ANAEROBIC MANGANESE- OR IRON-MEDIATED PHARMACEUTICAL DEGRADATION IN WATER

Wenbo Liu

Propositions

1. Anaerobic technology is the most sustainable and efficient application for Mn- or Fe-mediated pharmaceutical degradation.
   (this thesis)

2. The challenges of cultivating Mn(IV)- and Fe(III)-reducing bacteria is rewarded by their degradation of highly recalcitrant pharmaceuticals.
   (this thesis)

3. Priority for water safety goes to nutrient recovery instead of removing micropollutants.

4. Playing computer or video games solves scientific problems.

5. Remote control and communication systems promote the spread of knowledge but ruin sunny holidays.

6. Men remain kids throughout their life.

7. Fear of the unknown drives exploration.

Propositions belonging to the thesis, entitled

"Anaerobic manganese- or iron-mediated pharmaceutical degradation in water"

Wenbo Liu

Wageningen, 26 January 2018

Anaerobic manganese- or iron-mediated pharmaceutical degradation in water

Wenbo Liu

Thesis committee

Promotor

Prof. Dr H.H.M Rijnaarts

Professor of Environmental Technology

Wageningen University & Research

Co-promotors

Dr. A.A.M. Langenhoff

Associate professor, Sub-department of Environmental Technology

Wageningen University & Research

Dr. N.B. Sutton

Assistant professor, Sub-department of Environmental Technology

Wageningen University & Research

Other members

Prof. Dr R.N.J. Comans, Wageningen University & Research

Dr. K. Roest, KWR Watercycle Research Institute, the Netherlands

Dr. A. Butkovskyi, Helmholtz-Zentrum für Umweltforschung UFZ, Germany

Prof. Dr P.J. van den Brink, Wageningen University & Research

This research was conducted under the auspices of the Graduate School for Socio-Economic and Natural Sciences of the Environment (SENSE)

Anaerobic manganese- or iron-mediated pharmaceutical degradation in water

Wenbo Liu

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 26 January 2018
at 1:30 p.m. in the Aula

Wenbo Liu

Anaerobic manganese- or iron-mediated pharmaceutical degradation in water

224 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2017)

With references, with summaries in English and Chinese

ISBN: 978-94-6343-221-4

DOI: 10.18174/425246

To my parents and family

谨以此书献给我的父母和家人

Contents

Summary

Summary

Pharmaceutical compounds, originating mainly from industrial production and public consumption, are detected at extremely low levels ($ng \cdot L^{-1}$ –$\mu g \cdot L^{-1}$) in groundwater, surface water, and wastewater. So far, the adverse effects of pharmaceuticals and their intermediates have been widely reported, and include toxicity to humans and ecosystem, and enhancement of antimicrobial resistance. These effects call for the elimination of pharmaceuticals from water. This can be done by both abiotic and biotic degradation in the presence of oxygen (aerobic conditions) or in the absence of oxygen (anaerobic conditions). The technologies under anaerobic conditions are generally more sustainable and attractive because they require less energy and produce less pollutants, such as greenhouse gas, compared to technologies under aerobic conditions. Anaerobic degradation with metal oxides such as manganese (Mn) or iron (Fe) oxides has clear advantages in both drinking water treatment and wastewater treatment. Therefore, anaerobic degradation of pharmaceuticals in water with Mn or Fe is promising to study and develop into applicable techniques. This thesis investigates the feasibility of anaerobic degradation of pharmaceuticals in Mn- and Fe-mediated systems via both abiotic removal processes and by biodegradation. In Chapter 1, the scientific and technological motivation of the thesis is proposed.

Applications and scientific developments of Mn- or Fe-based technologies to remove pharmaceuticals from water are reviewed and discussed in Chapter 2. Based on the removal mechanisms found in nature and technical systems, these Mn- or Fe-based technologies can be classified into 3 groups – physico-chemical removal, chemical removal, and biologically-related removal. A review of previous research indicates that pharmaceutical removal with Mn- or Fe-based technologies from water is efficient, and the removal efficiency varies whit the different technologies applied. Positive and negative aspects of these processes, such as (non-)specificity, treatment conditions, formation of and effects of

intermediates and by-products, and effects of Mn or Fe compounds were evaluated. Based on that, new and promising Mn- or Fe-based technologies are proposed as future potential effective and sustainable pharmaceutical removal technologies. Among these proposed technologies, the dissimilatory Mn or Fe reduction is identified as a most attractive, sustainable, and low-cost technology because this novel technology requires neutral conditions and the bacteria involved are able to completely mineralize the pharmaceuticals.

The anaerobic biodegradation of pharmaceuticals coupled to dissimilatory Mn(IV) or Fe(III) reduction is tested with different types of Mn(IV) and Fe(III) (Chapter 3). With a mixture of adapted sediment to metoprolol and chemically synthesized Mn(IV), anaerobic biodegradation with amorphous, chemically synthesized Mn(IV) can effectively remove caffeine (26%) and naproxen (52%) after 42 days of incubation. Further experiments with Mn(IV) obtained from drinking water treatment plants show that this type of Mn(IV) can be used to remove metoprolol and propranolol, with respectively 96% and 31% after 72 days of incubation. The inoculum can also use Fe(III) as alternative electron acceptor to degrade metoprolol. Results show that metoprolol degradation with insoluble chemically synthesized Fe(III) and soluble Fe(III)-citrate reaches 57% and 52%, respectively. No significant removal is observed in all the abiotic controls, showing that the biodegradation is the main removal mechanism in pharmaceutical removal with Mn(IV) or Fe(III).

Abiotic removal of selected pharmaceuticals with MnO_2 is compared under aerobic conditions and anaerobic conditions (Chapter 4). Results show that anaerobic conditions promote diclofenac removal, while it inhibits removal of metoprolol and propranolol. In demineralized water (demiwater), diclofenac removal under anaerobic conditions is 78%, and higher than the 59% found under aerobic conditions. In 50 mM phosphate buffer, and under aerobic conditions, the diclofenac removal achieves complete removal. Under anaerobic conditions the observed removal is similar as in demiwater. Preliminary investigation shows that diclofenac removal with MnO_2 under anaerobic condition is better at acidic pH (pH 4 – 5) and the removal is higher when applying amorphous MnO_2 compared to applying crystalline MnO_2. The key factors determining the extent of pharmaceutical removal with MnO_2 under anaerobic conditions are the following: the chemical structure and molecular properties of the pharmaceuticals, and the properties and activity of reactive sites on the MnO_2 surface.

Applying MnO_2 under anaerobic conditions to remove diclofenac from water is further investigated (Chapter 5). Results show that increasing the temperature from 10 to 30°C leads to an increase in the diclofenac removal, whereas further increase of temperature to 40°C results in a decrease in the removal. The latter effect is possibly due to Ostwald ripening and/or aging processes. Increasing the amount of MnO_2 increases the diclofenac degradation, as this provides more reactive sites for diclofenac conversions. Further shifting the molar ratio of MnO_2 and diclofenac from 2200:1 to 8900:1, however, does not further increase diclofenac removal, probably due to limited oxidation capacity of MnO_2. The presence of metal ions strongly inhibits the diclofenac removal following the order of $Mn^{2+} > Ca^{2+} \approx Mg^{2+} > Fe^{3+}$. The metal ions appear to adsorb onto the MnO_2 surface and compete with diclofenac for reactive sites. Phosphate has a diverse effect on diclofenac degradation: low concentrations inhibit and high concentrations promote the removal. The humic acids significantly promotes diclofenac

removal, probably caused by affecting MnO_2 reactive surface sites.

To reuse the Mn or Fe during pharmaceutical removal under anaerobic conditions, biological production of Mn(IV) or Fe(III) is investigated under oxygen-limiting conditions, or with nitrate as electron acceptor (Chapter 6). Mn(IV) is successfully produced with Mn(II)-oxidizing bacteria under O_2-limiting conditions, and the produced Mn(IV) is amorphous. Pharmaceutical removal with the Mn(II)-oxidizing bacteria is not observed. In abiotic pharmaceutical removal, using Mn(IV) from a drinking water production plant, is effective to remove metoprolol and propranolol. The successful production of Fe(III) is also observed under NO_3^--reducing conditions via biological processes. The biologically produced Fe(III) is also amorphous. There is no significant removal of pharmaceuticals coupled to the biological Fe(III) production. When comparing the biologically produced Fe(III) and other types of Fe(III), only Fe(III) from a drinking water production plant and one Fe(III)-based sorbent can remove propranolol.

Finally, the outcomes of this thesis are discussed and provide insights into the application of anaerobic degradation of pharmaceuticals with mediation of Mn and Fe oxides (Chapter 7). The removal mechanisms include adsorption, chemical oxidation, and biodegradation and are identified to contribute to the different removal processes. The anaerobic Mn(IV)- and Fe(III)-mediated pharmaceutical degradation processes are evaluated on the basis of removal performance, environmental and operational conditions, sustainability of the processes, as well as the Mn and Fe types involved. Results described in this thesis provide a proof of principal for anaerobic Mn(IV)- or Fe(III)-mediated degradation in removing pharmaceuticals from water. To translate the process into a pharmaceutical removal technology for water treatment, three steps are proposed including (1) exploring the limits of anaerobic Mn- or Fe-mediated pharmaceutical degradation processes; (2) simulating the process in practice with a controlled systems, and (3) translating the processes to a

pilot-scale system before a full-scale application. In addition, research topics are identified that can help to meet these challenges in the future. In summary, anaerobic Mn(IV)- or Fe(III)-mediated systems can remove pharmaceuticals from water through both abiotic removal and biotic degradation. These are promising processes which can be developed into a robust, sustainable, affordable, and environmentally friendly technology to remove pharmaceuticals from water.

Chapter 1

Introduction: Pharmaceuticals in the environment and pharmaceutical removal technologies

1.1 Pharmaceuticals in water

Pharmaceuticals, commonly known as medicines or drugs, are chemical compounds used in the medical diagnosis, cure, treatment, or prevention of disease. These compounds have been detected in the aquatic environment including surface water and groundwater. Pharmaceuticals in water originate from waste streams of human activities, such as industrial production and an increase in consumption and emissions due to population growth, aging, and rising levels of obesity [161]. In the European Union (EU), production by the pharmaceutical industry increased 76% from 2000 to 2014 [57]. More production leads to more wastewater from manufacturing containing the raw materials as well as pharmaceuticals. Moreover, pharmaceutical consumption keeps increasing. The World Health Organization (WHO) reports that the pharmaceutical consumption in the investigated countries increased 20 – 30% from 2000 to 2008 [181]. It is predicted that the global pharmaceutical consumption in 2020 will increase 24% from that in 2015, reaching 4.5 trillion dosages [2]. Pharmaceuticals consumed for curing illness or increasing animal productivity are generally not completely degraded in humans or animals. These pharmaceuticals, together with pharmaceuticals disposal from household, healthcare facilities, and industries, will enter the aquatic environment primarily through discharged wastewaters (Table 1.1, Figure 1.1) [30, 367, 369].

Pharmaceuticals can be transformed to intermediates in the body, water treatment facility, and in the environment, and both parent and intermediates can be toxic to ecosystems [41]. One well-documented case is the 34 – 95% decrease of vulture population in Pakistan between 2000 and 2003 due to the indirect consumption of diclofenac and its residues [219]. Studies also describe the "cocktail effects" of pharmaceuticals, which may lead to more significant effects than predicted based on the individual compounds. For example, a higher toxicity on *Daphnia magna* is observed with a mixture of diclofenac, ibuprofen, and clofibric acid, than the individual compounds [88].

While toxic effects on ecosystems are relevant, the public focuses more on human exposure to pharmaceuticals, especially the pharmaceuticals in drinking water. Some pharmaceuticals have mutagenic activity, which may cause a somatic mutation that can lead to cancer [234, 236]. Previous studies evaluated 1048 marketed pharmaceuticals, and nearly half of them are positive in the genotoxicity test or conflicting in results from different studies [24, 283]. Furthermore, despite the extremely low concentrations of which pharmaceuticals are found in water resources, concern can be raised. Considering the toxicity of the individual compound, one pharmaceutical can have negligible effects at this low concentration on human's health or an ecosystem. However, the multitude of these compounds present in water potentially inducing synergistic long-term toxic effects are still unclear [129]. Results show that the long-term toxicity (chronic toxicity) of pharmaceuticals occurred at lower concentration (at $\mu g \cdot L^{-1}$) than short-term toxicity (acute toxicity, at $mg \cdot L^{-1}$) [233]. The antimicrobial resistance developed by microorganisms when exposed to antimicrobial pharmaceuticals, cause an increase in human mortality as a result of antimicrobial resistant pathogens [343]. To deal with all these potential threats, it is vital to better understand the sources causing emissions of pharmaceuticals to the environment, the fate, and effects of these chemicals on the environment. Moreover, specific technologies should be developed to remove these compounds from water, which is the focus of this thesis.

1.2 Pharmaceutical degradation techniques

Due to their unclear eco- and human toxic effects and their association with antimicrobial resistance, pharmaceuticals are considered as unwanted chemicals, even at the low concentrations currently observed in water resources ($ng \cdot L^{-1}$ – $\mu g \cdot L^{-1}$) [297, 298]. Many processes have been used to remove pharmaceuticals from water [170, 255]. In some processes, physical or chemical mechanisms are used to remove the compound. These abiotic removal processes include various types of advanced oxidation, activated

carbon adsorption, or membrane filtration, and usually can achieve high removal efficiencies. For example, photodegradation catalysed by TiO_2 can completely remove diclofenac and propranolol, and remove over 70% of carbamazepine and ibuprofen in both demineralised water and WWTP effluent [95, 354]. Activated carbon (AC) adsorption including both powder and granular activated carbons is widely studied and used to remove pharmaceuticals as well as other micropollutants [50]. The AC adsorption capacities differ for specific pharmaceuticals. For less hydrophobic pharmaceuticals such as ibuprofen, AC has a relatively low adsorption capacity of 12 – 56 $mg \cdot g^{-1}$. For hydrophobic pharmaceuticals like trimethoprim and paracetamol, the AC adsorption capacity is much higher, in the range of 120 – 300 $mg \cdot g^{-1}$ [255, 280].

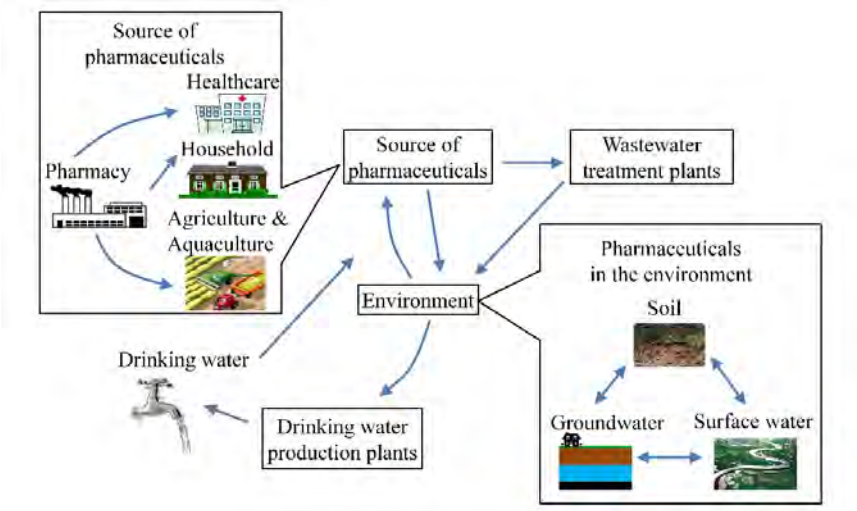
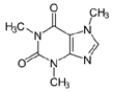
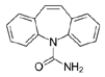
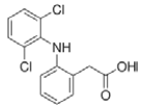
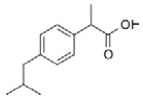
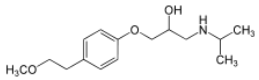
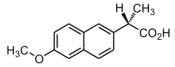
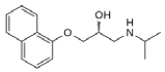


FIGURE 1.1 Source and pathways of pharmaceuticals in the environment

TABLE 1.1 Occurrence of selected pharmaceuticals in water cycles (μg·L^{-1}) [a]

Compounds (CAS-No.)	Structure	Therapeutic class	pKa	LogKow	Investigated countries and regions [b]	SW	GW	WWTP influent	WWTP effluent
Caffeine (58-08-2)		Nervous stimulant	0.4 [c]	-0.07	BRA, CAN, CHN, CRI, EU, GBR, KOR, SGP, USA, VNM	0.57	357	209	43.5
Carbamazepine (298-46-4)		Anticonvulsants	13.9	2.25	CAN, CHN, EU, GBR, JOR, KOR, SGP, USA, WB	1.15	1.1	9.42	22
Diclofenac (15307-86-5)		Anti-inflammatory	4.15	4.51	CAN, CHN, EU, GBR, JPN, KOR, SGP, USA, WB	0.84	0.93	94.2	11.0
Ibuprofen (15687-27-1)		Anti-inflammatory	4.91	3.97	CAN, CHN, EU, GBR, GRC, HRV, JPN, JOR, KOR, SER, USA	6.4	0.99	603	95
Metoprolol (37350-58-6)		beta-blocker	9.5	1.88	CAN, CHN, GBR, HRV, IND, KOR, USA	2.2	0.09	7.2	2.27
Naproxen (22204-53-1)		Anti-inflammatory	4.15	3	CAN, CHN, GBR, JPN, KOR, USA, WB	4.5	0.14	52.9	24.6
Propranolol (525-66-6)		beta-blocker	9.42	-0.45 [d]	CAN, CHN, GBR, HRV, SER, USA	0.22	0.10	10	1.11

[a] Data is the highest recorded concentrations from literature [62, 161, 182, 243, 258, 297]. The abbreviation in the table SW=Surface Water; GW=GroundWater; WWTP=WasteWater Treatment Plant

[b] The abbreviations are obtained from The World Bank (Table S1.1)

[c] Data is obtained at 40°C

[d] Data is obtained at pH 2.0

In contrast to abiotic processes, biotic processes are regarded as cost-effective and environmentally friendly measures for removing pharmaceuticals from the aquatic environment (Table 1.2). In conventional wastewater treatment processes, both activated sludge and membrane bioreactor systems proved efficient in removing ibuprofen, atenolol, and some other pharmaceuticals [151, 198, 289]. A wide range of biological species including bacteria, plants, fungi, and algae can remove pharmaceuticals from water. Pure microbial cultures like *Rhodococcus rhodochrous* or *Pseudomonas* have been reported to efficiently degrade various types of medical chemicals [72, 364]. Mixed microbial cultures, for instance, obtained from activated sludge and adapted to the specific pharmaceuticals, show an improved removal compared to unadapted groups, such as for ibuprofen and diclofenac [151]. Complete removal of ibuprofen has been demonstrated by the plant *Phragmites australis,* often used in wetlands [94]. The white-rot fungus *Trametes versicolor* has been tested, and it can degrade pharmaceuticals including ibuprofen, clofibric acid, and carbamazepine [192]. In the algae-based water treatment systems, both biodegradation and photolysis contributed to the complete removal of ibuprofen [49].

TABLE 1.2 Comparison of abiotic and biotic processes in pharmaceutical removal

	Advantages	Challenges
Abiotic processes (chemical and physical removal)	• High and fast removal • Relatively high mineralization • Efficient for nearly all pharmaceuticals • Efficient for other (micro)pollutants • Improve the biodegradability	• High cost • Special operation conditions • Radical formation • Toxic intermediates
Biotic processes (biodegradation)	• Commonly moderate operation conditions • Less energy input, cost-effective • Less toxic intermediates • Resistance to shock	• Slow removal & mineralization • Developing antimicrobial resistant • Only efficient for selected pharmaceuticals • Produce waste sludge

1.3 Pharmaceutical degradation techniques using metal species

The role of metal species in organic compound degradation is widely studied, and can either have a purely chemical, heterogenic catalytic nature or can occur in interaction with biotic processes [248]. In previous studies, manganese (Mn)- or iron (Fe)-based technologies have been shown to remove pharmaceuticals via both abiotic removal and biodegradation [170]. Using Mn or Fe to remove pharmaceuticals has some clear advantages (Table 1.3). Mn and Fe are abundant, and they are essential to transforming organic contaminants in nature [175]. Hence, these materials can be easily and cheaply obtained from natural resources. Furthermore, Mn is unwanted in drinking water because it is toxic to humans [226] and needs to be removed from drinking water resources. Fe is an unwanted species in drinking water as well, since it affects the appearance and taste of the water, and can lead to clogging of water pipes and hinder the functioning of faucets and valves. Using these waste metal species to remove pharmaceuticals is economically and environmentally attractive in drinking water and wastewater treatment. Therefore, the abiotic removal and biodegradation of pharmaceuticals by these metals in various speciation are worth studying. Studying the Mn- and Fe-mediated pharmaceutical removal will contribute to an increased basic understanding of the fate, and specifically the transformation of pharmaceuticals in the environment. Also, investigating Mn or Fe species to remove pharmaceuticals from water is promising for the development of a novel cost-effective water treatment technology. To achieve this goal, further research is required to meet the challenges mentioned for various processes (Table 1.3).

TABLE 1.3 Comparison of using Mn or Fe, and other chemicals in pharmaceutical removal processes

	Advantages	Challenges
Using Mn or Fe	• Abundant in nature, easy to obtain • Cheap • Reuse Mn or Fe • High removal efficiency	• Extra pollutants Mn^{2+} or Fe^{2+} • Strict anaerobic condition requires (biodegradation) • Slow growth of bacteria (biodegradation)
Using other chemicals (O_3, NO_3^-, etc.)	• High & rapid removal efficiency • Bacteria are easy to cultivate (biodegradation)	• High cost of materials • High operational skills

Eliminating pharmaceuticals from water is an important step to reduce the negative effects of these compounds on ecosystem and human. The ideal pharmaceutical removal technology should produce less direct and indirect pollution including toxic intermediates and greenhouse gases, and consume less energy. The technologies under anaerobic conditions (absence of oxygen) are most suitable for this purpose. Previous studies with various metal species have shown pharmaceutical degradation under anaerobic conditions via abiotic and biological processes [74, 170, 182].

Using Mn or Fe in anaerobic abiotic processes to remove pharmaceuticals is considered as more sustainable compared to aerobic processes (presence of oxygen) due to the low energy input needed for aeration. Some studies find that the presence oxygen can both promote the Mn or Fe mediated removal of sulfamethazine [69], inhibit the removal of metoprolol and propranolol [172], or does not affect the removal of levofloxacin [163]. In the absence of oxygen, the use of Mn oxides to remove pharmaceuticals from water is widely studied [248], and the role and function of oxygen vary among the different investigations. Mohatt, et al. [206] have reported the abiotic removal of sulfamethoxazole in soil by the mediation of Fe species. Further detailed investigations on the underlying mechanisms are missing. Therefore, the abiotic removal of pharmaceuticals with Mn or Fe under anaerobic conditions is essential for further studies.

Biodegradation under anaerobic conditions is known to be more efficient to degrade some recalcitrant pollutants, like highly halogenated aromatic compounds [324]. Under anaerobic conditions, microorganisms can use different alternative electron acceptors, including nitrate, Mn, Fe, sulphate, and CO_2 [74]. The biodegradation of various pharmaceuticals has been observed under these different redox conditions [74, 339]. However, in comparison with other redox conditions, biodegradation of pharmaceuticals under Mn and Fe reducing conditions has been insufficiently addressed. The biodegradation of organic compounds under Mn and Fe reducing conditions is through dissimilatory Mn or Fe reduction [175]. A promising process is dissimilatory Mn(IV) or Fe(III) reduction process. In these anaerobic Mn- or Fe-mediated systems, bacteria use Mn(IV) or Fe(III) as the electron acceptor to remove organic pollutants like aromatic compounds [152, 175, 335]. Since pharmaceuticals are mainly aromatic compounds, these processes potentially can also remove pharmaceuticals. Therefore, studying the process can form the basis for developing a specific removal technology for aromatic structure based pharmaceuticals. Previous studies on pharmaceutical degradation with different electron acceptors show that pharmaceuticals like naproxen can be efficiently removed under Mn and Fe reducing conditions [268]. The limited studies so far indicate that further studies on anaerobic biodegradation of pharmaceuticals with Mn or Fe are desired.

In the Mn- and Fe-mediated system, the Mn and Fe species is important. The Mn(IV)- and Fe(III)-(hydr)oxides are widely studied Mn and Fe species, and they can efficiently remove pharmaceuticals in both abiotic and biotic processes [65, 170, 327]. The Mn and Fe species from drinking water production are also (hydr)oxides, and could be suitable metal species to start detailed studies on anaerobic abiotic removal and anaerobic biodegradation of pharmaceuticals with Mn or Fe.

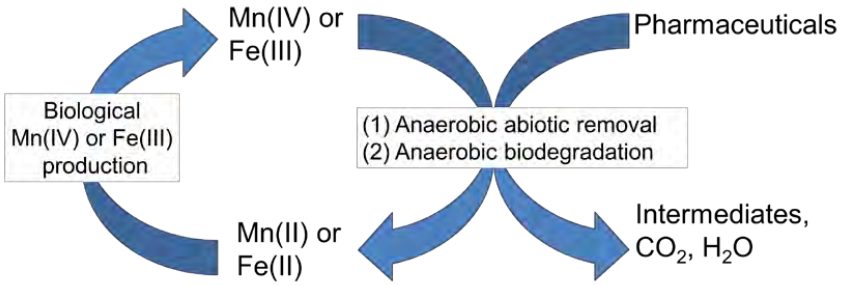


FIGURE 1.2 Anaerobic Mn(IV)- or Fe(III)-mediated pharmaceutical removal processes through (1) anaerobic abiotic removal and (2) anaerobic biodegradation

In summary, anaerobic Mn(IV) or Fe(III) mediated abiotic and biotic degradation (Figure 1.2) offers the potential for developing a sustainable technology to remove pharmaceuticals from water. Such a technology requires limited energy and therefore produces little indirect pollution in the form of greenhouse gasses (CO_2). Moreover, Mn(IV) and Fe(III) containing waste sludge of the drinking water industry may be reused for the degradation of pharmaceuticals.

1.4 Aim and Scope of this thesis

As discussed, Mn(IV) or Fe(III) mediated abiotic and biotic anaerobic degradation of pharmaceuticals is promising in water treatment and attractive to both drinking water and wastewater treatment. The objective of this dissertation is to first provide a proof of principle and subsequently further explore anaerobic Mn(IV)- or Fe(III)-mediated pharmaceutical degradation processes (Figure 1.3). This pharmaceutical removal process needs to be based on new fundamental insights into the transformation of pharmaceuticals in the Mn(IV)- or Fe(III)-mediated systems. Both Mn(IV) and Fe(III) are studied in abiotic and biotic systems to assess the potential of the various processes to remove pharmaceuticals as well as the potential of the recycling of the Mn(IV) and Fe(III) used.

Developing Mn- or Fe-based technologies for pharmaceutical removal is discussed by reviewing current and past studies reported in the literature

(Chapter 2). All reported Mn- or Fe-mediated systems are classified into three groups according to their removal mechanisms – physico-chemical processes, chemical processes, and biological processes. Removal efficiency and relevant parameters of these technologies and processes are put in inventory, and the advantages and challenges of these technologies and processes are discussed. Based on the review, several promising Mn- and Fe-mediated systems are proposed, which are currently not yet used to remove pharmaceuticals. The dissimilatory Mn(IV) or Fe(III) reduction process is identified and expected to have a high potential for removing pharmaceuticals from water.

After that, the biodegradation of pharmaceuticals in the anaerobic Mn- or Fe-mediated systems is tested by laboratory research (Chapter 3). The degree to which Mn and Fe species can function as electron acceptors in pharmaceutical biodegradation is studied. Batch experiments are carried out with pharmaceutical mixtures in water, using a microbial inoculum adapted to Mn(IV) and metoprolol. The removal efficiency of selected pharmaceuticals with Mn(IV) is subsequently investigated. The same microbial inoculum ((Mn(IV) adapted) is cultivated with metoprolol and Fe(III) species, namely Fe(III) hydroxides and Fe(III)-citrate, to test Fe(III) as an electron acceptor. Further tests with Mn(IV) and Fe(III) from drinking water treatment plants, and two Fe(III)-based sorbents are performed to find the best source of Mn(IV) and Fe(III) for pharmaceutical degradation.

After the biological studies, the studies into the potential of abiotic Mn and Fe mediated removal processes are described in Chapter 4. The anaerobic abiotic removal of pharmaceuticals is compared to that under aerobic conditions, to test and reveal the influence of oxygen. In addition, the effects of process parameters such as phosphate, pH, and MnO_2 morphologies are investigated. To this end batch experiments are carried out in the different matrices including solutions of the seven test pharmaceuticals as a mixture and as single compound (diclofenac) in demineralized water or phosphate buffer. The abiotic removal of diclofenac

in the absence of oxygen is described in detail because anaerobic conditions promote diclofenac removal, which has not been reported before. The next chapter (Chapter 5), continues with the MnO_2 based abiotic processes to further elucidate the effects of parameters relevant for technology application. In this chapter, diclofenac is selected as a model pharmceutical to study the influence of temperature, amount of MnO_2 per volume or per amount of pharmaceutical dosed, and co-solutes such as metal ions, phosphate, and organics in wastewater treatment plant effluents.

The Mn(IV) and Fe(III) can be regenerated from the Mn(II) and Fe(II) produced during the anaerobic Mn- or Fe-mediated pharmaceutical degradation. The biological production of Mn(IV) and Fe(III) is tested (Chapter 6). To better reuse, the regenerated Mn(IV) and Fe(III), the production of these metals is carried out under oxygen-limiting conditions for Mn(IV), and nitrate-reducing conditions for Fe(III). Removal of pharmaceuticals is tested during the biological metal production processes. In addition, anaerobic abiotic removal of pharmaceuticals with Mn(IV) and Fe(III) from different sources are compared.

In the final synthesis chapter, all processes described in the thesis are discussed regarding scientific progress, technology development and application potential (Chapter 7). A system for evaluating the performance of metal-oxide based pharmaceutical removal from water is presented. The potential remaining challenges of this proposed pharmaceutical removal technology are identified and discussed. Finally, an outlook and recommendation for research and application in water reuse are presented.

