Selective natural induction of laccases in *Pleurotus sajor-caju*, suitable for application at a biofuel cell cathode at neutral pH

Oleksandra Fokina a, Jens Eipper b, Sven Kerzenmacher b, Reinhard Fischer a,⇑

⇑Corresponding author at: Karlsruhe Institute of Technology (KIT), Institute for Applied Biosciences, Department of Microbiology, Hertzstrasse 16/B, 06.40, D-76187 Karlsruhe, Germany.

E-mail address: Reinhard.fischer@kit.edu (R. Fischer).

**HIGHLIGHTS**

- Selective laccase induction in *Pleurotus sajor-caju*.
- Crude culture supernatant yields electrocatalytic activity for oxygen reduction.
- *Pleurotus sajor-caju* laccases work at pH 5–7 in biofuel cells.

**GRAPHICAL ABSTRACT**

**ABSTRACT**

Laccases are multicopper oxidoreductases with broad substrate specificity and are applied in biofuel cells at the cathode to improve its oxygen reduction performance. However, the production of laccases by e.g. fungi is often accompanied by the need of synthetic growth supplements for increased enzyme production. In this study we present a strategy for the white-rot fungus *Pleurotus sajor-caju* for natural laccase activity induction using lignocellulose substrates and culture supernatant of *Aspergillus nidulans*. *P. sajor-caju* laccases were secreted into the supernatant, which was directly used at a carbon-nanotube bucky-paper cathode in a biofuel cell. Maximal current densities of \(148 \pm 3 \, \text{mA cm}^{-2}\) and \(102 \pm 9 \, \text{mA cm}^{-2}\) at 400 mV were achieved at pH 5 and 7, respectively. Variations in cathode performance were observed with culture supernatants produced under different conditions due to the induction of specific laccases.

**1. Introduction**

Biofuel cells (BFC) provide an ecologically clean alternative to conventional energy production methods. They allow the direct conversion of organic substrates into electrical energy using biological catalysis (Bullen et al., 2006). Microorganisms or enzymes act as biocatalysts, oxidizing organic materials such as biomass, lignocellulose or glucose from extracellular fluids, creating electron flow that can be used to produce electricity (Logan et al., 2006; Minteer et al., 2007; Pant et al., 2010). In BFCs the electron flow is generated at the anode through oxidation of organic substrate followed by the transfer of the produced electrons to the cathode where usually molecular oxygen is reduced.

Laccases (EC 1.10.3.2) have been established as a popular biocatalyst for cathodic oxygen reduction due to their high redox potential, the ability to perform a direct electron transfer from the electrode, and the fact that they neither require nor produce toxic chemical compounds such as for instance \( \text{H}_2\text{O}_2 \) (Christenson et al., 2004; Zheng et al., 2008). Laccases are...
multicopper oxidoreductases with a wide range of substrates and a conserved reaction mechanism. One-electron oxidation of the substrate is performed at the type-1 (T1) copper site in the active center of the enzyme. The electron is then transferred to the trinuclear cluster, containing typically one type-2 (T2) and two type-3 (T3) copper ions. The internal electron transfer is followed by the four-electron reduction of molecular oxygen to water (Christenson et al., 2004). In BFCs carbon-based electrodes serve as electron donor instead of the native laccase substrate (e.g. phenols, lignin).

Laccases are found in bacteria, fungi and plants and fulfill a wide range of functions from lignin polymerization/degradation to spore pigmentation (Baldrian, 2006). In particular, white-rot fungi are capable of secreting large quantities of laccases to degrade lignin in wood. Natural lignocellulose-containing substrates like wheat bran, bagasse or corn cob have been shown to increase laccase levels in fungal cultures (Sahay et al., 2008; Singh et al., 2013). Moreover, co-cultivation of different white-rot fungi also has positive effect on laccase production, probably due to interspecies competition (Baldrian, 2004). A further advantage for the application of renewable biological substrates is the induction of natural mediators that act as electron carriers between laccase and its substrate, increasing the efficiency of the enzyme (Li et al., 1999). The secretion of these compounds improves lignin biodegradation in fungi and is induced in the presence of lignocellulose (Eggert et al., 1996; Li et al., 2014; Riva, 2006). In addition, natural mediators have several advantages over synthetic ones: lower toxicity, fewer or no by-products and low production costs (Christopher et al., 2014).

