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Degradation of metoprolol from wastewater in a bio-electro-Fenton system

Xiaoyong Yang, Rusen Zou, Kai Tang, Henrik Rasmus Andersen, Irini Angelidaki, Yifeng Zhang*

Department of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark

*Corresponding author:

Dr. Yifeng Zhang

Department of Environmental Engineering, Technical University of Denmark, Denmark

Tel: (+45) 45251410

Fax: (+45) 45933850

E-mail address: yifz@env.dtu.dk; yifzmfc@gmail.com
Abstract

Advanced oxidation processes (AOPs) have been intensely studied for the removal of refractory pollutants because of the strong oxidizing capacity of hydroxyl radical. One of the emerging AOP methods gaining increased attention is bio-electro-Fenton (BEF) which can generate hydroxyl radical in-situ in the cathode chamber using the energy harvested by exoelectrogenic bacteria in the anode. In this study, the feasibility of BEF technology for the removal of metoprolol, a typical micropollutant widely found in the water environment, was for the first time investigated. It was found that applied voltage and working pH had a significant effect on removal efficiency while Fe$^{2+}$ dosage as catalyst showed a little effect. Besides removal by hydroxyl radical, metoprolol might be absorbed on the surface of the reactor, electrode, and precipitated with iron sludge, especially at neutral pH. In a batch experiment with a supplied voltage of 0.2 V, pH 3, and a Fe$^{2+}$ dose of 0.2 mM, the removal rate of metoprolol in the BEF for the synthetic wastewater and the real effluent from the secondary sediment tank was 66% and 55% within 12 hours, respectively. A possible degradation pathway was proposed. Then the removal of metoprolol in a continuous flow BEF system was further studied at different hydraulic retention times (HRTs) of 2, 4, and 6 h, about 77%, 92%, and 95% removal was observed. A toxicity test (less than 20% inhibition on bioluminescence) during treatment and energy cost analysis ($5.269 \times 10^{-3}$ kWh/order/m$^3$) in treating 10 μg/L of metoprolol containing wastewater effluent at continuous flow mode implied that the proposed BEF has a potential for wastewater treatment.

**Keywords:** Advanced oxidation process; Bio-electro-Fenton system; Micropollutants; Metoprolol; Degradation pathway; Wastewater treatment
1. Introduction

Micropollutants (also termed as emerging pollutants) have been found ubiquitous in water environments and therefore become a worldwide concerning issue recently. Hospital wastewaters and even the effluents from wastewater treatment plants (WWTPs) have been found contain a variety of micropollutants and the concentrations vary from ng/L to µg/L (Deblonde et al., 2011; Verlicchi et al., 2010). To improve the removal efficiency for these pharmaceuticals, several technologies have been developed including chlorination, UV irradiation, coagulation-flocculation, activated carbon adsorption, and membrane filtration (Khetan and Collins, 2007). However, with these technologies, many pollutants still cannot be fully eliminated, or they just were transferred from one phase to another phase. Meanwhile, pollutants enriched side streams, used adsorbents, and sludge were generated which need to be further collected and treated (Dhodapkar and Gandhi, 2019). Advanced oxidation processes (AOPs) have been developed recently to effectively mineralize organic pollutants because of its strong oxidation capacity and non-selectivity to organics (Oturan and Aaron, 2014). Among diverse AOPs, electro-Fenton (EF) process is widely used to treat pharmaceuticals contained wastewaters (García-Montoya et al., 2015; J Radjenovic et al., 2011). The EF process is an indirect electrochemical AOP during which hydroxyl radicals are generated in the solution from Fenton’s reaction (Eq. (1)) instead of directly produced at the electrode surface from charge transfer. Afterward, the pollutants can be destroyed by the hydroxyl radicals. H₂O₂ is supplied according to Eq. (2). Besides, sufficient Fe²⁺ also needs to be added to ensure the occurrence of the reaction. Thereafter, Fe²⁺ can be cycled through ferric reduction (Eq. (3)).

Metoprolol, one of the most commonly used β-blockers for treating cardiovascular diseases (Escher et al., 2006), is quite frequently detected in the water environment (Hughes et al., 2012).
The removal efficiency of metoprolol in WWTPs was quite different depending on the design and operation parameters of WWTPs (Falås et al., 2012). Generally, metoprolol was regarded as poorly removed by WWTPs with a removal efficiency from negative value to less than 60% (Luo et al., 2014). The concentration of metoprolol might vary from \(10^{-2}\) to \(10^1\) \(\mu\)g/L in urban wastewaters and \(10^{-1}\) to \(10^2\) \(\mu\)g/L in hospital wastewaters (Verlicchi et al., 2010). The degradation efficiency of several β-blockers such as atenolol, metoprolol, and propranolol has been investigated in the EF process. Most of these compounds can be degraded in 5 to 30 min and completely mineralized in several hours (Isarain-Chávez et al., 2010a, 2010b). Notably, the current density applied was in a wide range of 40-10000 A m\(^{-2}\) which means considerable energy consumption was involved in the treatment (Isarain-Chávez et al., 2010a, 2010b). It was pointed out that though EF process owns high pollutants removal efficiencies, the inherent shortages including high energy input hindered its application to real wastewater treatment (Olvera-Vargas et al., 2017; Ribeiro et al., 2015).

