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Enzymatic Remediation of Bisphenol A from Wastewaters: Effects of Biosurfactant, Anionic, Cationic, Nonionic, and Polymeric Additives

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Abstract One of the limitations compromising the utilization of enzymes for the remediation of phenolic wastewaters is enzyme activity loss during the treatment. Some surface active additives have the potential to protect enzymes and, thus, improve their performance. In this study, the removal of bisphenol A from synthetic wastewater samples by laccase has been studied in the presence of rhamnolipid biosurfactant (RL), polyethylene glycol (PEG), Triton X-100, cetyltrimethylammonium bromide (CTAB), and sodium dodecylbenzenesulfonate (SDBS). The results demonstrated that the addition of 1 ppm RL provides the highest removal rate and removal extent of BPA. In the case of PEG and Triton X-100, the results showed that both additives have almost similar positive effects on the enzymatic remediation of BPA. However, unlike RL, the positive effects of PEG and Triton X-100 were appreciable only at higher concentration (i.e., 25 ppm). On the other hand, the addition of the two ionic surfactants (SDBS and CTAB) resulted in a negative effect on the enzyme activity and, thus, the remediation of BPA, demonstrating the undesirable interactions of these ionic surfactants with laccase. The negative effect of the charged additives was more pronounced for the case of the positively charged additive (i.e., CTAB).

Keywords Wastewater treatment · Bisphenol A · Laccase enzyme · Rhamnolipid biosurfactant · PEG · Surfactant

1 Introduction

Water pollution is one of the most monumental issues that the globe is currently facing. A major source of this pollution is the release of large quantities of phenolic components to the environment. One of the commonly encountered phenols in the generated wastewaters is BPA (Corrales et al. 2015). BPA plays a vital role in a number of industries such as the production of polycarbonates and epoxy resins (Kim and Nicell 2006). Other BPA-containing materials include, but are not limited to, pipe linings, papers, optical lights, plastic packing, and paints (Corrales et al. 2015; Husain and Qayyum 2013). Despite the widespread use of BPA, it is a very harmful wastewater pollutant. In addition to being an endocrine-disrupting compound, it also produces other noxious health effects in humans and other animals. For instance, the exposure to BPA can lead to excessive malfunctions in the hormonal systems (Zdarta et al. 2018), resulting in adverse effects on reproduction organs (Alshabib and Onaizi 2019b). As reported by Canesi and Fabbri (2015), BPA can also hinder the protective action of white blood cells in fish against pathogens and foreign substances.

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Owing to the negative impacts of BPA and other phenolic pollutants on environment and public health, various methods including adsorption (Yao et al. 2018), photo-oxidation (Abo et al. 2016), membrane separation (Yüksel et al. 2013), and biological degradation (Husain and Qayyum 2013) have been extensively exploited to treat phenolic (including BPA) wastewater streams. However, most of these techniques are plagued by economic infeasibility, complexity, ineffectiveness, negative environmental impacts, and/or the generation of secondary toxic pollutants (Daâssi et al. 2016). Enzymes, which are widely used in several industries (He et al. 2011; Onaizi et al. 2009a, 2012), have been proposed as potential biocatalysts for the degradation of organic pollutants present in wastewaters. Accordingly, the utilization of extracellular enzymes for the enzymatic wastewater treatment has received a huge attention in the past decades (Mukherjee et al. 2013; Sukan and Sargin 2013). One of these extracellular enzymes is laccase (EC 1.10.3.2), which has been reported to be capable of degrading a wide range of chemicals including BPA (Fernández-Fernández et al. 2013). However, it has been reported that laccase is highly susceptible to activity loss due to the interactions with the generated free radicals from the cleavage of the phenolic compounds and/or the produced polymeric products (Viswanath et al. 2014).

Although the mechanism of enzyme deactivation is still ambiguous, some studies have attempted to elucidate the causes of such deactivations. For example, it has been hypothesized that some free radicals, which are generated as intermediates during the enzymatic degradation of phenols, tend to interact with laccase molecules, resulting in the formation of covalent bonds between the free radicals and the enzyme molecules. The formation of such enzyme-radical conjugates compromises the enzyme activity (Bratkovskaja et al. 2004). Another possible scenario is the adsorption of laccase molecules on the surface of the formed charged microparticles (i.e., polymeric products). As a result, a diffusion layer around the micro-aggregates is established, which limits the access of BPA to the active site of laccase, rendering the enzymatic remediation of phenolic wastewater less active or even completely inactive (Alshabib and Onaizi 2019a).

With a view to preserve enzyme activity, surface active additives have been attempted and some of them were reported to be effective in protecting the enzyme molecules against the inhibitory components present in the enzymatic reaction medium (Alshabib and Onaizi 2019a). One of the most widely utilized additive is PEG (Kimura et al. 2016; Modaressi et al. 2005a). Triton X-100 has been also used to enhance the enzymatic degradation of various phenols (Feng et al. 2013; Torres et al. 2016). However, there is a debate in the published literature on the effectiveness of these additives. For instance, some studies reported a significant enhancement of the enzymatic remediation of phenolic pollutants from wastewater samples upon the addition of PEG (Ghosh et al. 2008; Tonegawa et al. 2003; Yamada et al. 2010) while other studies found no positive gain (González et al. 2008; Kurnik et al. 2017) or even a negative effect (Steevensz et al. 2009). Another serious concern reported in the literature is the increase in toxicity of the treated wastewater upon the addition of PEG and other chemical-based surface active agents (D'Annibale et al. 2012; Kim and Nicell 2006). Such toxicity issues can be eliminated if the chemically synthesized additives are replaced by bio-based additives. However, there is a lack of information in the published literature on the performance of bio-based additives.