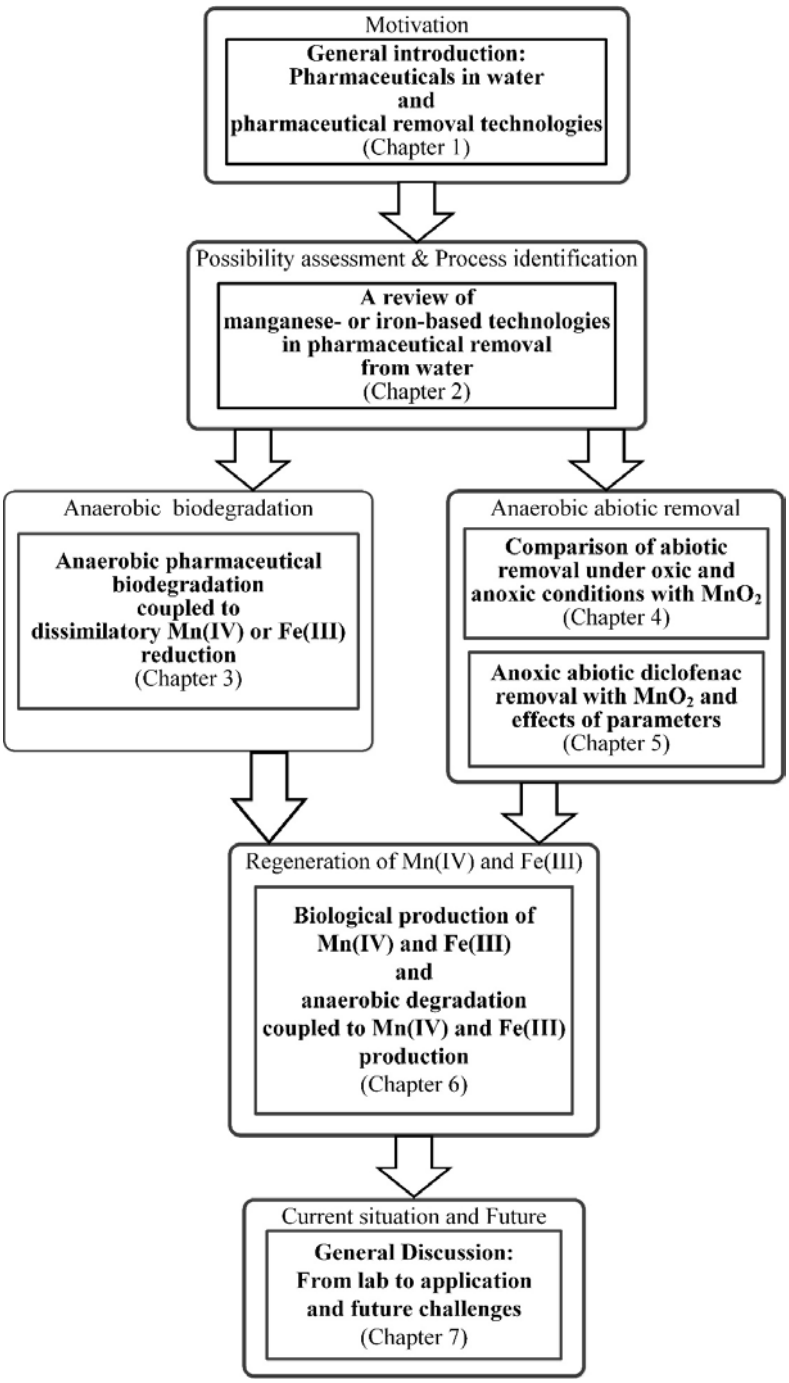


FIGURE 1.3 Schematic outline of the research topics in this thesis

Chapter 2

Pharmaceutical removal from water with manganese- or iron-based technologies: A review

A modified version of this chapter is published as

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Pharmaceutical removal from water with iron- or manganese-based technologies: A review. Critical Reviews in Environmental Science and Technology 2016, 46, (19-20), 1584-1621, DOI: 10.1080/10643389.2016.1251236

ABSTRACT

Pharmaceuticals are detected at trace levels in waters. Their adverse effects on aquatic ecosystems and human health demand novel pharmaceutical removal technologies for treating wastewater effluents. Manganese (Mn) or iron (Fe) may play important roles in these new technologies since these metals are abundantly available at low costs and are known to contribute to organic conversions via physico-chemical, chemical, and biologically related processes. Few reviews describe and discuss Mn- or Fe-based technologies for the purpose to remove pharmaceuticals from water. Therefore we review the current literature sorted into the three removal mechanisms, that is, through physico-chemical, chemical and biological processes. The principals, performance, and influential parameters of these three types of technologies are described. Current and potential applications of these technologies are critically evaluated in order to identify advantages and challenges. In addition, the Fe- or Mn-based technologies which are currently not used but promising to further develop to remove pharmaceuticals cost efficiently are proposed.

KEYWORDS: biologically-related removal; chemical removal; iron- or manganese-based technology; pharmaceutical removal; physico-chemical removal; water

2.1 Introduction

Removing pollutants from water is crucial to human as well as ecosystem health. Micropollutants are a growingly popular subject due to their low concentration and potential hazard. Among the micropollutants, pharmaceuticals are well studied and can be used as a representative case for a variety of micropollutants. Pharmaceuticals, commonly known as medicines or drugs, are a vast array of chemical compounds used for medical diagnosis, cure, treatment, or prevention of disease [255]. Improvements in analytical technologies have resulted in detection techniques to quantify pharmaceuticals at trace concentrations ($ng \cdot L^{-1}$ – $\mu g \cdot L^{-1}$) in wastewater, groundwater and surface water, and even in drinking water [38, 54, 106, 194]. Pharmaceuticals in water can threaten the quality of drinking water resources, can lead to spread of antibiotic resistance [241, 348] and can be toxic to some aquatic organisms [147, 263]. The presence and fate of these compounds have become a growing worldwide concern for politicians and the general public [118, 342]. For example, U.S. Environmental Protection Agency and European Union have added pharmaceuticals to their watch list for water quality [60, 314]. Due to adverse effects of pharmaceuticals in water on the quality of drinking water and food supply to humans, and on the functioning of ecosystems, regulators start to call for cost-efficient removal technologies to reduce emissions of such compounds.

Pharmaceuticals from human consumption, excretion, and disposal mainly enter the environment via wastewater [113]. If they are not removed, these compounds will pass through wastewater treatment trains and enter water bodies directly [207, 306]. Advanced technologies are employed to remove these pharmaceuticals from water [11, 68, 95, 139, 270, 357]. Manganese (Mn) and iron (Fe) play important roles in many water treatment technologies, including physico-chemical removal (e.g. polyferric coagulation) [345], chemical removal (e.g. Fenton) [160, 186], and biologically related removal (e.g. biogenic Mn oxides oxidation) [65, 67]. Mn- or Fe-based technologies for

treating organic chemicals and heavy metals in general are reviewed in the literature [45, 158, 300]. None of these is specifically oriented on Pharmaceuticals. The number of studies that have examined the processes and applications of Mn- or Fe-based technologies to remove pharmaceuticals is growing. These studies indicate the special character of pharmaceuticals, that is, their complex chemical structures, which strongly influence compound specific physico-chemical and biological processes related to Mn or Fe reactive species. Therefore, an integrated review of this literature, on Mn- or Fe-based technologies and assessment of their scientific soundness, application potential, and main prevailing knowledge gaps is highly needed.

This paper reviews Mn- or Fe-based technologies which are used to remove pharmaceuticals from water. All these technologies are sorted based on 3 removal mechanisms: physico-chemical removal, chemical removal, and biologically related removal. We evaluate the benefits and limitations of these technologies and discuss their application. In addition, we suggest promising Mn- or Fe-based technologies that should be explored for pharmaceutical removal. The results of this review are thus a critical assessment of the currently available and future potential Mn- or Fe-based technologies for pharmaceutical removal.

2.2 Processes review

2.2.1 Physico-chemical removal

Mn and Fe compounds are used in water treatments, which involve physical and physico-chemical processes such as flocculation and coagulation, adsorption, and co-precipitation. These processes will be discussed in more detail in the following sections. Generally, pollutants are immobilized by interaction with Mn or Fe particles, which are subsequently settled. These physico-chemical technologies are used to remove pharmaceuticals from the water phase but not to convert or to degrade them.

2.2.1.1 Flocculation and coagulation

In flocculation and coagulation, the soluble or colloidal compounds are taken out of solution or suspension in the form of a floc or flake by using chemical flocculants or coagulants; subsequently, the formed particles are settled. Flocculation is the aggregation of particles while coagulation is a physico-chemical destabilization of the colloidal system [18]. In literature, the flocculation and coagulation is interchangeable, and in this review, we will use the term "flocculation and coagulation" to refer to either or both of these related processes as used in Bratby [25]. These flocculants and coagulants can be organic polymers, metal salts such as $FeCl_3$, and prehydrolized metal salts such as polyferric sulphate [18, 203].

Ferric salts, including $FeCl_3$ and $Fe_2(SO_4)_3$, are commonly used in flocculation and coagulation processes for organic matter removal from drinking water and wastewater [28, 168, 199]. Excess Fe(III) is generated by zero-valent iron/H_2O_2 system and improves flocculation and coagulation of organic pollutants [131, 212]. Recently, polyferric sulphate (PFS), an inorganic polymeric flocculant, is used as a new coagulant to remove pharmaceuticals. In wastewater from a pharmaceutical production facility where the main organic COD (chemical oxygen demand) consisted of the pharmaceuticals, such as cefpirome, latomoxef, aztreonam, cefoperazone, cefatridine, ceftazidime, and other chemicals like propylene glycol, over 70% of 3300 $mg \cdot L^{-1}$ COD was removed by flocculation and coagulation with PFS [345]. Results indicate that both the pH and PFS concentration influenced the removal, with the highest removal being obtained at an optimum pH of ~ 4 with 300 $mg \cdot L^{-1}$ PFS. At pH 4, the COD removal increases from 0 to 80% with increasing PFS concentrations from 0 to 200 $mg \cdot L^{-1}$; when 200 – 900 $mg \cdot L^{-1}$ PFS was dosed, COD removal only increased 10% [345]. PFS contains large amounts of polynuclear complex ions such as $(Fe_2(OH)_3)^{3+}$, $(Fe_2(OH)_2)^{4+}$, $(Fe_8(OH)_{20})^{4+}$, which leads to higher removal performance of organic compounds [318, 379], as compared to conventional flocculation and coagulation like $FeSO_4$ and $FeCl_3$.

2.2.1.2 Adsorption

Mn, Fe and their oxides can be used as adsorbents, especially when present as nanoparticles, that is, particles ranging in size between 10 and 100 nm. During adsorption, the pollutant is removed from the liquid phase through transfer to the surface of adsorbent. Especially nanoscale metal oxides, have an extremely large specific surface area supporting efficient removal of pharmaceuticals [310]. If the adsorbent has a preferential affinity for certain compounds in the liquid phase, the efficiencies can then be further enlarged with orders of magnitude [203].

MnO_2 can be used to remove pharmaceuticals and other pollutants by adsorption including antibacterial agents like sulfonamides and tetracycline, and endocrine disruptors [19, 248]. Results showed that clarithromycin and roxithromycin can be rapidly absorbed onto this amorphous MnO_2 at pH 5 [61], but adsorption did not contribute to carbamazepine removal with amorphous MnO_2 [93, 248]. Adsorption of pharmaceuticals onto MnO_2 is through surface complex forming. The different properties of pharmaceuticals affect the surface complex and will lead to different performances of adsorption.

The performance of pharmaceutical adsorption to Fe(III) has been evaluated by previous studies [26, 61, 358]. Two human-used macrolide antibacterial agents, clarithromycin and roxithromycin, are strongly adsorbed (> 90%) to Fe(III) in the form of ferrihydrate. This is probably due to the macrolide antibacterial agents that can form a complex on the surface of Fe(III) [61]. Due to the different properties of pharmaceuticals, the ability to form a complex with Fe(III) is different for each pharmaceutical, which leads to selective adsorption. The results indicate that the efficiency of adsorption is closely related to the process condition. For example, at weakly acidic pH (~ 6), the highest adsorption is achieved due to changes in the Fe(III) surface chemistry, thus making Fe(III) more selective for the carboxylic group of the investigated pharmaceuticals such as [358].

Nanoparticles and nanoscale materials including nano Fe species can also remove pollutants by adsorption. Due to their small size, ranging from 10 to 100 nm, and large specific surface area, nanoscale materials such as nanoscale zero-valent iron (nZVI) can efficiently remove pharmaceuticals via adsorption [138]. For example, nZVI adsorption can contribute to the removal of carbamazepine [277], amoxicillin, and ampicillin [76]. Supportive materials such as polyethylene glycol (PEG) and zeolite are used to improve the performance of nZVI as an adsorbent. Results show 10% more amoxicilline removal and 30% more ampicilline removal by PEG-nZVI, while nearly 30% more ampicilline removal is found by zeolite-nZVI [76]. Another nanoscale Fe-related adsorbent in pharmaceutical removal is magnetic permanently confined micelle arrays (Mag-PCMAs), which has a magnetite core confined in a silica porous layer [104, 331]. Pharmaceuticals including atenolol, gemfibrozil, and sulfamethoxazole can be absorbed and removed at $mg \cdot L^{-1}$ level by these Mag-PCMAs from water [104]. Magnetic iron oxide nanoparticles ($M_xFe_{3-x}O_4$) are used to remove organic pollutants via adsorption, where M represents one or more components from Fe, Mn, Co, Li, Ni, Zn, etc. MFe_2O_4 (M=Fe, Mn, Co, Zn) can remove more than 96% of tetracycline, oxytetracycline, and chlortetracycline at 100 $\mu g \cdot L^{-1}$ by adsorption within 5 min [12]. Incorporation of $M_xFe_{3-x}O_4$ into other chemicals like activated carbon (AC) can also be used to remove pharmaceuticals. For example, the maximum adsorption capacity of $MnFe_2O_4$/AC to sulfamethoxazole is 159 $mg \cdot g^{-1}$ at pH 7 [325], and maximum adsorption of hierarchically porous $MgFe_2O_4$/γ-Fe_2O_3 magnetic microspheres to minocycline is 201 $mg \cdot g^{-1}$ [180]. Metal-organic framework (MOF) is a class of highly crystalline porous materials consisting of a metal ion and an organic linker molecule [136]. It is used to remove hazardous organics from water by adsorption and photocatalysis [136, 332]. MIL-100-Fe is a type of Fe-organic framework, and it is used in adsorption processes to remove pharmaceuticals including naproxen and clofibric acid [91], as well as other organic pollutants like bisphenol A [110, 242]. The adsorption capacity of MOF

MIL-100-Fe can reach 100 $mg \cdot g^{-1}$, which is even higher than granular activated carbon (~50 $mg \cdot g^{-1}$) [91].

2.2.1.3 Co-precipitation

Coprecipitation is a process in which soluble compounds are removed by sequestration in a precipitating phase [218, 240]. Pharmaceutical removal through coprecipitation is observed with Fe species including Fe corrosion products [63, 76, 218]. For example, amoxicillin and ampicillin can be sequestered by precipitation with $Fe(OH)_3$ [76]. Triazole, the raw material to produce antimycotic drugs such as terconazole, is also removed with zero-valent iron (ZVI) by co-precipitation [120, 216].

2.2.2 Chemical removal

In chemical removal of pharmaceuticals, Mn and Fe play important roles as oxidants, reductants, or catalysts such as Fe(II) in Fenton processes [31, 87, 140, 248, 271]. Chemical removal of pharmaceuticals in water treatment occurs through chemical oxidation via oxidizing agents (Fe(III), Fe(VI), Mn(IV), Mn(VII)) or chemical reduction via reducing agents like nZVI to degrade a compound or a group of compounds [87, 155]. In addition to conventional oxidation processes, advanced oxidation processes (AOPs) including Fenton, photolysis, and ozonation are used to remove pharmaceuticals. In these processes, Mn and Fe species work as catalysts in the formation of free radicals such as hydroxyl radicals ($OH^{\bullet}$) and sulfate radicals ($SO_4^{\bullet-}$), which are used as strong oxidants to destroy organic compounds.

2.2.2.1 Chemical oxidation

(1) Mn and Fe as oxidants

Mn(VII) and Mn(IV) are used to remove pharmaceutical compounds and other pollutants in water and wastewater due to their high standard redox potential (+ 1.23 V for Mn(IV) and + 1.52 V for Mn(VII), see Figure 2.1(a)) [69, 93, 163, 200, 248, 312]. Permanganate (MnO_4^-) can be used to remove pharmaceuticals and other micropollutants containing electron-rich moieties [87, 96]. One study found that ciprofloxacin, lincomycin, and trimethoprim are removed in a second-order reaction from drinking water by Mn(VII), with rate constants of 0.61, 1.6, 3.6 $M^{-1}\cdot s^{-1}$, respectively [102, 103]. In a second study, more than 90% of sulfamethoxazole at concentrations between 0.5 and 5 $mg\cdot L^{-1}$ is removed with 2 $mg\cdot L^{-1}$ Mn(VII) at pH 7 [70]. Mn(VII) is also used to oxidize nonsteroidal anti-inflammatory drugs ibuprofen, diclofenac, naproxen, ketoprofen, fenoprofen, indomethacime, and salicylic acid [256]. Results showed diclofenac and indomethacime were completely removed, while the others decrease less than 30% [256].

Mn(IV) (+ 1.23 V) has a stronger oxidation potential than the Fe(III) (+ 0.77 V). This is observed in the faster transformation of clarithromycin and roxithromycin by Mn(IV), which has a 2 – 3 times higher reaction rate per surface unit than Fe(III) [61]. Studies show that amorphous MnO_2 can be used to remove pharmaceuticals such as carbamazepine, sulfamethazine, and diclofenac [69, 93, 163, 200, 312]. This chemical removal process is pH-dependent, and effective when pH < 6 [69, 93]. Over 70% of diclofenac at about 3 $mg\cdot L^{-1}$ is removed by oxidation in a MnO_2 bed filter with amorphous MnO_2 in natural environment, where adsorption of both parent compound and by-products of diclofenac oxidation is observed [108, 109]. Similarly, 95% of amoxicillin at around 360 $mg\cdot L^{-1}$ is oxidised in 4 hours with 1×1 molecular sieve-structured MnO_2 while only 4.5% of amoxicillin is removed by adsorption [146].

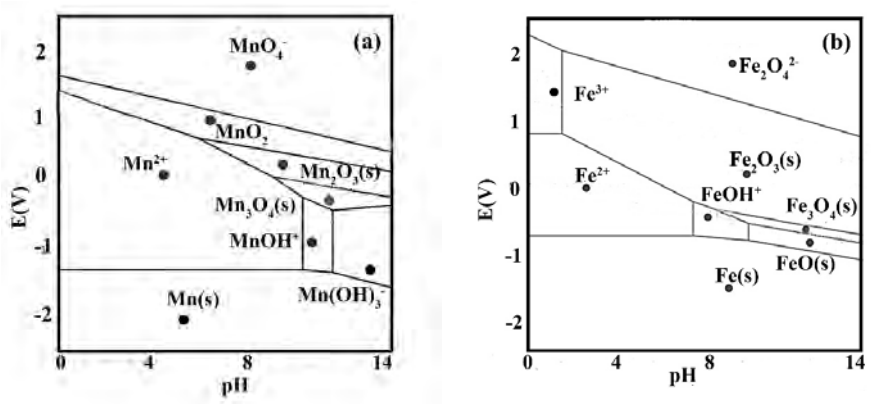


FIGURE 2.1 Eh-pH diagram for Metal in water at 25˚C ((a) for Mn; (b) for Fe). Adapted from previous data [294] using Material Project website [116]

In addition to pH, other environmental parameters also affect chemical oxidation of pharmaceuticals by MnO_2. For instance, MnO_2 oxidation is negatively influenced by cosolute compounds, such as metal ions and natural organic compounds which inhibit the chemical reaction by occupying the reactive surface site of MnO_2 [93, 96, 167, 360]. In addition, reactant loading has influence on this oxidation process. For example, loading for triclosan, ciprofloxacin, and carbadox has no effect on reaction rate constants because of the fixed number of reactive surface sites on MnO_2 surface [359]. In contrast, higher loading for tetracycline leads to lower reaction rate constants because of the self-competition for the same reactive surface sites of MnO_2 [359].

Most studies using MnO_2 to remove pharmaceuticals are carried out under aerobic condition. There are only two published studies using anaerobic condition as control group. Results show that anaerobic condition has no influence on fluoroquinolone removal [361] while it can inhibit the sulfamethazine removal [69].

Fe(III), Fe(V) and Fe(VI) are used to oxidize pharmaceuticals from water, including tetracycline, clarithromycin, and roxithromycin [328]. Both Fe(V) and Fe(VI) have a higher standard redox potential of + 2.2 V under acidic pH 4 – 5 than under neutral pH 7 (Figure 2.1(b)) [34, 122, 123]. These ferrate compounds can react with pharmaceuticals which contain electron-rich moieties [121, 124, 155, 349], which leads to the formation of nontoxic by-products and Fe(III) [121, 274]. The removal efficiency varies for the different compounds at 10 – 100 $\mu g \cdot L^{-1}$ were: ciprofloxacin > 60%, naproxen > 40%, and n-acetyl sulphamethoxazole < 10% [121, 124, 349]. In addition, 88% removal efficiencies were achieved for selected pharmaceuticals and other micropollutants by combining oxidation, and flocculation and coagulation with Fe(VI) [349]. Owing to the high standard redox potential of Fe(III) species (+ 0.770 V, Figure 2.1(b)), the removal of tetracycline antibiotics, including tetracycline, oxytetracycline, and chlorotetracycline, with Fe(III) is 2 – 20 times higher than without Fe(III) [328]. This process is influenced by pharmaceutical concentrationas well as the concentration of Fe(III) [328]. At pH 7 and 20ºC, increasing of initial tetracycline concentration caused a decrease in the reaction rate, while increasing the initial Fe(III) concentration resulted in an increases in the reaction rate.

(2) Mn and Fe as catalysts

Mn can function as catalyst to remove pharmaceuticals. Manganese oxide supported or doped by other compounds has been used in catalytic ozonation process. Alumina-supported manganese oxide suspension is used as catalyst in ozonation to remove phenazone, ibuprofen, diphenhydramine, phenytoin, and diclofenac. Results show that more than 90% TOC of pharmaceuticals mixture is removed with alumina-supported manganese oxide whereas only 20% is removed without a catalyst [350]. Carbon nanotube-supported manganese oxides are used to assist ozone to remove ciprofloxacin. Ciprofloxacin removal increases within 15 min from 26.7% of 10 $mg \cdot L^{-1}$ to 87.5% when the catalyst was added [296]. MnO_2-CuO/γ-Al_2O_3 catalyst is also used to remove ibuprofen in ozonation [21]. Ibuprofen

removal increases from 27% of 5 $mg \cdot L^{-1}$ to 55% in the presence of the catalyst. A novel Ce-doped manganese oxide octahedral molecular sieve is generated recently and used as a catalyst in ozonation [365]. 81% ciprofloxacin of 10 $mg \cdot L^{-1}$ was removed in the ozonation process with the catalyst. Both Mn-Ce-O catalyst prepared in the laboratory and commercial Fe-Mn-O catalyst are used in catalytic ozonation process and show similar catalytic capacity [195]. Approximately 60% COD of the mixture of sulfamethoxazole and diclofenac is removed in the presence of either catalyst within 120 min. Tetracycline can complex with dissolved Mn^{2+} ions, thus improving tetracycline removal by oxygen. At pH 8 – 9.5, over 90% removal of tetracycline is achieved in Mn^{2+}-mediated system, and the reactivity trend is as follows: oxytetracycline > tetracycline >> isochlorotetracycline [35].

In classic Fenton's reagent (Fe/H_2O_2 system), a mixture of Fe(II) and hydrogen peroxide generates $OH^{\bullet}$ (Figure 2.2) [212, 238]. $OH^{\bullet}$ can oxidize pharmaceuticals [10, 111, 160] together with another oxidant produced in the process, for instance Fe(IV) species at pH >5 (Equation 2.1) [107, 221]. For example, more than 90% berberine at around 1000 $mg \cdot L^{-1}$ and over 70% metronidazole at 1 $mg \cdot L^{-1}$ are removed in Fe/H_2O_2 oxidation systems [44, 275]. In addition, nearly complete removal of paracetamol, chloramphenicol, and diclofenac is obtained [10]. In Fenton's reaction, ethylenediaminetetraacetic acid (EDTA) is used to improve the removal performance [15, 133, 150, 375]. The complex of Fe(II) and EDTA reduces dissolved O_2 and produces H_2O_2 and $OH^{\bullet}$ [15, 150]. In addition, the presence of EDTA enhances the Fe(III) solubility, thus preventing Fe(III) precipitation as well as possible Fe(II) coprecipitation with Fe(III) [15, 133]. Additional flocculation and coagulation by Fe(III) improves removal in Fenton's reaction [302].

$$Fe(II) + H_2O_2 \longrightarrow Fe(IV)\ (e.g.\ FeO^{2+}) + H_2O \qquad (2.1)$$

Other Fe species or compounds containing Fe can work as catalysts in Fenton's reactions. In these processes, pharmaceuticals and other micropollutants can be removed via oxidation [48, 270, 277]. For example, ZVI and nZVI are used as a catalyst in Fenton's reaction with H_2O_2 to remove pharmaceuticals [270, 277] and other pollutants [145, 159, 271]. Under acidic conditions (pH < 3), ZVI is oxidized to Fe(II) on the surface of the metal and then Fe(II) catalyzes the Fenton process. In the presence of 1.2 $g \cdot L^{-1}$ ZVI, 3.2 $g \cdot L^{-1}$ H_2O_2, and 0.1 $g \cdot L^{-1}$ total organic carbon (TOC), 80% of a mixture of pharmaceuticals and other organic pollutants are removed in 1 hour from pharmaceutical wastewater with a pH of 3 [270]. Similarly, carbamazepine can be totally removed from both distilled water and groundwater with 20 $mg \cdot L^{-1}$ nZVI and 25 $mg \cdot L^{-1}$ H_2O_2 [277].

Furthermore, the Fenton's reaction can be enhanced by ultraviolet–visible photo, electronic, and ultrasonic irradiation [8, 9], which are photo/Fe/H_2O_2 system, electro/Fe/H_2O_2 system, and sono/Fe/H_2O_2 system, respectively. In the photo/Fe/H_2O_2 system, it is reported that a wavelength below 254 nm, or higher than 300 nm enhances the photolysis of Fe(III) to Fe(II) (Figure 2.2) [47, 311, 316]. Via photo/Fe/H_2O_2 system, completely removal of 16 $mg \cdot L^{-1}$ metoprolol and 17.6 $mg \cdot L^{-1}$ atenolol are obtained within 150 min [316], while 76% of 10 $mg \cdot L^{-1}$ sulfamethoxazole is removed within 7 hours [311].

Electro-Fe(II)/H_2O_2 system is another advanced process in which H_2O_2 is generated continuously if sufficient O_2 is present at a suitable cathode (Equation 2.2, Figure 2.2) [27]. In addition, the production of $OH^{\bullet}$ is accelerated by the regeneration of Fe(II) from soluble Fe(III) (Equation 2.3).

$$O_2(g) + 2H^+ + 2e^- \longrightarrow H_2O_2 \quad (2.2)$$

$$Fe^{3+} + e^- \longrightarrow Fe^{2+} \quad (2.3)$$

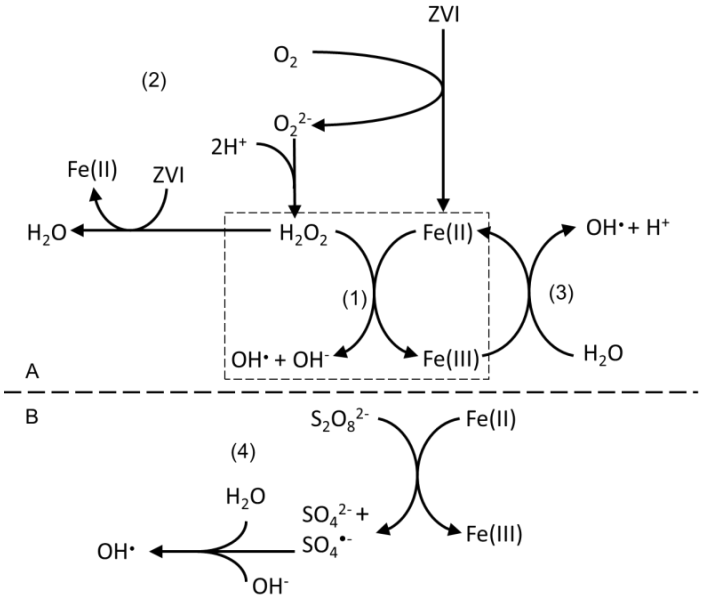


FIGURE 2.2 Activated species generated in Fe-based advanced oxidation processes (AOPs) where (A) is for $OH^{\bullet}$ based AOPs [48, 90, 212, 238] and (B) is for $SO_4^{\bullet-}$ based AOPs [75, 119], (1) generated activated species from Fe/H_2O_2, photo/Fe/H_2O_2, electro/Fe/H_2O_2, sono/Fe/H_2O_2, etc.; (2) generated activated species from ZVI/O_2; (3) generated activated species from photo degradation, electro degradation, sono degradation, etc.; (4) generated activated species from Fe/persulfate

Experiments with electro/Fe/H_2O_2 system indicate that this process is efficient for pharmaceutical removal [114, 224, 282]. For example, 40% mineralization of atenolol at 158 mg·L^{-1} [114] and nearly 30% mineralization of ranitidine at 33.8 mg·L^{-1} [224] are obtained. Additionally, a combination of photo and electro irradiation for photo- electro/Fe/H_2O_2 system increases the performance in pharmaceutical removal even further [114, 224]. Results showed the complete removal of atenolol and 80% mineralization of ranitidine by photo-electro/Fe/H_2O_2 system with solar radiation. This is due to (1) the production of more $OH^{\bullet}$ than photo/Fe/H_2O_2 system or electro/Fe/H_2O_2 system alone (Figure 2.2), and (2) the regeneration of Fe(II) also contributes to the removal [114, 224]. Sono/Fe/H_2O_2 system has been employed to remove pharmaceuticals as ultrasonic irradiation improves the Fenton's reaction. Complete removal of levofloxacin is obtained by sono/Fe/H_2O_2 system in the presence of Fe_3O_4 magnetic nanoparticles [336].

