Continuous transformation of chiral pharmaceuticals in enzymatic membrane bioreactors for advanced wastewater treatment
Luong N. Nguyen, Faisal I. Hai, James A. McDonald, Stuart J. Khan, William E. Price and Long D. Nghiem

ABSTRACT
This study demonstrates continuous enantiomeric inversion and further biotransformation of chiral profens including ibuprofen, naproxen and ketoprofen by an enzymatic membrane bioreactor (EMBR) dosed with laccase. The EMBR showed non-enantioselective transformations, with high and consistent transformation of both (R)- and (S)-ibuprofen (93 ± 6%, n = 10), but lower removals of both enantiomers of naproxen (46 ± 16%, n = 10) and ketoprofen (48 ± 17%, n = 10). Enantiomeric analysis revealed a bidirectional but uneven inversion of the profens, for example 14% inversion of (R)- to (S)- compared to 4% from (S)- to (R)-naproxen. With redox-mediator addition, the enzymatic chiral inversion of both (R)- and (S)-profens remained unchanged, although the overall conversion became enantioselective; except for (S)-naproxen, the addition of redox mediator promoted the degradation of (R)-profens only.

Key words | biotransformation, chiral pharmaceuticals, enantiomeric inversion, enantioselective, enzymatic membrane bioreactor, redox mediator

INTRODUCTION
Pharmaceuticals are continuously released into the environment from households, animal husbandries, aquacultures, and the pharmaceutical manufacturing industry either in unmetabolized or partially metabolized forms (Mompelat et al. 2009). They eventually end up in wastewater and the aquatic environment in the concentrations of a few nanograms per litre to several micrograms per litre (Tran et al. 2013; Hai et al. 2014; Luo et al. 2014). More than 50% of pharmaceuticals are chiral. In other words, they exist as either (R)- or (S)-enantiomers (Nguyen et al. 2006; Khan 2014). Although chiral compounds have similar physical and chemical properties, the biodegradability and toxicity of enantiomers can differ significantly (Nguyen et al. 2006; MacLeod et al. 2007; Khan 2014). For instance, biotransformation of (S)-ibuprofen was faster than (R)-ibuprofen in a constructed wetland (Hijosa-Valsero et al. 2010). The (S)-enantiomer of ibuprofen, naproxen, and propranolol had higher rates of biotransformation than the corresponding (R)- enantiomer in an aerobic wastewater treatment plant (Fono & Sedlak 2005). The toxic effects of fluoxetine, a chiral pharmaceutical widely used for treatment of depression, are enantiomer-dependent with (S)-exhibiting greater toxicity to Pimephales promelas than (R)-fluoxetine (MacLeod et al. 2007).

When assessing the effectiveness of wastewater treatment systems, most studies have monitored the removal of racemic mixtures without consideration of their enantiomeric forms (Luo et al. 2014). Only a few studies have attempted to elucidate chiral inversion using pure enantiomer (Hashim et al. 2011; Kasprzyk-Hordern & Baker 2011). The few available studies have tended to highlight the preferential degradation of one enantiomer (e.g., (S)- over (R)-ibuprofen, and (R)-over (S)-ketoprofen).
An enzymatic membrane bioreactor (EMBR) combines enzymatic treatment and ultrafiltration (UF) to completely retain the enzymes within the reactor and enable enzyme replenishment during continuous operation (Modin et al., 2014; Ji et al., 2016). Among the oxidoreductase enzymes, laccases (E.C. 1.10.3.2) have received much attention due to their capacity to degrade phenolic and certain non-phenolic compounds. An EMBR dosed with laccase was reported to remove a broad spectrum of pharmaceuticals that are resistant to conventional biological processes (Nguyen et al., 2015). The degradation efficiency and substrate spectrum of laccase can be enhanced by the addition of redox mediators, which form reactive radicals upon oxidation by laccase. The reactive radicals act as ‘electron shuttles’ between laccase and the substrate, thus overcoming any effects of steric hindrance (Yang et al., 2013).

Laccase and mediators have been used in the pharmaceutical industry, for example, as a mild aid in the deracemization process to synthesize pure enantiomers (Galletti et al., 2014; Diaz-Rodriguez et al., 2015). Diaz-Rodriguez et al. (2015) used laccase and the mediator 2,2,6,6-tetramethylpiperidin-1-yloxy in the first step of deracemization of racemic 2-phenyl-1-propanol. A laboratory-scale multiphase hollow fibre membrane reactor was employed to produce optically pure butyrate by the lipase-catalysed reaction of racemic glycidyl butyrate (Wu et al., 1993). However, enzymatic chiral inversion and further transformation of pharmaceuticals in water have not been investigated yet.

