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Integration of an enzymatic bioreactor with membrane distillation for enhanced biodegradation of trace organic contaminants

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ABSTRACT

A novel membrane distillation – enzymatic membrane bioreactor (MD-EMBR) system was developed for efficient degradation of trace organic contaminants (TrOCs). Degradation of five TrOCs, namely carbamazepine, oxybenzone, diclofenac, atrazine and sulfamethoxazole was examined using two commercially available laccases (from *Trametes versicolor* and *Aspergillus oryzae*). The MD system ensured complete retention (>99%) of both enzyme and TrOCs. Of particular interest was that the complete retention of the TrOCs resulted in high TrOC degradation by both laccases. Oxybenzone and diclofenac degradation in the MD-EMBR ranged between 80 and 99%. Compared to previously developed EMBRs, as much as 40% improvement in the removal of resistant non-phenolic TrOCs (*e.g.,* carbamazepine) was observed. Laccase from *A. oryzae* demonstrated better TrOC degradation and enzymatic stability. With the addition of redox mediators, namely 1-hydroxybenzotriazole (HBT) or violuric acid (VA), TrOC degradation was improved by 10–20%. This is the first demonstration of a laccase-based high retention membrane bioreactor for enhanced biodegradation of TrOCs.

1. Introduction

Trace organic contaminants (TrOCs) include a wide array of natural or anthropogenic chemicals including pesticides, pharmaceuticals and personal care products. Recent studies have confirmed the potentially harmful effects of TrOCs on the growth and reproduction patterns of aquatic flora and fauna and also on human health due to prolonged ingestion (Flint et al., 2012; Gavrilescu et al., 2015). Conventional wastewater treatment processes cannot effectively remove certain groups of TrOCs, resulting in their widespread occurrence in freshwater sources (Deblonde et al., 2011; Luo et al., 2014). Therefore, the scientific community is in constant pursuit of an effective wastewater treatment process for TrOC removal.

Different physicochemical and biological wastewater treatment processes have been investigated over the years for TrOC removal (Gao et al., 2012; Hai et al., 2014; Navaratna et al., 2017; Silva et al., 2012). TrOC degradation by biocatalysts such as laccase, peroxidase and proteases is a promising eco-friendly technique (Demarche et al., 2012; Hai et al., 2013). Enzymatic transformation of TrOCs is governed by a number of factors such as pH, temperature, TrOC properties and characteristics of enzymes (Yang et al., 2013). Laccase is an oxidase enzyme that can degrade a broad spectrum of TrOCs over a wide range of pH by utilizing the dissolved oxygen in water (Chea et al., 2012; Nguyen et al., 2015). Particularly mention worthy is the ability of laccase to oxidize the phenolic TrOCs including aromatic/aliphatic amines, diphenols and methoxy-substituted monophenols (Yang et al., 2013). Molecular structure, namely distribution of the functional groups, *i.e.*, electron withdrawing group (EWG) and electron donating groups (EDG), governs the extent of TrOC removal by laccase. The oxidation of TrOCs containing EWGs such as amide, halogen and nitro groups is slower as compared to those containing EDGs (Asif et al., 2017; Yang et al., 2013). TrOC oxidation can be enhanced by introducing a redox mediator, which can act as an electron shuttle between the target compounds and enzyme. Depending on the type of mediator and TrOC structure, laccase-mediator systems can achieve significant improvement in the removal of target compounds (Ashe et al., 2016; Nguyen et al., 2014a).

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Enzyme washout is a major constraint in the large scale application of an enzymatic bioreactor. To mitigate this problem, laccase can be immobilized onto or entrapped within different supports (Ba et al., 2013; Yang et al., 2013). Alternatively, enzymatic bioreactor can be coupled with a membrane having a suitable molecular cutoff. For example, Nguyen et al. (2015) and Lloret et al. (2012) achieved complete retention of laccase with ultrafiltration (UF) membranes. The use of enzymatic membrane bioreactor (EMBR) can avoid the mass transfer limitations associated with laccase immobilization onto support media. Although TrOCs are not expected to be retained by UF membranes, Nguyen et al. (2015) observed the formation of an enzyme gel layer on the surface of the membrane that effectively adsorbed non-phenolic hydrophobic TrOCs such as octocrylene, amitriptyline and benzophenone. This resulted in enhanced degradation of these compounds. However, enzyme gel layer could not adsorb hydrophilic non-phenolic TrOCs such as atrazine and carbamazepine, and their overall removal was less than 10% (Nguyen et al., 2015). Hence, it was postulated that the use of high retention membranes, which will retain both laccase and TrOCs, can facilitate the degradation of resistant TrOCs.

