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Anaerobic biodegradation of pharmaceutical compounds coupled to dissimilatory manganese (IV) or iron (III) reduction

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GRAPHICAL ABSTRACT

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ABSTRACT

Pharmaceuticals in water have adverse effects on aquatic environment. Anaerobic pharmaceutical biodegradation coupled to dissimilatory manganese(Mn) (IV)- or iron(Fe) (III)-oxides reduction is potentially efficient but unexplored. In this study, batch experiments were performed using different Mn(IV) and Fe(III) species with a microbial inoculum pre-cultivated with 15 mM chemically-synthesized Mn(IV) and 10 mg L⁻¹ metoprolol. Results show 26% caffeine and 52% naproxen are degraded with Mn(IV) as terminal electron acceptor and insignificant biodegradation for other pharmaceuticals tested. Reduction of Mn(IV) from drinking water treatment is coupled to anaerobic biodegradation of metoprolol and propranolol, resulting in removal efficiencies of 96% and 31%, respectively. The results indicate that adsorption contributes to the pharmaceutical removal during the first 10 days of incubation, while biodegradation is the main removal mechanism in the whole period. Fe(III) can also be used as electron acceptor in anaerobic pharmaceutical biodegradation. Over half of the added metoprolol is degraded with both chemically-synthesized Fe(III) and Fe(III)-citrate as terminal electron acceptors. However, this process did not occur when using Fe(III) from drinking water treatment or Fe(III)-based sorbents. This study indicates that anaerobic pharmaceutical biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction is possible, and promising for application to cleaning wastewater treatment plant effluents.

1. Introduction

In the last decades, there is concern about pharmaceutical chemicals

in water due to the increased consumption of these compounds and improvements in the detection of these compounds. These chemicals are observed in surface water, groundwater, wastewater, and even in

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drinking water in the range from ng L^{-1} to $\mu\text{g L}^{-1}$ [1,2]. Previous studies have shown that pharmaceuticals have adverse effects. For example, after 4 week of exposure to propranolol at concentrations as low as $0.5 \mu\text{g L}^{-1}$, a decrease in the reproduction of fish (*Oryzias latipes*) has been observed [3]. Consequently, pharmaceuticals as emerging contaminants are unwanted even at low level concentrations, and compounds like diclofenac or clarithromycin have been classified as a priority substances in water quality watch lists [4,5].

Conventional wastewater treatment facilities insufficiently remove pharmaceuticals such as diclofenac, carbamazepine, and β -blockers. The removal efficiency is generally less than 50% [6]. To eliminate these pharmaceuticals from water, technologies such as advanced oxidation processes, activated carbon adsorption, and membrane filtration are applied. For example, complete removal of the pharmaceuticals propranolol and diclofenac as well as 75% carbamazepine removal can be achieved in catalytic photodegradation [7,8]. However, some intermediates from these advanced technologies can be more toxic than their parent compounds, which is undesired [9]. Furthermore, the high energy consumption will increase the ecological footprint and potential cost, making these technologies less attractive [10].

Biodegradation can be a less energy consuming way to efficiently remove pharmaceuticals and different redox conditions can be applied [10–12]. Under aerobic conditions, biodegradation of ibuprofen and diclofenac can achieve 75–100% removal [10]. Under nitrate-reducing conditions, biodegradation is efficient for β -blockers like atenolol and propranolol [13]. Under sulphate-reducing conditions, over 50% atenolol can be biodegraded within one week [14]. Under methanogenic conditions, more than 80% of diclofenac, ibuprofen, naproxen, and ketoprofen were found to be biodegraded [15]. However, alternative electron acceptors such as Mn(IV) or Fe(III) are less studied compared to the other redox conditions.

Applying Mn(IV) or Fe(III) as electron acceptors in anaerobic biodegradation of pollutants has clear advantages. In comparison to other electron acceptors like nitrate or sulphate, adding solid Mn(IV) and Fe(III) could avoid increasing wastewater salinity, and it is easy in application. The reduced products, Mn(II) and Fe(II), can be easily re-oxidized via a biological low-energy process. In addition, using Mn(IV) or Fe(III) could also remove other pollutants like ammonia or heavy metals simultaneously [16,17].