Persulfate can be activated at pH 6 by different Fe species, such as ZVI, Fe(II), and Fe_3O_4. This process generates free $SO_4^{\bullet-}$ as well as other active species, such as $OH^{\bullet}$ (Figure 2.2) [75, 119, 347]. Standard redox potential of $SO_4^{\bullet-}$ is + 2.6 V, which is similar to $OH^{\bullet}$ with + 2.72 V [211]. Sulfate radical-based advanced oxidation processes (SR-AOPs) are new methods to oxidize pharmaceuticals in water [75, 119, 273, 347].

In previous studies, Fe-activated persulfate has been used to remove pharmaceuticals. Fe(II)-activated persulfate oxidation removes 50% carbamazepine at 6 $mg \cdot L^{-1}$ [245], and 96% ciprofloxacin and 75% sulfamethoxazole both at 7.6 $mg \cdot L^{-1}$ [119]. In micrometric ZVI-activated persulfate oxidation, 10 $mg \cdot L^{-1}$ sulfamethoxazole is completely removed after 60 min [75]. Additionally, more than 90% removal efficiency of sulfamonomethoxine at 16.8 $mg \cdot L^{-1}$ is observed with Fe_3O_4-activated persulfate oxidation [347]. Together with simulated solar light irradiation, complete removal of carbamazepine is obtained by an Fe(II)/persulfate/UV-Vis system, in which persulfate is activated by Fe(II) [1]. Furthermore, Fe can activate peroxymonosulfate ($KHSO_5$, PMS) to generate $SO_4^{\bullet-}$. One study shows that this Fe(II)-activated PMS can completely remove triclosan, sulfamethoxazole, and acetaminophen, which is more effective than Fe(II)-activated persulfate under the same conditions [213].

$M_XFe_{3-X}O_4$, including incorporation of $M_XFe_{3-X}O_4$ to other chemicals, has also been used in catalytic oxidation processes to remove pharmaceuticals. A new synthetic material, core-shell structured Fe_3O_4/α-MnO_2 microspheres (Fe_3O_4/α-MnO_2), is used to remove pharmaceuticals via catalytic oxidation with persulfate. In one previous study, removal is observed for ciprofloxacin: 90% of 50$mg \cdot L^{-1}$ by Fe_3O_4/α-MnO_2-activated persulfate, 80 % by α-MnO_2-activated persulfate, and almost 30% by Fe_3O_4-activated persulfate [370].

Similarly to Fenton's reaction, Fe can work as a catalyst in other advanced oxidation processes including photo degradation, sono degradation, and ozonation. However, as opposed to Fenton or Fenton-like processes, these AOPs do not use H_2O_2 to generate $OH^{\bullet}$ [5, 31, 51, 112]. Fe-ZnO is used in catalytic photo degradation, catalytic sono degradation, and catalytic photo-sono degradation, in which irradiation of ultrasonic frequencies or/and visible light can generate reactive radicals from water through forming vapor cavities [187]. Results show that the removal rates for diclofenac increase in the following order: catalytic photo degradation (0.4×10^{-7} $M\cdot min^{-1}$) < catalytic sono degradation (14.3×10^{-7} $M\cdot min^{-1}$) < catalytic photo-sono degradation (15.3×10^{-7} $M\cdot min^{-1}$) [187].

Even without H_2O_2, ZVI can catalyse processes in the presence of O_2 (Figure 2.2) [15, 48, 84]. 96% diazepam at 25 $mg\cdot L^{-1}$ is removed by the ZVI/O_2 system after 60 min. Moreover, 60% diazepam is mineralized [15]. However, complexation of Fe may increase yield of H_2O_2 and change the nature of the reactive oxidant such as Fe(V) [133].

Ozonation effectively removes pharmaceuticals because ozone is a strong oxidant with a standard redox potential of + 2.08 V [17, 105]. Fe species are used in catalytic ozonation process and showed a high, stable catalytic activity [183]. Removal efficiencies of more than 90% of herbicides and pharmaceuticals were achieved within 40 min with magnetic cobalt-doped Fe_3O_4 (FeCo), whereas less than 40% is removed without the catalyst. Hematite (α-Fe_2O_3) and Fe_3O_4 show catalytic activity in ozonation as well. The order of catalytic activity is FeCo > Fe_3O_4 > α-Fe_2O_3 [184].

2.2.2.2 Chemical reduction

Chemical reduction is often used to remove pollutants such as heavy metals [320] and nitrate [100]. However, its application for pharmaceutical removal has not been extensively studied. During chemical reduction, pharmaceuticals receive electrons from other chemical compounds. For example, (n)ZVI can reduce pharmaceuticals, resulting in the oxidation of Fe; this occurs alone or in conjunction with other removal processes such as adsorption [76, 217, 247, 277, 376]. Previous studies showed that pharmaceutical compounds with certain functional groups (such as C-N, N=N, nitro or halogens) can be reduced by (n)ZVI via chemical reduction [247]. Results indicated rapid reduction of amoxicillin and ampicillin, while less than 12.6% removal of carbamazepine is observed [76]. Diazepam is removed by chemical reduction for almost 65% [15]. In addition, complete removal of chloramphenicol is obtained [344].

2.2.3 Biological-related removal

There are pharmaceutical removal processes related to the metal-related activity of different types of microorganisms, including bacteria and fungi [130, 225]. In these processes, microorganisms can produce Mn- or Fe-oxides, which are used to remove pharmaceuticals by chemical oxidation. In addition, bacteria or fungi can work in advanced oxidation together with Fe which is known as biologically catalyzed advanced oxidation [86, 192].

Biogenic Mn oxides (bioMnOx, also known as Mn bio-oxides) can efficiently remove pharmaceuticals [64, 67] and other pollutants [97, 202]. These bioMnOx are produced by oxidation of Mn(II) by bacteria such as *Pseudomonas putida* MnB6 (BCCM/LMG 2322) and *Bacillus sp.* SG-1, or fungi [97, 301]. Enrichment of *P. putida* which was used to generate bioMnOx was achieved under nitrifying conditions in a down flow sponge reactor with artificial wastewater [29]. As compared to synthetic MnO_2, microbially produced bioMnOx has a unique structure, yielding a variety of advantages for oxidation. BioMnOx are reactive under neutral pH (~7), instead of the

acidic conditions required for abiotically produced MnOx [146, 248]. Additionally, the bacteria can bind the intermediate Mn(III) compounds via ligands, thus effectively increasing the oxidative power of a Mn-bacteria mixture [200]. As a result, diclofenac removal via adsorption and chemical oxidation with bioMnOx is 10-times faster than with synthetic MnO_2 [65]. Additionally, complete removal of ciprofloxacin [312], and 17% removal of carbamazepine is obtained [64]. Studies also show that the performance will increase in the presence of trace metals, such as Ag or Pb. The interaction between the metals and the microorganism cell leads to formation of reactive oxygen species [200]. These reactive species can improve the pharmaceutical removal [200, 321].

Manganese peroxidase (MnP) is an extracellular enzyme from the lignin-degrading basidiomycetes fungi such as *Phanerochaete chrysosporium*, which can oxidize Mn^{2+} to Mn^{3+}. Mn^{3+} can act as a mediator to oxidize phenolic compounds. The presence of Mn^{2+} also stimulates the MnP production and functions as a substrate for MnP. The MnP is a key factor for fungi to remove pharmaceuticals including carbamazepine [80, 265]. Recently, a crude MnP is produced by white-rot fungi *Phanerochaete chrysosporium* and used as a biocatalyst to remove tetracycline and oxytetracycline via enzymatic degradation [337]. Results show that 72.5% of 50 $mg \cdot L^{-1}$ tetracycline and 84.3% of 50 $mg \cdot L^{-1}$ oxytetracycline are removed at 40 $U \cdot L^{-1}$ within 4 h.

Biological Fenton-like system is a recently discovered biological advanced oxidation process in which radicals are generated in the presence of white-rot fungi *Trametes versicolor*. The biological Fenton-like reaction occurs in the presence of lignin-derived quinone (2,6,-dimethoxy-1,4-benzoquinone, DBQ) and Fe(III) (Figure 2.3) [86, 192]. In this process, DBQ is reduced to hydroquinone ($DBQH_2$) in the presence of an intracellular quinone reductase produced from the fungi, followed by the generation of semiquinone radicals ($DBQ^{\bullet -}$) via subsequent oxidation of $DBQH_2$ by lignin-modifying enzymes (laccases and peroxidases) which are also from

the white-rot fungi. Fenton's reagent is formed by $DBQ^{\bullet -}$ auto-oxidation catalyzed by Fe(III), and $OH^{\bullet}$ is produced via this cycling [81, 193]. In this system, removal efficiencies of 80% were achieved for lofibric acid, carbamazepine, atenolol, and propranolol; more than 20% of the observed carbamazepine removal could be attributed to biological activity by this white-rot fungi [193].

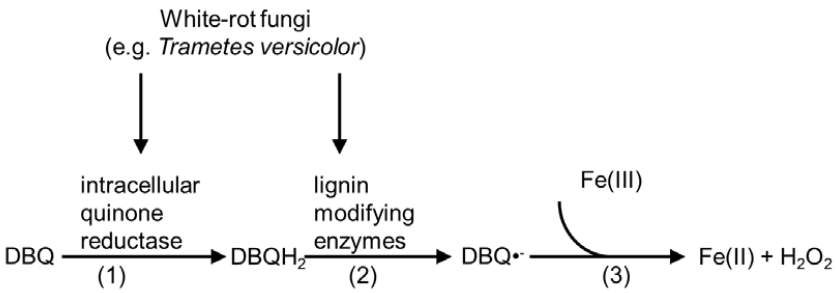


FIGURE 2.3 Biological Fenton's-like system catalysed by white-rot fungi [193] (1) fungi catalyse the conversion of BDQ to $DBQH_2$ by an intracellular quinone reductase from the fungi; (2) oxidation of $DBQH_2$ to $DBQ^{\bullet -}$ by lignin modifying enzymes (laccases and peroxidases) from the white-rot fungi; (3) Fenton's reagent is formed by semiquinone radicals autoxidation catalyzed by Fe(III)

TABLE 2.1 Pharmaceutical removal from water by Mn- or Fe- based technologies

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
Polyferric sulphate (PFS)	Flocculation and coagulation	Antibiotic fermentation wastewater	Batch experiment	33% – 84% (COD)	Aztreonam, cefatridine, cefoperazone, ceftazidime, latomoxef, and ropylene glycol	[345]
Fe(III) oxidation	Chemical oxidation (adsorption)	Distilled water or ultrapure water; surface water; municipal wastewater (secondary clarification effluent)	Batch experiment	45% – 90%	Chlorotetracycline, clarithromycin, roxithromycin, tetracycline, and oxytetracycline	[61, 328]
ZVI/H_2O_2 system (including ZVI/H_2O_2, ZVI/H_2O_2/O_2)	Catalytic oxidation (coprecipitation)	Pharmaceutical wastewater	Lab-scale reactor	7% – 100% (TOC)	No details [c]	[270, 271]
ZVI/O_2 system	Catalytic oxidation (coprecipitation)	Ultrapure water	Batch experiment	50% – 96%	Diazepam	[15]
nZVI/H_2O_2 system (including nZVI/H_2O_2, nZVI/O_2	Catalytic oxidation (adsorption and coprecipitation)	Distilled water; groundwater	Batch experiment	95% – 99%	Carbamazepine, and chloramphenicol	[277, 344]
(n)ZVI reduction	Reduction (adsorption and corecipitation)	Water; ultrapure water or distilled water	Batch experiment	2.6% – 100%	Amoxicillin, ampicillin, carbamazepine, and diazepam,	[15, 76, 277, 344]
Mag-PCMAs	Adsorption	Water	Batch experiment	2.7 –15mg·g^{-1} [d]	Atenolol, gemfibrozil, and sulfamethoxazole	[104]

Continued Table 2.1

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
$M_xFe_{3-x}O_4$ (including $M_xFe_{3-x}O_4$ complex, M = Mn, Fe, Co, Zn, etc.)	Adsorption,	Water	Batch experiment	> 90% (<159mg·g^{-1}[d])	Chlortetracycline, minocycline, oxytetracycline, sulfamethoxazole, and tetracycline	[12, 180, 325]
MIL-100-Fe	Adsorption	Deionized water	Batch experiment	75 − 100 mg·g^{-1}[d]	Naproxen	[91]
Ferrate (Fe(V) and Fe(VI))	Chemical oxidation (flocculation and coagulation)	Deionized water; second sedimentation effluent	Batch experiment	0 − 100%	Atenolol, bezafibrate, carbamazepine, ciprofloxacin, cyclophosphamide, diclofenac, enrofloxacin, ibuprofen, ifosfamide, indometacin, lidocaine, meclofenamic acid, mefenamic acid, metoprolol, n-acetyl sulphamethoxazole, naproxen, paracetamol, propranolol, tolfenamic acid triclosan, and lidocaine	[121, 124, 155, 338, 349]
Mn(IV) oxidation	Chemical oxidation (adsorption)	Influent sewage water; distilled water or ultrapure water; tap water	Batch experiment; column experiment; lab-scale reactor	3% − 99%	Amoxicillin, carbamazepine, clarithromycin, diclofenac, levofloxacin, paracetamol, roxithromycin, sulfamethazine, and triclosan	[61, 69, 93, 96, 108, 109, 146, 163, 360]

Continued Table 2.1

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
Permanganate (Mn(VII))	Chemical oxidation	Sewage water; deionized water	Batch experiment	30% – 100%	Ciprofloxacin, diclofenac, fenoprofen, ibuprofen, indomethacime, ketoprofen, lincomycin, naproxen, salicylic acid, sulfamethoxazole, and trimethoprim,	[70, 102, 103, 256]
Fe/H_2O_2 system	Catalytic oxidation	Deionized water; industrial berberine wastewater (secondary effluent); pharmaceutical wastewater	Batch experiment; lab-scale experiment; pilot-scale reactor	45% – 100%	Acetaminophen (i.e. paracetamol), atenolol, berberine, caffeine, diclofenac, fluoxetine, gemfibrozil, iopromide, metronidazole, naproxen, and sulfamethoxazole	[10, 44, 111, 160, 275, 302, 345]
photo/Fe/H_2O_2 system	Catalytic oxidation	Distilled water; seawater; ultrapure water	Lab-scale experiment; pilot-scale reactor	30% – 100%	Atenolol, metoprolol, metronidazole, and sulfamethoxazole,	[275, 311, 316]
electro/Fe/H_2O_2 system	Catalytic oxidation	Ultrapure water	lab-scale experiment; pilot-scale reactor	23% – 90%	Atenolol and ranitidine	[114, 224]
(solar) photoelectro/Fe/H_2O_2 system	Catalytic oxidation	Ultrapure water	Lab-scale experiment; pilot-scale reactor	44% – 97%	Atenolol and ranitidine,	[114, 224]
Fe_3O_4 MNP/H_2O_2	Catalytic oxidation	Ultrapure water	Lab-scale reactor	71%	Levofloxacin	[336]

Continued Table 2.1

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
sono-Fe_3O_4 MNP/H_2O_2	Catalytic oxidation	Ultrapure water	Lab-scale reactor	30% − 99%	Levofloxacin	[336]
Fe-ZnO catalysed photocatalysis	Catalytic oxidation	Water	Lab-scale experiment	20%	Diclofenac	[187]
Fe-ZnO catalysed sonocatalysis	Catalytic oxidation	Water	Lab-scale experiment	> 90%	Diclofenac	[187]
Fe-ZnO catalysed sonophotocatalysis	Catalytic oxidation	Water	Lab-scale experiment	> 90%	Diclofenac	[187]
Fe-catalysed ozonation	Catalytic oxidation	dionized water	Semibatch experiments	79% − 98%	Phenazone	[184]
Fe-activated persulfate	Catalytic oxidation	Distilled and deionized water; ultrapure water; river water	Batch experiment; batch reactor	6% − 100%	Acetaminophen (i.e.paracetamol) carbamazepine, ciprofloxacin sulfamethoxazole, sulfamonomethoxine, and triclosan	[119, 213, 245, 347]
Fe-activated persulfate	Catalytic oxidation	Distilled and deionized water; ultrapure water; river water	Batch experiment; batch reactor	6% − 100%	Acetaminophen (i.e.paracetamol) carbamazepine, ciprofloxacin sulfamethoxazole, sulfamonomethoxine, and triclosan	[119, 213, 245, 347]
Fe-activated PMS	Catalytic oxidation	Water	Batch reactor	100%	Sulfamethoxazole and triclosan	[213]

Continued Table 2.1

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
Mn-catalysed ozonation	Catalytic oxidation	Distilled water	Semibatch experiment	10% – 90%	Ciprofloxacin, diclofenac, diphenhydramine, ibuprofen, phenazone, phenytoin, and sulfamethoxazole	[21, 195, 296, 350, 365]
Mn(II)-mediated system	Catalytic oxidation	Water	Batch experiment	35% – 90%	Chlorotetracycline , tetracycline, and oxytetracycline	[35]
Fe_3O_4/α -MnO_2 -activated persulfate	Catalytic oxidation (adsorption)	Water	Lab-scale experiment	90%	Ciprofloxacin	[370]
BioMnOx oxidation	Chemical oxidation (adsorption, biological degradation)	Deionized water; sewage treatment plant effluents	Batch experiment; lab-scale reactor	1% – 100%	Bezafibrate, carbamazepine, 10,11-dihydroxy-10,11-dihydrocarbamazepine, 10,11-dihydrocarbamazepine, ciprofloxacin, clarithromycin, codeine, dihydrocodeine, diazepam, diclofenac, erythromycin, ibuprofen, methadone, morphine, naproxen, nordiazepam, oxazepam, primidonesulfamethoxazole , n-acetyl-sulfamethoxazole, tramadol, and trimethoprim	[64, 65, 67, 200, 312]

Continued Table 2.1

Process	Removal mechanism[a]	Matrix	Scale	Performance[b]	Pharmaceuticals	Reference
Biological Fenton-like system	Catalytic oxidation (biological degradation by *Trametes versicolor*)	Ultrapure water	Batch experiment	> 80%	Atenolol, carbamazepine, and propranolol	[193]
Crude MnP degradation	Catalytic oxidation (enzymatic degradation by *Phanerochaete chrysosporium*)	Water	Batch experiment	72% – 84%	Tetracycline and oxytetracycline	[337]

[a] The removal mechanism in parentheses is the secondary mechanism or responsible microorganism in the processes
[b] COD=Chemical Oxygen Demand, TOC=Total Organic Carbon. The percentage gives the removal efficiency range of tested pharmaceuticals. If no pharmaceuticals are mentioned, the removal is based on TOC or COD removal efficiencies
[c] No details means there are no specific pharmaceuticals mentioned in the papers
[d] The number is material adsorption capacity of pharmaceuticals instead of the removal efficiency

2.3 Evaluation and discussion

Mn- and Fe-based water treatment technologies have the potential to play important roles in pharmaceutical removal processes. However, there are some challenges to the direct application of available water technologies for the removal of pharmaceuticals. The four main challenges, including advantages and challenges, will be explained in detail in the section 2.3.1 – 2.3.4.

First of all, most described technologies are nonspecific, meaning that they can remove various pharmaceuticals as well as other pollutants. While this versatility can be an advantage, it can also lead to more consumption of reagents in order to also remove pharmaceuticals. Secondly, the high removal efficiency obtained with Mn- or Fe-based technologies require specific reaction conditions, which may result in more consumption of energy or chemicals. Finally, some Mn- or Fe-based technologies can oxidize the pharmaceuticals into by-products or intermediates. While these intermediates may be more biodegradable than the parent pharmaceutical, in some cases, these daughter compounds are either toxic or recalcitrant to further removal. Finally, the crystal structures and morphologies will also affect pharmaceutical removal performance. Additionally, using these Mn- and Fe-based technologies will introduce Mn- or Fe-compounds into the environment. In general, the presence of Fe or Mn in the environment is safe. However, some compounds used in Mn- or Fe-based technologies such as nZVI or persulfate can be toxic to the native microorganisms.

In the following section, we will evaluate the positive and negative aspects of using the currently available Mn- or Fe-based technologies for pharmaceutical removal, highlighting the challenges of application but also the potential of these technologies.

2.3.1 Nonspecificity

Many physico-chemical, chemical, and biologically related pharmaceutical removal processes with Fe or Mn are nonspecific. This improves the robustness and versatility of the technology, as most of these processes can remove various pharmaceuticals (Table 2.1). For example, Fe/H_2O_2/system is suitable for removing compounds with a range of chemical properties, including antibiotics, analgesics, beta-blockers, and lipid-lowering drugs. However, this versatility means that pharmaceuticals will be removed together with other organic and inorganic pollutants. For instance, bioMnOx is used to remove pharmaceuticals, and also heavy metals, such as Pb and As [97]. This nonspecificity towards pharmaceuticals can reduce the removal efficiency, as other pollutants will be removed together with pharmaceuticals, thus competing for the available Mn or Fe. For example, organic compounds and colloids can compete for the surface of amorphous MnO_2, leading to lower removal of carbamazepine [93]. Even with selective oxidation processes like ferrate and permanganate oxidation, they will not only attack pharmaceuticals containing electron-rich organic moieties, but also aliphatic and aromatic organic compounds [77, 305], phosphate [155], and taste and odor compounds [287]. Thus, other compounds in the matrix compete with the pharmaceuticals for Mn or Fe. While this can achieve the goal of removing both common pollutants and pharmaceuticals, the nonspecificity of the process will increase the reagent consumption for removal of a certain amount of pharmaceuticals.

2.3.2 Treatment conditions

There are a variety of robust technologies that can achieve high pharmaceutical removal efficiencies. However, efficient and reliable removal can only be obtained under specific treatment conditions. Almost all pharmaceuticals can be completely removed by all AOPs, including SR-AOPs (Table 2.1). In addition, direct chemical oxidation (e.g. Fe(III), Mn(IV), ferrate, and permanganate oxidation) and chemical reduction (ZVI and nZVI reduction) result in more than 90% removal of pharmaceuticals. In order to achieve optimal removal, chemical treatments usually require acidic conditions (pH < 6). The sterilized condition for pure culture cultivation is used in biologically related pharmaceutical removal process. BioMnOx used for pharmaceutical removal can be produced by *P. putida* MnB6 (BCCM/LMG 2322), which grows in synthetic medium [64, 65, 67, 200]. Similarly, the biological Fenton-like system with the white-rot fungi *T. versicolor* [192] requires an acidic medium (pH=4.5) for the cultivation of this fungi [43, 86, 192, 193]. Thus, specific cultivation equipment and sterile conditions are needed, and this might limit further application of these technologies in pharmaceutical removal technologies.

2.3.3 Intermediates and by-products

In most pharmaceutical removal processes with Fe or Mn, incomplete mineralization may occur. For example, only 30% – 40% mineralization is obtained in electro/Fe/H_2O_2 system processes [114, 224]. The presence and accumulation of by-products can be advantageous for further downstream treatment. For example, processes like Fe/H_2O_2/system will result in partial oxidation of compounds, thus improving the biodegradability of the pharmaceuticals in wastewater [10, 302]. A previous study using Fe/H_2O_2/system as pretreatment shows improved BOD/COD ratios from 0.25 to 0.50 following chemical oxidation, indicating improved biodegradability [10]. Thus, combining chemical oxidation of the pharmaceutical with biological treatment of the by-products can result in

complete mineralization. Similarly, after ferrate treatment, biodegradation of water containing beta-blockers improves from nonbiodegradable to 14% – 70% biodegradation [338].

While improved biodegradability can be observed, some by-products will be similarly or even more toxic or recalcitrant as their parent compounds, especially by-products from the oxidation process. In a previous study, the toxicity of atenolol's intermediates from photo/Fe/H_2O_2 system process is higher than the parent compound [316]. Moreover, sulfamethoxazole removal from wastewater by solar photo/Fe/H_2O_2 system increases the toxicity of the wastewater from 16 to 86%, as assessed by *Vibrio fischeri* bioassays [311]. Another important product in these pharmaceutical removal processes is Mn(II) or Fe(II). Even though they are less threat to human health, they can have a negative effect on the taste, appearance, and staining of water. These results indicate that (1) these treatment technologies must go beyond merely considering removal of the parent pharmaceutical, and (2) many treatment technologies will require a downstream step to ensure complete removal of all toxic compounds.

2.3.4 Effects of Fe or Mn compounds

Mn or Fe compounds exist in different crystal structures and morphologies, and this can lead to different pharmaceutical removal performance. For example, the oxidation efficiency of naproxen with α-MnO_2 nanomaterials follows the order of commercial particles < nanorods < flower-like nanostructures < nanoparticles [368]. Similarly, comparing different types of iron particles for pharmaceutical removal, ZVI has a faster rate for removal of ampicillin than nZVI [76].

In general, Mn or Fe compounds in pharmaceutical removal processes are safe to human or ecosystem. For example, nanomaterials like nZVI and MNPs are promising materials for pharmaceutical removal, but they are also potentially toxic to the environment. Previous studies show that nZVI results in ecotoxicity to organisms in both fresh and marine water [134, 235].

Daphnia magna survival exposed to nZVI (Fe ≥ 5mg·L^{-1}) drops within 96 h [134]. In addition, nZVI is also potentially toxic to nerve cells, animal cells, and human cells [42, 137, 235]. One possible explanation is the physical disruption of cell membranes by nZVI. It can also enhance the biocide effect of Fe [154]. Toxicity to microorganisms is also observed with other Mn- or Fe-containing nanoparticles [16]. Other compounds used in Mn- and Fe-based technologies like peroxymonosulfate are also harmful to human [222].

2.4 Outlooks

The challenges and limitations of current Mn- or Fe--based pharmaceutical technologies discussed above require either further optimizing current technologies or developing new technologies (Figure 2.4). These new technologies have many of the treatment advantages mentioned in section 2.2.3, including nonspecificity, high removal efficiency, and/or potentially biodegradability improvements. These promising technologies require less specific treatment conditions and/or the products will be less toxic, thus addressing some of the challenges mentioned in section 2.2.3.

2.4.1 Fe- or Mn-enhanced processes

There are some existing technologies for pharmaceutical removal which could be further enhanced through the addition of Mn and Fe. After improvement or enhancement, these technologies are likely to more efficiently remove pharmaceuticals.

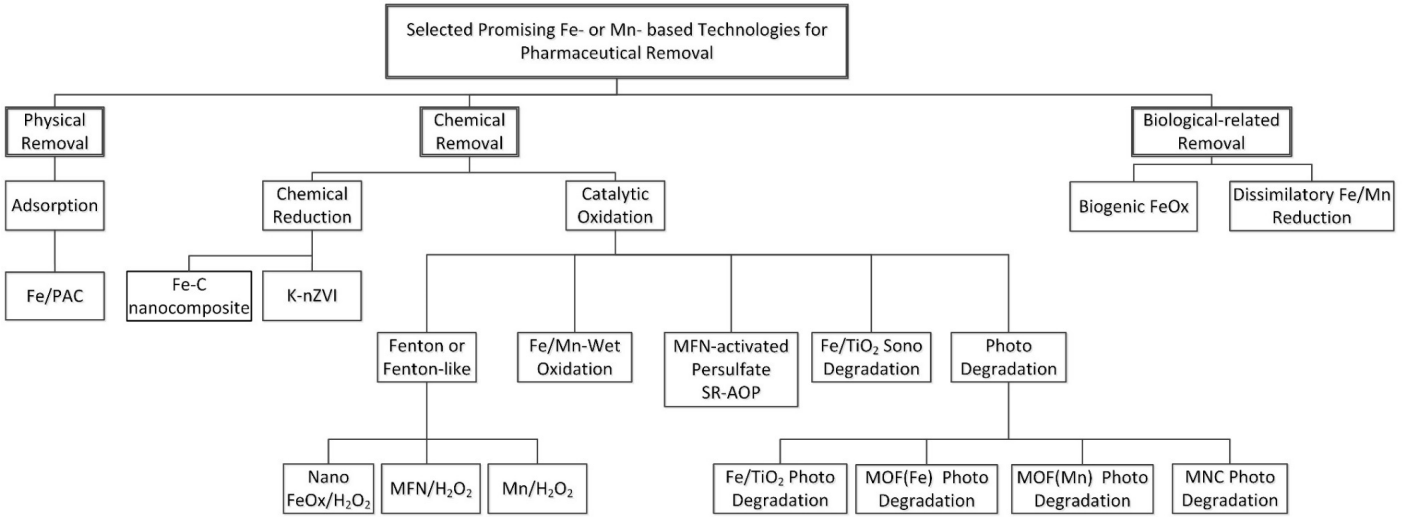


FIGURE 2.4 Promising Mn- or Fe-based technologies to remove pharmaceuticals

Powdered activated carbon (PAC) adsorption is a physico-chemical removal process for pharmaceuticals [166, 191] and other pollutants [73, 98, 244]. Studies show that addition of Fe species can improve the performance of PAC adsorption [89, 231]. Research investigating the removal of bisphenol A and natural organic matter shows that the adsorption capacities were in the following sequence: bare PAC < hematite/PAC < magnetite/PAC < ferrihydrite/PAC [231]. These results show that adding Fe species can improve the adsorption of compounds onto PAC, which is a known physico-chemical process for pharmaceutical removal. While the adsorption on Fe/PAC is a nonspecific pharmaceutical removal process, this physio-chemical technique does not result in toxic products formation.

