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Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from Pleurotus ostreatus

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Publication Details

Chapple, A., Nguyen, L. N., Hai, F. I., Dosseto, A., Rashid, M., Oh, S., Price, W. E. & Nghiem, L. D. (2019). Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from Pleurotus ostreatus. Biocatalysis and Biotransformation, 37 (1), 10-17.

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Abstract
This study investigated the influence of inorganic salts on enzymatic activity and the removal of trace organic contaminants (TrOCs) by crude laccase from the white-rot fungus *Pleurotus ostreatus*. A systematic analysis of 15 cations and anions from common inorganic salts was presented. Laccase activity was not inhibited by monovalent cations (i.e. Na^+ , NH_4^+ , K^+), while the presence of divalent and trivalent cations showed variable impact - from negligible to complete inhibition - of both laccase activity and its TrOC removal performance. Of interest was the observation of discrepancy between residual laccase activity and TrOC removal in the presence of some ions. Mg^{2+} had negligible impact on residual laccase activity but significant impact on TrOC removal. Conversely, F^- showed greater impact on residual laccase activity than on TrOC removal. This observation indicated different impacts of the interfering ions on the interaction between laccase and TrOCs as compared to that between laccase and the reagent used to measure its activity, implicating that residual laccase activity may not always be an accurate indicator of TrOC removal. The degree of impact of halides was in the order of $F^- > I^- > Br^- > Cl^-$. Particularly, the tolerance of the tested laccase to Cl – has important implications for a range of industrial applications.

Disciplines
Engineering | Science and Technology Studies

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This journal article is available at Research Online: https://ro.uow.edu.au/eispapers1/985

Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from *Pleurotus ostreatus*

Revised Manuscript Submitted to

Biocatalysis and Biotransformation

November 2017

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Abstract:

This study investigated the influence of inorganic dissolved constituents (i.e., cations and anions) on enzymatic activity and trace organic contaminants (TrOCs) removal by crude laccase from the white-rot fungus *Pleurotus ostreatus*. A systematic analysis of 15 cations and anions from common inorganic salts was presented. Laccase activity was not inhibited by monovalent cations (i.e., Na^+, NH_4^+, K^+), while the presence of divalent and trivalent cations showed variable impact – from negligible to complete inhibition – of both laccase activity and its TrOC removal performance. Of interest was the observation of discrepancy between residual laccase activity and TrOC removal in the presence of some ions. Mg^{2+} had negligible impact on residual laccase activity but significant impact on TrOC removal. Conversely, F^- showed greater impact on residual laccase activity than on TrOC removal. This observation indicated different interactions of the interfering ions with laccase and TrOCs as compared to laccase and the reagent used to measure its activity, meaning that residual laccase activity may not always be an accurate indicator of TrOC removal. The degree of impact of halides was in the order of $F^- > I^- > Br^- > Cl^-$. Particularly, the tolerance of the tested laccase to Cl^- has important implications for a range of industrial applications.

Keywords: Laccase; trace organic contaminants (TrOCs); inorganic salts; halides; metals

Introduction

Trace organic contaminants (TrOCs) include, among others, pharmaceuticals and industrial chemicals. (Hoeger et al. 2005; Lee et al. 2014; Luo et al. 2014). Conventional wastewater treatment processes are not designed to remove TrOCs and thus wastewater treatment plant effluent is considered as a major source of TrOC into the environment. TrOCs often occur in various aquatic environment at concentrations ranging from few ng/L to µg/L (Luo et al. 2014). Due to the persistence and/or bioaccumulative properties, many of the TrOCs pose risk to ecosystem and public health.

Enzymatic transformation of TrOCs has recently attracted much attention as a promising eco-friendly concept (Yang et al. 2013). Laccase (Benzenediol: oxygen oxidoreductase; EC 1.10.3.2) is of interest as it only requires molecular oxygen as a co-substrate, unlike the peroxidases which require H_2O_2. Laccase catalysed oxidation mechanism includes the reduction of molecular oxygen to water and one electron oxidation of various aromatic substrates such as TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; Yang et al. 2013). Laccase has been found to be abundant in many white-rot fungi. The potential of laccase for the removal of TrOCs has been investigated intensively by various researchers. Results have demonstrated that laccase can effectively degrade a range of TrOCs (Majeau et al. 2010; Nguyen et al. 2014b; Spina et al. 2015; Tran et al. 2010; Yang et al. 2013). TrOC removal by laccase is governed by factors such as pH, temperature, and physicochemical properties of TrOCs (Asif et al. 2017a). For example, optimum pH for the removal of triclosan was in the range of 5 to 5.5 (Kim & Nicell 2006b), while diclofenac was found to be removed at the highest rate under acidic conditions (pH 3 - 4.5) (Nguyen et al. 2014a). TrOC properties can also strongly influence laccase performance. The compounds which contain phenolic moiety are more amendable to laccase. On the other hand, compounds which contains functional group such as carboxylic, amide, and chloride are resistant to laccase oxidation (Asif et al. 2017b; Shi et al. 2017; Tran et al. 2010; Yang et al. 2013).

