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Voltammetric detection of As(III) with *Porphyridium cruentum* based modified carbon paste electrode biosensor

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ABSTRACT

A novel biosensor based on carbon paste electrode modified with *Porphyridium cruentum* biomass was developed for the determination of As(III) in contaminated water. As(III) was first biosorbed–accumulated on the electrode surface at open circuit potential and then stripped off by applying anodic scan range of −0.8 to +0.8 V using differential pulse anodic stripping voltammetric technique. The best result was obtained at pH 6.0 with 0.1 M HNO_3 solution as stripping medium, allowing biosorption–accumulation time of 8 min using 5% *P. cruentum* biomass in graphite–mineral oil paste. Linear range for As(III) detection with the modified electrode-biosensor was observed between 2.5 and 20 μg L^{-1}. The FTIR spectrum of *P. cruentum* biomass confirmed the presence of active functional groups that participate in the binding of As(III). Scanning Electron Microscopy (SEM) indulged the surface morphology of modified electrode-biosensor before and after As(III) adsorption. Similarly, Atomic Force Microscopy (AFM) showed that the average roughness of the modified electrode decreased indicating the successful incorporation of *P. cruentum* biomass. Efficiency of the biosensor in the presence of different interfering metal (Na^+, K^+, Ca^{2+}, and Mg^{2+}) ions were also evaluated. The application of *P. cruentum* modified biosensor was successfully used for the detection of As(III) in the binary metal (Fe^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}) contaminated system. The accuracy of application of biosorption based biosensor for the detection of As(III) is as low as 2.5 μg L^{-1}.

1. Introduction

Arsenic naturally exists in the earth's crust in low concentrations (Hussain et al., 2012). It has received much attention during recent years due to toxicity it may cause to humans (Bissen and Frimmel, 2003). The metalloid, on leaching from its geochemical formations, becomes a natural component of soil, water, and living organisms in a definite ratio (Mandal and Suzuki, 2002). Included among the anthropogenic origin of arsenic are insecticides (Welch et al., 2000), herbicides (Baker et al., 1969), wood preservatives (Bhattacharya et al., 2002), feed additives (Pergantis et al., 1997), and drugs (Bates et al., 1992), though its use in agrochemicals has been declining (Sanok et al., 1995). Arsenic may cause acute toxicity resulting in dysphasia, profuse diarrhea, dehydration, muscular cramps, and facial edema, or sub-acute toxicity as the loss of appetite, erythema, jaundice, nervous weakness, and tingling of hand and feet (Jain and Ali, 2000; Choong et al., 2007). The element exists in the oxidation states of 0, −3, +3, and +5 (Bissen and Frimmel, 2003). However, As(III) and As(V) are of greater significance due to their presence in the drinking water, which is regarded as the major route of inorganic arsenic entry into the human body (Khan and Ho, 2011). Depending on the groundwater pH, As(III) and As(V) may exist as the chemical species H_3AsO_3, H_2AsO^{3-}, $HAsO_3^{2-}$, H_2AsO^{4-}, H_3AsO_4, $HAsO_4^{2-}$ and AsO_4^{2-} (Yan et al., 2000).

Most heavy metals exist in their cationic form and can be removed by conventional methods, including membrane filtration, precipitation, and ion-exchange (Arief et al., 2012). These methods, however, are not suitable for removing oxyanion forming elements such as arsenic (Dzombak and Morel, 1990). Arsenic removal is even more difficult as it has greater mobility under both the oxidizing and reducing conditions (Smedley and Kinniburgh, 2002). The process of biosorption, using the biomass of algae (Taboada-de la Calzada et al., 1998), bacteria (Stocker et al., 2003),

