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Degradation of a broad spectrum of trace organic contaminants by an enzymatic membrane reactor: Complementary role of membrane retention and enzymatic degradation

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ABSTRACT

Laccase-catalysed degradation of 30 trace organic contaminants (TrOCs) with diverse chemical structure was investigated in an enzymatic membrane reactor (EMR) equipped with an ultrafiltration membrane. Compared to the results from batch incubation tests, the EMR could facilitate degradation of some phenolic and a number of non-phenolic TrOCs. Laccase, which was completely retained by the membrane, formed a dynamic gel layer on the membrane surface onto which TrOCs were adsorbed. EMR investigations with active and heat-inactivated laccase confirmed that the TrOCs retained by the active laccase gel layer were eventually degraded. Redox-mediator addition to the EMR significantly extended the spectrum of efficiently degraded TrOCs, but a limited improvement was observed in batch tests. The results demonstrate the important role of TrOC retention by the enzyme gel layer dynamically formed on the membrane in achieving improved degradation of TrOCs by the mediator-assisted laccase system. Despite following the same hydrogen atom transfer pathway, the mediators tested (syringaldehyde and 1-hydroxybenzotriazole) exhibited TrOC-specific degradation improvement capacity.

Introduction

Trace organic contaminants (TrOCs) are ubiquitous in wastewater, and water sources polluted by wastewater. These TrOCs include, but are not limited to, pharmaceutically active compounds, industrial chemicals, pesticides, and natural and artificial hormones. Some TrOCs have been observed to bring about detrimental physiological changes in aquatic fauna. Under prolonged exposure, TrOCs may also affect human health (Schwarzenbach et al., 2006). Conventional wastewater treatment plants do not effectively remove TrOCs (Luo et al., 2014). Thus for both safe discharge into the environment and wastewater reuse it is important to develop effective wastewater treatment processes.

Enzymatic degradation of wastewater-borne resistant pollutants has gained much attention in recent years. Compared to conventional chemical oxidation, enzymatic degradation can be achieved under milder conditions, while realizing higher rates and reaction specificity. Laccases (EC 1.10.3.2) are copper-containing oxidoreductase enzymes that can use atmospheric oxygen as the terminal oxidant. Laccase has been used in various industrial biotechnology processes such as denim bleaching and pulp delignification. It has also been reported to efficiently degrade resistant compounds including aromatic hydrocarbons and dyes (Modin et al., 2014). Recent studies demonstrate that laccase can efficiently degrade a broad spectrum of TrOCs that are hardly degradable by conventional biological processes (Cabana et al., 2007; Yang et al., 2013). Notably, most of the available studies on enzymatic degradation of resistant compounds in general and TrOCs in particular have been conducted in small scale and batch mode.

Enzymatic TrOC degradation may depend on various factors such as chemical structure of the TrOCs, chemistry of the reaction

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media (*i.e.*, pH, temperature and ionic strength), and the characteristics of the enzyme applied (Yang et al., 2013). Laccase can efficiently degrade compounds with phenolic moeity including diphenols, methoxy-substituted monophenols as well as aromatic/aliphatic amines. For compounds which possess higher redox potential than laccase, or are too large to gain access to the active sites of the enzyme, addition of redox-mediators may facilitate their oxidation. Mediators are low-molecular weight substrates of laccase which can act as "electron carrier" between the enzyme and the target pollutant. However, the efficiency of a laccase—mediator system depends largely on mediator type and the molecular structure of TrOCs (Yang et al., 2013).

Enzyme-washout with treated effluent is a critical problem encountered during their application in continuous systems such as wastewater treatment plants. By using a membrane with an appropriate pore size relative to the enzyme molecule, an enzymatic membrane reactor (EMR) can prevent enzyme washout. This approach offers several advantages over other alternatives: (i) EMR retains enzymes more effectively than conventional packed bed reactors, (ii) operation with free enzyme avoids limitation of mass transfer associated with immobilization on carriers, and (iii) enzyme can be easily replenished during long term operation (Modin et al., 2014). To date, only a few studies have explored continuous biotransformation of TrOCs by EMR (Lloret et al., 2012; Nguyen et al., 2014a,b). Lloret et al. (2012) reported high removal of phenolic TrOCs, namely estradiol and estrone; however, that study was conducted for only 8 h. High and stable biotransformation of both bisphenol A and diclofenac by an EMR was demonstrated by Nguyen et al. (2014a). Nguyen et al. (2014b) investigated the removal of four non-phenolic compounds, namely diclofenac, carbamazepine, sulfamethoxazole, and atrazine, and proposed simultaneous dosing of mediator and activated carbon to enhance their removal efficiencies.

