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Short communication

A microbial biosensor based on bacterial cells immobilized on chitosan matrix

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ABSTRACT

A bio-electrochemical system consisting of *Gluconobacter oxydans* DSM 2343 cells as a biological material and carbon nanotube (CNT)-free and CNT-modified chitosan as immobilizing matrices has been developed. The measurement was based on the respiratory activity of the cells estimated by the oxygen consumption at −0.7 V (versus the Ag|AgCl reference electrode) due to the metabolic activity in the presence of substrates. The system was calibrated and dependence of signal amplitude on the measuring conditions and cell amount was studied as well as the substrate specificity, pH, temperature and working potential. The biosensors (CNT-modified and unmodified) were demonstrated for the quantification of glucose in the range of 0.05–1.0 mM, at 30 °C and pH 7.0 with the 40 s of response time. The linear relationships between sensor response (y; μA/cm^2) and substrate concentration (x; mM) were defined by the equations of $y=1.160x+0.151$ ($R^2=0.990$) and $y=1.261x+0.197$ ($R^2=0.982$), respectively. All other data were also given as comparison of two systems one with CNT-modified and CNT-free.

1. Introduction

Gluconobacter oxydans is an obligate aerobe, having a respiratory type of metabolism using oxygen as the terminal electron acceptor and a gram-negative bacterium belong to the *Acetobacteraceae* [1]. These organisms have been used since ancient times in biotechnological processes like the vinegar and vitamin C production, [2] and are still used for industrial applications [3,4]. *G. oxydans* has been previously used industrially to produce polysaccarides, cellulose, dextran [5], L-sorbose from D-sorbitol [6], dihydroxyacetone from glycerol [7], L-riboluse from ribitol [8], tagatose from D-galactitol [9], gluconic acid from glucose [10]. Moreover, *G. oxydans* has been identified as a prospective biocatalyst for saccharide and alcohol biosensors because of the membrane localization of oxidative enzymes [11]. Most of the reactions are catalyzed by membrane-bound dehydrogenases, whose reactive centers are oriented to the periplasmic space so that transport of substrates into the cell is not necessary [12].

Since cofactor and recycle systems form part of the cells' metabolism, application of whole microbial cells can be very attractive when oxidations are involved [13]. Whole cell microbial sensors have received recent attention; because enzyme purification is unnecessary, whole microbial sensors are simple and inexpensive systems to construct and [14] enzymes are usually more stable in their natural environment in the cell [15]. On the other side, microbial biosensors have some disadvantages in compare to the enzyme biosensors: in many cases they have a low selectivity and longer response time [16]. When viable microbial cells are involved a gentle immobilisation technique should be used to keep the cells in their metabolic active forms. Entrapment into the natural polymers prepared from alginate/pectate, κ-carrageenan, collagen, gelatin, chitosan and agar (agarose) can be performed under mild conditions with high viability of cells entrapped [17].

Chitosan (CHIT), the primary derivative of chitin, is obtained by *N*-deacetylation to a varying extent that is characterized by the degree of deacetylation, and is consequently a copolymer of *N*-acetylglucosamine and glucosamine [18,19]. Increasingly over the last decade chitin- and chitosan based materials have been examined and a number of potential products have been developed for areas such as wastewater treatment, the food industry, agriculture, pulp and paper industry, cosmetics and toiletries, medicine and biotechnology, in biosensors as an immobilization platform [20]. Recently, CHIT has been used as an effective dispersant of carbon nanotubes (CNTs). The resulting biocompatible composite of CHIT–CNT has been applied as matrix to immobilize biological materials [21–25].

CNTs are built from sp^2 carbon units and present a seamless structure with hexagonal honeycomb lattices, being several nanometers in diameter and many microns in length [26]. CNTs have attracted tremendous interest in analytical chemistry because of their unique properties, such as high electrical conductivity, high mechanical properties, and ability to grown on different substrates, and nanoscale size with a high aspect ratio [27]. In recent years, many works have been published using CNTs together with biological materials in biosensors, drug and vaccine delivery vehicles and novel biomaterials [28].

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In the present study, *G. oxydans* cells were immobilized onto the graphite electrode via CHIT matrix. The response characteristics, stabilities and substrate specificities were investigated as well as the effect of the presence of CNT as a modifier on the CHIT membrane.

2. Experimental

2.1. Reagents

D(+)-glucose, D(+)-mannose, D(+)-galactose, D(+)-xylose, β-lactose, chitosan (CHIT, from crab shells, minimum 85% deacetylated), glutaraldehyde were purchased from Sigma Chem. Co. (St. Louis, MO, USA), multiwalled carbon nanotube (MWCNT, diameter; 110–170 nm, length; 5–9 μm, 90%) were obtained from Aldrich (Dorset, UK), D(−)-fructose, N-acetyl D-glucosamine, maltose monohydrate were purchased from Fluka (Steinheim, Germany). All other chemicals were analytical grade.

To prepare CHIT–CNT solutions, different amounts of MWCNTs were dispersed in CHIT solutions and the blends were treated under an ultrasonic field for 10 min. This CHIT–CNT blend was then stirred for 1 h. During this step, chitosan macromolecules were adsorbed on the surface of the CNTs thereby acting as polymer cationic surfactants to stabilize the CNTs [29].