This study investigates the fate of three chiral fungus profens, namely ibuprofen, naproxen, and ketoprofen, during EMBR treatment. Pure (R)- and (S)-enantiomers were used to facilitate the observation of chiral inversion. A preliminary assessment of enantiomeric transformation of the compounds by laccase was performed in a series of batch tests. This was followed by long-term observation of the fate of the profens during continuous treatment by an EMBR. The impact of addition of a redox mediator, 1-hydroxybenzotriazole (HBT), on the enzymatic transformation of the profens in the EMBR was also evaluated. Overall, this study provides unique insight into the fate of chiral pharmaceuticals with respect to enantiomeric transformation.

MATERIALS AND METHODS

Crude laccase preparation

Crude laccase solution was collected from a culture of white-rot fungus Pleurotus ostreatus (ATCC 34675) grown in malt extract broth (2 g/L) (Merck, Germany). The fungi were incubated under sterile conditions on a rotary shaker at 70 rpm and 28 °C. After 3 days of incubation, the crude enzyme solution (i.e., the liquid portion of the culture) was extracted and stored at 4 °C. The laccase activity in crude enzyme solution was 50–60 μM_{DMP}/min (measured using 2,6-dimethoxyphenol (DMP) as substrate).

To allow EMBR operation with nominal addition of laccase, the crude enzyme solution was concentrated at a volumetric ratio of 20:1 using a spiral wound, tangential flow filtration membrane module (Merck Millipore, Australia) with a molecular weight cutoff of 1 kDa and surface area of 0.23 m². The crude laccase solution was recirculated through the membrane at a cross-flow velocity of 0.007 m/s. The retentate was returned to the enzyme container, and the permeate was discarded. The final solution showed an activity of 500 μM_{DMP}/min, which is less than that expected based on the 20:1 concentration ratio. It was confirmed that the membrane effectively retained laccase (40 kDa); i.e., no enzyme activity was detected in the membrane permeate; however, laccase denaturation may have occurred due to shear stress during the filtration process (Modin et al., 2014; Ji et al., 2016).

Chemicals

Pure (R)- and (S)-enantiomers of ibuprofen, naproxen, and ketoprofen (Supplementary Data Table S1, available with the online version of this paper) and the redox mediator 1-HBT were purchased from Sigma-Aldrich (Australia). The profens were dissolved in pure methanol to make up stock solutions of 0.2 g/L each and stored at −18 °C until use. HBT was dissolved in Milli-Q water to make up a stock solution of 50 mM and stored at 4 °C.

Batch tests

A crude enzyme solution with an initial laccase activity of 50 μM_{DMP}/min was used for the batch tests. Profens were incubated with the crude enzyme with a nominal concentration of 5 μg/L for each compound (actual concentrations were in the range of 1 to 3 μg/L). The first set contained only the (R)-enantiomers. Similarly, the second set contained (S)-profens and the final set included the racemic form of the compounds. All the beakers were covered with aluminium foil and incubated in a rotary shaker at 70 rpm and 28 °C (Bioline Shaker Incubator BL 8600, Edwards Group Pty Ltd, NSW, Australia). The samples were collected at the intervals of 4, 8, 12, 24, and 48 h for profen analysis. Control samples containing only profens in Milli-Q water were
incubated in parallel. The whole test medium was diluted to 500 mL with Milli-Q water and filtered through a 0.45 μm glass fibre filter (Filtech, Australia). All samples were then spiked with a standard solution of isotopically labelled versions of each analyte (50 ng) for isotope dilution quantification. The samples were loaded to solid-phase extraction cartridges under vacuum and maintained with a constant flow rate of 15 mL/min, rinsed with 5 mL Milli-Q water, and finally dried under a gentle nitrogen gas flow for 30 min. The samples were then analysed by gas chromatography tandem mass spectrometry (GC-MS/MS).