AOPs such as photo degradation and sono degradation can be accelerated by catalysts. Fe can work as a doping agent for catalysts such as TiO_2 in catalytic photo degradation and sono degradation. In catalytic photo degradation, Fe-doped catalyst can improve the removal of volatile organic compounds acetone and benzyl alcohol [286, 373]. Similarly, sono degradation catalysed by Fe-doped TiO_2 shows notably higher removal of the dyes Blue 4 dye and azo fuchsine than undoped TiO_2 [117, 329].

Wet oxidation is the oxidation of organic and inorganic substances in an aqueous solution or suspension by means of oxygen or air at 200˚C – 320˚C and 2 – 20 MPa either with or without [139, 142, 157, 378]. Wet oxidation includes wet air oxidation and wet peroxide oxidation and is used as a pretreatment technology for pharmaceutical wastewater [99, 201, 357, 371]. Results show that Mn and Fe (together with Ce, Pt or C) can catalyse wet oxidation for phenolic compounds removal [40, 71, 251, 264]. AOPs can form $OH^{\bullet}$ and other active oxygen species which are efficient and nonspecific in pharmaceutical removal processes. In previous studies, Mn is used as a catalyst in supercritical water oxidation to treat pharmaceutical laboratory wastewater [259]. In addition, results show wet oxidation can significantly improve the biodegradability of pharmaceutical wastewater from pharmaceutical industry [82, 230, 357]. For example, the ratio of BOD_5/COD in

pharmaceutical wastewater increases from 0.1 to 0.75 by catalytic wet air oxidation at 220 ˚C [357].

2.4.2 Advanced Mn or Fe compounds

Advanced Mn or Fe compounds such as Fe-organic framework, nZVI, and bioMnOx are used to remove pharmaceuticals. These Fe or Mn compounds will be synthesized by combining with other materials as well as developing new generation processes. These advanced Fe or Mn compounds may lead to better pharmaceutical removal.

Some advanced Mn or Fe compounds are used as catalysts in AOPs (Fenton, photo degradation, and SR-AOPs), which are currently employed to remove pharmaceuticals. Nano particulate Fe–oxides (nano FeOx) is a newly synthetic Fe compound which can catalyse Fenton's reaction [355, 356]. Results show that higher concentrations of nano FeOx leads to a higher reaction rate [355, 356]. Moreover, nano FeOx can improve the removal of pollutants via adsorption because of the large specific surface area of these nanoparticles [355, 356]. Metal-organic framework containing Fe or Mn is used as a catalyst in photo degradation with both visible and UV irradiation [326]. Another new synthetic AOPs catalyst are ferrite nanoparticles ($M_xFe_{3-x}O_4$ nanoparticles, M=Mn, Fe, Co, etc.). For example, $MnFe_2O_4$ (MFN) can catalyse photo degradation or Fenton's reactions. Over 90% removal of dye compounds was obtained [190]. MFN can also activate PMS, which is used to remove pollutants via SR-AOPs [353]. In this removal process, adsorption is an important mechanism [333]. Incorporation of $M_xFe_{3-x}O_4$ to metal oxides or carbon is also promising in pharmaceutical removal. For example, Fe_3O_4-Cr_2O_3 magnetic nanocomposite (MNC) is used to catalyse photo degradation. This MNC shows efficient removal of 4-chlorophenol under UV irradiation, good magnetic separation for recovery, and recyclability [281]. Kaolinite-supported nZVI (k-nZVI) is recently synthesized [36, 315]. It is proven that nZVI can- remove pharmaceuticals through adsorption and chemical reduction [76, 277], and it can catalyse Fenton-like processes to

remove pharmaceuticals. In addition, kaolinite alone can remove the pharmaceutical metoprolol by adsorption together with talc [165]. Thus, this newly synthesized k-nZVI is a promising reagent for pharmaceutical removal.

Biologically produced MnOx have been shown to remove pharmaceuticals. Similar to bioMnOx, Fe oxides can also be generated by microbial activity, resulting in bioFeOx [97]. Their potential to remove pollutants has been observed. The large surface area of bioFeOx leads to the adsorption of heavy metals as well as potential adsorption of pharmaceuticals [64, 97]. In addition, bioFeOx is effective in dehalogenation reactions of organic pollutants, including chlorinated solvents, pesticides and freons [97, 141]. For pharmaceuticals containing halogens like diclofenac, applicability of dehalogenation by bioFeOx should be investigated.

2.4.3 Other promising processes

Some novel processes with Mn or Fe compounds are also promising in pharmaceutical removal. These processes should be explored to determine their application for pharmaceutical removal. Due to the similar properties between Mn and Fe (Figure 2.1), Mn can work as a catalyst in Fenton's reaction [143, 185, 353]. In Mn/H_2O_2 system, both amorphous and crystalline Mn(IV) compounds are used as catalysts to remove carbon tetrachloride, with removal efficiencies of ~ 90% at pH 6 [334]. $OH^{\bullet}$ is generated in Mn/H_2O_2 system and it is applicable to remove pharmaceuticals. Moreover, the $OH^{\bullet}$ is generated at nearly neutral conditions (pH 6), instead of the acidic conditions at pH 3 that are needed for Fe/H_2O_2 system [238]. Consequently, Mn/H_2O_2 will be more cost-efficient for pharmaceutical removal in practice.

Dissimilatory Mn or Fe reduction is demonstrated as a process to remove organic pollutants [33, 175, 179, 261]. In this process, dissimilatory Fe-reducing bacteria (DIRB) such as *Geobacter metallireducens*, metabolically degrade organic matter while respiring on Fe(III) or Mn(IV). Dissimilatory Mn(IV) or Fe(III) reduction has been applied for the removal of

monoaromatic compounds, such as benzene, toluene, ethylbenzene and xylene isomers (BTEX) [4, 115, 153, 175, 323]. A previous study shows the complete removal of BTEX with Fe(III) at a rate of 0.19 $\mu mol \cdot L^{-1} \cdot d^{-1}$ [323]. Dissimilatory iron reduction has been shown for a variety of soluble and insoluble Fe(III) forms, indicating the versatility of this process [176, 177]. We assume that biological processes which can remove phenolic or aromatic compounds might have the potential to remove pharmaceuticals, as many pharmaceuticals contain at least one aromatic ring. Thus, dissimilatory Fe reduction was potential to remove pharmaceuticals.

Mn(IV) has similar characteristics and an even higher standard redox potential compared to Fe(III). In addition, Mn(IV) can be used as an alternative electron acceptor by some DIRB [175]. Together with Mn(IV)-reducing bacteria, the process is employed to remove aromatic compounds, e.g. BTEX [53, 323]. Results show that complete biodegradation of BTEX [323] and > 60% naphthalene is mineralized to CO_2 with Mn(IV) as the terminal electron acceptor [153].

The mentioned dissimilatory Mn or Fe reduction requires a neutral pH and anaerobic conditions, so there are no needs to adjust the pH or add large amounts of oxygen. These prerequisites make it an attractive, sustainable and low-cost technology. This biological removal process will remove not only pharmaceuticals but also other organic materials. If given enough time, bacteria will degrade and eventually completely mineralize the pharmaceuticals. Products of the process, Mn(II) or Fe(II), can be oxidized into Mn(IV) or Fe(III), thus be recycled and reused for the removal process.

2.5 Conclusions

Mn- or Fe-based technologies are capable of removing pharmaceuticals in water systems. Removing pharmaceuticals decrease the possibility of developing antibiotic-resistant gene or antibiotic-resistant bacteria. Furthermore, the AOPs using Mn or Fe compounds can also remove antibiotic-resistant gene and antibiotic-resistant bacteria [204, 285]. These technologies are efficient for a wide variety of pharmaceuticals, as well as other pollutants under favourable conditions. As discussed in this review, the removal mechanism involved physico-chemical, chemical, and biologically-related processes. So far, current Mn- or Fe-based technologies to remove pharmaceuticals focus on chemical removal. The Mn or Fe compounds are generally safe to the human and environment, but there are still some of them like nZVI can be toxic. Even when these technologies partially remove pharmaceuticals, their by-products can be more biodegradable than the parent compounds. These challenges require further attention to optimize these technologies. We have also discussed that Fe- or Mn-based technologies look promising in pharmaceutical removal processes and are worth studying, for example, based on combined technologies and/or biological processes. Especially biological processes are interesting, due to their mild operational conditions and environmental-friendly characteristics.

The combination of current and potential Fe- or Mn-based processes is therefore promising to develop novel technologies for the removal of pharmaceuticals from (waste)water. Furthermore, all these Mn- or Fe-based technologies are also valuable for removal both conventional pollutants and micropollutants.

ACKNOWLEDGEMENT

The authors thank Dr. Thomas ter Laak (KWR Watercycle Research Institute, the Netherlands), Justine van Enennaam and Thomas Wagner (both from Wageningen University) for valuable discussions. Funding is supported by China Scholarship Council (CSC, File No.201308610161) and Wageningen University, the Netherlands.

Chapter 3

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

A modified version of this chapter is in preparation for publication as

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anaerobic biodegradation of pharmaceutical compounds coupled to dissimilatory manganese (IV) or iron (III) reduction.

ABSTRACT

Pharmaceutical compounds in water are regarded as emerging contaminants due to their adverse effects on aquatic ecological systems. Anaerobic pharmaceutical biodegradation coupled to dissimilatory manganese (Mn(IV))- or iron (Fe (III))- (hydr)oxides reduction is a potentially efficient but relatively unexplored process to remove these pharmaceuticals from water. In this study, batch experiments were carried out using different types of Mn(IV) and Fe(III) species with a microbial inoculum adapted to pharmaceutical biodegradation with 15 mM chemically synthesized Mn(IV) and 10 $mg \cdot L^{-1}$ metoprolol. Results show an anaerobic degradation of 26% for caffeine and 52% for naproxen with Mn(IV) as a terminal electron acceptor and insignificant biodegradation for other pharmaceuticals tested. Reduction of Mn(IV) from Mn-rich sludge from a drinking water treatment plant 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. More than half of the added metoprolol is degraded with both chemically synthesized Fe(III) and Fe(III)-citrate as terminal electron acceptors. However, such a process did not occur when using Fe(III) in the form of Fe-rich sludge from drinking water treatment processes or Fe(III)-based sorbents. This study indicates that anaerobic pharmaceutical biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction is possible, which is promising for application to cleaning wastewater treatment plant effluents.

KEYWORDS: anaerobic conditions; biodegradation; Mn(IV) or Fe(III) reduction; pharmaceuticals

3.1 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 drinking water in the range from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ [6, 164]. Previous studies have shown that pharmaceuticals have adverse effects. For example, propranolol could be harmful to aquatic organisms [197]. 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 [20, 60].

Conventional wastewater treatment facilities insufficiently remove pharmaceuticals such as diclofenac, carbamazepine, and β-blockers. The removal efficiency is generally less than 50% [330]. 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 [95, 354]. However, some intermediates from these advanced technologies can be more toxic than their parent compounds, which is undesired [223]. Furthermore, the high energy consumption will increase the ecological footprint and potential cost in construction and operation, making these technologies less attractive [151].

Biodegradation can be a less energy consuming way to efficiently remove pharmaceuticals, and different redox conditions can be applied in biodegradation [71, 74, 151]. Under aerobic conditions, biodegradation of ibuprofen and diclofenac can achieve 75 – 100% removal [151]. Under nitrate-reducing conditions, biodegradation is efficient for β-blockers like atenolol and propranolol [268]. Under sulphate-reducing conditions, over 50% atenolol can be biodegraded within one week [13]. Under methanogenic

conditions, more than 80% of pharmaceuticals such as diclofenac were found to be biodegraded [262]. However, alternative electron acceptors such as Mn(IV)- or Fe(III) are less studied compared to the other redox conditions.

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 acceptor in the anaerobic biodegradation of organic compounds [175]. Previous studies show that anaerobic biodegradation with Mn(IV) as terminal electron acceptor can efficiently remove aromatic compounds such as benzene, toluene, and poly aromatics: for example, naphthalene is mineralized to CO_2 and water for more than 60% [153]. To date, anaerobic degradation of naproxen and atenolol under Mn(IV)- or Fe(III)-reducing conditions is described [13, 268]. These results indicate that anaerobic biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction can 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) (labelled as $Mn(IV)_{chem\text{-}synthesis}$ and $Fe(III)_{chem\text{-}synthesis}$) as well as the Mn(IV) or Fe(III) from natural source are commonly used, and reported in literature [153, 175]. 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 [135]. Thus, these materials could be a cheap alternative for $Mn(IV)_{chem\text{-}synthesis}$ and $Fe(III)_{chem\text{-}synthesis}$. For example, $Fe(III)_{DWTP}$ usually consist of FeOOH, which is a suitable electron acceptor in anaerobic biodegradation processes [175, 220]. In addition, different types of Mn(IV) or Fe(III) can serve as electron acceptor during anaerobic biodegradation of organic compounds [175]. 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 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.

3.2 Methods and materials

3.2.1 Chemicals

Anaerobic water was prepared as previously described by boiling either ultrapure water (18.2 MΩ·cm, 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 [172]. 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 [172]. A stock solution of the pharmaceutical mixture was prepared with anaerobic ultrapure water, at a concentration of 20 $mg \cdot L^{-1}$ for each pharmaceutical. Stock of metoprolol and propranolol (1 $g \cdot L^{-1}$) were prepared separately with anaerobic ultrapure water.

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.

3.2.2 Preparation of Mn(IV) or Fe(III)

Different types of Mn(IV) and Fe(III) were used in this study (Table S3.1). The $Mn(IV)_{chem\text{-}synthesis}$ was prepared as described previously [172]. The $Mn(IV)_{DWTP}$ (Figure S3.1(a)) originated from drinking water production plant 'Noordbargeres' in Emmen, kindly provided by Water Laboratorium Noord (WLN, the Netherlands) [23].

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 1N NaOH and diluted to 40 mM. The Fe(III)-citrate solution was stored in a closed glass bottle and covered with aluminium foil to avoid photodegradation.

$Fe(III)_{chem\text{-}synthesis}$ was prepared based on the method described previously [153]. In brief, 0.4 M $FeCl_3$ was neutralized with 1 N NaOH until pH 7. Thereafter, $Fe(III)_{chem\text{-}synthesis}$ was washed and stored as $Mn(IV)_{chem\text{-}synthesis}$. $Fe(III)_{DWTP}$ was obtained from Evides Waterbedrijf (the Netherlands, Figure S3.1(b)). 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, $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$, were both obtained from HeGo Biotec GmbH in granular form (Germany, Figure S3.1(b)).

3.2.3 Experimental setup

(1) Inoculum

Inoculum used in the experiments with Mn(IV), $Fe(III)_{chem\text{-}synthesis}$ and Fe(III)-citrate consisted of a mixture of anaerobic sediments in the effluent channel of wastewater treatment plants in the Netherlands (Text S3.1). This sediment mixture was adapted with 10 $mg \cdot L^{-1}$ metoprolol and 15 mM $Mn(IV)_{chem\text{-}synthesis}$ over 800 days in cultivation medium described previously (Table S3.2) [178]. In the experiments with $Fe(III)_{chem\text{-}synthesis}$, the mixture samples were taken out after 100 day incubation. These samples were used as a new inoculum for the experiments with $Fe(III)_{DWTP}$, $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$.

(2) Experimental preparation

All experiments were performed in the same medium, as previously described [178]. 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 $Mn(IV)_{chem\text{-}synthesis}$ 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 $Mn(IV)_{chem\text{-}synthesis}$ was distributed to the bottles, achieving a final concentration of 15 mM Mn(IV). The stock solution of pharmaceutical mixture was added to reach a final concentration of 10 $mg \cdot L^{-1}$ for each pharmaceutical. 10 mL inoculum mixture was transferred to the experimental bottles.

The experiments with $Mn(IV)_{DWTP}$ (granule and grinded powder) were performed in duplicate in 120 mL bottles, filled with 50 mL medium inside an anaerobic glovebox. About 4 g dry $Mn(IV)_{DWTP}$ was added into the bottles, resulting in a final concentration of 15 Mm Mn(IV). Only metoprolol and propranolol were tested to minimalize 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 $mg \cdot L^{-1}$. 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. With $Fe(III)_{chem\text{-}synthesis}$, the experiments were repeating the adaption of the inoculum but in the process, 40 mM $Fe(III)_{chem\text{-}synthesis}$ was used instead of 15 mM $Mn(IV)_{chem\text{-}synthesis}$. The experiments with Fe(III)-citrate were also the same but the 40 mM Fe(III)-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 $mg \cdot L^{-1}$.

The experiments with $Fe(III)_{DWTP}$, $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$ (all ground powder) were the same as those with $Mn(IV)_{DWTP}$ 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 $mg \cdot L^{-1}$.

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 aluminium crimp caps, and the headspace was exchanged to N_2/CO_2 (80%/20%). All experiments were conducted at 30ºC in the dark without shaking. Abiotic controls were prepared similarly, with additional aliquots of $HgCl_2$ (0.1 $gHg \cdot L^{-1}$) and NaN_3 (0.3 mM) to inhibit biotic activity.

3.2.4 Sample preparation and analysis

Liquid samples for pharmaceutical analysis were taken every 3 weeks. After sampling, the samples were centrifuged for 10 min at 10000 rpm. The supernatant was diluted and transferred to amber vials. All the samples were stored at -20ºC before analysis. Pharmaceutical analysis was performed by an ultra-performance liquid chromatography with a diode array detector (UPLC, ultimate 3000, Thermo, USA), as previously described [95].

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 $Mn(IV)_{DWTP}$ were reduced by 0.5 mM $NH_2OH \cdot HCl$ (1 $mL \cdot mg^{-1}$ sample). The samples for Mn(II) measurement were also taken at the end of the experiments. The samples were centrifuged at 10000 rpm for 10 min. The supernatant was collected and stored at -4ºC 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,VISTA-MPX CCD Simultaneous, VARIAN co.).

Samples for Fe analysis were prepared by a modified HCl extraction method [52]. Briefly, 0.5 mL samples were mixed with 0.5 mL 1 M HCl to fix Fe(II). Thereafter, Fe(II) was analyzed directly by colourmetric methods (Hach Dr. Lange Kit 340). The samples were analysed immediately. The Fe content in $Fe(III)_{DWTP}$, $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$ 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).

3.3 Results and discussion

3.3.1 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 $Mn(IV)_{chem\text{-}synthesis}$ is amorphous with two small but broad XRD peaks, which is similar as previously described by others (Figure 3.1) [93]. The morphology of $Mn(IV)_{DWTP}$ could not be clarified by an XRD analysis. Since $Mn(IV)_{DWTP}$ is MnO_2-coated sand, the XRD pattern highly match the pattern of quartz (Figure 3.1 (a), Figure S3.2(a)).

The XRD pattern of $Fe(III)_{chem\text{-}synthesis}$ shows amorphous Fe(III) with two small but broad XRD peaks, which is a similar pattern as $Fe(OH)_3$ (Figure 3.1(b), Figure S3.2(b)). The XRD patterns of $Fe(III)_{DWTP}$, $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$ are all partially similar to those of Fe_2O_3 and Fe_3O_4, indicating a semi-crystalline morphology. The morphology of $Fe(III)_{DWTP}$ is closest to an amorphous one, and $Fe(III)_{FerroSorp®RW}$ is closer to a crystalline morphology.

Based on the literature, the amorphous Mn(IV) and Fe(III) are commonly used Mn and Fe species in dissimilatory Mn(IV) or Fe(III) reduction, and different types of Mn(IV) and Fe(III) such as crystalline hematite, goethite, akaganeite, and magnetite, are also suitable for the process[175, 177]. Therefore, the amorphous and semi-crystalline Mn(IV) and Fe(III) in this study are expected to remove pharmaceuticals coupled to the dissimilatory Mn(IV) or Fe(III) reduction.

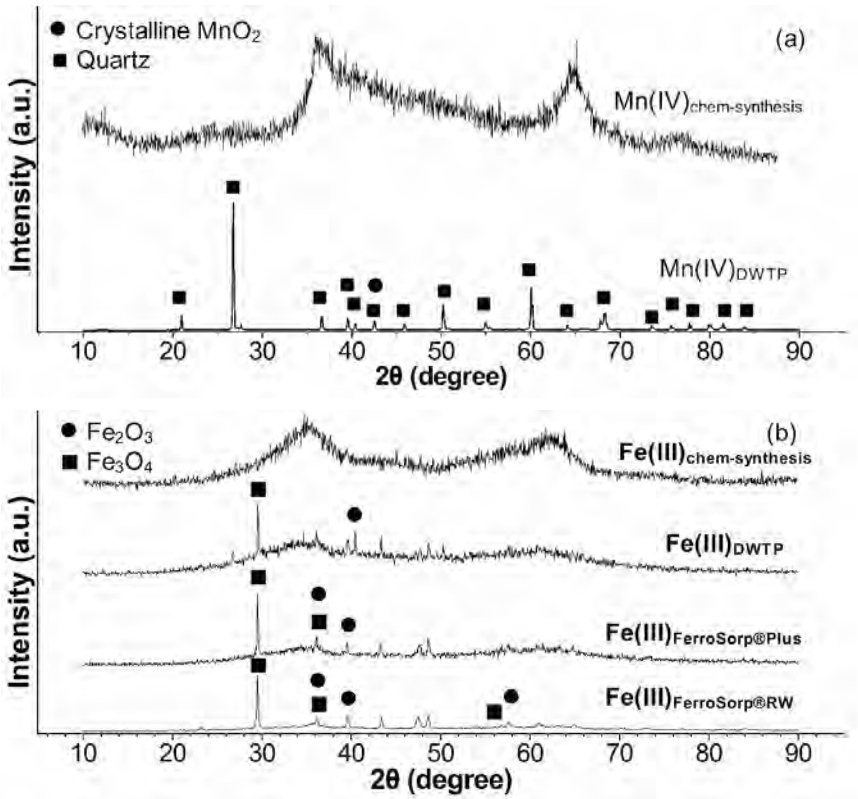


FIGURE 3.1 XRD patterns of different types of (a) chemically synthesised Mn(IV) ($Mn(IV)_{chem\text{-}synthesis}$) and Mn(IV) from a drinking water treatment plant ($Mn(IV)_{DWTP}$); and (b) chemically synthesised Fe(III) ($Fe(III)_{chem\text{-}synthesis}$), Fe(III) from a drinking water treatment plant ($Fe(III)_{DWTP}$), and two Fe(III)-based sorbents $Fe(III)_{FerroSorp®Plus}$ and $Fe(III)_{FerroSorp®RW}$

3.3.2 Anaerobic biodegradation of pharmaceuticals with Mn(IV)

(1) Chemically synthesized Mn(IV)

Anaerobic biodegradation with $Mn(IV)_{chem\text{-}synthesis}$ was tested with a mixture of six commonly used pharmaceuticals. Our results show that the anaerobic biodegradation is effective within 40 days (Figure 3.2). 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, namely12% 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 3.1), indicating that the Mn(IV) is the electron acceptor in the anaerobic biodegradation. In the abiotic controls, no degradation of pharmaceuticals is observed (Figure 3.2), 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 [175]. 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 [175, 196]. 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 matallereducens*. Mn(IV) reduction in this process provides energy for these bacteria [175].

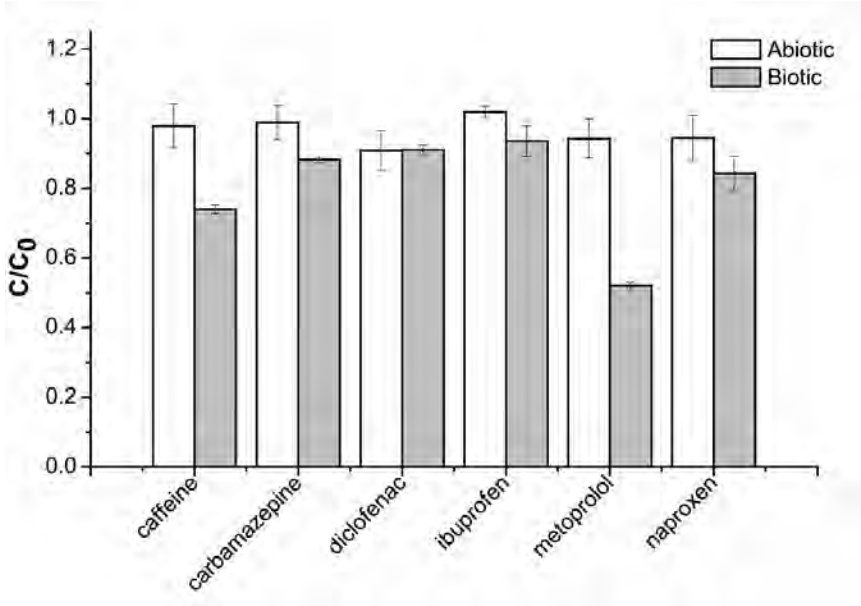


FIGURE 3.2 Biodegradation of pharmaceuticals with chemically synthesized Mn(IV) oxides after 42 days incubation. Experimental conditions: $[MnO_2]_0$=15 mM, [pharmaceutical]$_0$=10 mg·L^{-1}, pH 6.5 – 7.5, T= 30ºC. Error bars are the difference between duplicate

Since the pharmaceutical removal coupled to dissimilatory Mn(IV) 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 [66, 268] while the rest of pharmaceuticals are resistant [268, 290]. 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 $Mn(IV)_{chem\text{-}synthesis}$ as shown here.

Mn(IV) reduced in the experiments is more than two time higher than that in theoretical calculation (Table 3.1), taking the depleted pharmaceutical amounts as the quantity of consumed electron donor (Text S3.2, Table S3.3). 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.

TABLE 3.1 The reduction of Mn(IV) or Fe(III) 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]
$Mn(IV)_{chem\text{-}synthesis}$	1.8	5.4
$Mn(IV)_{DWTP}$	2.2	4.9
$Fe(III)_{chem\text{-}synthesis}$	1.7	1.8
Fe(III)-citrate	1.2	8.8

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

[b] The calculation methods of Mn(IV) or Fe(IV) reduced described in Text S3.2 and Table S3.3

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

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

$Mn(IV)_{DWTP}$, both as granules and ground powder, is tested whether it is suitable to be used in anaerobic pharmaceutical biodegradation coupled to dissimilatory Mn(IV) reduction. Results show that $Mn(IV)_{DWTP}$ granules can remove these two β-blockers via biodegradation. In the first 10 days, the two pharmaceuticals are removed with $Mn(IV)_{DWTP}$ from the medium in both biotic experiments and abiotic controls (Figure 3.3). 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 3.1). 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).

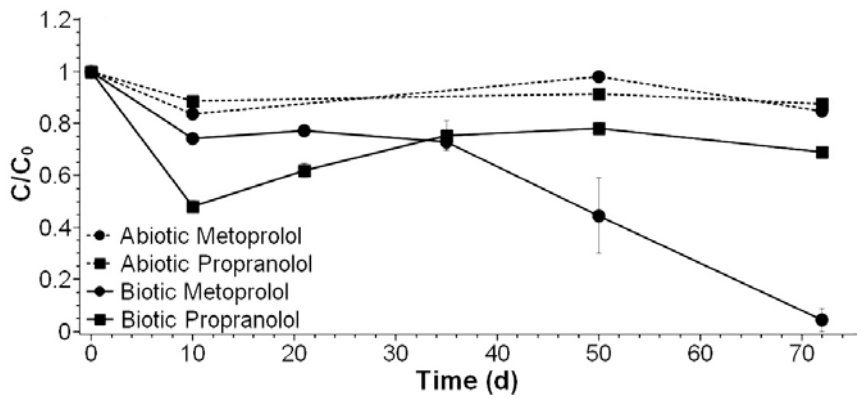


FIGURE 3.3 Biodegradation of metoprolol (●) and propranolol (■) with Mn(IV) oxides from drinking water treatment plants ($Mn(IV)_{DWTP}$). Experimental conditions: $[MnO_2]_0$=15 mM, [pharmaceutical]$_0$=10 mg·L^{-1}, pH 6.5 – 7.5, T= 30ºC. Error bars are the difference between duplicate

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 the two pharmaceuticals 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 shows 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. The removal of propranolol shows that the adsorption of this pharmaceuticals onto MnO_2 could be reversible, leading to the decrease of removal from 52% to 31% after 10 days incubation. In addition, metoprolol biodegradation consumes $Mn(IV)_{DWTP}$ during the removal process, probably releasing the propranolol that is adsorbed onto Mn(IV). The Mn(IV) reduced in this experiments is also higher than expected due to the presence of yeast extract.

3.3.3 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) [175]. We used both insoluble $Fe(III)_{chem\text{-}synthesis}$ and soluble Fe(III)-citrate to test the anaerobic biodegradation of metoprolol coupled to dissimilatory Fe(III) redcution. Our results show that Fe(III) is an alternative electron acceptor for dissimilatory Mn(IV) reduction, which can be coupled to biodegradation of metoprolol (Figure 3.4). Within 162 days, 57% metoprolol is degraded with $Fe(III)_{chem\text{-}synthesis}$ and about 52% with Fe(III)-citrate. Based on the theoretical calculation, when 1 mM metoprolol is totally mineralized, 85 mM Fe(III) is reduced (Text S3.2, Table S3.3). During the anaerobic metoprolol biodegradation with Fe(III), 1.8 mM Fe(III) was reduced when

$Fe(III)_{chem\text{-}synthesis}$ is applied, and 8.8 mM Fe(III) is reduced when Fe(III)-citrate is applied.

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 adapted to Mn(IV). Previous studies have reported that some bacteria can use both Mn(IV) and Fe(III) as electron acceptor [175, 177]. Therefore, the inoculum adapted to Mn(IV) is also active with Fe(III), indicating that Mn(IV) and Fe(III) are exchangeable in application.

