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Elimination and detoxification of sulfathiazole and sulfamethoxazole assisted by laccase immobilized on porous silica beads

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ABSTRACT

This study assessed the immobilization of laccase on CPC silica beads and removal of two sulfonamides, sulfathiazole (STZ) and sulfamethoxazole (SMZ), using free and immobilized laccase. The results showed that free laccase (1 U ml^{-1}) removed 66% and 77% of SMZ and STZ, respectively, in the presence of 1-hydroxybenzotriazole (1 mM) as a laccase mediator after incubation at 40 °C for 1 h. Maximum enzymatic removal of both applied sulfonamides was achieved at a temperature of 50 °C and a pH of 5. At the elevated temperature of 70 °C, immobilized laccase removed 71.7% of STZ and 53% of SMZ. After ten cycles of removal experiments, 63.3% and 82.6% of the initial activity of the immobilized laccase toward SMZ and STZ remained, respectively. A lower K_m value for STZ (0.056 mM) compared to that of SMZ (0.096 mM) confirmed a higher affinity of immobilized laccase toward STZ. A microtoxicity study of the inhibition of bacterial growth showed a decrease in toxicity of the laccase-treated sulfonamide solution.

Introduction

Due to their low cost and broad spectrum of activity, sulfonamides are among the most prescribed antibacterial agents (Deck and Winston, 2012; Weng et al., 2012). Sulfamethoxazole (SMZ, found mostly in combination with trimethoprim as co-trimoxazole) and sulfathiazole (STZ, commonly used in combination with sulfacetamide and sulfathiazole under the name of Sultrin) are two important sulfa drugs applied in the treatment of gastrointestinal and urogenital infections, respectively (Rybak and Aeschlimann, 2005). In addition, the ability of some sulfonamides, such as sulfasalazine and mesalamine, to reduce the synthesis of inflammatory mediators makes them the first choice in treatment of chronic diseases such as inflammatory bowel disease (IBD) and ulcerative colitis (UC) (Deck and Winston, 2012). The extensive use of sulfonamides in human and veterinary medicine and, as a result, the excretion of parent compounds or metabolites of sulfonamides in human and animal urine and feces, together with the discharging of sulfonamides from pharmaceutical manufacturing wastewaters, make this issue an environmental concern (Sukul and Spiteller, 2006). Releasing antibacterial agents into the environment can increase the risk of development of resistant bacteria (Weng et al., 2013). The degradation of pharmaceuticals and personal care products (PCPs) using either physicochemical means (such as reverse osmosis, electrochemical oxidation, and carbon adsorption) (Yang et al., 2008) or biological ones (using various microorganisms and/or their purified enzymes) (Garcia-Galan et al., 2011; Ostadhadi-Dehkordi et al., 2012; Zhang et al., 2012, 2013a,b) has been evaluated in many studies. The remarkable advantages of enzymatic removal methods, such as higher efficiency and lower toxicity of produced metabolites, have encouraged researchers to apply this eco-friendly method (Ashrafi et al., 2013; Mirzadeh et al., 2014).

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multi-copper-containing oxidases produced mainly by fungal (especially white-rot basidiomycetes) and bacterial strains (Forootanfar et al., 2011; Aghaie-Khouzani et al., 2012; Heidary et al., 2014; Mogharabi and Faramarzi, 2014). The ability of laccases

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to oxidize a broad range of organic pollutants (such as chlorinated phenol and polycyclic aromatic hydrocarbons [PAHs]) (Forootanfar et al., 2012; Dehghanifard et al., 2013) and synthetic dyes (Gholami-Borujeni et al., 2011; Ashrafi et al., 2013; Mirzadeh et al., 2014) as well as some pharmaceutical agents (such as diclofenac, naproxen, ketoprofen, oseltamivir, sulfonamides, erythromycin, and estrogenic hormones) has been well-documented (Lloret et al., 2010, 2013; Rodríguez-Rodríguez et al., 2012; Sathishkumar et al., 2012; Suda et al., 2012). However, application of these eco-friendly biocatalysts for elimination of xenobiotics is limited by two main bottlenecks: low stability and low production yield (Sathishkumar et al., 2012). Such constraints could be overcome by the development of immobilized laccases on solid supports, which enables the reusing of immobilized enzymes and, as a result, reduces the overall cost of enzymatic elimination in addition to the improvement of enzyme stability (Fernandez-Fernandez et al., 2013; Sadighi and Faramarzi, 2013). Among different techniques that have been applied for the immobilization of biocatalysts, covalent binding supplies significantly more stable enzymes due to increasing the rigidity of enzyme structure and reducing protein unfolding (Fernandez-Fernandez et al., 2013). Improvement of thermal and operational stabilities of laccases after immobilization on silica-based supports has been confirmed by recent studies (Champagne and Ramsay, 2010; Dehghanifard et al., 2013).

