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Removal of trace organic contaminants by enzymatic membrane bioreactors: Role of membrane retention and biodegradation

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Abstract

Performance of an enzymatic membrane bioreactor (EMBR) equipped with either an ultrafiltration (UF) or a nanofiltration (NF) membrane was explored for the degradation of a set of 29 chemically diverse trace organic contaminants (TrOCs). The NF membrane provided effective retention (90-99%) of TrOCs within the NF-EMBR. On the other hand, partial retention of charged and significantly hydrophobic (log >3) TrOCs was achieved by the UF membrane via charge repulsion and adsorption on the enzyme gel-layer formed on the membrane surface during UF-EMBR operation. Laccase achieved TrOC-specific degradation in both EMBRs. The extent of TrOC degradation was significantly (5 to 65%) better by NF-EMBR as compared to that achieved by UF-EMBR. Addition of a redox-mediator (violuric acid) at concentrations ranging from 10-100 μM improved the degradation of non-phenolic TrOCs, but degradation efficiency reached a plateau when its concentration was increased beyond 25 μM. Although the permeate flux of the UF/NF membranes dropped with time due to membrane fouling caused by enzyme gel-layer and/or concentration polarization, membrane flushing with water was effective in recovering the flux by up to 95%.

Keywords

trace, bioreactors:, role, retention, biodegradation, organic, contaminants, enzymatic, removal, membrane

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Removal of trace organic contaminants by enzymatic membrane bioreactors: role of membrane retention and biodegradation

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Abstract:

Performance of an enzymatic membrane bioreactor (EMBR) equipped with either an ultrafiltration (UF) or a nanofiltration (NF) membrane was explored for the degradation of a set of 29 chemically diverse trace organic contaminants (TrOCs). The NF membrane provided effective retention (90-99%) of TrOCs within the NF-EMBR. On the other hand, partial retention of charged and significantly hydrophobic (log >3) TrOCs was achieved by the UF membrane *via* charge repulsion and adsorption on the enzyme gel-layer formed on the membrane surface during UF-EMBR operation. Laccase achieved TrOC-specific degradation in both EMBRs. The extent of TrOC degradation was significantly (5 to 65%) better by NF-EMBR as compared to that achieved by UF-EMBR. Addition of a redox-mediator (violuric acid) at concentrations ranging from 10-100 µM improved the degradation of non-phenolic TrOCs, but degradation efficiency reached a plateau when its concentration was increased beyond 25 µM. Although the permeate flux of the UF/NF membranes dropped with time due to membrane fouling caused by enzyme gel-layer and/or concentration polarization, membrane flushing with water was effective in recovering the flux by up to 95%.

Keywords: Enzymatic membrane bioreactor (EMBR); Laccase; Membrane properties; Redox-mediators; Trace organic contaminants (TrOCs)

1. Introduction

Trace organic contaminants (TrOCs) such as pharmaceuticals, pesticides, steroid hormones and industrial chemicals are commonly detected in different environmental systems including surface water and groundwater due to the discharge of secondary treated wastewater [1, 2]. In addition, agricultural run-off, combined sewer overflow and stormwater run-off can significantly increase the concentration of TrOCs in freshwater bodies [1, 3]. Since TrOCs can be potentially harmful to the aquatic ecosystem and human health [3], an efficient treatment system is required for effective TrOC removal.

Conventional activated sludge process and membrane bioreactors (using micro- or ultrafiltration membranes) have been reported to be ineffective for the removal of a range of TrOCs [4, 5]. Bioreactors equipped with high retention membranes (*e.g.*, nanofiltration, membrane distillation, forward osmosis) can be a promising alternative. Among different types of high retention membrane separation processes, nanofiltration (NF) membranes have been studied extensively for the removal of TrOCs from secondary treated wastewater and freshwater [6-8]. However, following membrane separation, an additional process is required for the treatment of the membrane-concentrate containing high concentrations of TrOCs. Instead of providing a separate treatment process for the degradation of TrOCs, it is a sensible approach to integrate a TrOC degradation process with the NF membrane. In this context, an enzymatic bioreactor can be combined with an NF membrane, which will provide complete retention and TrOC biodegradation in a single step. TrOC degradation by fungal enzymes in enzymatic bioreactors is a promising eco-friendly technique. Compared to the conventional activated sludge process, fungal enzymes can degrade TrOCs with diverse physicochemical properties. Depending on their physicochemical properties, TrOC degradation by fungal enzymes has been reported over a wide range of pH (*e.g.*, 4 to 9) [9, 10]. Notably, enzymatic processes do not produce a large amount of chemical sludge, which is a key attribute of chemical treatment processes [9, 11]. Among different fungal enzymes, laccase is interesting as, unlike peroxidases, it does not require an external co-factor such as hydrogen peroxide (H_2O_2) to catalyze the degradation or oxidation of TrOCs [9, 12]. Laccase has three actives sites. TrOC degradation involves the reduction of

Type I active site due to the transfer of an electron from TrOC to laccase, which promotes the transfer of an electron from Type I to Type II and Type III actives sites. This is followed by the reduction of the co-factor (*i.e.,* dissolved oxygen) and the release of water [13, 14].

Performance of laccase is governed by the operating conditions (*e.g.,* pH and temperature) and molecular properties of pollutants (*e.g.,* molecule structure and hydrophobicity). Typically, laccase can efficiently catalyze the degradation of TrOCs containing strong electron-donating functional groups (EDGs) such as hydroxyl (–OH) and amine (–NH) functional groups. By contrast, degradation of TrOCs containing strong electron-withdrawing functional groups (EWGs) such as amide and halogen is incomplete [13, 15]. To improve the degradation of resistant TrOCs, a 'redox-mediator' can be introduced into the enzymatic bioreactor. Redox-mediators are readily oxidized by laccase and produce highly reactive radicals that can either directly degrade or polymerize resistant TrOCs [16, 17].

To-date, the performance of NF based enzymatic membrane bioreactor (NF-EMBR) has been reported only twice [16, 18]. For example, Escalona et al. [18] reported the removal of an industrial chemical (bisphenol A) by NF-EMBR operated over a short duration of only 5 h [18]. Because the available studies focused on a few compounds, it is imperative to investigate the degradation of TrOCs with diverse physicochemical properties at their environmentally relevant concentrations in an NF-EMBR. This will facilitate advancement of the current state of knowledge of the fate of TrOCs in NF-EMBR and will expand the current knowledge on TrOC removal mechanisms in enzymatic membrane bioreactors to establish a comprehensive database for future predictions.

This study was conducted with the aim to assess the degradation of a set of 29 chemically diverse TrOCs in an enzymatic bioreactor coupled to the NF membrane (NF-EMBR). Additionally, the performance of a "control" UF based EMBR, that can only retain laccase but not TrOCs, was investigated and compared to that achieved by NF-EMBR. Importantly, this study analyses the factors governing the performance of NF and UF membranes as well as laccase and, thereby, elucidates the mechanisms responsible for TrOC degradation in the NF-EMBR. To further improve the degradation of TrOCs, impact of a naturally occurring

redox-mediator (violuric acid) at different concentrations was systematically studied. Finally, variations in membrane flux and changes in membrane properties were assessed and their implications explained.

2. Materials and methods

2.1. Enzyme solution, redox-mediator and trace organic contaminants

Laccase from genetically modified *Aspergillus oryzae* supplied by Novozymes Australia Pty. Ltd. (Sydney, NSW, Australia) was used in this study. According to the supplier, composition (w/w) of enzyme solution was as follows: 66% water, 25% propylene glycol, 4% glucose, 3% laccase and 2% glycine. The purpose of adding propylene glycol, glucose and glycine is to stabilize the enzyme solution. The enzyme solution had an enzymatic activity of 190,000 $\mu M_{(DMP)}$/min, which was measured before the commencement of this study using 2,6-dimethoxy phenol (DMP) as substrate at room temperature and pH =4.5 (*see* Section 2.4.2).

A naturally occurring redox-mediator, namely violuric acid (VA), was used in this study because it has been reported to significantly improve degradation of TrOCs that are resistant to laccase-catalyzed degradation [19]. Analytical grade VA was purchased from Sigma Aldrich (Sydney, NSW, Australia). A stock solution of VA was prepared in Milli-Q water and stored at -4 ºC in dark.

Various categories of TrOCs such as pharmaceuticals, personal care products, pesticides, steroid hormones and industrial chemicals are ubiquitously detected in municipal wastewater and sewage-impacted bodies [1, 2]. Therefore, synthetic wastewater was prepared by adding a mixture of 29 TrOCs in Milli-Q water at a concentration of 5 µg/L to stimulate the composition of TrOCs in sewage-impacted water bodies. These TrOCs include ten pharmaceuticals, seven pesticides, five naturally-occurring steroid hormones, three industrial chemicals, three ingredients of personal care products and one phytoestrogen (*see* Supplementary Data Table S1). Relevant physicochemical properties of TrOCs are given in Table 1. Analytical grade TrOCs (purity >98%) were purchased from Sigma Aldrich (Sydney, NSW, Australia), and a stock solution containing the mixture of 29 TrOCs was prepared in methanol. The stock solution was stored at -18 ºC in dark for use within one month.

[Table 1]

2.2. Experimental setup

A laboratory-scale cross-flow filtration system coupled to an enzymatic bioreactor (3 L) was used in this study (Figure 1). A detailed description of the filtration system is given elsewhere [20]. Briefly, this system mainly consists of a stainless steel enzymatic bioreactor, high-pressure pump (Hydra-Cell, Wanner Engineering Inc., Minneapolis, MN, USA), stainless steel membrane cell, and bypass and back-pressure valves (Swagelok, Solon, OH, USA). The membrane cell with a channel height of 2 mm holds the flat-sheet NF or UF membrane with a surface area of 40 cm^2. A digital flow meter (FlowCal, GJC Instruments Ltd, Chester, CH, UK) was connected to the permeate line for monitoring the permeate flux. The cross-flow velocity and temperature were maintained at 40.2 cm/s and 25°C, respectively in all experiments.

[Figure 1]

Commercially available flat-sheet UF and NF membranes were used in this study. The UF membrane was purchased from Sterlitech (WA, USA). The active layer of the UF membrane is made of polyvinylidene fluoride (PVDF), and its molecular weight cut-off (MWCO) is 30,000 Da. The UF membrane was not expected to retain TrOCs by size exclusion, because the molecular weight of the selected TrOCs ranged between 138-361 Da in this study (Table 1). On the other hand, the NF membrane (NF90, Dow chemicals, MI, USA) had an MWCO of 200 Da. It was a polyamide thin film composite (TFC) membrane that has been studied extensively for the rejection of recalcitrant pollutants from surface water and secondary treated wastewater [7]. However, the performance of the polyamide-TFC NF membrane has not been studied for the removal of a broad spectrum of TrOCs following its integration with an enzymatic bioreactor.

2.3. Enzymatic membrane bioreactor operation and experimental protocols

Each experiment was initiated with membrane compaction. The NF membrane was compacted at a pressure and cross-flow velocity of 10 bar and 40.2 cm/s, respectively, using Milli-Q water until the permeate flux stabilized. Similarly, the UF membrane was also compacted but without applying any pressure. This is because the cross-flow velocity of 40.2 cm/s was enough to generate a permeate flux equivalent to that

achieved by the NF membrane. A series of experiments were conducted by operating UF/NF-EMBR separately to assess: TrOC degradation by laccase; and TrOC removal by the UF and NF membrane as explained in the following sections.

2.3.1. Confirmation of laccase retention

Laccase stock solution (4 mL) was diluted to a final volume of 3 L in an enzymatic bioreactor using Milli-Q water to achieve an enzymatic activity of 180 $\mu M_{(DMP)}$/min. To assess the effective retention of laccase, the NF-EMBR was operated for a period of 24 h under full recirculation mode by continuously returning the membrane permeate to the enzymatic bioreactor at a cross-flow velocity of 40.2 cm/s. Consistent with the previously reported pressure range of 6 to 12 bar [6, 18], NF-EMBR was operated at a pressure of 8 bar, which corresponds to an average initial permeate flux of 6.9 L/m^2.h bar. On the other hand, UF-EMBR was operated without applying pressure to produce a permeate flux comparable to that achieved by NF-EMBR for maintaining an equivalent hydraulic retention time (HRT). Other operating conditions such as cross-flow velocity, temperature and enzymatic activity for UF-EMBR were identical to that of NF-EMBR. Duplicate samples at regular intervals (2, 4, 8 and 24 h) were collected from the permeate of UF/NF-EMBRs to confirm effective retention of laccase by the membranes (*see* Supplementary Data Figure S2).

2.3.2. Continuous operation of EMBRs

The NF-EMBR and UF-EMBR ("control") were operated under continuous mode to systematically investigate the effect of TrOC retention on their degradation. Under continuous mode, the synthetic wastewater described in Section 2.1, *i.e*., TrOC mixture in Milli-Q water, was continuously fed to UF/NF-EMBR separately for a period of 68 h using peristaltic pumps (Masterflex, Vernon Hills, IL, USA). The operating conditions for UF/NF-EMBR are described in section 2.3.1. Based on the initial permeate flux of the membranes, the HRT for both EMBRs was approximately 16 h. Duplicate samples from enzymatic bioreactor were collected at 32 and 68 h for analysis to assess TrOC degradation by laccase. At same intervals (*i.e.*, 32 and 68 h), duplicate samples from permeate were also collected to analyze the overall

removal of TrOCs (*i.e*., biodegradation+membrane retention). At the end of each experiment, UF and NF membranes were backwashed with Milli-Q water for 1 h, and the clean water flux of the membranes was measured to assess flux recovery.

2.3.3. Effect of redox-mediator on TrOC degradation

Redox-mediators are low molecular weight phenolic compounds that can facilitate the degradation of TrOC by acting as an electron shuttle between laccase and target pollutant [21]. In this study, the NF-EMBR was operated with and without mediator dosing to investigate the influence of mediator dosing on TrOC degradation. A single dose of violuric acid (VA) was introduced at different concentrations (*i.e.*, 10, 25, 50 and 100 µM) separately to the NF-EMBR. Duplicate samples from enzymatic bioreactor and permeate were collected at 32 and 68 h for TrOC analysis.

2.3.4. Laccase stability and maintenance in EMBRs

During the operation of EMBRs, laccase activity may diminish due to various physical, chemical and biological inhibitors such as shear stress caused by membrane filtration [21, 22]. Moreover, the transformation products formed following TrOC degradation in an EMBR can also inhibit laccase by blocking the active sites of enzymes [22]. Therefore, laccase activity was regularly monitored during the operation of EMBRs. Based on laccase activity drop (see Supplementary Data Figure S3), a protocol of re-injecting a small dose of laccase (250 µL per litre of bioreactor media) was developed to maintain a laccase activity of 170-185 $\mu M_{(DMP)}$/min.

