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Overcoming Bottlenecks of Enzymatic Biofuel Cell Cathodes: Crude Fungal Culture Supernatant Can Help to Extend Lifetime and Reduce Cost

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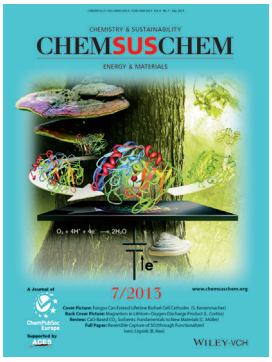
Enzymatic biofuel cells (BFCs) show great potential for the direct conversion of biochemically stored energy from renewable biomass resources into electricity. However, enzyme purification is time-consuming and expensive. Furthermore, the long-term use of enzymatic BFCs is hindered by enzyme degradation, which limits their lifetime to only a few weeks. We show, for the first time, that crude culture supernatant from enzyme-secreting microorganisms (*Trametes versicolor*) can be used without further treatment to supply the enzyme laccase to the cathode of a mediatorless BFC. Polarization curves show that there is no significant difference in the cathode performance when using crude supernatant that contains laccase compared to purified laccase in culture medium or buffer solution. Furthermore, we demonstrate that the oxygen reduction activity of this enzymatic cathode can be sustained over a period of at least 120 days by periodic resupply of crude culture supernatant. This is more than five times longer than control cathodes without the resupply of culture supernatant. During the operation period of 120 days, no progressive loss of potential is observed, which suggests that significantly longer lifetimes than shown in this work may be possible. Our results demonstrate the possibility to establish simple, cost efficient, and mediatorless enzymatic BFC cathodes that do not require expensive enzyme purification procedures. Furthermore, they show the feasibility of an enzymatic BFC with an extended lifetime, in which self-replicating microorganisms provide the electrode with catalytically active enzymes in a continuous or periodic manner.

Introduction

The depletion of fossil fuels and the concerns regarding their CO_2-release upon combustion, stress the strong need to improve technologies that provide energy from sustainable resources. Therefore, research efforts are increasing to find alternative methods for converting biomass resources into electrical energy.[1–3] In this context, biofuel cells (BFCs) show great potential for the direct conversion of biochemically stored energy into electricity by using biological catalysts.[4] They are eco-friendly because they can utilize a wide range of organic substrates from abundant sources as fuels, for example, blood sugar (glucose) for implantable BFCs,[5,6] biological waste materials, and lignocellulose biomass as nutrition for microorganisms (e.g., bacteria).[7,8] In addition, renewable materials such as carbon can be used as electrodes, circumventing the need to apply resource-limited noble metal catalysts.[7] Furthermore, BFCs operate under mild conditions with water as a solvent at near-neutral pH and at room temperature.[4]



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BFC types include microbial BFCs that use living microorganisms and enzymatic BFCs in which isolated enzymes employed.[4] Although microbial BFCs have the advantage of long-term operational stability (five years have been reported),[9] they show lower power densities in comparison to enzymatic BFCs.[10] The latter can provide higher power densities because the isolated enzymes can more efficiently access an electrode at high loading.[2,11] A microbial anode can also be combined with an enzymatic cathode to improve the performance of the oxygen reduction reaction at the cathode.[13]

In any case, the bottlenecks of enzymatic electrodes are the limited lifetime caused by enzyme degradation as well as the

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high cost and the time that is needed for enzyme purification[13]

Approaches for extending the enzymatic BFC lifetime have mainly considered the stabilization of enzymes to decrease denaturation and the associated loss of activity.[14] This has included methods to increase the number and strength of intramolecular interactions,[15] and to immobilize the enzymes through entrapment, chemical bonding, and crosslinking.[12,16] However, these approaches can affect the activity of the enzyme, for example, by introducing additional mass-transfer limitations of the substrate, and they involve additional costs.[17] Furthermore, the loss of activity caused by enzyme inactivation can only be slowed down and not fully prevented. Alternative approaches aim to achieve theoretically unlimited lifetimes by using in situ enzyme regeneration. So far, this has been reported for biosensors with indirect electrochemical conversion,[18] and for mediated enzyme electrodes in which microorganisms at the electrode express redox enzymes at their surface.[19,20]

