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A highly efficient buckypaper-based electrode material for mediatorless laccase-catalyzed dioxygen reduction

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ABSTRACT

The redox enzyme laccase from *Trametes versicolor* efficiently catalyzes the oxygen reduction reaction (ORR) in mediatorless biofuel cell cathodes when adsorbed onto multi-walled carbon nanotubes (MWCNTs). In this work we demonstrate that the fabrication of MWCNTs in form of buckypaper (BP) results in an excellent electrode material for laccase-catalyzed cathodes.

BPs are mechanically stable, self-entangling mats with high dispersion of MWCNTs resulting in easy to handle homogeneous layers with highly mesoporous structures and excellent electrical conductivities.

All biocathodes have been electrochemically investigated in oxygen-saturated buffer at pH 5 by galvanostatic polarization and potentiodynamic linear sweep voltammetry. Both methods confirm an efficient direct interaction of laccase with BP with a high open circuit potential of 0.882 V vs. normal hydrogen electrode (NHE). The high oxygen reduction performance leads to high current densities of $422 \pm 71\,\mu A\,cm^{-2}$ at a typical cathode potential of 0.744 V vs. NHE.

When the current density is normalized to the mass of the electrode material (mass activity), the BP-based film electrodes exhibit a 68-fold higher current density at 0.744 V vs. NHE than electrodes fabricated from the same MWCNTs in a non-dispersed agglomerated form as packed electrodes. This clearly shows that MWCNTs can act more efficiently as cathode when prepared in form of BP. This can be attributed to reduced diffusional mass transfer limitations and enhanced electrical conductivity.

BP is thus a very promising material for the construction of mediatorless laccase cathodes for ORR in biofuel cells. In addition we demonstrated that these electrodes exhibit a high tolerance towards glucose, the most common bioanode fuel.

1. Introduction

Glucose/oxygen biofuel cells are able to harvest ambient energy by using glucose and oxygen from their environment. High overpotentials at the cathode (Schaetzle et al., 2009) quite often limit their power output, making the ORR a major challenge towards application (Habrioux et al., 2010).

The redox enzyme laccase (EC 1.10.3.2, benzenediol:oxygen oxidoreductase) from the white rot fungus *Trametes versicolor* is an attractive biocatalyst for the cathodic oxygen reduction in enzymatic biofuel cells (Zheng et al., 2008). Laccase does not only catalyze selectively the four electron reduction of dioxygen directly to water (Yaropolov et al., 1994) without hydrogen peroxide as toxic and corrosive intermediate, but also exhibits a high redox potential of 0.785 V vs. NHE at pH 5.5 (Reinhammar, 1972). A further advantage is that laccase adsorbed to carbon materials shows the ability of direct electron transfer (DET) (Christenson et al., 2004) due to a copper atom close to the enzyme surface channeling the electrons to the tri-nuclear copper cluster at the active center (Yaropolov et al., 1994). This eliminates the need for an electron mediator (MET – mediated electron transfer) or redox polymers to electrically 'wire' the enzyme to the electrode, thus facilitating electrode design and fabrication and enhancing electrode stability (Barton et al., 2004; Binyamin et al., 2001; Kamitaka et al., 2007). At DET conditions, however, laccase can only be adsorbed

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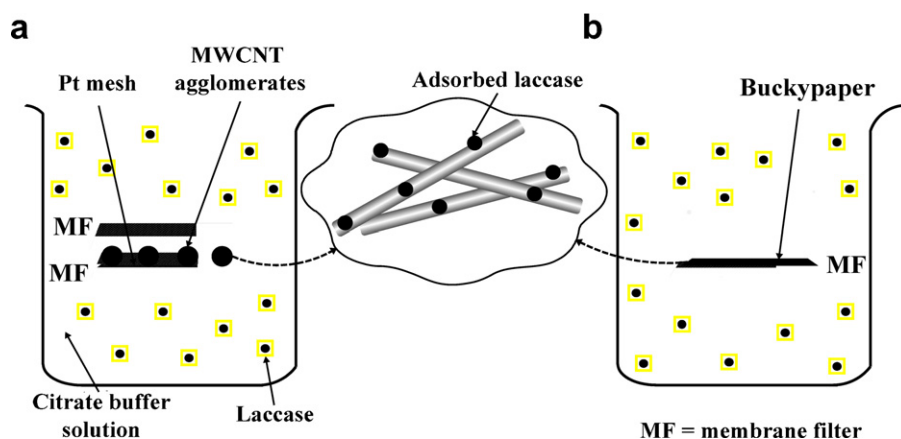[1] Both authors contributed equally to the work.