\[
\begin{align*}
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{O}_2^- \quad (1) \\
\text{O}_2 + 2\text{H}^+ + 2\text{e}^- & \rightarrow \text{H}_2\text{O}_2 \quad (2) \\
\text{Fe}^{3+} + \text{e}^- & \rightarrow \text{Fe}^{2+} \quad (3)
\end{align*}
\]

In a bio-electro-Fenton (BEF) system, microorganisms are employed to catalyze the oxidation of organic substrates at the anodic compartment while the electrons generated are then transferred to cathode electrodes for the in-situ synthesis of \(\text{H}_2\text{O}_2\) according to Eq. (2). Subsequently, hydroxyl radicals are formed in-situ of the cathode (Eq. (1) and (3)). Compared with the EF process for pharmaceuticals removal, BEF can greatly reduce energy cost and is regarded as an emerging sustainable technology for the treatment of persistent organic pollutants from
wastewater. Several attempts have been made to remove refractory organic compounds like dyes, pharmaceuticals, industrial pollutants using the BEF process (Li et al., 2017; Yong et al., 2017; Zou et al., 2020). However, degradation of metoprolol using BEF has not been investigated. Therefore, in this present study, the removal efficiency of metoprolol in a lab-scale BEF system at room temperature was investigated. The effect of applied voltage, pH, and ferrous ions concentration on metoprolol degradation was firstly explored at batch mode. Afterwards, the system was changed to continuous flow mode to verify its feasibility of treating real wastewater at a more realistic concentration. Moreover, the degradation byproducts during the treatment process were detected and the toxicity of the treated water was evaluated. Besides, the energy consumption aspects of the proposed technology were compared with other existing methods.

2. Materials and methods

2.1 Chemicals

Metoprolol tartrate purchased from Sigma-Aldrich (USA) was dissolved in methanol as 1.00 g metoprolol/L stock solution. Sodium sulfate anhydrous (purity ≥ 99%) and ferrous sulfate heptahydrate (purity ≥ 99%) were used as electrolytes and catalysts, respectively. Sulfuric acid (95 ~ 97% purity, Sigma-Aldrich) was used to adjust the electrolyte pH to the desired value. All solvents including methanol, acetonitrile as well as tertiary butanol were HPLC-grade. All the other chemicals used were analytical grade. Solutions were prepared with deionized water.

2.2 BEF system setup and operation

The BEF consisted of two equal rectangular polymethyl methacrylate frames assembled by stainless bolts and nuts (Fig. 1). A cation exchange membrane (CEM) (CMI 7001, Membrane International, NJ, USA) was used to separate the anode and cathode chambers. The membrane
was immersed in a 5% NaCl solution overnight before use. Rubber gaskets were inserted between each part to prevent leakage. The internal dimensions of each frame were 8 × 5 × 5 cm. A carbon brush (Mill-Rose, USA) preheated in a muffle furnace at 450 °C for 30 min was used as an anode electrode. A cubic graphite plate (4.2 × 4.3 × 0.3 cm) was used as the cathode electrode. The two electrodes were connected through a 1000 Ω resistance during the exoelectrogens enrichment stage as a microbial fuel cell system (otherwise a 10 Ω external resistance was used during the BEF process).

During the enrichment period, 180 mL of sewage collected from a municipal wastewater treatment plant (Lyngby, Denmark) was filled into anode chamber together with 0.18 g sodium acetate, 0.82 g Na₂HPO₄ and 0.41 g NaH₂PO₄·2H₂O. The anode chamber was then purged with nitrogen gas (99.99% purity) for 15 minutes to obtain an anaerobic atmosphere. In cathode, 180 mL of 50 mM of sodium sulfate solution was used as the electrolyte. A peristaltic pump (BT100-2J/DG-2-B, Longer Precision Pump Co., Ltd, China) set at 12 rpm (2 mL air/min) was employed for electron acceptor oxygen supply in the cathode. The solution in both chambers was kept being stirred using a magnetic stirrer. The voltage across the resistance was recorded every half an hour by a digital multimeter (model 2700, Keithley Instruments, Inc.; Cleveland, OH, USA). The solution in both chambers was refreshed after 4-5 days when the voltage began to decrease quickly. When the voltage became stable at around 280 mV, the system was ready for the BEF test.

2.3 Removal of metoprolol in synthetic wastewater

To study the removal of metoprolol, the system was switched to BEF mode powered by a power supply. The removal experiments were performed at batch mode and the reaction time was 12 hours. The synthetic wastewater in the cathode was made up of 200 mL deionized water,
dissolved with metoprolol (500 μg/L), ferrous sulfate heptahydrate (0.05 ~ 1 mM), and sodium sulfate (50 mM). The cathode chamber was washed using 10 mL of the solution twice. Afterward, the remaining 180 mL solution was filled into the cathode chamber for the tests. Subsequently, the effect of external voltage, cathode electrolyte pH, and catalyst concentration on metoprolol removal was investigated using a variable-controlling approach. In the external voltage supply test, the initial pH of the catholyte was adjusted to 2.0. Afterward, the catholyte pH was adjusted to the desired value using sulfuric acid to investigate the effect of initial pH on metoprolol removal. To diminish the pH change during the process, a pH automatic controller (ProSystem Aqua, Spain) was used to maintain the catholyte pH constant at the set value. In the catalyst dosage test, the catholyte pH was set at 3.0 with pH control. The details of operation parameters were described in Table S1.