Recently, our group demonstrated that laccase from *Trametes versicolor* (*T. versicolor*) has a mean residence time of only two days on a buckypaper electrode, and that the lifetime of an enzymatic oxygen reduction cathode could be extended 2.5-fold by resupplying fresh enzymes.[21] In this case, the electrolyte with laccase from *T. versicolor* (Figure 1) spiked in citrate

Figure 1. The white-rot fungus *T. versicolor*. Photograph by Martina Berg.

buffer was manually replenished periodically at the cathode compartment. The white-rot fungus *T. versicolor* secretes a variety of enzymes for lignin degradation, dependent on the strain, culture medium, and aromatic compounds added.[22–24] Among these lignolytic enzymes, laccase (Lac, EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13), and lignin peroxidase (LiP, EC 1.11.1.14) are suitable for cathodic oxygen reduction in a BFC. All three enzymes are capable of direct electron transfer (DET),[25] rendering the supply of a mediator unnecessary. Laccase is one of the best investigated enzymes for the use in enzymatic BFC cathodes,[1] and laccase from *T. versicolor* catalyzes the four-electron reduction of dioxygen to water.[40] For the two peroxidases, hydrogen peroxide is used as the substrate.[23] Previously, it was shown that *T. versicolor* produced the enzyme pyranose oxidase, which is a major source of H_2O_2 that supplies the peroxidases during wood degradation.[26]

Herein, we present the first use of untreated culture supernatant from the enzyme-secreting fungus *T. versicolor* to supply laccase to the cathode of a mediatorless enzymatic BFC. We also show that long-term stability of a cathode can be achieved with periodic resupply of laccase in crude culture supernatant.

Results and Discussion

Identification of secreted enzymes

To identify the major enzymes secreted by *T. versicolor*, we analyzed the supernatant of *T. versicolor* grown in synthetic complete laccase (SCL) medium by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Four major bands could be distinguished, corresponding to apparent molecular weights of approximately 66, 50, 45, and 34 kDa (Figure 2). To identify the most abundant enzymes contained

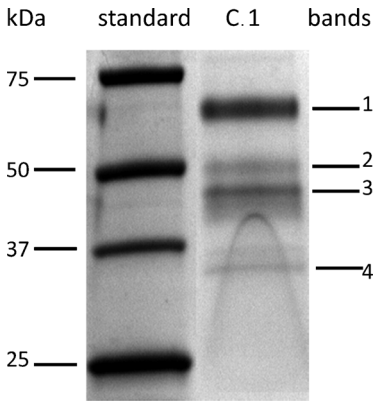

Figure 2. SDS gel after Coomassie staining of proteins in crude *T. versicolor* culture supernatant batch C.1. Four major bands are visible in the range of 30 to 70 kDa.

within these bands, mass spectrometry (MS) was performed after excising the bands. Table 1 lists the enzymes (laccase, manganese peroxidase, and lignin peroxidase) known to be capable of DET on a cathode, and which were identified in the MS analyses (the five most abundant enzymes detected in each band, based on the total number of peptide-hits are shown in Table S1 in the Supporting Information). The presence of peptides derived from the same enzyme in more than one band could most likely be attributed to the secretion of multiple isoforms of the enzyme. The identified proteins are known to be highly glycosylated, which could be one reason why not all of the peptides for each protein were detected.

To investigate which one of these enzymes was responsible for the oxygen reduction performance of the cathode, the enzyme activity was recorded with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and corrected for peroxidase activity by adding catalase. A decrease in

Table 1. Analyses of crude *T. versicolor* culture supernatant. The four main bands visible in the SDS–PAGE were excised and analyzed by LC–MS.

