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Graphite epoxy composite electrodes modified with bacterial cells

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Abstract

The modification of a graphite–epoxy composite electrode (GECE) with bacterial cells along with an analytical application are presented. *Pseudomonas putida* DSM 50026 was used as a biological component and the measurement was based on the respiratory activity of the cells. The optimization of working conditions of resulting biosensor (including pH and temperature) was conducted and the limit of detection was calculated as 7 μM phenol based on the signal to noise ratio. Then the system was applied for xenobiotic detection. Resulting sample signals were found to be very similar with the standard solutions having the same concentration while the recoveries of the spiked samples were close to 100%.
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Keywords: Microbial biosensor; Graphite–epoxy composite electrode; *Pseudomonas putida*

1. Introduction

It is well known that biosensors are small devices that combines a biological component with a physico-chemical transducer. Carbon based electrodes like carbon paste and glassy carbon electrodes have been widely used for this purpose [1]. Carbon, in many respects, is an ideal electrode substrate due to its wide anodic potential range, low residual current, chemical inertness, and low cost. In addition, carbon electrodes exhibit fast response and can easily be fabricated in different configuration and sizes.

Another alternative to the previous carbon electrodes are the rigid carbon-polymer composite (or biocomposite if a biological molecule is included inside) based electrodes that can often be fabricated with great flexibility in size and shape, permitting easy adaptation to a variety of electrode configurations (conventional, flow-through, screen-printed etc.) [2–9]. Their surfaces can be smoothed or polished to provide fresh active material ready to be used in a new assay (an interesting issue for biosensor devices). Each new surface yields reproducible results because all individual compounds are homogeneously dispersed or compressed in the bulk of the composite. Moreover the composite electrodes have higher signal-to-noise (S/N) ratio (due to their random assembly of microelectrode – RAM behaviour – of the exposed surface) compared to the corresponding pure conductors, that accompanies an improved (lower) detection limit [7].

(Bio)composite materials have proved to be robust, easily machinable and excellent reservoir for biological materials. These materials are suitable for the fabrication of thick-film biosensors in a single step [7].

In this study, for the first time a graphite epoxy composite electrode (GECE) is modified with bacterial cells and its utility as a microbial biosensor is checked out. The use of microbial biosensors to determine the concentrations of substances is based on the presence of specific enzyme systems in microorganisms which transform certain chemical compounds [10]. The transformation processes can be accompanied by the appearance of electrochemically active products or utilization of reaction co-substrates, which enable the use of standard electrochemical techniques—amperometry or potentiometry [11]. As judged by their sensitivity, time of response and stability of signals, microbial sensors are similar to enzyme based sensors but are less selective. This may be due to the complexity of the elements of the enzyme apparatus of cells. Insignificant amount of biomass as well as high stability makes

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the use of microbial sensors preferable in some cases compared to enzyme sensors. This is especially true in the detection of a pool of toxic compounds showing similar composition, or the assessment of comprehensive indices of the condition of the environment as, for instance, biological oxygen demand (BOD) [12,13].

Pseudomonas putida DSM 50026 which is one of the well-known phenol degrading organisms was used as a biological component and the measurement was based on the respiratory activity of the cells. For this purpose, the cells were grown in the presence of phenol as the source of organic carbon. The characterization of the system was performed and then resulting biosensor was applied for xenobiotic detection.

2. Experimental

2.1. Apparatus

Chronoamperometric experiments were carried out with a Radiometer electrochemical measurement unit (France). The electrodes were inserted into the cell through its Teflon cover. Ag/AgCl (including 3 M KCl with saturated AgCl as an internal solution, Radiometer Analytical, REF321) and Pt (Radiometer Analytical, M241PT) were used as reference and counter electrodes, respectively.

2.2. Reagents

Phenol was purchased from Merck AG (Darmstadt, Germany) while 300 bloom calf skin gelatin and glutaraldehyde were obtained from Sigma Chem. Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Mineral salts medium (MSM) with the following composition was used as a growth medium; 0.1% NH_4NO_3, 0.05% $(NH_4)_2SO_4$, 0.05% NaCl, 0.05% $MgSO_4$ $7H_2O$, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.0014% $CaCl_2$ $2H_2O$, 0.001% $FeSO_4$ $7H_2O$ and trace element solution (1 mL/L). The pH of the medium is 6.9 [14].Synthetic waste (phenol containing) water composition; 50 g/L NaCl and 100 g/L phenol in 1.0 M HCl solution [15].