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fungi (Ridvan et al., 2003), and plant residues (Kamala et al., 2005) has been successfully used as an alternative for the removal of As (III) from water. The mechanism of this process is related to the ability of these biological materials to adsorb metal ions on their active sorption sites (Wang and Chen, 2009) mainly due to the presence of functional moieties, such as carboxyl, hydroxyl and amino groups on their surface (Volesky and Holan, 1995). The biosorption process is further useful as it performs efficiently at low metal levels, at which the conventional methods are not feasible, and is appropriately termed as 'metal-polishing' (Volesky, 2001). Most commonly used methods for the estimation of As(III) include UV–vis spectroscopy, inductively coupled plasma spectroscopy, hydride generation atomic absorption spectroscopy, and neutron activation analysis. Although these techniques are reliable for detection at low concentrations, they cannot be routinely used for *in-situ* analysis or screening as the instruments and their running and maintenance costs are high, and require trained technicians for their handling (Mays and Hussam, 2009). The field method used for arsenic detection in potable water is based on color development on mercuric bromide strips. This method has nevertheless given false results, specifically at levels below 70 $\mu g\ L^{-1}$ (Stocker et al., 2003) and is thus not reliable for arsenic detection at a concentration below 10 $\mu g\ L^{-1}$, a maximum permissible limit set by WHO for safe drinking water (WHO, 2006). An alternative method is electrochemistry-based, in which electrodes are used to measure, detect, and differentiate between various oxidation states of heavy metals (Mays and Hussam, 2009). The technique offers several benefits over others, such as simple sample preparation, easy to operate, and low in running and maintenance cost. However, commercial electrodes used for electrochemical As(III) analysis require extensive pretreatment and exhaustive adsorption or coating procedures that are time-consuming, costly and require technical handling skills (Giacomino et al., 2011). The ability of metal ions to interact with functional groups on the biomass surface, particularly at low concentrations, combined with the electrochemical technique offer the potential to develop a biosorption based biosensor for heavy metal analysis. Biosensors have been used in recent years for the detection of several metals (Yuce et al., 2010a; Alpat et al., 2008). Performance of biosensors has been further improved by using microorganisms as the electrode-modifying agents, as reported for the determination of Pb(II) with a biosensor modified with the biomass of *Rhizopus arrhizus* (Yuce et al., 2010b).

Pakistan is located in a region of arsenic-rich geochemical formations, which has resulted in high levels of arsenic in drinking water (Nickson et al., 2005). This alarming situation demands the development of a simple method for the estimation of arsenic in aqueous medium. The present study reports a low-cost, easy to make and operate, environment-friendly, and efficient sorption-based biosensor technique that uses the biomass of a unicellular red alga *Porphyridium cruentum* dispersed in a graphite–mineral oil paste made into a modified electrode. The main objective of the study was to develop a carbon paste electrode-biosensor able to detect As(III) in drinking water within the WHO limits of below 10 $\mu g\ L^{-1}$. Various experimental conditions, such as deposition potential, pre-concentration time, pH of the arsenic-accumulating medium, biomass quantity, and metal ion concentration were optimized. The studies were further extended to investigate the working ability of biosensor in the presence of interfering alkali metal (AM) and alkaline earth metal (AEM) ions. The selectivity of the developed biosensor towards As(III) in the presence of other metal/heavy metal ions were also studied. The microalga *P. cruentum* (Rhodophyta) used in the present study to develop biosensor is one of the most widely studied microalgae due to its high contents of fatty acids, lipids, polysaccharides and pigment (Wijffels et al., 2013). However, its environmental application as biosorbent/biosensor for the removal/detection from polluted water has not been studied yet. Therefore, present study is the first report of the use of *P. cruentum* to develop modified carbon paste electrode (PC-MCPE)-biosensor for the detection of As(III) in polluted water. This novel hand-made biosensor is expected to become a useful tool for arsenic-free water quality assurance in the arsenic-rich belt running through the developing-country region in south-Asia.

2. Materials and method

2.1. Chemicals

Mineral oil (light, white; Sigma-Aldrich, USA), graphite powder (7–11 μ particle size, 99%; Alfa Aesar, Germany), and analytical grade (Merck, Germany) AsO_3, HCl, NaOH and HNO_3.

2.2. Microalgal biomass production

Biomass used for the preparation of a modified carbon paste electrode-biosensor was of the unicellular red alga *P. cruentum* (CCAP 1380/1A) obtained from the Culture Collection of Algae and Protozoa, Switzerland. The microalga was grown in Erlenmeyer flasks in axenic culture in artificial sea water (ASW) medium (Jones et al., 1963). The pH of ASW medium was adjusted at 7.6. The culture flasks were incubated at 25 ± 1 °C under cool white light continuous illumination (75 $\mu E\ m^{-2}\ s^{-1}$). After 25 day of growth, the microalgal biomass was harvested, washed with double-distilled water, freeze-dried, ground, passed through 250 μ particle size sieves, and stored in desiccators till further use.