Band	Gene	Accession no.[a]	Coverage[b] [%]	Digest[c]
1	laccase I	AAC49828	38	T, CT
	laccase B	AAL07440	26	T, CT
2	manganese peroxidase	CAA54398	35	T, CT
		CAG32981.2		
		AAB63460.1		
3	manganese peroxidase	CAG33918.4	58	CT
	lignin peroxidase	LIGC-trave	36	V8
4	manganese peroxidase	AAT90351.1	10	CT
		CAG33918.1		

[a] GenBank accession number for the peptides with identified genes from *T. versicolor*. [b] Coverage refers to the percentage of the full length protein represented by the peptides identified through LC–MS. [c] Digest refers to the enzymes used to digest the proteins, which were trypsin (T), chymotrypsin (CT), and endoproteinase GluC (V8).

Table 2. Batches of *T. versicolor* with their respective culturing time and enzyme activity.

Batch	Culturing time [days]	Enzyme activity [U mL^{-1}]
A.1[a]	41	1.3
A.2[a]	41	1.0
B	10	0.8
C.1[a]	33	3.6
C.2[a]	33	2.0

[a] Aliquots from batches A.1 and C.1 were thawed, samples taken, and then frozen again. This procedure decreased the enzyme activity resulting in batches A.2 and C.2, respectively.

activity is expected when the peroxidase is inhibited by removal of peroxide because the peroxidase activity is dependent on the presence of peroxide. Our results showed no change in enzyme activity; therefore, we concluded that even though the existence of peroxidases could be detected in the supernatant, there was little or no peroxidase activity at pH 5 and 30 °C in the culture supernatant without further supply of peroxide. This suggested that the cathode performance, when using crude culture supernatant, primarily resulted from the redox-enzyme laccase activity in the crude culture supernatant of *T. versicolor*.

pH and enzyme activity over a 74 day culture period

For the development of a self-regenerating enzymatic BFC, a culture with a stable pH and sustained enzyme activity is important. Both parameters were monitored during the culture time course (see Figure S1 and S2 in the Supporting Information). The results showed that the culture supernatant remained stable between pH 4 and 5 without adjustment. The enzyme activity increased until about day 40 to values around 2.0 U mL^{-1}, at which it remained relatively constant until the end of the measurement. As shown below, a good cathode performance could be achieved with an enzyme activity as low as 0.8 U mL^{-1}. This activity was already reached by day 10. These results showed that a pH value, as well as an enzyme activity, suitable for a good cathode performance was reached over a period of more than two months, suggesting that continuous supply was possible on the long term.

Comparison of polarization curves

To test whether it was possible to use the crude supernatant of *T. versicolor* in the cathode compartment, polarization curves of the crude culture supernatant, with an enzyme activity of 3.6 U mL^{-1} towards ABTS (Table 2), were compared to polarization curves of commercially available purified *T. versicolor* laccase with the same activity spiked into either SCL medium or sodium acetate buffer. As a control experiment, a polarization curve in SCL medium without enzyme was also recorded. Three polarization curves were recorded for each catholyte composition, and that shown in Figure 3 is the mean polarization curve. The maximum and minimum bars represent the two other polarization curves recorded under the same condition. All three enzymatic cathodes showed an open circuit potential of 856 mV ±30 mV (vs. a normal hydrogen electrode, NHE). In contrast, cathodes that were supplied with SCL medium without enzymes showed an open circuit potential of only 412 mV versus NHE, demonstrating the catalytic effect on oxygen reduction at the buckypaper cathode. When increasing the current upload, a slight decrease in the potential was initially observed (activation and Ohmic losses), then a pronounced drop in the electrode potential with increasing current density occurred at around 644 mV (400 mV vs. a saturated calomel electrode, SCE), which could be attributed to mass-transport limitations. At higher load currents, a rapid drop in potential to values of about −500 mV versus NHE was ob-