Accordingly, the focus of this study is on the assessment of the effectiveness of a common biosurfactant (i.e., RL) in enhancing the enzymatic remediation of BPA. The performance of this biodegradable additive will be benchmarked to those of four chemical-based additives having different characteristics. The first additive (i.e., PEG) is a nonionic polymer while the second one (Triton X-100) is a nonionic surfactant. In addition to these nonionic additives, anionic (i.e., SDBS) and cationic (i.e., CTAB) additives are also utilized. Unlike PEG and Triton X-100, the effects of SDBS and CTAB on the enzymatic remediation of phenolic pollutants, BPA in particular, are still not documented in the published literature. The changes in BPA concentration will be followed in time in order to get insights into the effects of these additives on both the rate and the extent of BPA removal.

2 Materials and Methods

2.1 Materials

BPA (as a model pollutant), laccase (EC 1.10.3.2) from *Trametes versicolor*, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), RL, CTAB, Triton X-100, and SDBS were purchased from

Sigma-Aldrich. PEG, with a molecular weight of 2025, was purchased from BDH Chemicals. The utilized RL is a mixture of mono- and di-rhamnolipid; the ratio of the former to the latter is 3:2. The purity of this RL is 90 wt.%; the remaining fraction represents non-canonical rhamnolipids (unsaturated rhamnolipid molecules and/or those with a lipid chain of 8 or 12 carbons rather than the standard 10 carbon atoms). All other reagents used in this study were of analytical grades.

2.2 Methods

2.2.1 Laccase Activity Assay

The activity assay of laccase was carried out as follows. A specific volume of laccase solution (freshly prepared before the activity assay experiment) was mixed with a specific volume of ABTS solution. The final concentrations of laccase and ABTS (in 0.1 M acetate buffer, pH 4.5) were 0.025 mg/mL and 0.2 mM, respectively. Immediately after adding laccase to the ABTS solution, the change in absorbance at 420 nm wavelength was recorded as a function of time using UV-Vis spectroscopy. With the known extinction coefficient (36×10^{-3} M^{-1} cm^{-1}) of ABTS at 420 nm and the path length (1 cm) (Mohammadi et al. 2018), the rate of ABTS degradation and, thus, the laccase activity were calculated. The enzyme activity was expressed in terms of activity unit, where one unit (U) of laccase activity was defined as the amount of laccase required to oxidize 1 μmol of ABTS per min. The above activity assay was repeated in the separate presence of RL, CTAB, SDBS, Triton X-100, and PEG. All laccase activity assay experiments were conducted in duplicate with a standard deviation in terms of laccase activity unit ranging from 0.94 to 3.18 U/L (corresponding to a relative standard deviation of 1.8 to 6.5%).

2.2.2 Critical Micelle Concentration Determination of RL

To determine the CMC of RL, dynamic surface tension measurements were carried out as described elsewhere (He et al. 2011; Onaizi 2018; Onaizi et al. 2012, 2014, 2016). Briefly, a specific volume (~ 8 mL) of RL solution at a given concentration was placed in a cuvette and, then, a small air bubble was created in the RL solution. Then, the change in the surface tension of the air bubble was measured as a function of time using KRUSS DSA 25S until an equilibrium surface tension value was obtained. The above procedure was repeated using different RL concentrations. Each experiment was carried out at least twice and the reproducibility was quite good (i.e., error was less than 5%). The collected values of the equilibrium surface tension were plotted against the logarithmic values of RL concentrations, enabling the determination of the RL CMC.

2.2.3 Dynamic BPA Degradation Studies

Degradation experiments were conducted in 50-mL batch reactors using fixed BPA and laccase concentrations of 50 ppm and 0.05 mg/mL, respectively. All enzymatic reactions were carried out at the optimal pH value (i.e., pH 5.8) for laccase, which has been determined in preliminary tests. This optimal pH value is in agreement with the findings of other researchers (Ghosh et al. 2008; Liu et al. 2012; Modaressi et al. 2005b). A fresh enzyme sample was prepared and immediately used in each experiment in order to eliminate the effect of enzyme solution aging on its activity. Initially, BPA degradation was carried out in the absence of surface active additives. In each experiment, the reaction mixture was stirred upon the addition of laccase and aliquots were withdrawn from the reaction mixture at different time intervals for analysis. Upon the withdrawal of an aliquot (3 mL), it was immediately quenched with 1 mL of 0.5 M hydrochloric acid to stop the reaction. In addition to these BPA degradation experiments in the absence of additives, its degradation in the presence of RL, CTAB, Triton X-100, SDBS, and PEG was also studied. The BPA degradation experiments in the presence of each of these additives were conducted in a similar manner as described above. However, a specific amount of each of the above additives was dissolved in the BPA solution before the addition of laccase. Two premicellar concentrations (1 and 25 ppm) of each additive were utilized. It is noteworthy to mention that all experiments were conducted in duplicate. The reported values in this study are the averages of the repeated runs. The standard deviations of the results in terms of remaining BPA concentrations ranged from 0.027 to 1.99 ppm (corresponding to a relative standard deviation from 0.1 to 10.1%).