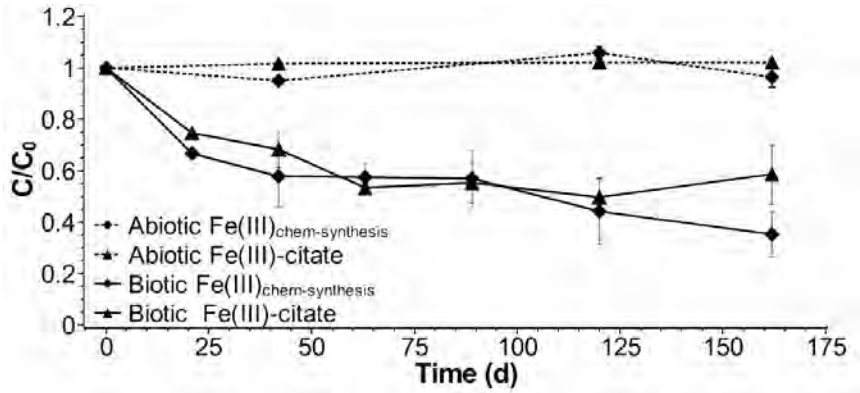


FIGURE 3.4 Biodegradation of metoprolol with chemically produced Fe(III) hydroxides ($Fe(III)_{chem\text{-}synthesis}$) (♦) and Fe(III)-citrate (▲). Experimental conditions: $[Fe(III)]_0$=40 mM, $[metoprolol]_0$=10 mg·L^{-1}, pH 6.5 – 7.5, T= 30ºC. Error bars are the difference between duplicate

In this study, the use of soluble Fe(III)-citrate is expected to result in more removal than insoluble $Fe(III)_{chem\text{-}synthesis}$, 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 the electron shuttle [175]. 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 3.1). Based on the theoretical calculation, to remove 52% metoprolol from 10 $mg \cdot L^{-1}$ 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 outcompete 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 (Figure S3.3). This indicates that the metoprolol biodegradation with two Fe(III) may be through different pathway. The reason could be that citrate also participates in the biodegradation of metoprolol, and/or that bacteria use soluble Fe(III) and insoluble Fe(III) in different ways.

(2) Other Fe(III) types

Inoculum obtained from the experiments with $Fe(III)_{chem\text{-}synthesis}$ 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) from drinking water treatment plants (labelled as $Fe(III)_{DWTP}$) and two commercial Fe(III)-based sorbents 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 (Figure 3.5).

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) [175]. In addition, the microbial inoculum is adapted to amorphous Fe(III), indicating that it could be less active with semi-crystalline Fe(III). As a results, no anaerobic metoprolol degradation via dissimilatory Fe(III) reduction is observed with these widely available Fe (III) forms.

FIGURE 3.5 Degradation of metoprolol with Fe(III) from drinking water treatment plants (labelled as $Fe(III)_{DWTP}$) and two Fe(III)-based sorbents, FerroSorp®Plus (labelled as $Fe(III)_{FerroSorp®Plus}$), and FerroSorp®RW (labelled as $Fe(III)_{FerroSorp®RW}$) within 62 days. Experimental conditions: $[Fe(III)]_0$=40 mM, $[metoprolol]_0$=10 mg·L^{-1}, pH 6.5 – 7.5, T= 30ºC. Error bars are the difference between duplicate

3.4 Conclusion

Our study shows that anaerobic biodegradation coupled to dissimilatory Mn(IV) or Fe(III) reduction is promising to remove pharmaceuticals. Results show that dissimilatory Mn reduction with $Mn(IV)_{chem\text{-}synthesis}$ can remove 26% caffeine and 52% naproxen within 42 days. Based on previous studies, both naproxen and caffeine can be efficiently removed under anaerobic conditions while the other selected pharmaceuticals are resistant to biodegradation. $Mn(IV)_{DWTP}$ granule can also be used to remove metoprolol and propranolol. After 72 days, an almost

complete removal of metoprolol, and 31% propranolol is obtained. The differences between the biotic groups and abiotic controls clearly show that biodegradation of two pharmaceuticals is important.

Fe(III) can also be used as electron acceptor during anaerobic metoprolol biodegradation. Through anaerobic biodegradation, 57% metoprolol is removed with $Fe(III)_{chem\text{-}synthesis}$ and 52% with Fe(III)-citrate. Anaerobic metoprolol biodegradation with $Fe(III)_{DWTP}$, $Fe(III)_{FerroSorp®Plus}$, and $Fe(III)_{FerroSorp®RW}$ is insufficient, probably due to these semi-crystalline form instead of amorphous Fe(III).

Yeast extract, citrate and other (metal complexing) organic components are electron-donor substrates that compete with pharmaceuticals for iron or manganese reduction, and may thereby inhibit de dissimilatory removal of pharmaceuticals. Care should be taken of competing organic substrates during removal of pharmaceuticals from waste water treatment plant effluents.

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. This study contributes to providing fundamental insight for a more sustainable pharmaceutical removal technology in wastewater treatment processes, as well as to understanding biotransformation of pharmaceuticals in the environment.

ACKNOWLEDGEMENT

The study is finical supported by China Scholarship Council (File No. 201308610161) and Wageningen University & Research. The authors appreciate the help from Laura Piai (WUR) and Peter van der Maas (WLN) for the experimental materials and additional information. The authors also appreciate the help from Hans Beijleveld, Livio Carlucci, Ilse Gerrits, Jean Slangen, and Vinnie de Wilde for assistance in analysis, and Marijn van Son for the work with Mn(IV) oxides from drinking water treatment plants.

Chapter 4

Anaerobic conditions are favourable for abiotic diclofenac removal from water with manganese oxides

A modified version of this chapter is submitted as

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anoxic conditions are favourable for abiotic diclofenac removal from water with manganese oxides.

ABSTRACT

This is the first study published addressing pharmaceutical removal under anaerobic conditions with MnO_2. This study compares the abiotic removal of seven pharmaceuticals with reactive MnO_2 particles in the presence oxygen (aerobic conditions) and in the absence of oxygen (anaerobic conditions). Due to the novelty of pharmaceutical removal under anaerobic conditions, the influence of phosphate buffer, pH, and MnO_2 morphologies are also examined. Results show that over 90% of diclofenac are removed under anaerobic conditions. Additionally, we found that: (1) anaerobic conditions promote diclofenac removal with MnO_2; (2) phosphate buffer affects the pharmaceutical removal efficiencies; (3) higher pharmaceutical removal is obtained at acidic pH compared to neutral or alkaline conditions; and (4) amorphous MnO_2 removes pharmaceuticals better than crystalline MnO_2. The pharmaceutical molecular structure and properties, MnO_2 properties especially reactive sites of the MnO_2 surface, are important for degradation kinetics. This study provides a fundamental basis towards understanding pharmaceutical degradation with MnO_2 under anaerobic conditions, and development of a cost-effective, sustainable technology for removal of pharmaceuticals from water.

KEYWORDS: manganese oxides; abiotic pharmaceutical removal; in the absence of O_2; pH effects; MnO_2 morphologies; MnO_2 reactivity mechanism

4.1 Introduction

Discharging pharmaceuticals into the water cycle threatens the aquatic environment and drinking water resources. Already at low levels ($ng \cdot L^{-1}$ – $\mu g \cdot L^{-1}$) [280, 303], pharmaceuticals can be toxic to aquatic organisms [78, 149]. As a result, pharmaceuticals discharged to water systems are seen as a priority concern of environmental regulators, and the European Union has added one of them, diclofenac, to the "Watchlist" [60].

Pharmaceutical removal in conventional wastewater treatment is poor, due to the low biodegradability and limited sorption properties of many pharmaceuticals [319]. Advanced technologies such as ozonation or photodegradation successfully remove selected pharmaceuticals from water and wastewater [17, 95]. However, these technologies require more energy inputs and operational costs, in addition to often high construction and maintenance costs, and unknown environmental effects of formed intermediate compounds.

A promising alternative method may be based on using manganese oxides (MnO_2) to remove pharmaceuticals from water. MnO_2 is a common oxidant in soil, sediment, and marine environments which include local environments with either presence of oxygen (aerobic conditions) or absence of oxygen (anaerobic conditions) [146, 276, 359]. Most studies using MnO_2 to remove pharmaceuticals and other micropollutants are conducted under aerobic conditions [248]. Previous studies also show that pharmaceutical removal with MnO_2 is more effective under acidic conditions [248]. Little is known about the abiotic removal of pharmaceuticals under anaerobic conditions with MnO_2. Furthermore, the effect of oxygen on pharmaceutical removal is inconsistent in different studies. For example, oxygen can accelerate sulfamethazine oxidation by participation in the formation of intermediates [69], while for levofloxacin removal rates under aerobic and anaerobic conditions are indifferent [163]. Therefore, more studies are required to address pharmaceutical removal with MnO_2 under

both aerobic and anaerobic conditions, and to improve the understanding of the removal mechanisms. From an application perspective, water treatment technologies commonly include aerobic and anaerobic steps. Investigating pharmaceutical removal under anaerobic conditions with MnO_2 may extend the application of this technology. Additionally, applying anaerobic conditions can reduce the construction and operation cost of maintaining aerobic conditions in water treatment systems.

Phosphate, pH, and MnO_2 morphologies are known to affect the removal of organic compounds with MnO_2 [69, 276, 352]. For example, various MnO_2 morphologies have been tested to remove pharmaceuticals and other organic compounds, with amorphous MnO_2 (birnessite) as most effective and most used [248]. However, little is known about how these parameters affect the removal process under anaerobic conditions.

In this study, a series of batch experiments with pharmaceuticals were conducted under aerobic and anaerobic conditions simulating the conditions encountered in nature as well as in wastewater treatment facilities. Seven widely used pharmaceuticals were selected and tested in the experiments. The effects of oxygen, phosphate, pH, and MnO_2 morphologies were studied to better understand the degradation processes involved and to optimize these towards the application of technology using reactive MnO_2 for pharmaceutical removal.

4.2 Methods and materials

4.2.1 Chemicals

Caffeine, carbamazepine, diclofenac, metoprolol, naproxen, and propranolol were purchased from Sigma-Aldrich while ibuprofen was purchased from MP Biomedicals (detailed information in Table S4.1). Other chemicals were purchased from Sigma-Aldrich at 98% purity (for solids), or at HPLC or UPLC quality (for solvents). Pharmaceutical stocks were prepared with ultrapure water (18.2 MΩ·cm, TOC=18 ppb, Millipore, USA) and stored in amber glass bottles at -20℃. Other solutions were prepared with demineralised water (demiwater). Details are described in Text S4.1.

4.2.2 MnO_2 preparation

Amorphous MnO_2 was obtained by freshly synthesizing prior to experiments as described [152]. Briefly, equal amounts of $MnCl_2$ and $KMnO_4$ were mixed, pH was adjusted to ~10 with NaOH, and MnO_2 was washed by centrifugation (Text S4.2). Amorphous MnO_2 was used in all experiments unless specification. Crystalline MnO_2 was purchased from Sigma-Aldrich (Figure S4.1, S4.2).

4.2.3 Batch experiments

Glass bottles (125 mL) were filled with 50 mL MnO_2 suspension (7 mM) in demiwater. Aerobic experiments were prepared at atmospheric oxygen level. Experiments under anaerobic conditions were prepared in the anaerobic glovebox with anaerobic water and closed with a rubber stopper and aluminum cap before taking them out of the anaerobic glovebox. Outside the glovebox, the headspace was exchanged with 100% N_2. All the experimental bottles were closed with rubber stoppers, crimped with aluminum caps, wrapped in aluminum foil to prevent photodegradation and incubated without shaking at 30℃.

Experiments were started by spiking bottles to achieve the final pharmaceutical concentration of 1 $mg \cdot L^{-1}$. Aliquots were collected, and reactions were quenched immediately for analysis by centrifugation (10000 rpm for 10 min). Blank experiments without MnO_2 were prepared and conducted simultaneously with each batch of experiments. Sample collection and preparation before analysis are detailed described in supplementary materials (Text S4.3).

Experiments in 50 mM phosphate buffer with only diclofenac were conducted to compare the process under aerobic and anaerobic conditions. In addition, effects of pH and MnO_2 morphologies under anaerobic conditions were investigated with phosphate buffer solutions at pH 4 – 5 (4.5), pH 7.0, and pH 8 – 9 (8.5) (Text S4.1).

4.2.4 Analysis

The pharmaceutical analysis was conducted as described previously using an ultra-performance liquid chromatography with a diode array detector (UPLC, ultimate 3000, Thermo, USA) [95]. pH was determined by a pH meter (PHM210, MeterLab, Radiometer analytical). The Mn^{2+} analysis was conducted by an inductively coupled plasma spectrometer with optical emission spectroscopy (ICP-OES). MnO_2 morphologies were characterized by X-ray diffraction. The MnO_2 before and after the reaction with diclofenac and metoprolol was characterized via a Fournier-Transform Infra-Red spectrometer (FTIR, Bruker TENSOR 27). Details are described in supplementary materials (Text S4.3).

4.3 Results and discussion

4.3.1 Pharmaceutical removal under aerobic versus anaerobic conditions

In the absence of MnO_2, no removal is observed for all seven pharmaceuticals within 24 hours under both aerobic and anaerobic conditions in all experiments (Table S4.2). In the presence of MnO_2, metoprolol, propranolol, and diclofenac are removed within 24 hours in both demiwater (Figure 4.1(a) and (b)) and phosphate buffer (Figure 4.1(c)), while no removal is observed for the other four pharmaceuticals (Figure S4.3). Furthermore, the results show that anaerobic conditions promote the removal of diclofenac, while higher removal is observed under aerobic conditions for metoprolol and propranolol. Diclofenac removal efficiencies of 78% under anaerobic conditions and 59% under aerobic conditions were observed after 24 hours incubating a solution of mixed pharmaceuticals in demineralized water (Figure 4.1(a)). However, only 33% metoprolol was removed under anaerobic conditions compared to 69% under aerobic conditions. Similarly, 51% propranolol was removed under anaerobic conditions compared to 84% under aerobic conditions. Diclofenac degradation in a mixture together with other six pharmaceuticals (Figure 4.1(a) was found to be lower than in a demiwater system which only diclofenac was present. Under anaerobic conditions, 92% diclofenac is removed with MnO_2. While under aerobic conditions, 69% diclofenac removal is observed (Figure 4.1(b)).

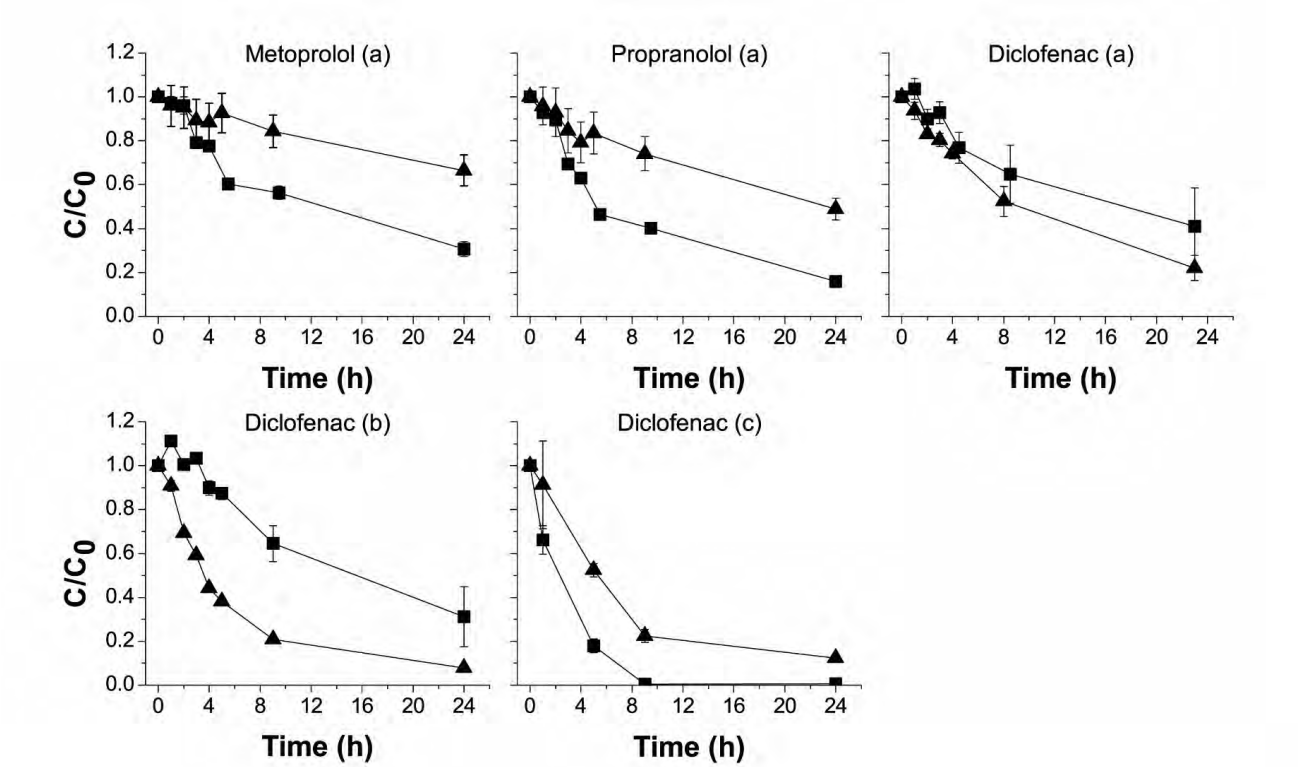


FIGURE 4.1 Pharmaceutical removal with MnO_2 in (a) demiwater with pharmaceutical mixture; (b) demiwater with only diclofenac solution; (c) phosphate buffer with only diclofenac solution under aerobic conditions (■) and anaerobic conditions (▲). Experimental conditions: $[MnO_2]_0$=7 mM, [pharmaceutical]$_0$=1mg·L^{-1}, pH ~8.5. In phosphate buffer with diclofenac solution, [phosphate]=50mM, [ionic strength]=0.1 M. Error bars are standard deviations

TABLE 4.1 Initial removal rate ($r_{obs,\ init}$, $mg \cdot L^{-1} \cdot h^{-1}$, R^2=0.80 – 0.97) and initial removal rate constant ($k_{obs,\ init}$, h^{-1}, R^2=0.85 – 0.99) of pharmaceutical removal with MnO_2 based on pseudo-first-order in first 5 hours

Experimental solution	Matrix	pH	Compound	$r_{obs,\ init}$ (10^{-2} $mg \cdot L^{-1} \cdot h^{-1}$)		$k_{obs,\ init}$ (10^{-2} h^{-1})	
				Aerobic	Anaerobic	Aerobic	Anaerobic
Mixture of seven pharmaceutical	Demiwater	~8.5	Metropolol	7.39	2.98 [a]	9.21	3.18
			Propranolol	10.10	4.02	14.18	4.48
			Diclofenac	5.33	6.48	5.96	7.49
Only diclofenac present in solution	Demiwater	~8.5	Diclofenac[b]	4.70	9.06	5.56	18.13
Only diclofenac present in solution	50 mM PO_4^{3-} buffer	~7.0	Diclofenac[b]	10.48	8.73	57.32	16.60

[a] Both $r_{obs,\ init}$ and $k_{obs,\ init}$ were calculated within 4 hours
[b] Both $r_{obs,\ init}$ and $k_{obs,\ init}$ were calculated within 9 hours

Our results show that anaerobic conditions promote (are favourable for) diclofenac removal with MnO_2. In contrast, previous studies show either no effect or lower removal efficiencies under anaerobic conditions [14, 69, 361]. This unique result directs our further studies on the mechanism of pharmaceutical removal under anaerobic conditions with MnO_2. In order to eliminate the effects of pH and ionic strength on pharmaceutical removal with MnO_2 [69, 108], we control pH (~7) with 50 mM phosphate buffer and maintain the ionic strength (0.1 M) with NaCl. In further experiments with phosphate buffer, 90% of diclofenac is removed under anaerobic conditions while nearly complete degradation of diclofenac is observed under aerobic conditions (Figure 4.1 (c)).

TABLE 4.2 Diclofenac removal efficiency under anaerobic conditions at different pH conditions with two MnO_2 morphologies after 48 hours. Experimental conditions: $[MnO_2]_0$=7 mM, $[diclofenac]_0$=1$mg \cdot L^{-1}$, [ionic strength]=0.1 M

MnO_2 morphologies	~ pH 4.5	~ pH 7.0	~ pH 8.5
Amorphous MnO_2	100%	100%	71 %
Crystalline MnO_2	21%	Not detected	Not detected

A pseudo-first-order model with initial incubation period was applied to analyze the removal kinetics (Table 4.1), as performed in previous studies under aerobic conditions [125, 359, 361]. Comparison of the initial removal rate

($r_{obs,\ init}$) and the initial removal rate constant ($k_{obs,\ init}$) of different pharmaceuticals shows that oxygen affects pharmaceutical removal with MnO_2. In demiwater with the pharmaceutical mixture and with only diclofenac, diclofenac removal is accelerated under anaerobic conditions; metoprolol and propranolol removal rates are lower under anaerobic conditions. Furthermore, diclofenac was removed at the highest rate when dissolved as a sole compound in aerobic phosphate buffer containing MnO_2.

4.3.2 Influence of pH and MnO_2 morphologies on diclofenac removal

Pharmaceutical removal with MnO_2 is affected by pH. Previous studies show that MnO_2 morphologies also influence pharmaceutical removal [276]. However, our novel observation of diclofenac removal under anaerobic conditions with MnO_2 indicate that the removal mechanisms of pharmaceuticals with MnO_2 under anaerobic conditions might be different from removal under aerobic conditions. Therefore, it is important to investigate the effect of pH and MnO_2 morphologies on diclofenac removal to understand the removal mechanism. We investigate the effect of pH and MnO_2 morphologies using both amorphous MnO_2 and crystalline MnO_2 under anaerobic conditions at pH ~4.5, pH~7.0, and at pH ~8.5 established with a 50 mM phosphate buffer.

Diclofenac removal efficiencies with MnO_2 under anaerobic conditions are inversely related to pH (Table 4.2). Within 48 hours, diclofenac removal under anaerobic conditions varies from 100% at around pH ~4.5 and pH ~7.0, to 70% at pH ~8.5 with amorphous MnO_2. In contrast, diclofenac removal is notably lower with crystalline MnO_2. Only 21% of diclofenac is removed with crystalline MnO_2 at pH ~4.5. In the experiments carried out at pH ~7.0 and pH ~8.5, no removal is with crystalline MnO_2.

4.3.3 Discussion

Under aerobic conditions, pharmaceutical removal can be accelerated

by oxygen [69]. However, this fails to explain why anaerobic conditions promote diclofenac removal when oxygen is not present to participate in the removal process (Text S4.4, Figure S4.4). There are different intermediates formed under aerobic and anaerobic conditions during diclofenac removal with MnO_2 (Text S4.4, Figure S4.4). These intermediates have different adsorption affinities for the reactive sites on the MnO_2 surface, which is possibly the key to explaining the differences between aerobic and anaerobic conditions. Based on the results, two factors appear to influence the efficiency of pharmaceutical removal and are elaborated below: (1) pharmaceutical molecular structure and chemical properties, and (2) the MnO_2 properties.

4.3.3.1 Pharmaceutical molecular structure and chemical properties

The molecular structure and chemical properties of pharmaceuticals are important in organic compound removal with MnO_2. Previous studies show that oxidation with MnO_2 in the presence of oxygen involves cleavage of the C-N bond of the organic compound. Metoprolol and propranolol have C-N bonds, in which the N atom is bound to an alkyl group. These compounds are similar to those tested in previous studies (Table S4.1, S4.3) in which aerobic conditions promote the removal. This C-N bond cleavage can result in the formation of radicals in the presence of oxygen [14, 69]. Oxidation of diclofenac involves hydroxylation and decarboxylation instead of C-N cleavage [108], which is a different mechanism than that of metoprolol and propranolol. This shows that the removal mechanism is closely related to pharmaceutical molecular structure and chemical properties.

The pharmaceutical's properties are also affected by pH. Due to the low pKa of diclofenac (pKa =4.15), lower pH results in a less negatively charged compound. This leads to less electrostatic repulsion between diclofenac and MnO_2, which is also negatively charged. As compared to the other two compounds, diclofenac has a higher affinity to adsorb at low pH onto MnO_2 surface and therefore has a more favourable first step in removal with MnO_2.

4.3.3.2 MnO_2 properties.

The properties of MnO_2 are affected by pH as well. At acidic pH, MnO_2 is also less negatively charged due to its isoelectric point, resulting in less electrostatic repulsion and better adsorption of organic compounds. In addition, the MnO_2 redox potential increases from 0.76 V at pH 8.0, to about 0.99 V at pH 4.0 [167]. Thus, the degradation reaction is energetically more favorable at lower pH. Both factors may lead to faster degradation, as shown in our study (Table 4.2). pH used in this experiment is at neutral pH, which was found unfavourable for oxidation of pharmaceuticals in previous studies [32, 93, 346]. In addition, there are less protons at the low redox potential of MnO_2 at higher pH, which is crucial for the electron transfer from Mn(IV) to Mn(II). As a result, no removal of caffeine, carbamazepine, ibuprofen, and naproxen was observed in this study, while the removal efficiency of metoprolol and propranolol is low under both aerobic and anaerobic conditions.

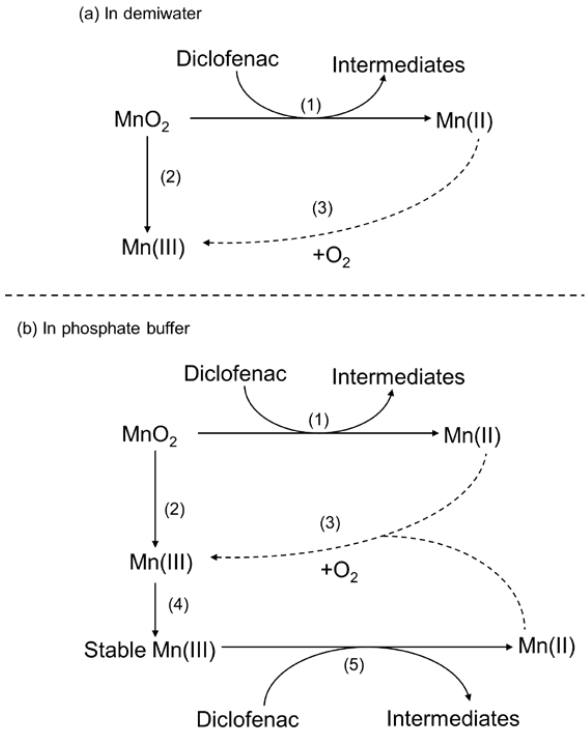


FIGURE 4.2 The effects of phosphate on diclofenac removal with MnO_2 under aerobic and anaerobic conditions. Solid lines are processes under both aerobic and anaerobic conditions; dashed lines are the processes only under aerobic conditions. (1) MnO_2 removes diclofenac via oxidation and produces Mn(II) [65, 108]; (2) Mn(III) comes from MnO_2 synthesis process [248]; (3) Mn(II) is oxidised to Mn(III) by O_2; (4) Mn(III) from MnO_2 was stabilized by $Mn_3(PO_4)_2$ formed via Eq.1 [127]; (5) Mn(III) oxidises diclofenac and produces Mn(II)

Different MnO_2 morphologies have different properties affecting diclofenac removal. In our research, diclofenac removal is better with amorphous MnO_2 than with crystalline MnO_2, which is in line with previously reported findings [248, 276, 313]. Amorphous MnO_2 particles are usually smaller than crystalline particles. Thus the amorphous MnO_2 particles have the larger surface area, which increases pharmaceutical removal. Unfortunately, due to the analytical limits, size analysis of amorphous MnO_2 appeared technically not feasible (Text S4.5, Figure S4.5). In addition, amorphous MnO_2 contains small amounts of Mn(III) which can increase MnO_2 reactivity and oxidizing ability [248], thus promoting pharmaceutical removal even further.

In the presence of phosphate, diclofenac removal with MnO_2 is better under aerobic conditions than that under anaerobic conditions. Using O_2 to oxidize Mn(II) to Mn(III) is a thermodynamically favorable reaction. In the presence of phosphate buffer, phosphate can form $Mn_3(PO_4)_2$ with Mn(II) from diclofenac oxidation (Equation 4.1) [127].

$$3Mn^{2+} + 2PO_4^{3-} \rightarrow Mn_3(PO_4)_2 \quad (4.1)$$

Computations show that the chemical structure of $Mn_3(PO_4)_2$ can stabilize Mn(III) and thereby facilitate Mn(II) oxidation to Mn(III) under aerobic conditions [127]. The Mn^{2+} analysis shows that the presence of higher Mn(II) concentrations in phosphate buffer than in demiwater, which we explain as a result of larger amounts of Mn(III) formed under aerobic conditions. More Mn(III) is likely the reason that more diclofenac is removed than under anaerobic conditions, as we observed (Figure 4.1) and mechanistically present in Figure 4.2.

4.3.3.3 Reactive sites on MnO_2 surface

The adsorption of organic molecules onto a reactive metal oxide surface is found to be the key parameter dictating removal of many organic compounds, and specifically to the reactive sites on MnO_2 surface [93, 346, 362]. Our results with the mixed pharmaceutical solution in the demiwater show competition for reactive sites between diclofenac and the other different pharmaceuticals. This is evidenced by the lower diclofenac removal in the presence of other pharmaceuticals (Figure 4.1(a), (b)).

Based on our FTIR results, there was no obvious disappearance of reactive sites during diclofenac removal with MnO_2 under both aerobic and anaerobic conditions (TEXT S4.6, Figure S4.6), possibly due to a relatively high concentration of MnO_2 in the experiment. However, it is clearly that the FTIR spectrums are different between the MnO_2 before and after reacting with diclofenac, especially under anaerobic conditions. This indicates that the intermediates from diclofenac change the MnO_2. This change may contribute to the better diclofenac removal with MnO_2 under anaerobic conditions.

In phosphate buffer, phosphate can reduce the diclofenac removal by being adsorbed onto the MnO_2 surface and competing with DFC for the reactive sites of MnO_2 [352]. Consequently, although the lower pH in phosphate buffer should promote diclofenac removal (pH 7 in buffer versus pH 8 – 9 in demiwater), diclofenac removal is better in demiwater because MnO_2 reactive sites are not blocked by phosphate (Table 4.1). However, similar removal efficiencies and kinetics in demiwater and phosphate buffer under anaerobic conditions are observed (Figure 4.1). This indicates there is a mechanism promoting diclofenac removal in phosphate buffer, which competes with the inhibition by phosphate adsorbing and occupying the reactive sites on MnO_2 surface. From previous studies, it is known that Mn(II) can occupy reactive sites on MnO_2 surface and then inhibit pharmaceutical removal [93, 346]. Our removal results in phosphate buffer show that 1.54 μM Mn^{2+} was generated under aerobic conditions while 2.16μM was generated under anaerobic conditions. Less Mn(II) under aerobic conditions resulted in possibly less formation of $Mn_3(PO_4)_2$ via Eq.1, which presumably lead to more available reactive sites for diclofenac removal. Under anaerobic conditions, the balance of these promoting and inhibiting effects by adsorbing phosphate leads to similar diclofenac removal in demiwater and phosphate buffer.