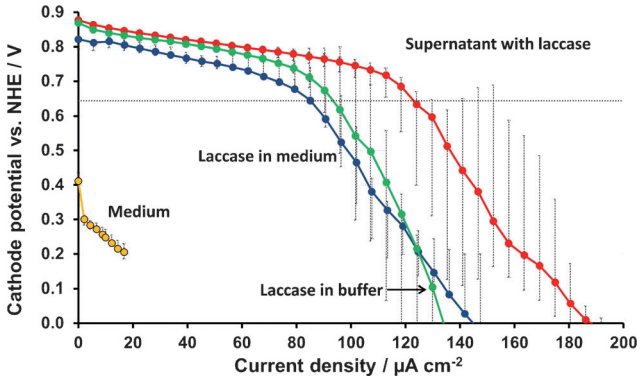

Figure 3. Polarization curves of three different catholytes with the same enzyme activity (3.6 U mL^{-1} towards ABTS): 1) crude culture supernatant of *T. versicolor* (●), 2) sodium acetate buffer spiked with laccase (●), 3) SCL medium spiked with laccase (●), and 4) SCL medium without enzymes as a reference (●). Potentials are shown versus a NHE reference. The depicted polarization curves are the mean values for each catholyte, and the error bars indicate maximum and minimum polarization curves.

served (not shown in Figure 3), which could be attributed to limitation in oxygen supply. If laccase was the limiting factor, the potential would have dropped to about −200 mV versus NHE.[33] Electrode performances in terms of current density were compared at 644 mV versus NHE (400 mV vs. SCE), to exclude mass-transport limitations. At this potential, the following current densities were recorded with the different catholytes: 1) $129 \pm 19\ \mu A\,cm^{-2}$ for laccase containing crude culture supernatant, 2) $97 \pm 11\ \mu A\,cm^{-2}$ for purified laccase spiked in sodium acetate buffer, and 3) $78 \pm 20\ \mu A\,cm^{-2}$ for purified laccase spiked in SCL medium (Figure 3). The variations between the polarization curves that were recorded under the same conditions could have been caused by inhomogeneity of the electrodes, or by small differences in the reactors and in the oxygen supply. The slightly improved performance of the electrodes with crude culture supernatant containing laccase demonstrated that enzyme purification was not necessary to operate an enzymatic laccase-based BFC. This opens new opportunities for the construction of a low-cost laccase-catalyzed BFC.

Cathode long-term stability with and without exchange of enzyme-containing supernatant

For the experiment involving the periodic exchange of enzyme-containing supernatant, the evolution of a cathode potential at a continuous load current density of $50\ \mu A\,cm^{-2}$ over time is shown in Figure 4a. For clarity, only the curve with the longest lifetime is shown (long-term graphs of all exchange experiments are provided in Figure S3 of the Supporting Information). As can be seen, the electrode exhibited a lifetime of 120 days. Furthermore, no progressive loss of potential over the time was observed. The rapid potential drop to values below −1 V (vs. NHE) after 120 days was not related to the enzyme activity at the electrode, but caused by drying out of the compartment (the other experiments stopped because of loss of oxygen supply, leakage, or drying out of the reactor). This suggested that, in principle, a lifetime longer than 120 days was feasible with periodic exchange of catholyte. As can be seen from Figure 4a, the periodic exchange of catholyte and the introduction of fresh enzymes caused large potential increases up to 200 mV. After the exchange, the potential dropped to an operating potential at which it remained relatively stable until the next exchange. This operating potential was almost the same for supernatant batches B and C.1 (Table 2). It was lower for culture supernatant batch A (Table 2) with longer culture duration and in spite of a higher laccase activity compared to batch B. This could be attributed to the secretion of more inhibitory byproducts by *T. versicolor* during the culturing period, which could have poisoned the electrode.