To investigate the adsorption of metoprolol, the concentration of metoprolol was measured at pH 2.0 and 7.0 in 12 hours under an open circuit. Therefore, the total adsorption of metoprolol was examined and compared. The distribution of absorbed metoprolol on each component (including reactor surface, CEM, rotor, and electrode) was further examined. The reactor was disassembled and each component was put in a sealed flask which was filled with methanol overnight and then the methanol was dried at 40 °C under a nitrogen flow environment. An adequate amount of fresh methanol was used to dissolve the absorbed metoprolol by washing the flask. The concentration of metoprolol was then measured by HPLC-MS.

2.4 Removal of metoprolol in the municipal effluent from WWTP

The municipal effluent of secondary sediment tank from the same WWTP was taken to investigate the degradation efficiency of metoprolol in the real wastewater matrix. The effluent was first filtered to remove big particles and then adjusted the pH to 3.0 which was proved as
optimal working pH based on the batch experiments using the synthetic wastewater. 0.2 mM Fe$^{2+}$ was added as the catalyst and the applied voltage was 3 V. The removal of metoprolol was then investigated under both batch and continuous flow mode. Under continuous flow mode, a peristaltic pump was used to control the flow speed so that the effect of different hydraulic residence times (HRTs) can be studied.

2.5 Sample preparation and chemical analysis

2.7 mL of sample from the cathode chamber was taken each time and mixed with 0.999 mL of acetonitrile. 40 μL of tert-butanol solution (mixed with acetonitrile, 1:1 v/v) was added to stop reaction immediately. Samples were stored at -20°C until analysis. Before analysis, samples were filtered through 0.22 μm PTFE filters and then 0.9 mL of filtrate was transferred into clean HPLC vials with 0.1 mL of propranolol (500 μg/L in methanol) as the internal standard. To quantify metoprolol and identify the intermediates formed a high-performance liquid chromatography (Agilent 1290 Infinity, USA, HPLC) system with a tandem mass spectrometer (Agilent 6470 series, USA, MS/MS) was used. The methods were described previously (Zou et al., 2020).

2.6 Toxicity assessment

The toxicity of wastewater during treatment was tested using a standard Microtox® toxicity test system (Microbics Corp., Carlsbad, CA, USA) (Libralato et al., 2010). 1.5mL of sample was taken directly from the cathode and supplied NaCl to reach 2% final concentration. The pH of samples was adjusted to 7.0 ± 0.2 using NaOH solution. The luminescence intensity was measured at time zero and after exposure for 10 min and 20 min, respectively.
3. Results and discussion

3.1 Removal of metoprolol in synthetic wastewater

The effects of several key parameters including applied voltage, working pH, and catalyst dosage on the removal of metoprolol from synthetic wastewater were first investigated in the BEF system at batch mode. The degradation of metoprolol followed pseudo-first-order kinetics (Table S1). The apparent rate constant ($k_{app}$) and Metoprolol removal efficiency were related to different operating parameters (Table S1).

3.1.1 Effect of external voltage on the removal of metoprolol

Different voltages from 0 to 0.6 V were applied to investigate its effect on metoprolol removal in the BEF system with the same initial pH of 2.0 (Fig. 2A). The pH of the synthetic wastewater in the cathode increased to 2.2 ~ 2.6 slowly along the reaction time except at open circuit condition (Fig. S1A). When the applied voltage was 0 V (open circuit), 29% of metoprolol was removed from the aqueous phase, which was ascribed to the adsorption since no electrons can be used to drive Fenton reaction. A similar metoprolol removal rate (37% and 21%) was reported through adsorption by using aluminum salt and iron salt as a coagulant, respectively (Estrada-Arriaga et al., 2016). The removal rate and apparent rate constant ($k_{app}$) increased from 54% and 0.0664 min$^{-1}$ to 65% and 0.0869 min$^{-1}$, respectively, when the applied voltage was improved from 0.1 to 0.2 V (Table S1). However, both the removal rate and $k_{app}$ began to decrease when further increasing external voltage (from 0.2 to 0.6 V).

The external voltage related to current density is one of the key parameters that could affect the system performance. The current density directly affects the electrochemical generation of H$_2$O$_2$ (Eq. (2)) and catalyst (Fe$^{2+}$) regeneration (Eq. (3)). Furthermore, hydroxyl radical production efficiency can be regulated and the pH may increase as the accumulation of hydroxyl (Eq. (1)).
was expected that the system performance would be improved along with the current since more hydroxyl radicals could be formed. However, both the removal rate and $k_{\text{app}}$ began to decrease with further increasing external voltage. The results indicated that increasing the external voltage to a level above a certain limit may in turn lower the pollutant removal efficiency. The results were consistent with previous research in which the highest $k_{\text{app}}$ and removal efficiency was achieved at 0.3 V for all chemicals though higher current density was observed at 0.9 V (Nadais et al., 2018). This can be explained that the cathode potential might be changed by the applied voltage, which may further lead side reactions (e.g., Eq. (4) – (8)), and thus lower current and pollutants removal efficiency (Li et al., 2018). The results indicated that the optimal applied voltage varies with different pollutants and should be verified in different cases. Thereafter, 0.2 V was chosen for the next experiments.