2.4. Analytical methods

2.4.1. TrOC analysis

TrOC concentration was measured using a method previously described by Hai et al. [23]. This method involves the extraction of TrOC by solid-phase extraction (SPE) technique followed by their quantification using a GC/MS system (QP5000, Shimadzu, Japan). Recovery of TrOCs by SPE ranged between 70 and

90%. TrOCs were quantified by an internal standard method. Bisphenol A-$_{d16}$ was added to each sample and standard as a surrogate to account for any error in the injection volume of the samples. The limit of detection for this method was compound-specific and ranged between 1-20 ng/L (see Supplementary Data Table S1). Removal efficiency by laccase ($R_{degradation}$) and the membrane ($R_{membrane}$) was measured as:

$$R_{degradation} = 100 \times (1 - C_{EBR}/C_f) \quad (1)$$

$$R_{membrane+degradation} = 100 \times (1 - C_p/C_f) \quad (2)$$

where, C_f, C_{EBR} and C_p are the concentration (ng/L) of a specific TrOC in feed, enzymatic bioreactor and permeate, respectively. The mass of TrOCs degraded by laccase was calculated as follows:

$$C_f \times V_f = (C_{EBR} \times V_{EBR}) + (C_p \times V_p) + \text{biodegradation/biotransformation} \quad (3)$$

where, V_f, V_{EBR} and V_p represents the volume of feed, enzymatic bioreactor and permeate, respectively.

2.4.2. Laccase activity assay and ORP

Laccase activity was measured by using a method previously reported by Paszczynski et al. [24]. Briefly, the change in absorbance of 2,6-dimethoxyl phenol (DMP) in sodium citrate buffer (pH = 4.5) was recorded over a duration of 2 min at room temperature using a UV-Vis spectrometer (DR3900, HACH, Colorado, USA). A molar extinction coefficient of 49.6/mM cm was used to calculate laccase activity. Oxidation-reduction potential (ORP) of laccase with and without the addition of redox-mediator was measured using an ORP meter (WP-80D dual pH-mV meter, Thermo Fisher Scientific, Australia).

2.4.3. Analysis of membrane properties and surface morphology

Surface charge and hydrophobicity was analyzed to assess the effect of laccase on membrane properties. Membrane hydrophobicity in terms of contact angle was measured by the standard sessile drop method using a Rame-Hart Goniometer (Model 250, Rame-Hart, Netcong, New Jersey, USA) as previously described [25].

For assessing the change in surface charge of the membranes, the zeta potential was measured at room temperature using a SurPASS elctrokinetic analyzer (Anton Par GmbH, Graz, Austria). Analytical grade potassium hydroxide and hydrochloric acid were used to adjust the pH of the electrolyte solution. The zeta potential was calculated from the steaming potential using the Fairbrother-Mastin approach [25].

NF and UF membranes collected at the end of experiments were air-dried in a desiccator. After coating the membranes with a gold layer by using a sputter coater (SPI Module, West Chester, PA, USA), the surface morphology of the membranes was characterized with scanning electron microscopy (SEM) (JCM-600, JEOL, Tokyo, Japan).

3. Results and discussion

3.1. Degradation by laccase in EMBRs

In this study, two EMBRs –one equipped with UF and another equipped with NF membrane – were operated. The UF/NF EMBRs were operated under identical HRT and TrOC loading rate of 16 h and 7.2 µg/L d, respectively. Laccase was effectively retained by both the membranes (*see* Supplementary Data Figure S2).

The mechanisms of TrOC removal by EMBR include enzymatic degradation and retention by a membrane. Of the two membranes used in this study, the NF membrane was expected to retain TrOCs more effectively, and this aspect will be discussed further in Section 3.2. In addition to retention by the membrane, the current study also intends to examine the extent of enzymatic degradation achieved by laccase in both UF/NF-EMBRs. Laccase-catalyzed biodegradation of TrOCs was assessed as explained in Section 2.4.1.

Laccase-catalyzed degradation occurs due to the transfer of a single electron from a substrate to laccase [26]. With some exceptions, phenolic TrOCs have been reported to be effectively degraded by laccase [13, 26]. On the other hand, degradation of non-phenolic TrOCs by laccase can be highly variable and may depend on the difference of ORP between laccase and the non-phenolic TrOCs, as well as the TrOC

molecular properties such as the presence of an EWGs or EDGs [27, 28]. Therefore, here the degradation of the phenolic and non-phenolic TrOCs is discussed separately.

3.1.1. Degradation of phenolic TrOCs

In this study, laccase achieved efficient degradation (>80%) for four out of 12 phenolic TrOCs, namely 17ß–estradiol-17-acetate, 4-tert-octylphenol, triclosan and salicylic acid in both UF- and NF-EMBRs (Figure 2). Efficient degradation of these TrOCs by laccase has been reported previously in both batch and continuous-flow enzymatic bioreactors [10, 27, 29].

[Figure 2]

As mentioned above, phenolic pollutants are typical substrates of laccase. However, the concomitant presence of EWGs in the molecule of phenolic TrOCs can cause steric hindrance, thereby delaying the access of a pollutant to the active sites of laccase for effective degradation [28]. For phenolic TrOCs containing EWG(s), the extent of degradation by laccase in NF-EMBR was observed to vary depending on the type of EWGs. For example, NF-EMBR achieved 80% degradation of estrone that contains the carbonyl (=O) functional group as an EWG in its molecule. On the other hand, degradation of pentachlorophenol, containing a halogen (−X) functional group (an EWG), was observed to be 60% in NF-EMBR.

Notwithstanding the above-mentioned variations in the degradation of the phenolic TrOCs containing EWG(s), NF-EMBR achieved from 5 up to 60% better degradation for eight out of the 12 investigated phenolic TrOCs as compared to the UF-EMBR (Figure 2). When an NF membrane is attached to an enzymatic bioreactor, the HRT of the bioreactor can be decoupled from the organic retention time due to effective TrOC retention. This leads to increased contact time between laccase and TrOC and can thus facilitate TrOC degradation. Indeed, in a study by Lloret et al. [30], enhanced removal (33-37%) of two phenolic TrOCs, namely estrone and 17ß–estradiol, was achieved by increasing the HRT of an enzymatic bioreactor coupled to a UF membrane. It is important to note that prolonged contact time might not be the

only reason for improved degradation of TrOCs in the NF-EMBR. This aspect is discussed further in Section 3.1.2.

Degradation of six phenolic TrOCs including four steroid hormones (17ß–estradiol and 17α–ethinylestradiol and estriol) and two industrial chemicals (4-tert-butylphenol and bisphenol A) by laccase was 70-90% in NF-EMBR, while UF-EMBR achieved 10-40% degradation (Figure 2). Although these TrOCs have been generally reported to be well removed by laccase in batch enzymatic bioreactors [10, 31], the lower performance of UF-EMBR in this study can be attributed to the continuous TrOC loading that has been reported to affect the extent of TrOC degradation [29, 30]. The NF-EMBR was better suited to withstand the continuous loading of the phenolic TrOCs to the enzymatic bioreactor.

3.1.2. Degradation of non-phenolic TrOCs

In this study, the following three trends were observed in the degradation profile of 17 non-phenolic TrOCs (Figure 2): (i) From 5 up to 65% better degradation in NF-EMBR of six pharmaceuticals (*i.e.*, ketoprofen, naproxen, primidone, gemfibrozil, amitriptyline and metronidazole) and five pesticides, namely fenoprop, clofibric acid, propoxur, pentachlorophenol, N, N-diethyl-meta-toluamide (DEET) and ametryn; (ii) efficient degradation (>80%) of two ingredients of personal care products (*i.e.*, benzophenone and octocrylene) in both UF- and NF-EMBR; and (iii) poor removal (5-15%) of a pesticide (*i.e.*, atrazine) and three pharmaceuticals (*i.e.*, carbamazepine, diclofenac and ibuprofen) in both UF- and NF-EMBR.

Only around 5-15% degradation of atrazine, carbamazepine, diclofenac and ibuprofen by the EMBRs can be attributed to the presence of strong EWGs such as amide (–C(=O)N), carboxylic (–COOH) and halogen (–X) functional groups (see Supplementary Data Table S1), which makes them resistant to laccase-catalyzed degradation [29, 31]. On the other hand, in line with a previous report [29], benzophenone and octocrylene were well removed by laccase in this study. Irrespective of their degradation in EMBR, overall removal (degradation + membrane retention) of TrOCs in NF-EMBR was observed to range between 90 and 99% as explained in Section 3.2.

As also noted in Section 3.1.2, the significantly better degradation of 11 non-phenolic TrOCs following their retention within the NF-EMBR can be attributed to the increased reaction time between laccase and the TrOCs. Asif et al. [32] reported high TrOCs degradation in membrane distillation (MD)-EMBR, where the studied TrOCs and laccase were retained by the MD membrane. However, in that study the performance of the MD-EMBR was not compared to a suitable "control" *i.e.*, an EMBR that will retain laccase but not the TrOCs. By comparing UF- vs. NF- EMBR, the current study demonstrates that effective TrOC retention within the bioreactor facilitates their degradation.

It is important to note that TrOCs containing hydroxyl and amine functional groups such as bisphenol A and steroid hormones can also play an important role in the degradation of non-phenolic TrOCs by acting as redox-mediators [10, 33]. The secondary radicals or coupling agents, which are formed following the oxidation of TrOCs containing hydroxyl and amine functional groups, are highly reactive and could directly oxidize or polymerize other TrOCs. For instance, lignin is a plant polymer with a highly complex chemical structure. The degradation pathway for lignin reveals that laccase directly oxidizes the phenolic components of lignin, and produces highly reactive phenoxyl radicals, which then oxidize the non-phenolic components of lignin [28, 34]. Similarly, Hachi et al. [33] demonstrated that the degradation of acetaminophen by laccase formed a coupling agent (*i.e.*, dimer). This coupling agent reacted with carbamazepine to form oligomers, thereby improving carbamazepine removal from 10 to 40% [33]. In another study by Jahangiri et al. [35], removal of triclosan was reported to improve in a batch enzymatic bioreactor following the addition of the phenolic compound acetaminophen. Enhanced removal of triclosan was attributed to the formation of acetaminophen-triclosan cross-coupling products [35]. In the current study, the synthetic wastewater that was continuously fed to the UF/NF-EMBR contained a mixture of 29 TrOCs including 12 phenolic and 17 non-phenolic TrOCs. Since these TrOCs were effectively retained by the NF membrane but not by the UF membrane (see Section 3.2), it is possible that the radicals or coupling agents formed after the oxidation of some phenolic TrOCs by laccase contributed to better degradation of the non-phenolic TrOCs in NF-EMBR as compared to UF-EMBR. On the other hand, the extent of degradation (90-99%)

for several TrOCs such as those containing hydroxyl, alkyl or amine functional groups (e.g., triclosan, octocrylene and salicylic acid) by laccase in the UF-EMBR was comparable to that achieved by NF-EMBR because these TrOCs are readily amenable to laccase-catalyzed degradation [29, 36], and thus membrane retention plays a less significant role for their overall removal by an EMBR.

A close look at the trend of laccase-catalyzed degradation in both UF- and NF-EMBR indicates that the improvement in degradation could be correlated with the molecular weight of TrOCs. In the current study, the extent of improvement in degradation was significantly higher for TrOCs with a molecular weight above 200 g/mol (Figure 3). This is probably because the presence of more branches and/or functional groups in TrOCs with high molecular weight would create more opportunities of their interaction with laccase, secondary radicals and coupling agents [5].

[Figure 3]

3.2. Overall removal of TrOCs in EMBRs

TrOC degradation in the enzymatic bioreactor ranged between 10-99% (Figure 2). However, the overall TrOC removal (calculated based on TrOC concentration in membrane permeate) by the NF-EMBR was 90-99%, demonstrating the significant contribution of the NF membrane to the overall removal.

NF membranes can reject TrOCs via following mechanisms: (i) size exclusion; (ii) charge repulsion; and (iii) adsorption [7, 37]. In general, the NF membrane used in this study has been reported to effectively retain TrOCs with a molecular weight of greater than 200 g/mol (i.e., MW > MWCO) via size exclusion mechanism [7]. In a previous study by Alturki et al. [6] an NF membrane with a MWCO of less than 200 g/mol achieved 85 to 100% removal for different groups of TrOCs such as pharmaceuticals, personal care products, pesticides and industrial chemicals. Consistent with the available literature [6, 7], TrOCs with MW above 200 g/mol were effectively removed (>90%) by the NF-EMBR in this study (Figure 4). Notably, some of the TrOCs having MW of less than 200 g/mol also showed removals of 95-99% in the NF-EMBR (Figure 4). These TrOCs include salicylic acid (138.12 g/mol), metronidazole (171.15 g/mol),

benzophenone (182.22 g/mol), DEET (191.27 g/mol) and 4-tert-butylphenol (150.22 g/mol). Previously, electrostatic interactions between the charged TrOCs and NF membrane have been reported to improve the extent of TrOC rejection [38]. In this study, since salicylic acid, atrazine and DEET are negatively charged (pK_a < pH) at the operating pH of the NF-EMBR (i.e., 6.7-6.9), charge repulsion between the negatively charged NF membrane and anionic TrOCs is likely responsible for their removal by the NF membrane [7, 39].

[Figure 4]

Hydrophobic TrOCs (log D > 3) can adsorb on the membrane surface, thereby resulting in their high initial rejection by the NF membrane [7], but this may reduce with time due to their diffusion into membrane permeate [7, 32]. For instance, removal of estrone (log D = 3.62) by NF membrane reduced from 90% at the start of the experiment to approximately 20% at the end of 24 h [40]. Nevertheless, in this study, the hydrophobic TrOCs were effectively degraded by laccase in the enzymatic bioreactor (80-99%, Figure 2). Thus, the overall removal of hydrophobic TrOCs (log D > 3) was above 99%. Previous studies reported that a combination of activated sludge [41] or enzymatic bioreactor [32] with a high retention membrane (e.g., MD membrane) can improve the overall removal of TrOCs as compared to a stand-alone high retention membrane system. However, this is the first study that demonstrates the performance of an NF-based EMBR for a set of 29 TrOCs.

UF membranes cannot reject TrOCs *via* size exclusion. Thus, as expected, the overall TrOC removal by the NF-EMBR was 10-80% higher than the UF-EMBR (Figure 4). However, it is noteworthy that, for the UF-EMBR, the overall removal efficiency of a few TrOCs was significantly better than that suggested by biodegradation efficiency (Figure 2). This indicates that the UF membrane provided partial retention of those TrOCs. To facilitate the discussion on TrOC removal by the UF membrane, the ratio of the concentration of selected TrOCs in membrane permeate and bioreactor (*i.e.*, P/S ratio) is shown in Figure 5.

[Figure 5]

Indeed, the permeate/bioreactor concentration ratio for significantly hydrophobic TrOCs (log D>3) including 17ß–estradiol-17-acetate, triclosan and 17α-ethinylestradiol was significantly below 1 and ranged between 0.3-0.6 (Figure 5). Previously, Nguyen et al. [29] observed adsorption of TrOCs on the enzyme gel-layer formed on the surface of a polyacrylonitrile hollow fiber UF membrane within an EMBR. They also reported that the adsorbed TrOC was subsequently degraded by laccase, and this prevented the accumulation of TrOCs on the membrane surface. In this study, the formation of enzyme gel-layer on membranes surface during EMBR operation was confirmed by characterizing the surface morphology of both the UF and NF membranes by SEM (Figure 6). Importantly, a stable concentration of TrOCs in the enzymatic bioreactor and membrane permeate indicates that the TrOCs adsorbed on enzyme gel-layer were subsequently degraded by laccase, which is consistent with the findings of Nguyen et al. [29].