Fig. 1. Schematic illustration of laccase biocathodes based on differently prepared MWCNT electrodes. The electrodes are submerged in laccase solution to allow the enzyme to adsorb to the MWCNT surface. (a) MWCNT agglomerates are packed by clamping them between membrane filters with an embedded Pt-mesh as current collector (conventional technique), (b) homogeneously dispersed MWCNTs are deposited on a membrane filter as BP (present work).

as monolayer, demanding for high surface area electrode materials and efficient electron transfer between the catalytic active center of the enzyme and the electrode support material.

In a previous study (Rubenwolf et al., 2010) we systematically compared the oxygen reduction performance of laccase adsorbed to different carbon-based electrode materials. We demonstrated that electrodes, fabricated by simply clamping single-, double- or multi-walled carbon nanotubes together with a platinum mesh current collector between filter membranes (Fig. 1a), exhibit greatly enhanced performance (e.g. higher volumetric current densities at the same electrode potential) as compared to electrodes fabricated from macroscopic materials such as graphite felt or porous carbon tubes. However, the agglomerated, as-received carbon nanotubes are not only difficult to handle, but their high specific surface area is not as effectively used for the electrochemical reaction as with electrodes made from the macroscopic materials (Rubenwolf et al., 2010). We attributed this to nanotube aggregation (Gong et al., 2005) in the non-dispersed powdery material, resulting in mass-transfer related effects such as hindered oxygen diffusion or reduced surface availability for enzyme adsorption. To fully utilize the high specific surface area of nanotube materials and further improve electrode performance it would thus be desirable to form electrodes with a high degree of nanotube dispersion.

One way to increase dispersion and surface area accessibility is the fabrication of ordered arrays of MWCNTs, directly grown on a substrate via chemical vapor deposition (Antiohos et al., 2010; Withey et al., 2006). However, this approach requires precise control of process parameters as well as expensive equipment. Despite being supported on a substrate, the arrays remain fragile.

Another method is the incorporation of the MWCNT arrays into membranes (Pushparaj et al., 2007). This yields stable and flexible electrodes, but the presence of polymer also decreases the surface area accessible for redox enzymes. Similarly, by nanocomposite formation of MWCNTs with polymers free-standing, flexible, and porous electrodes can be obtained. But also in this case the presence of polymers decreases electrode conductivity (Gong et al., 2005) and may represent a diffusion barrier as well as decrease enzyme activity (Cooney et al., 2008).

A higher degree of accessible surface area can be achieved when carbon nanotubes are deposited on 2-dimensional carbon materials like spectrographic graphite or glassy carbon after homogenization with water, ionic liquids, or polar organic solvents (Liu and Dong, 2007; Yao and Shiu, 2007; Zheng et al., 2008). However, due to the flat carbon substrate, this results in non-porous, inflexible and thick electrodes covered with only a thin layer of MWCNTs. Recently, MWCNTs have also been deposited onto carbon paper

with the help of *N,N*-dimethyl formamide (Ramasamy et al., 2010) to form a porous, 3-dimensional laccase-catalyzed cathode for oxygen reduction.

The use of a binding agent in electrode fabrication can be circumvented by the dispersion and deposition of the carbon nanotubes in the form of so-called buckypapers (BPs). BP is a flexible self-supporting mat of entangled assemblies (ropes and bundles) of MWCNTs, forming well-defined membrane-type black films that find application also in catalysis, filtration, actuators, or energy storage devices such as capacitors and batteries (Pushparaj et al., 2007; Wang et al., 2008; Zhang et al., 2010; Zhu et al., 2010). The high specific surface of the nanotubes in combination with inherent electrical conductivity renders BP a promising material for catalyst support for electrochemical applications (Bahr et al., 2001; Endo et al., 2005; Liu et al., 1998; Zhu et al., 2010). Recently, we have successfully introduced BP as reliable and stable carbon based electrode material for abiotic (based on gold and platinum nanoparticles) and biotic (based on bilirubin oxidase) electrodes (Hussein et al., 2009, 2011).