Only a few studies have provided some insight into the effect of heavy metals on the removal of dye by laccase (Murugesan et al. 2009; Rodríguez Couto et al. 2005). Murugesan et al. (2009) reported that metal ions such as Ca^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} at a concentration of 1 mM did not have impact on laccase performance. However, it is hypothesized that some ions may block or interfere with the active sites of laccase and thus decrease its activity (Asif et al. 2017b; Tran et al. 2010; Yang et al. 2013). Notwithstanding the available studies, the impact of

wastewater-derived dissolved interfering compounds on the removal of TrOCs by laccase has not been fully elucidated. Dissolved organic (e.g., humic substance, organic matters) and inorganic constituents (cations and anions) widely occur in water and wastewater. Therefore, to fully uncover the potential of laccase for the removal of TrOCs, the effects of these constituent ions need to be studied.

The aim of this study was to investigate the impact that a range of dissolved inorganic ions impose on laccase including its activity level and its removal of two TrOCs, namely, bisphenol A (BPA) and diclofenac (DCF). The experiment will cover a range of common salts at different concentrations, with the objective of not only representing wastewater streams that may be encountered, but also gaining an understanding of relative ionic influences from which extrapolations can be made to predict the influence of certain wastewater components. The results will allow development of enzyme based treatment systems to be optimised, especially around TrOC removal.

Materials and Methods

Materials

TrOCs and dissolved interfering salts

Two TrOCs, namely, bisphenol A (BPA) and diclofenac (DCF) (Sigma–Aldrich, USA) were selected in this study because of their ubiquitous presence in wastewater and wastewater-impacted waterbodies.

A set of cations and anions, which occur widely in water and wastewater, were selected to test their impact on laccase activity and its TrOC removal performance. Table 1 presents the selected cations and anions and the associated original salts.

Crude laccase preparation

A white-rot fungus *Pleurotus ostreatus* (ATCC 34675) was incubated in malt extract broth (2 g/L) at a pH of 4.5 to produce crude enzyme solution. The culture was kept in a rotary shaker at 28 °C and 70 rpm for 5 days. The crude enzyme was obtained by decanting the liquid portion into a sterile container. Under these culture conditions, the enzyme preparation exhibited predominantly laccase activity (70 to 90 $\mu M_{(DMP)}$/ min) and negligible peroxidase activity. The crude laccase preparation thus obtained was stored at 4 °C until use.

Batch test description

A strategic experimental plan was implemented in this study. Kim and Nicell (2006a), reported negligible impact of Na^+ and NH_4^+ on laccase activity. Therefore, at first, SO_4^{2-} dissolved from sodium and ammonium sulfate salts was tested to elucidate if SO_4^{2-} has low impact so that sulfate salts can be used as a source of cations. After confirming the low impact of SO_4^{2-}, a range of sulfate salts (Table 1) was used.

To test the variance in influence of the anions, tests were conducted to include PO_4^{3-} and NO_3^-, and four halides i.e., F^-, I^-, Br^- and Cl^-. The anions were matched with either Na^+ or K^+ to select the salt to be added to the test solution (Table 1).

The test solution was prepared in sterile test tubes. The impact of each ion was tested at the following concentrations: 1, 10, 100 and 250 mM. TrOCs were each added at a nominal concentration of 100 μg L^{-1} (actual measured concentrations of 116 ± 10 μg/L and 109 ± 5 μ/L (n = 14) of BPA and DCF, respectively).

A set of control tubes were prepared in the same fashion but excluding the interfering cations and anions. The test tubes were capped and incubated in a rotary shaker at 70 rpm and 25 °C for 24 h, following which the residual laccase activity and TrOC concentration were measured.

Analytical methods

Laccase activity

Laccase activity was measured by monitoring the change in absorbance at 468 nm due to the oxidation of 2,6-dimethoxy phenol (DMP) at room temperature over 2 min using a spectrophotometer (Spec UV-1700, Shimadzu, Kyoto, Japan) (Hai et al. 2009). Laccase activity was calculated from the molar extinction coefficient ε = 49.6 /mM.cm and expressed in μM substrate/min. Lignin and manganese peroxidase activity were determined as described elsewhere (Camarero et al. 1999).

TrOC analysis

A HPLC system (Shimadzu, Kyoto, Japan), equipped with a Supelco Drug Discovery 300 × 4.6 mm C-18 column (5 μm pore size) and a UV–vis detector, was used to measure the TrOC concentrations. The detection wavelength was 280 nm and the column temperature was 20 °C. The sample injection volume was 50 μL. The mobile phase comprised acetonitrile and

Milli-Q water buffered with 25 mM KH_2PO_4 (pH = 4.8). Two eluents, A (80% acetonitrile + 20% buffer, v/v) and B (20% acetonitrile + 80% buffer, v/v) were delivered at 0.7 mL/min through the column for 30 min in the following time-dependent gradient proportions: [Time (min), % of B] = [0, 80], [12, 80], [20, 0], [25, 0], [25, 80]. Under the operating conditions, the retention time of BPA and DCF was 23 and 26 min, respectively. The limit of quantification for the analytes under investigation using these conditions was approximately 10 µg/L. HPLC samples were prepared by diluting the samples two-fold by adding methanol to immediately stop any residual enzyme activity in the sample (Nguyen et al. 2014a).

