Simultaneous laccase production and transformation of bisphenol-A and triclosan using *Trametes versicolor*

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Abstract

New age micro-pollutants, bisphenol-A (BPA) and triclosan (TCA), known for their carcinogenic effects in living organisms can effectively be removed from water using laccase from *Trametes versicolor*. Laccase was produced from *T. versicolor* JSRK13 in both submerged and solid-state fermentation (SmF and SSF) conditions. In SmF, *T. versicolor* JSRK13 gave the maximum production of laccase on the 10th day with an activity of 22 U mL⁻¹, whereas, in SSF 185 U g⁻¹ of the enzyme was produced on the 17th day. Maximum production of laccase was observed with Parthenium as substrate. Parthenium, with a particle size of 3–5 mm having 60% moisture was found to be a suitable substrate for laccase production and simultaneous transformation (LPST) of BPA in a synergistic manner. A one-step concentration using 85% ammonium sulphate followed by dialysis was sufficient to give 6.7-fold purification of laccase from the crude culture filtrate. Transformation of BPA was achieved in both SmF and SSF conditions along with the production of laccase, whereas TCA was degraded with free enzyme only. Above 90% of BPA (55–5 mg L⁻¹) was degraded using the LPST strategy with HBT acting as a mediator in the reaction. LPST strategy did not work for TCA as it completely inhibits the growth of *T. versicolor* JSRK13. TCA was degraded up to 75% (1.5–0.375 mg L⁻¹) by the free enzyme. Our study of simultaneous laccase production and transformation proved to be efficacious in case of BPA. The results indicate that industrial and sewage wastewater containing BPA can potentially be treated with *T. versicolor* JSRK13 laccase. The described strategy can further be used to develop a bioprocess which can work both on solid and liquid wastes containing BPA.

Keywords Bisphenol-A · Triclosan · Laccase · Laccase production and simultaneous transformation · Solid-state fermentation · Submerged fermentation · *Trametes versicolor* JSRK13

Introduction

Since the late twentieth century, certain new age micro-pollutants such as bisphenol-A (BPA), triclosan (TCA), chloroxylenol (PCMX) and carbamazepine (CBZ), sulfamethoxazole, diclofenac, cefepime, iomeprol, metazachlor, and acesulfame

(Zhou et al. 2019a, b; Williams et al. 2019; Nagy-Kovacs et al. 2018) and many other endocrine-disrupting chemicals (EDCs) have been reported to have a negative effect on the endocrine systems of various animals including human beings (Zhou et al. 2019; Yu et al. 2019; Puig 2012; Annamalai and Namasivayam 2015). Many of these micro-pollutants mimic the role of endogenous hormones and their corresponding receptors. Such pollutants are broadly named as endocrine-disrupting chemicals (Skledar et al. 2019; Lecomte et al. 2017). Their harmful effects are reported to cause chromosomal abnormalities, diabetes, reproduction defect (Sifakis et al. 2017; Lind and Lind 2018) and early onset of diseases such as mammary and prostate cancers (Ghazarian et al. 2018; Siracusa et al. 2018). Bisphenol-A and triclosan are widely used EDCs. Bisphenol is utilized as an ingredient for the manufacturing of phenol and epoxy resins, polyester, polycarbonates and coatings or polishing on food cans. BPA is released into environment from bisphenol-A-producing factories, leachate from thermal

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receipts and newspapers, etc. (Bernier and Vandenberg 2017), and leaching of various household and industrial plastic wastes and landfill sites (Noonan et al. 2011). TCA which has been used as an antibacterial agent in various cosmetic and personal care products is now reaching our water bodies (Chattu et al. 2016; Liu and Wu 2012; Chiang et al. 2017; Oates et al. 2017; Aparicio et al. 2018; Koumaki et al. 2018; Nag et al. 2018; Yao et al. 2018). Because of the potential adverse health and ecological effects, the removal of EDCs including bisphenol-A and TCA is very important and has been extensively studied (Sifakis et al. 2017; Koumaki et al. 2018). For the removal of these EDCs, a number of physicochemical methods are already in practice such as adsorption, electrochemical and photochemical degradation, membrane-based filtration, and ozonation (Sin et al. 2012; Singh et al. 2014). Biological methods are reported to be promising and helpful in the absolute degradation of phenols and related EDCs. Chandra et al. (2018) reported a high rate of degradation of BPA and TCA with potential microbial strains and their consortium. More recently enzyme-based methods have gained popularity for the treatment of EDCs (Singh et al. 2017; Sharma et al. 2018; Baweja et al. 2016). Lassouane F et al. (2019) observed 99% removal of BPA in 2 h of treatment with the immobilized crude laccase from *Trametes pubescens* operating at optimal conditions (i.e. pH 5, 30 °C, 20 mg L⁻¹ BPA and 1500 U L⁻¹ laccase). Researchers have exploited numerous classes of enzymes and found that oxidoreductases including laccases (EC 1.10.3.2), manganese peroxidase (EC 1.11.1.13) and lignin peroxidase (EC 1.11.1.14), horseradish peroxidase (EC 1.11.1.7) and polyphenol oxidases are capable of degradation of EDCs (Becker et al. 2017; Llorca et al. 2017; Kumar et al. 2017). The rate of degradation of EDCs can be increased by various redox mediators, inducers, surfactants and other additives. White-rot basidiomycetous fungi are the most common source of enzymes capable of mineralizing a wide variety of toxic xenobiotic compounds (Singh and Kapoor 2014; Kapoor et al. 2015; Singh et al. 2017).

In this study, *T. versicolor* JSRK13 isolated from the northern region of India is tested for the biotransformation of bisphenol-A and triclosan. The process of transformation was investigated simultaneously with the production of laccase enzyme from *T. versicolor* JSRK13 in liquid medium as well as on solid state using agrowastes and weeds as solid substrates.

Materials and methods

Chemicals and reagents

Bisphenol-A (BPA), triclosan, 1-hydroxybenzotriazole (HBT), vanillin were purchased from Sigma-Aldrich, India (now Merck). Guaiacol and HPLC-grade organic solvents

were procured from HiMedia laboratories Ltd, Mumbai, India. All other reagents and chemicals utilized in research work were procured from SD-Fine chemicals Ltd, Maharashtra, India.

Solid substrate for fermentation

Sugarcane leaves, Parthenium (common name: Congress grass), rice straw and wheat straw were collected locally from farms in Rohtak district of Haryana, India, and used as lignocellulosic substrates after washing and drying at 40 °C to 4% moisture content in an incubator.

Fungal strain and its inoculum preparation

Trametes versicolor JSRK13 was isolated from Rajaji National Park, Haridwar, Uttarakhand, India (29°58'N, 78°13'E), and identified (Gene Accession Number: MH656757). The fungus was cultured on MEA plates comprising (g L⁻¹) malt extract 20, MgSO₄·7H₂O 0.5, KH₂PO₄ 0.5, Ca (NO₃)₂ 0.5, and Agar 20, and 400 µM CuSO₄ with pH 4.4 at 30 °C for 7 days, and was stored at 4 °C. 250-mL Erlenmeyer flasks containing 50 mL MEB (malt extract broth) inoculated with four discs (8.0 mm dia. each) of 7-day-old fungal culture grown on the MEB plates and incubated at a static condition in BOD incubator at 30 °C for 10 days. After 10 days of static growth, the cultures were harvested; their mycelial mat was mixed with 10 mL of sterilized distilled water and macerated in a sterilized blender for 2 min inside the laminar hood to make a slurry, which was later used as inoculum for SSF.