Here, we demonstrate the application of BP as electrode material for biofuel cell cathodes using adsorbed laccase as biocatalyst for ORR. The cathodes are electrochemically characterized as half-cell electrodes by potentiodynamic and galvanostatic methods, and compared to electrodes fabricated from the same MWCNTs, but using a different technique. Moreover, we investigate the cathodes' tolerance towards glucose, which is the most important fuel in biofuel cell applications.

2. Experimental

2.1. Buckypaper fabrication

BP-electrodes were fabricated as described in previous work (Hussein et al., 2011). In brief, 100 mg of agglomerated as-received MWCNTs (Baytubes C 150-HP, Lot # D0002AAB07, Bayer Material Science AG, Leverkusen, Germany) were dispersed in 200 ml aqueous solution containing 1 wt.% of the non-ionic surfactant Triton X-100 (Sigma Aldrich, Taufkirchen, Germany). This was achieved firstly by mechanical stirring for 30 min, and secondly using ultrasonication for 3 h. Subsequently, the resulting suspension was centrifuged at 1107 g to remove bigger agglomerates of MWCNTs. The remaining supernatant, containing a stable MWCNT-suspension, was then deposited on a nylon membrane filter (0.45 μm pore size, Whatman, Maidstone, UK) by vacuum-filtration. The resulting homogeneous black film was repeatedly washed with excess of deionized water, isopropyl alcohol and ace-

tone in order to remove the remaining surfactant. The as-prepared BP-films were kept at room temperature for 30 min and then dried overnight in a vacuum oven at 50 °C. BP-electrodes with thicknesses of 30 μm and 80 μm were prepared by using different amounts of the stable MWCNT-suspension.

2.2. Characterization of electrode morphology

The morphology of agglomerated MWCNTs as well as BP-electrodes was investigated by scanning electron microscopy (SEM, Quanta 250 FEG, FEI, Oregon, USA) on uncoated samples.

Nitrogen adsorption/desorption isotherms were measured at −196 °C on an automatic analyzer (Sorptomatic 1990, Porotec GmbH, Hofheim/Ts., Germany). Prior to the experiment, the samples were degassed by heating at 250 °C for 5 h under high vacuum condition. The textural properties were analyzed using the advanced data processing software (ADP version 5.1) provided by Thermo Fisher Scientific (Milan, Italy). The BJH-mesoporous median pore diameters (given in nm) were deduced from the desorption branch of the isotherm with the procedure developed from the Barrett–Joyner–Halenda model equation (Barrett et al., 1951; Lowell et al., 2004) at a relative pressure range of 0.4–0.999 $P/P°$.

The sheet resistance measurements of BPs were carried out on a four-point probe instrument (QuadPro Resistivity System, Lucas Signatone, Gilroy, California) and the thickness of BP was measured using a micrometer gauge (resolution 1 μm, Mitutoyo, Neuss, Germany).

2.3. Potentiodynamic electrode characterization

To characterize their DET and MET behavior, free-standing pieces of 80 μm thick BP (geometric area 0.95 cm^2) were mounted on a glassy carbon electrode tip (Ø 3 mm, Radiometer Analytical SAS, Villeurbanne, France) by using conductive carbon cement (Leit-C, Plano, Wetzlar, Germany). Rotating Disc Electrode (RDE) measurements were performed using a RDE set-up (Radiometer, Lyon, France) together with a computer-controlled potentiostat (PCI4/300, Gamry, Warminster, PA, USA) in a conventional three-electrode electrochemical cell with a Ag/AgCl reference electrode (3 M KCl, 0.210 V vs. NHE, Metrohm, Filderstadt, Germany) and a platinized titanium counter electrode (Bank Elektronik, Pohlheim, Germany). Linear sweep experiments (3 mV s^{-1}, from 0.848 V to 0.000 V vs. NHE) were performed firstly in O$_2$-saturated 0.1 M citrate buffer solution containing 7 U ml^{-1} laccase (activity according to Sigma Aldrich: 21.8 U mg^{-1}, Lot 100891817, # 53739) in the absence of a mediator, and secondly after the addition of 0.02 M 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) as mediator to the above testing solution. All chemicals were purchased from Sigma Aldrich, Taufkirchen, Germany. Bubbling with oxygen was stopped during the electrochemical measurements to avoid any disturbance of the measurement. All experiments were conducted at room temperature.