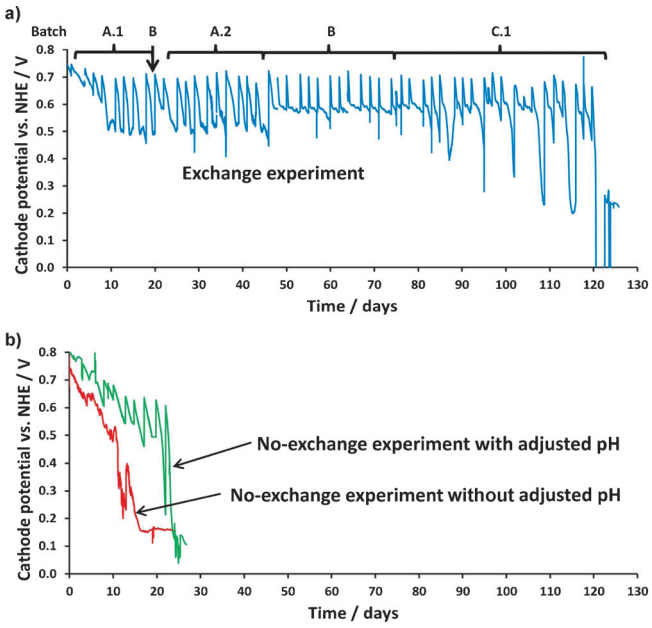

Figure 4. Long-term stability of the electrodes operated at $50\ \mu A\,cm^{-2}$. a) Representative curve for a setup with exchange of supernatant. During the time of operation four different batches were used. Gaps in the curve are attributed to measurement artifacts (such as measurements recorded during the exchange procedure, contact losses, and shorts). b) two curves are shown for a setup without exchange, without pH adjustment (batch C.1, $3.6\ U\,mL^{-1}$, ——) and with pH adjustment (sample C.2, $2.0\ U\,mL^{-1}$, ——). Peaks show the exchange with new catholyte.

The long-term performance of cathodes containing crude culture supernatant and operated without periodic exchange of the catholyte at $50\ \mu A\,cm^{-2}$ are shown in Figure 4b, with and without pH adjustment. Only the curves with the longest lifetime for each condition are shown (long-term graphs of all setups are shown in Figure S3 of the Supporting Information). In contrast to the exchange experiments, the experiments with no exchange showed a gradual decay of electrode potential over time. According to Figure 4b, the regular replenishment of evaporated water resulted in small potential peaks of typically less than 50 mV. Without catholyte exchange and without pH control, the longest lifetime that could be achieved was 14 days. As already reported for microbial BFCs, such a decrease in the cathode efficiency could be attributed to the difficulty in providing protons to the catalyst site, resulting in an increased pH in the cathode chamber.[37] To test this hypothesis, an additional experiment was performed in which the pH was adjusted to a value of 5 when refilling the cell after water evaporation. Under these conditions, a lifetime of 23 days was observed. The lifetime was thus increased by 9 days compared to the experiments without any pH adjustment. The highest pH value observed (before adjustment to pH 5) was 7.4, and in separate experiments (data not shown), we observed that an increase to pH 7.4 over a period of 2 days had no irreversible effect on laccase activity at pH 5.

The electrode potential in the experiments with no exchange ultimately reached about 150 mV versus NHE (Figure 4b), which corresponded to the potential range at which oxygen reduction on the buckypaper electrode without enzymes occurred. Therefore, the drop in electrode potential could be attributed to enzyme degradation and not to a limited availability of oxygen.[33] This was in agreement with the half-life of laccase activity of 9 days at room temperature, which was reported by Rubenwolf et al.[21] Our results showed that the resupply of fresh laccase at the cathode resulted in an extended cathode lifetime.