2.3. Biological material

Pseudomonas putida DSM 50026 was sub-cultured on Nutrient Agar. Adaptation of the cells to phenol (250 mg/L) was performed with the following steps; organism was inoculated into MSM containing gradually increasing phenol and decreasing glucose concentrations by daily inoculations until medium contained 250 mg/L phenol (without glucose). After adaptation was completed, the cellular paste was obtained. After 24 h, when cells were grown, the biomass was harvested by centrifugation at 10 000 rpm suspended in MSM and then re-centrifuged. The supernatant was removed and the cellular paste was used as modifier of a GECE so as to build the biosensor. Cell growth was followed spectrophotometrically by measuring optical density at 560 nm. The relationships between optical density and living cells were also investigated [16]. In order to obtain reproducible results, daily inoculated bacteria were used in all experiments and all biosensors were daily prepared. Also, microbial electrodes were immersed into MSM (mineral salt medium) containing phenol between the measurements.

2.4. Preparation of biosensor

Pseudomonas putida cells which have 7×10^7 cell titer (100 μL) and 300 bloom gelatin (10 mg) were mixed at 38 °C in 50 mM phosphate buffer (pH 7.5), (150 μL). 50 μL of mixed solution was placed onto the GECE working electrode surface and allowed to dry at 4 °C for 45 min. Finally, it was immersed in 2.5% glutaraldehyde in 50 mM phosphate buffer (pH 7.5) for 5 min [17,18].

2.5. Measurements

All measurements were carried out at 30 °C under continuous and constant magnetic stirring. After each run, the electrode was washed with distilled water and kept in 50 mM phosphate buffer (pH 7.0) solution at 30 °C for 10 min. Standard additions of phenol into the reaction cell were performed. The current density (μA/cm^2) changes were registered with a potentiostat at −0.7 V. At the optimal working conditions, the current density was assumed as 100% and other values calculated relative to this value.

3. Results and discussion

In the previous works, GECEs were combined with various types of enzymes. Morales et al. describe the preparation and evaluation of reagentless biosensors based on NADP-dependent dehydrogenases using graphite–methacrylate biocomposites. The measurement was based on the oxidation of cofactor [19]. Moreover, glucose oxidase was mixed with tetrathiafulvalene–tetracyanoquinodimethane (TTF TCNQ) and epoxy resin for obtaining biocomposite for glucose detection both for batch and FIA systems [20]. In this study bacterial cells were immobilized onto the surface of GECE by means of gelatin membrane which is then cross-linked with glutaraldehyde. Phenol oxidation takes place after each addition causing a decrease of oxygen concentration in the bioactive layer sensed as a decrease in the current. The response of a 'blank' GECE (GECE with a similar modification but without bacterial cell) to dissolved oxygen level in the medium was checked at −0.7 V prior to the immobilization step. Instead of applying the biological material directly onto the graphite epoxy surface, gelatin membrane was used as immobilization matrix that can have profound effect on protecting metabolic activity of the cells.

3.1. Effect of pH

According to the optimization studies, the effect of pH on the electrode response was investigated by using phosphate buffer systems (50 mM) between pH 5.5 and 8.0 (increments of 0.5) for 20 μM phenol (Fig. 1). The response current of the biocomposite electrode to phenol increases significantly from

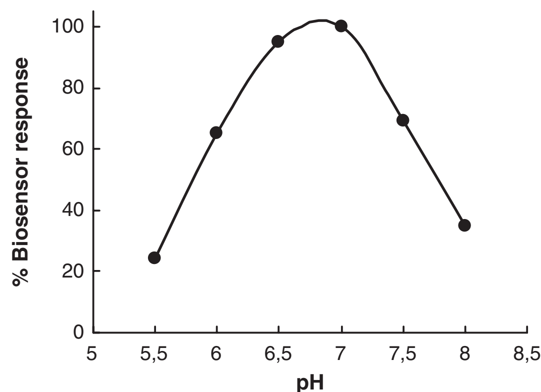

Fig. 1. The pH effect (increments of 0.5) on the biosensor response toward 20 μM phenol at 50 mM phosphate buffer and 30 °C.

pH 5.5 to 7.0, and then a sharp decrease is obtained at pH values higher than 7.0. As a result pH 7.0 was chosen as optimum pH and used for further studies.