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$ \hspace{1cm} (4)

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^- + H^+$$ \hspace{1cm} (5)

$$Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + O_2 + H^+$$ \hspace{1cm} (6)

$$H_2O_2 + \cdot OH \rightarrow H_2O + \cdot O_2$$ \hspace{1cm} (7)

$$Fe^{2+} + \cdot OH \rightarrow Fe^{3+} + OH^-$$ \hspace{1cm} (8)

3.1.2 Effect of pH on the degradation of metoprolol

The pH of the cathodic electrolyte can significantly affect the performance of the BEF system (Li et al., 2018). Hereby, the removal of metoprolol was examined at pH of 2.0, 3.0, 4.0, 5.0, and 7.0, respectively. Firstly, the experiments were conducted at different initial pH without pH control. The results showed that the fewest metoprolol (72%) was removed at the initial pH of
2.0 in 12 hours whilst similar removal of metoprolol (> 86%) was achieved when the initial pH was higher than 2.0 (Fig. 2B). The catholyte pH rapidly increased to above 10 in the first three hours when the initial pH was higher than 3.0 (Fig. S1B). When the initial pH was 3.0, the pH slowly rose to 3.6 in the first three hours and then went up to 9.5 quickly in the next three hours (Fig. S1B). The cathodic pH was still below 2.5 (2.3) after 12 hours when the initial pH was adjusted to 2.0 (Fig. S1B). It was reported that the efficiency of Fenton reaction decreases quickly at high pH, especially pH > 5.0 (Nidheesh and Gandhimathi, 2012). This is because H$_2$O$_2$ is not stable in alkaline solution, and on the other hand, the high pH leads to the precipitation of Fe$^{2+}$ and Fe$^{3+}$. Consistently, macroscopic iron precipitations were observed during reaction except at strong acidic conditions (pH 2.0). However, complex processes were involved during the removal of metoprolol in the system (e.g., advanced oxidation, adsorption and electrocoagulation). Firstly, metoprolol was able to be adsorbed on the inner surface of the reactor. Secondly, metoprolol was positively charged at acidic and neutral pH since its high p$K_a$ value (9.63). Therefore, metoprolol can be adsorbed on the cathodic electrode by charge attraction. Besides, iron salt is a commonly used coagulant. When the current was applied to the system, the iron ions formed different Fe(II) (and/or Fe(III)) species with hydroxide ion depending on the pH of the catholyte. These species act as coagulants so that retain micropollutants to form particles and finally producing some iron sludge. Therefore, the higher removal rates at higher pH were resulted from other processes (e.g., adsorption and electrocoagulation) besides the Fenton reaction (Nadais et al., 2018; Nidheesh and Gandhimathi, 2012).

To diminish the pH change during the reaction, the experiment was conducted again with pH control. Still, the lowest metoprolol removal (59%) was observed at the lowest pH (pH 2.0), and
70% of metoprolol was removed at pH 3.0 which was lower than that (90%) in the former experiment without pH control (Fig. 2C). The higher pharmaceutical removal (> 90%) at higher pH (pH 4.0, 5.0, and 7.0) (Fig. 2C) was still observed. Though the pH was controlled, Fe$^{2+}$ and Fe$^{3+}$ easily formed Fe(OH)$_n$ precipitations at such pH condition none the less (optical observed). Therefore, the higher removal rates were considered as the consequence of complex processes including Fenton reaction, adsorption, and electrocoagulation (Nadais et al., 2018; Nidheesh and Gandhimathi, 2012). The reduced pollutant removal efficiency at pH 3.0 with pH control compared to the one without pH control was probably ascribed to the alleviation of electroflocculation because less iron sludge was generated at pH 3.0. The result that the pollutant removal at pH 3.0 was higher than that at pH 2.0 was consistent to the finding that acidic pH of around 3 is the optimal condition for Fenton process which had been derived from most researches (Hartmann et al., 2010; Mirzaei et al., 2017; Velásquez et al., 2014). This may be due to that the Fe$^{2+}$ regeneration from Fe$^{3+}$ may be inhibited by the abundant H$^+$ according to Eq. (5) and Eq. (6) in the acidic environment (Hartmann et al., 2010). Furthermore, the generated hydroxyl radical would be scavenged by the excess of H$^+$ at pH below 3 (Feng et al., 2003). To prove the role of hydroxyl radicals during metoprolol removal, a quenching test was conducted (Fig. S3). When excess quencher (tert-butanol, TBA) was added to the system, 30.71% of metoprolol was removed from aquatic phase which was similar to the removal rate (28.74%) at open circuit (no generation of hydrogen peroxide and hydroxyl radicals). This portion of metoprolol removal was contributed by adsorption. In the control test, 65.75% of metoprolol was removed which indicated that more than 30% of metoprolol was destroyed by the strong oxidant hydroxyl radicals. During this process, hydrogen peroxide slowly accumulated in the system, 20.3 mg/L of hydrogen peroxide was detected after 12 hours. While when the catalyst (FeSO$_4$)
was absence, hydrogen peroxide accumulated constantly and finally reached to 66.2 mg/L in 12 hours. The results implied that at least 70% of hydrogen peroxide was converted into hydroxyl radicals and hydroxide through Fenton reaction. The results confirmed the role of Fenton reaction in metoprolol removal.