2.2.4 HPLC Analysis

The collected aliquots were analyzed using a high-performance liquid chromatography instrument (HPLC, Agilent, USA), equipped with a diode array detector (1290 Infinity II, Agilent, USA), and a ZORBAX Eclipse XDB-C18 column (Agilent, USA), which was thermostated at 40 °C. The quenched samples were filtered through 0.2-μm polyvinylidene fluoride (PVDF) membrane filters before subjecting them to the chromatographic analysis. A detection wavelength of 276 nm was utilized. The flow rate of the mobile phase was set to 1 mL/min (isocratic elution). The retention time of BPA and the injection volume were 3.80 min and 3.0 μL, respectively. The mobile phase consisted of ultra-pure water and acetonitrile (40%/60%, *v*/*v*). The extent of BPA degradation was calculated based on the peak area and the slope of a calibration curve that has been previously constructed using known BPA concentrations.

2.2.5 Statistical Analysis

All degradation experiments were performed in duplicate. For each set of data, the accuracy of the obtained results was assessed by calculating the standard deviation, variance, and standard error. The one-way analysis of variance (ANOVA) test was adopted to judge the statistical significance among the collected data. Measured values with $p < 0.05$ are considered to be significantly different while those with $p \geq 0.05$ are statistically indifferent. The results showed high significance with all p values less than 0.0001.

3 Results and Discussion

In this work, the performance of RL in enhancing the enzymatic degradation of BPA was compared to those of anionic (SDBS), cationic (CTAB), nonionic (Triton X-100), and polymeric (PEG) surface active additives. The chemical structures of these additives are presented in Fig. 1. Before conducting the enzymatic degradation of BPA in the presence of these surface active additives, the effect of these additives on the enzyme activity was assayed using ABTS substrate as described in the Sect. 2. The results of the enzyme activity assay in the presence of 1 ppm of each additive are shown in Fig. 2. A control experiment in the absence of these additives is also displayed in Fig. 2.

As shown in Fig. 2, the addition of 1 ppm RL increased the activity of laccase from 46.06 U/L (equivalent to 1.84 U/mg laccase) in the absence of RL (control experiment in Fig. 2) to 52.08 U/L (equivalent to 2.08 U/mg laccase). Thus, a more than 13% enhancement in the enzyme activity was achieved upon the addition of 1 ppm RL. The addition of 1 ppm PEG to the ABTS-laccase reaction mixture also increased the activity of laccase to 50.03 U/L (equivalent to 2.0 U/mg laccase); however, such activity in the presence of PEG is 4% lower than the laccase activity in the presence of the same quantity of RL. Additionally, the enzyme activity in the presence of 1 ppm Triton X-100 in the ABTS-laccase solution was about 48.64 U/L (roughly 1.94 U/mg laccase); this value is lower by 6.7 and 3% than those of RL and PEG, respectively. Unlike the enhancement of laccase activity (even though to different extents) in the presence of RL, PEG, and Triton X-100, the addition of 1 ppm SDBS or CTAB dropped the enzyme activity to 45.4 U/L (~ 1.82 U/mg laccase) and 44.54 U/L (~ 1.78 U/mg laccase), respectively, relative to the control experiment (i.e., in the absence of additives).

3.1 Effect of RL on BPA Conversion

Two concentrations (1 and 25 ppm) of each surfactant were utilized. These two concentrations were deliberately selected for two reasons: (1) RL was only effective below its CMC and (2) to make sure that all additives to be tested are below their CMCs. The CMC values of Triton X-100, SDBS, and CTAB were obtained from literature and they are 150–200 (Steevensz et al. 2014; Zhang et al. 2012), 976–1010 (Liu et al. 2014), and about 364 ppm (Liu et al. 2012), respectively. Since the reported CMC value for RL in the literature is widely scattered (i.e., from 10 to 120 μM) (Champion et al. 1995; Lebrón-Paler et al. 2006; Liu et al. 2018) due to the variations in the purity of RL and its producing microorganism, it was determined in this work as described in the Sect. 2. The obtained CMC value for RL was 50 ppm (results not shown).

The enzymatic removal of BPA in the presence and the absence of the above additives was investigated in this study. Since the focus of this study is on benchmarking the performance of RL to those of the other four additives in enhancing the enzymatic removal