Screening of solid substrates for laccase production in SSF condition

Screening of different air-dried substrates was carried out to select a substrate which supports fungal growth and produces maximum units of laccase in SSF condition. Sugarcane leaves, rice straw, wheat straw and Parthenium were used as the solid substrates. For moistening the substrate, 8 mL of moistening salt solution was added to 5 g of the uniformly sieved solid substrate in a 250-mL Erlenmeyer flask (Chhaya and Modi 2013). The 60% moistening salt solution contained (g L⁻¹): Ca (NO₃)₂·4H₂O 0.5, MgSO₄·7H₂O 0.5, KH₂PO₄ 0.5, pH 4.4. Flasks were sterilized by autoclaving at 121 °C and 100 psi for 15 min. After cooling the flasks to room temperature (~25 °C), they were inoculated with 2 mL of fungal inoculum followed by an incubation at 30 °C for 20 days (Zeng et al. 2017).

Optimization of process parameters in SSF

Initial moisture content and particle size were two important process parameters studied at different levels to optimize the laccase production. Five different moisture levels (40%, 50%, 60%, 70% and 80%) were investigated with 5 g of the solid substrate. Differently sized, air-dried substrate having a size range of below 0.5 mm (powder form), 0.5–1, 3–5 and 7–9 mm were separated by sieving through a nested column of sieves with wire mesh cloth (screen) (Niladevi et al. 2007; Chhaya and Modi 2013).

Laccase production in SmF

T. versicolor was cultured in 250-mL Erlenmeyer flasks containing 50 mL of MEB containing (g L^{-1}): malt extract 20, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, KH_2PO_4 0.5, $\text{Ca}(\text{NO}_3)_2$ 0.5, pH 4.4 (Vasdev and Kuhad 1994). The growing edge of 7-day-old fungal culture in a Petri plate was cut into 8.0-mm discs and each flask was inoculated with four such discs. Incubation was set for 10 days at 30 °C in static culture condition. Thereafter, contents of the flasks were vacuum filtered and centrifuged (Sigma 3K-30, Germany) at $2500 \times g$ for 8 min at 4 °C and the clear supernatant obtained was used for making a concentrated laccase preparation of the enzyme. The stepwise concentration was carried out at 4 °C. The clear supernatant was subjected to ammonium sulphate precipitation with 85% saturation level for concentration of the proteins. The flasks were left at 4 °C overnight, the content of the flasks was centrifuged at $4000 \times g$ for 10 min to obtain a pellet, and the pellet was re-suspended in 5.0 mL of 100 mM citrate–phosphate (CP) buffer, pH 4.0, and later subjected to dialysis against the same buffer.

Optimization of selected variables through the response surface methodology (RSM)

The optimization of laccase production was carried out using a statistical design of experiments in two steps. The first step involved the screening and selection of variables, and the second step involved the optimization of the significant variables. Plackett–Burman design, a widely used fractional factorial method, was adopted for the screening of parameters influencing laccase production by *T. versicolor* JSRK13 in submerged fermentation. For the present study, 11 variables such as pH, temperature, copper sulphate, malt extract, calcium nitrate, incubation time, Tween 80, MgSO_4 , FeSO_4 , DL-methionine and glycine were included. Each component was tested at two concentration levels, low (−1) and high (+1). The experiments were carried out according to the design matrix in 250-mL Erlenmeyer flasks.

The significant variables were identified by the analysis of the Plackett–Burman design and their levels were further

optimized in a second step, for enhanced laccase production by employing a central composite design (CCD) of RSM. In this experiment, a four-factor, five-level central composite design was employed to investigate statistically the main and interactive effects of the four potentially significant factors, namely A (pH), B (copper sulphate), C (calcium nitrate) and D (malt extract) (Table 1) on laccase production from *T. versicolor* JSRK13. After investigation of four different variables, i.e., A, B, C, D, the nonlinear quadratic model was generated in the form of Eq. 1:

$$Y = \alpha_0 + \sum_{i=1}^m \alpha_i Z_i + \sum_{i=1}^m \alpha_{ii} Z_i^2 + \sum_i \sum_j \alpha_{ij} Z_i Z_j + \epsilon. \quad (1)$$

In the above equation, Y , m , α_0 , α_i , α_{ii} , α_{ij} and Z_i are predicted response, number of factor variables, model constant, linear coefficient, quadratic coefficient, interaction coefficient and factor variables in coded form, respectively. The respective high and low levels with coded levels in parentheses for variables were used to design a total of 30 experiments.

Software and data analysis

Experimental designs were generated using the statistical software package ‘Design-Expert® 11.0’ Stat-Ease Inc., Minneapolis, MN, USA. Statistical analysis of experimental data was also performed using this software.

Enzyme extraction and assay in SSF

The enzyme was extracted by mixing the fermented substrate 5 g with 10 mL of 100 mM CP buffer, pH 4.0, containing 0.1% (w/v) Tween 80. The mixture was left for 2 h in room temperature with intermittent manual shaking. The solid residue was separated from the filtrate/enzyme solution by filtering through Whatman No. 1 filter paper. The filtrate was assayed for enzyme activity expressed in U g^{-1} .

Table 1 Range of variables used for response surface methodology

Independent variables	Levels			
	− α	−1	1.00	+ α
pH	3.00	4.00	6.00	7.00
CuSO ₄ (μM)	200	300	500	600
Temperature °C	20	25	35	40.00
Malt extract (%) w/v	0.50	1.50	2.50	3.00

Enzyme assay

Laccase activity was measured using the oxidative polymerization of guaiacol (Vasdev and Kuhad 1994). 100 μM of guaiacol in 100 mM CP buffer, pH 4.0, is the substrate solution. 1U of the enzyme activity is equal to absorbance change of $0.01 \text{ min}^{-1} \text{ mL}^{-1}$ measured at a fixed wavelength of 470 nm. All enzyme activity assays were performed in duplicates, and the reported data correspond to mean values with standard deviation.

Extraction of BPA and TCA from synthetic and sewage water sample

BPA was extracted from various day to day use items and sewage water in order to find the concentration of BPA and TCA that laccase will encounter in a contaminated water sample. BPA was extracted from food packs, newspapers, ATM slips and thermal receipt paper. For BPA extraction, 100 g of the mixture of all the four items were immersed in 1 L of boiled HPLC-grade water and kept for 5 days at 40 °C in a water bath. Similarly, aqueous extraction of TCA was carried out with 20 g each of toothpaste: (a) Colgate Total (b) Dabur Red and bath Soap (c) Lux International and liquid hand wash (d) Dial and (e) savlon hand wash mixed in 1 L of boiled water. A 10 mL aliquot was pipetted out from the mixture in a test tube and vortexed for 2 min. The sample was subjected to ultrasonic irradiation in an ultrasonic bath (PCI Analytics, India, Model 1.5L50, operating at 50 Hz) at room temperature for 10 min and centrifuged at $2500\times g$ for 15 min. The supernatants were collected and filtered using a 0.20- μm syringe filter. The sample of sewage water was prepared by first filtering it through Whatmann filter paper followed by evaporating it until its volume is 1/10th of its initial volume. The sample thus obtained was sonicated and filtered with a 0.20- μm syringe filter (Mohapatra et al. 2011; Dionex Corporation 2012).