[Figure 6]

In a study by Garcia-Ivars et al. [42], partial retention of anionic pharmaceuticals such as naproxen, diclofenac and ibuprofen by a flat-sheet ceramic UF membrane was attributed to charge repulsion mechanism. Similarly, in this study, despite being hydrophilic (log D <3), the partial retention of a few anionic TrOCs by the UF membrane was observed. These hydrophilic anionic TrOCs include naproxen (permeate/bioreactor ratio= 0.8), primidone (permeate/bioreactor ratio= 0.86), ibuprofen (permeate/bioreactor ratio= 0.9), propoxur (permeate/bioreactor ratio= 0.88) and diclofenac (permeate/bioreactor ratio= 0.94). Despite the higher MWCO of the UF membrane than the MW of TrOCs, data from the current study confirm that the flat-sheet PVDF UF membrane along with the enzyme layer on it can retain anionic TrOCs to some extent.

The discussion here suggests that UF membrane can contribute to the removal of TrOCs depending on their hydrophobicity and charge, thereby improving the overall performance of UF-EMBR. However, the overall

removal by the NF-EMBR was considerably better due to enhanced TrOC degradation (Figure 2) as well as effective TrOC removal (Figure 4) in a single step.

Biodegradation of TrOCs may produce degradation products or metabolites that could be more toxic than the parent compounds. However, previous studies suggest that toxicity of EMBR-permeate, after enzymatic treatment of a mixture of TrOCs, does not increase, particularly when a high retention membrane separation process, *e.g.*, membrane distillation, is integrated with an enzymatic bioreactor [19, 29]. In the current study, a high retention NF membrane combined with the enzymatic bioreactors effectively retained TrOCs (Figure 4). Thus, the permeate of NF-EMBR can be expected to be non-toxic.

The EMBRs were operated at the same permeate flux to maintain the same HRT, *i.e.*, TrOC loading rate. This was necessary to ensure the same baseline for laccase-catalyzed degradation of TrOCs. However, to achieve this, the NF membrane was operated at a transmembrane pressure of 8-10 bar, whereas the UF membrane was operated without applying additional pressure (*see* Section 2.3). This may have led to a different TrOC transport mechanism towards the UF membrane surface than usual, thus affecting TrOC deposition or retention by the UF membrane. However, because TrOCs removal by the UF membrane was expected to be inherently much lower than by the NF membrane, the impact of transmembrane pressure against the UF membrane on the comparative performance of the UF-EMBR and NF-EMBR may be considered minor.

Integration of an enzymatic bioreactor with an NF membrane can produce TrOC-free effluent. In addition, improved TrOC degradation achieved by laccase in NF-EMBR would lead to reduced concentrate treatment and disposal costs. However, laccase inactivation in EMBRs could be significant during the treatment of complex wastewater matrices such as raw wastewater, secondary treated effluent and sewage-impacted groundwater, which may affect the extent of TrOC degradation [9, 43]. According to available modelling studies [44, 45], for large-scale practical applications, EMBRs in series would be required for effective removal of TrOCs. These aspects including the strategies for improving laccase stability must be focused in future research.

3.3. Effect of redox-mediator addition on TrOC degradation by NF-EMBR

As noted in section 3.1, the degradation of several TrOC by laccase incomplete or ineffective (Figure 2). To improve the spectrum of efficiently degraded TrOCs, redox-mediators can be introduced to the reaction mixture. In a laccase-mediator system, laccase oxidizes the mediator to produce highly reactive radicals. Due to high redox-potential of these radicals, they can directly degrade or polymerize TrOCs, particularly those resistant to laccase-catalyzed degradation [46].

Since redox-mediators are not retained by the UF membrane [29], their continuous dosing to UF-EMBR would be required to achieve stable performance. This would significantly increase the operating cost of the treatment process. Therefore, in this study, the impact of redox-mediator addition on TrOC degradation was assessed in case of NF-EMBR only. Notably, the impact of a single dose of a redox-mediator on the performance of NF based high retention EMBR is demonstrated for the first time in this study.

Laccase can readily oxidize VA to form highly reactive aminoxyl (=N–O) radicals. The aminoxyl radicals degrade the target pollutants by following hydrogen atom transfer (HAT) mechanism [19, 47]. The driving force of HAT mechanism is the enthalpy balance between the forming bond (H–ON) and the dissociated C–H bond [19].

3.3.1. Overall improvement in TrOC degradation

Improvement in the degradation of TrOCs following the addition of a single dose of VA at a concentration of 10 µM is presented in Figure 7. Redox-mediators capable of generating radicals, which can degrade a substrate following the HAT mechanism, has been reported to be particularly effective for non-phenolic compounds, which are originally poorly removed by laccase [13, 19]. In this study, VA improved the degradation of six non-phenolic compounds by 10-50% (Figure 7). For example, diclofenac degradation increased from 13% in NF-EMBR to 42% in laccase-VA mediated NF-EMBR. Similarly, VA addition improved the degradation of the pesticide atrazine by 40%. The highest improvement (50%) was observed for ametryn (Figure 7). Laccase cannot efficiently degrade non-phenolic TrOCs with higher redox-potential

[28, 29]. The redox-potential of the media in enzymatic bioreactor increased from 300 to 390 mV following the addition of VA at a concentration of 10 μM (See Supplementary Data Figure S5), which is one of the reasons of the improved degradation in NF-EMBR. The concentration of redox-mediators is another influencing factor as explained in Section 3.3.2.

[Figure 7]

Laccase achieved almost complete (>99%) degradation of three phenolic TrOCs *viz* 4-tert-octylphenol, triclosan and salicylic acid in NF-EMBR. However, biodegradation of some phenolic compounds by laccase-only was incomplete. Six steroid hormones (estrone, 17β-estradiol, estriol 17α–ethinylestradiol and 17β-estradiol-17–acetate, enterolactone), two industrial chemicals (4-tert-octylphenol and bisphenol A) and a pesticide (pentachlorophenol) were degraded by laccase with an efficiency between 20 and 90%, and their degradation did not improve at a VA dose of 10 μM (Figure 7). Our observation is consistent with that by Nguyen et al. [29] who reported that the degradation of phenolic TrOCs such as estrone, estriol, 17β-estradiol 17–acetate, 4-tert-butylphenol and bisphenol A did not improve in UF-EMBR following the addition of another aminoxyl radical producing redox-mediator (*i.e.,* 1-hydroxybenzotriazole, HBT) at a concentration of 10 μM. This is probably because the laccase-VA system did not produce enough reactive aminoxyl radicals at such a trace concentration (*i.e.,* 10 μM) that would improve the degradation of the phenolic TrOCs tested here. Indeed, increasing the concentration of VA from 10 to 25 μM in NF-EMBR resulted in enhanced degradation of six phenolic TrOCs (*see* section 3.3.2).

It is noteworthy that redox-mediators have been reported to exhibit substrate specificity [48, 49]. In this study, VA (10 μM) was more effective in improving the degradation of the non-phenolic TrOCs, although it should be noted that the overall degradation of the phenolic compounds within the bioreactor was still significantly better than the non-phenolic TrOCs (Figure 7).

3.3.2. Effect of mediator concentration on TrOC degradation

The concentration of redox-mediators can influence the performance of the laccase-mediator system because TrOC degradation is affected by the abundance of highly reactive radicals. Hence, a single dose of VA at different concentrations (*i.e.,* 10, 25, 50 and 100 µM) was added separately to the NF-EMBR. To show different trends of improvement, degradation of 10 selected TrOCs at different VA concentrations is presented in Figure 8. The complete data set is available in Supplementary Data Figure S6.

[Figure 8]

Increasing the concentration of VA from 10 to 25 µM further improved the degradation of TrOCs by up to 10-25% (Figure 7). Although VA did not improve the degradation of phenolic TrOCs at 10 µM, an improvement of 10-25% was observed in the degradation of estrone, estriol, 17β-estradiol 17–acetate, 17β-estradiol, 4-tert-butylphenol and bisphenol A after adding VA at a concentration of 25 µM (Figure 8). Improvements were also noted in the case of non-phenolic compounds such as propoxur, ibuprofen, diclofenac, ametryn and atrazine. Despite a discernable increase in ORP (*see* Supplementary Data Figure S5), no further degradation improvement was observed by increasing the concentration of VA from 25 to 100 µM (Figure 8). Depending on mediator type, laccase source and the target pollutant, the improvement in TrOC degradation may reach a plateau beyond a certain mediator concentration [19, 50]. For instance, Ashe et al. [19] observed no improvement in atrazine and naproxen removal beyond 500 µM of VA in a batch enzymatic bioreactor. In another study, increasing VA concentration from 250 to 500 µM provided similar degradation for a few phenolic TrOCs such as bisphenol A and 4-tert-butylphenol [19]. The current study confirms this phenomenon for the first time in case of a continuous flow EMBR.

Although adding a redox-mediator can improve TrOC degradation, for some mediators, the radicals formed following the oxidation of redox-medaitors may cause toxicity. For example, in previous studies, addition of syringaldehyde [19] and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid [19] was reported to increase the toxicity of the treated effluent. However, in previous studies, following addition of VA (which was used in the current study) at concentrations ranging from 0.5–1 mM, toxicity of the treated effluent was reported not to increase significantly [19, 42].

In light of the mediator performance at different concentrations, VA at a concentration of 25 µM was the best for achieving improved TrOC degradation by the NF-EMBR. Three phenolic and 14 non-phenolic TrOCs were incompletely degraded even with redox-mediator dosing. However, the final treated effluent, *i.e*, NF-permeate achieved over 95% removal of all TrOCs (*see* Supplementary Data Figure S6).

3.4. Hydraulic performance of membranes

Variations in permeate flux during the operation of the laccase based EMBRs are presented as normalized permeate flux in Figure 9. Typically, a steep fall in the permeate flux of the NF and UF membranes is observed at the initial stage of their operation [18, 51]. Indeed, the permeate flux reduced rapidly in the first few hours of UF/NF-EMBR runs in this study (Figure 9). Given the MWCO of the membranes, *i.e*., 200 Da for the NF and 30,000 Da for the UF, the reduction in permeate flux for the NF membrane was steeper. The initial permeate flux of the UF membrane decreased by approximately 15%, and stabilized after 10 h of UF-EMBR operation. On the other hand, a progressive fall in the flux of the NF membrane was observed during the first 30 h of NF-EMBR operation. Despite this, the permeate flux at the end of NF-EMBR operation was still 65% of the initial flux (Figure 9).

[Figure 9]

The reduction in permeate flux in UF/NF-EMBR can be attributed to: (i) membrane fouling due to the adsorption of laccase on membrane surface forming an enzyme gel-layer (Figure 6); and/or (ii) concentration polarization due to the accumulation of TrOCs and transformation products on membrane surface [18, 52]. To assess whether the reduction in permeate was reversible or irreversible, permeate flux was measured after backwashing the UF and NF membranes with Milli-Q water for 1 h. Membrane cleaning recovered the permeate flux of the NF and UF membranes by 92 and 96%, respectively. The flux recovery was not 100% probably due to the irreversible adsorption of laccase on the membrane surface. This is also evident from changes in membrane properties, *i.e.*, contact angle and zeta potential as discussed in the following section.

3.5. Effect on membrane surface charge and hydrophobicity

The UF and NF membranes were negatively charged at the operating pH of the UF/NF-EMBRs (*i.e.*, approximately 7) as shown in Figure 10. The virgin NF membrane is negatively charged due to the protonation of carboxylic and amino functional groups of the active membrane layer [25]. On the other hand, the virgin PVDF UF membrane is usually not charged but it becomes negatively charged due to the adsorption of hydroxyl ions that originate from the self-ionization of water [25, 53].

[Figure 10]

The negative charge on the surface of UF and UF membranes in response to their operation with enzyme solution reduced as compared to the virgin membranes (Figure 10). These changes in membrane surface charge can be attributed to the adsorption of laccase on the membrane surface as shown in Figure 6. It was reported that adsorption of solutes on the membrane surface can change the surface roughness and chemistry of the active layer of the membrane, thereby altering their streaming potential [53].

Hydrophobicity of a membrane depends on its surface properties and hydrophilic functional groups [25]. Based on the contact angle, the UF membrane was significantly hydrophobic, while the NF membrane was moderately hydrophobic (Figure 10). However, hydrophobicity of both the UF and NF membrane reduced, which again confirms the adsorption of laccase on the membrane surface. Results from this study indicate that laccase adsorption can alter the properties of the membranes to some extent, although above 90% flux recovery can be achieved by flushing the membrane with ultrapure Milli Q water. While no effect of change in properties of the NF membrane was observed on TrOC removal, the formation of an enzyme-gel layer on the surface of the UF membrane following laccase adsorption can improve the overall performance of UF-EMBR by adsorbing hydrophobic TrOCs (*see* Section 3.2).

4. Conclusion

Enzymatic degradation of a broad spectrum of TrOCs including 12 phenolic and 17 non-phenolic compounds was compared in ultrafiltration (UF) vs. nanofiltration (NF) based enzymatic membrane

bioreactors (EMBR). This helped to assess the effect of effective TrOCs retention within enzymatic bioreactor on their degradation by laccase. As expected, the overall removal of TrOCs in NF-EMBR was better because the NF membrane achieved TrOC rejection ranging from 90-99%. Furthermore, mass balance analysis shows that as compared to the UF-EMBR, significantly better degradation (up to 65%) was achieved by laccase in NF-EMBR. A redox-mediator (violuric acid, VA) was dosed to NF-EMBR to further improve the degradation of TrOCs. VA achieved improved degradation for four phenolic and six non-phenolic TrOCs in NF-EMBR, at a concentration of 25 μM, beyond which the extent of degradation did not improve significantly. Change in membrane properties due to laccase adsorption along with concentration polarization can reduce the permeate flux of the UF and NF membrane, although flux can be recovered effectively by cleaning the membrane with water.

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References

[1] D. Lapworth, N. Baran, M. Stuart, R. Ward, Emerging organic contaminants in groundwater: a review of sources, fate and occurrence, Environmental pollution, 163 (2012) 287-303.

[2] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Science of the Total Environment, 473 (2014) 619-641.

[3] M. Gavrilescu, K. Demnerová, J. Aamand, S. Agathos, F. Fava, Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, New Biotechnology, 32 (2015) 147-156.

[4] J.T. Alexander, F.I. Hai, T.M. Al-aboud, Chemical coagulation-based processes for trace organic contaminant removal: Current state and future potential, Journal of environmental management, 111 (2012) 195-207.