Results and discussion

Impact of cations

Kim and Nicell (2006a) reported that sodium and ammonium ions had negligible impact on laccase activity. Therefore, sodium and ammonium sulfate salts were tested first to elucidate the impact of SO_4^{2-}. A negligible impact on laccase activity was observed until a SO_4^{2-} concentration of 250 mM, where a 23% drop in activity was observed. At this concentration, DCF removal efficiency showed a 30% decrease compared to the control (Figure 1). Based on this initial assessment of the impact of SO_4^{2-}, monovalent, divalent, and trivalent cations dissolved from sulfate salts were evaluated for their impact on laccase activity and TrOC removal performance.

[FIGURE 1]

Impact of type of cations

Figure 2 illustrates the impact of cations on both laccase activity and its performance on TrOC removal. The results show that monovalent cations (Na^+, $NH4^+$, and K^+) have low impact on both laccase activity and TrOC removal. At a concentration of 250 mM, Na^+ and NH_4^+ caused a 20% drop in laccase activity, whereas, K^+ showed positive impact with about 5% increment in observed laccase activity. Further discussion regarding this apparent increase in laccase activity is available in the penultimate section of the paper. The relative stability of laccase was reflected in the removal of BPA. On the other hand, Na^+ and NH_4^+ showed 50% and 30% decrease in DCF removal, respectively. Comparatively less impact of cations on BPA removal can be attributed to the fact that it is a phenolic compound which is highly amenable to laccase-catalysed degradation (Spina et al. 2015; Yang et al. 2013).

Divalent and trivalent cations (Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Al^{3+}) showed variable impact on laccase activity and TrOC removal. Mg^{2+} did not adversely affect laccase activity but showed significant impact on TrOC removal: 70 and 60% decreases in BPA and DCF removal, respectively was observed. It is possible that Mg^{2+} inhibited the catalytic activity of laccase on BPA and DCF but not on DMP (the substrate used in enzyme assay). The mismatch between impact on residual laccase activity and TrOC removal suggests that residual laccase activity may not be always used to indicate the impact of interfering cations on laccase-catalysed TrOC removal.

Ca^{2+} and Cu^{2+} showed significant impact on both laccase activity and TrOC removal. Up to 93% inhibition of laccase activity was observed in the presence of Cu^{2+}. This was accompanied by 90% and 70% drop in removal of BPA and DCF, respectively. This observation is consistent with that in the available literature. For example, Cu^{2+} has been shown to impose significant influence on laccase even at low concentrations (Hou et al. 2014; Lorenzo et al. 2005). Murugesan et al., (2009) reported a severe impact of Cu^{2+} on laccase from *Ganoderma lucidum*.

Zn^{2+} and Mn^{2+} demonstrated a moderate impact on both laccase activity and TrOC removal. Zn^{2+} caused about 30% and 40% reduction in laccase activity and TrOC removal, respectively. A stronger impact of Al^{3+} laccase activity and TrOC removal was noted (Figure 2). To date the mechanisms in which cations affect laccase activity and its TrOC removal performance have not been elucidated. Some possible mechanisms of metal-induced inactivation of laccase include modification of amino acid residue on enzyme, copper chelation or conformational change of the enzymes (Chmelová & Ondrejovič 2015; Johannes & Majcherczyk 2000).

[FIGURE 2]

Impact of cation concentrations

The cations selected in this study frequently occur in water and wastewater. Their concentration can vary with season, geographical location and type of water/wastewater. Therefore, the stability of laccase and its TrOC removal performance against several different concentrations (1-250 mM) of cations was assessed in this study (Figure 3).

As discussed before, the monovalent cations *i.e.*, Na^+, NH_4^+, and K^+ showed little impact on laccase activity over the whole concentration range (Figure 3). A previous study by Shankar et al. (2015) also reported no impact of Na^+ (0.5 - 15 mM) on laccase from *Peniophora sp*. On the other hand, Trovaslet et al. (2007) found that with an increase in Na^+ concentration from 0 – 1 M, the activity of the laccase from *T. versicolor* gradually decreases from 100% to 50%. Our results demonstrate that up to a concentration of 250 mM, laccase from *P. ostreatus* has strong tolerance to monovalent cations such as Na^+, NH_4^+, and K^+. Similar to laccase activity, the change in Na^+, NH_4^+, and K^+ concentration did not show any considerable impact on BPA removal. However, approximately 40-50% inhibition of DCF removal was observed at all tested concentrations of the monovalent ions Na^+, NH_4^+, and K^+, demonstrating again the resistance of DCF to laccase-catalysed degradation.

