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Development of a microbial biosensor based on carbon nanotube (CNT) modified electrodes

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Abstract

Pseudomonas putida DSM 50026 cells were used as the biological component and the measurement was based on the respiratory activity of the cells estimated from electrochemical measurements. The cells were immobilised on carbon nanotube (CNT) modified carbon paste electrodes (CPE) by means of a redox osmium polymer, viz. poly(1-vinylimidazole)$_{12}$-[Os-(4,4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$. The osmium polymer efficiently shuttles electrons between redox enzymes located in the cell wall of the cells and promotes a stable binding to the electrode surface. The effect of varying the amounts of CNT and osmium polymer on the response to glucose was investigated to find the optimum composition of the sensor. The effects of pH and temperature were also examined. After the optimisation studies, the system was characterised by using glucose as substrate. Moreover, the microbial biosensor was also prepared by using phenol adapted bacteria and then, calibrated to phenol. After that, it was applied for phenol detection in an artificial waste water sample.

Keywords: Biosensor; Osmium redox polymer; Carbon nanotube (CNT); *Pseudomonas putida*

1. Introduction

The recent discovery of the carbon nanotube (CNT) has attracted considerable attention due to their dimensions and structure sensitive properties. The high electrical conductivity of these nanoparticles allows the utilisation of CNTs as electrode material and in combination with its strong electrocatalytic activity offer the ability to mediate electron transfer reactions [1,2]. The facility of electron transfer between the electroactive species and the electrode offers great promise for fabricating chemical sensors or biosensors [3,4]. Besides using CNTs, the introduction of redox mediators (RM) i.e., redox active compounds able to shuttle electrons between the active site of redox enzymes and an electrode replacing the natural co-substrate of the enzyme and when incorporated into biosensor structures, reagentless second generation amperometric biosensors can be obtained.

RMs based on various $Os^{2+/3+}$-complexes have been successfully introduced into high molecular weight, yet highly flexible and aqueous soluble polymers [5] that form electrostatic complexes with redox enzymes to form hydrogels allowing both very efficient charge transfer reactions between the enzyme and the mediators as well as between the dispersed $Os^{2+/3+}$ redox centres and additionally allow fast diffusion of both enzyme substrate and product within the hydrogel. These hydrogels can be further stabilised by forming covalent linkages between the redox polymer and the protein promoting a stable immobilisation of both mediator and enzyme as well as the possibility for multiple layers of immobilised protein molecules on the electrode surface [6]. Moreover, it is possible to control through the use of different ligands the formal potential of the $Os^{2+/3+}$ redox functionality and hence the electron transfer properties [7,8]. For these reasons, since the first usage of

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osmium redox polymers for reagentless mediated biosensors [9–11], polymeric mediators still attract attention.

Whole microbial cells as well as isolated enzymes are frequently used for the construction of biosensors and transformation of organic compounds in bioreactors [12,13]. It is well-known that microbial cells are able to catalyse the oxidation of such organic compounds as glucose and ethanol via redox compounds acting as electron acceptors [14]. Mediated electron transfer from microbial systems to electrodes represents a promising alternative to the use of Clark electrodes [15,16]. The same basic principle can also be used in the operation of microbial fuel cells (MFCs), which are bio-electrochemical transducers that convert microbial reducing power (generated by the metabolism of organic substrates), into electrical energy [17,18]. Moreover, perturbations in microbial respiration due to changes in substrate or microbial concentration have previously been detected via the interaction of redox mediators at electrochemical transducers and are the basis for a number of devices [19–24].