Quantification of BPA and TCA in filtrate using reverse-phase isocratic HPLC

Samples of mixed product leachate and sewage wastewater were analyzed with HPLC [Young Lin (YL) model YL9120 having a YL9112 Isocratic pump and YL9120 UV/Vis detector] fitted with Column-C18, (250 $\times$ 4.60 mm). Elution of BPA was performed with a mobile phase containing water and acetonitrile in 60:40 ratios v/v at a flow rate of 1.0 mL min^{-1} at room temperature with 20 μL injection volume (Aristiawan et al. 2015; Aurand 2012). The mobile phase for triclosan was methanol, acetonitrile and sodium citrate buffer (100 mM) in 40:40:20 ratios v/v (Shimelis et al. 2014) keeping other HPLC conditions same. Absorbance maxima (λ_{max}) of BPA and triclosan were recorded

after spectrophotometric scanning of the standard solution of BPA and triclosan λ_{max} thus obtained was used in HPLC UV/Vis detector to analyze BPA and TCA.

Biotransformation and degradation of standard bisphenol-A and triclosan by free laccase

Spectrophotometric analysis was carried out to see the biotransformation rate of BPA and TCA, and then confirmed by HPLC. The laccase-catalyzed transformation was carried out in a glass tube containing 2 mL of the reaction mixture. The reaction mixtures contained 1 mL each of BPA (250 μM) and triclosan (5 μM), dissolved in methanol and water (1:1), and 1 mL of partially purified laccase (100 U/mL) dissolved in CP buffer (100 μM and pH 4.0). The reaction mixture was incubated for 24 h at 28 °C. The concentrations of BPA and TCA in the reaction mixture were based on the concentration found in mixed product leachate and sewage water used in the study. For setting a control, test lacking laccase but with the same concentration of BPA and TCA was kept.

Laccase production and simultaneous transformation of BPA and TCA with *T. versicolor* JSRK13 in SSF and SmF

A 2 mM stock solution of BPA and TCA was prepared in methanol and water (1:1) solution separately in a glass vial. The transformation of BPA and TCA was attempted in two formats:

- In SSF appropriately, sized *Parthenium* spiked with 250 μM of BPA and 5 μM of TCA was used as a substrate in SSF for cultivating *T. versicolor* JSRK13.
- In SmF 250 μM of BPA and 5 μM of TCA was added to 50 mL of MEB. The above-mentioned inoculation and incubation procedures were followed, and cultivation time was set according to fermentation strategies used and time course of laccase production.

The controls were also set with the same concentration of each EDCs but lacking the inocula in (a) and (b). After the respective incubation, the residual EDCs on a solid substrate was extracted by shaking a mixture of methanol and water (1:1) (1/10, W/V) on an orbital shaker at 200 rpm for 4 h followed by centrifugation at $1000\times g$ for 10 min. The clear supernatant was heated to 40 °C on a dry bath to remove volatile compounds and fragrances followed by measurement of residual EDC concentration.

In case of SmF, the remaining EDCs in the medium was extracted by a mixture of methanol and water (1:1) (1/1, V/V) followed by shaking (1.5 $\times g$ for 4 h) and centrifugation ($1000\times g$ for 10 min).

Effects of mediator on EDC transformation

The effects of synthetic and natural mediators in all the three formats (SSF, SmF and free enzyme) were studied. All the enzymatic reactions were carried out as described above at room temperature with 0.5 mM of synthetic 1-hydroxybenzotriazole (HBT) or vanillin as natural mediator dissolved in the CP buffer (pH 4.4), before the inoculation (Zeng et al. 2017).

Determination of BPA and TCA transformation from the samples

Both spectrophotometric and HPLC methods were used to calculate the percentage transformation of BPA and triclosan. Calculation of transformation using HPLC analysis was based on the equation (Eq. 2) given by (Zeng et al. 2017)

$$\text{Transformation (\%)} = \frac{\text{Peak area of control} - \text{Peak area of test sample}}{\text{Peak area of control}} \times 100. \quad (2)$$

Results and discussion

Effect of time course study

Laccase production by *T. versicolor* JSRK13 in SmF was maximum on the 10th day with enzyme activity 22 U mL⁻¹ and started decreasing thereafter (Fig. 1). In Ganoderma sp., also the maximum laccase activity was recorded on the 10th day of incubation (Sivakumar et al. 2010), whereas, *P. ostreatus* (ACCC 52857) was reported to give maximum laccase activity on the 11th day of incubation (Zhu et al.

Fig. 1 Time course of Laccase production by *T. versicolor* JSRK13 in solid-state fermentation and submerged fermentation condition

2016). In SSF, maximum enzyme activity of 185 U/g was achieved on the 15th day of cultivation (Fig. 1). Similarly, maximum laccase activity was reported on the 15th day of incubation with *P. ostreatus* at 25 °C using humidified sawdust as substrate (Jasim Hashim 2011). In another study, Sarnthima et al. (2009) reported maximum laccase activity of 1425 U L⁻¹ at 17th day of incubation using rice bran with rice husk (2:1 W/W). Other workers reported SSF of potato peel waste by *Pleurotus Kumm* (MCC16) with a laccase activity of 6708.3 ± 75 U L⁻¹ on day 17 (Ozciak and Ozturk 2017).

Optimization of process parameters in SSF

Screening of solid substrates for SSF

A very important factor for the viability and success of the SSF process is the selection of suitable and cheap substrate. To select the best substrate, laccase production was carried out on rice straw, wheat straw, sugarcane leaves, and Parthenium sp. Maximum production of laccase was observed (Fig. 2) with Parthenium sp. 185 U g⁻¹ and sugarcane leaves 165 U g⁻¹ followed by wheat straw 150 U g⁻¹ and rice straw 145 U g⁻¹. Substrate penetration and quick colonization is the key to success of SSF (Chhaya and Modi 2013). *T. versicolor* effectively colonized the solid substrate particles. Amongst all the substrate tested, Parthenium proved to be the most suitable for laccase production in SSF. It is worth mentioning here that congress grass is a devastating weed commonly distributed in North America, Asia, Australia, and Eastern and Southern Africa (Kaur et al. 2014; McConachie et al. 2011), and could be an easily available substrate for laccase production. Laccase is a ligninolytic extracellular enzyme that is extracellularly secreted on a lignocellulosic substrate in SSF condition. There are many studies on compositional analysis of Parthenium which report the higher

Fig. 2 Laccase production by *T. versicolor* JSRK13 on selected solid substrates

percent of lignin, compared to the conventional substrate (Murthy 2010; Patel 2011; Kim and Day 2011; Anwar et al. 2014; Pandiyan et al. 2014; Saini et al. 2014).

