# Impact of simultaneous retention of micropollutants and laccase on micropollutant degradation in enzymatic membrane bioreactor

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## ARTICLE INFO

## ABSTRACT

This study systematically compares the performance of ultrafiltration (UF) and nanofiltration (NF) based enzymatic membrane bioreactors (EMBRs) for the degradation of five micropollutants, namely atrazine, carbamazepine, sulfamethoxazole, diclofenac and oxybenzone to elucidate the impact of effective membrane retention of micropollutants on their degradation. Based on the permeate quality, NF-EMBR achieved 92–99.9% micropollutant removal (i.e., biodegradation + membrane retention), while the removal of these micropollutants by UF-EMBR varied from 20 to 85%. Mass balance analysis revealed that micropollutant degradation was improved by 15–30% in NF-EMBR as compared to UF-EMBR, which could be attributed to the prolonged contact time between laccase and micropollutants following their effective retention by the NF membrane. A small decline in permeate flux was observed during EMBR operation. However, the flux could be recovered by flushing the membrane with permeate.

## 1. Introduction

A wide range of micropollutants, e.g., pharmaceuticals, personal care products and pesticides, are detected in natural water bodies including surface water and groundwater at trace concentrations ranging from a few ng/L to a tens of μg/L. Because micropollutants are ineffectively removed from municipal wastewater via conventional wastewater treatment processes, wastewater treatment plant effluent is a major source of micropollutants in natural water bodies (Hai et al., 2014; Luo et al., 2014b). Owing to their potentially harmful effects on aquatic ecosystem and human health, in recent years, the widespread occurrence of micropollutants in freshwater sources has triggered specific water quality guidelines (Hai et al., 2018; Lapworth et al., 2012). Therefore, an efficient treatment process is required for the removal of micropollutants from water and wastewater.
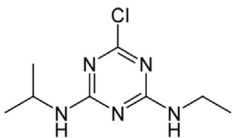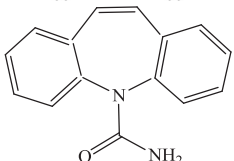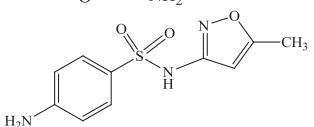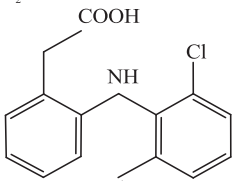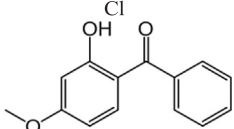
Several physicochemical and biological techniques such as membrane bioreactors, activated carbon and advanced oxidation processes have been assessed for effective removal of micropollutants (Luo et al., 2014b). Enzymatic degradation has gained significant attention in

recent years (Yang et al., 2013). Unlike the conventional biological treatment processes, the oxidoreductase enzyme laccase can catalyze the oxidation or degradation of recalcitrant micropollutants using dissolved oxygen as a co-factor. It typically involves the transfer of an electron from a substrate to the active sites of laccase followed by conversion of dissolved oxygen to water (Asif et al., 2017c; Gonçalves et al., 2015). The characteristics of active sites of laccase have been studied by using a combination of spectroscopic and crystallography techniques (Claus, 2004; Demarche et al., 2012). Briefly, laccase active sites consist of four copper atoms, and can be classified into following categories: (i) Type 1 containing one copper atom; (ii) Type II containing one copper atom; and (iii) Type III containing a pair of copper atoms. During the degradation process, reduction of Type I copper site occurs due to the transfer of an electron from a substrate to the laccase. This promotes the transfer of an electron to Type II and Type III active sites where dissolved oxygen is reduced, and release of water takes place (Claus, 2004; Gonçalves et al., 2015). In general, degradation of micropollutants by laccase is strongly influenced by their molecular properties. Micropollutants having strong electron donating functional

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**Table 1**
Physicochemical properties of the micropollutants used in this study.