2.2. Apparatus

Chronoamperometric measurements were carried out by Radiometer electrochemical measurement unit (Lyon, France) where *G. oxydans* modified graphite electrode were used as working electrode. Ag|AgCl electrode (with 3 M KCl saturated with AgCl as the internal solution, Radiometer Analytical, REF321) and platinum electrode (Metrohm, Switzerland) were used as reference and counter electrodes, respectively. The electrodes were inserted into a conventional electrochemical cell (20 mL) through its Teflon cover. Scanning electron microscope (JEOL JSM 5200 SEM, Tokyo, Japan) was used for surface imaging.

2.3. Biological material

G. oxydans DSMZ 2343 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and was sub-cultured in media containing (in g/L): glucose, 100; yeast extract, 10; calcium carbonate, 20; agar, 20. Then, cells were cultivated in shake flasks on a shaker (175 rpm) at 28 °C, in media containing (in g/L): yeast extract, 5; carbon source: glucose, 5 [30]. Cells at late exponential phase were harvested by centrifugation at 6 000 rpm for 15 min. Cells were washed by 0.9% NaCl and re-centrifuged. Obtained cellular paste was used for biosensor construction. Cell growth was followed spectrophotometrically by measuring the optical density at 600 nm [31].

2.4. Biosensor preparation

For the construction of the bioactive layer on the graphite electrode, the spectrographic graphite rods (Ringsdorff Werke GmbH, Bonn, Germany, 3.05 mm diameter and 13% porosity) were polished and washed thoroughly with distilled water and sonicated for 2 min.

20 μL of CHIT or CHIT–CNT solutions were placed on the electrode and allowed to dry at +4 °C for 18 h. Then 10 μL of the cellular paste and 20 μL of glutaraldehyde solution (in potassium phosphate buffer, pH 7.0) spread evenly onto the chitosan modified graphite electrode and allowed to stand at ambient conditions for 30 min. Then, it was washed with distilled water to remove unbound cells [32]. During the experimental steps microbial electrodes containing daily inoculated cells were prepared every day.

2.5. Measurements

The principle of measurement was based on the following of oxygen consumption due to the respiratory activity of microorganisms in the presence of glucose. Oxygen was reduced electrochemically by the working electrodes by applying a potential of −0.7 V (versus the Ag|AgCl reference electrode). After each measurement, the electrodes was washed with distilled water and kept in working buffer solution at 30 °C for 3 min. When the current density reached a steady state, the substrate solution was added to the working buffer solution (50 mM PBS (phosphate buffer saline), pH 7.0) until reached a new steady state. The sensor response was defined as the difference between first and second steady state currents and registered as current density (μA/cm^2).

3. Results and discussion

The major motivation behind the use of intact cells as biocatalysts in biosensors is the ease of preparation and the accessibility of enzymatic activities not available otherwise. *Gluconobacter sp.* is particularly suitable for biosensor construction due to the some attractive features that these species grow very fast in simple cultivation media, contain high activities, and relatively stable during immobilization, as well as their PQQ dependent dehydrogenases do not require an external cofactor to be added [33]. In previous works, *G. oxydans* cells were immobilized on the surface of different transducers by means of various polymeric membranes such as osmium redox polymers [34,35], cellulose acetate [30,36], gelatin cross-linked with glutardialdehyde [33] etc. and mediated and non-mediated microbial sensors were fabricated. Additionally, another type of system was characterized by physical adsorption of the bacterial cells to Whatman GF/A chromatographic paper as a support matrix [14,37,38]. On the other hand, a fuel cell assembly was constructed using *G. oxydans* cells

Table 1
Comparison of analytical characteristics of various *G. oxydans* biosensors reported in literature

Electrode	Analyte	Immobilization method	Working potential (mV)	Analytical performances		Reference
				Linear range	Operational stability	
GCE	PDO	DM with a silicone rubber O-ring	+300 (in the presence of the ferricyanide)	0.3 mg/L–1.2 g/L	Stable over the 10 h (0.66% h^{-1})	[35]
GCE	PDO	OP with a silicone rubber O-ring	+150	0.8 mg/L–1.5 g/L	70% decrease after 10 h (0.47% h^{-1})	[35]
CE	Glc	By adsorption onto CP	–	0.0–10.0 mM	–	[38]
CE		By adsorption onto CP	–	0.0–10.0 mM	–	[38]
CE	EtOH	By physical adsorption, using CP	–	0.05–10 mM	Stable for no less than 10–12 h	[37]
GE	Glc	DM with a O-ring	+300	0.002–2.2 g/L	No decrease over 9 consequent measurements	[11]
GDE	EtOH	OP with a silicone rubber O-ring	+300	0.25–2.5 mM	50% decrease after 51 h	[34]
GDE	GOH	OP with a silicone rubber O-ring	+300	2.5–45.0 mM	50% decrease after 68 h	[34]
GDE	Glc	OP with a silicone rubber O-ring	+300	0.25–2.0 mM	50% decrease after 3 h	[34]
CE	Xyl	On CP by simple physical adsorption	–	0.5–20 mM	–	[14]
GCE	EtOH	CA membrane	+300	2–270 μM	75% decrease after 4.5 h,	[30]
GE	Glc	Unmodified CHIT	−700	0.05–1.0 mM	24% decrease after 5 h	This work
GE	Glc	CNT-modified CHIT	−700	0.05–1.0 mM	15% decrease after 5 h	This work