**Enzymatic membrane reactor design and operation protocol**

A laboratory-scale EMBR system consisting of a 0.8 L (active volume) glass reactor was used (Supplementary Data Figure S1, available with the online version of this paper). A hollow fibre UF membrane module made of polyacrylonitrile was submerged in the reactor. The membrane was supplied by Microza Membrane (Pall Corporation, NSW, Australia) and had a molecular weight cutoff, surface area and clean water flux of 5 kDa, 0.2 m² and 25 L/(h bar), respectively. The membrane was operated at a flux of 0.5 L/(m² h) via a peristaltic pump (Masterflex L/S, USA) with 8 min on and 1 min off cycles. The reactor was placed in a water bath with a temperature controller unit (Julabo, Germany) to maintain the temperature at 28 °C. Dissolved oxygen concentration was maintained at 5 mg/L via an air pump (ACO-002, Zhejiang Sensen Industry Co. Ltd, Zhejiang, China) connected to a stone diffuser at the bottom of the reactor. The pH of the reactor supernatant remained at 5.6 ± 0.2 (n = 16) without any specific control. The EMBR was operated at a hydraulic retention time of 8 h. Transmembrane pressure was continuously monitored using a high-resolution (±0.1 kPa) pressure sensor (Speri scientific 840064, Extech Equipment Pty Ltd, Victoria, Australia) connected to a computer for data logging.

The EMBR was first operated to confirm retention of the enzyme and the stability of enzymatic activity under the applied hydraulic conditions in this study. Crude laccase solution (12 mL) was diluted to a final volume of 0.8 L in the reactor by Milli-Q water to obtain an initial enzymatic activity of 50 μM(DMP)/min. Enzymatic activity in the permeate and supernatant was measured every 5 h to confirm that the membrane effectively retained the enzyme (i.e., no laccase activity was observed in the permeate). In addition to enzyme retention, the maintenance of enzymatic activity level during the EMBR operation is an important factor.

Denaturation of enzyme due to various factors including physical, chemical and biological inhibitors and the effect of shear stress during filtration has been reported in the literature (Mendoza et al. 2017). In this study, the enzymatic activity was maintained stable at 50 μM(DMP)/min within the reactor by addition of concentrated crude laccase solution at 0.8% of the reactor volume every 12 h.

The EMBR was then operated to investigate enantiomeric transformation for around 72 days (i.e., 18 days each for EMBR-(R)-profens, EMBR-(S)-profens, EMBR-(R)-profens with HBT, and EMBR-(S)-profens with HBT). A synthetic wastewater (i.e., profens in ultrapure water) was used to precisely assess the enzymatic conversion of the profens by the EMBR. In all experiments, each profen was added at an initial concentration of approximately 2.5 μg/L (actual measured concentrations of 2.1 ± 0.2 μg/L (R)-ibuprofen, 2.2 ± 0.2 μg/L (R)-naproxen, 2.8 ± 0.1 μg/L (R)-ketoprofen, 2.4 ± 0.1 μg/L (S)-ibuprofen, 2.4 ± 0.1 μg/L (S)-naproxen, and 2.7 ± 0.1 μg/L (S)-ketoprofen, n = 20). The concentration was selected based on the commonly reported concentration of profens in municipal wastewater. HBT was added continuously via a peristaltic pump at a flow rate of 0.5 mL/min to achieve a mediator concentration of 10 μM in the reactor. Following each operation regime, the EMBR supernatant was discarded and the UF membrane was subjected to backwashing with 1 L Milli-Q water at a flux of 5 L/(m² h). The subsequent operation regime commenced with freshly introduced test solution to the reactor. Although the feed contained either (R)- or (S)-profen, the concentration of both (R)- and (S)-profens in the permeate of the EMBR was monitored to calculate the removal efficiency and quantify any chiral inversion.

**Analytical methods**

**Enzyme assay**

Laccase activity was determined by monitoring the oxidation of 10 mM DMP in 100 mM sodium citrate buffer solution (pH 4.5) over 2 min at room temperature. The measurement was based on monitoring the change in absorbance at a wavelength of 468 nm by a spectrophotometer (UV-visible UV-1700, Shimadzu, Kyoto, Japan). Laccase activity was calculated from the molar extinction coefficient $\varepsilon = 49.6$/mM.cm and expressed in μM(DMP)/min.

**Chiral pharmaceutical analysis**

The method used for GC-MS/MS analysis has been previously described and validated (Hashim & Khan 2011).
Briefly, the enantioseparations of analytes were performed on an HP5-MS fused silica capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) with 0.8 mL/min helium flow. The injector, interface and source temperature were 270, 260 and 280 °C, respectively. Samples were injected (1 μL) in splitless mode with a purge delay of 1 min. GC oven temperature was programmed initially at 120 °C for 1 min, then increased by 40 °C per min to 240 °C and finally by 5 °C per min to 300 °C and maintained for 4 min. The total run time was 18 min per sample. The quantitative detection limit of this analytical method was 5 ng/L. The removal of (R)- and (S)-profens was calculated using Equations (1) and (2).