[5] F.I. Hai, L.D. Nghiem, S.J. Khan, M.B. Asif, W.E. Price, K. Yamamoto, Removal of emerging trace organic contaminants (TrOC) by MBR, in: F.I. Hai, K. Yamamoto, C. Lee (Eds.) Membrane Biological Reactors: Theory, Modeling, Design, Management and Applications to Wastewater Reuse, IWA publishing, London, United Kingdom 2019, pp. 413-468.

[6] A.A. Alturki, N. Tadkaew, J.A. McDonald, S.J. Khan, W.E. Price, L.D. Nghiem, Combining MBR and NF/RO membrane filtration for the removal of trace organics in indirect potable water reuse applications, Journal of Membrane Science, 365 (2010) 206-215.

[7] M. Taheran, S.K. Brar, M. Verma, R.Y. Surampalli, T.C. Zhang, J.R. Valéro, Membrane processes for removal of pharmaceutically active compounds (PhACs) from water and wastewaters, Science of The Total Environment, 547 (2016) 60-77.

[8] M.B. Asif, Z. Fida, A. Tufail, J.P. van de Merwe, F.D.L. Leusch, B.K. Pramanik, W.E. Price, F.I. Hai, Persulfate oxidation-assisted membrane distillation process for micropollutant degradation and membrane fouling control, Separation and Purification Technology, 222 (2019) 321-331.

[9] M.B. Asif, F.I. Hai, J. Hou, W.E. Price, L.D. Nghiem, Impact of wastewater derived dissolved interfering compounds on growth, enzymatic activity and trace organic contaminant removal of white rot fungi – A critical review, Journal of Environmental Management 201 (2017) 89-109.

[10] J. Margot, J. Maillard, L. Rossi, D.A. Barry, C. Holliger, Influence of treatment conditions on the oxidation of micropollutants by Trametes versicolor laccase, New Biotechnology, 30 (2013) 803-813.

[11] H. Claus, Laccases: structure, reactions, distribution, Micron, 35 (2004) 93-96.

[12] I. Gonçalves, C. Silva, A. Cavaco-Paulo, Ultrasound enhanced laccase applications, Green Chemistry, 17 (2015) 1362-1374.

[13] S. Yang, F.I. Hai, L.D. Nghiem, W.E. Price, F. Roddick, M.T. Moreira, S.F. Magram, Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review, Bioresource technology, 141 (2013) 97-108.

[14] C. Ji, J. Hou, K. Wang, Y. Zhang, V. Chen, Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor, Journal of Membrane Science, 502 (2016) 11-20.

[15] N.H. Tran, T. Urase, O. Kusakabe, Biodegradation characteristics of pharmaceutical substances by whole fungal culture Trametes versicolor and its laccase, Journal of Water and Environment Technology, 8 (2010) 125-140.

[16] M.B. Asif, F.I. Hai, B.R. Dhar, H.H. Ngo, W. Guo, V. Jegatheesan, W.E. Price, L.D. Nghiem, K. Yamamoto, Impact of simultaneous retention of micropollutants and laccase on micropollutant degradation in enzymatic membrane bioreactor, Bioresource technology, 267 (2018) 473-480.

[17] A.I. Cañas, S. Camarero, Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes, Biotechnology Advances, 28 (2010) 694-705.

[18] I. Escalona, J. de Grooth, J. Font, K. Nijmeijer, Removal of BPA by enzyme polymerization using NF membranes, Journal of membrane science, 468 (2014) 192-201.

[19] B. Ashe, L.N. Nguyen, F.I. Hai, D.-J. Lee, J.P. van de Merwe, F.D. Leusch, W.E. Price, L.D. Nghiem, Impacts of redox-mediator type on trace organic contaminants degradation by laccase: Degradation efficiency, laccase stability and effluent toxicity, International Biodeterioration & Biodegradation, 113 (2016) 169–176.

[20] M.B. Asif, J.P. van de Merwe, F.D.L. Leusch, B.K. Pramanik, W.E. Price, F.I. Hai, Elucidating the performance of an integrated laccase- and persulfate-assisted process for degradation of trace organic contaminants (TrOCs), Environmental Science: Water Research & Technology, 6 (2020) 1069-1082.

[21] O. Modin, F.I. Hai, L.D. Nghiem, A. Basile, K. Fukushi, Gas-diffusion, extractive, biocatalytic and electrochemical membrane biological reactors, in: F.I. Hai, K. Yamamoto, C. Lee (Eds.) Membrane Biological Reactors: Theory, Modeling, Design, Management and Applications to Wastewater Reuse, IWA Publishing, London, United Kingdom, 2014, pp. 299-334. (ISBN: 9781780400655).

[22] R. Khlifi-Slama, T. Mechichi, S. Sayadi, A. Dhouib, Effect of natural mediators on the stability of Trametes trogii laccase during the decolourization of textile wastewaters, The Journal of Microbiology, 50 (2012) 226-234.

[23] F.I. Hai, K. Tessmer, L.N. Nguyen, J. Kang, W.E. Price, L.D. Nghiem, Removal of micropollutants by membrane bioreactor under temperature variation, Journal of membrane science, 383 (2011) 144-151.

[24] A. Paszczynski, M. Pasti, S. Goszczynski, D. Crawford, R. Crawford, New approach to improve degradation of recalcitrant azo dyes by Streptomyces spp. and Phanerochaete chrysosporium, Enzyme and Microbial Technology, 13 (1991) 378-384.

[25] A. Simon, W.E. Price, L.D. Nghiem, Influence of formulated chemical cleaning reagents on the surface properties and separation efficiency of nanofiltrationmembranes, Journal of membrane science, 432 (2013) 73-82.

[26] M. Naghdi, M. Taheran, S.K. Brar, A. Kermanshahi-pour, M. Verma, R. Surampalli, Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes, Environmental Pollution, 234 (2018) 190-213.

[27] L.I. Ramírez-Cavazos, C. Junghanns, N. Ornelas-Soto, D.L. Cárdenas-Chávez, C. Hernández-Luna, P. Demarche, E. Enaud, R. García-Morales, S.N. Agathos, R. Parra, Purification and characterization of two thermostable laccases from Pycnoporus sanguineus and potential role in degradation of endocrine disrupting chemicals, Journal of Molecular Catalysis B: Enzymatic, 108 (2014) 32-42.

[28] F. d'Acunzo, C. Galli, P. Gentili, F. Sergi, Mechanistic and steric issues in the oxidation of phenolic and non-phenolic compounds by laccase or laccase-mediator systems. The case of bifunctional substrates, New Journal of Chemistry, 30 (2006) 583-591.

[29] L.N. Nguyen, F.I. Hai, W.E. Price, J. Kang, F.D. Leusch, F. Roddick, J.P. van de Merwe, S.F. Magram, L.D. Nghiem, Degradation of a broad spectrum of trace organic contaminants by an enzymatic membrane reactor: complementary role of membrane retention and enzymatic degradation, International Biodeterioration & Biodegradation, 99 (2015) 115-122.

[30] L. Lloret, G. Eibes, G. Feijoo, M.T. Moreira, J.M. Lema, Degradation of estrogens by laccase from Myceliophthora thermophila in fed-batch and enzymatic membrane reactors, Journal of Hazardous Materials, 213–214 (2012) 175-183.

[31] F. Spina, C. Cordero, T. Schilirò, B. Sgorbini, C. Pignata, G. Gilli, C. Bicchi, G.C. Varese, Removal of micropollutants by fungal laccases in model solution and municipal wastewater: evaluation of estrogenic activity and ecotoxicity, J. Clean. Prod., 100 (2015) 185-194.

[32] M.B. Asif, F.I. Hai, J. Kang, J.P. Van De Merwe, F.D. Leusch, W.E. Price, L.D. Nghiem, Biocatalytic degradation of pharmaceuticals, personal care products, industrial chemicals, steroid hormones and pesticides in a membrane distillation-enzymatic bioreactor, Bioresource Technology, 247 (2018) 528-536.

[33] M. Hachi, A. Chergui, A.R. Yeddou, A. Selatnia, H. Cabana, Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from Trametes hirsuta, Biocatalysis and Biotransformation, 35 (2017) 51-62.

[34] A.I. Castro, D.V. Evtuguin, A.M. Xavier, Degradation of biphenyl lignin model compounds by laccase of Trametes versicolor in the presence of 1-hydroxybenzotriazole and heteropolyanion [SiW 11 VO 40] 5−, Journal of Molecular Catalysis B: Enzymatic, 22 (2003) 13-20.

[35] E. Jahangiri, I. Thomas, A. Schulze, B. Seiwert, H. Cabana, D. Schlosser, Characterisation of electron beam irradiation-immobilised laccase for application in wastewater treatment, Science of The Total Environment, 624 (2018) 309-322.

[36] M.B. Asif, F.I. Hai, L. Singh, W.E. Price, L.D. Nghiem, Degradation of Pharmaceuticals and Personal Care Products by White-Rot Fungi—a Critical Review, Current Pollution Reports, 3 (2017) 88-103.

[37] A. Simon, J.A. McDonald, S.J. Khan, W.E. Price, L.D. Nghiem, Effects of caustic cleaning on pore size of nanofiltration membranes and their rejection of trace organic chemicals, Journal of Membrane Science, 447 (2013) 153-162.

[38] Y.-l. Liu, X.-m. Wang, H.-w. Yang, Y.F. Xie, Quantifying the influence of solute-membrane interactions on adsorption and rejection of pharmaceuticals by NF/RO membranes, Journal of Membrane Science, 551 (2018) 37-46.

[39] T. Fujioka, S.J. Khan, J.A. McDonald, L.D. Nghiem, Rejection of trace organic chemicals by a nanofiltration membrane: the role of molecular properties and effects of caustic cleaning, Environmental Science: Water Research & Technology, 1 (2015) 846-854.

[40] J.Y. Hu, X. Jin, S.L. Ong, Rejection of estrone by nanofiltration: Influence of solution chemistry, Journal of Membrane Science, 302 (2007) 188-196.

[41] K.C. Wijekoon, F.I. Hai, J. Kang, W.E. Price, W. Guo, H.H. Ngo, T.Y. Cath, L.D. Nghiem, A novel membrane distillation–thermophilic bioreactor system: Biological stability and trace organic compound removal, Bioresource technology, 159 (2014) 334-341.

[42] J. Garcia-Ivars, J. Durá-María, C. Moscardó-Carreño, C. Carbonell-Alcaina, M.-I. Alcaina-Miranda, M.-I. Iborra-Clar, Rejection of trace pharmaceutically active compounds present in municipal wastewaters using ceramic fine ultrafiltration membranes: Effect of feed solution pH and fouling phenomena, Separation and Purification Technology, 175 (2017) 58-71.

[43] A. Chapple, L.N. Nguyen, F.I. Hai, A. Dosseto, M.H.-O. Rashid, S. Oh, W.E. Price, L.D. Nghiem, Impact of inorganic salts on degradation of bisphenol A and diclofenac by crude extracellular enzyme from Pleurotus ostreatus, Biocatalysis and Biotransformation, 37 (2019) 10-17.

[44] R. Abejón, M. De Cazes, M.P. Belleville, J. Sanchez-Marcano, Large-scale enzymatic membrane reactors for tetracycline degradation in WWTP effluents, Water Research, 73 (2015) 118-131.

[45] M. de Cazes, M.P. Belleville, E. Petit, M. Llorca, S. Rodríguez-Mozaz, J. de Gunzburg, D. Barceló, J. Sanchez-Marcano, Design and optimization of an enzymatic membrane reactor for tetracycline degradation, Catalysis Today, 236 (2014) 146-152.

[46] F. d'Acunzo, P. Baiocco, C. Galli, A study of the oxidation of ethers with the enzyme laccase under mediation by two N–OH–type compounds, New Journal of Chemistry, 27 (2003) 329-332.

[47] R. Bernini, F. Crisante, P. Gentili, F. Morana, M. Pierini, M. Piras, Chemoselective C-4 aerobic oxidation of catechin derivatives catalyzed by the Trametes villosa laccase/1-hydroxybenzotriazole system: synthetic and mechanistic aspects, The Journal of organic chemistry, 76 (2011) 820-832.

[48] H. Mizuno, H. Hirai, S. Kawai, T. Nishida, Removal of estrogenic activity of iso-butylparaben and n-butylparaben by laccase in the presence of 1-hydroxybenzotriazole, Biodegradation, 20 (2009) 533-539.

[49] L.N. Nguyen, F.I. Hai, J. Kang, F.D. Leusch, F. Roddick, S.F. Magram, W.E. Price, L.D. Nghiem, Enhancement of trace organic contaminant degradation by crude enzyme extract from Trametes versicolor culture: Effect of mediator type and concentration, Journal of the Taiwan Institute of Chemical Engineers, 45 (2014) 1855-1862.

[50] M.B. Asif, F.I. Hai, J. Kang, J.P. Van De Merwe, F.D. Leusch, K. Yamamoto, W.E. Price, L.D. Nghiem, Degradation of Trace Organic Contaminants by a Membrane Distillation—Enzymatic Bioreactor, Applied Sciences, 7 (2017) 879.

[51] R. Guha, B. Xiong, M. Geitner, T. Moore, T.K. Wood, D. Velegol, M. Kumar, Reactive micromixing eliminates fouling and concentration polarization in reverse osmosis membranes, Journal of Membrane Science, 542 (2017) 8-17.

[52] D. Breite, M. Went, A. Prager, A. Schulze, Tailoring membrane surface charges: A novel study on electrostatic interactions during membrane fouling, Polymers, 7 (2015) 2017-2030.

[53] C. Schnitzer, S. Ripperger, Influence of surface roughness on streaming potential method, Chemical engineering & technology, 31 (2008) 1696-1700.