Anaerobic biodegradation under Mn(IV)- or Fe(III)-reducing conditions is also known as dissimilatory Mn(IV) or Fe(III) reduction. During the process, Mn(IV)- or Fe(III)- (hydr)oxides are used as terminal electron acceptors in the anaerobic biodegradation of organic compounds [17]. Previous studies show that anaerobic biodegradation with Mn(IV) as terminal electron acceptor can efficiently remove aromatic compounds such as benzene, toluene, and polyaromatics: for example, naphthalene is mineralized to CO_2 and water for more than 60% [18]. To date, anaerobic degradation of naproxen and atenolol under Mn(IV)- or Fe(III)-reducing conditions is described [13,14]. These results indicate that anaerobic biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction can be effective to remove pharmaceuticals as a wastewater treatment technology. During this anaerobic pharmaceutical biodegradation, Mn(IV) and Fe(III) are consumed as terminal electron acceptor.

In addition to pharmaceutical removal efficiency, the influence of Mn(IV) or Fe(III) from different sources are important for application. Chemically synthesized Mn(IV) or Fe(III) (labeled as Mn(IV)_{chem-synthesis} and Fe(III)_{chem-synthesis}) as well as the Mn(IV) or Fe(III) from natural source are commonly used, and reported in literature [17,18]. Compared to these compounds, Mn(IV) or Fe(III) produced during the drinking water treatment (labelled as Mn(IV)_{DWTP} and Fe(III)_{DWTP}) could be a more desirable source. Mn(IV)_{DWTP} and Fe(III)_{DWTP} are waste streams from the drinking water treatment plants [19]. Thus, these materials could be a cheap alternative for Mn(IV)_{chem-synthesis} and Fe(III)_{chem-synthesis}. For example, Fe(III)_{DWTP} usually consist of FeOOH , which is a suitable electron acceptor in anaerobic biodegradation

processes [17,20]. In addition, different types of Mn(IV) or Fe(III) can serve as electron acceptor during anaerobic biodegradation of organic compounds [17]. To the best of our knowledge, there are no studies reported directly using Mn(IV)_{DWTP} or Fe(III)_{DWTP} in dissimilatory metal reduction, nor using it coupled to the removal of pharmaceuticals with these Mn(IV) or Fe(III) species as terminal electron acceptor.

In this study, we tested the anaerobic biodegradation of pharmaceuticals with different types of Mn(IV) or Fe(III). Since anaerobic biodegradation of aromatic compounds with Mn(IV) or Fe(III) as electron acceptor has been observed, the process is also expected to be effective in removal of aromatic pharmaceuticals. The results presented provide information on anaerobic pharmaceutical biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction and that contributes to understanding the fate and transfer of pharmaceuticals in the environment, where Mn(IV) or Fe(III) are ubiquitous, and which opens new ways towards application in waste water treatment.

2. Methods and materials

2.1. Chemicals

Anaerobic water was prepared as previously described by boiling either ultrapure water ($18.2 \text{ M}\Omega\text{-cm}$, Total Organic Carbon (TOC) = 18 ppb, Millipore, USA) or demineralized water (demiwater) for 5 min, followed by cooling down to room temperature under a gentle N_2 flow [21]. The anaerobic water was stored in closed glass bottles at room temperature. All the solutions in this study were prepared with anaerobic water unless specified.

Six pharmaceuticals were purchased from Sigma-Aldrich or MP Biomedicals including caffeine, carbamazepine, ibuprofen, metoprolol, naproxen, and propranolol. The chemical structure and properties of these compounds have been described previously [21]. A stock solution of the pharmaceutical mixture was prepared with anaerobic ultrapure water, at a concentration of 20 mg L^{-1} for each pharmaceutical. Metoprolol stock and propranolol stock were prepared separately with anaerobic ultrapure water at 1 g L^{-1} .

Other chemicals were purchased from Sigma-Aldrich. For solid chemicals, the purity was greater than 98% while the liquids had a purity at HPLC or UPLC quality.

2.2. Preparation of Mn(IV) or Fe(III)

Different types of Mn(IV) and Fe(III) were used in this study (Table S1). The Mn(IV)_{chem-synthesis} was prepared as described previously [21]. The Mn(IV)_{DWTP} (Fig. S1) originated from drinking water production plant ‘Noordbargeres’ in Emmen, kindly provided by Water Laboratorium Noord (WLN, the Netherlands) [22].

Soluble Fe(III)-citrate was dissolved with boiled demiwater. After cooling down under N_2 flush to room temperature, the pH of Fe(III)-citrate was adjusted to pH 7 with 1 N NaOH and diluted to 40 mM. The Fe(III)-citrate solution was stored in a closed glass bottle and covered with aluminum foil to avoid photodegradation.