The adsorption behavior of metoprolol on each component was examined at pH 7.0 and pH 2.0 (for comparison) under open circuit (Fig. S2). The results showed that more metoprolol was absorbed at pH 7.0 (26%) compared with that at pH 2.0 (15%). It was revealed that the reactor surface contributed the largest adsorption at both the two pH conditions under open circuit (17% at pH 7.0 and 12% at pH 2.0). The adsorption by rotors was negligible (< 0.01%) and the adsorptions on CEM were almost same (1%) at pH 2.0 and pH 7.0. Results also elucidated that the electrode absorbed three times more metoprolol at pH 7.0 (3%) than that at pH 2 (1%). K_{ow} is the octanol/water partition coefficient which can be used to predict the adsorption of chemicals on the solids phase. It has been reported that the chemical has a low sorption potential when its logK_{ow} is lower than 2.5 (Rogers, 1996). The logK_{ow} of metoprolol is 1.88; therefore, few sorption tests were reported among published works on metoprolol removal. However, results from Stein et al. and Schaffer et al. implied that cation exchange processes with the negatively charged surface with beta-blockers played an important role in the chemical’s distribution (Schaffer et al., 2012; Stein et al., 2008). Romero et al. reported 0.1% and 11% of metoprolol was absorbed at free pH and pH 9 (Romero et al., 2013). These results implied that the adsorption of metoprolol at high pH should be noticed because the pH goes up fast in the Fenton process without pH control (Fig. S1B).

It is worth mentioning that 4% of metoprolol was absorbed by iron at pH 7.0 under open circuit because the Fe(OH)$_n$ precipitations could slowly be formed as the spontaneous occurrence of the
hydrolytic process. In the actual MEC-Fenton process, the formation of such precipitations could be significantly enhanced with the generation of hydroxyl (Eq. (1)). Therefore, more metoprolol was removed through electrocoagulation at higher operational pH (Fig. 2B and C). In total, the results obtained in the present study indicated that the attention should be paid on adsorption and electrocoagulation when studying the removal of metoprolol at high pH or in long operation time. However, through adsorption and electrocoagulation, contaminants are just transferred from one phase to another phase (iron sludge) other than incinerated and the efficiencies of adsorption and electrocoagulation strongly depend on the characteristics of the pollutants (Estrada-Arriaga et al., 2016). The dispose of these generated excess sludge rich in micropollutants would cause other complex issues. Therefore, in the following experiments, the catholyte pH was controlled at 3.0 all the time to diminish the effect of adsorption and electrocoagulation.

3.1.3 Effect of Fe$^{2+}$ concentration on the degradation of metoprolol

Fe$^{2+}$ as catalyst plays an important role in Fenton reaction since it can considerably affect the formation of hydroxyl radicals according to Eq. (1). Either the deficiency or the excess of Fe$^{2+}$ dosage leads to a decline in the performance of the Fenton system. Moreover, the excess of Fe$^{2+}$ will increase the cost of downstream treatment after the BEF process. Therefore, the metoprolol removal efficiency at different Fe$^{2+}$ concentration (0.05, 0.1, 0.2, 0.5, and 1 mM) was examined (Fig. 2D). The removal of metoprolol increased when the Fe$^{2+}$ dosage was increased from 0.05 to 0.2 mM, and afterward, it began to decrease with higher Fe$^{2+}$ concentration. The highest removal and $k_{app}$ observed was 66% and 0.0881 min$^{-1}$ at 0.2 mM Fe$^{2+}$, respectively (Table S1). The results implied that the Fe$^{2+}$ was insufficient when it was less than or equal to 0.1 mM, and was excess when it was higher than or equal to 0.5 mM. The COD removal efficiencies of pharmaceuticals treated by the conventional Fenton oxidation showed a similar trend when the Fe$^{2+}$ dosage was
increased from 0.003 to 0.04 M (Tekin et al., 2006). The decrease in removal efficiency probably because superfluous Fe$^{2+}$ can consume hydroxyl radicals according to Eq. (8) (Li et al., 2018). Besides, the generated hydroxyl radicals might be consumed by some side reactions when the Fe$^{2+}$ concentration was higher than optimum dosage (Yang et al., 1998). The findings indicate that the optimal Fe$^{2+}$ dosage should be determined in the BEF process to get the highest removal efficiency and simultaneously get rid of squander of catalyst.

3.1.4 Degradation pathway

Samples taken along processing time were examined in the TIC full-scan mode of HPLC-MS/MS. Several new peaks appeared were considered as byproducts from metoprolol degradation based on the change of peak area (Fig. S4). Their mass to charge ratio (m/z) and possible molecular formulas proposed according to previous literatures were summarized in Table S2.