In recent years, high retention membranes, namely membrane distillation (Wijekoon et al., 2014b), nanofiltration (Phan et al., 2016) and forward osmosis (Alturki et al., 2012; Holloway et al., 2014; Luo et al., 2015), have been integrated with the conventional activated sludge bioreactors to achieve complete TrOC retention, resulting in their high aqueous phase removal. However, these short term studies have revealed accumulation of membrane-retained recalcitrant compounds in the bioreactor, indicating the need for enhancement of biodegradation. Although laccase has been reported to achieve better biodegradation than conventional activated sludge, no study has explored a high retention membrane - enzymatic bioreactor.

In this study, a laccase-based high retention membrane bioreactor was investigated for the first time to achieve enhanced degradation of five hardly degradable TrOCs. For this, a membrane distillation system was integrated with an enzymatic bioreactor (MD-EMBR). A series of experiments were performed to elucidate the performance of two commercially available laccases – one from genetically modified *Aspergillus oryzae* (*A. oryzae*) and the other from *Trametes versicolor* (*T. versicolor*). Impacts of two N=OH type redox mediators, namely 1-hydrozybenzotriazole (HBT) and violuric acid (VA) on TrOC degradation as well as on enzyme stability were also studied.

2. Materials and methods

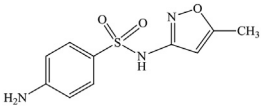
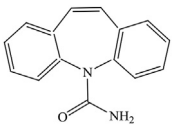
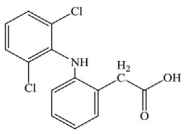
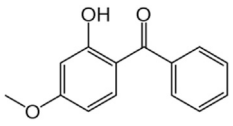
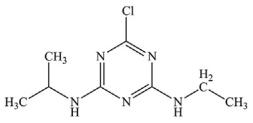
2.1. Trace organic contaminants

Four pharmaceutical and personal care products, namely sulfamethoxazole, carbamazepine, diclofenac and oxybenzone, and one pesticide (atrazine) were selected for this study due to their widespread occurrence in environmental systems (Luo et al., 2014). Analytical grade (>98% purity) standards of these TrOCs were purchased from Sigma–Aldrich (Australia). The physicochemical properties including molecular weight, chemical structure, hydrophobicity (log D) and volatility (pK_H) of the tested TrOCs are given in Table 1. A stock solution (2 g/L) of these compounds was prepared and stored at −18 °C in the dark.

2.2. Enzyme solutions and mediators

Commercially available laccase purified from *T. versicolor* (CAS No. 80498-15-3) purchased from Sigma–Aldrich (Australia) was used in this study. Laccase from genetically modified *A. oryzae* (Novozym 51030) was the second source of laccase used in this study, and it was supplied by Novozymes Pty. Ltd, Australia. These

Table 1
Physicochemical properties of selected TrOC.

Compound	Molecular structure	Molecular weight (g/mol)	Log D at pH 7	Vapor pressure (mmHg)	Water solubility at 25 °C (mg/L)	H (atm m^3/mol)	pK_H at pH 7
Sulfamethoxazole		253.28	−0.22	1.87×10^{-09}	410	1.52×10^{-12}	11.81
Carbamazepine		236.27	1.89	5.78×10^{-07}	220	8.17×10^{-10}	9.08
Diclofenac		296.15	1.77	1.59×10^{-07}	30	2.06×10^{-09}	8.68
Oxybenzone		228.24	3.99	5.26×10^{-06}	100	1.58×10^{-08}	7.80
Atrazine		215.68	2.64	1.27×10^{-05}	69	5.22×10^{-08}	7.28

Note: Henry's law constant (H) at 25 °C (atm m^3/mol) = Vapor pressure × molecular weight/water solubility. The pK_H value is defined as $pK_H = -\log_{10}H$. Chemical structure, molecular weight (MW), log D, vapor pressure and water solubility values were taken from SciFinder Scholar.