2.3. Preparation of the microalgal biomass-based biosensor

Biomass quantity of *P. cruentum* was varied between 0.5% and 7.5% to optimize for maximum arsenic biosorption. The microalgal biomass was well-dispersed in appropriate quantities of graphite powder (64.25–67.25%) and mineral oil (28.25–32.25%) using pestle and mortar to obtain a homogenized paste of good consistency. A polyethylene plastic syringe (internal dia 5 mm × 6 cm length) was chosen as the electrode body (Guo et al., 2011). Nozzle-tip of the syringe was removed with a razor blade before preparing the *P. cruentum*-modified carbon paste electrode (PC-MCPE). The biomass-graphite powder–mineral oil (B-GP–MO) paste was firmly packed into the syringe body and compressed with the syringe plunger to 1 cm height to make the modified sorbent-based biosensor. A copper wire (2 mm external dia × 7 cm length) was inserted into the B-GP–MO paste inside the modified carbon paste electrode-biosensor body to allow the conductance of electric current. A schematic representation of this biosensor is shown as Fig. 1a. Electrical conductivity was checked using a multimeter. After preparing the modified carbon paste electrode-biosensor, its surface was smoothened and cleaned of any residual paste on the sidewalls using a shiny weighing paper. A fresh surface was obtained by removing some of the top-surface paste with the help of the syringe plunger. Unmodified carbon paste electrode (UMCPE) was prepared by mixing graphite powder (90%) and mineral oil (10%) only.

2.4. Experimental procedure

Electrochemical experiments were performed using a Reference 600 TM Potentiostat (Gamry, Germany) equipped with Gamry framework and Echem Gamry Analyst software to perform differential pulse anodic stripping voltammetry (DPASV). The equipment has three electrode cells containing supporting