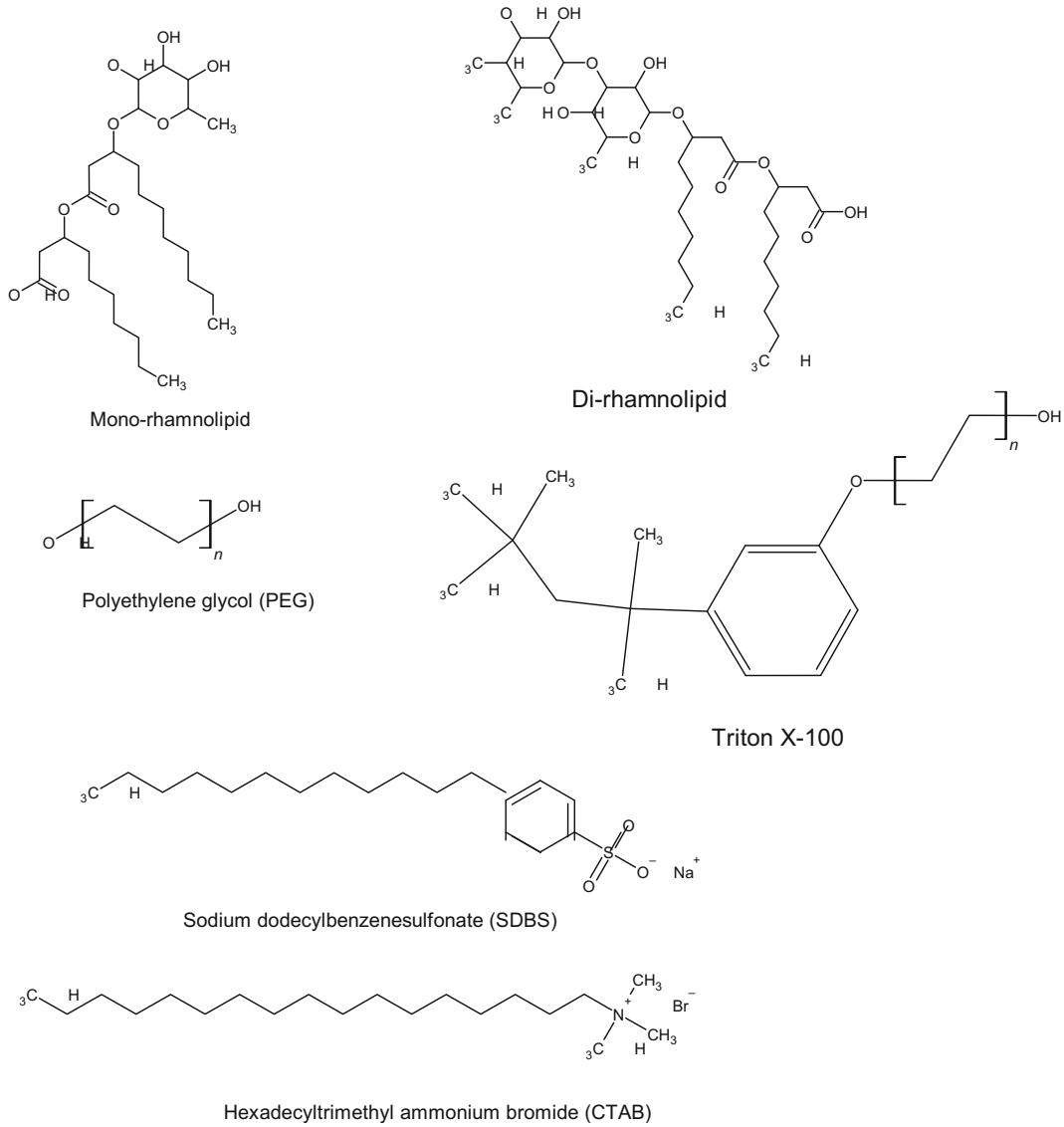

Fig. 1 Chemical structures of RL, PEG, Triton X-100, SDBS, and CTAB

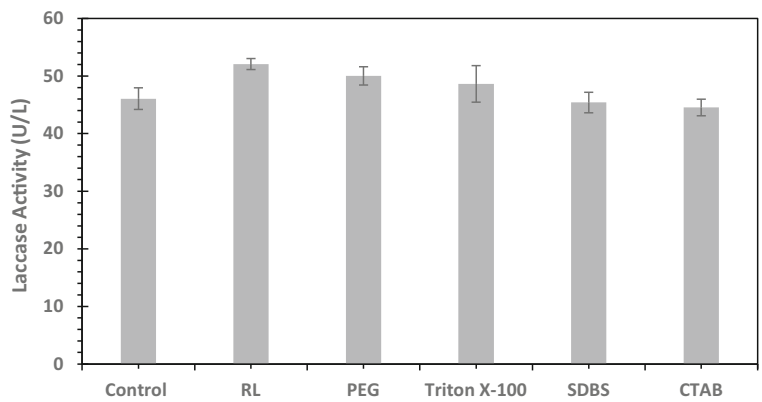

Fig. 2 Laccase activity in the absence (i.e., control) and the presence of additives. Conditions: 0.025 mg/mL laccase, 0.2 mM ABTS, 0.1 M acetate buffer (pH 5), and 22 °C. The concentration of each additive (used separately) was 1 ppm

of BPA from wastewater, the concentrations of the pollutant (i.e., BPA) and the enzyme (i.e., laccase) were fixed at 50 ppm and 0.05 mg/mL, respectively. Unlike other studies on the enzymatic removal of phenols from wastewater (Ji et al. 2009; Modaressi et al. 2005a; Torres et al. 2016), we address the rate of BPA degradation and not just the extent of BPA degradation after a fixed treatment time.

Figure 3 shows the enzymatic removal of BPA from a synthetic wastewater sample as a function of time in the presence and the absence of RL. At any point in time, the enzymatic degradation of BPA in the presence of RL exceeds its degradation in the absence of the biosurfactant. Interestingly, only a tiny amount of the biosurfactant (i.e., 1 ppm) could provide a significant enhancement in the enzymatic removal of BPA. This is an important economic factor. Surprisingly, higher RL concentration (i.e., 25 ppm) was less effective despite that this concentration is still much lower than the CMC of RL.