2.4. Galvanostatic electrode polarization

To investigate the oxygen reduction performance with adsorbed laccase as bioelectrocatalyst, 30 μm thick BP-electrodes were characterized in galvanostatic experiments in terms of open circuit potential and current density–potential behavior. Electrodes with an active area of 0.9 cm^2 (0.6 cm × 1.5 cm) were contacted with two separate platinum wires (0.1 mm diameter, 99.9%, Chempur, Karlsruhe, Germany) using conductive carbon cement (Leit-C, Plano, Wetzlar, Germany), and cured for at least half an hour at 70 °C. For comparison, also conventionally prepared electrodes with laccase adsorbed to packed agglomerated MWCNTs were characterized. Thereto, 20 mg MWCNTs (Baytubes C 150-HP, Lot # E0009AAD07,

Bayer Material Science AG, Leverkusen, Germany) were immobilized by clamping them between two sheets of cellulose acetate filters (pore size 0.45 μm, Sartorius, Göttingen, Germany), together with a platinum mesh (Goodfellow, Huntingdon, UK) as current collector (Rubenwolf et al., 2010). Prior to the measurements, all electrodes were wetted with 2-propanol (Carl Roth, Karlsruhe, Germany) and washed thoroughly with deionized water.

The electrochemical testing system for galvanostatic polarization has been reported previously (Kerzenmacher et al., 2009). Briefly, load currents for polarization curves were applied by an electronic load, and the cathode potential was recorded in intervals of 5 min. A saturated calomel electrode (SCE) was used as reference electrode (0.244 V vs. NHE) and plain platinum mesh served as counter electrode. The general design of the electrochemical test cell was described in previous work (Rubenwolf et al., 2010). However, the following modifications to the setup were introduced: (a) To avoid proton transport limitations a single compartment setup with reference electrode and platinum mesh counter electrode was used. (b) To increase the dissolved oxygen concentration, humidified oxygen was bubbled through optimized nozzles ("nozzle+") which improved the oxygen transfer to the solution by generating smaller bubbles (Kloke et al., 2010).

The testing solution was exchanged depending on the purpose of experiment. To investigate the inherent oxygen reduction performance of the electrode material in the absence of laccase, 4 ml of freshly prepared 0.1 M citrate buffer solution was used. The DET between adsorbed laccase and the electrode material in the absence of a mediator was investigated by using 4 ml citrate buffer solution as above, but additionally containing 7 U ml^{-1} laccase. In order to record comparable current density–cathode potential curves under conditions of MET, only enzyme previously adsorbed to the electrode should be involved in the reaction. Thereto laccase in citrate buffer was allowed to adsorb to the electrode as described above for at least 1 h. Subsequently, the testing solution was exchanged against laccase-free citrate buffer containing 0.02 M ABTS as mediator.

After stabilization of the open circuit potential (potential drift < 5 mV h^{-1}) the load current was stepwise increased from 0 μA to 800 μA (steps of 8 μA, 1 h each) to record the cathodic polarization curves after 1 h of operation at a given current density. To reduce experimental time, 50 μA steps were used in current ranges with small voltage changes (i.e. in the current range 0–300 μA for laccase catalyzed electrodes, 100–800 μA for bare carbon electrodes). Within the experimental time range of up to 3 days, no inactivation effects have been observed. The polarization curves were constructed from the cathode potentials recorded vs. the reference electrode after 1 h of operation at a given current density. To obtain representative values for quantitative analysis the electrode current densities exhibited at a cathode potential of 0.500 V vs. SCE (0.744 V vs. NHE) are compared, since in this range the potential stabilizes with drifts typically less than 5 mV h^{-1} and at maximum 17 mV h^{-1}. All experiments were performed at room temperature and were repeated at least three times to verify reproducibility. To exclude the possible influence of electrode aging on the performance, always freshly prepared electrodes were used. All potentials values are quoted against the normal hydrogen electrode (NHE).