Fe(III)_{chem-synthesis} was prepared based on the method described previously [18]. In brief, 0.4 M FeCl_3 was neutralized with 1 N NaOH until pH 7. Thereafter, Fe(III)_{chem-synthesis} was washed and stored as Mn(IV)_{chem-synthesis}. Fe(III)_{DWTP} was obtained from Evides Waterbedrijf (the Netherlands). These Fe(III)_{DWTP} granules are a mixture of Fe(III) from different drinking water treatment plants and then pelletized. Two types of Fe(III)-based sorbents, FerroSorp®Plus and FerroSorp®RW (labelled as Fe(III)_{FerroSorp®Plus} and Fe(III)_{FerroSorp®RW}), were both obtained from HeGo Biotec GmbH in granular form (Germany).

2.3. Experimental setup

(1) Inoculum

Inoculum used in the experiments with Mn(IV), Fe(III)_{chem-synthesis}

and Fe(III)-citrate consisted of a mixture of anaerobic sediments in the effluent channel of wastewater treatment plants in the Netherlands (Text S1). This sediment mixture has been incubated in the presence of 10 mg L^{-1} metoprolol and $15 \text{ mM Mn(IV)}_{\text{chem-synthesis}}$ for over 800 days in cultivation medium described previously (Table S2) [23]. In the experiments with $\text{Fe(III)}_{\text{chem-synthesis}}$, the mixture samples were taken out after 100-day incubation. These samples were used as a new inoculum for the experiments with $\text{Fe(III)}_{\text{DWTP}}$, $\text{Fe(III)}_{\text{FerroSorp}^{\text{Plus}}}$ and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$.

(2) Experimental preparation

All experiments were performed in the same medium, as previously described [23]. The medium was prepared under anaerobic conditions in an anaerobic glovebox. In the experiment with Mn(IV) , 0.02% yeast extract was added to the medium, in order to provide sufficient nutrient compounds but in the experiments with Fe(III) , no yeast extract is in the medium.

The experiments with $\text{Mn(IV)}_{\text{chem-synthesis}}$ were carried out in duplicate in 250 mL bottles, filled with 100 mL anaerobic medium. The experimental bottles were prepared in the anaerobic glovebox. A selected amount of $\text{Mn(IV)}_{\text{chem-synthesis}}$ was distributed to the bottles, achieving a final concentration of 15 mM Mn(IV) . 10 mL inoculum mixture was transferred to the experimental bottles. Since the experiments were carried out as a primary study for proof-of-principle, a relatively high pharmaceutical concentration was used. In the experiments, the stock solution of pharmaceutical mixture was added to reach a final concentration of 10 mg L^{-1} for each pharmaceutical.

The experiments with $\text{Mn(IV)}_{\text{DWTP}}$ (granule and ground powder) were performed in duplicate in 120 mL bottles, filled with 50 mL medium inside an anaerobic glovebox. About 4 g dry $\text{Mn(IV)}_{\text{DWTP}}$ was added into the bottles, resulting in a final concentration of 15 mM Mn(IV) . Only metoprolol and propranolol were tested to minimize the potential influence of pharmaceuticals on inoculum. 0.5 mL metoprolol stock solution and 0.5 mL propranolol stock solution were added to the bottles. The final concentration for each pharmaceutical was 10 mg L^{-1} . 5 mL inoculum mixture was transferred to the experimental bottles.

Fe(III) is hypothesised as an alternative electron acceptor of Mn(IV) in anaerobic pharmaceutical biodegradation. The experiments with $\text{Fe(III)}_{\text{chem-synthesis}}$ were repeating the adaption of the inoculum but in the process, $40 \text{ mM Fe(III)}_{\text{chem-synthesis}}$ was used instead of $15 \text{ mM Mn(IV)}_{\text{chem-synthesis}}$. The experiments with $\text{Fe(III)}_{\text{citrate}}$ were also the same but the $40 \text{ mM Fe(III)}_{\text{citrate}}$ solution replaced the anaerobic water during the medium preparation. Only 1 mL metoprolol stock solution was added to reach a final concentration of 10 mg L^{-1} .

The experiments with $\text{Fe(III)}_{\text{DWTP}}$, $\text{Fe(III)}_{\text{FerroSorp}^{\text{Plus}}}$ and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$ (all ground powder) were the same as those with $\text{Mn(IV)}_{\text{DWTP}}$ in a 120 mL bottles in duplicate. The final concentration of Fe(III) in these experiments was 40 mM . Only metoprolol was added into the experiments at a final concentration of 10 mg L^{-1} .