[FIGURE 3]

The divalent cations showed a strong effect at a concentration of 250 mM (Figure 2). Therefore, it was interesting to observe their impacts over a lower concentration range. The residual laccase activity in the presence of Mg^{2+} appear to be higher than that of the control. Mg^{2+} was also found to increase laccase activity in a study by Shankar and Nill (2015). Further discussion regarding this apparent increase in laccase activity is available in the penultimate section of the paper.

In contrast to laccase activity, the removal of BPA and DCF gradually dropped with Mg^{2+} concentration-increase. Ca^{2+} affected laccase activity at 100 mM. However, the effect of Ca^{2+} on TrOC removal was significant even at 1 mM (Figure 3). Murugesan et al. (2009) observed a concentration-dependent effect of Ca^{2+} on dye decolourisation by laccase from *Ganoderma lucidum*. Our study not only confirms such salt concentration dependent effect on TrOC but also highlights different extents of impact on laccase activity and TrOC removal.

Murugesan et al. (2009) observed a minor impact of Zn^{2+} on laccase and its dye decolourisation capacity. Similarly, in the current study, irrespective of its concentration, Zn^{2+} showed moderate inhibition of laccase activity and TrOC removal (Figure 3). Mn^{2+} also exhibited a moderate impact on laccase activity with around 20% inhibition at all tested concentrations. It is noteworthy that a 10% increase in BPA removal was achieved in presence of 1 and 10 mM Mn^{2+} (Figure 3). This is consistent with a few other studies who report enhanced

enzymatic dye decolourisation in presence of metal ions in low concentration range, generally below 15 mM (Majeau et al. 2010; Murugesan et al. 2009; Shankar & Nill 2015).

Cu^{2+} is of special interest as it is a key component in laccase structure. Previous studies showed no effect of Cu^{2+} (Murugesan et al. 2009; Shankar & Nill 2015) up to a concentration of 15 mM. The observation of significant inhibition at 250 mM in this study (Figure 2) necessitated testing at lower concentration range. As showed in Figure 3, Cu^{2+} has strong impact on both laccase stability and TrOC removal. Even at 1 mM, laccase activity was reduced by 50%. TrOC removal illustrated an interesting response to Cu^{2+}. Throughout this study, compared to BPA, DCF removal was observed to be affected more by different salts. As noted before, this can be explained by the fact that BPA has a phenolic moiety which makes it especially amenable to laccase catalysis (Majeau et al. 2010; Nguyen et al. 2014b; Tran et al. 2010; Yang et al. 2013). However, in the current study, in presence of 1 mM Cu^{2+}, BPA removal was down to 10% compared to control, while DCF removal appeared less affected. It is probably because the interaction between BPA and laccase was affected in the presence of Cu^{2+}, but not that of DCF and laccase. It is also possible that a different mode of interaction is involved here: Cu^{2+} is considered as a pro-oxidant and it can act as a catalytic oxidant which may form a copper-DCF complex, making DCF more amenable to degradation (Yang et al. 2013).

Impact of anions

Impact of halides

Halides are found in many industrial products and have been associated with significant environmental pollution and toxicity. Compared to Cl^- and F^-, the impact of halides Br^- and I^- on laccase have received much less attention in recent literature. Thus, the selection of all four halides in this study fills an important research gap.

In a similar approach to that taken with using sulfate salts to assess the impact of cations, the halides were matched with cations such as Na^+ or K^+ whose low impact had already been established (Figure 3). Cl^- showed about 20% reduction in laccase activity at a concentration of 250 mM (Figure 4). The extent of laccase activity reduction due to sodium chloride was even smaller than that for sodium sulfate (Figure 3). Consistent with laccase stability, Cl^- effect on BPA removal was negligible. However, up to 40% removal of DCF was observed. The effect of Cl^- on laccase stability and its TrOC removal performance has not been thoroughly tested in the

literature. Champagne et al. (2013) reported that Cl^- strongly affected both the laccase activity and its dye decolourisation efficiency. However, our study confirms that Cl^- does not impose as high of a negative influence on laccase secreted by *P. ostreatus*.

In this study, Br^- showed no significant impact on laccase activity. The activity dropped by 10% at 1 mM, and then levelled off at 20% for 10, 100 and 250 mM. However, BPA removal efficiency gradually decreased with the increase of Br^- concentration. It is noted that the Br^- ion was dissolved from potassium bromide. In comparison with the impact of potassium sulfate (Figure 3), BPA removal decreased more in case of potassium bromide (Figure 4), which confirmed some impact of Br^-. On the other hand, I^- exhibited a strong impact on laccase activity. The residual laccase activity dropped by about 50 % in the presence of 1 mM I^- and then reduced to 16% at higher concentrations. However, only about 30% reduction in BPA removal was observed at the highest I^- concentration i.e., 250 mM. This study is the first to report the impact of Br^- and I^- on laccase stability and its TrOC removal performance.