List of Tables

Table 1: Physicochemical properties of the selected 29 TrOCs

TrOCs	Chemical formula	Molecular weight g/mole	Log D at pH=7	pK_a	Charge at pH=7
Non-phenolic TrOCs					
Clofibric acid	$C_{10}H_{11}ClO_3$	214.65	-1.06	3.18	-ve
Metronidazole	$C_6H_9N_3O_3$	171.15	-0.14	14.44	Neutral
Fenoprop	$C_9H_7Cl_3O_3$	269.51	-0.13	2.93	-ve
Ketoprofen	$C_{16}H_{14}O_3$	254.28	0.19	4.23	-ve
Naproxen	$C_{14}H_{14}O_3$	230.26	0.73	4.84	-ve
Primidone	$C_{12}H_{14}N_2O$	218.25	0.83	12.26	Neutral
Ibuprofen	$C_{13}H_{18}O_2$	206.28	0.94	4.14	-ve
Propoxur	$C_{11}H_{15}NO_3$	209.24	1.54	12.28	Neutral
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.15	1.77	4.18	-ve
Carbamazepine	$C_{15}H_{12}N_2O$	236.27	1.89	13.94	Neutral
Gemfibrozil	$C_{15}H_{22}O_3$	250.33	2.07	4.75	-ve
Amitriptyline	$C_{20}H_{23}N$	277.4	2.28	9.18	Neutral
N, N-Diethyl-meta-toluamide (DEET)	$C_{12}H_{17}NO$	191.3	2.42	1.37	-ve
Atrazine	$C_8H_{14}ClN_5$	215.68	2.64	2.27	-ve
Ametryn	$C_9H_{17}N_5S$	227.33	2.97	3.71	-ve
Benzophenone	$C_{13}H_{10}O$	182.22	3.21	7.5	Neutral
Octocrylene	$C_{24}H_{27}N$	361.48	6.89	-	-
Phenolic TrOCs					
Salicylic acid	$C_7H_6O_3$	138.12	-1.13	3.01	-ve
Estriol	$C_{18}H_{24}O_3$	298.33	1.89	10.25	Neutral
Enterolactone	$C_{18}H_{18}O_4$	288.38	2.53	9.93	Neutral
Pentachlorophenol	C_6HCl_5O	266.34	2.85	4.68	Neutral
4-tert-Butylphenol	$C_{10}H_{14}O$	150.22	3.4	10.13	Neutral
Estrone	$C_{18}H_{22}O_2$	270.37	3.62	10.25	Neutral
Bisphenol A	$C_{15}H_{16}O_2$	228.29	3.64	10.29	Neutral
17α– Ethinylestradiol	$C_{20}H_{24}O_2$	269.4	4.11	10.24	Neutral
17β–Estradiol	$C_{18}H_{24}O_2$	272.38	4.15	10.27	Neutral
17β-Estradiol-17-acetate	$C_{20}H_{26}O_3$	314.42	5.11	10.26	Neutral
4-tert-Octylphenol	$C_{14}H_{22}O$	206.32	5.18	10.15	Neutral
Triclosan	$C_{12}H_7Cl_3O_2$	289.54	5.28	7.8	Neutral

Note: Data collected from SciFinder database, Taheran et al. (2016); and Fujioka et al. (2015)

Figure Caption

Figure 1. A schematic of the Lab-scale cross-flow filtration system attached to an enzymatic bioreactor. Flow direction is represented by the arrows. EMBRs were operated in continuous-flow mode for a duration of 68 h (approximately 4×HRT). In the schematics, the concentration (ng/L) of a specific micropollutant in the feed, enzymatic bioreactor and permeate is denoted by C_f, C_{EBR} and C_p, respectively, while V_f, V_{EBR} and V_p represent the volume of feed, enzymatic bioreactor and permeate, respectively.

Figure 2. Degradation of TrOCs in enzymatic bioreactor coupled to the UF or NF membrane for showing the effect of effective TrOC retention on degradation. Both enzymatic membrane bioreactors were operated at an initial laccase activity of 180 $\mu M_{(DMP)}$/min, TrOC concentration of 5 µg/L, HRT of 16 h and cross-flow velocity of 40.2 cm/s. The temperature of the enzymatic bioreactor was kept at 25 ºC. Data is presented as average ± standard deviation (n=4).

Figure 3. Enzymatic degradation in both UF- and NF-EMBR as function of TrOC molecular weight, showing that the extent of degradation was significantly higher for TrOCs with a molecular weight above 200 g/mol. Experimental conditions are given in the caption of Figure 2.

Figure 4. Overall TrOC removal in enzymatic bioreactor coupled to the UF or NF membrane. Data is presented as the average ± standard deviation (n=4). Experimental conditions are given in the caption of Figure 2.

Figure 5. Permeate to supernatant (P/S) ratio of the selected TrOCs to show their partial retention by the UF membrane in UF-EMBR. Data is presented as average ± standard deviation (n=4). Experimental conditions are given in the caption of Figure 2.

Figure 6. SEM images of the NF and UF membranes, confirming the formation of enzyme gel-layer on the surface of membranes. The formartion of an enzyme gel-layer on the surface of membranes could improve overall performance of EMBRs *via* TrOC adsorption

Figure 7. Effect of adding a naturally occurring redox-mediator, violuric acid VA, on the degradation of TrOCs in NF-EMBR. VA was added at a concentration of 10 µM at the start of the experiment. Data is presented as the average ± standard deviation (n=4). Experimental conditions are given in the caption of Figure 2.

Figure 8. Effect of different mediator concentration on the degradation of selected TrOCs in NF-EMBR. Data is presented as the average ± standard deviation (n=4).

Figure 9. Variations in the permeate flux presented as a normalized flux as a function of operating time. The reduction in the permeate flux was attributed to: (i) membrane fouling following the adsorption of laccase on membrane surface forming an enzyme gel-layer (*see* Figure 6), and/or (ii) concentration polarization due to the accumulation of TrOCs and transformation products on membrane surface. Cleaning the membranes with clean water for one hour was enough to recover the permeate flux by more than 90%.

Figure 10. Effect of laccase on the properties of the NF and UF membranes. Error bars represent the standard deviation among triplicate measurements. Although change in properties of the NF membrane did not affect TrOC removal by NF-EMBR, the formation of an enzyme-gel layer on the surface of the UF membrane following laccase adsorption can improve the overall performance of UF-EMBR by adsorbing hydrophobic TrOCs (*see* Figure 5)

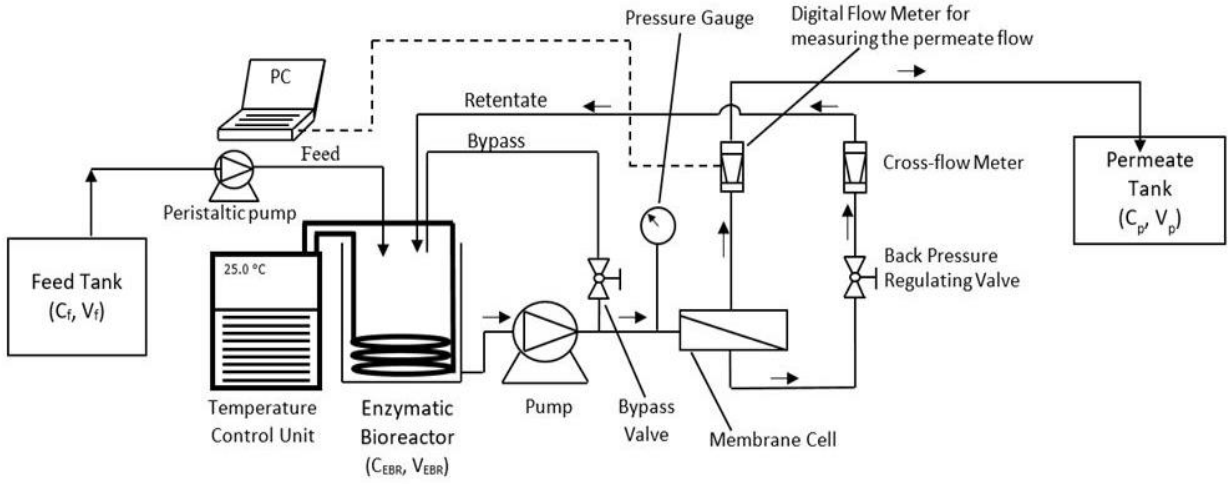

Pressure Gauge
Digital Flow Meter for measuring the permeate flow
PC
Retentate
Bypass
Feed
Peristaltic pump
Cross-flow Meter
Permeate Tank (C_p, V_p)
Feed Tank (C_f, V_f)
25.0 °C
Back Pressure Regulating Valve
Temperature Control Unit
Enzymatic Bioreactor (C_{EBR}, V_{EBR})
Pump
Bypass Valve
Membrane Cell

Figure 1.

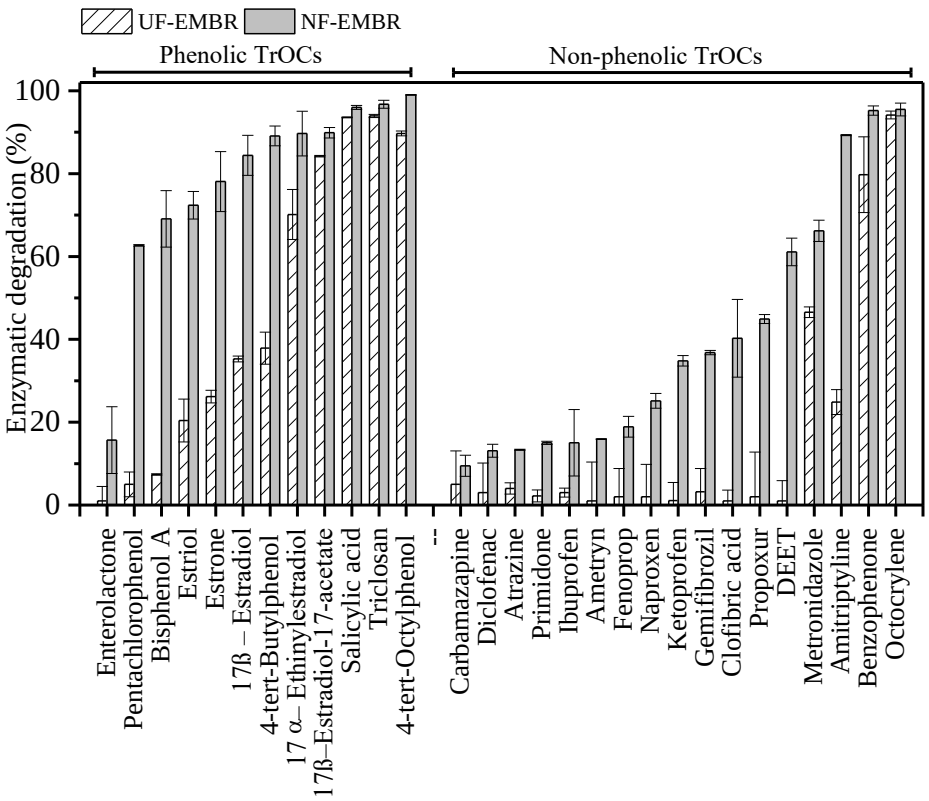

UF-EMBR
NF-EMBR
Phenolic TrOCs
Non-phenolic TrOCs
Enzymatic degradation (%)
0
20
40
60
80
100
Enterolactone
Pentachlorophenol
Bisphenol A
Estriol
Estrone
17ß – Estradiol
4-tert-Butylphenol
17 α– Ethinylestradiol
17ß–Estradiol-17-acetate
Salicylic acid
Triclosan
4-tert-Octylphenol
--
Carbamazapine
Diclofenac
Atrazine
Primidone
Ibuprofen
Ametryn
Fenoprop
Naproxen
Ketoprofen
Gemifibrozil
Clofibric acid
Propoxur
DEET
Metronidazole
Amitriptyline
Benzophenone
Octocrylene

Figure 2.

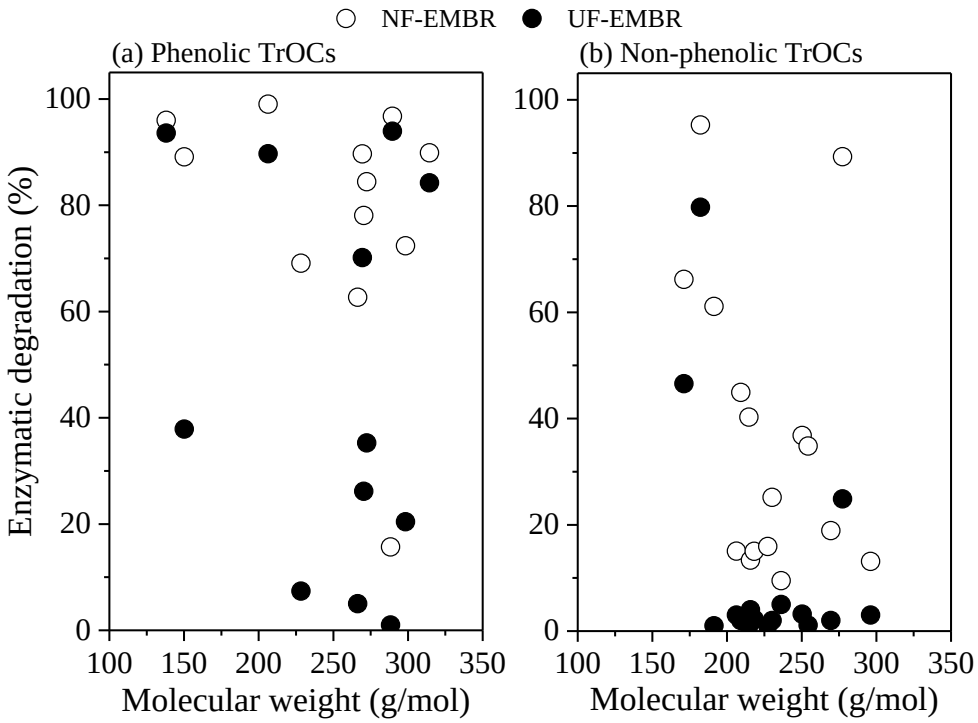

NF-EMBR
UF-EMBR
(a) Phenolic TrOCs
(b) Non-phenolic TrOCs
Enzymatic degradation (%)
Molecular weight (g/mol)
Molecular weight (g/mol)
0
20
40
60
80
100
100
150
200
250
300
350

Figure 3.

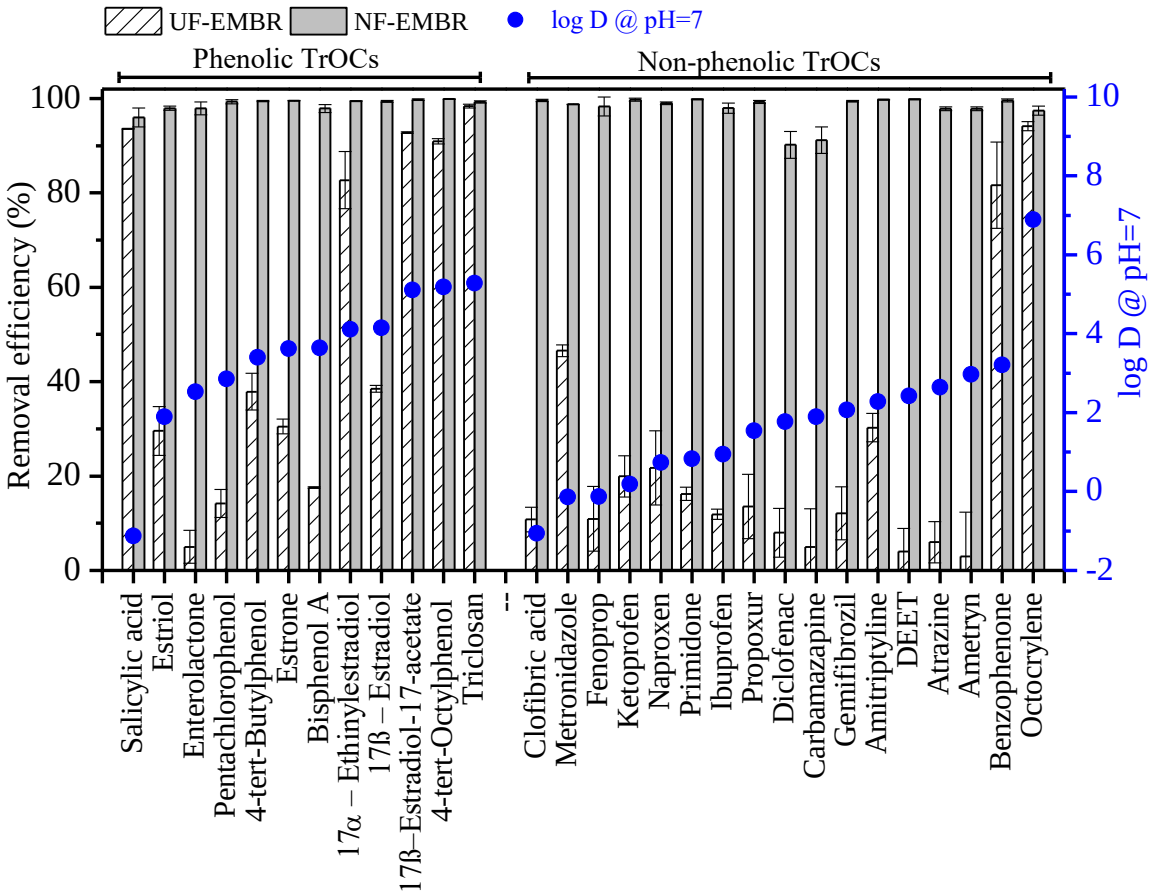

UF-EMBR
NF-EMBR
log D @ pH=7
Phenolic TrOCs
Non-phenolic TrOCs
Removal efficiency (%)
log D @ pH=7
Salicylic acid
Estriol
Enterolactone
Pentachlorophenol
4-tert-Butylphenol
Estrone
Bisphenol A
17α – Ethinylestradiol
17ß – Estradiol
17ß–Estradiol-17-acetate
4-tert-Octylphenol
Triclosan
--
Clofibric acid
Metronidazole
Fenoprop
Ketoprofen
Naproxen
Primidone
Ibuprofen
Propoxur
Diclofenac
Carbamazapine
Gemifibrozil
Amitriptyline
DEET
Atrazine
Ametryn
Benzophenone
Octocrylene

Figure 4.