When the bottles were ready, all the bottles were closed with butyl-rubber stoppers and taken out of the glovebox. The bottles were sealed with aluminum crimp caps, and the headspace was exchanged to N_2/CO_2 (80%/20%). All experiments were conducted at 30°C in the dark without shaking. Abiotic controls were prepared similarly, with additional aliquots of HgCl_2 (0.1 gHg L^{-1}) and NaN_3 (0.3 mM) to inhibit biotic activity.

2.4. Sample preparation and analysis

Liquid samples for pharmaceutical analysis were taken every 3 weeks. After sampling, the samples were centrifuged for 10 min at 10,000 rpm. The supernatant was diluted and transferred to amber vials. All the samples were stored at -20°C before analysis. The overall recovery rate of the sample preparation is $> 98\%$ as previously described [24]. Pharmaceutical analysis was performed by an ultra-performance liquid chromatography with a diode array detector (UPLC,

ultimate 3000, Thermo, USA), as previously described [7]. The detection limit of UPLC was $50 \mu\text{g L}^{-1}$.

The samples for pH were taken at the beginning and at the end of the experiments, and were analysed immediately with a pH-meter (MeterLab PHM210, Radiometer Analytical).

The samples for morphologies of the Mn(IV) and Fe(III) were prepared by grinding if the raw materials were granular. The morphology was analysed by an X-ray powder diffraction (XRD, Bruker D8 advance).

Samples for Mn content in $\text{Mn(IV)}_{\text{DWTP}}$ were reduced by $0.5 \text{ mM NH}_2\text{OH}\cdot\text{HCl}$ (1 mg sample/mL). The samples for Mn(II) measurement were also taken at the end of the experiments. The samples were centrifuged at 10,000 rpm for 10 min. The supernatant was collected and stored at -4°C before analysis. The Mn content and Mn(II) were both measured at wavelength 257.610 nm by an inductively coupled plasma optical emission spectrometry (ICP-OES) equipped with a MPX megapixel detector (VISTA-MPX CCD Simultaneous, VARIAN co.).

Samples for Fe analysis were prepared by a modified HCl extraction method [25]. Briefly, 0.5 mL samples were mixed with $0.5 \text{ mL } 1 \text{ M HCl}$ to fix Fe(II) . Thereafter, Fe(II) was analyzed directly by colourimetric methods (Hach Dr. Lange Kit 340). The samples were analysed immediately. The Fe content in $\text{Fe(III)}_{\text{DWTP}}$, $\text{Fe(III)}_{\text{FerroSorp}^{\text{Plus}}}$ and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$ was measured by the same ICP-OES at the wavelength 238.204 nm , while the Fe(II) generated during the experiments was measured using a Hach Dr. Lange Kit (LCK 340).

3. Results and discussion

3.1. X-ray diffraction of Mn(IV) and Fe(III)

First, the Mn(IV) and Fe(III) were characterized by X-ray diffraction (XRD), to identify the morphologies of these compounds and to assess if they are able to be used in dissimilatory Mn(IV) or Fe(III) reduction. The XRD pattern shows that the $\text{Mn(IV)}_{\text{chem-synthesis}}$ is amorphous with two small but broad XRD peaks, similar as previously described by others (Fig. 1) [26]. The morphology of $\text{Mn(IV)}_{\text{DWTP}}$ could not be clarified by an XRD analysis. Since $\text{Mn(IV)}_{\text{DWTP}}$ is MnO_2 -coated sand, the XRD pattern highly matches the pattern of quartz (Fig. 1(a), Fig.

Fig. 1. XRD patterns of different types of (a) chemically synthesised Mn(IV) ($\text{Mn(IV)}_{\text{chem-synthesis}}$) and Mn(IV) from a drinking water treatment plant ($\text{Mn(IV)}_{\text{DWTP}}$); and (b) chemically synthesised Fe(III) ($\text{Fe(III)}_{\text{chem-synthesis}}$), Fe(III) from a drinking water treatment plant ($\text{Fe(III)}_{\text{DWTP}}$), and two Fe(III) -based sorbents $\text{Fe(III)}_{\text{FerroSorp}^{\text{Plus}}}$ and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$.

S2(a)).