Mediated whole-cell biosensors based on *Gluconobacter oxydans* and poly(1-vinylimidazole)$_{12}$-[osmium-(4, 4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ [25] and also based on the bacteria *Pseudomonas putida* ATCC 126633 and *Pseudomonas fluorescens* were previously developed using two different flexible osmium redox polymers; poly(1-vinylimidazole)$_{12}$-[osmium-(4, 4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ and poly(vinylpyridine)-[osmium-(*N*,*N*′-methylated-2,2′-biimidazole)$_3$]$^{2+/3+}$ by our group and the efficiency of these polymers for "bacterial wiring" was investigated [26]. In this work, CNT and poly(1-vinylimidazole)$_{12}$-[osmium-(4,4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ (Fig. 1) were utilised together as a part of a microbial biosensor and the possibility to facilitate electron transfer reactions by these two systems were explored for the first time as far as we know. The integration of an osmium redox polymeric mediator in combination with CNTs as parts of a mediated microbial biosensor has not been accomplished before to the best of our knowledge.

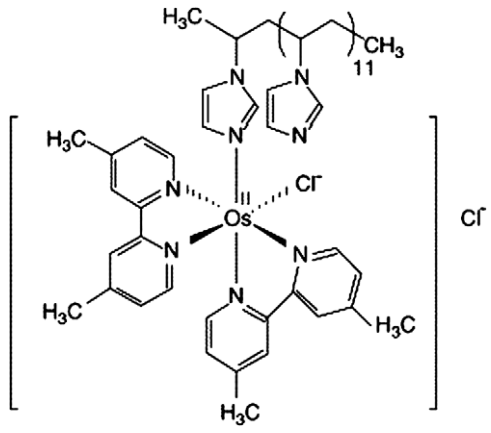

Fig. 1. Chemical structure of osmium redox polymer [poly(1-vinylimidazole)$_{12}$-[Os-(4,4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$].

In the present work, whole viable *P. putida* DSM 50026 cells were immobilised on CNT modified carbon paste electrode (CPE) using the osmium redox polymer. After the investigating the effect of the amount of CNT and Os redox mediator on the sensor response, the biosensor was characterised using glucose as substrate. Moreover, the system was also calibrated for phenol using bacterial cells adapted to phenol as the biological material.

2. Material and methods

2.1. Reagents and materials

Poly(1-vinylimidazole)$_{12}$-[Os-(4,4′-dimethyl-2, 2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ was generously provided as a gift from TheraSense (Alameda, CA, USA). Multiwall CNT (diameter; 110–170 nm, length; 5–9 μm), mineral oil and graphite powder were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without any pre-treatment since further purification with hot strong acids and treatment with ultrasonic bath had no significant effect on the sensor response. Standard solutions of D-(+)-glucose, D-(+)-galactose, and phenol were of analytical grade and were prepared by dissolving appropriate amounts in the working buffer solutions. Dialysis membranes with a cut-off of 6000–8000 Da were used.

Mineral salt medium (MSM) with the following composition was used as growth medium for *P. putida*; 0.1% NH_4NO_3, 0.05% $(NH_4)_2SO_4$, 0.05% NaCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.0014% $CaCl_2 \cdot 2H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$ and trace element solution (1 ml/L). The pH of the growth medium was adjusted to 6.9 [27].

P. putida DSMZ 50026 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and was sub-cultured in nutrient agar. Then, the cells were inoculated into 50 mL of MSM containing 250 mg/L glucose and incubated at 28°C on an orbital shaker at 150 rpm. Cells adapted to 250 mg/L phenol were obtained by gradually increasing the phenol and decreasing the glucose (250 mg/L) concentrations by daily inoculations until the medium contained 250 mg/L phenol. When the cells were grown, the biomass was harvested by centrifugation at 10000×*g*, suspended in MSM and then re-centrifuged. The cellular paste was used for making biosensors [28]. All optimisation studies were performed with non-adapted cells using glucose as substrate. However, the adapted cells were used only to calibrate the system to phenol and for sample application. Cell growth was followed spectrophotometrically by measuring the optical density at 560 nm and the relationship between the optical density and bacterial mass was also investigated [25]. In all experiments, log-phase bacterial cells were used. Daily prepared electrodes with fresh cells were used in all experimental steps.

Artificial waste water of a highly acidic and salty nature included 50 g/L NaCl and 100 g/L phenol in 1.0 M HCl solution [27].