Effect of particle size and moisture content

Physical properties of the solid substrate such as particle size, topology, surface area, porosity, moisture absorbing capacity, etc. play a crucial role in the SSF process. The most significant physical property is particle size because porosity, surface area, moisture retention are all dependent on the size of the substrate. In this study, we observed optimum laccase production at a particle size in the range of 3–5 mm (Fig. 3a) with all the four substrates. The enzyme yield was substantially reduced when the substrate is in a pulverized form with a particle size of 0.5 mm or below. This is because SSF demands higher levels of aeration to ensure efficient heat exchange (Rodríguez-Fernández et al. 2012), whereas, the particle size of 5–10 mm caused a drop in the enzyme yield. Larger particle size results in low retention of moisture and offers less surface area for colonization. SSF using cotton stalks resulted in highest ligninolytic enzyme activities, at a particle size of 5 mm (Meehnan et al. 2016). Another

important key parameter during SSF process is moisture content which greatly affects the growth of microorganism as well as metabolite production. More of moisture in the substrate leads to poor mass transfer which depletes product formation while low moisture level causes reduced solubility due to which nutrient availability for the growth of fermenting microorganism is decreased (Vastrad et al. 2012). In our study, 60% moisture content retained by all the four solid substrates was found to be optimum for maximum laccase production by *T. versicolor* (Fig. 3b). Moisture content of more than 60% resulted in decreased enzyme activity. This decrease might be due to the reduction in the diffusion of oxygen and porosity of the substrate (Niladevi et al. 2007; Chhaya and Modi 2013). Therefore, Parthenium or congress grass with a particle size of 3–5 mm having a moisture content of 60% was most suitable for the production of laccase through SSF.

Optimization of SmF through response surface methodology (RSM)

Placket–Burman design revealed that 7 out of 11 factors were affecting the production of the enzyme positively and out of the seven, four parameters, viz. pH, Temperature, malt extract and CuSO_4 , were selected for further optimization with RSM as these factors significantly affected production. The factors which were having a negative impact on the enzyme production were excluded.

The RSM based on CCD has been applied in which each parameter varied over five levels, a very low P value (<0.0001) along with the computed F value of 26.07 implies that the quadratic regression model was extremely significant for the analysis of variance (ANOVA). The coefficient of variation indicated a high correlation between experimentally observed and predicted values (complete data provided in the supplementary material) and the coefficient of determination (R^2) for laccase production was 0.923 (above 0.75 values designates fitness of the model). The P value is less than 0.05 indicates that the model terms are significant. The ANOVA results based upon the P value suggested that model terms A , C , D , A^2 , B^2 , C^2 and D^2 were significant. The lack of fit for the model designed was not significant indicating the model is fit for prediction and hence the statistical implication of this theoretical account is turned over by the equations for coded factors.

Laccase production R

$$\begin{aligned}
 R = & -252.05823 + 32.16479A + 12.48729B \\
 & + 39.93125C + 0.408427D + 0.383875AB \\
 & + 2.43875AC + 0.005331AD + 0.485750BC \\
 & - 0.002011BD + 0.018287CD - 6.19365A^2 \\
 & - 0.237796B^2 - 12.26958C^2 - 0.000511D^2,
 \end{aligned} \quad (3)$$

Fig. 3 Effect of particle size (a) and initial moisture content (b) of Parthenium on the production of laccase by *T. versicolor* JSRK13

where R , A , B , C and D are response, pH, temperature, malt extract and CuSO_4 , respectively.

The interaction between the variables predicted a suitable model with a pH of 4.3, temperature of 28 °C, malt extract 2% and CuSO_4 at a concentration of 410 μM , to be optimal for enzyme production. The predicted value for laccase enzyme units according to the model was 87.75 U mL^{-1} of laccase. The validation experiment with the predicted values resulted in the production of 85.91 U mL^{-1} .

Partial purification of laccase from SmF

An 85% ammonium sulphate precipitation cut followed by dialysis resulted in 6.7-fold purification and 49% yield with a specific activity of 148.80 U/mg (Table 2).

Determination of BPA and TCA concentrations in mixed product leachate and sewage wastewater

Maximum absorbance (λ_{max}) for the standard solution of BPA and triclosan were found to be 278 and 282 nm, respectively (Fig. 4a, b). Table 3 shows the concentration of BPA and TCA in mixed product leachate and sewage water samples.

Quantification of BPA and TCA was done for mixed product leachate (Fig. 5a, b) by HPLC. A standard curve for both the compounds was generated by plotting increasing concentrations of pure standard compounds against their average peak areas. Mixed product leachate was detected with 260 μM of BPA and 3.5 μM of TCA, whereas, in sewage water, 20 μM of BPA and a negligible amount of triclosan were detected. Cao et al. (2009) measured 0.032–4.5 $\mu\text{g L}^{-1}$ of BPA in all 72 samples of canned soft drink products, whereas, Yalcin et al. (2016) determined the concentration of BPA in all the randomly collected 12 thermal paper receipt samples from various workplaces. 13.83 mg BPA/g paper was found as the average value of BPA concentration for the ten selected samples. Xu et al. (2016) determined the BPA concentration by a spectrophotometric technique using diazotization-coupling reaction and then also with the HPLC method. 0.5–4 ng mL^{-1} concentration of BPA was detected in the water extracted samples of three brands of baby bottles.

Although the reported levels of BPA in the sewage water are low, their presence is high enough to raise immediate concern because long-time exposure even at trace quantities can create potential health issues in human and animals. Yang et al. (2019) analyzed the potential health risks of BPA and they estimated daily intake of BPA (0.025 $\mu\text{g kg}^{-1}$ bw day $^{-1}$) through the handling of thermal paper for the general population of China. Recently, Zhou et al. (2019) investigated the EDCs in three wild freshwater fishes from Xiangjiang river and found that the mean liver/muscle concentration ratios of 4-*n*-nonylphenol, BPA, estrone and 17 α -ethynylestradiol confirmed the prolonged exposure of the fish to these EDCs. Most importantly, they reported that the total estimated daily intakes from consuming the three freshwater fish species from the Xiangjiang River were higher than the acceptable daily intake. Therefore, the use of such chemicals should be restricted to save living beings from their potential hazard.

According to Danish Environmental Protection Agency, eight cosmetic product types: Toothpaste, Hand Soap, Body soaps/Shower gel, Face powder, Blemish Concealer, Nail products, Deodorant (non-spray) and Mouthwash contain triclosan in the range of 0.60–3.4% per product (Dhillon et al. 2015). Parenti et al. (2019) reported a significant increase of cell necrosis in Zebrafish embryos which highlighted a strong cytotoxic potential for TCS. Chattu et al. (2016) reported 10–4741 ppm as the average concentration of triclosan in the personal care product. The average concentrations in the pool water and river water samples were 49 and 72 ppb, respectively. The aquatic environments are also getting polluted by the continuous discharge of domestic wastewater, where 460 ng mL^{-1} of TCA was traced in bile from fish collected from the Yangtze River.