As shown in Fig. 3, at RL concentration of 1 ppm, the biosurfactant was able to boost the degradation rate of BPA within the first 10 min, allowing laccase to remove over 30% of the initial amount of BPA in the wastewater sample; this is about 50% higher than that (21%) obtained in the absence of RL within the initial 10 min of treatment. Extended treatment of BPA in the presence of RL (up to 2 h) led to an almost 23% enhancement in the removal efficiency of BPA (i.e., 65% removal in the presence of RL relative to only about 52.7% in its absence). The positive impact of RL might be linked to its high capability to mitigate the loss of enzyme activity by preventing the access of the formed free radicals/polymeric products into the active site of laccase (Alshabib and Onaizi 2019a). This is also in line with the findings of Ruta and Juozas (2013), who reported that the addition of RL (i.e., 0.1 to 2 ppm) enhanced the oxidation rate of 2-naphthol by almost 1.5-fold as compared to the control reaction after 10 min of treatment with peroxidase.

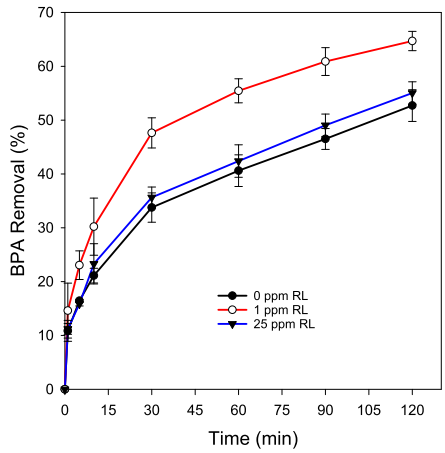

Fig. 3 Kinetics of BPA removal in the absence and the presence of RL. Conditions: 50 ppm BPA, 0.05 mg/mL laccase, pH 5.8, and 22 °C. The BPA degradation was followed for 2 h

As stated above, increasing RL from 1 to 25 ppm compromised the rate and the extent of BPA removal. As it is clear from Fig. 3, 55% of the initial amount of BPA in the wastewater sample was removed in the presence of 25 ppm RL. Despite that the BPA removal in the presence of 25 ppm is still higher than in the base case where no biosurfactant was added, it is lower than the extent of BPA removal obtained with 1 ppm RL under the same experimental conditions. It has been reported that some surfactants (Triton X-100 as an example) might form pseudo-micellar phase at relatively high premicellar concentrations (Ji et al. 2009). If this is the case with RL at 25 ppm, it would be expected that some BPA molecules might be sequestered into the pseudo-micelle and, therefore, prohibited the sequestrated BPA molecules from the interaction with laccase, leading to a lower BPA degradation. Another plausible explanation for the drop in BPA removal with increasing RL concentration is the occurrence of some undesirable RL-laccase interactions, which become more significant at higher RL concentrations. The pKa value of RL is about 4.28 (Lebrón-Paler et al. 2006) and, thus, such undesirable interactions might stem from the accumulation of an appreciable population of negatively charged RL molecules in the system at relatively high RL concentrations, compromising the RL enhancement effect obtained at low concentrations. This plausible charge-related effect is in line with the activity assay results and the subsequent discussion related to the enzymatic degradation of BPA in the presence of SDBS and CTAB where these charged additives have detrimental effects on laccase activity and BPA removal. Therefore, our findings indicate that RL in its premicellar form, particularly at low premicellar concentrations, is sufficient to induce a significant enhancement effect on the enzymatic removal rate and extent of BPA.

3.2 Effect of PEG on BPA Conversion

For the sake of benchmarking the performance of RL with that of PEG, which is widely used as an additive for enhancing the enzymatic remediation of phenols, we studied the rate and the extent of the enzymatic removal of BPA in the presence of PEG. Figure 4 shows the time-course of BPA conversion in the absence and the presence of 1 and 25 ppm PEG. As in the case of RL, the concentrations of BPA and laccase were fixed at 50 ppm and 0.025 mg/mL, respectively. Figure 4 demonstrates that the polymeric additive improved the enzymatic removal efficiency of BPA. However, the addition of 1 ppm PEG did not alter the degradation rate of BPA and also the BPA removal extent during the first 10 min. Furthermore, only small improvements on the removal rate and extent of BPA were attained with the extended treatment duration to 2 h. More specifically, 55.2% of BPA was removed in the presence of 1 ppm PEG after 2 h treatment relative to 52.7% in the absence of the polymeric additive. Increasing the concentration of PEG to 25 ppm significantly increased the BPA removal (both the rate and the extent). For example, the degradation efficiencies of BPA in the presence of 1 ppm and 25 ppm PEG after 2 h of laccase treatment were 55.2% and 61.1%, respectively. The enhancement of BPA removal using laccase in the presence of PEG was also observed by Kim and Nicell (2006). These researchers reported that PEG at a concentration of 5 ppm induced a positive impact on the enzymatic treatment of BPA and was able, under optimized conditions (i.e., 27.39 ppm BPA, 0.3 U/mL laccase, pH 5, and 25 °C), to remove over 95% of the initial BPA amount after 2 h of treatment.