Currently, purified laccases from the white-rot fungi of the genus *Trametes* are mainly used in biofuel cells for electron transfer from the cathode (Milton et al., 2013; Rubenwolf et al., 2010; Zheng et al., 2008). Recent studies even showed that direct application of crude culture supernatant of *Trametes versicolor* leads to slightly better oxygen reduction performance at the cathode compared to purified enzyme (Sané et al., 2013). Based on this finding we started to study the electrochemical characteristics of laccase-containing crude culture supernatants from less characterized fungi (Fokina et al., 2014).

One of the key operating conditions of BFCs with *T. versicolor* laccase is an acidic pH value around 5, which allows maximum electrocatalytic activity of the enzyme at the cathode (Hussein et al., 2011a; Martinez-Ortiz et al., 2011; Rubenwolf et al., 2010). The scientific interest in oxidases operating at higher pH values for induction in a 1:10 (v/v) ratio. For laccase production in liquid culture the *Pleurotus sajor-caju* DSM 8265 strain (Leibnitz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was grown in yeast extract-agar-glucose medium (YAG), containing 0.5% yeast extract, 1% glucose, 2 mM MgCl₂, vitamins and trace elements (Barratt et al., 1965) at 28 °C, 120 rpm in 100 mL Erlenmeyer flasks. Laccase induction was performed 3 days after inoculation of *P. sajor-caju* cultures by addition of 1 mM CuSO₄, 2 g/L straw, 2 g/L hay, 2 g/L pine wood dust or filtered *A. nidulans* culture supernatant. For the induction of laccase production straw and hay were cut in 0.1–1 cm pieces. Pine wood dust, straw and hay were autoclaved prior to application. For induction via interspecies interaction 50 mL *Aspergillus nidulans* FGSC A4 (Pontecorvo et al., 1953) culture was inoculated with 10⁶ spores and cultivated in liquid YAG medium for 3 days. Afterwards, the culture was filtered through miracloth (Merck KGaA, Darmstadt, Germany) and used for induction in a 1:10 (v/v) ratio.

2.2. Laccase activity assay

The laccase activity in culture supernatant was assayed using 1 mM ABTS (2,2′-azino-bis-[3-ethylthiazoline-6-sulfonate]) as described previously with an Ultrospec III Spectrophotometer (Pharmacia) (Fokina et al., 2014). The reaction velocity was calculated with a molar absorption coefficient of ABTS (ε₄₉₀ = 36,000 L mol⁻¹ cm⁻¹) from the slope of the change of absorbance per time. Laccase activity was expressed in units per mL culture supernatant, with one unit catalyzing the oxidation of 1 μmol ABTS per minute.

2.3. Identification of laccases in culture supernatant

The culture supernatants of *P. sajor-caju* after the induction with straw and A. nidulans were analyzed using 12.5% SDS-PAGE followed by Coomassie staining and 7.5% non-denaturing PAGE with 0.025% Nonidet P-40 (w/v). Native gels were incubated in 50 mM sodium acetate buffer pH 5 after the protein separation and stained with 1 mM ABTS.
For laccase identification in culture supernatants and electrochemical characterization of enzymes the proteins were purified from culture supernatants using anion exchange chromatography with DEAE (diethylaminoethyl) Sepharose as described previously (Fokina et al., 2014). Laccases in elution fractions were detected using ABTS assay and analyzed by SDS-PAGE. The gel bands, corresponding to the approximate molecular mass of *P. sajor-caju* laccases, were excised and sent for peptide mass fingerprinting analysis using MALDI-TOF/MS (TOPLAB GmbH, Martinsried, Germany). Laccase sequence alignment was performed with Clustal Omega multiple alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalo).

2.4. Electrochemical characterization

The electrochemical characterization of *P. sajor-caju* culture supernatant and purified laccases was performed in half-cell configuration using linear sweep voltammetry and a step-wise galvanostatic polarization curves measurements. Buckypaper electrodes, made from multi-walled carbon nanotubes on a nylon filter support (Whatman, Dassel, Germany), were prepared as reported previously (Hussein et al., 2011a,b) and applied as cathodes. A platinum mesh electrode was used as the counter electrode (Goodfellow, Huntingdon, UK).