Conclusions

Our experiments have demonstrated for the first time that the lifetime of an enzymatic BFC cathode could be extended by resupply of crude culture supernatant from *T. versicolor* that contained laccase enzymes. We have shown an electrode lifetime that reached 120 days before the experiment had to be stopped because of a dried out cathode compartment. No significant decrease in the potential at a constant current was observed during this operation period; this suggested that lifetimes significantly longer than 120 days could be achieved. In comparison, the electrode lifetime in no-exchange setups was no more than 14 days without controlling the pH, and no more than 23 days when the pH was adjusted to around pH 5. Furthermore, our experiments showed that the cathode performance of untreated crude culture supernatant of *T. versicolor* was comparable to the performance of purified laccase with the same activity in sodium acetate buffer or in SCL medium. Therefore, an enzymatic BFC cathode can be established without expensive and time-consuming purification of the enzymes. To increase the mass-transfer-limited current density, the implementation of air-breathing gas-diffusion cathodes should also be considered.

Our results show the possibility for self-regenerating enzymatic BFC cathodes with extended lifetimes. For large scale applications, the enzyme-containing culture supernatant can be produced in a separate bioreactor, and as demonstrated in our work, added to the fuel cell cathode at regular time intervals to achieve longevity. Such an enzymatic cathode can, for instance, be combined with a microbial anode in waste-water treatment plants. In this respect, further engineering efforts are required to find optimized membranes that act as the separator between our cathode and the particular anode. Enabling sufficient ion exchange between the compartments to minimize pH drift and preventing cross-over of substances that can cause biofouling at the electrodes must be considered.

For miniaturized BFCs,[38] for example those which power autonomous robots,[39] it may be desirable to have an enzyme-secreting fungus growing directly in the cathode compartment, which will require future research and optimization.

Experimental Section

Microorganism and culture conditions

The *T. versicolor* strain ATCC 32745 was used throughout this study. The fungus was maintained on yeast extract peptone dextrose (YPD) agar plates (10 $g\,L^{-1}$ yeast extract, 20 $g\,L^{-1}$ vegetable peptones, 20 $g\,L^{-1}$ agar, and 20 $g\,L^{-1}$ glucose; Sigma–Aldrich, Taufkirchen, Germany).

Cultures were prepared as follows: YPD plates were inoculated and incubated for 5 days in the dark. Three plugs (about 0.5 cm^2) from the grown edge of the mycelium were transferred into liquid medium (150 mL) in a 250 mL Erlenmeyer flask. We cultured *T. versicolor* three times with different culturing periods. For each culturing period, three flasks were inoculated and subsequently combined to one batch, resulting in batches A–C (Table 2). For the liquid medium, a synthetic complete laccase medium (SCL, pH 5.0) was used containing yeast nitrogen base (6.7 $g\,L^{-1}$; Carl Roth, Karlsruhe, Germany), synthetic drop out medium supplement without uracil (1.92 $g\,L^{-1}$), glucose (10 $g\,L^{-1}$), 2,2-dimethyl succinic acid (20 mM; Sigma–Aldrich, Taufkirchen, Germany), and $CuSO_4$ (0.25 $g\,L^{-1}$; Merck KGaA, Darmstadt, Germany). Aliquots of each batch (A–C) were stored at −20 °C.

To record the long-term stability of the pH value and the enzyme activity of *T. versicolor*, three more cultures were prepared as described above in 150 mL medium and monitored for 74 days. At each time point, 1 mL of culture supernatant was taken with a sterile serological pipette, and the pH value as well as the enzyme activity were analyzed. The amount of supernatant that was taken was replaced by fresh SCL medium.

The supernatant of batch C.1, with the highest enzyme activity of 3.6 $U\,mL^{-1}$, was used for electrophoresis and MS analyses.