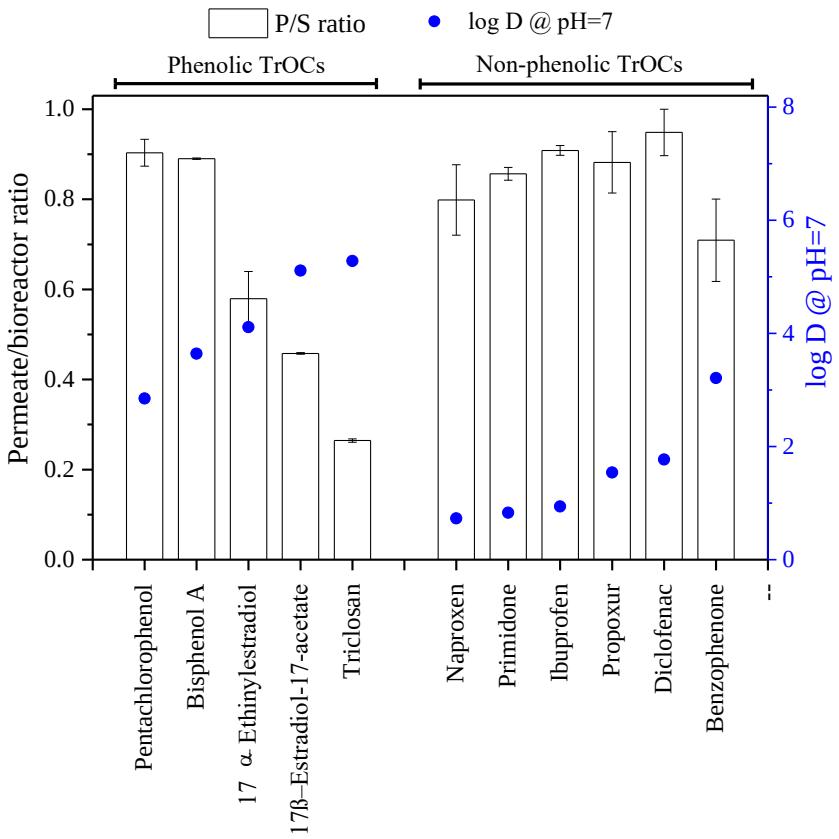

P/S ratio
log D @ pH=7
Phenolic TrOCs
Non-phenolic TrOCs
Permeate/bioreactor ratio
log D @ pH=7
1.0
0.8
0.6
0.4
0.2
0.0
8
6
4
2
0
Pentachlorophenol
Bisphenol A
17 α-Ethinylestradiol
17β-Estradiol-17-acetate
Triclosan
Naproxen
Primidone
Ibuprofen
Propoxur
Diclofenac
Benzophenone

Figure 5.

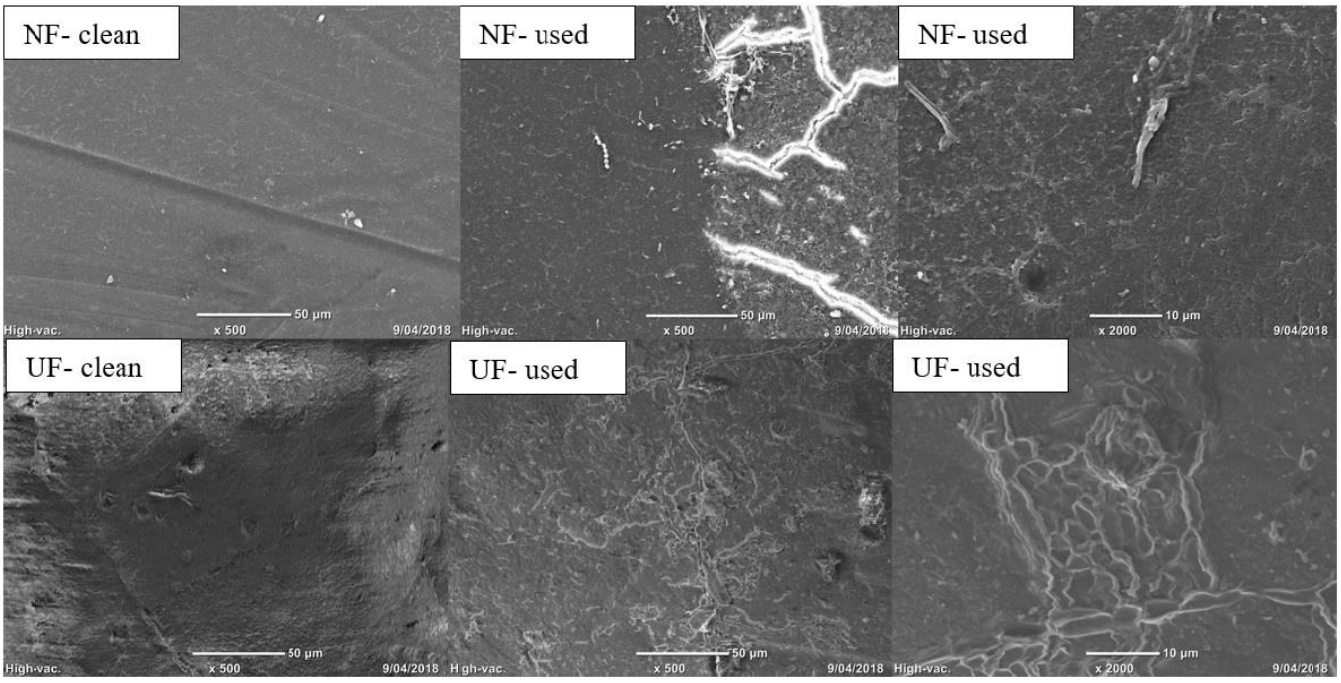

NF- clean
NF- used
NF- used
UF- clean
UF- used
UF- used
High-vac.
x 500
50 µm
9/04/2018
High-vac.
x 500
50 µm
9/04/2018
High-vac.
x 2000
10 µm
9/04/2018
High-vac.
x 500
50 µm
9/04/2018
H gh-vac.
x 500
50 µm
9/04/2018
High-vac.
x 2000
10 µm
9/04/2018

Figure 6.

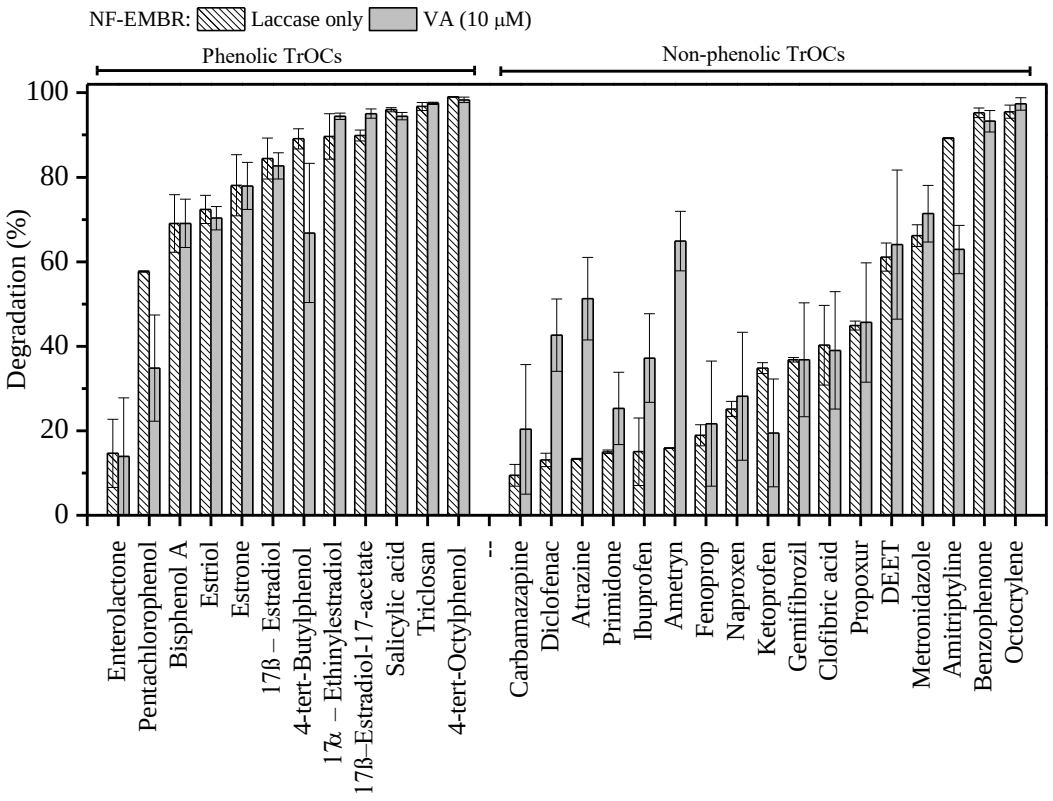

NF-EMBR: Laccase only VA (10 μM)
Phenolic TrOCs
Non-phenolic TrOCs
Degradation (%)
0
20
40
60
80
100
Enterolactone
Pentachlorophenol
Bisphenol A
Estriol
Estrone
17ß – Estradiol
4-tert-Butylphenol
17α – Ethinylestradiol
17ß–Estradiol-17-acetate
Salicylic acid
Triclosan
4-tert-Octylphenol
--
Carbamazapine
Diclofenac
Atrazine
Primidone
Ibuprofen
Ametryn
Fenoprop
Naproxen
Ketoprofen
Gemifibrozil
Clofibric acid
Propoxur
DEET
Metronidazole
Amitriptyline
Benzophenone
Octocrylene

Figure 7.

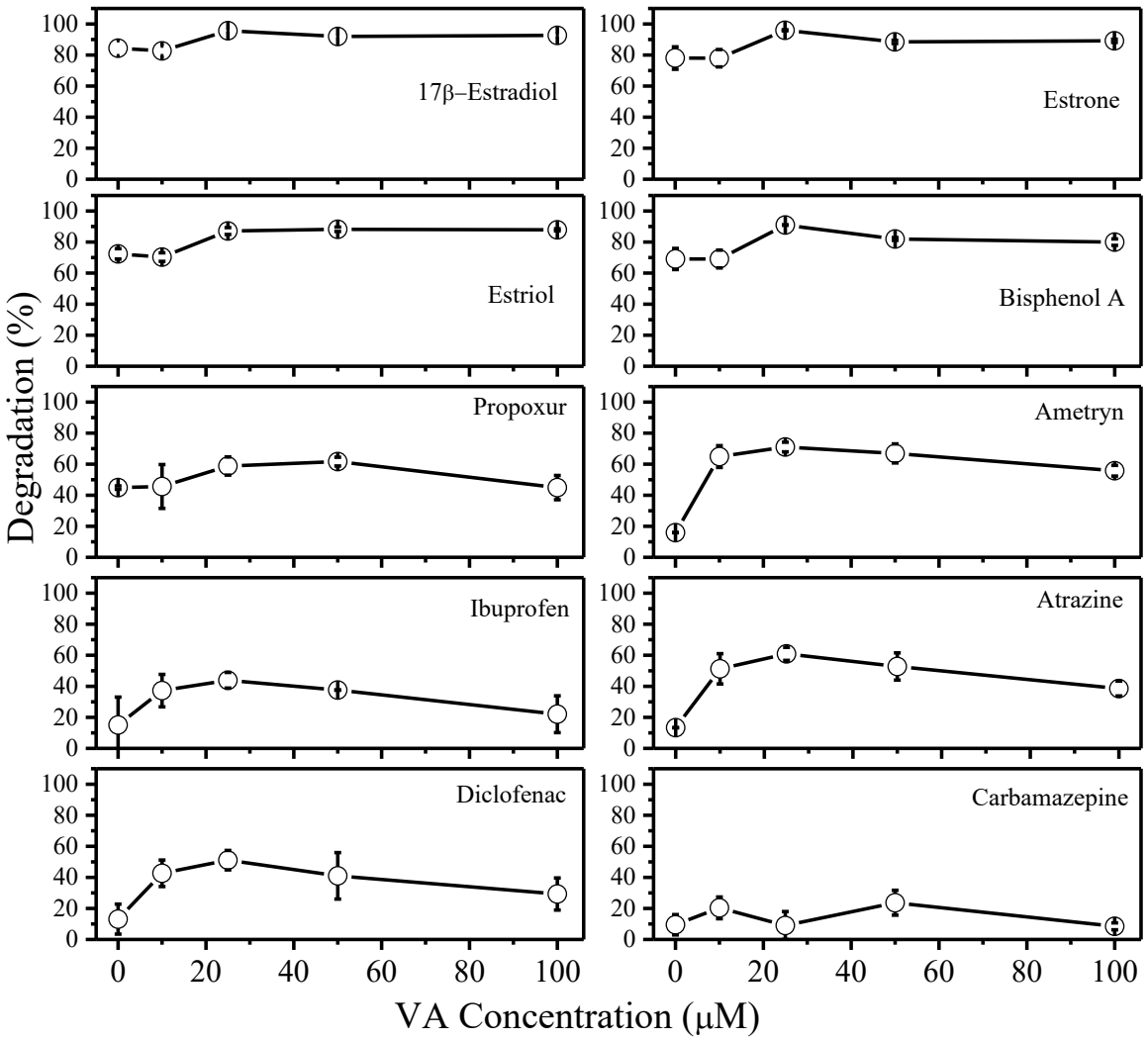

17β–Estradiol
Estrone
Estriol
Bisphenol A
Propoxur
Ametryn
Ibuprofen
Atrazine
Diclofenac
Carbamazepine
Degradation (%)
VA Concentration (μM)
0
20
40
60
80
100

Figure 8.