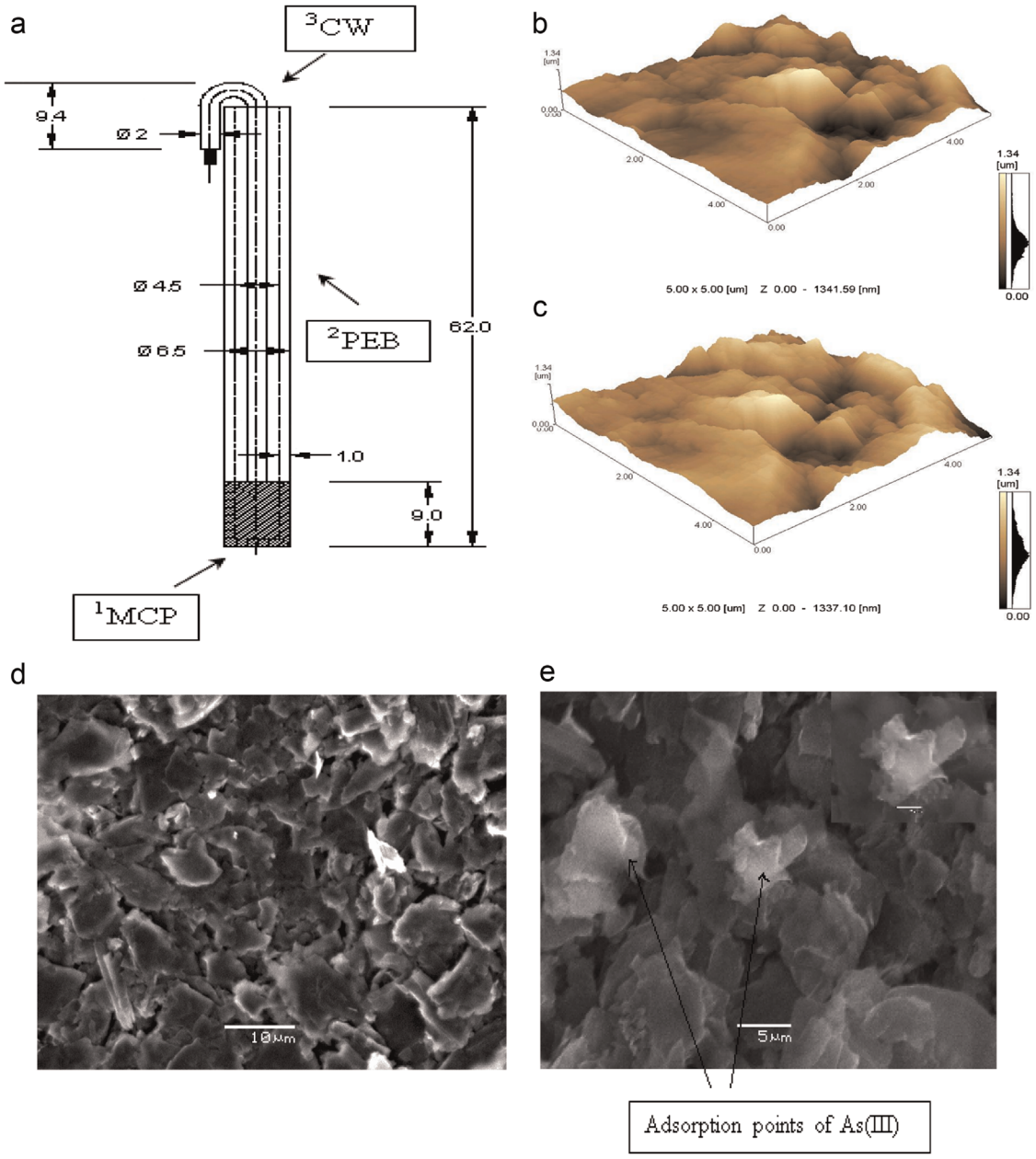

Fig. 1. Schematic representation of the constructed biosensor (a) where 1=modified carbon paste electrode. 2=plastic electrode body and 3=copper wire. All the dimensions are given in mm unit. AFM images of (b) unmodified carbon paste electrode; (c) *P. cruentum* modified carbon paste electrode; SEM of (d) unloaded *P. cruentum* modified carbon paste electrode biosensor; and (e) As(III) loaded *P. cruentum* modified carbon paste electrode biosensor.

electrolyte (0.1 M HNO_3), a working electrode (PC-MCPE), and a reference electrode (saturated calomel electrode) with platinum wire as the counter electrode. Various experimental procedures for determining As(III) were done to optimize working conditions of the PC-MCPE biosensor. In the arsenic-accumulation step, PC-MCPE was immersed in 20 mL of As(III) solution of varying concentrations (2.5–200 $\mu g\,L^{-1}$; prepared from stock $1000 \pm 2\ mg\,L^{-1}$, Merck) having different pH values (4–9) at an open circuit potential (−0.3 to −0.8 V) for various intervals of time (2–15 min). The PC-MCPE biosensor was then removed from the arsenic-accumulation medium, thoroughly washed with double-distilled water and transferred to 0.1 M HNO_3. Finally, an anodic scan was run in the range of −0.8 to +0.8 V. Alongside all experimental procedures, the PC-MCPE biosensor was replaced with a UM-CPE control. A reference stripping peak was obtained at −0.3 V shown in Fig. 2, as was observed for As(III) in a previously reported study (Simm et al., 2005). Thus the working principal of the technique used for As(III) measurement involves two steps, firstly As (III) is reduced to As^0 by applying potential of known intensity followed by stripping off or oxidation of As^0 to As(III) state using an anodic scan range of −0.8 to +0.8 V (Svancara et al., 2002).

Step I

$$As(III) + 3e^- \rightarrow As^0 \quad (1)$$

Step II

$$As^0 \rightarrow As(III) + 3e^- \quad (2)$$

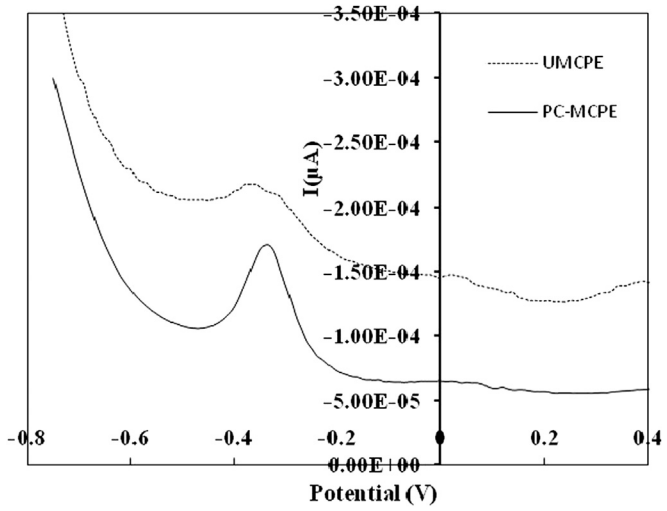

Fig. 2. Stripping peak of 10 μg L^{-1} As(III) solution for UMCPE and PC-MCPE at −0.3 V with accumulation time 8 min, pH 6.0, biomass 5%, −0.8 V deposition potential and 0.1 M HNO_3 supporting electrolyte using differential pulse anodic stripping voltammetry (DPASV).

The efficiency of PC-MCPE biosensor was investigated in the presence of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and anions (NO_3^-, F^-, Cl^- and SO_4^{2-}) at varying concentrations between 75 and 500 mg L^{-1} using 10 μg L^{-1} of As(III) solution. The selectivity of PC-MCPE towards As(III) in the presence of other metal ions such as Fe^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+} and Pb^{2+} at a concentration of 100 μg L^{-1} was also evaluated.

