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The effect of operational parameters on the biodegradation of bisphenols by *Trametes versicolor* laccase immobilized on *Hippospongia communis* spongin scaffolds

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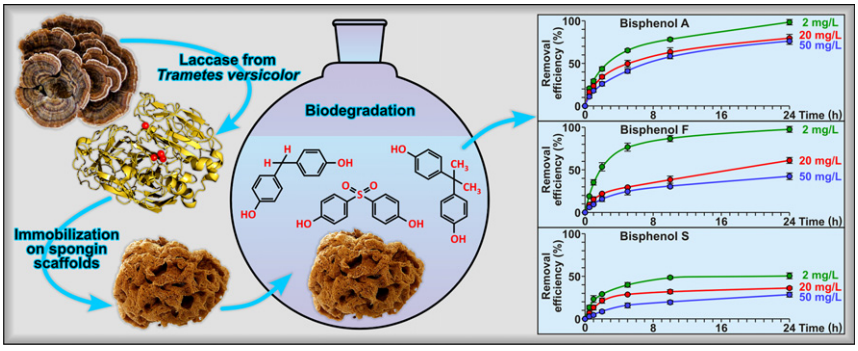
HIGHLIGHTS

- Application of *H. communis* scaffolds as effective support for laccase immobilization.
- Enhancement of the pH, temperature and storage stability of immobilized laccase.
- Evaluation of optimal biodegradation conditions for the highest removal efficiency.
- Formation of dimers and trimers as main products of bisphenols enzymatic oxidation.
- Degradation over 95% of bisphenol A and bisphenol F at pH 5 and temperature of 30 °C.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to the rapid growth in quantities of phenolic compounds in wastewater, the development of efficient and environmentally friendly methods for their removal becomes a necessity. Thus, in a presented work, for the first time, a novel material, *Hippospongia communis* spongin-based scaffold, was used as a biopolymeric support for the immobilization of laccase from *Trametes versicolor*. The resulting biocatalytic systems were used for the biodegradation of three bisphenols: bisphenol A (BPA), bisphenol F (BPF) and bioremoval-resistant bisphenol S (BPS). Optimization of the immobilization and biodegradation methodologies was performed to increase bisphenols removal. The effect of temperature, pH and initial pollutant concentration was evaluated. It was shown that under optimal conditions, almost 100% of BPA (pH 5, 30 °C) and BPF (pH 5, 40 °C), and over 40% of BPS (pH 4, 30 °C) was removed from the solution at a concentration of 2 mg/mL. Furthermore, the immobilized laccase exhibited good reusability and storage stability, retaining over 80% of its initial activity after 50 days of storage. In addition, the main biodegradation products of BPA and BPF were identified. It was shown that mainly dimers and trimers were formed following the oxidation of bisphenols by the immobilized laccase.

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1. Introduction

Bisphenols are a class of compounds that contain two phenol rings connected by a short spacer group. The most commonly known and widely used is bisphenol A (BPA), which is an intermediate product in the manufacture of polycarbonates, epoxy resins and flame retardants. There are many everyday products which may contain BPA, including compact discs, optical lenses, powder paints, thermal paper and food packaging (Staples et al., 1998). BPA can contaminate food or beverages by migrating from the container (Krishnan et al., 1993; EFSA, 2013). An even greater concern is the discovery that BPA is an endocrine disruptor. Endocrine disrupting compounds (EDCs) are substances or mixtures that can alter functions of the hormonal system and cause adverse effects in an intact organism, such as obesity, diabetes, cardiovascular diseases, breast cancer and reproductive disorder, and even exhibit genotoxic activity (Tiwari et al., 2012; Rochester, 2013; Rezg et al., 2014; Bilal et al., 2017a). Because of these properties, industry is beginning to look for substitutes for BPA in polymer production. Options include other bisphenols, such as bisphenol F (BPF) and bisphenol S (BPS), which are gradually replacing BPA (Eladak et al., 2015). Unfortunately, studies have shown that both BPF and BPS also have endocrine-disrupting and toxic properties (Chen et al., 2002; Eladak et al., 2015; Kitamura et al., 2005), although diverse results were presented by different authors. Chen et al. (2002) found BPF to have similar endocrine-disrupting activity to BPA, while BPS showed weak activity, and both BPF and BPS were less toxic than BPA. Eladak et al. (2015) reported that both BPF and BPS have anti-androgenic effects similar to those of BPA. On the other hand, Kitamura et al. (2005) showed BPA, BPF and BPS to have similar estrogenic activity, and BPF and BPS to have lower anti-androgenic activity than BPA.

Reported concentrations of BPA in river water vary between not detected and 68 ng/L in European countries (Kasprzyk-Hordern et al., 2008; Jonkers et al., 2009) and between 7 and 79 ng/L in Asia (Narasaki, 2002). Studies reported that BPA, alongside BPS and BPF, can be found not only in water and sediment (Song et al., 2012) but also in food and drinks (Grumetto et al., 2008; Liao and Kannan, 2013), in dust (Liao et al., 2012) and even in human urine samples (Yang et al., 2014).