F^- inhibited laccase activity significantly (Figure 4): the activity reduced by 50% and 95% at F^- concentrations of 1 mM and 10 mM, respectively. At 100 and 250 mM, no laccase activity was detected, which highlights the magnitude of the influence of F^-. From literature, a complete inhibition of enzymatic activity can be seen at F^- levels as low as 1 mM (Jung et al. 2002), but the available studies did not investigate TrOC removal. The current study additionally confirms that F^- strongly affects the laccase-catalysed degradation of both BPA and DCF.

In this study, the halides were observed to have an impact on laccase activity and TrOC removal in the following order: $F^- > I^- > Br^- > Cl^-$. The difference in the extent of inhibition by halides is probably due to the different mechanisms in which each halide interact with laccase. Morozova et al. (2007) suggested that these anions bind with the Type 2 and 3 copper atoms of laccase, preventing the electron to transfer from the Type 1 site, consequently inhibiting the oxidation pathway. Farnet et al. (2008) reported that Cl^- and Br^- ions act as competitive inhibitors with the electron donor while F^- acts as a non-competitive inhibitor.

[FIGURE 4]

Impact of PO_4^{3-} and NO_3^-

PO_4^{3-} and NO_3^- are commonly present in wastewater and wastewater-impacted natural waterbodies. N and P-species are responsible for eutrophication and other environmental hazards. Thus, their impact on laccase activity was tested in this study. Sodium phosphate and

297 sodium nitrate were used to assess the impact of PO_4^{3-} and NO_3^-, respectively as the effect of Na^+
298 was shown to be low (Figure 3). Kim and Nicell (2006a) reported insignificant impact of NO_3^- at
299 low concentrations (around 1-2 mM) on laccase from *T. versicolor*. By investigating a broader
300 range of concentration, our study confirms that the impact of both NO_3^- and PO_4^{3-} on laccase
301 activity can be significant at higher concentrations (Figure 5).
302 [FIGURE 5]

303 ***Laccase activity vs TrOC removal performance***
304 There are several key observations made from this study that may be critical in implementing
305 laccase treatment for TrOC removal. The first observation is the discrepancy between the
306 impacts of the salts on laccase activity and the specific TrOC removal in some cases. This
307 observation can be highlighted with the comparison of two sets of results, those of Mg^{2+} and F^-.
308 Mg^{2+} exhibited stable residual enzymatic activity over the 1-250 mM range, but suffered from a
309 70 and 60 % drop in removal efficiency for BPA and DCF, respectively (Figure 3). In this case
310 the laccase remains active, but the mechanism used to oxidise the target contaminant is
311 compromised. This is contrasted with the behaviour of F^-, which showed negligible residual
312 enzyme activity at 250 mM, but still managed to achieve 35 and 11% removal of BPA and DCF,
313 respectively. This emphasizes the different ways in which each of the ions interact and
314 potentially affect laccase activity.
315 [FIGURE 6]
316 The second observation is the elevated residual laccase activity in the presence of some
317 ions (Figure 6). Increased activity in whole-cell preparation in presence of some metals is well
318 established, but there have only been a few mentions of increasing the activity of the isolated
319 laccase by the addition of metal salts. For example, Shankar & Nill. (2015) reported an
320 enhancement of residual laccase activity in the presence of Ca^{2+}, Co^{2+} and Mg^{2+}. When testing
321 $CuSO_4$ and $MnSO_4$, Farnet et al. (2008) observed an apparent increase in enzyme activity for
322 Cu^{2+} (1 mM) and Mn^{2+} (20 mM). It is possible that the salts could increase the solubility of the
323 substrate used for laccase activity measurement (Farnet et al. 2008), therefore increasing its
324 exposure to laccase, allowing greater oxidation.

325 **Conclusion**

The inorganic salts evaluated in this study help isolating the relative impact of a range of ions on laccase from *Pleurotus ostreatus*. A variable impact on laccase and its TrOC removal performance was observed. Monovalent cations such as Na^+, NH_4^+ and K^+ had no or low impact on laccase activity and TrOC removal at all the tested concentrations, indicating strong tolerance of this laccase. On the other hand, divalent and trivalent cations showed different degree of influence. Specific halides also had different impacts on laccase performance: the degree of impact was in the order of $F^- > I^- > Br^- > Cl^-$. In particular, the tolerance of the tested laccase to Cl^- has important implications for a range of industrial applications.

Disclosure of conflict of interest

The authors report no conflicts of interest.

Funding

This study was partially funded by the GeoQuEST Research Centre, UOW, Australia. A part of this study was conducted during Dr Nguyen's career launch fellowship at UOW. Dr Nguyen also acknowledges a postdoctoral fellowship at the Nanyang Technological University, Singapore.