2.5. Surface characterization of P. cruentum and PC-MCPE

Detection of functional moieties present on the surface of *P. cruentum* biomass was performed via Fourier Transform Infrared Spectroscopy with attenuated total reflectance (ATR) in solid phase in the range of 400–4000 wavenumbers cm^{-1} (Alpha Bruker, Germany). The data obtained from FTIR-ATR spectra was evaluated with OPUS 5.5 software. The UMCPE and PC-MCPE electrodes were characterized for average roughness by AFM (Shimadzu, SPM 9500J3). The SEM of unloaded and As(III) loaded PC-MCPE biosensor was done at 1900× magnification with 10 μm marker and 3000× magnification with 5 μm marker respectively for surface morphology (JEOL, JSM-6480). The samples were placed in sample chamber and evacuated to high vacuum (2×10^6 Torr) and bombard with finely focused electron beam and the image formed by the secondary electrons generated by the primary beam was recorded.

3. Results and discussion

3.1. Physico-chemical characterization of P. cruentum biomass and PC-MCPE biosensor

IR spectrum of *P. cruentum* biomass was done to determine the functional moieties present on the surface of the biomass that possibly participate in As(III) binding. Fig. S1 showed the existence of several functional groups present on the surface of algal biomass. Major stretch bands were noted between the regions 2600 and 3600 cm^{-1}. A broad and strong band at 3000–3600 cm^{-1} indicated the presence of free or hydrogen bonded (–H) groups of alcohols and carboxylic acids (Iqbal et al., 2009). The peaks appearing at 1541.73 cm^{-1} and 1150.35 cm^{-1}, respectively, indicated the stretching of N–H and C–O groups (Murphy et al., 2007). Similarly, it can be noted from Fig. 1b and c respectively that the average roughness of the UMCPE decreased from 93.75 nm to 82.72 nm with the addition of *P. cruentum* indicating the successful incorporation of the biomass to prepare PC-MCPE biosensor. SEM image of the unloaded PC-MCPE (Fig. 1d) revealed that its surface contains several micro-cavities, irregularities or uneven surface with depressions on it. These pores were filled (Fig. 1e) in As (III) loaded PC-MCPE biosensor showing the attachment of As(III) to the electrode surface (Pandey and Banerjee, 2012).

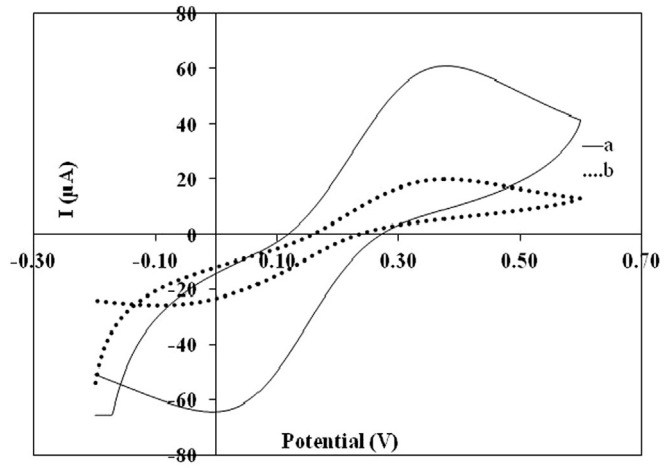

Fig. 3. Cyclic voltammograms of (a) *P. cruentum* modified carbon paste electrode (PC-MCPE) and (b) unmodified carbon paste electrode (UMCPE) in 5 mM $K_3[Fe(CN)_6]$.

3.2. Electrochemical characterization of PC-MCPE

Cyclic voltammetry analysis was carried out for the electrochemical characterization of modified and unmodified electrodes. Fig. 3 shows the cyclic voltammograms of both PC-MCPE and UM-CPE electrodes in 5 mM $K_3[Fe(CN)_6]$ solution. A well defined oxidation and reduction peaks were obtained using *P. cruentum* modified electrode. A greater electrochemical area of PC-MCPE was obtained showing the successful assembling of *P. cruentum* biomass on the electrode surface compared to UM-CPE.

3.3. Optimization experiments for As(III) detection by PC-MCPE biosensor

3.3.1. Stripping conditions for As(III) measurement

Deposition potential is an important parameter and has a significant effect on the anodic peak sensitivity of As(III). The effect of different deposition potentials was studied on the handmade PC-MCPE biosensor within the range of −0.3 to −0.8 V using 10 μg L^{-1} As(III) solution with 0.1 M HNO_3 stripping reagent. Fig. S2a shows that the biosensor response was increased when the potential was increased from −0.3 V to −0.8 V. Similar observations have been reported for *Circinella* sp. modified microbial biosensor for Cu^{2+} determination (Alpat et al., 2008). Further increase in the potential above −0.8 V showed that the surface of PC-MCPE was destroyed due to hydrogen gas generation resulted from the reduction of water molecules (Jahandari et al., 2013). Therefore, −0.8 V was chosen as the optimum deposition potential for further analytical procedure.