4.4 Conclusion

In conclusion, this study addresses the gap of understanding pharmaceutical removal in the absence of oxygen (anaerobic conditions) with MnO_2. Anaerobic conditions show higher diclofenac removal compared to aerobic conditions in demiwater. In phosphate buffer, aerobic conditions resulted in about 10% more diclofenac removal. pH and MnO_2 morphologies influence the removal process and its efficiency. Our results in both demiwater and phosphate buffer suggest that the process is promising in treating water and wastewater containing pharmaceuticals. The results show that amorphous MnO_2 is the most suitable material for further research and application, and the most optimal and applicable conditions are at neutral pH in anaerobic systems. By using a more favourable pH (acidic pH), the removal of all the pharmaceuticals can be expected under anaerobic conditions. To our knowledge, this is the first study discussing pharmaceutical removal with MnO_2 under anaerobic conditions. Using anaerobic conditions is less energy-consuming compared to aerobic conditions (aeration), and Mn can be regenerated and recycled via a biological or chemical process [126, 301]. Overall, this study makes a contribution to (1) understanding pharmaceutical removal in the absence of oxygen; (2) improving the knowledge of pharmaceutical removal mechanisms with MnO_2; and (3) providing fundamental insight into a MnO_2-based process which may lead to a more sustainable technology for pharmaceutical removal.

ACKNOWLEDGEMENT

The work was supported by China Scholarship Council (File No.201308610161) and Wageningen University & Research. The authors appreciate the support from Hans Beijleveld, Jan Kubiak, Ilse Gerrits, and Jean Slangen and their help with the chemical analyses. Special thanks go to Sidiharam Pujari for the FTIR analysis and Harry Baptist for the freeze-drying procedure.

Chapter 5

Application of manganese oxides under anaerobic conditions to remove diclofenac from water

A modified version of this chapter is submitted as

Liu, W.; Langenhoff, A. A. M.; Sutton, N. B.; Rijnaarts, H. H. M., Application of manganese oxides under anoxic conditions to remove diclofenac from water.

ABSTRACT

This study investigates diclofenac (DFC) removal with manganese oxides (MnO_2) under anaerobic conditions (absence of oxygen). DFC removal is quantified in terms of efficiency and observed initial kinetics ($k_{obs,\ init}$) to investigate the effects of temperature, MnO_2 amount, and co-solutes. Overall, DFC removal and $k_{obs,\ init}$ both increase upon changing temperature from 10 to 30ºC and both decrease after further increasing temperature to 40ºC. Increasing the amount of MnO_2 relative to diclofenac improve degradation, as this provides more reactive surface sites for DFC conversions. However, increasing the molar ratio of MnO_2 and DFC from 2200:1 to 8900:1 does not further increase diclofenac removal. The presence of metal ions inhibits DFC removal, possibly because the ions adsorb onto the reactive sites at the MnO_2 surface and compete with DFC. Phosphate has a diverse effect on diclofenac degradation: low concentrations inhibit and high concentrations promote removal. The presence of humic acids significantly promotes diclofenac removal, which is probably caused by quickly absorbing DFC and attaching to MnO_2 surface. Also, humic acids can bind the Mn^{2+} produced during the DFC removal. These findings are a first step towards further developing pharmaceutical removal technology using MnO_2 under anaerobic conditions.

KEYWORDS: manganese oxides; abiotic pharmaceutical removal; anaerobic condition; temperature; MnO_2 amount; co-solutes

5.1 Introduction

Diclofenac ([2-(2,6-Dichloroanilino)phenyl]acetic acid, DFC) is a commonly used pharmaceutical known to have the highest acute toxicity to wildlife among the non-steroidal anti-inflammatory drugs [174, 279]. In Europe, the mean DFC consumption is around 1300 $\mu g \cdot capita^{-1} \cdot d^{-1}$ [128]. As a result, DFC has been detected in surface water, groundwater, and even in drinking water in the range of 0 – 1000 $ng \cdot L^{-1}$ [304]. Due to its toxic effects to the environment and ecosystem, the European Union (EU) has added DFC into the "Watchlist" [60]. The European Community suggests DFC concentration for the environmental quality threshold for good water quality a value of 100 $ng \cdot L^{-1}$ (annual average) [56]. Various technologies can remove DFC, such as advanced oxidation processes like photo-degradation, or algae treatment systems, but these technologies often either produce toxic intermediates or are too costly [49, 170, 174].

Using manganese oxide (MnO_2) to remove pharmaceuticals can achieve high removal efficiencies and may produce less and lower amounts of toxic intermediates [32, 108]. Our previous study showed that anaerobic conditions (absence of oxygen) are favorable for DFC removal with MnO_2, opening the way to a new cost-efficient and sustainable pharmaceutical removal process based on applying MnO_2 under anaerobic conditions, not yet studied by others [172]. In order to develop this process into an applicable technology, a number of operational parameters were studied, namely temperature, the amount of MnO_2, and the presence of co-solutes (metal ions, phosphate, humic acid).

Reactions between MnO_2 and organic compounds are endothermic, and therefore increasing temperature can accelerate the rates of the removal processes [32, 260]. However, observed initial rate constant of oxytetracycline removal with MnO_2 under aerobic conditions is reported to be lower at 40ºC than at 5 to 30ºC [260]. These contradicting results to theory and the lack of investigations under anaerobic conditions lead to the necessity of further investigating temperature effects. From an application perspective, the results will partially determine the applicability of MnO_2 technologies in different seasons and climates.

Another important parameter in the application is the amount of MnO_2, which can be expressed as the molar ratio between the pharmaceutical(s) and MnO_2. Previous studies show that increasing the ratio between MnO_2 and chlortetracycline in the range of 4:1 to 32:1 leads to faster removal [32]. However, no significant change is observed in triclosan, carbadox, and ciprofloxacin removal with MnO_2 when the ratio changes from 10:1 to 300:1 [359]. In addition, the ratios studied previously are usually low while they can be much higher in application due to the low concentration of pharmaceuticals. From an application perspective, optimizing MnO_2 amount can decrease operation costs, by applying minimum amounts of MnO_2 at maximum DFC removal.

Co-solutes in water including metal ions, anions (like phosphate), and organic compounds are commonly observed. The EU allows 3.57 μM iron and 0.91 μM manganese in drinking water [59]. In urban wastewater effluent, the phosphate concentration should be < 2 mg $P \cdot L^{-1}$ while the organic compounds (expressed as Chemical Oxidation Demand, COD) are allowed at 125 mg$\cdot L^{-1}$ based on EU regulation [58]. All these co-solutes can affect pharmaceutical removal with MnO_2 in WWTP effluents under aerobic conditions. Previous studies under aerobic conditions showed that pharmaceutical removal could be inhibited by metal ions and phosphate (PO_4^{3-}) [32, 167] while humic acid representing organic matters can both promote and inhibit this process [132, 167]. However, none of these parameters

are investigated under anaerobic conditions. In this study, four metal ions (Mn^{2+}, Ca^{2+}, Mg^{2+}, and Fe^{3+}), PO_4^{3-}, and different concentrations of humic acid are selected to investigate the effects of co-solutes on the application of MnO_2 under anaerobic to remove DFC.

In this study, we investigate the efficiencies and reaction kinetics of DFC removal from water by MnO_2 under anaerobic conditions. The effects of temperature, MnO_2 amount, and co-solutes were investigated to evaluate the application potential and optimal conditions. Removal mechanisms under anaerobic conditions are affected by the pharmaceutical chemical structure and properties of both pharmaceuticals and MnO_2 [69, 172]. The results provide the basis for developing a new treatment technology for removal of trace organic contaminants from WWTP effluent.

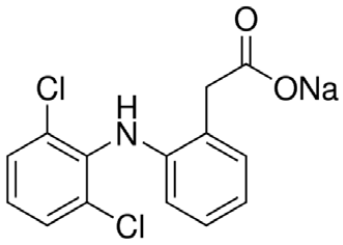
5.2 Materials and methods

5.2.1 Chemicals

Diclofenac sodium salt (DFC) was purchased from Sigma-Aldrich (Table 5.1). Other chemical reagents used in the experiments were purchased from either Sigma-Aldrich or Merck KGaA (Germany). All the chemicals had > 97% purity. The liquid used in these experiments was at either high performance liquid chromatographic grade or ultra-performance liquid chromatographic grade. Millipore water purification system was used to prepare ultrapure water (18.3 MΩ·cm, TOC=18 ppb). Anaerobic water was prepared by boiling demineralized water (demiwater) for 5 min and cooling under N_2-flow to the room temperature. All solutions were prepared with anaerobic water unless specified otherwise. DFC stock solution was prepared in ultrapure water to reach a final concentration of 157 μM in a 50-mL amber glass bottle. This stock was stored at -20ºC to avoid potential decomposition. Humic acid (HA) was purchased from Sigma-Aldrich (product No. 53680). HA stock solution was prepared at a final concentration of 1000 $mg \cdot L^{-1}$ in anaerobic water with 1 – 3 drops of 1M

NaOH. This stock was stored in a 50 mL glass bottle at 4ºC to avoid decomposition.

TABLE 5.1 Chemical structure and properties of diclofenac sodium (DFC)[a]

	CAS No.	15307-79-6
	Empirical Formula	$C_{14}H_{10}Cl_2NNaO_2$
	Molecular Weight	318.13 g·mol^{-1}
	pKa	4.15
	logK_{ow}	1.13
	Solubility in water	50mg·mL^{-1}

[a] The data are collected from Sigma-Aldrich and literature [308]

5.2.2 Synthesis of manganese oxides

Manganese oxides (MnO_2) were freshly synthesized by a modified method as previously described [172]. In brief, equal volumes of 0.4 M $MnCl_2$ and 0.4 M $KMnO_4$ were mixed and adjusted to pH 10 with 1M NaOH. The MnO_2 was washed six times with anaerobic demiwater via centrifugation (15 min at 5000 rpm) and decanting. MnO_2 was stored at 4ºC as a suspension with N_2 headspace and diluted to an appropriate concentration before using.

5.2.3 Experimental setup

Batch experiments were prepared under anaerobic conditions in 125 mL glass bottles. All bottles were sealed with rubber stoppers and aluminum crimp-caps, settled at 30ºC without shaking, and covered with aluminum foil to avoid photodecomposition. The pH was maintained at 7 for all experiments with a 10 mM MOPS (4-morpholinepropanesulfonic acid) buffer solution. Sodium chloride (NaCl) was added to adjust the ionic strength (I=0.01M). The final concentration of MnO_2 in the bottle was 7 mM. The 50 ml buffer containing NaCl was added into the experimental bottles. The headspace of all bottles was exchanged with N_2 to keep the system anaerobic.

The experiments were started by adding 0.25 – 1 mL DFC stock solution (157 μM) into the experimental bottles. All the experiments were carried out in triplicate and lasted for 33 hours in most cases. 1.2 mL mixture samples were taken regularly during the experimental period. The samples were centrifuged immediately at 10000 rpm for 10 min. The supernatant was collected and stored at -20ºC until analysis. Sorption of DFC onto MnO_2 were not taken into account because it is insignificant based on previous studies [65, 172], but it is still an important removal mechanism.

Control experiments without MnO_2 were prepared and conducted simultaneously with each batch of reactions. The effect of temperature was investigated by conducting the experiments at 10, 20, 30, or 40ºC. The effect of MnO_2 amount was investigated by applying different molar ratio between MnO_2 and DFC from 480:1 to 8900:1 at 20ºC. The effect of co-solutes was investigated at 30ºC with different concentrations of metal ions (Mn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+} at 0.1 and 0.1 mM), the anion (PO_4^{3-} at 0.5, 1, and 116 mgP·L^{-1}), and dissolved organic compound (HA at 5, 10, 15, and 25 mg·L^{-1}). In the experiments with HA, DFC was added before adding HA. Samples were taken before and after adding HA at the beginning of the experiments.

5.2.4 Analysis

DFC was measured by ultra-performance liquid chromatography with a diode array detector (Ultimate 3000, Thermo co. Ltd.). 10 μL of samples were automatically injected into a CSH phenyl-Hexyl column (1.7 μm, 130 Å, 2.1 × 150 mm, Waters co., USA). The details of analytical methods and setting of the machine were described previously [95, 172].

Phosphate concentration was analyzed by colorimetric method using cuvettes from Hach Lange (LCK 349). All metal ions were analyzed by an inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin Elmer Instruments, USA). pH was determined by a pH meter (PHM210, MeterLab, Radiometer Analytical).

5.2.5 Kinetics study

In literature, different models have been used to describe organic compound removal with MnO_2: pseudo-first-order model, pseudo-first-order model with data from the first several hours of the experiments, pseudo-second-order model, and mechanism-based kinetics models [32, 260, 359]. The pseudo-first-order kinetic model has been used to evaluate the reaction kinetics of micropollutants and other organic compounds with MnO_2 [125]. However, in experimental results deviation from this model after a certain reaction time was also reported. To better predict long-term kinetics of organic compound removal with MnO_2, the pseudo-second-order model was used [32]. The pseudo-second-order model usually gave a better fit during the long period. The mechanism-based model was proposed to fit data obtained in long-term experiments. Rubert and Pedersen [260] developed the mechanism-based kinetics model to better describe oxytetracycline removal with MnO_2. Similarly, Zhang, Chen and Huang [359] described another kinetic model based on the removal mechanism to describe antibacterial agent removal with MnO_2. In this study, all four models were validated with the results obtained at 30ºC with 7 mM MnO_2, 3.14 µM DFC, and no co-solutes.

5.3 Results and discussion

5.3.1 Kinetic results

Our results show that diclofenac (DFC) can be removed by applying MnO_2 under anaerobic conditions. The pseudo-first-order model fits the data poorly (R^2=0.84) while the other three models fit the data better and are useful for evaluating our kinetic studies (R^2=0.96 – 0.99) (Text S5.1, Table S5.1, Figure S5.1,). The results show that anaerobic MnO_2 mediated DFC removal follows a two-stage kinetic process under all experimental conditions. Most DFC conversion is obtained in the first 9 hours (following the pseudo-first-order reaction) and only about 10% DFC removal is obtained in the subsequent 24 hours (from t=9 h to t=33 h, following the pseudo-zero-order reaction). Therefore, the pseudo-first-order model with data from the first 9 hours was used to calculate the observed removal rate constants ($k_{obs,init}$) (Table 5.2). Without MnO_2, no DFC removal is observed (Text S5.2, Table S5.2, Figure S5.2).

5.3.2 Effect of temperature

Results show that the application of manganese oxides (MnO_2) to remove diclofenac (DFC) under anaerobic conditions is generally an endothermic process (Table 5.2, Figure 5.1). DFC removal increases from 85 to 90%, and the $k_{obs,init}$ increases from 0.14 to 0.19 h^{-1}, when increasing the temperature from 10 to 30ºC. However, when the temperature is further increased to 40ºC, both removal and $k_{obs,\ init}$ drop dramatically to respectively 38% and 0.04 h^{-1} (Figure 5.1, Table 5.2). When the temperature is subsequently reduced from 40 to 20ºC, only a slight increase in total removal is observed and $k_{obs,\ init}$ remained low.

At 40ºC, MnO_2 particle aggregation is observed (Figure S3(a-d), Figure S5.4). To investigate whether this aggregation plays a role in the unexpected drop of DFC removal at 40ºC, an additional experiment was performed (Figure 5.1, dashed line). The experiment was started at 40ºC, but the temperature was changed to 20ºC t=9 h. The temperature of the bottles dropped to 20ºC within 1 hour. Results show that only 22% DFC is removed from t=9 h to t=33 h, which is lower than the 48% DFC removed in this time period if the experiment is started at 20ºC. The results show that the process is irreversible.

Aggregation of MnO_2 particles was observed at 40ºC which can decrease the amount and availability of reactive surface sites on MnO_2 for DFC removal. This may be a reason for the reduced DFC removal at high temperature. The aggregation may be caused by two processes: Ostwald ripening and/or aging. Ostwald ripening, also called coarsening or competitive growth, occurs in the suspension where large particles grow at the cost of small particles [246]. Amorphous particles (or small crystals) dissolve and re-precipitate on the largest crystals initially present. As a result, the amorphous particles or small crystals grow to relatively large crystals [229]. Ostwald ripening is promoted by elevated temperature, as demonstrated in previous studies [188, 284]. The aging process is the physical change of minerals over time. Aggregation of MnO_2 is observed to be a fast process at 40ºC (hours – days), and a slow process at 20ºC (6 months, the duration of our experiments, Text S5.3, Figure S5.3 (e, f)). This indicates that MnO_2 aging process is accelerated when changing the temperature to 40ºC, which quickly changes MnO_2 morphology irreversibly [293]. As a result, active MnO_2 precipitates into a more stable form, leading to less DFC removal [208], and the reactivity cannot be restored when changing again to lower temperatures, i.e. 20ºC (Figure 5.1, dashed line). Overall, the aggregation of MnO_2 particles leads to less DFC removal.

TABLE 5.2 Calculated observed removal rate constants ($k_{obs,init}$) for DFC removal by applying MnO_2 under anaerobic conditions based on a pseudo-first-order kinetic model with data from first 9 hours under different experimental conditions (pH~7, I=0.01M)

T(ºC)	Matrix	$[MnO_2]_0$ (mM)	$[DFC]_0$ (μM)	Molar ratio ($[MnO_2]_0$:$[DFC]_0$)	$k_{obs,init}$ [b] (h^{-1})	R^2
10	10 mM MOPS	7	3.14	2200:1	0.14 ± 0.01	0.99
20	10 mM MOPS	7	3.14	2200:1	0.16 ± 0.00	0.96
30	10 mM MOPS	7	3.14	2200:1	0.19 ± 0.00	0.98
40	10 mM MOPS	7	3.14	2200:1	0.04 ± 0.00	0.83
20	10 mM MOPS	1.5	3.14	480:1	0.05 ± 0.01	0.90
20	10 mM MOPS	3	3.14	950:1	0.07 ± 0.01	0.96
20	10 mM MOPS	6	3.14	1900:1	0.12 ± 0.00	0.98
20	10 mM MOPS	7	3.14	2200:1	0.16 ± 0.00	0.96
20	10 mM MOPS	7	1.57	4500:1	0.13 ± 0.00	0.92
20	10 mM MOPS	7	0.79	8900:1	0.12 ± 0.01	0.96
30	10 mM MOPS, no metal ions	7	3.14	2200:1	0.19 ± 0.00	0.98
30	0.1 mM $MnCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.12 ± 0.01	0.99
30	0.1 mM $CaCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.15 ± 0.01	0.98
30	0.1 mM $MgCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.12 ± 0.01	0.98
30	0.1 mM $FeCl_3$ + 10 mM MOPS	7	3.14	2200:1	0.12 ± 0.01	0.96
30	1 mM $MnCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.06 ± 0.01	0.90
30	1 mM $CaCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.09 ± 0.01	0.96
30	1 mM $MgCl_2$ + 10 mM MOPS	7	3.14	2200:1	0.09 ± 0.01	0.96[a]
30	1 mM $FeCl_3$ + 10 mM MOPS	7	3.14	2200:1	0.12 ± 0.01	0.99

[a] The observed removal rate constant ($k_{obs,init}$) was calculated based on the first 7 hours data

[b] Uncertainty of 0.00 varied between 0.001 – 0.005

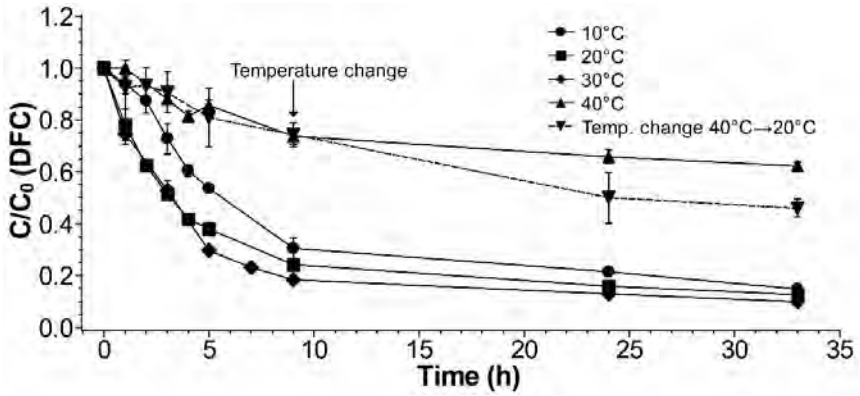


FIGURE 5.1 DFC removal by applying MnO_2 under anaerobic conditions at different temperatures. The solid lines represent the experiments maintained at one temperature while the dashed line represents the experiment in which the temperature changed. Experimental conditions: $[MnO_2]_0$=7 mM, $[DFC]_0$=3.14 μM, pH~7, I=0.01 M. Error bars are standard deviations of triplicate experiments

5.3.3 Effect of MnO_2 amount

The amount of MnO_2, expressed as the molar ratio between MnO_2 and DFC, determines the efficiency of DFC removal under anaerobic conditions. Results show that a higher molar ratio between MnO_2 and DFC leads to higher and faster removal (Table 5.2, Figure 5.2). DFC removal decreases from 52% at a molar ratio of MnO_2:DFC of 480:1 to 87% at a molar ratio of 2200:1, while the $k_{obs,init}$ increases from 0.05 to 0.16 h^{-1}. Further increasing the amount of MnO_2 to DFC/MnO_2 changes from 2200:1 to 8900:1, both the removal efficiency and $k_{obs,init}$ are slightly decreased and the $k_{obs,init}$ values levels off to 0.13 h^{-1}.

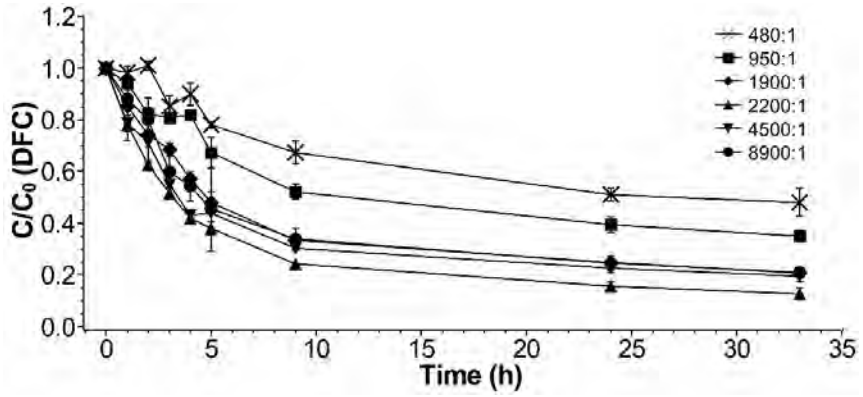


FIGURE 5.2 DFC removal by applying different amounts of MnO_2 (molar ratio $[DFC]_0/[MnO_2]_0$) under anaerobic conditions. Experimental conditions: 20ºC, pH~7, I=0.01 M. Error bars are the standard deviation of triplicate experiments

When the MnO_2 particles are applied in a reactor for treating WWTP effluent, the amount of MnO_2 is in the mM range while the concentrations of DFC and other pharmaceuticals are extremely low, in the nM to μM range. Therefore, high ratios between MnO_2 and DFC were used in this study to mimic those reactor conditions. Results show that increasing the amount of MnO_2 improves pharmaceutical removal with MnO_2. Increasing the amount of MnO_2 provides relatively more reactive surface sites. As a result, DFC can better attach to the MnO_2 surface, the first step in its removal. After adsorprion, the DFC is removed via chemical oxidation. During this process, increasing surface reactive sites will not improve the oxidation of DFC. In addition, due to the properties of pharmaceuticals and MnO_2, adsorption will not be improved by simply increasing the reactive surfaces sites because of increasing MnO_2 amount. As a result, both the removal and $k_{obs,init}$ are stable when the MnO_2 : DFC changes from 2200:1 to 8900:1.

5.3.4 Effect of metal ions

Under anaerobic conditions, inhibition of DFC removal is observed in the presence of four metal ions at two different concentrations (Table 5.2, Figure 5.3). In the absence of metal ions, after 33 hours, 90% of the DFC is removed. In the presence of 0.1 mM Mn^{2+}, Ca^{2+}, Mg^{2+}, or Fe^{3+}, 80 – 84% of DFC is removed. Results also show that Ca^{2+} has the least inhibition on DFC removal, with a decrease in $k_{obs,init}$ from 0.19 to 0.15 h^{-1}. For the other metal ions, $k_{obs,init}$ decreases to about 0.12 h^{-1}. Increasing the concentration of metal ions to 1 mM results in further inhibition effects in the order of "metal free" < Fe^{3+} < Ca^{2+} ≈ Mg^{2+} < Mn^{2+}. Only 58% of DFC is removed in the presence of Mn^{2+}, followed by 77% with Mg^{2+} and 74% with Ca^{2+}. The least inhibitory effect is observed in the presence of Fe^{3+} with about 88% of DFC removed, which is close to the removal (90%) in the absence of metals. The inhibition in the kinetics follows the same order as the removal efficiency, namely "metal free" < Fe^{3+} < Ca^{2+} ≈ Mg^{2+} < Mn^{2+} (Table 5.2).

The inhibitory effect of metal ions is most likely due to competition by ions with DFC for reactive surface sites on MnO_2. Since the pH characterizing the point-of-zero-charge of MnO_2 (birnessite) is 0.97 [299], MnO_2 is negatively charged at neutral pH. Similarly, DFC is also negatively charged (pKa=4.15) under the experimental conditions. Therefore, the positively charged metal ions are more easily adsorbed onto MnO_2 surface via electrostatic interactions than DFC via forming complex, resulting in fewer reactive surface sites available for DFC removal. The adsorption of metal ions onto MnO_2 is related to the radius of hydrated ions [83]. At low concentration (0.1 mM), abundant reactive sites for DFC removal are present, even though some reactive surface sites are used by metal ions. Therefore, there is little difference between metal ions and their inhibition of DFC removal. However, at a higher concentration (1 mM), the inhibition effect of metal ions follows the order of Mn^{2+} > Ca^{2+} ≈ Mg^{2+} > Fe^{3+}. This order is roughly the same as the adsorption affinity described previously [209].

The least inhibition of Fe^{3+} is probably because the radius of Fe^{3+} (0.064 nm) is the smallest among the four metal ions (Mn^{2+} : 0.080 nm, Ca^{2+} : 0.103 nm, Mg^{2+} : 0.070 nm). It is reported that the decreasing ionic radius will decrease adsorption affinity [269]. In addition, even though Fe^{3+} was added as ion into the bottle, some of them will precipitate at pH 7 in the experiments, which also decreases the inhibition effect of Fe.

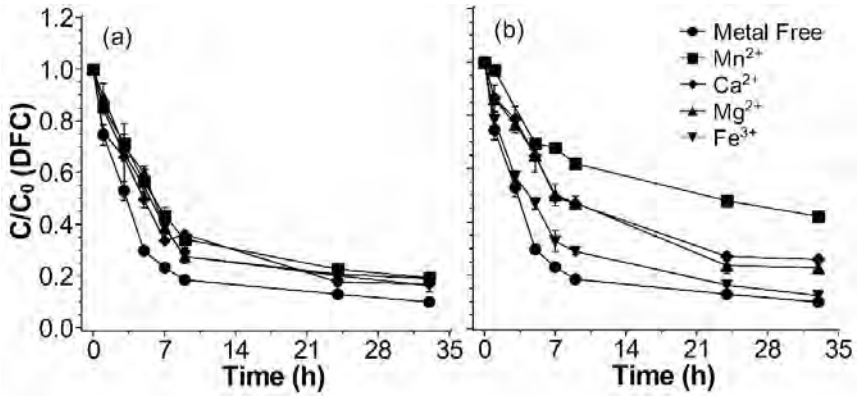


FIGURE 5.3 DFC removal by applying MnO_2 under anaerobic conditions in the presence of different metal ions at (a) 0.1 mM; and (b) 1 mM. Experimental conditions: $[MnO_2]_{0=}$7 mM; $[DFC]_{0=}$3.14 μM, 30ºC, pH~7, I=0.01 M. Error bars are standard deviations of triplicate experiments

5.3.5 Effect of phosphate

Phosphate (PO_4^{3-}) is a common pollutant in wastewater which affects the removal of micropollutants including pharmaceuticals and other organic compounds with MnO_2 [32, 85, 248]. Of the studies carried out under aerobic conditions, a limited number of studies have looked into the effects of PO_4^{3-}, and no studies are reported for anaerobic conditions [32, 248]. Therefore, we investigated the effects of phosphate (PO_4^{3-}) on the application of MnO_2 under anaerobic conditions to remove DFC (Table 5.3). At low PO_4^{3-} concentration (< 1 mg $P{\cdot}L^{-1}$), DFC removal decreases from 76% with no PO_4^{3-} to 46% at 0.5 mg $P{\cdot}L^{-1}$. When the PO_4^{3-} concentration further increases to 1 mg $P{\cdot}L^{-1}$, DFC removal increases slightly to 54%. Complete DFC removal is obtained when the concentration is 116 mg $P{\cdot}L^{-1}$.