The XRD pattern of $\text{Fe(III)}_{\text{chem-synthesis}}$ shows amorphous Fe(III) with two small but broad XRD peaks, which is a similar pattern as Fe(OH)_3 (Fig. 1(b), Fig. S2(b)). The XRD patterns of $\text{Fe(III)}_{\text{DWTP}}$, $\text{Fe(III)}_{\text{FerroSorp}^{\text{Plus}}}$ and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$ are all partially similar to those of Fe_2O_3 and Fe_3O_4 , indicating a semi-crystalline morphology. The morphology of $\text{Fe(III)}_{\text{DWTP}}$ is closest to an amorphous one, and $\text{Fe(III)}_{\text{FerroSorp}^{\text{RW}}}$ is closer to a crystalline morphology.

Based on the literature, the amorphous Mn(IV) and Fe(III) are commonly used Mn and Fe species, and different types of Mn(IV) and Fe(III) such as crystalline hematite, goethite, akaganeite, and magnetite, are suitable for dissimilatory Mn(IV) or Fe(III) reduction [17,27]. Therefore, the amorphous and semi-crystalline Mn(IV) and Fe(III) in this study are expected to be able to be used in the dissimilatory Mn(IV) or Fe(III) reduction.

3.2. Anaerobic biodegradation of pharmaceuticals with Mn(IV)

(1) Chemically synthesized Mn(IV)

Anaerobic biodegradation with $\text{Mn(IV)}_{\text{chem-synthesis}}$ was tested with a mixture of six commonly used pharmaceuticals. Our results show that the anaerobic biodegradation is effective within 40 days (Fig. 2). In this period, 26% of the applied caffeine, and 52% of the dosed naproxen is removed. Carbamazepine and propranolol are removed within 42 days at lower levels, namely 12% and 16%, respectively. No removal is observed for ibuprofen and metoprolol. During the anaerobic biodegradation of pharmaceuticals, 5.4 mM Mn(IV) is reduced (Table 1), indicating that the Mn(IV) is the electron acceptor in the anaerobic biodegradation. In the abiotic controls, no degradation of pharmaceuticals is observed (Fig. 1), further indicating that the observed degradation is linked to Mn(IV) reduction.

Based on previous studies, anaerobic biodegradation of pharmaceuticals coupled to dissimilatory Mn(IV) reduction may occur via two pathways [17]. First of all, the pharmaceuticals could be hydrolysed or fermented by different bacteria to easier degradable components, such as sugars, amino acids, long chain fatty acid, and/or simpler aromatic intermediates [17,28]. These components can be further converted to fermented acids and hydrogen and then consumed by bacteria. In addition, the pharmaceuticals, as well as the potential aromatic intermediates, can also be oxidised directly by bacteria like *Geobacter metallireducens*. Mn(IV) reduction in this process provides energy for these bacteria [17].

Since the pharmaceutical removal coupled to dissimilatory Mn(IV)

Fig. 2. Biodegradation of pharmaceuticals with chemically synthesized $\text{Mn(IV)}_{\text{chem-synthesis}}$ after 42 days incubation. Experimental conditions: $[\text{MnO}_2]_0 = 15 \text{ mM}$, $[\text{pharmaceutical}]_0 = 10 \text{ mg L}^{-1}$, pH 6.5–7.5, $T = 30^\circ\text{C}$. Error bars stand for the difference between the duplicate experiments.

Table 1

Mn(IV) or Fe(III) reduction during the anaerobic biodegradation of pharmaceuticals.

Mn or Fe used in experiments ^a	Theoretical Mn(IV) or Fe(III) reduced (mM) ^b	Measured Mn(IV) or Fe(III) reduced (mM) ^c
$\text{Mn(IV)}_{\text{chem-synthesis}}$	1.8	5.4
$\text{Mn(IV)}_{\text{DWTP}}$	2.2	4.9
$\text{Fe(III)}_{\text{chem-synthesis}}$	1.7	1.8
Fe(III)-citrate	1.2	8.8

^a $\text{Mn(IV)}_{\text{chem-synthesis}}$ = Chemically produced Mn(IV) oxides, $\text{Mn(IV)}_{\text{DWTP}}$ = Mn(IV) oxides from drinking water treatment plants, $\text{Fe(III)}_{\text{chem-synthesis}}$ = Chemically synthesized Fe(III) hydroxides.

^b The calculation methods of Mn(IV) or Fe(IV) reduced described in Text S2 and Table S3.