3.3.2. Biomass to carbon ratio

The effect of the concentration of algal biomass in carbon paste electrode is shown in Fig. S2b. The biomass ratio affects the peak current intensity as it is directly related to the number of binding

sites available on the surface of electrode. Different biomass concentrations in the range of 0.5–7.5% were used to analyze the response of *P. cruentum* in PC-MCPE using 10 μg L^{-1} As(III) solution. It is seen from Fig. S2b that maximum peak current intensity was obtained when the algal to carbon powder to mineral oil ratio was 5:65:30 (w/w %). However, the peak current intensity was decreased with further increase in algal biomass. The decreased peak response with increased biomass ratio may be attributed to the increased number of excess/surplus binding sites thus decreasing the conductive area of electrode (Estevez-Hernandez et al., 2007; Marino et al., 2003). Thus, 5% *P. cruentum* biomass was used in further experiments.

3.3.3. Effect of pH

pH of the solution is the most critical factor as it affects not only the overall charge present on the biomass surface but also the degree of ionization of the metal ion (Hashim and Chu, 2004; Rahman and Islam, 2009). The effect of pH of accumulating medium using 10 μg L^{-1} As(III) solution is shown in Fig. S2c. The anodic stripping intensity was increased as the pH of the solution was increased from 4 to 6 while decreased as the pH reached to 9. This might be due to the fact that at low pH values (> 4) As(III) exists in neutral form (H_3AsO_3) that restrict its availability for adsorption (Manju et al., 1998). However, as the pH increased up to 6, As(III) ionized to its anionic form as $H_2AsO_3^-$ thus facilitating its accumulation on the surface of PC-MCPE biosensor (Pokhrel and Viraraghavan, 2006). It is clearly seen from Fig. S2c that the anodic peak response was decreased as the pH of the solution raised above 6. This might be due to the increased number of OH^- ions that restricts the availability of $H_2AsO_3^-$ anions (Sari et al., 2011). Thus the repulsive forces exists between the negatively charged functional groups present on the biomass surface and anionic species of arsenite ($H_2AsO_3^-$, $HAsO_3^{2-}$, and AsO_3^{3-}) under alkaline conditions (Rahman et al., 2008).

3.3.4. Effect of accumulation time

Accumulation time plays a key role to investigate the rapidness of response of the handmade PC-MCPE. To optimize the time required for the accumulation of 10 μg L^{-1} As(III) solution on PC-MCPE at −0.8 V deposition potential, different time intervals between 2 and 15 min were investigated. It is seen from Fig. S2d that a characteristic peak appeared within first 2 min of the contact of PC-MCPE and As(III) solution. An increase in the peak response was noted when accumulation time was increased from 2 to 8 min after which a significant decrease was observed on further increase in the accumulation time to 15 min. The increasing in contact time between adsorbate and adsorbent may favors the desorbing process of the bonded metal ions. This desorbing of bonded metal ions thus cause a decrease in peak current intensity of As(III). Similar results have been reported by Al-Ghamdi et al. (2012). The rapid appearance of the peak within 2 min may be attributed to the functional moieties present on the *P. cruentum* surface that have might participated in As(III) binding.

3.4. Analytical characteristics of PC-MCPE biosensor

Fig. 4 shows the dependence of the PC-MCPE biosensor response on different As(III) concentrations ranged between 2.5 and 200 μg L^{-1} at −0.8 V deposition potential, pH 6, time 8.0 min and biomass concentration 5.0% with 0.1 M HNO_3 as stripping media. The PC-MCPE biosensor showed a linear range between 2.5 and 20 μg L^{-1} As(III). However, at concentrations higher than 20 μg L^{-1}, a deviation from linearity was noted, which may have been due to saturation of the binding sites on the electrode surface. Similar results have been reported for voltammetric biosensor developed using carbon paste electro modified