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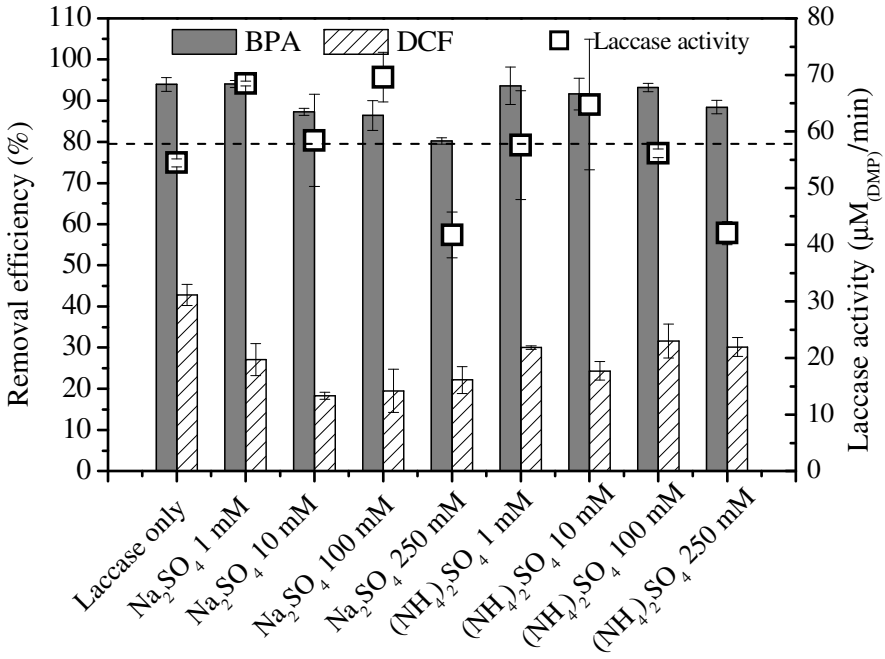

Figure 1: Impact of sulfate (SO_4^{2-}) on laccase stability and removal of BPA and DCF. Error bars present the standard deviation of duplicate samples. The dotted line indicates the initial laccase activity.

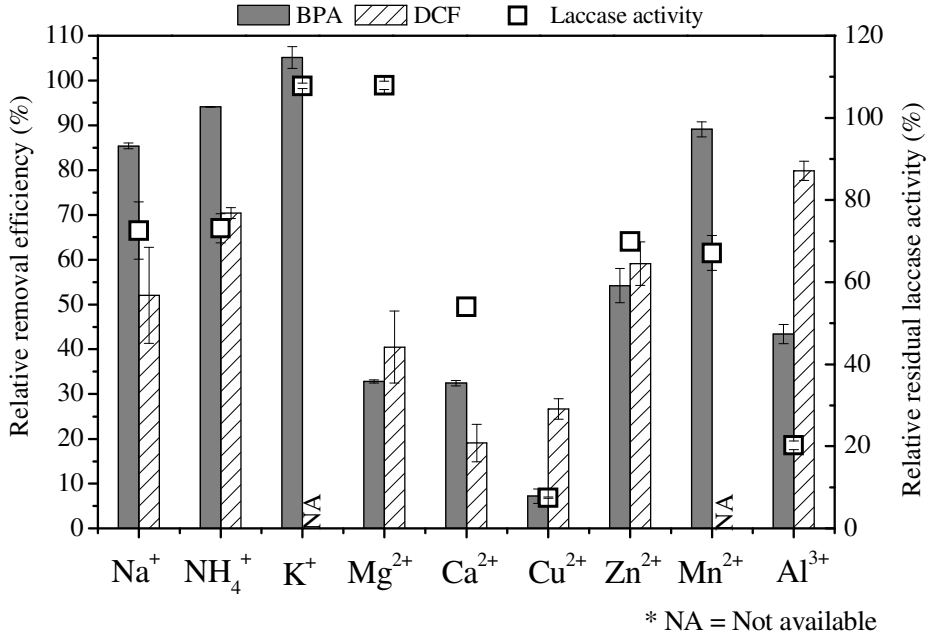

Figure 2: Effect of different cations on laccase activity and BPA and DCF removal. The cations were dissolved from sulfate salts at a concentration of 250 mM. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