2.5. Investigation of glucose tolerance

To investigate the glucose tolerance of the BP-electrodes, the glucose concentration in the testing solution was increased stepwisely every 2 h at a continuous galvanostatic load of 111 μA cm^{-1} (BP-electrodes were prepared as described in Section 2.4) Total concentrations of 0.001 and 0.01 M glucose were achieved by adding a stock solution with 470 g/l α-D(+)-glucose monohydrate (Carl Roth, Karlsruhe, Germany); by further addition of glucose monohydrate

powder directly to the electrolyte the concentration was increased to 0.1 M and 1 M.

3. Results and discussion

3.1. Electrode morphology

In Fig. 2 SEM images of untreated MWCNT-agglomerates are compared to the BP-electrode prepared from the same raw-material. As can be seen from Fig. 2a, the agglomerated MWCNT-particles have diameters in the range of 500 μm, which is within the manufacturer's specification (agglomerates of 0.1–1 mm diameter according to the Baytubes datasheet). In contrast, BP consists of a homogenous film of entangled MWCNTs evenly dispersed over the area of the supporting filter membrane. On the nanoscale both, the agglomerated MWCNT-particles and BP seem to exhibit a comparable apparent porous structure on their surface as visualized in Fig. 2. However, following the BJH-model one can calculate a median pore diameter of 48 nm for BPs and 23 nm for MWCNT-particles. This can facilitate a better mass transport within the BP-based electrode for enzymes, reactants and products, compared to the untreated MWCNT agglomerates. In summary, this indicates that the homogeneous dispersion of MWCNTs in BP may allow enhanced laccase adsorption and reduce diffusional resistances.

In terms of electrical conductivity, the entangled MWCNT-film of a 30 μm thick BP-electrode (typical MWCNT-loading: 0.9 ± 0.1 mg cm^{-2}) exhibits a specific electrical resistance ρ of 0.0405 (±0.0007) Ω cm. This value is sufficient for the low current densities in biofuel cells and thus no additional current collector is needed in electrode fabrication. Moreover, deposited on the supporting membrane filter the BP-electrodes exhibit excellent mechanical stability in aqueous solution. In contrast, the conventionally prepared electrodes require an embedded platinum mesh as current collector to achieve sufficient electrical conductivity over the electrode area and a second membrane filter on top of the electrode in order to pack and clamp the MWCNT-agglomerates (as illustrated in Fig. 1) which could act as diffusion barrier.