Removal efficiency of (R)-enantiomer (%)
\[ \text{Removal efficiency of } (R)\text{-enantiomer} = \frac{R_F - R_P}{R_F} \times 100 \]  

Removal efficiency of (S)-enantiomer (%)
\[ \text{Removal efficiency of } (S)\text{-enantiomer} = \frac{S_F - S_P}{S_F} \times 100 \]  

where \( R_F \) and \( S_F \) are the concentration of (R)- and (S)-enantiomer respectively in the feed and \( R_P \) and \( S_P \) are the concentration of (R)- and (S)-enantiomer respectively in the permeate.

The relative concentration of enantiomers of the chiral compounds was expressed as the enantiomeric fraction (EF). The EF of the feed was calculated using Equations (3) and (4) where the feed contained (R)-enantiomer and (S)-enantiomer, respectively.

\[ EF_R = \frac{R_F}{R_F + S_F} \]  
\[ EF_S = \frac{S_F}{S_F + R_F} \]  

The same calculations were used to calculate EF for the permeate as the feed.

For enantiomerically pure starting materials EF equals 1 and in that case reduction in EF following treatment can be used to identify chiral inversion.

The Student’s t-test was performed to verify if the removal efficiency of the enantiomers of a specific compound was statistically different (i.e., \( P \) value < 0.05).

RESULTS AND DISCUSSION

Laccase degradation of profens in batch experiments

The removal of an enantiomer (i.e., disappearance of the parent molecule) implies its chiral inversion and/or transformation to other metabolites. Figure 1 presents the removal of profens in the batch mode after 8 h of incubation.

*Figure 1* | Removal of (R)- and (S)-profens and enantiomeric fraction (EF) of profens following laccase treatment over 8 h in batch test conditions. The error bars represent the standard deviation (n = 10).
Under the laccase dose of 50 μM(DMP)/min and profen loading of 5 μg/L, the removals of all profens were below 30%. No improvement in the removal efficiency was observed when the incubation time was increased to 48 h (Figure 2). This is consistent with previous reports regarding batch enzymatic degradation systems where degradation products may interfere with further enzymatic degradation of the parent compound (Tauber et al. 2005; Cajthaml 2015). More importantly, as indicated by EF, no significant chiral inversion was observed in batch experiments (Figure 1). In other words, the pure enantiomers were oxidized mainly to other metabolites than the opposite enantiomer. Possible metabolites of profens were not analysed in this study; however, these can be identified from the relevant literature. For example, 1-hydroxyibuprofen, 1,2-dihydroxyibuprofen and 2-hydroxyibuprofen are known metabolites from laccase treatment of racemic ibuprofen (Marco-Urrea et al. 2009). Notably, additional batch tests with racemic solutions showed a change in EF for naproxen (Figure 2). Because the pure enantiomers did not show chiral inversion in the batch tests, this change in EF of the racemic solution following enzymatic treatment does not mean chiral inversion; rather it implies different biodegradation rates for the (R)- and (S)-enantiomers.

Overall, the batch tests with pure enantiomers demonstrated that the laccase used in this study could oxidize both (R)- and (S)-profens without any apparent chiral inversion or variability in removal rates. However, the reaction dynamics in a continuous flow EMBR are different from the batch test conditions. For example, in an EMBR,
Degradation products are continually removed from the reaction site. Indeed, Nguyen et al. (2015) demonstrated that an enzyme gel layer formed on the membrane retained pharmaceuticals and thus facilitated their degradation. Thus, further investigations with continuous flow EMBRs were conducted and the results are discussed in the following sections.

Removal of profens by EMBR

Figure 3 shows the removal efficiency of (R)- and (S)-profens during continuous treatment. ‘Removal’ here means chiral inversion and/or transformation to other products. Similar removal was observed for (R)- and (S)-ibuprofen (90 ± 5 and 93 ± 6% (n = 10) removal, respectively). The ibuprofen removal observed in the current study is almost 20% higher than that in a previous study (Nguyen et al. 2013) where racemic ibuprofen instead of pure enantiomers was used. It is possible that the low removal of racemic ibuprofen in the previous study (Nguyen et al. 2013) was due to the competition of the two enantiomers for the active sites of laccase, or the inhibition of one enantiomer for the enzyme for which the other enantiomer is a substrate (Kasprzyk-Hordern & Baker 2011). However, this cannot be confirmed because in addition to the difference in feed (racemic mixture vs pure enantiomers) a different laccase preparation was used in the previous study.