The laccate containing culture supernatant was filtered through miracloth prior to application and diluted to 1.8 U/mL laccase concentration vs. ABTS in 100 mM sodium acetate buffer pH 5 or 100 mM sodium phosphate buffer pH 7 and titrated to pH 5 or 7 respectively. For electrochemical characterization of purified laccases the laccate-containing elution fractions after fast protein liquid chromatography (FPLC) purification were pooled and concentrated with Vivaspin centrifugal concentrators (Sartorius AG, Göttingen, Germany). Subsequently, the laccase solution was diluted to 2.5 U/mL with sodium-acetate buffer or sodium phosphate buffer and titrated to pH 5 or 7.

Linear sweep experiments were performed with 50 mL culture supernatant or laccase from *T. versicolor* (Sigma-Aldrich, Taufkirchen, Germany) in electrode compartment using WaveNow USB Potentio-/Galvanostat (Pine Research Instrumentation Durham, USA) as described previously (Hussein et al., 2011a). The current measurements were performed from 20 mV above OCP (open circuit potential) to −244 mV vs. SCE or −199 mV vs. Ag/AgCl (0 mV vs. NHE) reference electrode (Sensortechnik Meinsberg, Ziegra-Knobelsdorf, Germany) with a scan rate of 3 mV/s.

Galvanostatic polarization curves under quasi-steady-state conditions were measured using the electrical testing setup and electrochemical reactor as described previously (Kerzenmacher et al., 2009; Kloké et al., 2010). Before the measurement the enzyme was incubated in electrode compartment with 0.9 cm² for 12 h at open circuit conditions. During the recording the current density was increased in 5.5 μA cm⁻² h⁻¹ steps to the maximum of 225 μA cm⁻² and the electrode potential were recorded vs. a SCE reference electrode.

3. Results and discussion

3.1. Induction of laccases from *Pleurotus sajor-caju*

Production of laccases in fungi is mostly dependent on the growth conditions. The stimulation with various growth supplements like copper sulfate or aromatic compounds combined with longer incubation periods are often required to reach laccase concentrations sufficient for direct and efficient application of crude culture supernatant (CS) in biofuel cells (Sané et al., 2013).

*P. sajor-caju* responded best to natural lignocellulose-containing substrates like straw and hay in standard growth medium and reached the culture laccase activity levels, required for BFCs, one week after induction (Fig. 1). In the absence of inducers extracellular laccase activity levels remained low during the cultivation (70 U/L). Maximal laccase activities of 2350 U/L and 2070 U/L were achieved 7 days after the induction with straw and hay, respectively. Also, probably due to the shearing forces, caused by the straw/hay particles in shaken cultures, the formation of larger mycelial agglomerates, typical for submerged fungal cultures, was avoided in these cultures. The addition of cellulase- and laccase-free supernatant from *A. nidulans* culture also had a positive effect on laccase production (1285 U/L), though its effect was weaker. Surprisingly, wood dust had a much weaker effect on laccase production and was comparable with the effect of copper sulfate. These results suggest that natural lignocellulose-containing substrates can be used in large-scale laccase production instead of synthetic/toxic supplements. Also *P. sajor-caju* could be applied in other biotechnological branches like for example lignocellulose waste treatment, with lignocellulose serving as both substrate and inducer of enzyme activity. Additionally, presence of other microorganisms in the waste could further induce the enzyme production, as demonstrated on the example of *A. nidulans* in the laccase induction experiments.

3.2. Electrochemical characterization of laccase-containing culture supernatants

The cathodic oxygen reduction performance of laccase in crude culture supernatant at the buckypaper electrode (BP) was analyzed using linear sweep voltammetry (LSV) and by recording polarization curves in a step-wise galvanostatic technique. Cultures from induction experiments with straw and *A. nidulans* culture supernatant, representing two different induction conditions, were chosen for these experiments.

Linear sweep voltammetry (LSV) measurements with 1.8 U/mL laccase-containing *P. sajor-caju* culture supernatant showed that the presence of the enzyme strongly increased the cathode performance compared to the control measurement without laccase (Fig. 2a). At pH 5 the open circuit potential was raised from 168 ± 6 mV vs. SCE to 554 ± 2 mV vs. SCE for *A. nidulans* induction and to 574 ± 3 mV vs. SCE for induction with straw, which is slightly lower compared to the known laccase-containing culture supernatants of *T. versicolor* and *P. sanguineus* with OCP values of 612 mV vs. SCE and 582 mV vs. SCE respectively (Fokina et al., 2014; Sané et al., 2013). Laccases in *P. sajor-caju* culture
supernatants were capable to perform electron transfer from the cathode without the addition of mediators, as indicated by the change in current densities (Fig. 2a). Current densities of 

\[-148 \pm 3 \mu \text{A cm}^{-2}\] and 

\[-129 \pm 18 \mu \text{A cm}^{-2}\] were achieved at 400 mV with straw and A. nidulans induction supernatants respectively.