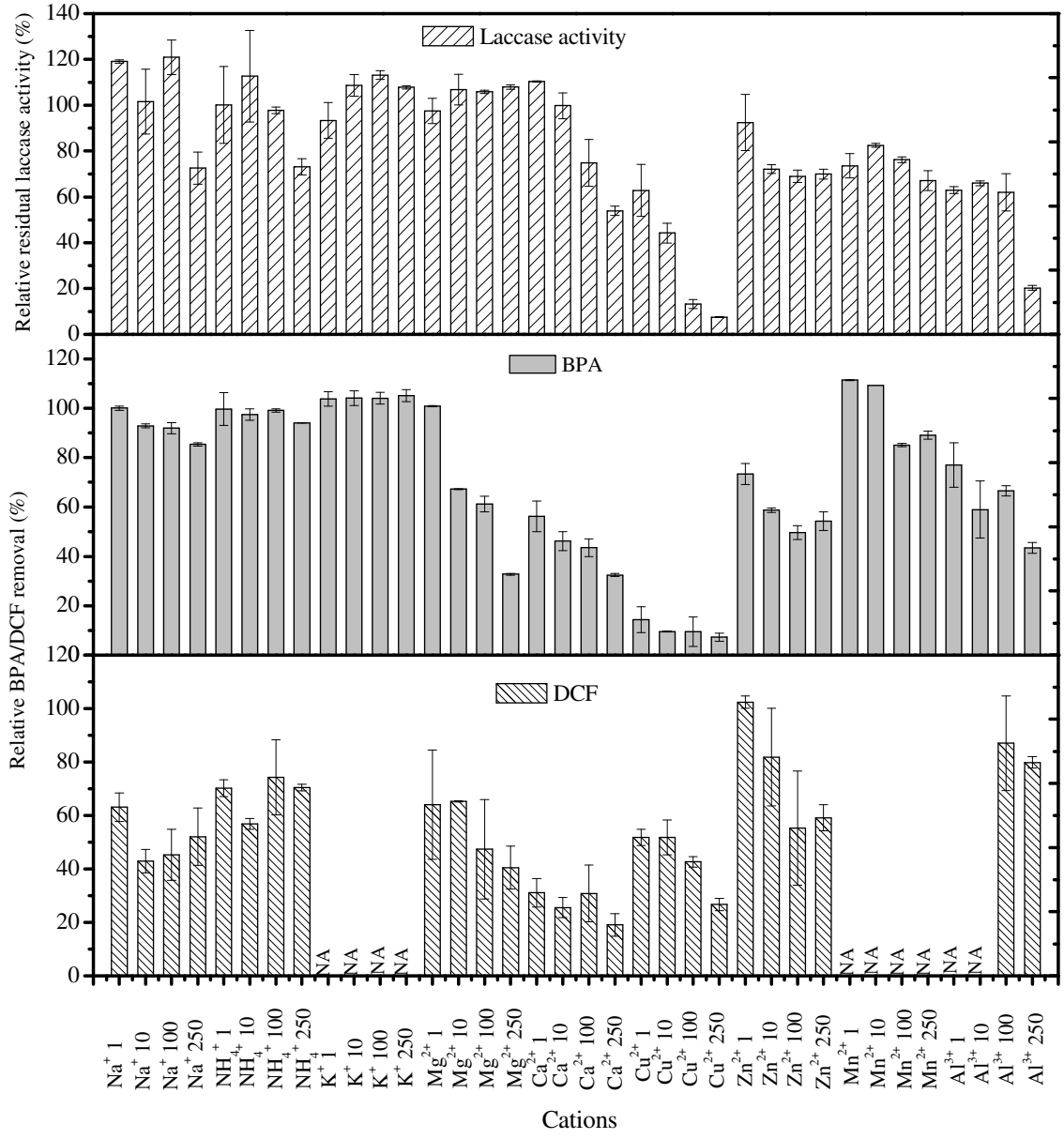

Figure 3: Effect of cation concentrations on laccase activity and BPA and DCF removal. The cations were dissolved from sulfate salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

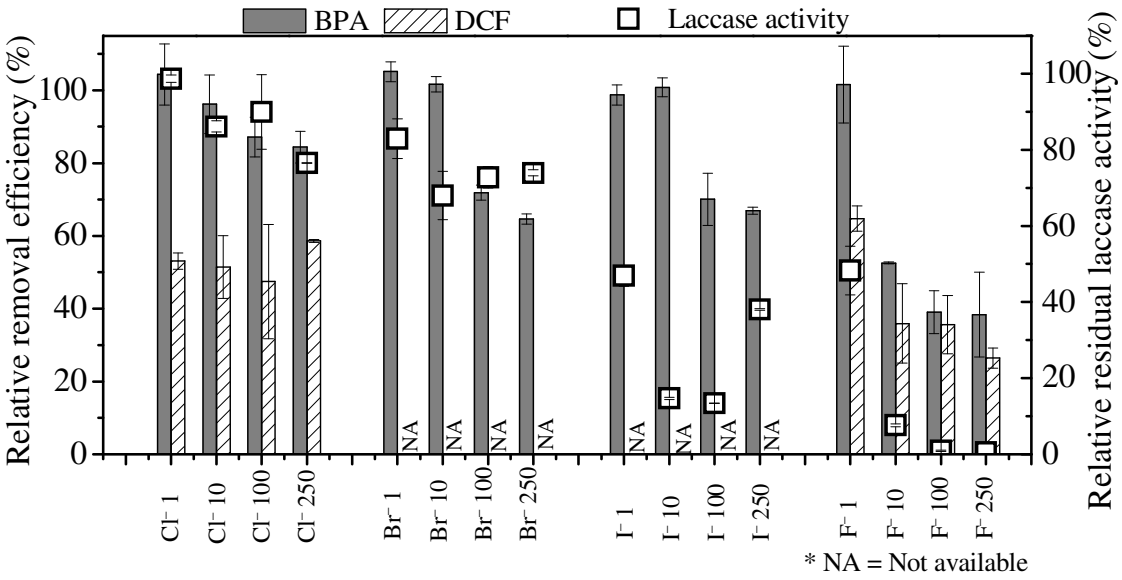

Figure 4: Effect of halides on laccase activity and BPA and DCF removal. The anions were obtained from sodium and potassium salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