The present study was designed to immobilize laccase on silica beads; this is followed by the application of free and immobilized laccase for elimination of two sulfonamides, sulfamethoxazole and sulfathiazole, and evaluation of the effect of temperature, pH, and presence of a mediator (1-hydroxybenzotriazole) on laccase-assisted removal of sulfonamides. The enzyme kinetic parameters (K_m and V_{max}) on the applied sulfonamides were also calculated. In addition, the toxicity of laccase-treated samples was evaluated using microtoxicity studies.

Materials and methods

Chemicals

The purified laccase of *Trametes versicolor* (activity of 10 U mg^{-1}), pre-silanized (3-aminopropyltriethoxysilane [APTES]) CPC silica beads, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1-hydroxybenzotriazole (HBT), and a glutaraldehyde solution were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfamethoxazole and sulfathiazole (Table 1) were kindly donated by Tehran Daru and Sina Daru (pharmaceutical companies in Tehran, Iran), respectively. All other reagents and chemicals were of the highest purity available.

Laccase immobilization

Immobilization of laccase on CPC silica beads was performed based on the method described by Champagne and Ramsay (2010) and Mirzadeh et al. (2014). In brief, CPC silica beads (5 mg) were added to 1 ml of 2.5% glutaraldehyde solution (previously degassed under 2.0 bar vacuum pressure for 2 h) and stirred for 60 min, followed by washing the silica beads using a citrate buffer (0.1 M, pH 5) and drying in a vacuum oven at room temperature. The activated silica beads were then mixed with the laccase solution (prepared in citrate buffer 0.1 M, pH 5) and incubated at 25 °C for 120 min under low agitation. The immobilized enzyme was then washed with citrate buffer (0.1 M, pH 5) three times and stored at 4 °C before being used.

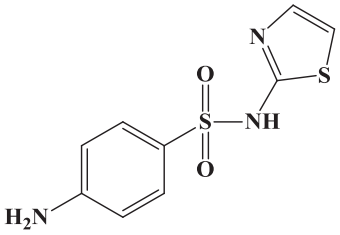
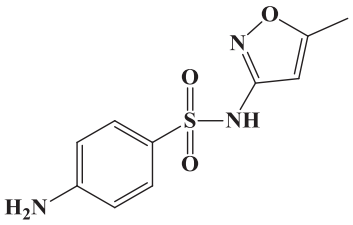
Temperature, pH, and storage stability of free and immobilized laccase

The stability of the free or immobilized laccase (mixed in 0.1 M of citrate buffer pH 5) at different temperatures was investigated by preincubation of free or immobilized enzymes at a temperature range of 20–80 °C for 2 h, followed by measuring the residual enzyme activity. The effect of pH on enzyme stability was determined after introducing free or immobilized enzymes into 50 mM of citrate–phosphate buffer (pH 2–7) or Tris–HCl buffer (50 mM, pH 8–9), and incubation for 1 h at 40 °C, followed by measuring the residual enzyme activity. Storage stability was evaluated by incubating free or immobilized laccase (in citrate buffer 0.1 M, pH 5) for 14 days at 25 °C and analysis of residual activity in samples.

Determination of laccase activity

Oxidation of ABTS as a laccase substrate was used in order to determine the laccase activity of the free or immobilized laccase

Table 1
Chemical structure and kinetic parameters of STZ and SMZ in presence of free and immobilized laccase.

Substrate	Structure	K_m (mM)	V_{max} (mM min^{-1} mg^{-1})
Sulfathiazole		0.056[a] 0.082[b]	240[a] 190[b]
Sulfamethoxazole		0.096[a] 0.16[b]	67.3[a] 47.2[b]

[a] Calculated K_m and V_{max} in presence of immobilized laccase.
[b] Calculated K_m and V_{max} in presence of free laccase.