Biotransformation of BPA and TCA with partially purified free laccase

Figure 6a, b proves the biotransformation of BPA and TCA after enzymatic treatment through spectrophotometric analysis in the UV region. Figure 6 shows shifts in UV spectra of both the compounds after a 10-h treatment with partially purified free laccase. After 24 h, the spectra for the test or the blank analytes (control) did not show any significant changes. The spectra were recorded for both

Table 2 Partial purification of laccase using ammonium sulphate precipitation

S. no.	Purification steps	Volume (mL)	Laccase (U)	Total protein (mg)	Specific activity (U mg^{-1})	Yield (%)	Fold purification
1	Crude enzyme	300	25,500	1150	22.17	100	1
2	85% ammonium sulphate precipitation	20	12,500	84	148.80	49	6.7

Fig. 4 a, b Absorption spectra of BPA and TCA **Table 3** Detected concentrations BPA and TCA in the respective sample

S.no.	Sample	BPA concentration	Triclosan concentration
1	Synthetic or mixed product leachate	60 mg L ⁻¹ or 260 µM or 300 mg kg ⁻¹	1 mg L ⁻¹ or 3.5 µM or 4 mg kg ⁻¹
2	Sewage water sample	4.5 mg L ⁻¹ or 20 µM	Nd

the compounds with bathochromic shifts and hypochromic shifts due to the increase and reduction in the absorbance intensity. Shifting of peaks around 270–295 nm gives a clear indication of biotransformation by laccase. Shifting of peaks and appearance of new absorbance peaks indicate the possible formation of new break down products, having different absorbance.

The high rate of biotransformation of BPA and TCA after 24 h of treatment was evident from the HPLC chromatograms which showed a decrease in the signal intensity. Around 24 h of enzymatic oxidation is sufficient to achieve transformation higher than 70% for BPA and about 60% for TCA (Fig. 7b, d). Morales et al. showed qualitative biotransformation of EDCs by UV–Vis spectrophotometry and quantitative biotransformation by HPLC–UV chromatography methods. They reported that laccase cocktail in culture filtrate was much effective for elimination of common endocrine disruptors including BPA, TCA, 4-nonylphenol and 17- α -ethynylestradiol. They reported 89–100% transformation of said synthetic samples after the crude laccase treatment (Garcia et al. 2015). The results obtained after HPLC analysis support the observation made through absorption spectrometry of BPA and TCA.

Both these compounds showed significant changes in their concentration after incubation of 10–24 h with the

Fig. 5 HPLC chromatograms of mixed product leachate samples of **a** BPA and **b** TCA

Fig. 6 **a** Biotransformation of BPA (**a**) and TCA (**b**) with partially purified laccase with 24-h treatment

partially purified enzyme (Fig. 7). In Fig. 7b, the appearance of a new peak at retention time of 2.7 min could be a degraded product of BPA. Laccase enzyme performs one-electron oxidation of its phenolic substrates, and the catalysis potential depends upon the presence of electron-donating functional groups such as hydroxyl ($-OH$), alkyl ($-R$), alkoxy ($-OR$), acyl ($-COR$) and amines ($-NH_2$)

which induce the electrophilic attack (Nguyen et al. 2013; Yang et al. 2013), whereas, the presence of electron withdrawing groups such as amide halogen ($-X$), ($-CONR_2$) and nitro ($-NO_2$) reduces the efficiency of enzymes for attacking the substrate by producing an electron deficiency (Yang et al. 2013; Asif et al. 2018).

Fig. 7 HPLC chromatograms of BPA and TCA control tube (a, c) and reaction tube (b, d) after a 24-h treatment with partially purified free laccase

Table 4 HPLC chromatogram data for control and laccase treated test samples

Sample	Average RT(retention time) (min)	Average peak area (mV s)	Removal (%)
Control BPA	7.21	2221.2121	00
Test 24-h BPA	7.18	665.2523	70
Control TCA	10.18	2650.5050	00
Test 24-h TCA	10.23	1050.3256	60

The presence of two electron-donating hydroxyl and alkyl groups results in ~70% transformation of polyphenolic BPA after enzymatic treatment, whereas TCA is transformed to an extent of 60% due to the presence of both electrons withdrawing and donating groups, which results in its lower transformation percentage (Table 4). There are various studies which endorse the results of enzyme-catalysed transformation of BPA and TCA. Previously, Garcia et al. (2015) reported that TCA, BPA and two other EDCs were eliminated up to 89% by crude laccase preparation from *Pycnoporus sanguineus* CS43, without using any mediators. The removal efficiency of EDC by an enzyme depends upon types of the microbial strain, media composition used for the production of the enzyme and the nature of the compound.

LPST of BPA and TCA with *T. versicolor* in SSF and SmF

A known concentration of BPA was added to both SmF and SSF. Analysis of the methanolic extracts was carried out at different intervals. In SmF, 10% of BPA removal was observed on 6th day and 75% after 10th day of fermentation by *T. versicolor* (Figs. 8a, 9b), whereas, for SSF 12% on the 7th day, 50% on 11th day and a maximum of 70% on the 15th day of fermentation was observed (Figs. 8b, 9d). In both the fermentation strategies, maximum laccase activity coincided with the highest BPA transformation. The transformation was maximum on 8th–10th days in SmF and 13th–15th days in SSF (Fig. 8c). LPST strategy worked best for BPA using *T. versicolor* cultivated on a lignocellulosic substrate. At the end of the experiment, the laccase activities were 95 U mL⁻¹ in SmF and 220 U g⁻¹ in SSF suggesting that the disappearance of the BPA is due to the oxidative action of enzymes and more specifically laccase though, the contribution of other ligninolytic enzymes such as MnP for the transformation cannot be neglected (Falade et al. 2018).

The actual concentration of BPA in wastewater sample ranges from 0.07 to 1.68 µg L⁻¹ (Mohapatra et al. 2011) and for TCA it is 2.01–17.6 µg L⁻¹ (Raisibe et al. 2017) which is far lower than the concentration of BPA (55 mg L⁻¹) and

Fig. 8 Percentage of transformation of BPA in SmF (a), SSF (b) and enhanced laccase activity in the presence of BPA (c) in both SmF and SSF conditions

Fig. 9 HPLC chromatograms of BPA transformation with *T. versicolor* JSRK13 in **a** SmF on 10th day and **b** SSF on 15th day of incubation

TCA (1.5 mg L^{-1}) used in the current study. Said concentration of BPA and TCA was chosen based on the results obtained after HPLC analysis of the synthetic mixed product leachate made for BPA and TCA separately. Zeng et al. (2017) also reported 90% of BPA (25 mg L^{-1}) transformation with simultaneous production of laccase from *T. versicolor* cultured in SSF on solid agricultural wastes. They concluded that the LPST of BPA with *T. versicolor* is more advantageous since BPA can act as an enhancer for laccase production without the requirement of laccase mediators. Tsioulpas et al. (2002) also confirmed the higher laccase activity by *Pleurotus* spp. in the growth medium in the presence of phenolic compounds resulting in 69–76% removal of such compounds from olive mill wastewater. However, the simultaneous transformation strategy did not work for the triclosan as it was toxic for the growth of *T. versicolor*.