TABLE 5.3 DFC removal by applying MnO_2 under anaerobic conditions in presence of phosphate at different concentrations after 33 hours. Experimental conditions: T=30 ℃, $[MnO_2]_0$ =7 mM; $[DFC]_0$=3.14 μM, pH~7, *I*=0.1 M

PO_4^{3-} (mg $P \cdot L^{-1}$)	DFC Removal
0	76%
0.5	46%
1	54%
116	100%

PO_4^{3-} can adsorb onto the MnO_2 surface, which decreases the reactive surface sites for DFC removal [228]. On the other hand, PO_4^{3-} can decrease the inhibition effects of Mn^{2+} generated from DFC removal by forming $Mn_3(PO_4)_2$ [127]. This mitigates inhibition of DFC removal by Mn^{2+}, leading to higher conversion efficiencies at higher PO_4^{3-} levels. In addition, the structure of $Mn_3(PO_4)_2$ can help in Mn(III) stabilization. As Mn(III) is a more active oxidant than Mn(IV), stabilization of Mn(III) by PO_4^{3-} also results in enhanced DFC removal. When the PO_4^{3-} concentration is low, the inhibition effect of PO_4^3 on DFC removal is dominant, resulting in lower DFC removal efficiencies. However, increasing PO_4^{3-} concentration leads to a decreasing inhibition effect of Mn^{2+} and even a stimulation effect due to Mn(III) stabilization resulting in more DFC removal.

5.3.6 Effect of humic acid

Humic acid (HA), chosen as a representative of organic matter, significantly promotes DFC removal under anaerobic conditions (Table 5.4). Based on the fast DFC removal observed in the presence of humic acid (HA) in a pre-test (data are not shown), initial DFC concentration was increased to 5.57 μM. Even when concentration of HA is only 5 $mg \cdot L^{-1}$, 80% DFC is removed within 1 hour. When HA concentration increases from 10 $mg \cdot L^{-1}$ to 20 $mg \cdot L^{-1}$, DFC removal increases from 87 to 96% within 1 hour.

TABLE 5.4 DFC removal by applying MnO_2 under anaerobic conditions at different humic acid (HA) concentrations. Experimental conditions: $[MnO_2]_0$=7 mM; $[DFC]_0$=5.57 µM, 30 ℃, pH~7, I=0.01 M

HA ($mg \cdot L^{-1}$)	t=0 h, no HA	t=0 h, with HA	t=0.5 h	t=1 h
0[a]	0%	0%	n.a.[b]	25%
5	0%	33%	71%	80%
10	0%	19%	76%	87%
15	0%	48%	90%	95%
20	0%	75%	91%	96%

[a] $[DFC]_0$=3.14 µM
[b] n.a.= not analysed

In this study, DFC removal is promoted in the presence of HA. Under aerobic conditions, it is reported that HA can form a complex with Mn^{2+} produced from MnO_2 during DFC removal [248]. Thus the strong binding ability of HA leads to more attractive sorption/complexation sites for Mn^{2+} than MnO_2 reactive sites, thus reducing blockage of the reactive surface site by Mn^{2+}. As a result, more sites are made available for DFC removal in the presence of HA.

5.3.7 Application potential

Applying MnO_2 under anaerobic conditions to remove DFC is a promising and efficient process. This study presents basic boundary conditions for application, providing the first step towards transferring this process from the lab into a feasible technology. Based on the results, the optimal conditions of applying MnO_2 under anaerobic conditions to remove DFC will be neutral pH, moderate temperature (10 – 30ºC), MnO_2:DFC ratio ≥ 2200:1, no metal ions, that may be compensated by the presence of organic matter (humic acids), and no or very high concentration of PO_4^{3-}, namely ≥ 116 mg $P \cdot L^{-1}$.

Experiments varying the temperatures show that DFC removal efficiency is high and stable in the range 10 – 30ºC. In most temperate and subtropical climate areas, the water or wastewater temperature is within this range all the year round. Therefore, no extra heating or temperature-control systems are necessary, leading to less cost in construction and operation.

Supplying MnO_2 during this process is another potential cost for operation. As mentioned in this paper, amount of MnO_2 needed in the application can be high under both aerobic and anaerobic conditions due to the low pharmaceutical concentrations and possible reactor form (like bed filter). In this study, results provide information on optimizing MnO_2 dosage based on pharmaceutical removal. These results show that the application of MnO_2 at a certain amount is possible to maintain the high pharmaceutical removal efficiency for a long time.

We studied the effects of typical co-solutes to assess the application of this process both in drinking water treatment and in wastewater treatment processes. The four selected metal ions are all ubiquitously present in the aquatic environment, especially in drinking water. The two concentrations tested in this study are at a similar level in drinking water based on World Health Organization [340, 341] and EU [59]. In addition, Mn^{2+} will be produced in the process, and this has the strongest inhibition effect among the four metal ions. Therefore, incorporation of metal removal processes and pharmaceutical removal with MnO_2 will be a wise option in application.

Both PO_4^{3-} and organic compounds, like HA, are commonly observed co-solutes in wastewater. The diverse effects of PO_4^{3-} on DFC removal indicate that the PO_4^{3-} should be taken into account in design and operation of full application technologies. HA can promote DFC removal. In addition, the HA can bind the metals including Mg, Ca, and Mn. This reduces the inhibition of metal ions on pharmaceutical removal with MnO_2. As a result, the application of manganese oxides under anaerobic conditions as a tertiary treatment has advantages in the application. The organic matter remaining after wastewater treatment processes contain often high levels of HA and may, therefore, promote DFC removal.

5.4 Conclusion

The application of manganese oxides (MnO_2) under anaerobic conditions to remove diclofenac (DFC) is effective. Compared to pharmaceutical removal technologies under aerobic conditions, anaerobic conditions do not require aeration systems to ensure oxygen supply. This decreases the costs of construction and operation. The results indicate that the optimal control of the MnO_2 properties, especially the MnO_2 reactive surface sites, are important in DFC removal under anaerobic conditions. Based on the experimental results, the optimal operational conditions are neutral pH, moderate temperature (10 – 30ºC), MnO_2/DFC ratio in the range 2200:1 – 8900:1, no metal ions, no or high concentration of PO_4^{3-}, and in the presence of humic acids. This study is the first step towards translating the process into the application. Results show the diverse effects of temperature, MnO_2 dosage, and phosphate on the DFC removal efficiency and the observed initial reaction rate constant ($k_{obs,init}$). While there is obvious inhibition by metal ions, the significant promotion of humic acid to DFC removal may be used to compensate for this inhibitory effect, including mitigating the inhibitory effect of Mn^{2+} formed during the process. In addition, Mn^{2+} produced in this process can be oxidized back to MnO_2 using permanganate, manganese oxidizing bacteria, or other oxidation processes. The agents, used in the re-oxidation of Mn^{2+} to MnO_2, can also remove Pharmaceuticals [67, 101]. The cycling of Mn decreases the concentration of Mn^{2+} in the effluent and recovers valuable manganese for reuse.

In summary, anaerobic MnO_2 mediated removal of pharmaceuticals from water is a potentially interesting, sustainable and promising technology. Therefore we plan to extend our future research to pharmaceuticals other than DFC, and to develop the technology further to scaling up and application.

ACKNOWLEDGEMENT

This work is finical supported by China Scholarship Council (CSC, File No. 201308610161), and Wageningen University & Research. The authors thank Menghan Sun and Lydia Senanu for assistance with the experiments and Hans Beijleveld, Livio Carlucci, Ilse Gerrits, and Jean Slangen for their help in analysis, and colleagues at our department for their valuable suggestions.

Chapter 6

Biological regeneration of manganese (IV) using oxygen and iron (III) using nitrate for anaerobic metal oxide mediated removal of pharmaceuticals from water

A modified version of this chapter is in preparation for publication as

Liu, W.; Langenhoff, A. A. M.; Sutton, N. B.; Rijnaarts, H. H. M., Biological regeneration of manganese (IV) using oxygen and iron (III) using nitrate for anaerobic metal oxide-mediated removal of pharmaceuticals from water.

ABSTRACT

Application of manganese (IV) (Mn(IV)) and iron (III) (Fe(III)) (hydr)oxides in removing pharmaceuticals from water is attractive for their capacity in degrading these chemicals, and their potential regeneration. Pharmaceutical degradation under anaerobic conditions is energetically most favorable. Thus, regeneration of the Mn(IV) or Fe(III) should also be produced under these conditions, or with a minimum oxygen dosage. In the study presented here, batch experiments were carried out to investigate this in-process production of Mn(IV) and Fe(III) from Mn(II) and Fe(II), as well as the removal of pharmaceuticals coupled to the biological Mn(IV) and Fe(III) regeneration. Results show that biological production of (re-oxidation to) Mn(IV) can be achieved under oxygen-limiting conditions. The biological production of Fe(III) can be achieved with nitrate under anaerobic conditions. Both biologically produced Mn(IV) and Fe(III) are amorphous. Pharmaceutical biodegradation during the regeneration is tested, as well as the abiotic removal of pharmaceuticals with different types of Mn(IV) or Fe(III). Biodegradation of pharmaceuticals with Mn(II)-oxidizing bacteria resulted in an insignificant removal of the selected pharmaceuticals. Using Mn(IV) produced from drinking water treatment plants achieves a removal of 23% of metoprolol and 44% of propranolol, similar to the removal with chemically synthesized Mn(IV). When Fe(III) was produced with the help of nitrate, pharmaceutical biodegradation is insignificant. Anaerobic abiotic pharmaceutical tests showed that about 31 – 43% of propranolol is removed when using Fe(III) originating from drinking water treatment plants, which is higher than with either biologically or chemically synthesized Fe(III). In addition, one of the commercially available Fe(III)-based sorbent tested, $Fe(III)_{FerroSorp®RW}$, can also remove propranolol. This study indicates that the biological regeneration of Mn(IV) and Fe(III), from their reduced species produced in an anaerobic pharmaceutical degradation set up, is feasible. Mn(IV) and Fe(III) produced and obtained from different processes can be used for this.

KEYWORDS: biological production; Mn(IV) and Fe(III) (hydr)oxides; abiotic removal; pharmaceuticals; biodegradation; anaerobic condition; nitrate

6.1 Introduction

Pharmaceutical compounds have been identified as important emerging environmental water contaminants in the last decades. Pharmaceuticals are detected not only in wastewater but also in groundwater, surface water, and even in drinking water [79]. The continuous discharge of these compounds to the water originating from industrial emissions and human consumption could lead to potential risks for ecosystems such as extended reproductive periods of aquatic organisms, and also for human health [79, 148]. Therefore, the presence of pharmaceuticals in aquatic environments is unwanted, even at the low concentrations currently encountered ($ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$) [6].

Conventional wastewater treatment processes are insufficient in removing pharmaceuticals such as carbamazepine, diclofenac, or metoprolol [46], so many advanced technologies such as photocatalysis are applied [95, 354]. Such methods usually require aerobic conditions (presence of oxygen), leading to high energy consumption for aeration or oxygen generation. Manganese (Mn)- or iron (Fe)- based technologies, especially those applying Mn(IV)- or Fe(III)-(hydr)oxides, successfully remove various pharmaceuticals via both abiotic and biotic processes under anaerobic conditions (without oxygen) [170]. Previous research showed that anaerobic abiotic diclofenac removal with MnO_2 is more efficient than under aerobic conditions [172]. The anaerobic biodegradation can also efficiently remove pharmaceuticals including naproxen with Mn(IV) or Fe(III) [171, 268]. Furthermore, the anaerobic conditions are more sustainable because they require less energy input for operations like aeration.

An additional outstanding advantage is that Mn(IV) and Fe(III) can be regenerated after use, decreasing waste sludge outputs and increasing the sustainability in potential upscaled application. As anaerobic pharmaceutical degradation is foreseen, Mn(IV) or Fe(III) production under anaerobic conditions is most attractive, allowing these metals to be re-used

directly in the process. Both chemical and biological methods can be used to regenerate Mn(IV) and Fe(III) from the Mn(II) and Fe(II) released during pharmaceutical degradation. Chemical production is achieved through direct oxidation using oxygen (O_2) or a strong oxidant such as chlorine, chlorine dioxide, ozone or $KMnO_4$ [135]. For example, chemically produced Mn(IV) can be obtained via the oxidation of $MnCl_2$ with $KMnO_4$ [65]. Fe(II) can also be directly oxidized in the presence of O_2.

The chemical synthesis of Mn(IV) or Fe(III) usually uses strong oxidants and these oxidants can also produce unwanted by-products during the synthesis. The biological production, however, can be cleaner with no or fewer by-products. Since pharmaceutical removal with Mn(IV) or Fe(III) is more attractive under anaerobic conditions, the regeneration of Mn(IV) and Fe(III) should be preferentially also under anaerobic conditions. However, the high redox potential of Mn(IV) (+ 1.23 V) indicates that biological Mn(IV) production can only occur in the presence of O_2 [301]. To limit the O_2 transfer to the anaerobic step of biological Mn(IV) production and pharmaceutical removal, minimal O_2-dosage need to be applied. Fe(III) can be produced by microorganisms under anaerobic conditions using NO_3^- as electron acceptor [292, 366]. In addition, biological Fe(III) production has the potential to remove pharmaceuticals via abiotic processes and/or biotic processes. Previous studies reported complete removal of atenolol and 70% removal of propranolol with NO_3^- via biological degradation [13, 268].

In addition to regeneration, Mn(IV) or Fe(III) can be obtained from commercial sources or from various waste streams in the water sector. This results in other properties of the two metal oxide species , which may lead to more or less effective pharmaceutical degradation. Biologically produced Mn(IV) and Fe(III) (labelled as $Mn(IV)_{bio\text{-}production}$ and $Fe(III)_{bio\text{-}production}$ throughout) have a larger specific surface area and higher binding energy than chemically synthesized Mn(IV) and Fe(III) (labelled here as $Mn(IV)_{chem\text{-}synthesis}$ and $Fe(III)_{chem\text{-}synthesis}$) [97, 301]. These properties are suitable for abiotic removal of pollutants such as pharmaceuticals or heavy metal. A

previous study showed that the removal of diclofenac with $Mn(IV)_{bio\text{-}production}$ at neutral pH is 10-fold faster compared to $Mn(IV)_{chem\text{-}synthesis}$ [65]. Another source of Mn(IV) and Fe(III) is waste from drinking water treatment plants. Mn(IV) or Fe(III) produced during drinking water treatment ($Mn(IV)_{DWTP}$ and $Fe(III)_{DWTP}$) are usually produced via chemical or biological processes, or the combination of these two processes [135, 144]. Thus, $Mn(IV)_{DWTP}$ and $Fe(III)_{DWTP}$ likely have different properties than Mn(IV) or Fe(III) produced solely via defined chemical or biological processes. Little is known about applying these types of Mn(IV) or Fe(III) oxide species to remove of pharmaceuticals under anaerobic conditions.

This paper aims to (1) investigate biological regeneration of Mn(IV) under O_2-limiting conditions from Mn(II) formed during pharmaceutical degradation or drinking water production; (2) investigate biological regeneration of Fe(III) by applying NO_3^- under anaerobic conditions from Fe(II) formed during anaerobic pharmaceutical degradation or drinking water production; (3) investigate biodegradation of pharmaceuticals during these metal oxide regeneration steps; and (4) compare the abiotic removal of pharmaceuticals under anaerobic conditions with different Mn(IV) and Fe(III) metal oxide species. The results together contribute to an understanding of essential aspects of Mn and Fe regeneration and recycling during metal oxide mediated pharmaceutical removal from water. This brings future application of such a technology into view.

6.2 Methods and materials

6.2.1 Chemicals

Anaerobic water was used in most of the experiments unless specified. Anaerobic water was prepared by boiling ultrapure water or demineralized water (demiwater) for 5 min and cooled to room temperature by flushing with N_2. Ultrapure water was collected from a Millipore system (18.2 MΩ·cm, TOC=18 ppb, USA).

Seven commonly used pharmaceuticals are tested in this study, including caffeine, carbamazepine, diclofenac, ibuprofen, metoprolol, naproxen, and propranolol, were purchased either from Sigma-Aldrich or from MP Biomedicals, as previously described [172].The stock solution of pharmaceutical mixture (20 $mg \cdot L^{-1}$ each) was prepared with ultrapure water. All other chemicals were purchased from Sigma-Aldrich at purity > 98% for solids and at HPLC or UPLC quality for liquids.

The Mn(IV) and Fe(III) involved in this study are the same materials used in a previous study (Table 6.1) [171]. $Mn(IV)_{bio\text{-}production}$ was collected during the experiments and prepared as described [65]. In short, $Mn(IV)_{bio\text{-}production}$ was centrifugation at 7,000 g for 15 min, and then was washed several times through centrifugation and resuspended with 10 mM anaerobic phosphate buffer (Na_2HPO_4/KH_2PO_4, Sørensen buffer). $Mn(IV)_{chem\text{-}synthesis}$ was prepared the same as previously described [172]. $Mn(IV)_{DWTP}$ was kindly provided by Water Laboratorium Noord (WLN, the Netherlands), originally from drinking water production plant 'Noordbargeres' in Emmen. The $Mn(IV)_{DWTP}$ was dried under N_2 flushing.

$Fe(III)_{bio\text{-}production}$ was collected and prepared by washing the same as $Mn(IV)_{bio\text{-}production}$ by centrifugation and resuspended with 10 mM anaerobic phosphate buffer (Na_2HPO_4/KH_2PO_4, Sørensen buffer). $Fe(III)_{chem\text{-}synthesis}$ was prepared based on a previous study [153]. Briefly, the 0.4 M $FeCl_3$ was neutralized with 1 N NaOH until pH 7. Then, the $Fe(III)_{chem\text{-}synthesis}$ was washed in the same way as $Mn(IV)_{chem\text{-}synthesis}$. $Fe(III)_{DWTP}$ was kindly provided by Evides Waterbedrijf (the Netherlands). Two Fe(III)-based sorbents, FerroSorp®Plus and FerroSorp®RW was obtained from HeGo Biotec GmbH.

6.2.2 Experimental setup

6.2.2.1 Biological production of Mn(IV)

(1) Inoculum

The Mn(II)-oxidizing bacteria was kindly provided by Utrecht University. The pre-cultivation of the bacteria was carried out in 150 mL flasks with 50 mL fresh Luria-Bertani (LB) medium at 30 ºC on a shaker (200 rpm) [295]. LB medium was freshly prepared before experiments consisted of (per liter of demiwater) 10 g tryptone, 10 g NaCl, and 5 g yeast extract [295]. The pH of the medium was adjusted to 7.0 with 10 N NaOH. The medium was sterilized by autoclaving at 120 ºC for 20 min prior to use. When the OD_{600} reached around 1.0, the bacterial culture was ready for use in our experiments.

(2) Batch experiments

The batch experiments to produce $Mn(IV)_{bio\text{-}production}$ were carried out in triplicate in 125 mL glass bottles containing 30 mL sterilized anaerobic LB medium. The preparation of the medium and the batch bottles was carried out in an anaerobic glovebox. Bottles were closed with butyl-rubber stoppers and then taken out of the glovebox. Thereafter, bottles were sealed with aluminum crimp caps. The headspace was exchanged with the N_2/O_2 gas mixture to 1.2 bar. The total O_2 amount, including gaseous O_2 and dissolved O_2, was expressed in the percentage in headspace, that is 0.3% (total O_2 7 μmol), 5.8% (178 μmol), 10.1% (330 μmol), 24.3% (697 μmol). The calculation process was described in Text S6.1.

The bottles were sterilized by autoclaving at 120ºC for 15 min. Bottles with cotton stoppers were carried as a positive control (no O_2 limitation). Blank controls without bacteria were taken along as well. Filter-sterilized $MnCl_2$ stock (9 mM) was added to the bottles to reach a final concentration of 0.3 mM Mn(II) (~9 μmol Mn). The experiments were started by adding 0.3 mL pre-cultivated Mn(II)-oxidizing bacteria. All bottles were incubated at 30 ºC on a shaker (200 rpm). Liquid samples were collected regularly for analysis of bacteria and Mn(II).

(3) Sample preparation

The samples for bacteria growth were taken and measured immediately without treatment. Liquid samples for Mn(II) analysis were centrifuged at 10,000rpm for 10 min. Then, the supernatant was collected and analyzed immediately based on standard methods [252].

6.2.2.2 Biological production of Fe(III)

(1) Inoculum

The inoculum was kindly provided by Wageningen University & Research, the Netherlands. The inoculum consisted of the pre-incubated activated sludge of WWTP Bennekom, the Netherlands in the presence of 48.5 $mg \cdot L^{-1}$ Fe(II) and 10.1 $mg \cdot L^{-1}$ NO_3^-.

(2) Batch experiments

The batch experiments to produce $Fe(III)_{bio\text{-}production}$ were carried out in triplicate in 125 mL bottles with 100 mL medium (Table S6.1). Sufficient NO_3^-, 3mM, was used to biologically produce Fe(III). The pH of the medium was maintained at pH 7.5. The preparation of the medium and batch bottles was carried out in the anaerobic glovebox. The bottles were closed with butyl-rubber stoppers, taken out of the anaerobic glovebox and sealed with aluminum crimp caps. The headspace of the bottles was exchanged with the N_2/CO_2 gas mixture to achieve 80% N_2 and 20% CO_2 (v/v) and brought to 1.2 bar. The experiments were started by adding 10 mL inoculum to all the

bottles. Abiotic controls were prepared with 3 mM NO_3^- along with the experiments without inoculum . The experiments were conducted in the dark at 30˚C, shaking at 120 rpm. Concentrations of NO_3^- and Fe were analyzed at the beginning and at the end of the experiments.

(3) Sample preparation

Samples for NO_3^- measurement were prepared exactly the same as those for pharmaceuticals. The samples for NO_3^- were stored at 4 ºC and analyzed within one month.

Samples for Fe analysis were prepared by a modified HCl extraction method [52]. Briefly, 0.5 mL samples were mixed with 0.5 mL 1M HCl to fix Fe(II). Thereafter, Fe(II) was analyzed directly by colorimetric methods (Hach Dr. Lange Kit 340). The total Fe was analyzed by the same procedure, with the exception that 1mL 0.5 M $NH_2OH{\cdot}HCl$ was added before analysis. The samples were stored at 4 ºC before analysis.

TABLE 6.1 Summary of Mn(IV)- and Fe(III)-(hydr)oxides involved in this study [a]

Mn(IV)- or Fe(III)-(hydr)oxides [b]	Synthesis methods	Specification	Morphology	Original form	Metal content in dry matter (DM)
$Mn(IV)_{bio\text{-}production}$	Biological production	Mn(IV) produced by bacteria oxidizing Mn(II) with O_2	Amorphous	Powder attached on bacteria	n.a. [d]
$Mn(IV)_{chem\text{-}synthesis}$	Chemical synthesis	Mn(IV) produced via reaction between $KMnO_4$ and $MnCl_2$	Amorphous	Slurry (powder)	n.a.
$Mn(IV)_{DWTP}$	Biological and/or chemical processes	Mn(IV) Originally from drinking water production plant 'Noordbargeres' in Emmen (Waterleiding Maatschappij Drenthe, the Netherlands)	Semi-crystalline	Granule	10.8 $mgMn \cdot gDM^{-1}$
$Fe(III)_{bio\text{-}production}$	Biological production	Fe(III) produced by bacteria oxidizing Fe(II) with NO_3^-	Amorphous	Powder attached on bacteria	n.a.
$Fe(III)_{chem\text{-}synthesis}$	Chemical synthesis	Fe(III) produced by neutralizing $FeCl_3$ solution with NaOH to pH 7	Amorphous	Slurry (powder)	n.a.
$Fe(III)_{DWTP}$	Biological and/or chemical processes	A mixture of Fe(III) from several drinking water treatment plants, obtained from Evides Waterbedrijf (the Netherlands)	Semi-crystalline	Granule	109.6 $mgFe \cdot gDM^{-1}$
$Fe(III)_{FerroSorp®Plus}$	Chemical synthesis	Fe(III)-based sorbent, FerroSorp®Plus, from HeGo Biotec (Germany)	Semi-crystalline	Granule	441.2 $mgFe \cdot gDM^{-1}$
$Fe(III)_{FerroSorp®RW}$	Chemical synthesis	Fe(III)-based sorbent, FerroSorp®RW, from HeGo Biotec (Germany)	Semi-crystalline	Granule	119.5 $mgFe \cdot gDM^{-1}$

[a] Part of the results is from a previous study [171]

[b] $Mn(IV)_{bio\text{-}production}$ = biologically produced Mn(IV) oxides, $Mn(IV)_{chem\text{-}synthesis}$ = chemically synthetic Mn(IV) oxides, $Mn(IV)_{DWTP}$ = Mn(IV) oxides from drinking water treatment plants, $Fe(III)_{bio\text{-}production}$ = biologically produced Fe(III) hydroxides, $Fe(III)_{chem\text{-}synthesis}$ = chemically synthetic Fe(III) hydroxides, $Fe(III)_{DWTP}$ = Fe(III) hydroxides from drinking water treatment plants, $Fe(III)_{FerroSorp®Plus}$ = Fe(III)-based sorbent, FerroSorp®Plus, $Fe(III)_{FerroSorp®RW}$ = Fe(III)-based sorbent, FerroSorp®RW

[c] n.a. = not analysed

6.2.2.3 Pharmaceutical removal

(1) Biodegradation during biological Mn(IV) production

Biodegradation of pharmaceuticals was carried out by the same method as biological Mn(IV) production process in and add filter-sterilized stock of pharmaceutical mixture (20 $mg \cdot L^{-1}$) at a final concentration 0.5 $mg \cdot L^{-1}$.

The samples for pharmaceutical measurement were centrifuged at 10,000 rpm for 10 min immediately after taken. The supernatant was collected and pre-treated by solid phase extraction (SPE) as described previously [339]. The recovery rate of SPE is 85 – 115%. Thereafter, the samples were stored in amber vials at -20 ºC before analysis.

(2) Abiotic removal with different Mn(IV)

The batch experiments on the abiotic removal of pharmaceuticals were carried out in duplicate under anaerobic conditions. Batch experiments were similar to previous studies [169, 172], and the pH of solutions was maintained by 10 mM phosphate buffer which is also used to prepare $Mn(IV)_{bio\text{-}production}$. Final concentrations in the batches were 7 mM $Mn(IV)_{bio\text{-}production}$. The experiments were initiated by adding 1.25 mL stock of pharmaceutical mixture into the experimental bottles, resulting in a final concentration of 0.5 $mg \cdot L^{-1}$. Samples were taken regularly to analyze the concentration of pharmaceuticals.

Since the $Mn(IV)_{DWTP}$ is produced main via biological process but the chemical process is also possibly involved [307], it can have different properties from $Mn(IV)_{chem\text{-}synthesis}$ or $Mn(IV)_{bio\text{-}production}$. Therefore, the abiotic removal of pharmaceuticals under anaerobic conditions with this $Mn(IV)_{DWTP}$ (granules and ground powder) was also tested.

The samples for pharmaceutical measurement were centrifuged at 10,000 rpm for 10 min immediately after taken. The supernatant was collected and stored in amber vials at -20 ºC before analysis. Parallel samples were taken to investigate the contribution of adsorption to the removal. 0.5 mL samples were taken, and then 1mL reducing agent (0.5 mM $NH_2OH \cdot HCl$) were added immediately. Then, the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and stored in amber vials at -20 ºC before analysis. The presence of $NH_2OH \cdot HCl$ has no effects on the analysis of pharmaceuticals.

(3) Biodegradation during biological Fe(III) production

Pharmaceutical biodegradation during biological Fe(III) production was also tested. The experiments were carried out the same as biological Fe(III) production. Before adding the inoculum, the pharmaceutical mixture was added to batch bottles at a final concentration of each pharmaceutical at 1 $mg \cdot L^{-1}$. Samples were taken every 30 days for analyzing the concentrations of pharmaceuticals, NO_3^- and Fe.

The samples for pharmaceutical measurement were prepared as those in experiments on biodegradation during biological Mn(IV) production.

(4) Abiotic removal with different Fe(III)

Abiotic removal of pharmaceuticals with different types of Fe(III) was carried out the same as with Mn(IV). The final concentration of Fe(III) is 0.4 mM, and that of pharmaceutical is 0.5 $mg \cdot L^{-1}$. Sample preparation was the same as those in biodegradation of pharmaceuticals during biological Mn(IV) production.

6.2.3 Analysis

The Mn analysis including total Mn and Mn(II) was performed by an inductively coupled plasma optical emission spectrometry (VISTA-MPX CCD Simultaneous, VARIAN co.) equipped with an megapixel (MPX) detector at wavelength 257.610 nm.

Fe analysis including total Fe or Fe(II) was performed by Hach Dr. Lange Kit (LCK 340). NO_3^- concentrations were measured by a Hach Dr. Lange Kit (LCK 320, 321). Morphologies of $Mn_{bio\text{-}production}$ and $Fe_{bio\text{-}production}$ were analyzed by an X-ray powder diffraction (XRD, Bruker D8 advance, Bruker, Germany). The pharmaceutical analysis was performed by an ultra-performance liquid chromatography with a diode array detector (UPLC, ultimate 3000, Thermo, USA), as previously described [95].

6.3 Results and discussion

6.3.1 Biological regeneration

(1) Biological Mn(IV) production under O_2-limiting conditions

The biological production of Mn(IV) under O_2-limiting conditions occurs under all O_2 concentrations (Figure 6.1). The initial O_2 amount varies from 178 to 700 μmol, and similar amounts of $Mn(IV)_{bio\text{-}production}$ are produced during the first 24 hours ($p > 0.05$). During the period from 24 to 48 hours, no further $Mn(IV)_{bio\text{-}production}$ production is observed, that resulting in a total $Mn(IV)_{bio\text{-}production}$ production of 0.7 μmol. In the group without O_2 limitation (positive control group), the $Mn(IV)_{bio\text{-}production}$ after 24 hours and 48 hours is much higher than in the O_2 -limiting groups. These results indicate that the amount of O_2 limits biological Mn production. Mn(II)-oxidizing bacteria are aerobic bacteria, and this limited initial amount of O_2 will affect bacterial activity. A certain level of O_2 is necessary for their growth and biological processes. Therefore, a certain amount of O_2 must be present during biological production of Mn, despite the inhibition of the biological Mn(IV) production.