^c Measured Mn(IV) or Fe(IV) reduced is calculated by the difference between the biotic experiments and abiotic controls.

reduction is a biological process, the process is expected to be more effective for readily biodegradable pharmaceuticals. In this study, the removal of naproxen and caffeine is higher than other compounds. Previous studies have shown that naproxen and caffeine are efficiently removed under anaerobic conditions [13,29] while the rest of pharmaceuticals are resistant [13,30]. That indicates that naproxen and caffeine could be more biodegradable than other pharmaceuticals. Apparently, removal of naproxen and caffeine is also more effective in anaerobic biodegradation with $\text{Mn(IV)}_{\text{chem-synthesis}}$ as shown here.

Mn(IV) reduced in the experiments is more than two times higher than that in theoretical calculation (Table 1), taking the depleted pharmaceutical amounts as the quantity of consumed electron donor (Text S2, Table S3). The dis-match is most likely due to the presence of yeast extract, which also contains a lot of organic compounds. Therefore, the bacteria will use yeast extract not only as a nutrient source, but also as electron donor to reduce Mn(IV) . As a result, more Mn(IV) reduction is observed than calculated for pharmaceutical removal.

Degradation of pharmaceuticals selected in this study under anaerobic conditions has been reported using electron acceptors other than Mn(IV) or Fe(III) . Degradation of caffeine, naproxen, and ibuprofen was observed previously under anaerobic conditions. High removal of caffeine and naproxen, both over 90%, has been obtained under nitrate-reducing, sulphate-reducing, and methanogenic conditions [13,29,31,32]. The removal efficiency and rate are both higher than those observed in this study. Nonetheless, the degradation of naproxen with Mn(IV) in this study is similar to the results obtained under Mn(IV) -reducing conditions [13], namely 50 – 60% naproxen is removed within about 1 month. Metoprolol and propranolol can be degraded under aerobic conditions [13,33] but are resistant to biodegradation under anaerobic conditions [13,31,32,34]. Only under nitrate-reducing conditions, propranolol can be degraded after 10 month. Similarly, the degradation of metoprolol and propranolol are not observed in this experiment. However, our following experiments (Section 3.2(2) and Section 3.3) indicated that metoprolol can be degraded with $\text{Mn(IV)}_{\text{DWTP}}$, $\text{Fe(III)}_{\text{chem-synthesis}}$ or Fe(III)-citrate . Similarly, ibuprofen can be readily biodegraded under aerobic conditions, its removal under anaerobic conditions is poor [10,35]. Even when the inoculum has been adapted, the removal of ibuprofen under nitrate-reducing conditions are less than 20% [10]. In our experiments, no ibuprofen degradation is observed. Carbamazepine is considered as a persistent compound under both aerobic and anaerobic conditions [12]. In this experiment, less than 15% carbamazepine degradation is observed.

(2) Mn(IV) from drinking water treatment plants

$\text{Mn(IV)}_{\text{DWTP}}$, both as granules and ground powder, is tested as whether it is suitable to be used in anaerobic pharmaceutical biodegradation coupled to dissimilatory Mn(IV) reduction. Results show that $\text{Mn(IV)}_{\text{DWTP}}$ granules can remove metoprolol and propranolol via biodegradation. In the first 10 days, the two pharmaceuticals are removed

Fig. 3. Biodegradation of metoprolol (●) and propranolol (■) with Mn(IV) oxides from drinking water treatment plants (Mn(IV)_{DWTp}). Experimental conditions: $[\text{MnO}_2]_0 = 15 \text{ mM}$, $[\text{pharmaceutical}]_0 = 10 \text{ mg L}^{-1}$, pH 6.5–7.5, $T = 30^\circ\text{C}$. Error bars stand for the difference between the duplicate experiments.

with Mn(IV)_{DWTp} from the medium in both biotic experiments and abiotic controls (Fig. 3). Thereafter, no removal of metoprolol is observed in biotic experiments between day 10 and day 35, while 72% is removed in the later 37 days incubation between day 35 and day 72. The removal of propranolol in biotic experiments is less pronounced, and resulted in a final removal of 31% after 72 days of incubation. However, the removal of propranolol is 52% in the first 10 days incubation. During the anaerobic biodegradation with metoprolol and propranolol, 4.9 mM Mn(IV) is reduced (Table 1). The abiotic controls remained stable after 10 days of incubation, with max. 15% metoprolol removal, and 9% propranolol removal. No removal of metoprolol or propranolol is observed in the presence of Mn(IV)_{DWTp} powder, (< 5% removal, data not shown).