Protein composition and enzyme activity

To characterize the protein molecular mass and the composition of the crude supernatant, the proteins were separated on polyacrylamide gels according to Laemmli's procedure.[27] SDS–PAGE was performed on a Mini*P*rotean tetra gel system (Bio-Rad, München, Germany). Protein bands were visualized by staining with Coomassie according to the manual of the Blue R staining kit (Serva Electrophoresis, Heidelberg, Germany), and compared with Precision Plus Protein Dual Color Standard (Bio-Rad, München, Germany). Gel bands were excised and the proteins were digested separately with trypsin (T), chymotrypsin (CT) proteases, and endoproteinase GluC (V8), as described by Shevchenko et al.[28] and Flach et al.[29] MS analysis was performed by using nanoscale reverse-phase LC (Agilent 1200 nanoLC, Waldbronn, Germany) coupled with a hybrid linear ion-trap mass spectrometer (LTQ-Orbitrap XL + ETD, Thermofisher, Germany) equipped with a nanoelectrospray source (Proxeon, Denmark). A 15 cm fused silica emitter packed with ReproSil-Pur C18-AQ3 μm resin (Dr. Maisch GmbH, Germany) was used with inner and spray tip diameters of 75 and 8 μm, respectively. Peptides were eluted with a 2–42% linear acetonitrile gradient at a flow rate of 250 $nL\,min^{-1}$, and sprayed directly into the mass spectrometer. Acquisition and processing of the spectra was performed as described by Waldmann et al.[30] The processed MS data (generic peak list files) were searched with Mascot 2.2 against the Joint Genome Institute Trametes versicolor Gene Catalog (version 1.0). Protein identifications were manually verified.

The laccase activity of the five different *T. versicolor* batches A.1–C.2 was quantified through photo-spectrometry by monitoring the oxidation of ABTS (Sigma–Aldrich) dissolved in 0.1 M sodium acetate buffer (pH 5.0). Triplicate aliquots of 100 μL sterile filtered culture supernatant were placed in 96 well microplates (Greiner bio-one, Frickenhausen, Germany). By using a Wallac Victor^2 microplate reader (PerkinElmer, Rodgau-Jügesheim, Germany), 100 μL of ABTS (2 mM) was injected and the absorption at 405 nm was measured at 0.17 s intervals. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute at pH 5 and 30 °C. Commercial purified laccase from *T. versicolor* was used as a reference (Lot BCBC1663, Sigma–Aldrich, 21.0 $U\,mg^{-1}$; 1 U corresponds to the amount of enzyme which converts 1 μmol catechol per minute at pH 4.5 and 25 °C).

To account for a possible reduction of ABTS by the peroxidases, the batches A.2–C.1 were additionally tested following the addition of catalase to remove H_2O_2. Catalase (1 mg) from bovine liver (Lot 010M7011V, 3809 $U\,mg^{-1}$, Sigma–Aldrich) was added to 1 mL

aliquots of batches A.2–C.1 and incubated for 1 h at 37 °C prior to performing the ABTS activity test as described above.[31]

To clarify if it was possible to irreversibly deactivate laccase at higher pH values, we adjusted an aliquot of supernatant to pH 7.4 by using 0.1 M NaOH (Merck KGaA, Darmstadt, Germany), and maintained another aliquot of the same supernatant at pH 5. The two aliquots were stored for 2 days at 30 °C, and the activity was then measured at 30 °C and pH 5, that is, the pH of the aliquot at pH 7.4 was readjusted to pH 5 with 10% acetic acid (Carl Roth, Karlsruhe, Germany).

Reactor set up

Freshly prepared buckypaper electrodes prepared from dispersed carbon nanotubes were used as cathodes. The cathodes were prepared as described in a previously published report with a carbon nanotube loading of 4 ± 1 mg cm^{-2} onto a nylon filter support.[32,33] Galvanostatic polarization curves and long-term behavior of the cathodes were tested in a half-cell configuration. The electrochemical reactor, described in detail by Kloke et al.,[34] consisted of polycarbonate frames alternatingly stacked with silicone gaskets. Vents and channels were incorporated as cavities or bore holes. The cathode compartment was separated from the reference electrode and counter electrode compartment by a cation exchange membrane (Fumapem F-950, FuMA-Tech, St. Ingbert, Germany). Sterile conditions were maintained in long-term experiments by protecting the medium exchange port with a septum closure (Greiner Bio-One, Frickenhausen, Germany), and the gassing channels were separated by using syringe filters (FP 30/0.2 CA-S, Whatman, Dassel, Germany). Reactors were equipped with buckypaper cathodes with separate platinum wire connections for the potential and the current. Electrodes were glued onto a silicon gasket by using a two-components-epoxide resin glue (UHU plus sofortfest 2K-Epoxidharzkleber, UHU, Vienna, Austria). Furthermore, they were equipped with platinum mesh counter electrodes (Goodfellow, Huntington, UK), filled with deionized water, and autoclaved (121 °C, 20 min). Afterwards, the water was exchanged with catholyte (4 mL) and purged with humidified air.