As shown in Fig. 2a the OCP of the cathodes at pH 7 with both P. sajor-caju culture supernatants decreased slightly to 470 ± 1 mV vs. SCE (A. nidulans) and 491 ± 1 mV vs. SCE (straw). However, they were still higher than previously shown OCP of 436 mV vs. SCE for P. sanguineus culture supernatant at pH 7 (Fokina et al., 2014). Since no performance data of T. versicolor laccase with buckypaper electrode at pH 7 were published so far, a reference measurement with purified T. versicolor laccase in phosphate buffer pH 7 were used as references. Average values of triplicate experiments are shown. (b) Polarization curves were recorded with 1.8 U/mL laccase concentration after the induction with straw and A. nidulans at pH 5 and 7, as indicated. Average values of triplicate experiments are shown. Bars represent maximum and minimum values.

As shown in Fig. 2a the OCP of the cathodes at pH 7 with both P. sajor-caju culture supernatants decreased slightly to 470 ± 1 mV vs. SCE (A. nidulans) and 491 ± 1 mV vs. SCE (straw). However, they were still higher than previously shown OCP of 436 mV vs. SCE for P. sanguineus culture supernatant at pH 7 (Fokina et al., 2014). Since no performance data of T. versicolor laccase with buckypaper electrode at pH 7 were published so far, a reference measurement with purified laccase in buffer was performed. It showed that the laccase from T. versicolor almost completely lost the ability to interact with the cathode at pH 7 (Fig. 2a). This result corresponds to a previously published study, showing relatively low activity of the T. versicolor laccase in a biofuel cell with a mediator-graphite electrode system at pH 7 (Barrière et al., 2006). A maximum current density of 

\[-102 \pm 9 \mu \text{A cm}^{-2}\] was achieved with the supernatant from straw-containing culture at 400 mV vs. SCE. Remarkably, the difference between current densities at different pH values of straw-induced culture supernatant was very low, while the laccases from A. nidulans induction showed a relatively strong decrease in cathode performance at pH 7 (–69 ± 3 \mu \text{A cm}^{-2} at 400 mV).

Cathode polarization curves with P. sajor-caju culture supernatants (1.8 U/mL laccase) were recorded to test the electrochemical activity of the laccases under quasi-steady-state conditions. Compared to the previously measured OCP of 156 ± 6 mV vs. SCE of the cathode without the laccase (Fokina et al., 2014), a significant increase in OCP in the presence of P. sajor-caju culture supernatants was detected (Fig. 2b), similarly to the LSV experiments. Laccase-containing culture supernatant from the straw-containing culture showed higher electrocatalytic activity than the CS from A. nidulans induction until approximately 200 mV vs. SCE at both pH 5 and 7. Significantly, the cathodes with straw-containing CS showed a slow gradual decrease of cathode potential with increasing current at both pH values, whereas the cathodes with culture supernatants from A. nidulans induction had a steep drop in potential around 155 mV, which is normally attributed to mass transport limitations or limited enzyme turnover numbers (Hussein et al., 2011a). It is possible that products of straw degradation by the fungus in culture supernatants aggregated at the cathode during the longer-time galvanostatic measurement and impaired cathode performance. Also, if the fungus secreted mediators into the culture medium due to the presence of straw, they would support the laccase activity, but they would also degrade slowly over time.

The electrocatalytic activity of P. sajor-caju laccases at pH 7 was significantly higher, than the activity measured with crude P. sanguineus CS (OCP, pH 7–436 mV vs. SCE) and multi copper efflux oxidase CueO (OCP, pH 7.4–229 mV vs. SCE) in the previous studies (Fokina et al., 2014; Sané et al., 2014). In both cases large activity losses were observed immediately after increasing the current density at neutral pH values.