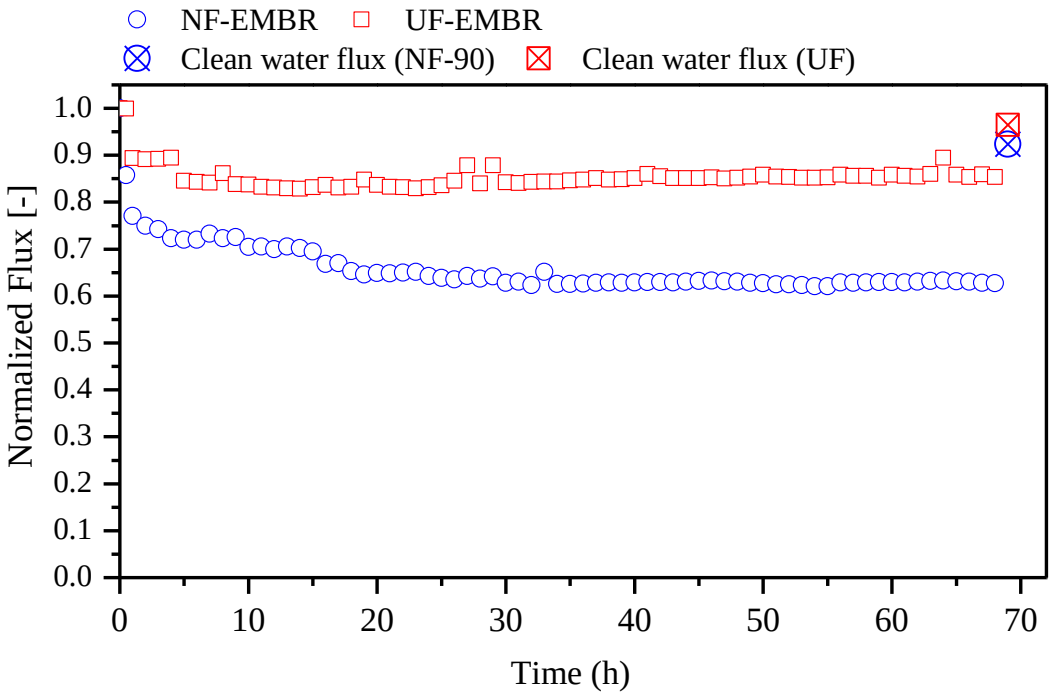

NF-EMBR
UF-EMBR
Clean water flux (NF-90)
Clean water flux (UF)
Normalized Flux [-]
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
Time (h)

Figure 9.

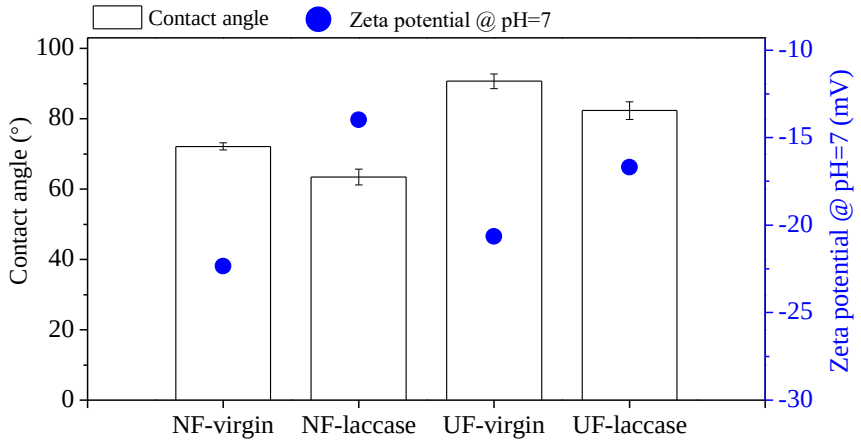
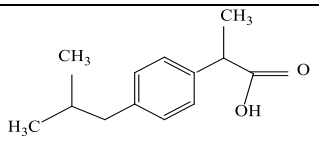
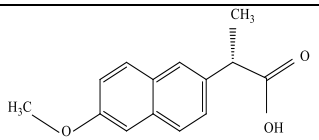
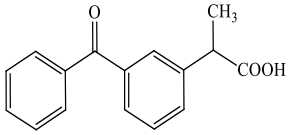
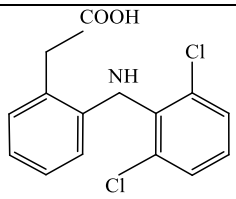
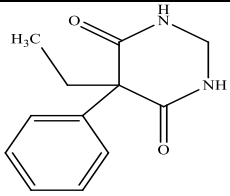
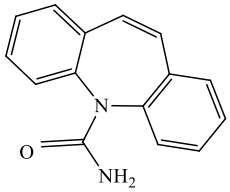
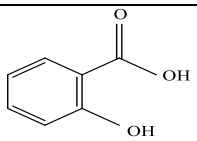

Contact angle
Zeta potential @ pH=7
Contact angle (°)
Zeta potential @ pH=7 (mV)
100
80
60
40
20
0
-10
-15
-20
-25
-30
NF-virgin
NF-laccase
UF-virgin
UF-laccase

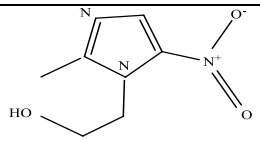
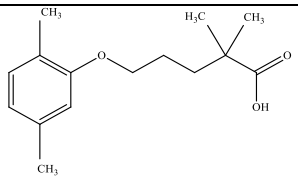
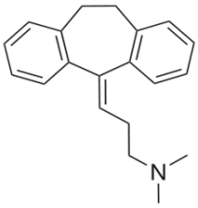
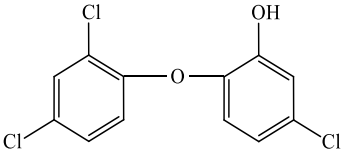
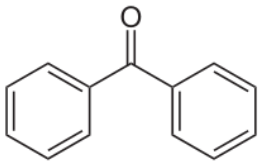
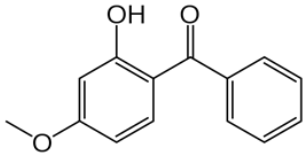
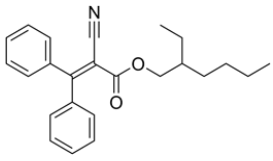
Figure 10.

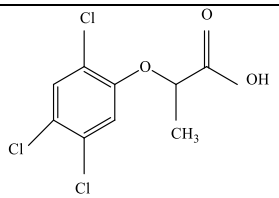
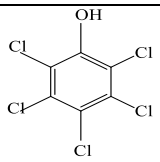
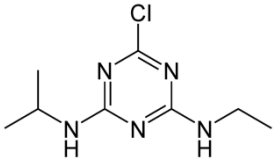
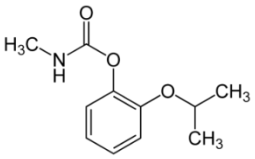
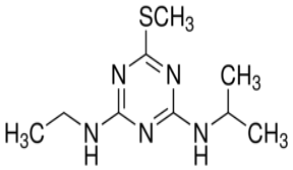
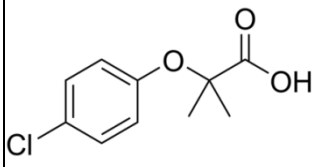
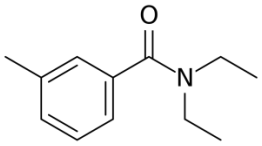
Supplementry information

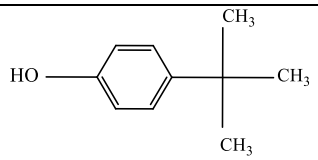
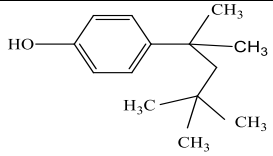
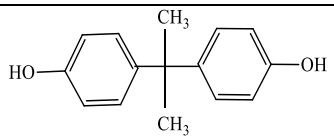
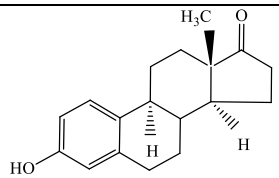
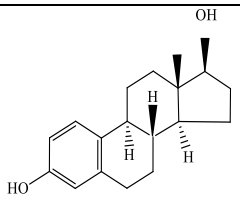
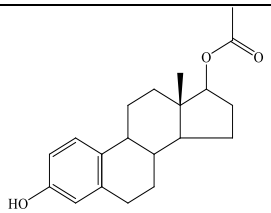
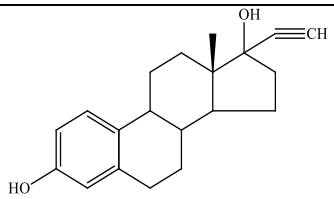
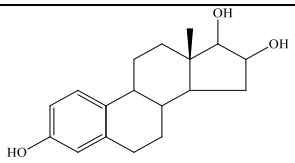
Table of Contents

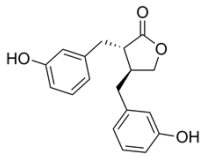
Table S1: Physicochemical properties of the selected trace organic contaminants (TrOCs)

Category	Compound (Formula) (CAS number)	Molecular weight (g/mol)	Log K_{OW}[a]	Limit of detection (ng/L)[b]	Chemical structure
Pharmaceuticals	Ibuprofen ($C_{13}H_{18}O_2$) (5687-27-1)	206.28	3.50 ± 0.23	20	
	Naproxen ($C_{14}H_{14}O_3$) (22204-53-1)	230.26	2.88 ± 0.24	1	
	Ketoprofen ($C_{16}H_{14}O_3$) (22071-15-4)	254.28	2.91 ± 0.33	20	
	Diclofenac ($C_{14}H_{11}Cl_2NO_2$) (15307-86-5)	296.15	4.55 ± 0.57	5	
	Primidone ($C_{12}H_{14}N_2O_2$) (125-33-7)	218.25	0.83 ± 0.50	10	
	Carbamazepine ($C_{15}H_{12}N_2O$) (298-46-4)	236.27	1.89 ± 0.59	10	
	Salicylic acid ($C_7H_6O_3$) (69-72-7)	138.12	2.01 ± 0.25	1	

	Metronidazole ($C_6H_9N_3O_3$) (443-48-1)	171.15	-0.14 ± 0.30	20	
	Gemfibrozil ($C_{15}H_{22}O_3$) (25812-30-0)	250.33	4.30 ± 0.32	1	
	Amitriptyline $C_{20}H_{23}N$ (50-48-6)	277.40	4.40±0.26	1	
Personal care products	Triclosan ($C_{12}H_7Cl_3O_2$) (3380-34-5)	289.54	5.34 ± 0.79	1	
	Benzophenone $C_{13}H_{10}O$ (119-61-9)	182.22	3.21 ± 0.29	5	
	Oxybenzone $C_{14}H_{12}O_3$ (131-57-7)	228.24	3.99±0.36	5	
	Octocrylene $C_{24}H_{27}NO_2$ (6197-30-4)	361.48	6.89±0.33	10	

Pesticides	Fenoprop ($C_9H_7Cl_3O_3$) (93-72-1)	269.51	3.45 ± 0.37	20	
	Pentachlorophenol (C_6HCl_5O) (87-86-5)	266.34	5.12 ± 0.36	1	
	Atrazine ($C_8H_{14}ClN_5$) (1912-24-9)	215.68	2.636±0.205	10	
	Propoxur ($C_{11}H_{15}NO_3$) (114-26-1)	209.24	1.538±0.229	1	
	Ametryn ($C_9H_{17}N_5S$) (843-12-8)	227.33	2.967± 0.12	10	
	Clofibric acid ($C_{10}H_{11}ClO_3$) (882-09-7)	214.65	2.425±0.273	1	
	DEET ($C_{12}H_{17}NO$) (134-62-3)	191.27	2.42 ± 0.23	1	

Industrial chemicals	4-tert-butylphenol ($C_{10}H_{14}O$) (98-54-4)	150.22	3.39 ± 0.21	1	
	4-tert-octylphenol ($C_{14}H_{22}O$) (140-66-9)	206.32	5.18 ± 0.20	1	
	Bisphenol A ($C_{15}H_{16}O_2$) (80-05-7)	228.29	3.64 ± 0.23	1	
Steroid hormones	Estrone ($C_{18}H_{22}O_2$) (53-16-7)	270.37	3.62 ± 0.37	5	
	17β-estradiol ($C_{18}H_{24}O_2$) (50-28-2)	272.38	4.15 ± 0.26	5	
	17β-estradiol 17–acetate ($C_{20}H_{26}O_3$) (1743-60-8)	314.42	5.11 ± 0.28	5	
	17α - ethinylestradiol ($C_{20}H_{24}O_2$) (57-63-6)	269.40	4.10 ± 0.31	10	
	Estriol (E3) ($C_{18}H_{24}O_3$) (50-27-1)	288.38	2.53 ± 0.28	10	

Phytoestrogens	Enterolactone $C_{18}H_{18}O_4$ (78473-71-9)	298.33	1.89± 0.37	10	

[a] Source: SciFinder database https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
Log *D* is logarithm of the distribution coefficient which is the ratio of the sum of concentrations of all forms of the compound (ionised and unionised) in octanol and water at a given pH.

[b]Limit of detection (LOD) of the compounds during GC-MS analysis as described in Section 2.5.2. LOD is defined as the concentration of an analyte giving a signal to noise (S/N) ratio greater than 3. The limit of reporting was determined using an S/N ration of greater than 10.