Galvanostatic cathode characterization

The polarization curves of four different catholytes were compared: 1) crude culture supernatant of *T. versicolor* grown in SCL medium (batch C.1 in Table 2, enzyme activity of 3.6 U mL^{-1} towards ABTS), 2) laccase from *T. versicolor* (Lot BCBC1663, 21.0 U mg^{-1} towards catechol, Sigma–Aldrich) with the same enzyme activity (3.6 U mL^{-1} towards ABTS) spiked in 0.1 M sodium acetate buffer or 3) laccase as described under (2) but spiked in SCL medium, and 4) SCL medium without enzyme as a control.

Electrode potentials were measured against a SCE reference (KE11, Sensortechnik Meinsberg, Germany, 244 mV vs. NHE). All potentials were referenced to the NHE. Load currents were applied by using an electronic load as described elsewhere.[35] Prior to all electrochemical experiments, the enzyme was allowed to adsorb for 12 h under open circuit conditions. For each of the four catholytes (see above), three BFCs were set up and galvanostatic electrode polarization curves were recorded. The current was increased in steps of 5.6 μA cm^{-2} h^{-1} in the range of 0–222 μA cm^{-2} for the enzyme-containing electrolyte. When the load curves were recorded in culture medium without enzymes, the current was increased in steps of 1.1 μA cm^{-2} h^{-1} in the range of 0–17 μA cm^{-2}. The electrode performance was evaluated by comparing the current densities at 644 mV versus NHE (400 mV vs. SCE) as described before,[36] and values were reported as mean ± sample standard deviation. All experiments were performed in triplicate at 30 °C and pH 5.

To record the long-term performance of the cathode, a constant current density of 50 μA cm^{-2} was applied in eight experimental setups. Cathode compartments were initially supplied with crude culture supernatant (4 mL). In the three exchange setups, supernatant (4 mL) was periodically exchanged (every second or third day) with a freshly thawed aliquot of supernatant. At the same time, the deionized water was refilled to replace any evaporated water in the three setups with no exchange. To assess the influence of the pH change on the lifetime of the supernatant-based experiments without exchange, when refilling the evaporated water in two no-exchange setups, the pH value was adjusted by using 10% acetic acid to a pH value of 5. For the no-exchange setups without adjusted pH, supernatant from batch C.1 (enzyme activity = 3.6 U mL^{-1}) was used. For the pH-adjusted setups, we used batch C.2 with an enzyme activity of 2.0 U mL^{-1}. During the course of the exchange experiments, culture supernatants from the four different batches were used (batch A.1–C.1, Table 2). The lifetime of the electrodes was defined as the time in which the electrode potential did not drop below 300 mV versus NHE for more than three days, because the enzyme-containing catholyte was exchanged at least every three days.

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Keywords: biofuel cell · electrochemistry · energy conversion · enzymes · laccase · microbial

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The authors regret that in the experimental section ("Reactor setup" on page 1214) of the published paper the carbon nanotube loading of the used buckypaper electrodes is given incorrectly. The correct loading is 2 ± 1 mg cm^{-2} instead of 4 ± 1 mg cm^{-2}.

This correction does not affect the results and conclusions of the paper, and the authors would like to apologize for any inconvenience caused. The editorial office apologizes for the oversight.