Effect of Laccase–mediator system (LMS) on BPA and TCA transformation

In this study, the effect of HBT and vanillin was studied to enhance the transformation of BPA and TCA. Reaction

mixtures, where HBT was used, could effectively improve the rate of transformation of BPA and TCA. BPA transformation of ~70% was observed without HBT and 90% with HBT (Figs. 10a, 11), and TCA transformation of ~60% was observed without HBT to ~75% with HBT (Fig. 11). Vanillin (VA) was not found to be much effective laccase mediator for enhancing the transformation rate. Vanillin could enhance the transformation of BPA by 8% and 5% for TCA with respect to free laccase treatment (Fig. 11). The effect of both the mediators on BPA transformation was also investigated in the SmF and SSF process with *T. versicolor* but the negligible increment in the transformation rate was observed compared to in vitro transformation with free laccase. Based on the previous reports (Zeng et al. 2017) and our results, it can be concluded that redox mediators are dispensable for BPA transformation in the LPST process format. Ashe et al. (2016) studied effects of seven redox mediators and found violuric acid (VA) and 1-hydroxybenzotriazole (HBT) as best mediators for the enhancement of complex compound transformation and also causing insignificant toxicity in the reaction mixture. Zeng et al. (2017) observed 72.20% transformation of BPA

Fig. 10 HPLC chromatograms of BPA and TCA control run (a, c) and reaction run (b, d) showing effect of laccase mediator (HBT) on BPA and TCA transformation after a 24-h treatment, respectively

Fig. 11 Effect of mediators on BPA and TCA transformation after 24 h of free laccase treatment

using HBT, whereas, without HBT the transformation efficiency was only 58%. Xie (2013) found laccase–vanillin system responsible for the transformation of chlorpyrifos and 98% transformation level was achieved with the two-step addition of vanillin.

Conclusions

The potential of enzyme laccase in degrading emerging EDCs including BPA and TCA can be used to develop a biological process to decontaminate our water bodies from EDCs. Weeds such as Parthenium can be advantageously utilized as substrates for developing an economically feasible process for laccase production by *T. versicolor* JSRK13 in SSF conditions. The simultaneous transformation of BPA with laccase production in both SmF and SSF conditions can be developed into a bioprocess which can work both on solid and liquid wastes containing BPA. Unfortunately, the said strategy did not work for the triclosan as it was toxic for the growth of *T. versicolor* JSRK13. Therefore, cell-free system which uses the free enzyme would be more desirable.

In LPST strategy, the use of mediators such as HBT and Vanillin is dispensable for the enzyme used in the current study. Further, bio-monitoring studies for the determination of trace amount of most common EDCs such as BPA and triclosan in our water bodies and domestic supplies are the need of the hour, so that their influence on human health

can be studied. The results suggest that removal of EDCs by laccase-catalyzed processes offers a promising way to treat contaminated water bodies. Future studies are required to develop an efficient enzyme immobilization system in the form of a cassette to decontaminate water samples.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Annamalai J, Namasivayam V (2015) Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife. *Environ Int* 76:78–97. <https://doi.org/10.1016/j.envint.2014.12.006>
- Anwar Z, Gulfranz M, Irshad M (2014) Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. *J Radiat Res Appl Sci* 7:163–173. <https://doi.org/10.1016/j.jrras.2014.02.003>
- Aparicio I, Martín J, Abril C et al (2018) Determination of household and industrial chemicals, personal care products and hormones in leafy and root vegetables by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1533:49–56. <https://doi.org/10.1016/j.chroma.2017.12.011>
- Aristiawan Y, Aryana N, Putri D, Styarini (2015) Analytical method development for Bisphenol-A in Tuna by using High-performance liquid chromatography—UV. *Proc Chem* 16:202–208
- Ashe B, Nguyen LN, Hai FI et al (2016) Impacts of redox-mediator type on trace organic contaminants degradation by laccase: degradation efficiency, laccase stability and effluent toxicity. *Int Biodeterior Biodegrad* 113:169–176. <https://doi.org/10.1016/j.ibiod.2016.04.027>
- Asif MB, Hai FI, Kang J, Price WE, Nghiem LD (2018) Biocatalytic degradation of pharmaceuticals, personal care products, industrial chemicals, steroid hormones and pesticides in a membrane distillation-enzymatic bioreactor. *Biores Technol* 247:528–536
- Aurand C (2012) Comprehensive SPE sample preparation and fast HPLC method for bisphenol-A in drinking water. *Supelco Analytical. Report US Rapid Determ Packag Contam Food Beverages* 30.1:8–9
- Baweja M, Nain L, Kawarabayasi Y, Shukla P (2016) Current technological improvements in enzymes toward their biotechnological applications. *Front Microbiol* 7:1–13. <https://doi.org/10.3389/fmicb.2016.00965>
- Becker D, Rodriguez-Mozaz S, Insa S et al (2017) Removal of endocrine disrupting chemicals in wastewater by enzymatic treatment with fungal laccases. *Org Process Res Dev* 21:480–491. <https://doi.org/10.1021/acs.oprd.6b00361>
- Bernier MR, Vandenberg LN (2017) Handling of thermal paper: Implications for dermal exposure to bisphenol-A and its alternatives. *PLoS One* 12:e0178449. <https://doi.org/10.1371/journal.pone.0178449>
- Cao X-L, Corriveau J, Popovic S (2009) Levels of Bisphenol-A in canned soft drink products in canadian markets. <https://doi.org/10.1021/jf803213g>
- Chandra R, Sharma P, Yadav S, Tripathi S (2018) Biodegradation of endocrine-disrupting chemicals and residual organic pollutants of pulp and paper mill effluent by biostimulation. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2018.00960>
- Chattu D, Supervisor C, Donkor K (2016) Determination of triclosan in personal care products and swimming pool samples by liquid chromatography-mass spectrometry. In: Proceedings of the annual thompson rivers university undergraduate research and innovation conference, vol 10(1), Article 3. <https://digitalcommons.library.tru.ca/urcproceedings/vol10/iss1/3>
- Chhaya R, Modi HA (2013) Comparative Study of laccase production by streptomyces chartreusis in solid state and submerged. *Indian J Fundam Appl Life Sci* 3:73–84
- Chiang C, Mahalingam S, Flaws J (2017) Environmental contaminants affecting fertility and somatic health. *Semin Reprod Med* 35:241–249. <https://doi.org/10.1055/s-0037-1603569>
- Dhillon GS, Kaur S, Pulicharla R et al (2015) Triclosan: current status, occurrence, environmental risks and bioaccumulation potential. *Int J Environ Res Public Health* 12:5657–5684. <https://doi.org/10.3390/ijerph120505657>
- Dionex Corporation (2012) Determination of inorganic anions in environmental waters using a hydroxide-selective column. Application Note 154; Sunnyvale, pp 42–50
- Falade AO, Mabinya LV, Okoh AI, Nwodo UU (2018) Ligninolytic enzymes: versatile biocatalysts for the elimination of endocrine-disrupting chemicals in wastewater. *MicrobiologyOpen* 7:1–17. <https://doi.org/10.1002/mbo3.722>
- Garcia-Morales R, Rodríguez-Delgado M, Gomez-Mariscal K et al (2015) Biotransformation of endocrine-disrupting compounds in groundwater: bisphenol-A, nonylphenol, ethynylestradiol and triclosan by a laccase cocktail from *Pycnoporus sanguineus* CS43. *Water Air Soil Pollut*. <https://doi.org/10.1007/s1127-0-015-2514-3>
- Ghazarian AA, Trabert B, Robien K et al (2018) Maternal use of personal care products during pregnancy and risk of testicular germ cell tumours in sons. *Environ Res* 164:109–113. <https://doi.org/10.1016/j.envres.2018.02.017>
- Jasim Hashim A (2011) Determination of optimal conditions for laccase production by *Pleurotus ostreatus* using sawdust as solid medium and its use in phenol degradation. *Baghdad Sci J* 9(3):491–498
- Kapoor RK, Rajan K, Carrier DJ (2015) Applications of *Trametes versicolor* crude culture filtrates in detoxification of biomass pretreatment hydrolyzates. *Bioresour Technol* 189:99–106
- Kaur M, Aggarwal NK, Kumar V, Dhiman R (2014) Effects and management of *Parthenium hysterophorus*: a weed of global significance. *Int Sch Res Not* 2014:1–12. <https://doi.org/10.1155/2014/368647>
- Kim M, Day DF (2011) Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. *J Ind Microbiol Biotechnol* 38:803–807. <https://doi.org/10.1007/s10295-010-0812-8>
- Koumaki E, Mamais D, Noutsopoulos C (2018) Assessment of the environmental fate of endocrine disrupting chemicals in rivers. *Sci Total Environ* 628–629:947–958. <https://doi.org/10.1016/j.scitotenv.2018.02.110>
- Kumar V, Baweja M, Liu H, Shukla P (2017) Microbial enzyme engineering: applications and perspectives. Recent advances in applied microbiology. Springer, Singapore, pp 259–273
- Lassouane F, Ait-Amar H, Amrani S, Rodriguez-Couto S (2019) A promising laccase immobilization approach for Bisphenol-A removal from aqueous solutions. *Bioresour Technol* 271:360–367. <https://doi.org/10.1016/j.biortech.2018.09.129>
- Lecomte S, Habauzit D, Charlier DC, Pakdel F (2017) Emerging estrogenic pollutants in the aquatic environment and breast cancer. *Genes (Basel)* 8:2–21. <https://doi.org/10.3390/genes8090229>