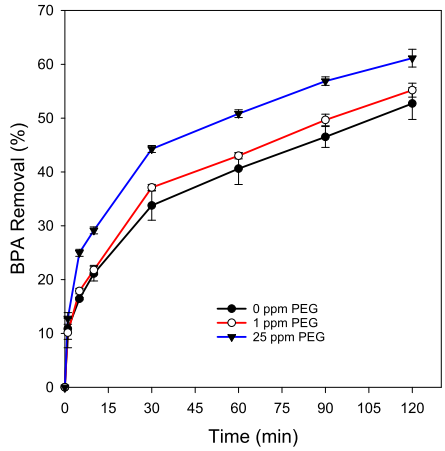

Fig. 4 Kinetics of BPA removal in the absence and the presence of PEG. Conditions: 50 ppm BPA, 0.05 mg/mL laccase, pH 5.8, and 22 °C. The BPA degradation was followed for 2 h

Such enhancement in the enzymatic removal of BPA upon the addition of PEG is most likely correlated to its protective effects on laccase activity (Alshabib and Onaizi 2019a; Modaressi et al. 2005a; Steevensz et al. 2012). In this regard, Ghosh et al. (2008) reported that PEG at a concentration of 1 ppm combated the deactivation of laccase, lowering the required enzyme amount by more than 2-fold to achieve the same removal extent of 2,4-dimethylphenol in the absence of PEG within 3 h of treatment. Compared to our findings at 1 ppm PEG, the higher improvement obtained in Ghosh et al. (2008) study might be attributed to the longer treatment time and the utilization of a different phenolic substrate. The type of phenolic pollutant has been reported to have a significant effect on its enzymatic removal from wastewater (Alshabib and Onaizi 2019b). It was postulated that PEG tends to preclude the entrapment of enzyme molecules within the oligomeric products, which are generated during the phenolic degradation reaction (Kimura et al. 2016). In accordance with this statement, PEG was found to bind with water, which results in the formation of a relatively bulky hydrated volume (Deva et al. 2014). PEG molecules, as stated by Steevensz et al. (2012), have the tendency to fold and capture more water molecules, creating a globular PEG structure. Such an interaction contributes to the protection of laccase against inhibitory products (Kim and Nicell 2006).

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3.3 Effect of Triton X-100 on BPA Conversion

The enzymatic degradation of BPA in the presence of PEG (nonionic polymer) and RL revealed that RL outperforms PEG at low concentrations while PEG is more effective at higher concentrations. It was also observed that 1 ppm RL is more effective than 25 ppm PEG. To benchmark the performance of RL to that of another nonionic additive (i.e., Triton X-100), the kinetics of the enzymatic degradation of BPA in the presence Triton X-100 was studied and the results are shown in Fig. 5. As displayed in Fig. 5, a positive impact on both the BPA degradation rate and extent was gained upon the addition of Triton X-100. In the absence of Triton X-100, laccase degraded less than 11 and 41% of the initial amount of BPA present in the wastewater sample within 1 and 60 min, respectively, of treatment. However, when

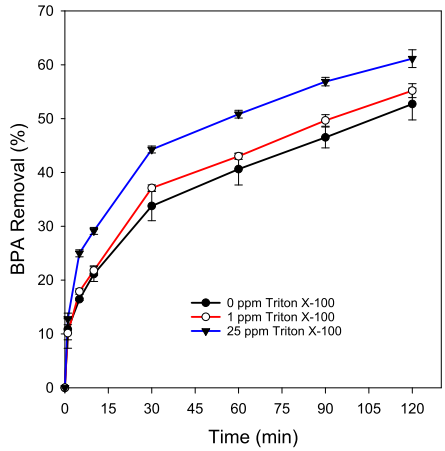

Fig. 5 Kinetics of BPA removal in the absence and the presence of Triton X-100. Conditions: 50 ppm BPA, 0.05 mg/mL laccase, pH 5.8, and 22 °C. The BPA degradation was followed for 2 h

25 ppm Triton X-100 was introduced into the enzymatic reaction medium, about 14% and 48% of the initial amount of BPA were removed within 1 and 60 min, respectively. After 2 h of treatment in the presence of 1 and 25 ppm Triton X-100, the enhancement in the enzymatic removal of BPA (relative to the case when no Triton X-100 was added) was about 3 and 14%, respectively. This is in agreement with the findings of Ji et al. (2009), who observed that increasing the concentration of Triton X-100 (when present in a premicellar concentration as is the case in our study) enhanced the BPA conversion catalyzed by laccase. The same trend was also reported in other studies (Steevensz et al. 2014; Zhang et al. 2012).

It has been proposed that the interaction between laccase and Triton X-100 is responsible for stabilizing the enzyme, and thereby enhancing the enzymatic conversion of BPA (Y. Liu et al. 2018). Other studies (Ji et al. 2009; Zhang et al. 2012) also demonstrated the protective effect of Triton X-100 on laccase. This positive impact is supported by the molecular analysis, which revealed that this nonionic surfactant induced some changes in laccase structure, and as a result, laccase was converted into a more active/stabilized form (Zhang et al. 2012). Such laccase-Triton X-100 interactions allowed the hydrophobic amino acid residues of laccase to be entrapped within the inner part of the enzyme confirmation (Ji et al. 2009). This action is thought to be useful for minimizing the rate of enzyme deactivation due to the attack of the free radicals generated from the degradation of phenolic components or the attachment of polymeric products to the surface of laccase (Liu et al. 2012).

