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Biocatalytic degradation of pharmaceuticals, personal care products, industrial chemicals, steroid hormones and pesticides in a membrane distillation-enzymatic bioreactor

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ABSTRACT

Laccase-catalyzed degradation of a broad spectrum of trace organic contaminants (TrOCs) by a membrane distillation (MD)-enzymatic membrane bioreactor (EMBR) was investigated. The MD component effectively retained TrOCs (94–99%) in the EMBR, facilitating their continuous biocatalytic degradation. Notably, the extent of TrOC degradation was strongly influenced by their molecular properties. A significant degradation (above 90%) of TrOCs containing strong electron donating functional groups (*e.g.,* hydroxyl and amine groups) was achieved, while a moderate removal was observed for TrOCs containing electron withdrawing functional groups (*e.g.,* amide and halogen groups). Separate addition of two redox-mediators, namely syringaldehyde and violuric acid, further improved TrOC degradation by laccase. However, a mixture of both showed a reduced performance for a few pharmaceuticals such as primidone, carbamazepine and ibuprofen. Mediator addition increased the toxicity of the media in the enzymatic bioreactor, but the membrane permeate (*i.e.*, final effluent) was non-toxic, suggesting an added advantage of coupling MD with EMBR.

1. Introduction

Membrane distillation (MD) is a low temperature distillation process in contrast to conventional distillation processes such as fractional or steam distillation. It essentially relies on the transport of water in the vapor phase from a feed solution through a microporous hydrophobic membrane to the permeate or distillate. Among different MD configurations, direct contact membrane distillation (DCMD) has been predominantly studied due to the ease of its operation (Alkhudhiri et al., 2012; Curcio and Drioli, 2005). In DCMD, the temperature of the feed solution is maintained at 15–20 °C higher than the permeate to create an adequate vapor pressure difference, which allows water to pass through a microporous membrane in vapor form *via* diffusion (Alkhudhiri et al., 2012; Duong et al., 2017). Since mass transfer occurs in gaseous phase, MD can theoretically achieve complete rejection of all non-volatile compounds (Martinetti et al., 2009; Wijekoon et al., 2014a).

Due to efficient separation efficiency, low fouling propensity and potentially low energy requirement (subject to the availability of low grade heat), stand-alone MD has been studied for applications such as protein recovery in dairy processing (Hausmann et al., 2013), treatment of industrial (Khaing et al., 2010) and municipal wastewater (Phattaranawik et al., 2008; Wijekoon et al., 2014b), as well as for the removal of trace organic contaminant (TrOCs), such as pharmaceuticals and personal care products, pesticides and industrial chemicals, from wastewater (Darowna et al., 2014; Wijekoon et al., 2014a). Recently, TrOC removal has also been investigated by coupling an activated sludge based bioreactor to MD that achieved excellent (95–99%) TrOC retention (Wijekoon et al., 2014b). Since effective retention of TrOCs by the MD theoretically decouples organic retention time from hydraulic retention time of a bioreactor, the degradation of TrOCs is expected to improve due to prolonged contact time between the recalcitrant compounds and the microorganisms (Luo et al., 2014a). However, it was found that the biodegradation of resistant TrOCs, such as those containing strong electron withdrawing functional groups (EWGs), by the activated sludge in the MD-coupled bioreactor did not improve, and eventually these TrOCs accumulated in the bioreactor (Hai et al., 2014; Wijekoon et al., 2014b). Hence, to realize the full potential of a

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combined biological – MD process, it is necessary to find the means to improve biodegradation of TrOCs retained in the bioreactor by the MD membrane. In this context, it is noteworthy that the oxidoreductase enzyme laccase (EC 1.10.3.2) can degrade TrOCs that are less susceptible to degradation by the activated sludge process (Cruz-Morató et al., 2014; Yang et al., 2013).

Laccase can catalyze the degradation of a broad spectrum of pollutants including aromatic hydrocarbons, aliphatic amines and TrOCs by using dissolved oxygen as a co-substrate (Asif et al., 2017b; Hai et al., 2007; Yang et al., 2013). However, its larger scale application is restricted by the lack of a reactor system, which can prevent washout of enzymes along with treated effluent. In a recent study, Asif et al. (2017c) combined an enzymatic bioreactor with the MD (MD – Enzymatic membrane bioreactor or MD-EMBR), which retained both laccase and the tested TrOCs (carbamazepine, sulfamethoxazole, diclofenac, atrazine and oxybenzone). During a short term (12 h) batch operation of the MD-EMBR (Asif et al., 2017c), degradation of the investigated TrOCs by laccase was found to improve significantly compared to that achieved by an activated sludge-based MD bioreactor (Wijekoon et al., 2014b). Furthermore, TrOC degradation by the MD-EMBR was better than those achieved by previously developed ultrafiltration (UF)-EMBRs, which retained laccase but not TrOCs (Asif et al., 2017c; Nguyen et al., 2016). Apparently, the effective retention of the TrOCs by the MD membrane also improved their biodegradation. The initial observations were promising but it is necessary to assess the performance of MD-EMBR for a wide range of TrOCs during continuous operation.