The cleavage of the C-O bond of the aromatic ether site was previously found in the metoprolol mineralization process led by the radical attack in EF and photoelectron-Fenton treatment (Isarain-Chávez et al., 2011; Olvera-Vargas et al., 2016). The cleavage resulted in the formation of 3-(propan-2-ylamino) propan-1,2-dion (molecular mass 133 g/mol) and 4-(2-methoxyethyl) phenol (molecular mass 152 g/mol). Though the two byproducts were not detected in the present study, the observed compound P3 (m/z 100.1/98) was regarded as the derivative formed from 3-(propan-2-ylamino) propan-1,2-dion which was also reported in other processes such as UV/chlorine, UV/H$_2$O$_2$, and stressed degradation processes (Borkar et al., 2012; Gao et al., 2020). Hence, the P4 (m/z 183) was assumed as the product of 4-(2-methoxyethyl) phenol after the further hydroxyl radical attack at the α-C position of the methoxy group and an attachment of a hydroxyl. Besides the cleavage of the C-O bond, the P1 (m/z 158.1/159.1) and P2 (158.1/159.1)
indicated the ·OH might attack the C-N bond at the secondary amino group. The cleavage during metoprolol degradation has been reported in EF, UV/chlorine, and UV/H₂O₂ processes (Gao et al., 2020; Jelena Radjenovic et al., 2011). Based on the above results, the possible degradation pathway of metoprolol in the present BEF process was proposed and exhibited in Fig.3.

3.2 Removal of metoprolol in municipal effluent

3.2.1 System performance at batch mode

Based on the results obtained from former experiments, operational condition with 0.2 V, pH 3.0 (with pH control), and 0.2 mM of Fe²⁺ was selected to test metoprolol removal using the municipal effluent from WWTP. The removal of metoprolol in the synthetic wastewater and municipal effluent was compared at different initial pollutant concentrations (500 μg/L and 10 μg/L) (Fig. 4). Generally, metoprolol was removed faster in synthetic wastewater than that in municipal effluent. At a higher pollutant concentration of 500 μg/L, the $k_{\text{app}}$ was 0.0717 h⁻¹ for the municipal effluent while the $k_{\text{app}}$ obtained from the synthetic wastewater was 21% lower. Similarly, when the initial metoprolol concentration was 10 μg/L, the $k_{\text{app}}$ was 1.6204 h⁻¹ in the municipal effluent and the one obtained using the synthetic wastewater was approx. 40% lower in the first one hour. After one hour, the $k_{\text{app}}$ began to decay fast. When the initial concentration of metoprolol was 500 μg/L, 66% and 55% of metoprolol were removed after 12 hours in the synthetic wastewater and municipal effluent, respectively. This indicated that to completely remove such pollutant, longer treatment time is needed. When the initial concentration of metoprolol was 10 μg/L, which was closer to the pollutant concentration in most aquatic environments, the metoprolol concentration in the municipal effluent was stable after 4 hours and 98% removal was observed (Fig. 4). In the synthetic wastewater, the metoprolol degradation rate was faster and the metoprolol removal rate reached more than 98% after 4 hours.
The difference in removal rate and $k_{app}$ may be caused by different conductivity. The municipal effluent had a much lower conductivity than the synthetic wastewater (0.97 vs 8.94 mS/cm). This led to a higher ohmic loss and lower current density of the system and thereby reducing the efficiency of hydrogen peroxide production (Li et al., 2017). Besides, the natural organic matter (NOM) presented in the municipal effluent (68 mg COD/L) could compete for hydroxyl radicals with metoprolol and thus the poor treatment performance was obtained in treating the real wastewater matrix.

3.2.2 Removal of metoprolol under the continuous flow mode

Most studies on pollutant removal through the electro Fenton process were conducted in batch mode; however, continuous flow is more realistic for treating real wastewater. Therefore, the system performance was further evaluated in continuous flow mode. HRT is one of the most important parameters in continuous flow mode. It can significantly affect treatment efficiency and capacity (Ramirez et al., 2009). The effect of different HRT of 2, 4, and 6 hours was examined under the operating condition of 0.2 V, pH 3 (with pH control), 0.2 mM Fe$^{2+}$, and 10 μg/L metoprolol (Fig. 5). The system reached a stable removal rate after one HRT period at all conditions. The shorter the HRT was, the lower the removal rate was obtained. About 80%, 92%, and 95% of metoprolol were removed at 2, 4, and 6 hours HRT, respectively. This was due to that the in-situ generated hydroxyl radicals can react with pollutants in a sufficient retention time at longer HRT while the radicals may be flushed out at shorter HRT. Similar results can be found in a continuous BEF system treating a mixture of pharmaceuticals (Zou et al., 2020). The results indicated that the system has the potential for real application at continuous flow mode.

3.2.3 Toxicity evaluation
The acute toxicity of the treated effluent from the cathode chamber feeding with municipal effluent was evaluated via the change of luminescence intensity of *Vibrio fischeri* after exposure for 10 and 20 min (Fig. 6). When the inlet metoprolol concentration was 10 μg/L and HRT was 2 hours, 15.4% inhibition on the luminescence after exposure for 10 min was observed. Thereafter, the inhibition changed with time because of the evolution of different byproducts. During the period equal to three HRTs, the inhibition on luminescence did not increase significantly and most of the samples (11 of 14) exhibited toxicity below 20%. Comparatively, the toxicity to *V. fischeri* after exposure for 20 min was similar or lower than exposure for 10 min and the inhibition rates were all below 20%. Different from our observation, a significant increment of inhibition (rose to over to 40%) during metoprolol treatment by EF process was discovered, which could be due to the higher concentration employed (26.7 mg metoprolol/L) (Olvera-Vargas et al., 2016). Notably, the inhibition on *V. fischeri* might also ascribe to the residual iron and hydrogen peroxide involved in samples therefore the removal of these compounds could further reduce adverse effects on aquatic beings. In total, the minor inhibition on the luminescence of *V. fischeri* indicated the BEF treated effluent would not significantly affect the microorganisms in aquatic environments.