3.4 Effect of SDBS on BPA Conversion

The influence of SDBS on the rate and the extent of BPA degradation by laccase is shown in Fig. 6. Unlike RL, PEG, and Triton X-100, the presence of the anionic surfactant in the enzymatic reaction medium imposed a negative impact on BPA degradation. Although SDBS at a concentration of 1 ppm induced an imperceptible decrement on BPA conversion, the addition of a higher SDBS concentration (i.e., 25 ppm) resulted in a significant drop in the rate and the extent of the enzymatic removal of BPA. For instance, in the SDBS-free reaction mixture, about 11% of the initial amount of BPA in the wastewater sample was enzymatically degraded in the first min relative to less than 7.7% in the presence of this anionic surfactant. Similarly, the extent of BPA degradation in the absence of SDBS after 2 h of treatment was about 53% compared to less than 49% in its presence. These observations are in line with the findings of Liu et al. (2012), who reported that the addition of sodium dodecyl sulfate (SDS), which has similar characteristics to SDBS, at a concentration of about 144 ppm led to a 13% reduction in the removal efficiency of phenol by laccase.

It has been reported in the published literature that anionic surfactants can act as inhibitors for laccases. For instance, it was found that 0.1 mM (= 28.84 ppm) of SDS was enough to lower the activity of laccase by almost 20% when compared to a control experiment

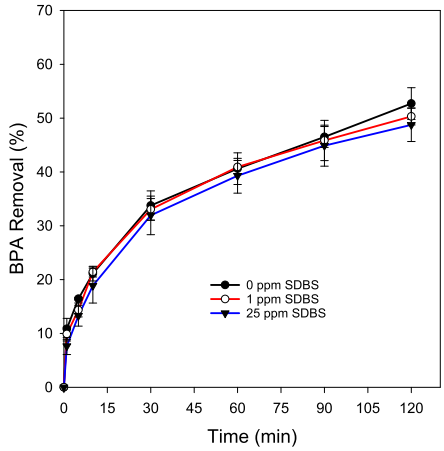

Fig. 6 Kinetics of BPA removal in the absence and the presence of SDBS. Conditions: 50 ppm BPA, 0.05 mg/mL laccase, pH 5.8, and 22 °C. The BPA degradation was followed for 2 h

carried out under the same conditions but in the absence of SDS (Zerva et al. 2019). Another study revealed that the addition of SDS at the same concentration (28.84 ppm) caused a more than 25% reduction in the activity of laccase (Gaur et al. 2018). These results are in agreement with our findings. The decrease in the enzymatic removal of phenols upon the addition of anionic surfactants (e.g., SDS or SDBS) might be attributed to the tendency of the sulfate group in SDS and SDBS to interact with the positively charged groups of the enzyme amino acids, leading to the alteration of the enzyme structure (Couto and Herrera 2006; Khlifi et al. 2010). This behavior might explain the detrimental effect of SDBS addition to the enzymatic reaction on the rate and the extent of the enzymatic removal of BPA.

3.5 Effect of CTAB on BPA Conversion

The time-course of BPA removal catalyzed by laccase in the absence and the presence of the cationic surfactant (i.e., CTAB) has been also investigated in this work. Figure 7 clearly reveals the negative effect of CTAB on the kinetics and the extent of BPA removal by laccase. Similar to SDBS, a very slight decrease (relative to the case when CTAB was not added) in BPA removal was noticed at a CTAB concentration of 1 ppm. However, the addition of 25 ppm CTAB to the enzymatic reaction mixture led to significant drops in the rate and also the extent of the enzymatic removal of BPA. After 2 h of the enzymatic treatment, the extent of BPA removal dropped from about 52.7% to about 43.8%. This is the lowest removal extent of BPA. The low removal rate

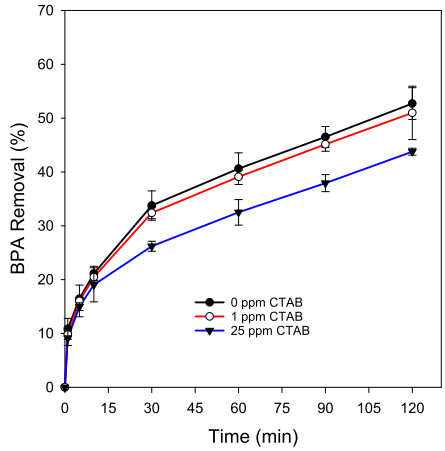

Fig. 7 Kinetics of BPA removal in the absence and the presence of CTAB. Conditions: 50 ppm BPA, 0.05 mg/mL laccase, pH 5.8, and 22 °C. The BPA degradation was followed for 2 h

and extent of BPA highlight the harmful effect of CTAB on laccase. Our observation that CTAB is the most harmful additive (among the ones studied in this work) is in line with the finding of Liu et al. (2012) where CTAB was found to be more detrimental to the removal of phenol than SDS. For instance, Liu et al. (2012) found that SDS at 28.84 ppm lowered the phenol removal extent by almost 13% while a 19% decrease in the phenol removal was observed when the same concentration of CTAB was used instead.

The observed decline in the BPA removal upon the addition of CTAB to the enzymatic reaction medium might be correlated to the unfavorable binding of this cationic surfactant to laccase. In this regard, it has been postulated that the positively charged hydrophilic head of CTAB (i.e., the ammonium) might strongly interact with the amino acid residues in the side chains of laccase (Azimi et al. 2016). As a result, undesirable alterations in laccase structure occur, which in turn, render the activity of laccase otiose. This finding is supported by the observation that a relatively high premicellar concentration of CTAB (i.e., 182.23 ppm) lowered the activity of laccase by almost 40% when compared to the control experiment in the absence of CTAB (Azimi et al. 2016).