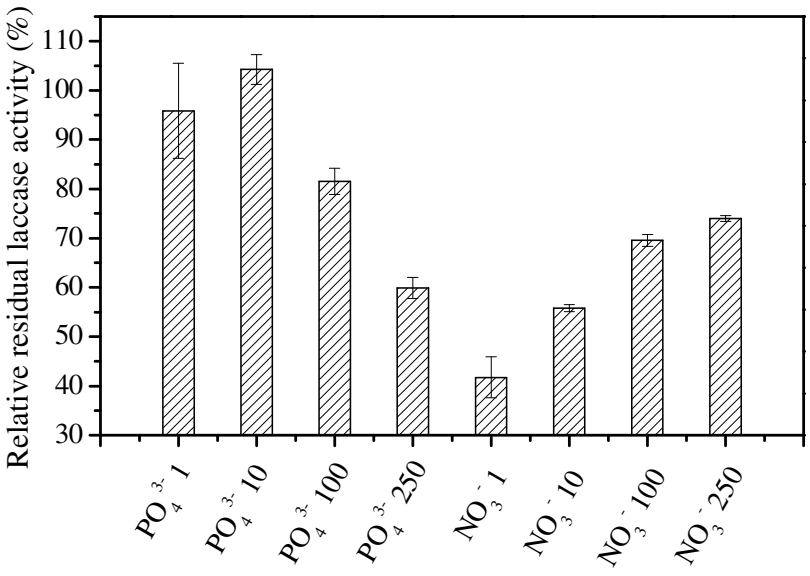

Figure 5: Effect of PO_4^{3-} and NO_3^- on laccase activity. The anions were dissolved from sodium salts. Error bars present the standard deviation of duplicate samples. The results are normalised against the respective values in the control experiment conducted in absence of salt.

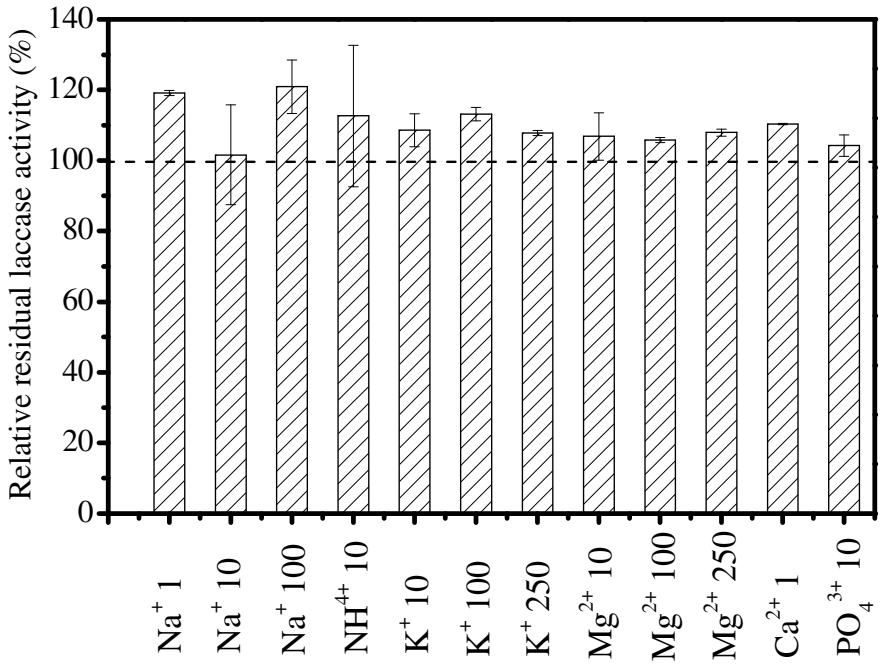

Figure 6: Relative residual laccase activity in presence of selected cations and anions showing an increase in laccase activity after incubation period. The results are normalised against the respective values in the control experiment conducted in absence of salt.

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Table 1: Selected cations and anions

Cations	Original salts		Anions	Original salts
Na^{+}	Na_2SO_4		NO_3^{-}	$NaNO_3$
Mg^{2+}	$MgSO_4$		PO_4^{3-}	Na_2PO_4
NH^{4+}	$(NH_4)_2SO_4$		Cl^{-}	NaCl
Ca^{2+}	$CaSO_4.2H_20$		F^{-}	NaF
Cu^{2+}	$CuSO_4.5H_20$		I^{-}	KI
Zn^{2+}	$ZnSO_4.7H_20$		Br^{-}	KBr
Mn^{2+}	$MnSO_4.4H_20$			
K^{+}	K_2SO_4			
Al^{3+}	$Al_2(SO_4)_3.16H_20$			