3.3 Perspectives

In this study, BEF was proved competent to treat metoprolol in wastewater in continuous flow mode. The electrical energy per order of magnitude per m³ (EE/O) was generally used to compare advanced oxidation processes for treating low concentration contaminants. It can be determined according to Eq.(9) and (10) for a batch mode and a continuous flow mode, respectively (Bolton et al., 1996).
In the equations, $P$ (kW) is the power input of the AOP system, $t$ (h) is the processing time, $V_0$ (m$^3$) is the volume of the water matrix in the batch mode, $V$ (m$^3$/h) is the volumetric flow rate in the continuous flow mode, $C_0$ and $C_f$ are the initial and final concentration of the target pollutant, respectively. In the present study, the EE/O for treating the real municipal effluent at the batch and continuous flow mode (0.2 V, pH 3 with control, Fe$^{2+}$ 0.2 mM, metoprolol 10 μg/L, reaction time 12 hours, HRT 2h) was $1.056 \times 10^{-3}$ and $5.269 \times 10^{-3}$ kWh/order/m$^3$, respectively.

Adsorption (by powdered activated carbon), ozonation, and solar photo-Fenton are the treatment processes which have been applied for micropollutants removal in wastewater treatment at full or pilot scale. The costs (including electricity, reagent, and investment cost) on micropollutants removal through adsorption, ozonation, solar photo-Fenton, and the BEF process presented in this study were compared in Table 1. It can be seen that the cost of electricity and reagents by ozonation and solar photo-Fenton were similar (0.16~0.24 €/m$^3$) and were 3 to 4 times higher than adsorption and BEF. The investment cost of adsorption, ozonation, and photo-Fenton was below 0.3 €/m$^3$. The information about the capital cost of microbial electrochemical systems is quite limited. It was reported that the cost of a bipolar membrane MEC reactor could reach 5544 €/m$^3$ in Denmark (Zhang and Angelidaki, 2016) which was extremely high compared with other costs. It was worth mentioning that the cost calculated in the case was based on laboratory data. Among the total cost, 88% cost was on the reactor construction (polycarbonate), and another 8% cost was on the platinum cathode. In the up-scaled application, the polycarbonate could be
replaced by concrete or other cheaper materials. The graphite plate cathode used in the present study was also much cheaper than the platinum cathode. Therefore, more investigations on the investment cost of the BEF systems are needed. Nevertheless, the economic analysis indicated that one of the most challenging drawbacks facing BEF is the capital cost including electrodes and exchange membranes. Based on the results reported previously, though ozonation and solar photo-Fenton cost comparatively on micropollutants removal, treatment by ozonation was superior to photo-Fenton by reducing overall environmental impacts based on the quantifying analysis through Life Cycle Assessment (Arzate et al., 2019). Therefore, in addition to the economic cost, the environmental assessment is also required to comprehensively evaluate the suitability and sustainability of treatment technologies. Besides, the local regulation standard on wastewater disposal, the economic development level, and the characteristics of the influents could also affect the choice of treatment technologies.

Though the BEF process manifests perspectives on refractory pollutants removal, it has to be admitted that the BEF technologies are still in the infant stage of development and most of the related researches are limited in lab scale. Several technical limitations including narrow working pH range, low oxidation efficiency, and addition of iron salts are challenging its practical applications. However, many of these challenges are promising to be overcome as the development in this area in the future: 1) cheaper membrane materials which can both lower the cost and increase the efficiency of mass transfer are being developed (Tiwari et al., 2016); 2) novel cathodic electrodes (e.g., carbon nanotube (CNT)/γ-FeOOH composite cathode (Feng et al., 2010)) which can both broaden the working pH range and avoid iron addition are developed; 3) heterogeneous Fenton catalysts are being synthesized which can obtain effective contaminants removal in a broader pH range (Wan and Wang, 2016; Xu and Wang, 2012); 4) high removal
efficiencies at natural pH are achieved by introducing some chelating reagents, ligands, and atomic H* to the systems (Liu et al., 2019; Wang et al., 2015; Zhang and Zhou, 2019). In conclusion, BEF can be a promising alternative as a treatment process and the combination of BEF and other existing technologies (e.g., BEF-Adsorption) can be an effective way to minimize the drawbacks of adopting one technology alone. Moreover, investigation on scaling-up has to be conducted to further optimize the process and validate the feasibility before final application.

4. Conclusions

The feasibility and efficiency of metoprolol removal in a BiF process were investigated in a lab-scale reactor under both batch mode and continuous flow mode. Applied voltage and pH had a significant effect on metoprolol removal while Fe**2+** dosage had a little effect. pH higher than 3 may lead to an increased absorbance of metoprolol on reactor surface, iron sludge, and electrode. Metoprolol containing wastewater can be effectively treated in the BEF reactor at continuous flow mode. The results of the toxicity of wastewater during treatment implied a minor effect on aquatic microorganisms.