The differences between biotic groups and abiotic controls of both propranolol and metoprolol clearly show that the removal of these two pharmaceuticals is via biodegradation. In addition, adsorption is also responsible for the removal of metoprolol and propranolol with Mn(IV)_{DWTp}. In the first 10 days, removal of two pharmaceuticals is observed in both abiotic controls and biotic experiments, but the removal in biotic experiments is higher. These findings show that the biodegradation of pharmaceuticals is the main removal mechanisms, but that adsorption also contributes. After, adsorption reached equilibrium biodegradation becomes the dominant removal process. During this process, metoprolol biodegradation consumes Mn(IV)_{DWTp}, probably releasing the propranolol that is adsorbed onto Mn(IV). This might explain the increased concentration of propranolol after 10 days. The Mn(IV) reduced in this experiments is also higher than expected due to the presence of yeast extract.

As mentioned, degradation of metoprolol and propranolol are negligible under anaerobic conditions [13,31,32,34]. In our experiment, nearly complete removal of metoprolol is obtained within 75 days, which is faster than some studies under aerobic conditions. For example, results show that under aerobic conditions, about 80% of metoprolol removal is obtained within 120 days [13].

3.3. Anaerobic biodegradation of metoprolol with Fe(III)

(1) Chemically synthesized Fe(III) and Fe(III)-citrate

Fe(III) is hypothesised as a suitable electron acceptor for anaerobic biodegradation of pharmaceuticals, because it has been reported that the bacteria involved can use both Mn(IV) and Fe(III) [17]. We used both insoluble Fe(III)_{chem-synthesis} and soluble Fe(III)-citrate to test the anaerobic biodegradation of metoprolol coupled to dissimilatory Fe(III) reduction. Our results show that Fe(III) is an alternative for

Fig. 4. Biodegradation of metoprolol with chemically produced Fe(III) hydroxides (Fe(III)_{chem-synthesis}) (◆) and Fe(III)-citrate (▲). Experimental conditions: $[\text{Fe(III)}]_0 = 40 \text{ mM}$, $[\text{metoprolol}]_0 = 10 \text{ mg L}^{-1}$, pH 6.5–7.5, $T = 30^\circ\text{C}$. Error bars stand for the difference between the duplicate experiments.

dissimilatory Mn(IV) reduction, which can be coupled to biodegradation of metoprolol (Fig. 4). Within 162 days, 57% metoprolol is degraded with Fe(III)_{chem-synthesis} and 52% with Fe(III)-citrate. Based on the theoretical calculation, when 1 mM metoprolol is totally mineralized, 85 mM Fe(III) is reduced (Text S2, Table S3).

Insignificant removal of metoprolol was found in the abiotic controls (< 5%). This shows that the removal of metoprolol with two different types of Fe(III) occurs through biodegradation and both can be used by our inoculum that was pre-cultivated with Mn(IV).

In this study, the use of soluble Fe(III)-citrate is expected to result in more removal than insoluble Fe(III)_{chem-synthesis}, because the soluble form is more accessible to bacteria. However, our results show no obvious difference between the soluble and insoluble Fe(III). Previous studies reveal that bacteria can directly transfer electrons from organic compounds to insoluble Fe(III) during dissimilatory Fe(III) reduction, without the formation of compounds that serve either as chelator or as electron shuttle [17]. As a result, the solubility of Fe(III) then does not influence its use by bacteria. In addition, Fe(III)-citrate contains an additional carbon source, citrate, which is an easier degradable organic compound than metoprolol. Results show that Fe(III) reduction during metoprolol biodegradation with Fe(III)-citrate is much higher than stoichiometrically needed for the degraded amount of metoprolol (Table 1). Previous studies have reported that some bacteria can use both Mn(IV) and Fe(III) as the electron acceptor [17,27]. Therefore, the inoculum adapted to Mn(IV) is also active with Fe(III), indicating that Mn(IV) and Fe(III) are exchangeable in application. Based on the theoretical calculation, to remove 52% metoprolol from 10 mg L^{-1} requires 1.2 mM Fe(III). If the citrate is also taken into account and assuming it is totally removed, the Fe(III) required is increased to 721.2 mM. The higher Fe(III) consumption indicates that citrate is also used as electron donor in the reduction of Fe(III). Citrate will out-compete metoprolol for Fe(III), leading to less Fe(III) available for biodegradation of metoprolol.