| Micropollutants | Molecular structure | Molecular weight (g/mole) | Vapor pressure (mmHg) | Water solubility at 25 °C (mg/L) | Acid dissociation coefficient (pKa) | log D at pH = 7 |
|---|---|---|---|---|---|---|
| Atrazine | | 215.68 | $1.27 \times 10^{-05}$ | 69 | 2.27 | 2.64 |
| Carbamazepine | | 236.27 | $5.78 \times 10^{-07}$ | 220 | 13.94 | 1.89 |
| Sulfamethoxazole | | 253.28 | $1.87 \times 10^{-09}$ | 410 | 5.6 | −0.22 |
| Diclofenac | | 296.15 | $1.59 \times 10^{-07}$ | 30 | 4.18 | 1.77 |
| Oxybenzone | | 228.24 | $5.26 \times 10^{-06}$ | 100 | 7.56 | 3.99 |

*Note*: molecular weight, acid dissociation coefficient, log D values, water solubility and vapor pressure values were taken from SciFinder Scholar.

groups (EDGs), particularly the phenolic group, are more susceptible to degradation by laccase as compared to those containing electron withdrawing functional groups (EWGs) (Ji et al., 2016; Yang et al., 2013).

Enzymatic degradation of micropollutants has been predominantly investigated in batch bioreactors due to the concern of enzyme washout along with the treated effluent from a continuous flow bioreactor. This problem could be addressed either by immobilizing the enzyme onto a carrier (Datta et al., 2013) or by coupling an enzymatic bioreactor to a membrane of suitable molecular weight cut-off (Nguyen et al., 2014a). The use of enzymatic membrane bioreactors (EMBR) offers several advantages over enzyme immobilization including negligible mass transfer limitations, effective enzyme retention, and ease of enzyme replenishment during long term operation (Modin et al., 2014).

Recent studies have explored ultrafiltration enzymatic membrane bioreactors (UF-EMBR) for micropollutants removal (Lloret et al., 2012; Nguyen et al., 2014a) because they can potentially retain the enzyme (*i.e.*, laccase), thus allowing continuous micropollutant degradation within the UF-EMBR without the requirement of continuous dosing of laccase. However, UF membranes in practice cannot effectively retain micropollutants. Thus, micropollutants that are not readily degraded by laccase can still pass through the UF membrane, consequently requiring an additional post-treatment process for their effective removal. An innovative approach to this is to combine a high retention membrane such as nanofiltration (NF) membrane with an enzymatic bioreactor. To date, performance of the NF-EMBR concept has not been systematically studied.

Standalone nanofiltration has been studied extensively for effective removal of micropollutants from secondary treated wastewater [HYPHEN]impacted natural water or freshwater. However, the concentrate produced during nanofiltration requires further treatment before safe disposal (García-Vaquero et al., 2014). Instead of an additional step for NF concentrate treatment, its combination with an enzymatic bioreactor would provide degradation and separation of micropollutants in a single step.

It is also possible that the prolonged contact time between laccase and micropollutants due to their effective retention in the enzymatic bioreactor by the NF membrane may facilitate enhanced micropollutant degradation. The beneficial effect of longer retention of micropollutants on their degradation has been alluded to for other designs of enzymatic bioreactors, but has not been clearly demonstrated. For example, Nguyen et al. (2016a) attributed enhanced degradation of micropollutants by an activated carbon-bound laccase system to their simultaneous adsorption (*i.e.*, retention) and degradation. In another study, efficient micropollutant degradation was achieved by integrating an enzymatic bioreactor with the membrane distillation process (Asif et al., 2018). Membrane distillation retained both laccase and micropollutants and thus facilitated their long contact time. However, since a suitable "control" EMBR, which can only retain laccase but not the micropollutants, was not operated, the mechanisms of enhanced micropollutants removal could not be elucidated in that study.

The study aims to elucidate the effect of simultaneous retention of micropollutants and laccase on micropollutant degradation. This is achieved by studying the performance of an UF-EMBR ("control") and NF-EMBR under identical operating conditions such as hydraulic retention time (HRT) and micropollutant loading rate. Overall this study systematically analyses the role of the UF and NF membranes for the removal of micropollutants, and also elucidates the micropollutant removal mechanism depending on the molecular properties of the micropollutants studied. Finally, the hydraulic performance of the membranes within the EMBRs is compared to confirm the stability of the process developed.