- Lind PM, Lind L (2018) Endocrine-disrupting chemicals and risk of diabetes: an evidence-based review. *Diabetologia* 61:1495–1502. <https://doi.org/10.1007/s00125-018-4621-3>
- Liu T, Wu D (2012) High-performance liquid chromatographic determination of triclosan and triclocarban in cosmetic products. *Int J Cosmet Sci* 34:489–494. <https://doi.org/10.1111/j.1468-2494.2012.00742.x>
- Llorca M, Badia-Fabregat M, Rodríguez-Mozaz S et al (2017) Fungal treatment for the removal of endocrine disrupting compounds from reverse osmosis concentrate: identification and monitoring of transformation products of benzotriazoles. *Chemosphere* 184:1054–1070. <https://doi.org/10.1016/j.chemosphere.2017.06.053>
- McConnachie AJ, Strathie LW et al (2011) Current and potential geographical distribution of the invasive plant *Parthenium hysterophorus* (Asteraceae) in eastern and southern Africa. *Weed Res* 51:71–84. <https://doi.org/10.1111/j.1365-3180.2010.00820.x>
- Meehnan H, Jana AK, Jana MM (2016) Effect of particle size, moisture content, and supplements on selective pretreatment of cotton stalks by *Daedalea flavida* and enzymatic saccharification. *3 Biotech* 6:235. <https://doi.org/10.1007/s13205-016-0548-x>
- Mohapatra DP, Brar SK, Tyagi RD, Surampalli RY (2011) Occurrence of bisphenol-A in wastewater and wastewater sludge of CUQ treatment plant. *J Xenobiot* 1:9–16. <https://doi.org/10.4081/xeno.2011.e3>
- Murthy K (2010) Chemical and biochemical properties of *Parthenium* and *Chormolaena* compost. *Int J Sci Nat* 1(2):166–171
- Nag SK, Das Sarkar S, Manna SK (2018) Triclosan—an antibacterial compound in water, sediment and fish of River Gomti, India. *Int J Environ Health Res*. <https://doi.org/10.1080/09603123.2018.1487044>
- Nagy KZ, László B, Fleit E, Czihat MK (2018) Behavior of organic micropollutants during river bank filtration in Budapest, Hungary. *Water* 10:2–13
- Nguyen LN, Hai FI, Yang S et al (2013) Removal of trace organic contaminants by an MBR comprising a mixed culture of bacteria and white-rot fungi. *Bioresour Technol* 148:234–241. <https://doi.org/10.1016/j.biortech.2013.08.142>
- Niladevi KN, Sukumaran RK, Prema P (2007) Utilization of rice straw for laccase production by *Streptomyces psammoticus* in solid-state fermentation. *J Ind Microbiol Biotechnol* 34:665–674. <https://doi.org/10.1007/s10295-007-0239-z>
- Noonan GO, Ackerman LK, Begley TH (2011) Concentration of Bisphenol-A in highly consumed canned foods on the U.S. market. *J Agric Food Chem* 59:7178–7185. <https://doi.org/10.1021/jf201076f>
- Oates RP, Longley G, Hamlett P, Klein D (2017) Pharmaceutical and endocrine disruptor compounds in surface and wastewater in San Marcos, Texas. *Water Environ Res* 89:2021–2030. <https://doi.org/10.2175/106143017X14902968254584>
- Ozgirak Ergun S, Ozturk Urek R (2017) Production of ligninolytic enzymes by solid state fermentation using *Pleurotus ostreatus*. *Ann Agrar Sci* 15:273–277. <https://doi.org/10.1016/J.AASCI.2017.04.003>
- Pandiyar K, Tiwari R, Singh S et al (2014) Optimization of enzymatic saccharification of alkali pretreated *Parthenium* sp. using response surface methodology. *Enzyme Res* 2014:1–8. <https://doi.org/10.1155/2014/764898>
- Parenti CC, Ghilardi A, Torre DC, Giacco DL, Binelli A (2019) Environmental concentrations of triclosan activate cellular defence mechanism and generate cytotoxicity on zebrafish (*Danio rerio*) embryos A. *Sci Total Environ* 650:1752–1758
- Patel S (2011) Harmful and beneficial aspects of *Parthenium hysterophorus*: an update. *3 Biotech* 1:1–9. <https://doi.org/10.1007/s13205-011-0007-7>
- Puig DRT (2012) Removal of endocrine disrupting chemicals by the ligninolytic enzyme versatile peroxidase. Group of Environmental Biotechnology Doctoral thesis
- Raisibe F, Adegbenro LP, DasoJonathan OO (2017) Occurrence and environmental levels of triclosan and triclocarban in selected wastewater treatment plants in Gauteng Province, South Africa. *Emerg Contam* 3:107–114. <https://doi.org/10.1016/j.emcon.2017.07.001>
- Rodríguez-Fernández DE, Rodríguez-León JA, de Carvalho JC et al (2012) Influence of airflow intensity on phytase production by solid-state fermentation. *Bioresour Technol* 118:603–606. <https://doi.org/10.1016/J.BIORTECH.2012.05.032>
- Saini A, Aggarwal NK, Sharma A et al (2014) Utility potential of *Parthenium hysterophorus* for its strategic management. *Adv Agric* 2014:1–16. <https://doi.org/10.1155/2014/381859>
- Sarnthima R, Khammuang S (2009) Laccase activity from fresh fruiting bodies of *Ganoderma* sp. MK05: purification and remazol brilliant blue R decolorization. *J Biol Sci* 9:83–87. <https://doi.org/10.3923/jbs.2009.83.87>
- Sharma B, Dangi AK, Shukla P (2018) Contemporary enzyme based technologies for bioremediation: a review. *J Environ Manag* 210:10–22. <https://doi.org/10.1016/j.jenvman.2017.12.075>
- Shimelis O, Halpenny M, Ken E, Schultz K (2014) Determination of triclosan in environmental waters using polymeric SPE cleanup and HPLC with mass spectrometric detection. *Supelco Anal Report* 32(2):22–23
- Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA (2017) Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environ Toxicol Pharmacol* 51:56–70. <https://doi.org/10.1016/J.ETAP.2017.02.024>
- Sin J-C, Lam S-M, Mohamed AR, Lee K-T (2012) Degrading endocrine disrupting chemicals from wastewater by photocatalysis: a review. *Int J Photoenergy* 2012:1–23. <https://doi.org/10.1155/2012/185159>
- Singh JP, Kapoor RK (2014) Microbial laccases: a mini-review on their production, purification and applications. *Int J Pharm Arch* 3:528–536
- Singh S, Moholkar VS, Goyal A (2014) Bioethanol production by pretreatment, hydrolysis and fermentation of *Parthenium Hysterophorus*. *Int J Appl Eng Res* 9:1149–1150
- Singh J, Saharan V, Kumar S, Gulati P, Kapoor RK (2017) Laccase grafted membranes for advanced water filtration systems: a green approach to water purification technology. *Crit Rev Biotechnol*. <https://doi.org/10.1080/07388551.2017.1417234>
- Siracusa JS, Yin L, Measel E et al (2018) Effects of bisphenol-A and its analogs on reproductive health: a mini review. *Reprod Toxicol* 79:96–123. <https://doi.org/10.1016/j.reprotox.2018.06.005>
- Sivakumar R, Rajendran R, Balakumar C et al (2010) Isolation, screening and optimization of production medium for thermostable laccase production from *Ganoderma* sp. *Int J Eng Sci* 2:7133–7141
- Skledar DG, Carino A, Trontelj J, Troberg J, Distrutti E, Marchionò S, Tomašič T et al (2019) Endocrine activities and adipogenic effects of bisphenol-AF and its main metabolite. *Chemosphere* 2015:870–880. <https://doi.org/10.1016/j.chemosphere.2018.10.129> (Epub 2018 Oct 18)
- Tsioulpas A, Dimou D, Iconomou D, Aggelis G (2002) Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. *Bioresour Technol* 84:251–257
- Vasdev K, Kuhad RC (1994) Induction of laccase production in *Cyathus bulleri* under shaking and static culture conditions. *Folia Microbiol* 39:326–330. <https://doi.org/10.1007/BF02814322>
- Vastrad BM, Neelagund SE (2012) Optimization of process parameters for rifamycin b production under solid state fermentation from amycolatopsis mediterranean MTCC 14. *Int J Curr Pharm Res* 4(2):101–106