There are two possible explanations for the difference in cathode performance with culture supernatants from two different induction experiments, observed in linear sweep measurements and polarization records. It has been shown previously that laccases from P. sajor-caju exhibit different expression levels depending on the growth conditions (Soden and Dobson, 2001). The induction with straw could cause secretion of a laccase that is more active at higher pH values than the other. Another explanation would be the secretion of natural electron mediators by the fungus in the presence of straw to make its assimilation as carbon source easier for the fungus. It would explain the similar current densities in the presence of straw culture supernatant at pH 5 and 7 in linear sweep measurements. The amount of mediator, similar in both experiments, would be the limiting factor for the cathodic oxygen reduction, not the amount/performance of the laccase itself. Degradation products and various proteins, secreted to digest straw, could also interfere with the cathode-laccase interaction and limit the maximum cathode performance.

In the case of the P. sajor-caju culture after the induction with A. nidulans, less components (e.g. mediators, interfering compounds) would be present in the medium and the laccase would be the limiting factor, determining the electron transfer rate from the cathode. Therefore, the system would be more vulnerable to pH variation, typical for laccases. Since less components would be present in the medium under these conditions, less interference with the laccase electron transfer from the cathode would occur and higher current densities could be achieved under optimal conditions at pH 5, explaining the results shown in Fig. 2a.

To find out, whether the observed effect was caused by mediators or laccases themselves, linear sweep voltammetry was performed with P. sajor-caju culture supernatant with an artificial mediator ABTS. Since ABTS was added in excess, it should have overlaid the effect of natural mediators. As shown in Fig. 3, ABTS had a strong effect on both cathode OCP values and current densities in both culture supernatants. The decrease in cathode OCP, compared to the culture supernatants without ABTS, can be attributed to a lower, compared to laccase, redox potential of ABTS (446 mV vs. SCE) (Branchi et al., 2005). ABTS also contributes...
significantly to higher current densities, by shuttling electrons from the electrode to the adsorbed and dissolved laccase molecules, as it was also shown for a *T. versicolor* laccase (Hussein et al., 2011a). Remarkably, in contrast to the measurements without ABTS, the culture supernatant after the induction with straw showed a large difference in current densities at pH 5 and 7 in the presence of ABTS (Fig. 3b). ABTS has probably overlaid the positive effect of natural mediators that previously allowed higher laccase activity at the cathode at pH 7. The overall effect of ABTS on current densities was slightly weaker in culture supernatant after *A. nidulans* induction (Fig. 3a) despite the same laccase concentration of 1.8 U/ml, suggesting that laccases with different properties were present in the two cultures.

3.3. Identification and electrocatalytic characterization of laccases in culture supernatant

Though several laccases have been reported and partially characterized in *P. sajor-caju* previously, various expression patterns could be expected in different strains (Fu et al., 1997; Soden and Dobson, 2001). To investigate the content of secreted proteins in cultures after the induction with straw and *A. nidulans* CS, electrophoretic analyses were performed. SDS-PAGE showed, as expected, that both inductions caused secretion of various proteins in culture medium (Fig. 4a). Several protein bands, corresponding to the calculated molecular masses of *P. sajor-caju* laccases, were present in the two cultures. In the presence of straw, produced mostly laccases 2 and 4 and contained only small amounts of laccase 3. On the other hand, the culture with *A. nidulans* CS secreted large amounts of laccase 3 and the protein band, corresponding to the laccases 2 and 4, was less prominent.

All of the *P. sajor-caju* laccases belong to the same class and should possess similar electrocatalytic properties, but they could exhibit differences in pH tolerance due to their individual structural characteristics. Therefore, the enzymes after FPLC purification were concentrated and analyzed electrochemically by recording polarization curves at pH 5 and 7 with 2.5 U/ml laccase concentration. As shown in Fig. 5, the maximum current density of the laccases at the cathode decreased, compared to the polarization curves measured with culture supernatants (Fig. 2b), even though more enzyme was used in the measurements. A similar effect was observed previously with *T. versicolor* and *P. sanguineus* laccases (Fokina et al., 2014; Sané et al., 2013). Culture supernatant clearly presents a more suitable environment for the laccase catalysis at the cathode even in the presence of various proteins and other compounds. The ion exchange chromatography, followed by dialysis not only separates proteins, but also removes compounds with low molecular weight that could act as mediators and support the laccase activity.