Most, if not all, studies to date on TrOC removal by EMR have focused on a few compounds at a time. For the establishment of a uniform database regarding EMR performance, investigation of a broader spectrum of TrOCs is imperative. Another aspect that requires further systematic investigation is the role of the membrane in enzymatic degradation of TrOCs in an EMR. Ultrafiltration membranes typically used in EMRs cannot retain TrOCs. However, enzyme gel layer, which typically forms on membrane surface, may adsorb the TrOCs. Thus the membrane may facilitate enzymatic degradation of TrOCs. A few studies have alluded to this aspect (Nguyen et al., 2014a,b), however, any systematic study elucidating the phenomena involved, particularly the extent of adsorption and biodegradation during prolonged operation, has not been reported.

The objective of this study was to assess the performance of an EMR utilizing a commercially available laccase from *Aspergillus oryzae* for the removal of 30 chemically diverse TrOCs (*e.g.*, phenolic/non-phenolic moieties and electron releasing/demanding substituent groups). Baseline batch tests provided valuable insight into the EMR performance. The effect of addition of redox-mediators, namely 1-hydroxybenzotriazole (HBT) or syringaldehyde (SA), on the enzymatic TrOC degradation was highlighted. Particularly, the complementary role of TrOC retention by the gel layer on the membrane and their enzymatic degradation was systematically elucidated.

Materials and methods

TrOCs, laccase and mediators

A synthetic wastewater containing a mixture of 30 TrOCs in Milli-Q water was prepared for this study. These compounds were selected due to their ubiquitous presence in wastewater and contaminated water bodies, and their range of chemical properties, *e.g.*, phenolic/non-phenolic moieties and electron releasing/demanding substituent groups. Relevant physicochemical properties of these TrOCs appear in Supplementary data Table S1. The TrOCs investigated were also chosen to represent various common classes of TrOCs, namely pharmaceutical and personal care products, industrial chemicals, steroid hormones and phytoestrogens, and pesticides. In the batch tests, each TrOC concentration was 100 μg/L, while the synthetic wastewater fed to the EMR contained each of the compounds at a concentration of 5 μg/L, except for one run which was conducted at a TrOC concentration of 100 μg/L.

Laccase, purified from genetically modified *A. oryzae*, was obtained from Novozymes Australia Pty Ltd. According to the supplier, the molecular weight of this laccase is 56 kDa. It has a purity of approximately 10% (w/w), density of 1.12 g/mL, and activity (measured using 2,6-dimethoxy phenol, DMP, as substrate) of 150,000 $\mu M_{(DMP)}$/min. Laccase catalysed degradation of a target compound ('substrate') depends on the relative oxidation reduction potential (ORP) of that compound and laccase (Xu et al., 2000). The laccase used in this study had an ORP of 0.27 mV.

The mediators selected for this study *i.e.*, syringaldehyde (SA) and 1-hydroxybenzotriazole (HBT), have been well characterized in the literature (Xu et al., 2000). SA is a small molecular weight phenolic compound, while HBT contains the structural group =N–OH. Both of these mediators work on hydrogen atom transfer mechanism. Oxidation of SA and HBT by laccase produces highly reactive radicals phenoxyl and aminoxyl, respectively.

TrOCs and mediators were obtained from Sigma–Aldrich (Australia). TrOC stock solution (1 g/L each) was prepared in pure methanol, and stored at −18 °C. The solution was used within a month. Separate stock solutions (50 mM) of SA and HBT were prepared in Milli-Q water and stored at 4 °C.

Batch tests

The test solution contained an enzymatic activity of *ca.* 180 $\mu M_{(DMP)}$/min (dilution of 30 μL stock laccase solution by Milli-Q water to 25 mL). An aliquot of the stock TrOC cocktail was added to the test solution to obtain an initial nominal concentration of 100 μg/L of each TrOCs. The impact of redox mediator addition on laccase degradation of TrOCs was assessed by adding SA or HBT (10 μM) to the test solution. The mediator concentration was selected following a baseline investigation with bisphenol A and diclofenac (Nguyen et al., 2014a). TrOCs in Milli-Q water (without laccase) served as control. The pH of the test solution was 6.8 ± 0.2. All the containers were covered and incubated at 25 °C for 24 h in a rotary shaker (70 rpm). The experiments were conducted in triplicate. The whole test solution was collected for TrOC analysis at the end of the incubation period. The samples were diluted to 500 mL, filtered through 0.45 μm glass fiber filter, and pH immediately adjusted to 2 by adding H_2SO_4 (4 M).

EMR system and operation protocol

EMR setup

The EMR system comprised a 1.5 L (active volume) glass reactor (Supplementary data Fig. S2). A hollow fiber membrane module (Microza Membranes, Pall Corporation, NSW, Australia) having a surface area of 0.19 m^2 was used in the submerged configuration. The membrane was made of polyacrylonitrile. It was an ultrafiltration (UF) membrane having a molecular weight cut off of 6 kDa. The temperature of the reactor was maintained at 28 °C by placing it in a water bath equipped with a temperature controller (Heating immersion circulator, Julabo, Germany). A dissolved oxygen (DO) concentration of 3 mg/L was maintained in the EMR via air bubbling