3.2. Potentiodynamic electrode characterization

In Fig. 3 the results from the linear sweep experiments both in the presence and absence of the mediator ABTS are shown. For clarity only the RDE-curves recorded at 100 rpm and 600 rpm are given. As can be seen, the RDE-curves coincide in the activation overpotential region above 0.800 V vs. NHE (i.e. kinetic region), indicating the occurrence of an efficient electron transfer between adsorbed laccase and the MWCNTs of the BP-electrode. At potentials below 0.800 V vs. NHE the electrode reaction becomes increasingly dominated by mass-transfer and the curves inflect towards the plateau-like cathodic diffusion-limited current with some deviations from ideal behavior (i.e. plateau) due to developing of non-smooth flow in front of carbon nanoporous electrode (Bron,
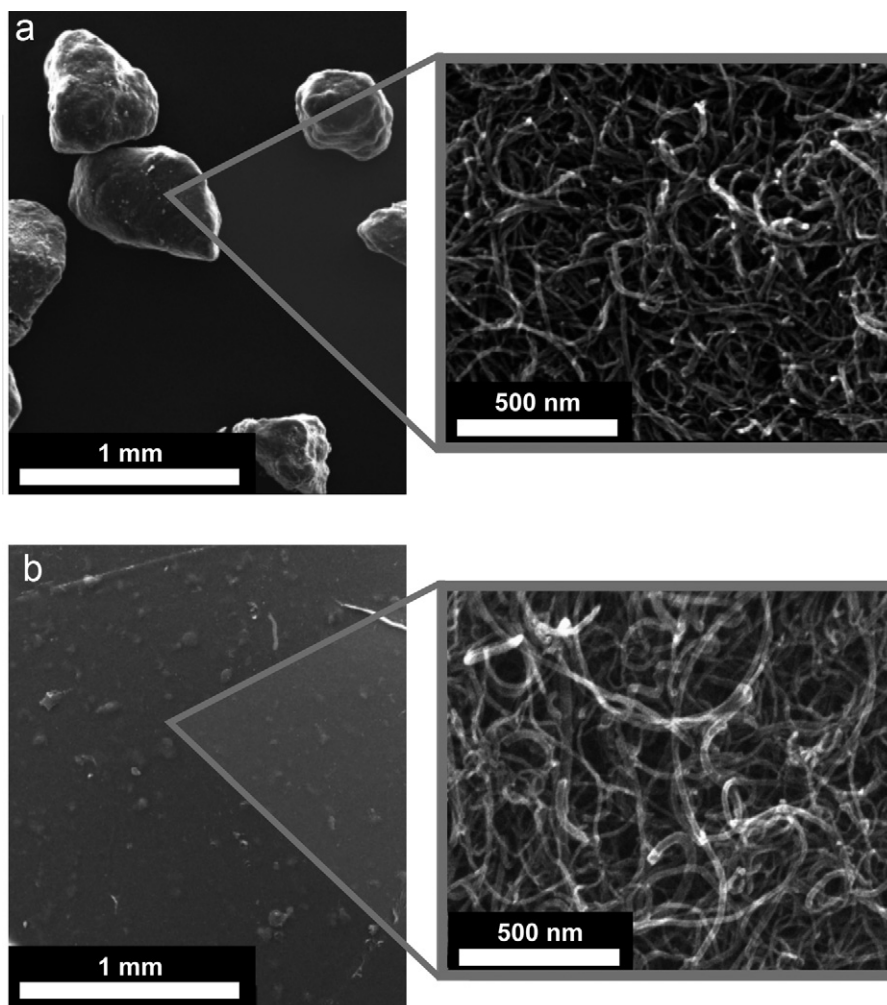

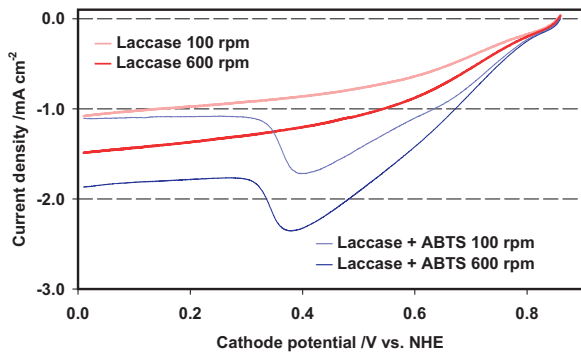**Fig. 2.** SEM images of the electrode surfaces fabricated from (a) agglomerated MWCNTs and (b) BP.

Fig. 3. Linear sweep voltammetry results from RDE experiments with BP-cathodes, recorded in O_2-saturated citrate buffer solution containing 7 U ml^{-1} laccase with and without ABTS (as indicated in the figure).

2008). Here the RDE-curves recorded with the mediator ABTS show significantly higher current densities than the curves recorded only with laccase, which is attributed to the higher concentration of ABTS (0.020 M) in the testing solution compared to dissolved oxygen (0.001 M, Lide, 2001). In the mass-transfer dominated regime thus ABTS contributes dominantly to the electrode current density by facilitating the shuttling of electron directly from the BP based-electrode to dissolved as well as adsorbed laccase molecules. Moreover, the reduction peak between 0.3 and 0.4 V vs. NHE, it is ascribed to ABTS reduction.