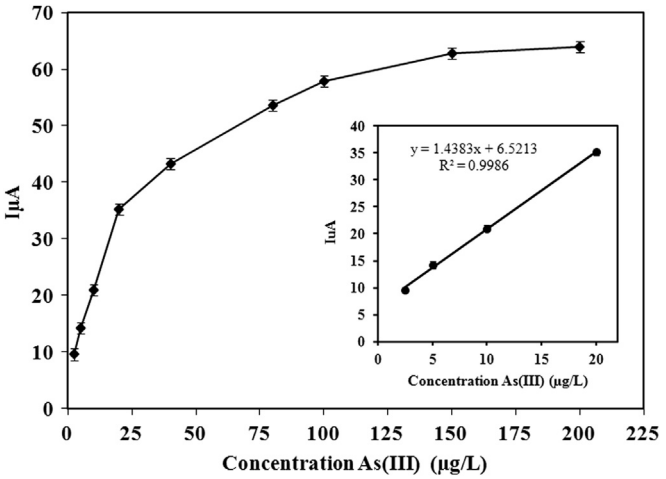

Fig. 4. The effect of different concentrations of As(III) solutions ranging between 2.5 and 150 μg L^{-1} on PC-MCPE. The operational conditions were deposition potential −0.8 V, pH 6.0, time 8.0 min and biomass concentration 5% with 0.1 M HNO_3 stripping media.

with *R. arrhizus* (Yuce et al., 2010b). Concentration calibration plots obtained from 2.5 to 20 μg L^{-1} is presented in Fig. 4 inset. The equation of calibration graph for As(III) is $y = 1.4383X + 6.5213$ ($r^2 = 0.999$). This is the first report on the development of biosensor for the determination of As (III). Some studies on the development of different electrochemical sensors for the detection of As(III) however have been reported in literature (Rajkumar et al., 2011; Kamenev et al., 2005; Sue et al., 2008). Comparison of these electrochemical sensors with the PC-MCPE biosensor developed during the present study shows that the detection limit of our biosensor for As(III) is 1.08 μg L^{-1} which is much lower than the electrochemical sensors. Limit of detection (LOD) value of PC-MCPE biosensor was calculated by using 3 S_b/m criteria (Akyilmaz et al., 2010). Other advantages of our reported biosensor over the electrochemical sensor are its simplicity, biological nature and cost effective technique. The PC-MCPE biosensor has also shown some superiority in terms of its sensitivity range over the biosensors, previously reported for other toxic metals. For example 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} (100 μg L^{-1}). They also found the linear range to be between 1 and 20 mg L^{-1}.

3.5. Working efficiency of the handmade biosensor

3.5.1. Interfering metal ions

It is known that chemically ground water contains major cations like Na^+, K^+, Ca^{2+}, Mg^{2+}, and anions such as NO_3^-, F^-, Cl^- and SO_4^{2-} (Smedley and Kinniburgh, 2002, Parkash et al., 2012). The effect of these alkali (AM—Na^+–K^+) and alkaline earth (AEM—Ca^{2+}–Mg^{2+}) metals ions on As(III) detection is reported in Table 1. No significant decrease was observed in As (III) peak intensity when the concentration of AM–Na^+–K^+ and AEM–Ca^{2+}–Mg^{2+} was set between 75 and 250 mg L^{-1} (sum equivalent to 300–1000 mg L^{-1}). Thus the developed biosensor showed promising results for the detection of As(III) in the presence of high concentrations of interfering cations (Parkash et al., 2012). However, significant decrease in As(III) detection was observed as the concentration increased up to 500 mg L^{-1} (sum equivalent to 2000 mg L^{-1}). This decrease in As(III) signal intensity might be due to the resistance created by the greater size of the ionic radii of Ca^{2+} and Mg^{2+} ions present in the medium. Metal ions with larger ionic radii show greater affinity towards the functional

Table 1
Detection of As(III) by PC-MCPE in the presence of cations and anions. The conditions applied were biomass concentration, 5%; As(III) concentration, 5 μg L^{-1}; cation/anion concentration, 300–2000 mg L^{-1}; pH 6; 0.1 M HNO_3 stripping media; and deposition potential, −0.8 V.

Target ion	Conc. (μg L^{-1})	Interfering medium		Current intensity (μA)	Efficiency (%)	RSD (%)
		Cations/anions	Conc. (mg L^{-1})			
As(III)	5	Control	–	25.25	–	–
		$Na^+ + K^+ + Ca^{2+} + Mg^{2+}$	75+75+75+75	25.24	−0.04	0.04
		$Na^+ + K^+ + Ca^{2+} + Mg^{2+}$	150+150+150+150	25.20	−0.20	0.27
		$Na^+ + K^+ + Ca^{2+} + Mg^{2+}$	250+250+250+250	25.14	−0.44	0.04
		$Na^+ + K^+ + Ca^{2+} + Mg^{2+}$	300+300+300+300	20.26	−19.76	0.05
		$Na^+ + K^+ + Ca^{2+} + Mg^{2+}$	500+500+500+500	19.15	−24.15	0.62
		$NO_3^- + F^- + Cl^- + SO_4^{2-}$	75+75+75+75	25.14	+0.43	0.1
		$NO_3^- + F^- + Cl^- + SO_4^{2-}$	150+150+150+150	25.28	+0.12	0.03
		$NO_3^- + F^- + Cl^- + SO_4^{2-}$	250+250+250+250	25.32	+0.27	0.1
		$NO_3^- + F^- + Cl^- + SO_4^{2-}$	300+300+300+300	25.37	+0.47	0.07
		$NO_3^- + F^- + Cl^- + SO_4^{2-}$	500+500+500+500	25.66	+1.62	0.1