3.6 Comparison of the Effects of Surface Active Additives

As shown in the previous sections, the effects of the tested five additives on the laccase-catalyzed removal of BPA from wastewater samples were significantly different (all p values are less 0.0001). For example, comparing the enhancement effect of RL and PEG (Figs. 3 and 4) reveals that their effects are opposite. While increasing the concentration of PEG results in a better removal of BPA, the opposite is true for RL. Such a trend might suggest that these two additives enhance the enzymatic degradation of BPA via different mechanisms. Among the attractive features of RL is its ability to enhance the enzymatic degradation of BPA at 1 ppm by about 1.2 times the enhancement obtained using 25 ppm. Clearly, this is a key important economic factor, which favors the use of RL over PEG despite the lower price of PEG. However, with the advancement in biotechnologies, the production cost of RL is expected to significantly drop (Onaizi et al. 2009b). Another important factor that must be taken into consideration is the environmental impacts of RL and PEG. While RL can be produced sustainably

using microorganisms (Lovaglio et al. 2015; Marchant and Banat 2012; Shekhar et al. 2015), PEG is produced via chemical synthesis, which is by far more harmful to the environment (Biondi 2002; Webster et al. 2009). Furthermore, RL is more biodegradable compared to PEG (Alshabib and Onaizi 2019a). Additionally, the effluent of treated water in the presence of PEG has been reported to be even more toxic than the untreated wastewater sample (D'Annibale et al. 2012; Kim and Nicell 2006). Such a toxicity issue is not encountered in the case of RL.

Benchmarking the performance of Triton X-100 with PEG reveals that the nonionic surfactant has comparable BPA removal enhancement effect to that of PEG at both concentrations (see Figs. 4 and 5). This is in line with the findings reported by Torres et al. (2016) where the addition of PEG and Triton X-100 into a reaction catalyzed by peroxidase enhanced the phenol removal efficiency by almost the same extent. This might be associated with the similarities in the chemical structures of PEG and Triton X-100 as shown in Fig. 1 (i.e., the hydrophilic group in both additives is comprised of poly(oxyethylene) residues). In contrast to Triton X-100 and PEG, the biosurfactant at a concentration of 1 ppm exhibited a more positive impact on the enzymatic degradation of BPA (see Table 1). The superiority of RL at low concentrations might be attributed to its low CMC (Alshabib and Onaizi 2019b), and more importantly, to its different chemical structure. Chemical structures of additives are likely to alter the mode and the extent of enzyme-additive, pollutant-additive, and product-additive interactions. This assertion might be justified by the decrease in the enzymatic degradation of BPA when RL concentration was increased from 1 to 25 ppm, unlike the case of Triton X-100 and PEG. This is worth of future studies on a molecular level to shed some light on the molecular interactions of such complex reactive systems.

In addition to their similar effect on the enhancement of the degradation of BPA by laccase, PEG and Triton X-100 are derived from fossil sources. Thus, in terms of sustainability and environmental impact, RL has the advantage over both chemical additives. The generation of secondary pollutions in the case of Triton X-100 and PEG, particularly that they provide appreciable enhancements only at relatively high concentrations, is a key concern. Thus, our findings reported so far support the utilization of RL over the nonionic additives (PEG and Triton X-100). The superior performance of RL relative to the other two chemical additives (SDBS and CTAB) is also shown in Table 1. In addition to the superior performance of RL relative to SDBS and CTAB, these chemical surfactants have also other drawbacks in terms of sustainability and environmental impact.

4 Conclusion

The effects of surface active additives (i.e., RL, PEG, Triton X-100, SDBS, and CTAB) on the rate and the extent of BPA degradation by laccase have been studied and benchmarked. RL displayed a significant enhancement in the enzymatic removal of BPA at as low concentration as 1 ppm. This is a highly desirable cost factor. A higher concentration of RL is less effective. RL is a weak acid and, thus, it slightly dissociates at the tested pH of 5.8 (the pKa of RL is 4.28) and the benefit of RL addition at higher concentrations is probably compromised by the accumulation of a sizable population of some negatively charged RL molecules. In line with this conclusion is the observation that the addition of the ionic surfactants (SDBS and CTAB) compromised the enzyme activity and, thus the BPA removal. The negative effect of the ionic additives was more severe at higher populations (i.e., concentrations) of

Table 1 Effect of the tested five additives on BPA removal catalyzed by laccase. The results shown in this table are for the following conditions: 50 ppm BPA, pH 5.8, 22 °C, 0.05 mg/mL laccase, and reaction duration of 2 h

Additive concentration	BPA removal efficiency (%)				
	RL	PEG	Triton X-100	SDBS	CTAB
0 ppm	52.7	52.7	52.7	52.7	52.7
1 ppm	64.7	55.2	54.3	50.3	51.0
25 ppm	55.1	61.1	59.9	48.8	43.8

the charged surfactant molecules. Positively charged surfactant molecules are more harmful to the enzyme activity and, thus, the enzymatic remediation of BPA from wastewaters. The addition of the neutral molecules (PEG and Triton X-100) improved the enzyme activity; higher improvement was obtained with increasing the concentration of these nonionic additives. Despite the big difference in their molecular size, PEG and Triton X-100 provided comparable enzyme activity enhancement in a demonstration that the molecular characteristics of additives are more important than the molecular size. The reported observations in this study lay the foundations for further studies (on a molecular level) in order to correlate the extent and the rate of enzymatic remediation of phenolic pollutants from wastewaters to the interactions between the components (additives, enzyme, pollutants, reaction products) present in the enzymatic reaction medium.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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