5.43 ± 0.02	76
Pb(II) + Zn(II) + Cu(II) + Co(II) + Ni(II)	10 + 10 + 10 + 10 + 10	11.67 ± 0.58	58

All analyses were performed two times and reported as average ± standard deviation.

asymmetric/symmetric stretching vibrations of ionic carboxylic groups (–COO^-) (Yun et al., 2001). The peak observed at 1740 cm^{-1} was the stretching vibrations of C=O bond indicating main lipid and is derived primarily from the ester linkage of the fatty acids (aliphatic monocarboxylic acids) (Kansiz et al., 1999).

The bands observed at 1541 cm^{-1} represented characteristic vibrations of the amide functional groups typical for the cyanobacterial cells (Benning et al., 2004; Kansiz et al., 1999). The peaks at 1354, 1034 and 836 cm^{-1} were assigned to the C–N, COO^-, P–O and S=O groups, respectively (Figueira et al., 2000; Pokrovsky et al., 2008).

Disappearance of the band at 836 cm^{-1} after Pb(II) accumulation, as seen in Fig. 4(b), indicated the intervention of sulphoxide groups during metal sorption. And the sharp decrease at the 1354 cm^{-1} after Pb(II) loading indicated the importance of the COO^- and C–N groups on the Pb(II) accumulation. Elongation of the band at 1740 cm^{-1} after loading Pb(II) also showed the role of these functional groups in the adsorption process.

Different physical and chemical treatments could improve metal uptake capacity of the biosorbents by removing or masking functional groups or exposing more metal binding cites. Therefore, cell surface modifications could greatly alter the binding of the metal ions. The appearances of new small bands after formaldehyde modification between 1250 and 900 cm^{-1} showed that formaldehyde caused a variation on the surface functional groups. The band at 1230 cm^{-1} may be assigned to deformation vibration of P=O and stretching formation of –OH of carboxylic acids or phenols.

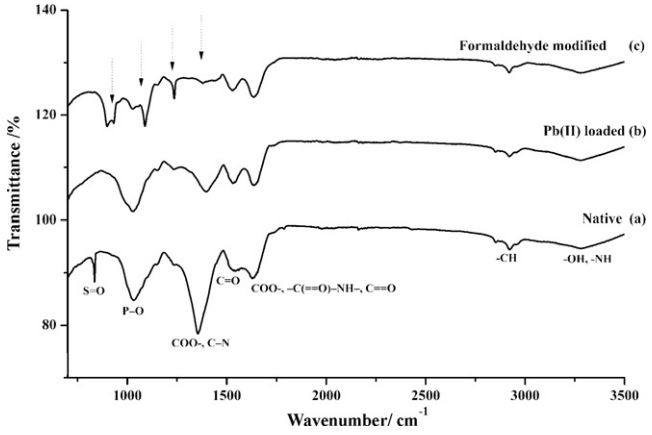

Fig. 4. FTIR-ATR results of native (a), Pb(II) loaded biomass (b) and chemically modified biomass (c).

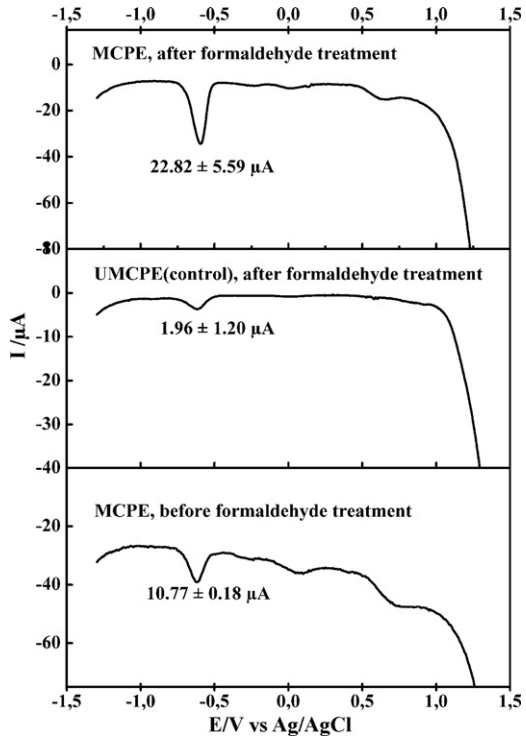

Fig. 5. Effect of formaldehyde modification on the electrode response (concentration of Pb(II): 1.0×10^{-5} M, electrolyte solution: 0.05 M Tris–HCl, electrolyte solution's pH: 8.0, modification solution: formaldehyde (1%), modification time: 90 min, detection: DPSV).

The peaks indicating P–OH links of the organic phosphorus groups could be found at 1092 cm^{-1}. Peak occurred at 1055 cm^{-1} could be assigned to stretching vibration of C–OH of alcoholic groups and carboxylic acids. Band at 1027 cm^{-1} indicated C–O stretching of alcoholic groups and the others at 903–934 cm^{-1} were assigned to C–O stretching vibrations.

In line with above information, a kind of electrode surface modification was tried by using the same formaldehyde solution in order to figure out both effect of new bands on the Pb(II) binding and to simplify modification process. The electrode surface modification tests were inspired by some biosorption studies (Chen and Yang, 2006; Majumdar et al., 2010). As seen in Fig. 5, electrochemical response doubled with the effect of formaldehyde modification, and a uniform baseline was reached. According to the literature, in the presence of formaldehyde, amine could react with the aldehyde groups or formaldehyde could react with an alcoholic group to form dative linked structure (Chen and Yang, 2006). The narrow width of the stripping peaks was one indication that both the electrochemical and subsequent chemical processes were fast. The ration of the stripping current to the background current was significantly better for the formaldehyde-treated electrode, as can be seen by comparing the voltammograms in Fig. 4. In principle, this positive difference could be due to either enhanced mass transport to the smoother electrode surface during the preconcentration step or to the presence of a higher coverage of binding site at the chemically modified electrode surface, or both. It was seen that CMCPE could be a better analytical tool for this application and lower detection limits can be expected by using CMCPE.

Consequently, formaldehyde modification has provided a considerable improvement on the algal biosensor's electrochemical response. This result could be explained by the increased functional group content on the surface that they allowed more Pb(II) ions to bind. In spite of some important groups disappeared, a consider-

able increase on the peak current has revealed the major roles of the new functional cites on the Pb(II) sorption.

4. Conclusions

The main goal of this study was to investigate whether dead *Phormidium* sp. biomass could serve as a recognizer component at Pb(II) ion selective algal sensor. The study was based on biosorption ability of the *Phormidium* sp. reported previously by other studies, and main results obtained were listed as below:

The MCPE and CMCPE exhibited a strong capacity for the Pb(II) determination from aqueous solutions.
Solutions' pH highly affected Pb(II) determination, higher solution pH caused higher metal accumulation on the electrode surface.
Hydroxyl, carboxyl, sulphoxide, amine and phosphate functional groups were found on the bare biomass. It was also shown that carboxyl and sulphoxide groups were predominant contributors in Pb(II) ion uptake.
Chemical modification by formaldehyde enhanced the electrochemical response and the content of the functional groups. It was found that new cites could also be highly responsible for Pb(II) accumulation on the *Phormidium* sp. by evidence of electrochemical results.
Consequently, these sensors could be an inexpensive and effective way to detect Pb(II), and should be investigated further for their practical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.08.022.

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