- Williams M, Kookana RS, Mehta A, Yadav SK, Tailor BL, Maheshwari B (2019) Emerging contaminants in a river receiving untreated wastewater from an Indian urban centre. *Sci Total Environ* 647:1256–1265. <https://doi.org/10.1016/j.scitotenv.2018.08.084>
- Xie H (2013) Production of a recombinant laccase from *Pichia pastoris* and biodegradation of chlorpyrifos in a laccase/vanillin system. *J Microbiol Biotechnol* 23:864–871. <https://doi.org/10.4014/jmb.1212.12057>
- Xu ZX, Wu Q, Duan Y et al (2016) Development of a novel spectrophotometric method based on diazotization-coupling reaction for determination of bisphenol-A. *J Braz Chem Soc* 28:1475–1482. <https://doi.org/10.21577/0103-5053.20160328>
- Yalçın MS, Geçgel C, Battal D (2016) Determination of bisphenol-A in thermal paper receipts. *J Turk Chem Soc Sect A Chem* 3:167–174. <https://doi.org/10.18596/jotcsa.21345>
- Yang S, Hai FI, Nghiem LD et al (2013) Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review. *Bioresour Technol* 141:97–108. <https://doi.org/10.1016/j.biortech.2013.01.173>
- Yang Y, Yang Y, Zhang J, Shao B, Yin J (2019) Assessment of bisphenol A alternatives in paper products from the Chinese market and their dermal exposure in the general population. *Environ Pollut* 244:238–246. <https://doi.org/10.1016/j.envpol.2018.10.049>
- Yao L, Lv Y-Z, Zhang L-J et al (2018) Determination of 24 personal care products in fish bile using hybrid solvent precipitation and dispersive solid phase extraction cleanup with ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. *J Chromatogr A* 1551:29–40. <https://doi.org/10.1016/j.chroma.2018.04.003>
- Yu H, Caldwell DJ, Suri RP (2019) In vitro estrogenic activity of representative endocrine disrupting chemicals mixtures at environmentally relevant concentrations. *Chemosphere* 215:396–403. <https://doi.org/10.1016/j.chemosphere.2018.10.067> (Epub 2018 Oct 11)
- Zeng S, Zhao J, Xia L (2017) Simultaneous production of laccase and degradation of bisphenol-A with *Trametes versicolor* cultivated on agricultural wastes. *Bioprocess Biosyst Eng* 40:1237–1245. <https://doi.org/10.1007/s00449-017-1783-1>
- Zhou Y, Cheng G, Chen K, Lu J, Lei J, Pu S (2019a) Adsorptive removal of bisphenol-A, chloroxylenol, and carbamazepine from water using a novel β -cyclodextrin polymer. *Ecotoxicol Environ Saf* 170:278–285. <https://doi.org/10.1016/j.ecoenv.2018.11.117>
- Zhou X, Yang Z, Luo Z, Li H, Chen G (2019b) Endocrine disrupting chemicals in wild freshwater fishes: species, tissues, sizes and human health risks. *Environ Pollut* 244:462–468. <https://doi.org/10.1016/j.envpol.2018.10.026>
- Zhu C, Bao G, Huang S (2016) Optimization of laccase production in the white-rot fungus *Pleurotus ostreatus* (ATCC 52857) induced through yeast extract and copper. *Biotechnol Biotechnol Equip* 30:270–276. <https://doi.org/10.1080/13102818.2015.1135081>