Even though the metoprolol removal with two Fe(III) is similar, the different intermediates formed in the processes are different (Fig. S2). During the anaerobic metoprolol biodegradation with Fe(III), 1.8 mM Fe(III) was reduced when Fe(III)_{chem-synthesis} is applied, and 8.8 mM Fe(III) is reduced when Fe(III)-citrate is applied. Since citrate can also be an electron donor, the incomplete biodegradation of citrate could produce intermediates which are expected to be different from that of metoprolol. Furthermore, the presence of citrate as an alternative electron donor could result in co-metabolic conversion of metoprolol, yielding different intermediate products.

(2) Other Fe(III) types

Inoculum obtained from the experiments with Fe(III)_{chem-synthesis}

Fig. 5. Degradation of metoprolol with Fe(III) from drinking water treatment plants (labelled as Fe(III)_{DWTP}) and two Fe(III)-based sorbents, FerroSorp®Plus and FerroSorp®RW (labelled as Fe(III)_{FerroSorp®Plus} and Fe(III)_{FerroSorp®RW}) within 62 days. Experimental conditions: [Fe(III)]₀ = 40 mM, [metoprolol]₀ = 10 mg L⁻¹, pH 6.5–7.5, T = 30 °C. Error bars stand for the difference between the duplicate experiments.

was tested for its ability to use other insoluble Fe(III) types to remove metoprolol, such as Fe(III) from drinking water treatment plants or commercial Fe(III) compounds. In this study, Fe(III)_{DWTP}, Fe(III)_{FerroSorp®Plus} and Fe(III)_{FerroSorp®RW} are selected because they are cheap, widely available, and theoretically suitable for bacteria in dissimilatory Fe(III) reduction. These Fe(III) compounds can be a good source for electron acceptors in anaerobic biodegradation of pharmaceuticals with Fe(III). Results show that in both biotic experiments and abiotic controls, the anaerobic metoprolol degradation is less than 10% after 62 days incubation, indicating no significant removal of metoprolol (Fig. 5).

The insignificant removal of metoprolol with these three types of Fe(III) might be due to their semi-crystalline form instead of having an amorphous nature. Previous studies show that reduction rate using crystalline Fe(III) in dissimilatory Fe(III) reduction is much slower compared to amorphous Fe(III) [17]. In addition, the microbial inoculum was cultivated with amorphous Fe(III) for a long period (over 200d) before transfer. When semi-crystalline Fe(III) was used instead of amorphous Fe(III), this inoculum may need a longer time before the degradation of metoprolol is initiated. As a result, no anaerobic metoprolol degradation via dissimilatory Fe(III) reduction is observed with these widely available Fe(III) forms.

4. Conclusion

Our study shows that anaerobic biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction is promising to remove pharmaceuticals. Based on these results, the following conclusions can be reached:

- (1) Anaerobic biodegradation with Mn(IV) can be used to remove pharmaceuticals including caffeine and naproxen, and the resistant pharmaceutical, metoprolol.
- (2) Fe(III) can be used as an alternative electron acceptor in anaerobic biodegradation of metoprolol with Mn(IV).
- (3) Different types of Mn(IV) or Fe(III) can be used in the anaerobic biodegradation of pharmaceuticals. The removal efficiency may be related to the morphologies of these Mn(IV) or Fe(III) compounds.
- (4) During the degradation process, the presence of yeast extract, citrate and other (metal complexing) organic components will compete with pharmaceuticals for iron or manganese reduction, and may thereby inhibit the dissimilatory removal of pharmaceuticals.

In comparison to previous studies under anaerobic conditions using different electron acceptors, biodegradation of pharmaceuticals with Mn(IV) or Fe(III) is more efficient for the removal of persistent compounds like metoprolol. In addition, the Mn(IV) or Fe(III) used in this

process is widely available, easy to acquire and cheap, and easy to recycled via low-energy process. It is a promising process in the future wastewater treatment removing pharmaceuticals.

In summary, anaerobic biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction can be used for pharmaceutical removal. This pharmaceutical removal process is effective with different types of Mn(IV) and Fe(III), including some widely available Mn(IV) and Fe(III) forms such as Mn(IV) present in sludge waste originating from drinking water treatment plants. Care should be taken of competing for organic substrates during removal of pharmaceuticals from wastewater treatment plant effluents. Further investigation based on the fundamental insight into this more sustainable pharmaceutical removal technology in wastewater treatment processes provided by this study is required. In addition, our study also contribute to understanding biotransformation of pharmaceuticals in the environment.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2018.04.078>.

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