Compared to the high and consistent transformation of both (R)- and (S)-ibuprofen (93 ± 6%, n = 10), lower removals of both enantiomers of naproxen (46 ± 16%, n = 10) and ketoprofen (48 ± 17%, n = 10) were observed. This observation is consistent with previous studies confirming higher resistance of naproxen and ketoprofen to enzymatic degradation compared to ibuprofen (Nguyen et al. 2015). Nguyen et al. (2015) reported that racemic naproxen and ketoprofen were resistant to EMBR treatment possibly due to the presence of the carboxylic group (-COOH), which is an electron demanding functional group.

Compared to the batch tests (Figure 1), the EMBR showed a better removal efficiency of all profens (Figure 3). The higher removal of the profens by the EMBR may be due to retention of profens by a dynamically formed enzyme gel layer on the membrane, which may have facilitated further degradation. During the filtration process the enzyme can form a thin gel layer on the membrane (Modin et al. 2014). This thin enzyme gel layer may adsorb or retain the pollutants in the reactor and degrade them further as demonstrated in a previous EMBR study (Nguyen et al. 2015), which, however, investigated racemic mixtures of profens, not their pure enantiomers.

Enantioselective degradation, i.e., preferential degradation of one enantiomer over the other, has been reported in previous studies, where racemic mixtures were used (Hanlon et al. 1994; Hung et al. 1996). For example, Hanlon et al. (1994) reported that the fungus Verticillium lecanii degraded (R)-ibuprofen selectively when they used racemic ibuprofen. On the other hand, Hashim et al. (2011) observed preferential degradation of (S)-ibuprofen by conventional activated sludge. Enantioselective degradation of chiral pharmaceuticals in the wastewater treatment plants can lead to the accumulation of certain enantiomers in the environment (Hashim et al. 2010). However, the current study confirms that laccase degrades both (R)- and (S)-profens with similar efficiencies.

It is noted that the EMBR technology has not been extensively evaluated using real wastewater. So far, only one study successfully operated an EMBR for 100 h with minimal repetitive addition of enzyme for the removal of oestrogenic compounds from filtered secondary effluent (Lloret et al. 2015). The physical and chemical properties of the wastewater to be treated could adversely affect the enzymatic activity, stability and substrate specificity. It is
important to consider these properties in EMBR process design and optimization for scale-up. However, this is beyond the scope of the current study.

**Chiral inversion of profens in EMBR**

The test solutions of (R)- and (S)-profens showed an EF of 0.99, indicating only trace impurities. Any decrease in EF during the EMBR treatment would indicate chiral inversion (Equations (3) and (4)). The concentrations of (R)- and (S)-profens and the EF in the permeate of the EMBR at different sampling events (Figure 4) illustrated a compound-specific bidirectional inversion of the chiral pharmaceuticals. This appears to be the first evidence of the bidirectional inversion of the tested profens, i.e., ibuprofen, naproxen and ketoprofen. Previous studies have mostly reported unidirectional inversion of profens by microbes (Hutt et al. 1995; Hashim et al. 2010; 2011).

**Figure 4** | The concentration of (R)- and (S)-enantiomers in the EMBR feed and permeate, and enantiomeric fractions (EF) of the profens in the permeate, showing chiral conversion. For the EMBR receiving (R)-profens, the EF was calculated by \( \frac{[R]}{[R] + [S]} \) and the EF in the influent was 0.99 ± 0.01 (n = 10). For the EMBR receiving (S)-profens, the EF was calculated by \( \frac{[S]}{[R] + [S]} \) and the EF in the influent was 0.99 ± 0.01 (n = 10).
The EF of ibuprofen in the permeate was $0.82 \pm 0.03$ and $0.75 \pm 0.04$ ($n = 10$), when the feed contained (R)-ibuprofen and (S)-ibuprofen, respectively. The concentrations of (R)- and (S)-enantiomer in the permeate was quantified and calculated to be less than 2% of initial (S)- or (R)-enantiomer, respectively. This is low compared to the overall removal of over 90% of ibuprofen, meaning that ibuprofen was predominantly biotransformed to other metabolites.