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Fig. 1. Graphical configuration of the BEF system.

Fig. 2. The effects of applied voltage (A), pH (without pH control (B) and with pH control (C)), and Fe$^{2+}$ concentration (D) on the removal of metoprolol from the synthetic wastewater in the BEF system.

Fig. 3. Proposed degradation pathway of metoprolol BEF treatment

Fig. 4. Removal of metoprolol in synthetic wastewater and municipal effluent at batch mode.

   Applied voltage 0.2 V, pH 3.0 (with pH control), Fe$^{2+}$ 0.2 mM, Na$_2$SO$_4$ 50 and 0 mM in synthetic wastewater and municipal effluent, respectively.

Fig. 5. Removal of metoprolol in the municipal effluent at continuous flow mode. The initial metoprolol concentration was 10 μg/L, applied voltage 0.2 V, pH 3.0 (with pH control), Fe$^{2+}$ 0.2 mM.

Fig. 6 Dynamic inhibition on luminescence of V. fischeri after 10 min and 20 min of assay time during 6 hours BEF treatment of 10 μg/L of metoprolol containing municipal effluent under continuous flow mode. Treatment condition: applied voltage 0.2 V, pH 3.0 (with pH control), Fe$^{2+}$ 0.2 mM, HRT 2 hours.
### Table 1 Cost comparison of micropollutants removal through various physicochemical treatment processes

<table>
<thead>
<tr>
<th>Treatment process</th>
<th>Scale</th>
<th>Removal rate</th>
<th>Electricity (€/m³)</th>
<th>Reagent (€/m³)</th>
<th>Investment (€/m³)</th>
<th>Total (€/m³)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption with sand filter</td>
<td>pilot</td>
<td>80%</td>
<td>0.006</td>
<td>0.054</td>
<td>0.107</td>
<td>0.167</td>
<td>(Margot et al., 2013)</td>
</tr>
<tr>
<td>Ozonation</td>
<td>pilot</td>
<td>98%</td>
<td>0.02</td>
<td>0.22</td>
<td>0.27</td>
<td>0.51</td>
<td>(Prieto-Rodríguez et al., 2013)</td>
</tr>
<tr>
<td>Solar photo-Fenton</td>
<td>pilot</td>
<td>98%</td>
<td>0.01</td>
<td>0.15</td>
<td>0.15</td>
<td>0.31</td>
<td>(Prieto-Rodríguez et al., 2013)</td>
</tr>
<tr>
<td>Bio-electro-Fenton</td>
<td>lab</td>
<td>99%</td>
<td>0.007</td>
<td>0.053</td>
<td>5.44</td>
<td>5.54</td>
<td>This study and (Zhang and Angelidaki, 2016)</td>
</tr>
</tbody>
</table>

Note: a indicates additional costs or factors.
CRediT authorship contribution statement

Xiaoyong Yang: Investigation, Methodology, Validation, Formal analysis, Writing - original draft. Rusen Zou: Investigation, Visualization, Writing – review & editing. Kai Tankg: Resources, Visualization, Writing – review & editing. Henrik Rasmus Andersen: Resources, Writing – review & editing. Irini Angelidaki: Supervision, Writing – review & editing. Yifeng Zhang: Conceptualization, Supervision, Funding acquisition, Writing – review & editing.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Highlights

- Highest removal of Metoprolol in bio-electro-Fenton at 0.2 V, pH 3 and 0.2 mM Fe$^{2+}$.
- Absorption and electrocoagulation during BEF process was significant at high pH.
- 77% of Metoprolol (10 μg/L) was removed in continuous mode with HRT 2 hours.
- Toxicity and degradation pathway of Metoprolol was determined for the BEF process.
Figure 1
Figure 2

(A) Effect of voltage on the degradation of a compound over time, with different voltages (0 V, 0.1 V, 0.2 V, 0.3 V) shown.

(B) Effect of pH without pH control, showing degradation over time with different pH values (pH 2, pH 3, pH 4, pH 5, pH 7).

(C) Effect of pH with pH control, showing degradation over time with different pH values (pH 2, pH 3, pH 4, pH 5, pH 7).

(D) Effect of concentration without pH control, showing degradation over time with different concentrations (0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM).

Figure 2
Figure 3

MTPL (m/z 268)

Cleavage at C-O bond (position A)

P4 (m/z 183)

Cleavage at C-N bond (position B)

P1, P2 (m/z 158.1/159.1)

P3 (m/z 100.1/98)

\( \text{CO}_2 + \text{H}_2\text{O} + \text{NH}_4^+ + \text{NO}_3^- \)
Figure 4

- Synthetic wastewater: 10 µg/L
- Municipal effluent: 10 µg/L
- Synthetic wastewater: 500 µg/L
- Municipal effluent: 500 µg/L

Graph showing the relationship between normalized concentration ($C_t / C_0$) and time (hour) for different concentrations of synthetic wastewater and municipal effluent.
Figure 5

(A) 2 h HRT

(B) 4 h HRT

(C) 6 h HRT

Graphs showing the change in $C_f/C_0$ over time (hour) with different HRTs (2 h, 4 h, and 6 h).
Figure 6

Inhibition on luminescence (%) vs. Time (h)

- Exposure time 10 min
- Exposure time 20 min

Figure 6