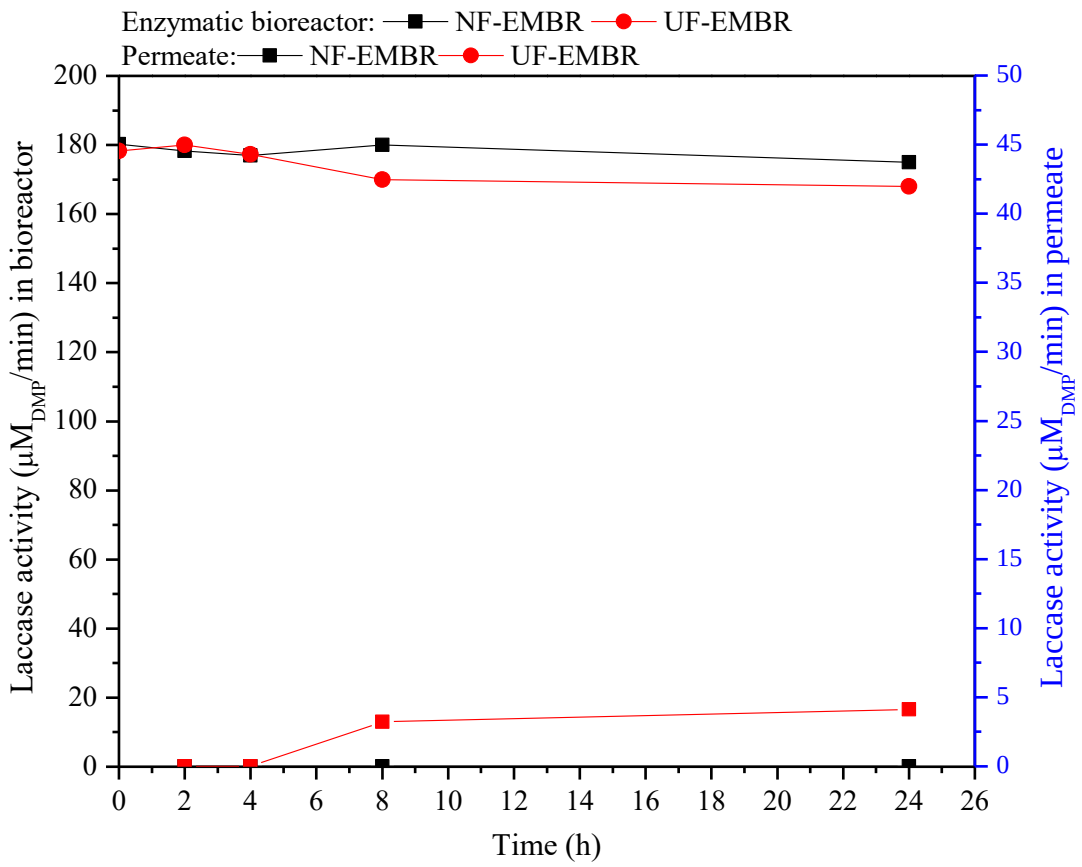


Figure S2: Confirmation of effective laccase retention by the NF and UF membrane. Laccase retention of >99% and 95% was achieved by the NF and UF membrane respectively. UF/NF-EMBR were operated for a period of 24 h in full recirculation mode without the addition of TrOCs.

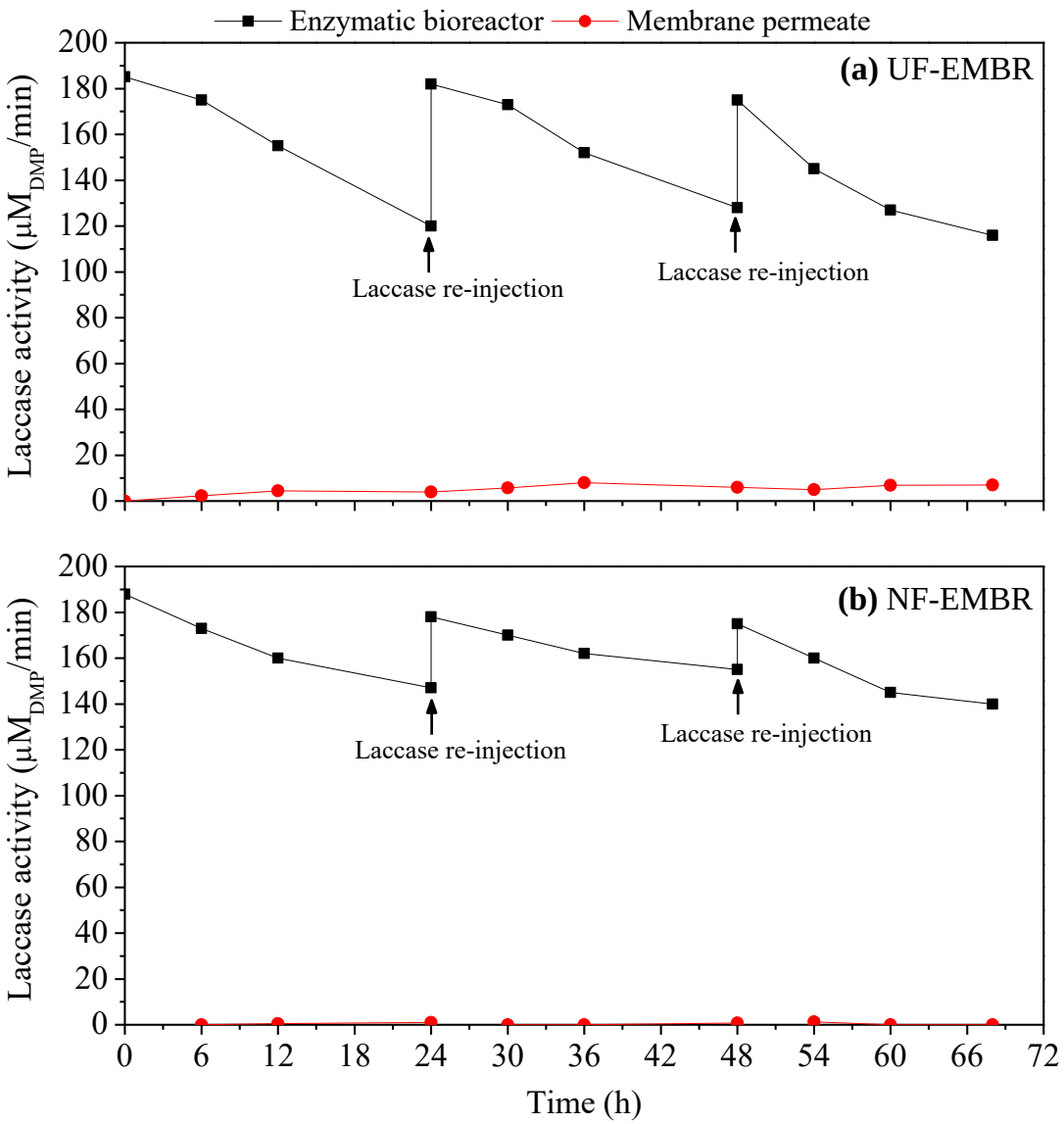


Figure S3: Laccase activity profiles in UF-EMBR (a) and NF-EMBR (b). Laccase activity was maintained by re-injecting a small dose of laccase (250 µL per litre of bioreactor volume) every 24 h.

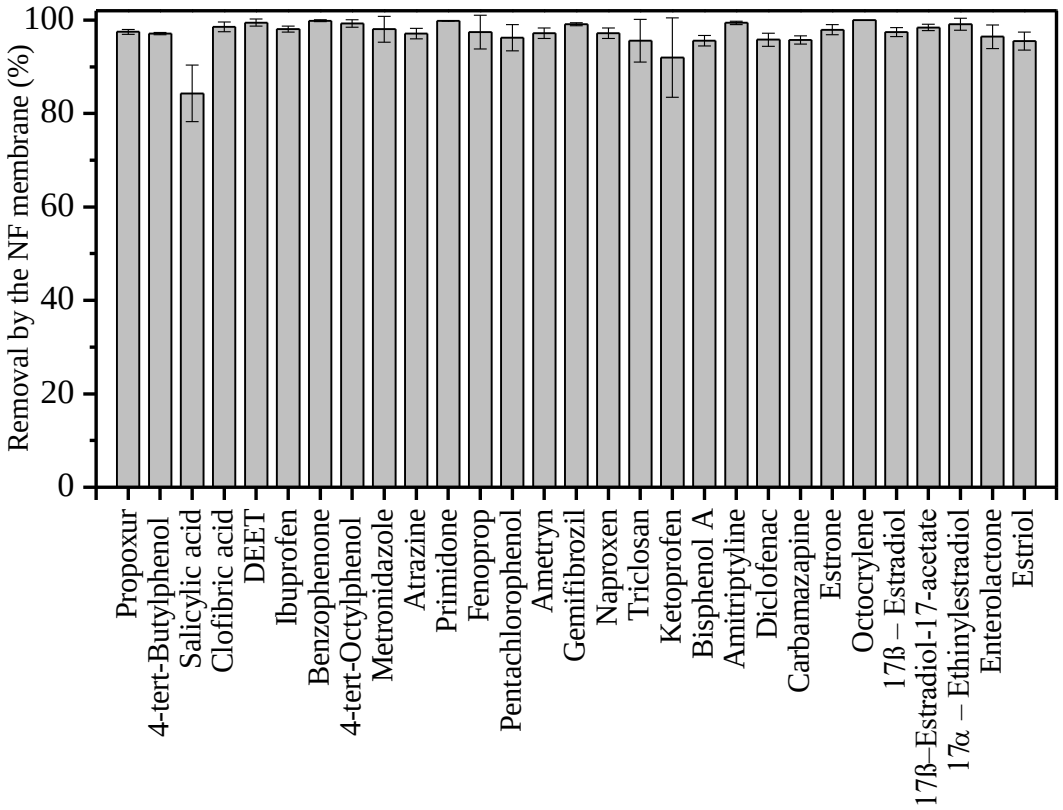


Figure S4: Removal of TrOC by the NF membrane to confirm their effective retention. NF-EMBR were operated for a period of 32 h in continuous mode without the addition of laccase. The UF-EMBR was not operated because the UF membrane was not expected to retain TrOCs. Data presented as average ± standard deviation (n=2).

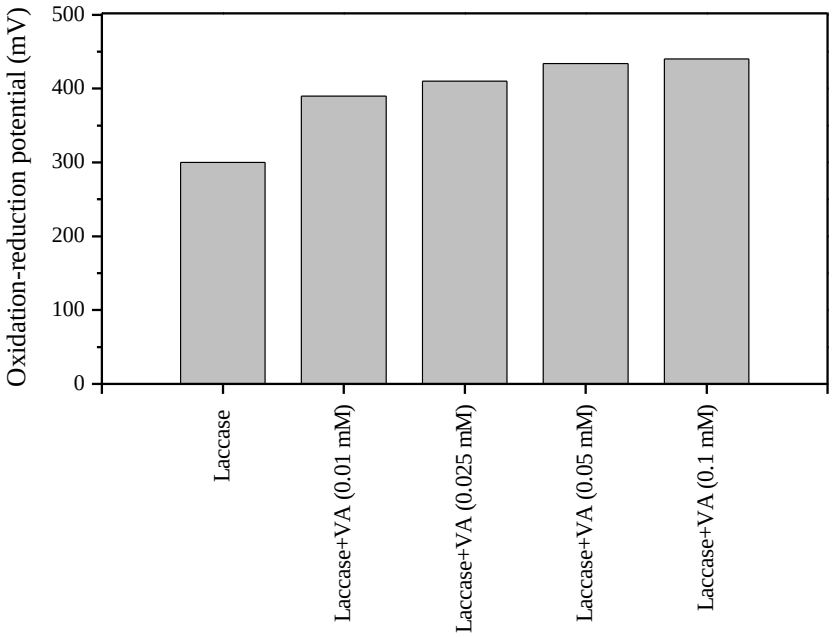


Figure S5: Impact of VA concentration on the oxidation reduction potential of media in NF-EMBR

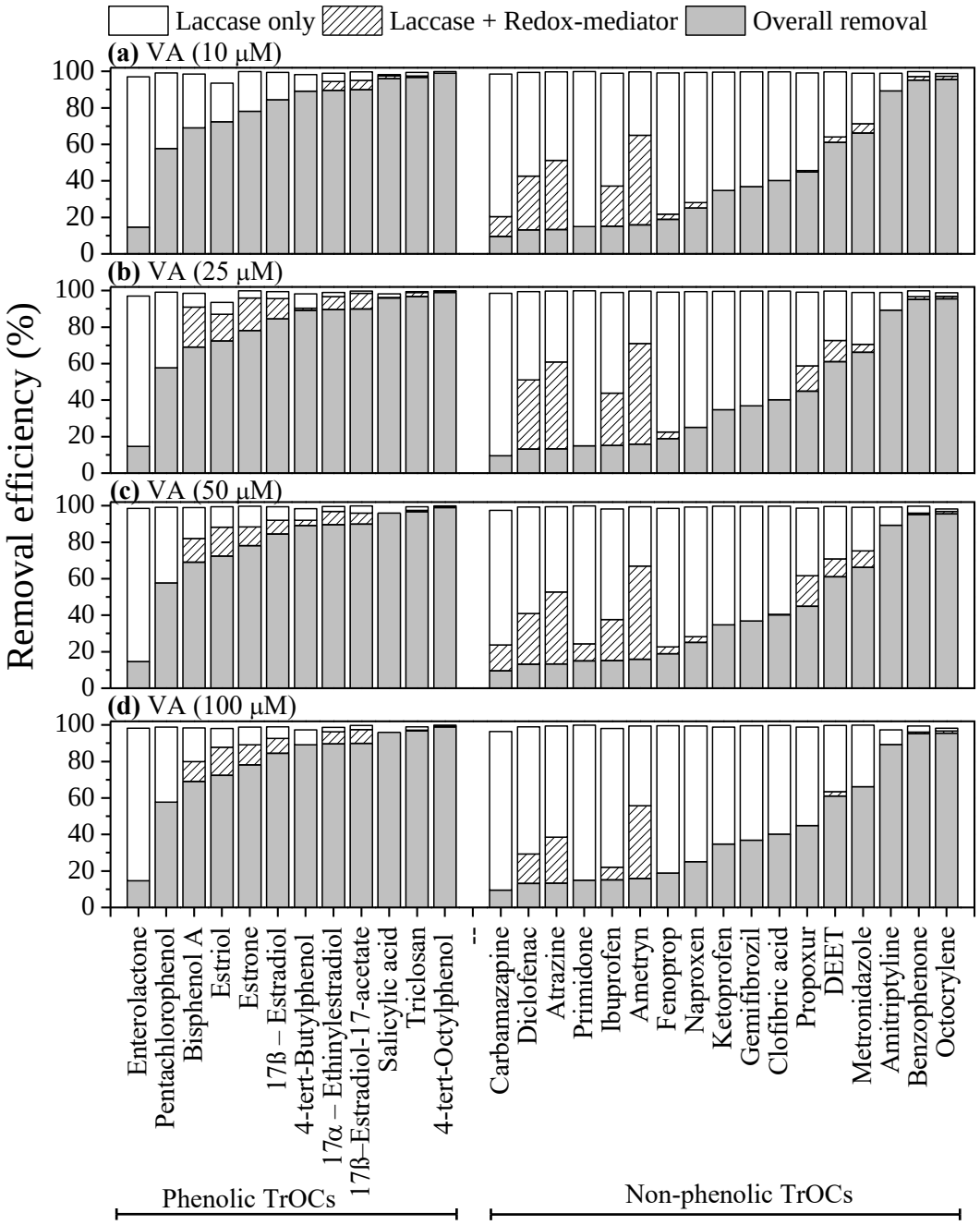


Figure S6: Improvement in TrOC degradation by adding single dose VA at different concentration separately at the start of NF-EMBR operation. VA showed compound-specific and concentration dependent improvement. The overall removal of TrOCs in NF-EMBR was >90%. The NF-EMBR were operated for a period of 68 h in continuous mode at an initial HRT of 16 h.