The EF of naproxen in the EMBR permeate was determined to be $0.74 \pm 0.02$ and $0.92 \pm 0.01$, ($n = 10$), when the feed contained (R)- and (S)-naproxen, respectively, confirming greater inversion of (R)- to (S)-naproxen. Compared to $46 \pm 18$ and $46 \pm 13\%$ overall removal of (R)- and (S)-naproxen (Figure 3), respectively, $14 \pm 4\%$ of (R)-naproxen in the influent was inverted to (S)-enantiomer, while only $4 \pm 1\%$ of (S)-naproxen was inverted to (R)-naproxen (Figure 4). The mode of enantiomeric inversion of naproxen by fungal laccase in this study appears to be somewhat different from that by bacteria. For example, a significant increase of (R)-naproxen in treated effluent was noticed after treatment of a synthetic wastewater containing...
(S)-naproxen by bacteria-dominated activated sludge (Hashim et al. 2011).

A minor change in the EF of ketoprofen was observed when the EMBRs were fed with its (R)- or (S)-enantiomers, confirming minimal chiral inversion (Figure 4) and non-enantioselective biodegradation (Figure 3). Indeed ketoprofen is widely administered as a racemic mixture, and minimal enantioselectivity during human metabolism has been reported (Jamali & Brocks 1990). Hashim et al. (2011) also observed no significant change in EF of ketoprofen during treatment by bacteria-dominated activated sludge.

**Impact of redox-mediator dosing**

In previous studies, laccase-mediator systems have been demonstrated to enhance the removal efficiency of pharmaceuticals (Yang et al. 2013; Nguyen et al. 2014). Two factors contribute to the enhanced performance of the mediator-amended enzyme systems: (i) reduction of steric hindrance, and (ii) higher redox potential of the hydroxyl radicals generated (Yang et al. 2013). As shown in Figure 5, in this study, HBT addition consistently improved the removal of (R)-profens: an improvement of 10 to over 50% was achieved ($p < 0.05$, Table 1). However, HBT addition had no effect on the chiral inversion of (R)-profens. Apparently, the radicals which are produced via oxidation of HBT by laccase could easily attack the (R)-enantiomers. Indeed Tanaka et al. (2001) reported that the addition of mediator hydroquinone to a reactor containing the yeast Trichosporon cutaneum was effective for the degradation of (R)-ibuprofen and the repression of (S)-ibuprofen degradation. Tanaka et al. (2001) explained that hydroquinone may have had inhibited mono- or dioxygenase enzymes which were essential for initial degradation of (S)-ibuprofen.

In this study, except for (S)-naproxen, the removal of (S)-profens remained unaffected by HBT addition. Additionally, HBT addition showed negligible impact on the conversion of (S)- to (R)-profens. This observation indicates that with HBT addition, degradation to other metabolites proceeds preferentially over enantiomeric inversion. This is probably because hydroxyl radicals generated by the laccase–HBT system promoted profen degradation.

It is noteworthy that operating conditions such as pH, enzyme loading, temperature, profen concentrations, and the presence of wastewater-derived inhibitory compounds could impact on the performance of the EMBR system. The effects of these conditions on the enzymatic degradation of pharmaceuticals including profens have been elucidated in the literature (Lloret et al. 2010; Wen et al. 2010; Zhang & Geißen 2010). However, there has been no study about their impact on the enzymatic inversion of chiral profens. This is the first study on the enzymatic chiral inversion and further transformation of pharmaceuticals in water. Future studies specifically focusing on the impact of relevant operating conditions on chiral transformation during EMBR treatment are recommended.

**CONCLUSION**

Enantiomeric inversion and further degradation of profens by an EMBR were investigated for the first time. Bidirectional inversion of the chiral pharmaceuticals, particularly for naproxen, was observed. Overall profen degradation was non-enantioselective, with high and consistent removal of both (R)- and (S)-ibuprofen but relatively lower levels of removal of the enantiomers of naproxen and ketoprofen. The influence of redox mediator on the enzymatic degradation of some profens appeared to be enantioselective. The addition of redox mediator promoted the degradation of (R)-profens mainly, while showing no impact on chiral inversion.

**ACKNOWLEDGEMENTS**

The University of Wollongong is thanked for a Career Launch Fellowship to Dr Luong N. Nguyen. This study was partially funded by a grant from the GeoQuEST Research Centre, University of Wollongong.

**REFERENCES**


First received 8 March 2017; accepted in revised form 22 May 2017. Available online 20 June 2017