3.3. Galvanostatic electrode polarization

Fig. 4a shows polarization curves of BP-cathodes with the enzyme laccase in the presence (MET approach) or absence (DET approach) of the mediator ABTS in O_2-saturated citrate buffer solution. In both cases, the electrodes exhibit similarly high current densities of $422 \pm 71\,\mu A\,cm^{-2}$ for DET and $383 \pm 69\,\mu A\,cm^{-2}$ for MET at 0.744 V vs. NHE. The variations may be caused by inhomogeneities of the respective BP-films. The lack of a significant difference between both electron transfer approaches indicates a very efficient DET between adsorbed laccase and the MWCNTs, and is confirmed by results from the RDE measurements (for Tafel plot analysis see Supplementary material). At higher current densities, the electrode potential stabilizes at around -0.2 V vs. NHE, and the polarization curves of BP-electrodes with and without enzyme coincide. This indicates that the rapid drop in electrode potential is not caused by limited oxygen availability, but rather by the limited turnover number of the laccase enzymes. At higher current density

thus the laccase-catalyzed reaction plays a minor role, and oxygen reduction occurring directly at the MWCNT-surface dominates the electrode potential.

3.4. Comparison of BP electrodes to conventional MWCNT electrodes

In Fig. 4b, the oxygen reduction performance of the novel BP-based cathodes is compared to electrodes prepared from agglomerated MWCNTs (Baytubes) in the recently used conventional technique (Rubenwolf et al., 2010). By normalizing the current density to the mass of the electrode material (mass activity), it can clearly be seen that MWCNTs act more efficiently as cathode when prepared in form of BP, instead of being used in the non-dispersed agglomerated form. The mean mass activity at 0.744 V vs. NHE of $471 \pm 64\,\mu A\,mg^{-1}$ for BP-electrodes is 68-fold higher than with electrodes fabricated from agglomerated MWCNTs (approx. $7 \pm 1\,\mu A\,mg^{-1}$). This can be attributed to smaller diffusion distances within the electrode, resulting from the better MWCNT dispersion and less MWCNT agglomeration in the BP film. For comparison, the mean diameter of the MWCNT agglomerates in conventionally prepared electrodes is in the range of 500 μm, which is an order of magnitude above the BP thickness. Conventionally prepared electrodes also require an additional membrane in front, which may further contribute to a mass transport-limited electrode reaction. Additionally, high electrical resistance between MWCNT-particles (i.e. electron hopping) could contribute to the reduced performance in contrast to the well-organized MWCNTs network of BP-films.

3.5. Glucose tolerance

The influence of glucose on the performance of cathodes operated at a constant current density of 111 $\mu A\,cm^{-1}$ was investigated by increasing the glucose concentration in the catholyte, and monitoring the consequent potential response of a cathode. As can be seen in Fig. 5, the BP-based cathodes exhibit virtually no sensitivity towards glucose in the concentration range up to 1 M.

4. Conclusions and outlook

In the present work we demonstrated that buckypaper (BP) fabricated from multi-walled carbon nanotubes (MWCNTs) is an excellent electrode material for laccase-catalyzed oxygen reduction cathodes in biofuel cell applications. The high dispersion of MWCNTs as mechanically stable, self-entangling mat results in easy to handle homogeneous layers with high electrical conductivity. So
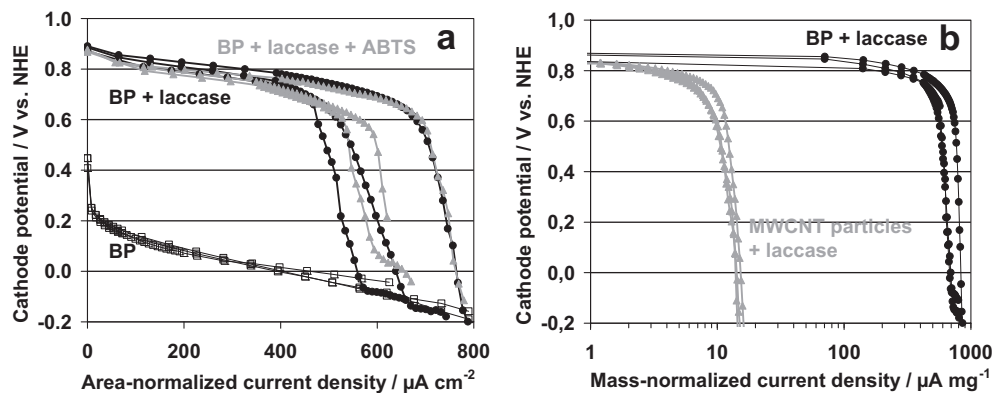