On the other hand, the polarization curves showed that laccases from *P. sajor-caju* were capable of direct electron transfer from the cathode and exhibited almost no decrease in OCP due to the changing pH after anion exchange chromatography (Fig. 5). At pH 5 laccase 3 had an OCP of 489 ± 21 mV and at pH 7–436 ± 46 mV. Similarly, the mixture of laccases 2 and 4 had an OCP of 520 ± 23 mV at pH 5 and 477 ± 1 mV at pH 7. The shape of the load curves, taken in the presence of laccases 2 and 4, resembles the ones measured with straw-induced culture supernatant, and the
shape of the curves with laccase 3 – the measurements with culture supernatant after the A. nidulans induction. This observation correlates with the results of the electrophoretical analysis of the culture supernatant protein content.

Evidently, the electrocatalytical activity of the laccases from P. sajor-caju is supported by compounds in culture supernatant, that are secreted by the fungus and can probably act as mediators.

However, the enzymes themselves obviously possess characteristics that grant them partial pH tolerance, when operating at the buckypaper cathode.

3.4. Correlation between structural characteristics of laccases and their pH tolerance

Based on the studies of the pH dependence of laccase electron absorption, inhibition, redox potential and catalyzed substrate oxidation, it was proposed that two opposing effects play a role in the pH optimum determination of laccases (Xu, 1997). First, the redox potential difference between the substrate and the T1 copper of the laccase could increase substrate oxidation at higher pH. Secondly, the increasing amount of hydroxide anion, binding to the T2/T3 coppers, could disrupt the electron flow inside the enzyme. The pH-induced changes may occur in the substrate, oxygen or the enzyme, in particular on its catalytic T1 and T2/T3 centers.

The comparison of the amino acid sequences of the high redox potential laccases with different pH activity profiles in enzymatic biofuel cells with the isolated laccases from P. sajor-caju showed that the copper-binding domains of these laccases are highly conserved in all three species (Fig. 6). Laccases 2 and 4 from P. sajor-caju have only two different residues in the T1 site compared to the laccases from T. versicolor and P. sanguineus, marked with arrows in Fig. 6. The F457 amino acid residue in T. versicolor laccase is changed to W462 in laccase 2 and W465 in laccase 4, F463 is...
Protein sequence alignment. Amino acid sequences of laccases 2, 3, 4 from *P. sajor-caju* (GenBank accession numbers CAD45378, CAD45379 and CAD45380), laccase 1 from *T. versicolor* (AAC49828) and laccase from *P. sanguineus* (ACZ37083) were aligned using Clustal Omega tool. The copper-binding consensus sequences are in bold blue, signal peptides in green. The differences in copper binding sites are indicated by arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
changed to L468 and L471 respectively. Laccase 3 further has two P. sajor-caju of this residue for pH tolerance of laccases has not been reported so far. Other three residues, which differ in P. sajor-caju laccase compared to the enzymes from Trametes and Pycnoporus, were not reported to be directly involved in the binding of coppers in the catalytic center.

Despite conserved active center, laccases from P. sajor-caju, T. versicolor and S. sanguineus exhibit broad variability in their amino acid sequences. Though crystal structures of the laccases from P. sajor-caju would deliver a clearer picture, it is probable that the pH tolerance of the laccases, observed in BFC in this study, is not a direct effect of copper ions coordination in the catalytic center. To determine, which structural features favor the laccase activity under neutral pH, more laccases with high pH tolerance should be isolated and analyzed. The structural similarities found in these enzymes compared to the pH sensitive laccase from for example T. versicolor, would allow further insights into the pH influence on laccase catalysis.

4. Conclusions

The results of this study show that natural substrates and co-cultivation can be used to selectively induce the production of specific laccases in P. sajor-caju. Moreover, compounds in the culture supernatant, secreted under these conditions, support laccase-mediated cathode oxygen reduction, probably due to their activity as natural mediators. Laccases from P. sajor-caju are also more active at pH 7 in BFCs than previously characterized laccases. They bring us one step closer to understanding the mechanisms behind laccase pH tolerance and are essential for the improvements in biotechnological branches with a requirement for pH insensitive laccases like dye decolorization or bioelectrochemistry.

Acknowledgement

This work was supported by the German Federal Ministry of Education and Research (BMBF) through the program “BioProFi” (FKZ: 03SF0424).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.06.126.

References