3.2. Effect of temperature

The amperometric response of the microbial electrode to 20 μM phenol was measured at different temperatures varying from 20 to 40 °C and the results are shown in Fig. 2. From 20 to 25 °C, a sharp increase is observed that lasts up to 30 °C which is very close to growth temperature and then the amperometric response decreases at 35 °C. As at 30 °C, best current value was observed, further experiments were conducted at this temperature.

3.3. Stability

The stability of cell based biosensor was investigated at working conditions (30 °C in phosphate buffer) and a 24% decrease of activity is observed after 5 h (results not shown). During this period approximately 20 measurements have been made and it could be possible to make more measurements in a more longer time period.

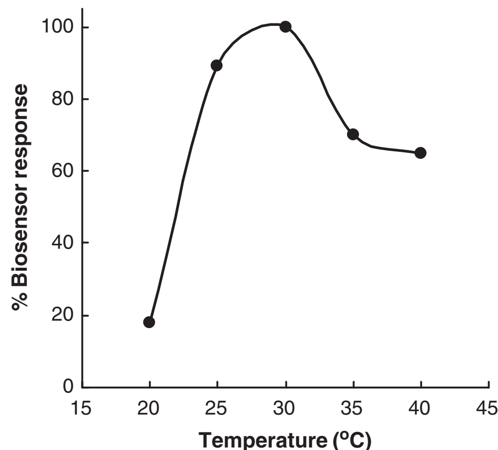

Fig. 2. The effect of temperature on the biosensor response at pH 7. Other conditions as in Fig. 1.

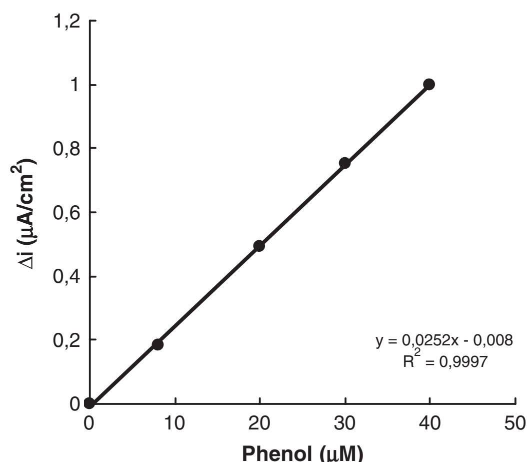

Fig. 3. The biosensor response to phenols at pH 7 and 30 °C. Other conditions as in Fig. 1.

3.4. Analytical characteristics

Linear range: A linearity for the biosensor was obtained in concentration range between 8 and 40 μM of phenol under the response time of 170 s. (Fig. 3). At higher concentrations, standard curve showed a deviation from linearity.

The repeatability of the biosensor was tested for 20 μM of phenol ($n=5$) and the standard deviation (S.D.) and variation coefficient (cv) were calculated as ±0.25 mM and 1.2%, respectively. The detection limit value was estimated as 7 μM phenol based on the signal to noise background characteristic of these data.

3.5. Sample application

Developed biosensor was used for phenol detection in waste water samples. Synthetic waste water samples with highly acidic and salty nature including 20.0 and 30.0 μM phenol were used as stock substrate solutions with different dilutions by working buffer and added to the reaction cell after equilibration and then the signal was measured. The phenol amounts in samples were calculated from calibration curve as 19.7±0.8 and 30.7±0.6 μM, respectively. Results are expressed as the mean±S.D., ($n=4$). The recoveries of the spiked samples were close to 100%. Our data showed that, no sample matrix effect due to the salty and acidic nature interfered in our measurements. The developed biosensor shows promising results to be used in the analysis of similar waste water samples without requiring previous pre-treatments.

4. Conclusion

Combination of microorganisms with graphite epoxy composite electrodes (GECEs) provides economic and practical disposable biosensors. In this study GECEs modified with bacterial cells were developed for determining phenol. The developed microbial biosensor is inexpensive and easily produced, and possesses an adequate sensitivity and stability

comparable with those of enzyme based biosensors. Additionally the use of this kind of biosensor avoids the purification of the enzyme rending the biosensors production easier.

The amperometric response current of the developed biosensor displays a linear relationship with respect to the concentrations of phenol in the range of 8–40 μM while the detection limit (LOD) was estimated as 7 μM. The DL is almost 1000 times lower when compared with the thick film microbial biosensor based on the same type of bacterial cells and conventional oxygen electrode [17]. The obtained biosensor seems to be an interesting, simple and rapid alternative to other methods regarding the direct determination of xenobiotics in waste water samples without requiring sample pre-treatment.

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