The biodegradation of BPA in river water is rapid, with a half-life often under one day after a lag period of 2–7 days (Klecka et al., 2001), although in seawater its degradation generally takes slightly longer (Danzl et al., 2009). These studies showed that microorganisms capable of degrading BPA are present in the aquatic environment, thus BPA is not a persistent compound. BPA and BPF exhibit fairly good biodegradability in the environment, but BPS shows some problems. In aerobic and anaerobic conditions, BPF, which seems to be a better choice for the replacement of BPA, offers the best degradability properties (Ike et al., 2006).

Various enzymes, including laccase, tyrosinase, and manganese and horseradish peroxidase, are used for the biodegradation of hazardous compounds such as phenol and its derivatives, or synthetic and natural dyes (Bilal et al., 2016, 2017b, 2017c, 2017d). Among the enzymes, laccases (EC 1.10.3.2) are the most frequently applied. These oxygen oxidoreductases catalyze a wide range of chemical reactions, mainly by one-electron oxidation of mono-, di- and polyphenols (Bronikowski et al., 2017). As a multi copper oxidase, laccase contains four copper atoms in its structure, which exhibit different properties. A type-1 (T1) copper atom causes the blue color of the laccase, while a type-2 (T2) atom and two atoms of type 3 (T3) participate in oxidation reactions (Abdel-Hamind et al., 2013). These enzymes have found applications in the textile, food, fuel and medical industries (Mogharabi and Faramarzi, 2014; Zeeb et al., 2014; Kumar et al., 2017). However, it should be noted that laccase has ubiquitous properties enabling its use in many processes related to the biodegradation of environmental pollutants and their removal from soils and wastewaters (Yadav and Yadav, 2015; Le et al., 2016; Chatha et al., 2017). Laccase biocatalysts occur in bacteria, plants and insects (Singh et al., 2010; Geng et al., 2016), but fungal laccases deserve special attention due to their high adaptability (Cha et al., 2017).

In view of the potential increase in thermal stability and pH range, enabling the use of laccase in a wider range of conditions, in recent years increasing interest in laccase immobilization processes is observed [Jesionowski et al., 2014, Ba and Kumar, 2017]. There are many published studies that describe the immobilization of fungal laccase on different carriers (Cabana et al., 2007, 2009; Skoronski et al., 2017). For instance, Georgieva et al. (2010) described laccase immobilization on polypropylene membranes in non-isothermal conditions. This biocatalytic system was used in the degradation of phenol and its derivatives. Singh et al. (2010) studied γ-proteobacterium JB laccase and its immobilization on nitrocellulose membrane. Compounds such as syringaldazine, catechol, guaiacol and hydroquinone were degraded by the obtained biocatalytic system. In another study, Lin et al. (2016) proposed the immobilization of laccase on Cu(II) and Mn(II) chelated magnetic microspheres. The obtained system was used for the degradation of BPA, which resulted in the removal of over 85% of that compound. Furthermore, Aydemir and Guler (2015) described the synthesis of magnetic chitosan composite beads for the immobilization of laccase. The support with the immobilized enzyme removed phenol with an efficiency of 80%. Apart from the above examples, it should be noted that immobilization of laccases on biopolymeric supports is of increasing interest (Duran et al., 2002; Kues, 2015; Bilal et al., 2017e).

In this work, for the first time, spongin-based skeletons of *H. communis* marine sponges were used as a novel carrier for laccase immobilization. Spongin, as a collagen-like protein, is compatible with enzymes, but importantly it is more resistant to enzyme degradation than collagen. Moreover, marine sponge skeletons are renewable and do not require complicated preparation procedures or additional surface functionalization, due to their unique chemical and structural properties. Synthesized systems combine the beneficial properties of both substrates: the degradation activity of laccase and the three-dimensional architecture of the skeletons, which allows easy access of hazardous compounds to the active sites of the immobilized biocatalysts.

In the present study, the effect of various process parameters on the removal efficiency of BPA, BPF and the rarely studied BPS was determined. As an efficient biocatalytic system, laccase from *Trametes versicolor* immobilized on *H. communis* spongin scaffolds was used. Effective enzyme immobilization was confirmed by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The effect of the initial immobilization conditions and the quantity of the biocatalytic system on the degradation of bisphenols was tested. Moreover, the storage stability of the free and immobilized enzyme was examined. Finally, the effect of pH, temperature, initial solution concentration and number of biodegradation cycles on the efficiency of removal of BPA, BPF and BPS was evaluated and compared.

2. Materials and methods

2.1. Materials

Hippospongia communis sponges, farmed on the Mediterranean coast in Tunisia, were purchased from INTIB GmbH (Germany). Laccase from *Trametes versicolor* (EC 1.10.3.2), bisphenol A (BPA, >99%), bisphenol F (BPF, >99%), bisphenol S (BPS, >98%),

2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS, 99%), Coomassie Brilliant Blue and sodium azide were received from Sigma-Aldrich (USA). Ethanol, methanol, hydrochloric acid and orthophosphoric acid (all laboratory grade) were purchased from Chempur (Poland). Acetate and phosphate buffers with specific pH were supplied by Sigma-Aldrich (USA). HPLC-grade methanol was purchased from Avantor (Poland). HPLC-grade water was prepared by reverse osmosis in a Demiwa system from Watek (Czech Republic), followed by double distillation from a quartz apparatus.