Table 2
Comparative sensitivities in peak intensities of As(III) by PC-MCPE biosensor in the presence of Ni^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Mn^{2+} and Fe^{3+} ions. The experimental conditions were biomass concentration, 5%; As(III) to other metal ions ratios, 1:20; pH 6; stripping media, 0.1 M HNO_3; and deposition potential, −0.8 V.

Target ion	Conc. (μg L^{-1})	Competitive selection		Current intensity (μA)	Decreased selectivity (%)	RSD (%)
		Metal ions	Conc. (μg L^{-1})			
As(III)	5	Control	–	25.25	–	–
		As(III)+Ni(II)	5+100	25.24	0.04	0.08
		As(III)+Cd(II)	5+100	22.07	12.59	0.22
		As(III)+Pb(II)	5+100	21.77	13.78	0.32
		As(III)+Hg(II)	5+100	21.00	16.83	0.05
		As(III)+Cu(II)	5+100	20.49	18.85	0.20
		As(III)+Mn(II)	5+100	11.24	55.48	0.08
		As(III)+Fe(III)	5+100	9.21	63.52	0.21

moieties present on the bio sorbent (Kotrba et al., 2011). This is the reason why Ca^{2+} and Mg^{2+} interfere in As(III) binding on PC-MCPE and decrease peak current intensity for As(III). On the other hand alkali metals like Na^+ and K^+ also have larger ionic radii than As^{3+}. However, these metal ions lack the ability to complex with a number of ligand/functional groups present on the surface of biosorbent (Kotrba et al., 2011). On the other hand anions did not show any significant interference in electrochemical detection of As(III) up to 300 mg L^{-1} (sum equivalent to 1200 mg L^{-1}). However, a slight increase in current intensity was observed as the concentration of anions raised up to 500 mg L^{-1} (sum equivalent to 2000 mg L^{-1}). This increase is related to the As(III)-anion complex formation during the accumulation step of voltametric determination of As(III) by PC-MCPE (Parkash et al., 2012).

3.5.2. Competitive response of PC-MCPE biosensor in the presence of other metallic ions

The competitive behavior of PC-MCPE biosensor towards As(III) in the presence of other metallic cations viz., Fe^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+} and Pb^{2+} was evaluated. Current intensity of 5 μg L^{-1} As(III) solution was measured in the presence of 20 times higher concentration of the corresponding competitive metal ion. It is seen from Table 2 that the intensity of As(III) peak decreased significantly in the presence of these metallic species expect for Ni^{2+} that did not cause any change in As(III) peak signal. The decrease in analyte signal in the presence of Cu^{2+} ions might be due to the formation of intermetallic compounds with As(III) (Giacomino et al., 2011). However, the decrease in As(III) signal intensity with respect to Cd^{2+}, Pb^{2+} and Hg^{2+} is related to the >fact that the competition exists between As(III) and these metal ions for the occupancy of the binding sites present on the biomass of *P. cruentum* incorporated in carbon paste (Dai et al., 2004). On the other hand, Fe^{3+} and Mn^{2+} have drastically decrease the As (III) peak intensity. This decrease occurred due to the fact that an inter-competition exists among the ions of iron, manganese and arsenic as they belong to the group of hard metal ions and compete for the same binding sites present on the electrode surface (Appenroth, 2010).

4. Conclusion

In the present study, algal biomass of *P. cruentum* was used to construct an electrochemical sensor that detects As(III) as low as 2.5 μg L^{-1} which is 4 times lower than the maximum allowable WHO limit of 10 μg L^{-1} in drinking water. This biosensor is a low cost, easy to prepare and operate and gives reliable results in the presence of several interfering cations and anions. Besides its low cost and easy preparation, it exhibits comparable efficiency to the other reported electrochemical sensors. Further application of the constructed PC-MCPE will be to detect As(III)contamination in ground water/real waste water samples.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at: http://dx.doi.org/10.1016/j.bios.2014.06.055

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