Fig. 4. Galvanostatic polarization curves of different electrodes in O_2-saturated citrate buffer. (a) 30 μm thick BP-cathodes without laccase (black squares), with adsorbed laccase (DET, black circles), and with adsorbed laccase and ABTS (MET, grey triangles). (b) Comparison of different MWCNT-based electrodes with the current density normalized to the mass of carbon nanotubes (note the logarithmic scale). Both, electrodes fabricated as BP (black circles) and in conventional technique (grey triangles) were investigated with adsorbed laccase at mediatorless conditions.
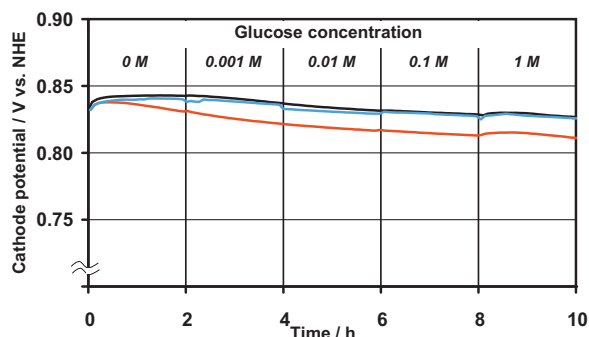
Fig. 5. Glucose-sensitivity of 3 identically prepared laccase cathodes with BP as electrode material at a constant current density of 111 µA cm^{-1}. The glucose concentration of the catholyte was increased every 2 h, as indicated in the figure.

the need for electrode-embedded current collectors, as required in previous approaches, can be circumvented.

Potentiodynamic measurements showed that after adsorption of laccase at BP-electrodes direct electron transfer (DET) occurs with a high open circuit potential of 0.882 V vs. NHE, which is close to the thermodynamic potential for oxygen reduction at the corresponding pH (0.934 V vs. NHE at pH 5) at 25 °C. Similarly, galvanostatic polarization experiments showed that electrode performance cannot be further enhanced by the addition of an electron mediator, which indicates an efficient DET process. The high nanotube dispersion of BP-electrodes is reflected in high current densities at mediatorless conditions. At the typical cathode potential of 0.744 V vs. normal hydrogen electrode (NHE), the BP-electrodes exhibit a 68-fold higher current density than electrodes fabricated from the same MWCNTs but in a packed agglomerated form. In comparison to agglomerated MWCNT particles, the BP electrodes showed a significant increase in BJH-mesoporous sizes, shorter diffusion pathways and do not have further membranes on top of the electrode, allowing for better enzyme accessibility and increased mass transfer.

Furthermore, our electrodes show a higher open circuit potential and enhanced performance than the recently reported cathodes fabricated from laccase chemically tethered to MWCNTs supported onto Toray carbon paper (~0.3 mg MWCNTs per cm^2, corresponding to a third of the loading used in the present work) (Ramasamy et al., 2010). Their cathodes showed an open circuit potential of only 0.799 V vs. NHE (pH 5.8). At a potential of 0.702 V vs. NHE their electrodes exhibited a current density of only 50 µA cm^{-2}, while the here presented BP-electrodes show a current density of 484 ± 17 µA cm^{-2}, which is by an order of magnitude higher.

BP is thus a very promising material for the construction of mediatorless laccase cathodes for oxygen reduction in biofuel cells. In addition, these electrodes exhibit a high tolerance towards glucose, the most common bioanode fuel. This renders the possible fuel cross-over from anode to cathode compartment insignificant and may facilitate biofuel cell construction.

In future, the BP concept may also be applied to fabricate nanobioelectrodes with other enzymes. In this context, a fabrication route that obviates the use of surfactants may be advantageous to improve enzyme adsorption and activity. While in the present work only the reduction of dissolved oxygen at an electrode submersed in aqueous solution was considered, the implementation of an air-breathing cathode design based on the BP-electrodes should be considered. This may lead to improved cathode performance due to the increased oxygen concentration and availability. Furthermore, an air-breathing cathode would circumvent the need for air sparging of the catholyte and thus allow for energy-efficient passive aeration.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.04.008.

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