This study aims to evaluate the performance of the MD-EMBR system for the degradation of a set of 30 TrOCs with diverse physicochemical properties following their effective retention by the MD membrane. Redox mediators, which are low molecular weight substrates of laccase, can enhance enzymatic degradation (Yang et al., 2013). Thus, additionally, the effect of dosing two redox-mediators *viz* violuric acid (VA) and syringaldehyde (SA), separately and as a mixture, on TrOC degradation and laccase stability was investigated. Redox mediators can improve degradation but may increase the toxicity of the treated effluent (Ashe et al., 2016; Nguyen et al., 2016), therefore, the toxicity of the bioreactor media and MD permeate (*i.e.*, final effluent) to bacteria was monitored to clarify the applicability of this treatment process. Finally, during continuous operation, TrOC retention by MD can decrease over time due to 'membrane wetting' or loss of hydrophobicity (Alkhudhiri et al., 2012; Duong et al., 2017). Accordingly, the effect of laccase and redox-mediators on the MD performance was also investigated.

2. Materials and methods

2.1. 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 to represent different common classes of TrOCs, *viz* pharmaceutical and personal care products, industrial chemicals, steroid hormones and pesticides, which are commonly detected in different environmental systems (Luo et al., 2014b). A complete list along with their chemical structures appears in Supplementary data. Relevant physicochemical properties of the selected TrOCs such as hydrophobicity (log D) and volatility (pK_H) are given in Table 1. Analytical grade TrOCs were purchased from Sigma Aldrich (Australia). A stock solution (25 mg/L) containing the mixture of 30 TrOCs was prepared in methanol, and kept in dark at −18 °C prior to use.

Laccase from genetically modified *Aspergillus oryzae* (Novozymes Australia Pty Ltd.) was used in this study. 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. One of the main factors governing the laccase-catalyzed degradation of a substrate is the relative oxidation reduction potential (ORP) of that substrate and laccase (Yang et al., 2013). In this study, the ORP of the laccase, measured using an ORP meter (see Section 2.4.2), was 0.3 V.

Two analytical grade natural redox-mediators, namely violuric acid (VA) and syringaldehyde (SA) (Sigma Aldrich, Australia), were used. A separate stock solution (50 mM) of each mediator was prepared in ultrapure Milli-Q water, and stored at 4 °C in the dark. SA and VA produce highly reactive phenoxyl and aminoxyl radicals, respectively. They can mediate TrOC degradation by following a hydrogen atom transfer pathway (Ashe et al., 2016; Asif et al., 2017b). The physicochemical properties of redox-mediators are presented in Supplementary data.

2.2. The MD-EMBR system

A laboratory scale MD-EMBR system was used comprising a glass enzymatic bioreactor (1.5 L) and an external direct contact membrane distillation (DCMD) module. A schematic of the setup is available in Supplementary data. The enzymatic bioreactor was covered with aluminum foil and was placed in a water bath maintained at 30 ± 0.2 °C using an immersion heating unit (Julabo, Germany). The enzymatic bioreactor was equipped with an air pump (ACO-002, Zhejiang Sensen Industry Co. Ltd., Zhejiang, China) to maintain the dissolved oxygen concentration at around 3 mg/L.

The external DCMD module contained an acrylic glass membrane cell, two circulation pumps (Micropump Inc., USA) and a glass permeate tank. Feed and permeate flow channels were engraved on each block of the membrane cell. The length, width and height of each flow channel were 145, 95 and 3 mm, respectively. The media from the glass enzymatic bioreactor and water from the permeate tank were passed through the membrane cell and then returned back to the enzymatic bioreactor and permeate tank, respectively. A chiller (SC100-A10, Thermo Scientific, USA) was used to regulate the temperature of the permeate tank at 10 ± 0.1 °C. The permeate tank was also placed on a precision balance (Mettler Toledo Inc, USA) to monitor permeate flux. The recirculation flow rate of both feed and the distillate was controlled at 1 L/min (corresponding to a cross flow velocity of 9 cm/s) using two rotameters.

A hydrophobic microporous flat-sheet polytetrafluoroethylene (PTFE) membrane (GE, Minnetonka, MN) was used in this study. The PTFE membrane had a nominal pore size of 0.2 μm, thickness of 175 μm, porosity of 70% and an active layer thickness of 5 μm (Nghiem and Cath, 2011).

2.3. Experimental protocol

After confirming the retention of laccase by the MD membrane, a series of experiments were conducted to investigate TrOC retention (by MD membrane) and enzymatic degradation with and without the addition of mediators (*i.e.,* SA and VA). The initial laccase activity and TrOC concentration in the enzymatic bioreactor of MD-EMBR was 95–100 $\mu M_{(DMP)}$/min and 20 μg/L, respectively. It is noteworthy that laccase activity in the enzymatic bioreactors may gradually diminish due to various physicochemical and biological inhibitors such as shear stress caused by membrane filtration (Asif et al., 2017a). Hence, the laccase activity was maintained at 95–100 $\mu M_{(DMP)}$/min by injecting a small dose of laccase (275 and 400 μL per liter of reactor volume for laccase and laccase-mediator, respectively) every 12 h to sustain MD-EMBR operation.

The MD-EMBR was first operated for a period of 60 h (*i.e.*, 2 × HRT) in a continuous mode (*i.e.*, continuous withdrawal of treated effluent) without the addition of mediators. The enzymatic bioreactor was replenished with synthetic wastewater every time the water recovery reached 70% (*i.e.,* approximately around every 24 h). Feed, bioreactor supernatant and treated effluent (*i.e.*, MD-permeate) samples were collected after 30 and 60 h of MD-EMBR operation for TrOC