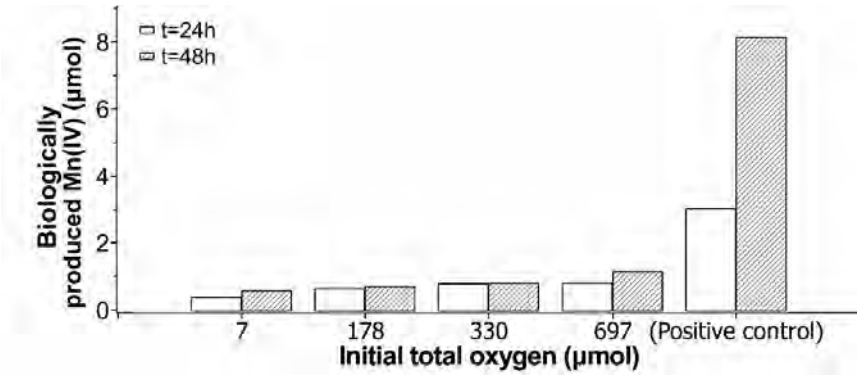


FIGURE 6.1 Biologically produced Mn(IV) oxides from Mn(II) under O_2-limiting conditions after 24 and 48 hours

(2) Biological Fe(III) production with NO_3^- under anaerobic conditions

During the biological production of Fe(III), 0.8 mM sodium acetate was added to promote the growth of the bacteria and biological production of Fe(III). The 3 mM NO_3^- is used in this experiment to make sure that NO_3^- is not the limiting factor. During the process, 0.61 mM Fe(III) biologically produced coincided with 0.59 mM NO_3^- consumption (Table 6.2). The molar ratio of $Fe(III)_{bio\text{-}production}$ and consumed NO_3^- in our study is around 1:1, while the ideal ratio is 5:1 (Equation 6.1) [215]. There are a number of processes that could contribute to additional nitrate consumption.

$$10Fe(II) + 2NO_3^- + 28H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+ \qquad (6.1)$$

Since the redox pairs at pH 7 of during the nitrate reduction are more positive than that of Fe(III)/Fe(II) (Ferrihydrite/Fe^{2+}, -100 to 100 mV) [291], they can all oxidise Fe(II) to Fe(III), forming different products. For example, when the final product is NO_2, the ratio between $Fe(III)_{bio\text{-}production}$ and NO_3^- consumption is around 1:1 (Equation 6.2).

$$Fe(II) + NO_3^- + 2H^+ \rightarrow Fe(OH)_3 + NO_2 + H_2O \qquad (6.2)$$

TABLE 6.2 Biological production of Fe(III) ($Fe(III)_{bio\text{-}production}$) with a different initial concentration of NO_3^-.

Initial NO_3^- (mM)	Fe(III) production (mM)	NO_3^- consumption (mM)	Molar ratio $[Fe(III)_{bio\text{-}production}]$: $[NO_3^-]_{consumption}$
0.08 (Blank controls)	n.d. [a]	0.02	n.a. [b]
2.8	0.61	0.59	1.04 : 1
2.7 (Abiotic controls)	n.d.	n.d.	n.a.

[a] n.d. = not detected
[b] n.a. = not applicable

In addition, the presence of acetate promotes denitrification under anaerobic conditions: a portion of the nitrate is used to oxidize the acetate. In the process, bacteria will consume the NO_3^- without producing Fe(III). Therefore, the observed molar ratio between $Fe(III)_{bio\text{-}production}$ and NO_3^- consumption is 1:1, less than the theoretical value 5:1.

(3) Properties

The properties of the Mn(IV) and Fe(III) used in this study are analyzed by X-ray diffraction (XRD). Based on the XRD pattern, $Mn(IV)_{bio\text{-}production}$ is amorphous (Figure 6.2). The morphology of the two Mn(IV) species produced within this study, $Mn(IV)_{bio\text{-}production}$ and $Mn(IV)_{chem\text{-}synthesis}$, are same [171]. Similar results are also reported in a previous study [322]. Furthermore, materials sourced from DWTP waste are also tested. From a previous study, the ground $Mn(IV)_{DWTP}$ powder has peaks which are also observed in crystalline MnO_2. This indicates that the $Mn(IV)_{DWTP}$ has a semi-crystalline nature (Table 6.1) [171]. However, the raw materials of $Mn(IV)_{DWTP}$ could be amorphous. During the grinding, the specific area of $Mn(IV)_{DWTP}$ powder will increase, but it might also change its morphology of $Mn(IV)_{DWTP}$ from amorphous to semi-crystalline. In addition, the long-term storage can slowly change the morphology of $Mn(IV)_{DWTP}$ via aging processes, for instance changing them from amorphous to crystalline [293].

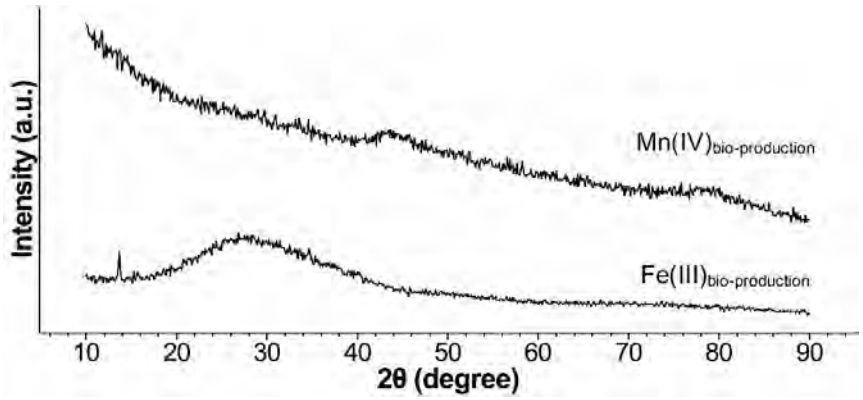


FIGURE 6.2 X-ray diffraction spectrum of biologically produced Mn(IV) oxides ($Mn(IV)_{bio\text{-}production}$) and biologically produced Fe(III) hydroxides ($Fe(III)_{bio\text{-}production}$)

XRD patterns show that $Fe(III)_{bio\text{-}production}$ is amorphous and similar to that used in our other study (Figure 6.2) [272]. The other Fe(III) used in this study are the same as used in a previous study [171]. The Fe(III) from drinking water treatment ($Fe(III)_{DWTP}$), and two Fe(III)-based sorbents are semi-crystalline. They are all a mixture of Fe_2O_3 and Fe_3O_4. Based on the XRD pattern, the $Fe(III)_{DWTP}$ appears to be the most amorphous among these three materials while the $Fe(III)_{FerroSorp®RW}$ is the most crystalline.

6.3.2 Removal of pharmaceuticals

(1) Pharmaceutical removal during biological Mn(IV) production

Mn(IV) production in this study occurs under oxygen-limiting, but nonetheless aerobic conditions. Pharmaceutical removal during Mn(IV) production is expected since aerobic biodegradation of pharmaceutical compounds has been reported previously [151]. In this study, biodegradation of pharmaceuticals with Mn(II)-oxidizing bacteria was tested. Results show that there is no significant removal of the selected pharmaceuticals (Table S6.2). Similar studies with *Pseudomonas putida* MnB6 (a strain of Mn(II)-oxidizing bacteria) also show that the removal of diclofenac and other micropollutants is insignificant [67, 200]. The Mn(II)-oxidizing bacteria used to produce Mn(IV) is a pure strain specialized in Mn(IV) oxidation. These bacteria are growing under O_2-limiting conditions. Thus, this individual

strain most likely had neither the degradation capacity nor optimal growth conditions to biodegrade challenging pharmaceutical structures.

(2) Abiotic pharmaceutical removal with Mn(IV)

Batch experiments were carried out to test the abiotic removal of pharmaceuticals with different types of Mn(IV). Due to experimental conditions, we were unable to produce sufficient $Mn(IV)_{bio\text{-}production}$ for our abiotic removal tests. Therefore, the experiments were carried out with $Mn(IV)_{DWTP}$ (granule and ground powder). The experiments on the abiotic removal of pharmaceuticals were carried out in anaerobic water in duplicate. Results show no abiotic anaerobic removal with the $Mn(IV)_{DWTP}$ granule but with ground $Mn(IV)_{DWTP}$ powder supported the efficient removal of metoprolol and propranolol (Table 6.3). With ground $Mn(IV)_{DWTP}$ powder , removal of metoprolol is 23% in 24 hours, of which 20% is removed due to adsorption. Similarly, 44% propranolol is removed, and 30% is a result of adsorption.

The experimental conditions are exactly the same as applied in a previous study with $Mn(IV)_{chem\text{-}synthesis}$. In that study, 78% diclofenac, 33% metoprolol and 51% propranolol are removed under anaerobic conditions with MnO_2 in anaerobic water (Table 6.3) [172]. In this current study, the removal of metoprolol and propranolol with two different Mn(IV) types is similar, but no diclofenac removal is observed with $Mn(IV)_{DWTP}$. No adsorption was observed during the abiotic removal of pharmaceuticals with $Mn(IV)_{chem\text{-}synthesis}$.

TABLE 6.3 Abiotic removal of pharmaceuticals (%) with Mn(IV) from drinking water treatment plants ($Mn(IV)_{DWTP}$) and manually synthesized, chemically produced Mn(IV) ($Mn(IV)_{chem\text{-}synthesis}$) under anaerobic conditions

	$Mn(IV)_{DWTP}$ granule [a]	$Mn(IV)_{DWTP}$ powder [a]	$Mn(IV)_{chem\text{-}synthesis}$ [b]
Diclofenac	3(±2)	-4(±0)	78(±6)
Metoprolol	5(±3)	23(±2)	33(±7)
Propranolol	9(±3)	44(±3)	51(±5)

[a] Data between brackets show the difference between the duplicates

[b] Data are from a previous study of the same group [172]. The data in the bracket is the standard deviation of the triplicate

Our research on anaerobic abiotic removal of diclofenac with Mn(IV) shows that the amorphous Mn(IV) is better than crystalline Mn(IV) [172]. Based on the XRD pattern, the $Mn(IV)_{DWTP}$ powder can be defined as semi-crystalline. Therefore, it appears to be less effective in anaerobic abiotic pharmaceutical removal. In addition, even though both $Mn(IV)_{DWTP}$ granule and $Mn(IV)_{DWTP}$ powder are semi-crystalline, the $Mn(IV)_{DWTP}$ powder has a larger specific area and potentially has more reactive surface and sites. Previous studies indicated that more reactive surface sites of Mn(IV) generally lead to more removal of pharmaceuticals [172, 248]. This may explain the better performance by the ground $Mn(IV)_{DWTP}$ powder than $Mn(IV)_{DWTP}$ granule in pharmaceutical removal.

The reason why $Mn(IV)_{DWTP}$ powder is only effective to remove two β-blockers but is inactive towards converting other pharmaceuticals could not yet be revealed. We hypothesize that there may be a relation with the molecular properties of the pharmaceuticals. The two β-blockers have quite a similar chemical structure, the aromatic ring, the oxygen molecule connected to the aromatic ring, and a long-chain structure. In addition, they have a similar pKa value (metoprolol 9.5, propranolol 9.42) [172]. During the abiotic removal, the compounds are firstly adsorbed onto MnO_2 surface or forming a complex [172]. The adsorption capacity is related to the chemical structure and properties. Thus, the two pharmaceuticals are better removed than others.

(3) Pharmaceutical removal during biological Fe(III) production

The pharmaceutical removal units in application will also transfer pharmaceuticals to the biological Fe(III) regeneration unit. Since organic compounds like acetate present in the media can lead to dentrification, the pharmaceuticals are likely to function in the same way, affecting biological Fe(III) production under anaerobic conditions. In addition, based on the literature, the degradation of pharmaceuticals in the presence of Fe(II) and NO_3^- could occur via (1) biodegradation with NO_3^-, (2) abiotic removal with Fe(II), or (3) abiotic removal with $Fe(III)_{bio\text{-}production}$ [206, 268, 327]. In this study, we aimed to better illuminate the removal processes by carrying out experiments without Fe(II) or NO_3^- to see the role of these compounds in pharmaceutical removal during the biological Fe(III) production step.

Results show that biological production of Fe(III) occurs in the presence of pharmaceuticals, but the removal of pharmaceuticals is inefficient during the production. During the experiments, 0.17 mM $Fe(III)_{bio\text{-}production}$ is produced and 0.14 mM NO_3^- is consumed, a similar ratio of 1:1 to that is observed with acetate instead of pharmaceuticals (Table 6.4). But the biological production of Fe(III) is inhibited by the presence of pharmaceuticals. Both $Fe(III)_{bio\text{-}production}$ production and NO_3^- consumption is much less than the experiments with acetate without pharmaceuticals. Without Fe(II), 0.24 mM NO_3^- is consumed with pharmaceuticals as the electron donor, indicating that there is also a denitrification process consuming pharmaceuticals. No $Fe(III)_{bio\text{-}production}$ is produced in the experimental groups with only NO_3^- or with only Fe(II) (Table 6.4).

TABLE 6.4 Biological production of Fe(III) with NO_3^- coupled to the biodegradation of pharmaceuticals

Experimental group	NO_3^- consumption (mM)	Fe(III) production (mM)
Fe(II) + NO_3^-	0.14	0.17
Fe(II)	n.a. [a]	n.d. [b]
NO_3^-	0.24	n.a.

[a] n.a.= not analysed
[b] n.d. =not detected

The degradation of pharmaceuticals is tested during the biological production of Fe(III) in the presence of Fe(II) and NO_3^-. Results show that the removal of pharmaceuticals is inefficient (Figure 6.3). During the biological Fe(III) production, about 20% diclofenac, 17% ibuprofen and 24% naproxen are removed, and the removal of caffeine, carbamazepine, and metoprolol is less than 10%. A slightly higher removal is seen in the experimental groups with only NO_3^-, e.g. with a removal of 34% for carbamazepine, 40% for diclofenac, 31% for metoprolol, and 26% for naproxen. Removal of pharmaceuticals with only Fe(II) is insignificant (< 15%). The abiotic controls were carried out without inoculum. Results show that the removal of six pharmaceuticals in all abiotic controls is negligible (Figure S6.1, Text S6.2).

Results show that biological Fe(III) production has no significant promoting effects on pharmaceutical removal. Pharmaceutical removal efficiency during biological Fe(III) production is similar, or even less than that with only NO_3^-. In previous studies, the biodegradation of six selected pharmaceuticals is inefficient with NO_3^- [268, 339], similar to the results in this study. In addition, the results indicate that in our experiments Fe(II) is a more reactive electron donor for denitrification than the pharmaceutical compounds tested.

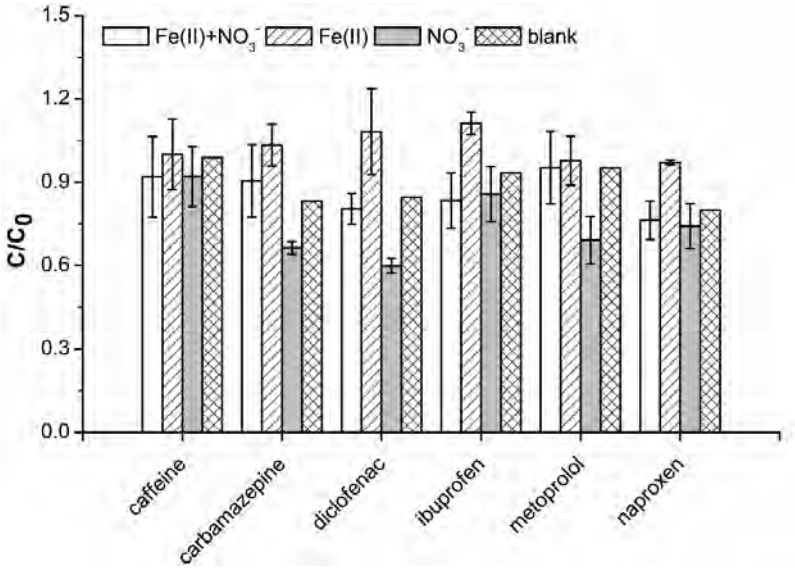


FIGURE 6.3 Biodegradation of pharmaceuticals during the experiments of biological production of Fe(III) within 150 days. Experimental conditions: $[Fe(II)]_0$= 1.2 mM , $[NO_3^-]_0$= 0.4 mM, $[pharmaceutical]_0$=1 mg·L^{-1}, T=30ºC, pH =7, shaking speed 120 rpm. Error bars stand for the standard deviation

Biodegradation of some pharmaceuticals with NO_3^- has been observed previously [13, 268]. However, removal of pharmaceuticals like metoprolol is reported to be inefficient [268, 339]. In our study, the biodegradation of all six pharmaceuticals with NO_3^- is low (< 40%). Therefore, the bacteria appear to preferentially oxidize Fe(II) instead of pharmaceuticals, using NO_3^- as the electron acceptor. In addition, the $Fe(III)_{bio\text{-}production}$ usually attaches on the surface of bacteria [237], which may be of influence on their removal performance in processes.

Previous studies reported that Fe(II) could also remove pharmaceuticals via abiotic processes [206, 327]. In this study, removal of pharmaceuticals with Fe(II) was found to be insignificant. Other authors revealed that removal of antibiotic pharmaceuticals with Fe(II) requires the presence of both Fe(II) and Fe(III) (goethite) [55, 206]. The amorphous $Fe(III)_{bio\text{-}production}$ produced in this process is different from highly crystalline goethite [377]. As a result, Fe(II) appears to have no contribution to the degradation of pharmaceuticals.

In summary, the biological production of Fe(III) from Fe(II) in the presence nitrate and pharmaceuticals can be successfully conducted, but the removal of pharmaceuticals during the process is inefficient and has no additional contribution to the anaerobic Fe(III) mediated pharmaceutical removal. These results also show that nitrate dosage should be controlled to prevent nitrate concentrations in the pharmaceutical degrading process step.

(4) Abiotic removal with Fe(III)

Abiotic removal of pharmaceuticals with Fe(III) compounds is also investigated in this study. Removal by $Fe(III)_{bio\text{-}production}$ is expected via adsorption since it has a large specific surface area [97]. $Fe(III)_{DWTP}$ is usually produced via chemical processes with O_2 (aeration) while biological processes are also observed under conditions where substantial organic matter is present [135, 144]. The production process leads to potentially different Fe(III) compounds and thus different pharmaceutical removal from $Fe(III)_{bio\text{-}production}$. In addition, Fe(III)-based sorbents also have large specific surface area. Similar to $Fe(III)_{bio\text{-}production}$, these sorbents may also be suitable to remove pharmaceuticals via adsorption. Thus, we investigate commercially available $Fe(III)_{FerroSorp®Plus}$, and $Fe(III)_{FerroSorp®RW}$.

The abiotic removal of pharmaceuticals under anaerobic conditions with different Fe(III) are tested. Both granule and ground powder of these Fe(III) are used to select the best materials for the future reactor development. Results show that 31% propranolol is removed within 5 days under anaerobic conditions with $Fe(III)_{DWTP}$ powder (Table 6.5). Furthermore, 20% of propranolol is removed with $Fe(III)_{FerroSorp®RW}$ powder. Like the results with powder, only propranolol is removed for 45% under anaerobic condition with $Fe(III)_{DWTP}$ granule and for 24% using $Fe(III)_{FerroSorp®RW}$ granule. This removal efficiency with the granules is higher than that with the same Fe(III) powder. Adsorption of pharmaceuticals is not observed with $Fe(III)_{DWTP}$ powder and $Fe(III)_{FerroSorp®RW}$ powder during the removal.

TABLE 6.5 Abiotic removal of propranolol under anaerobic conditions with different Fe(III) powder within 5 days. Experimental conditions: $[Fe(III)]_0$=20 mM. $Pharmaceutical]_0$=0.5 $mg \cdot L^{-1}$, phosphate buffer pH=7, T=30 °C [a]

Fe(III) (hydr)oxides	Removal efficiency (%) [b]	Fe(III) (hydr)oxides	Removal efficiency (%) [b]
$Fe(III)_{DWTP}$ granule	43(±2)	$Fe(III)_{FerroSorp®RW}$ granule	25(±2)
$Fe(III)_{DWTP}$ powder	31(±1)	$Fe(III)_{FerroSorp®RW}$ powder	21(±1)

[a] $Fe(III)_{DWTP}$ = Fe(III) (hydr)oxides from drinking water treatment plants, $Fe(III)_{FerroSorp®RW}$ = Fe(III)-based sorbent FerroSorp®RW
[b] The data in the bracket is the difference between the duplicate

No removal is observed with other types of Fe(III) or without Fe(III) (Figure S6.2, S6.3, Table S6.3). Pharmaceutical removal under anaerobic conditions with $Fe(III)_{bio-production}$ was found to be insignificant. Adsorption is not observed at high levels, while is a critical step during the reactive removal [61, 327, 328]: after adsorption onto Fe(III) through molecular complexation at the oxide surface, pharmaceuticals are chemically converted. The previous study shows that phosphate ions can be adsorbed onto $Fe(III)_{bio-production}$ [250], blocking pharmaceuticals to reach the sorption sites. In this study, phosphate is used as a buffer to maintain the pH ~ 7. As a result, there may be few $Fe(III)_{bio-production}$ reactive sites available for pharmaceutical adsorption and subsequent conversion, leading to the insufficient removal of pharmaceuticals.

Morphologies of Fe(III) may lead to the higher removal efficiency of propranolol with $Fe(III)_{DWTP}$ than with $Fe(III)_{FerroSorp®RW}$. The $Fe(III)_{DWTP}$ is more amorphous than $Fe(III)_{FerroSorp®RW}$. However, pharmaceutical removal with amorphous $Fe(III)_{chem-synthesis}$ is also inefficient. In addition, grinding the granule is expected to increase the specific surface area of Fe(III), potentially increasing the adsorption capacity and number of reactive sites per weight of Fe(III) oxide, and also increasing the corresponding abiotic removal. However, this study shows that abiotic pharmaceutical removal with Fe(III) granule is similar, even better than with powder. A detailed investigation is required to understand the abiotic pharmaceutical removal with different types of Fe(III) oxides under anaerobic conditions.

In this study, only anaerobic abiotic removal of propranolol with Fe(III) is observed. Base on the previous study, the pharmaceutical removal mechanisms with Fe(III) is similar to that with Mn(IV) [61]. Therefore, the removal of diclofenac, propranolol, and metoprolol is expected. Since the oxidant power of Fe(III) is weaker than Mn(IV), it is reasonable that pharmaceutical removal with Fe(III) is less efficient than that with Mn(IV), and that a less more extensive range of pharmaceuticals can be removed with Fe(III) than with Mn(IV). As a result, the only removal of propranolol is observed with Fe(III), that appears to have more suitable properties for the anaerobic abiotic removal of pharmaceuticals with Fe(III) than other pharmaceuticals. It is essential in future studies to reveal favourable properties present in the suit of pharmaceuticals to react with Fe(III).

6.4 Conclusion

This study aimed at investigating biological regeneration of manganese(IV) by oxygen and iron (III) by nitrate in support of the anaerobic metal oxide-mediated removal of pharmaceuticals from water.

Results of this study show that the biological production of amorphous Mn(IV) occurs under O_2-limiting conditions, despite the limited growth of the associated bacteria. There is no removal of pharmaceuticals mediated by Mn(II)-oxidizing bacteria. In the abiotic removal of pharmaceuticals with Mn(IV) under anaerobic conditions, ground $Mn(IV)_{DWTP}$ powder can remove pharmaceuticals like metoprolol and propranolol.

Under anaerobic conditions, amorphous $Fe(III)_{bio\text{-}production}$ is formed under nitrate-reducing conditions. The biological production of Fe(III) is obtained with and without pharmaceuticals. However, pharmaceuticals appear to inhibit Fe(III) production. During production of $Fe(III)_{bio\text{-}production}$, the degradation of pharmaceuticals is negligible. Abiotic removal with Fe(III) is only observed with propranolol. Based on the abiotic removal of propranolol under anaerobic conditions, $Fe(III)_{DWTP}$, both granule and ground powder, has the best performance, followed by the $Fe(III)_{FerroSorp®RW}$.

In conclusion, biological regeneration of Mn(IV) and Fe(III) is feasible with dosage of limited amounts of O_2 or nitrate not changing the anaerobic conditions needed for pharmaceutical degradation. $Mn(IV)_{DWTP}$ and $Fe(III)_{DWTP}$ show potential for abiotic anaerobic removal of propranolol. The abiotic anaerobic removal of metoprolol is also achieved with $Mn(IV)_{DWTP}$. These Mn(IV) and Fe(III) oxide species produced from biological, chemical or biological-chemical (like in drinking water treatment) procedures could be used to remove pharmaceuticals under anaerobic conditions. The results of this study indicate that biological processes can be used to produce or regenerate Mn(IV) and Fe(III). The proof of principle is given but scaling up towards a complete recycling of Mn or Fe in continuously operated reactor configurations is the next challenge for future research oriented at further development of this technology.

ACKNOWLEDGEMENT

The work was supported by China Scholarship Council (File No.201308610161) and Wageningen University & Research (WUR). The authors appreciate the help from Laura Piai (WUR), Yvonne Mos (WUR), Peter van der Maas (Water Laboratorium Noord), and Margot Koster (Utrecht University), for the experimental materials and the information. The authors appreciate the support from Hans Beijleveld, Livio Carlucci, Ilse Gerrits, and Vinnie de Wilde for their help with the chemical analyses, and Yiyuan Dong for collecting part of results in experiments on the biological production of Mn(IV) and Fe(III).

Chapter 7

General Discussion: Towards a sustainable technology for removing pharmaceuticals from water using metal oxides

7.1 Introduction

Concerns for the adverse effects of pharmaceutical compounds in water results in a societal demand for control and removal of these compounds. In this dissertation, anaerobic manganese (Mn)- or iron (Fe)-mediated pharmaceutical degradation is proposed as a treatment technology to remove these compounds from water. A series of experiments are designed and setup to study these processes, in which pharmaceuticals are converted via abiotic removal or biological degradation while in most cases, the Mn(IV) or Fe(III) is reduced to Mn(II) or Fe(II). Thereafter, these reduced metal ions can be re-oxidized via biological or chemical processes, and transferred back to the pharmaceutical removal processes for reuse (Figure 1.2). Since the Mn and Fe are abundant in the environment and are important to the transformation pathway of organic compounds, including contaminants, studying the anaerobic Mn- or Fe- mediated pharmaceutical degradation also contributes to understanding the fate of pharmaceuticals in the environments.

This dissertation focuses on the principals and application of anaerobic degradation of pharmaceuticals in Mn(IV)- or Fe(III)-mediated system. Investigations have been carried out on the pharmaceutical removal efficiency, different types of Mn(IV) and Fe(III), environmental and operational conditions, as well as removal mechanisms. Biological regeneration of Mn(IV) and Fe(III) are tested, as well as the potential biodegradation of pharmaceuticals coupled to the regeneration. The outcomes are a step towards translating the results from this study into an affordable, environmentally friendly technology to remove pharmaceuticals during water treatment, and to improve and optimize pharmaceutical removal technologies. This technology can be used in wastewater treatment plants and in drinking water treatment plants (Figure 7.1), preventing the presence and accumulation of pharmaceuticals in the aquatic environment and soil, therefore reducing of potential long-term effects of

pharmaceuticals on human's health [156].

The results of these studies are obtained through batch experiments under ideal conditions which can be different from the environmental and operational conditions in application. To apply in practice, these results need to be critically reviewed, and the influence of the actual situation on the pharmaceutical removal during the water treatment processes need to be taken into account. In this chapter, removal mechanisms and the removal efficiencies are summarized and compared. Finally, the gaps between the lab-scale experimental results and the potential full-scale treatment performance are emphasized, and the future research perspectives to cross these gaps are proposed.

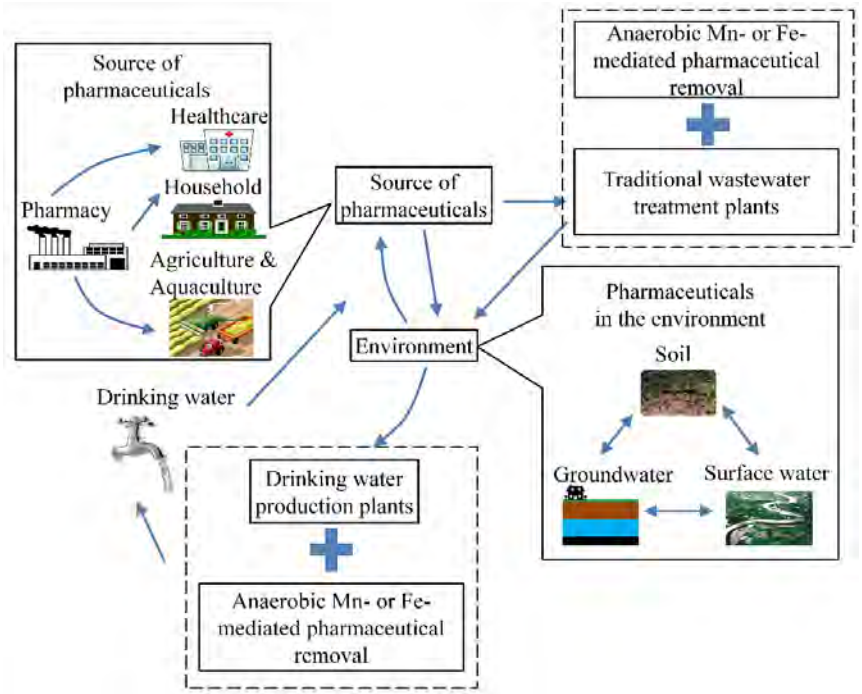


FIGURE 7.1 Source and pathway of pharmaceuticals in the environment controlled by the application of anaerobic Mn- or Fe- mediated pharmaceutical degradation

7.2 Outcomes

7.2.1 Removal mechanisms

The main mechanisms of anaerobic pharmaceutical removal in the Mn(IV)- or Fe(III)-mediated systems include adsorption, chemical oxidation, and biological degradation. Often, these mechanisms act simultaneously in reactors and the natural environment and contribute together to the overall removal of the pharmaceuticals. For example, the anaerobic pharmaceutical removal coupled to dissimilatory Mn(IV) reduction is mainly through biodegradation but adsorption is still an important process in the chain of interactions occurring leading to biodegradation.

(1) Adsorption

Adsorption is an important step during the pharmaceuticals removal in both abiotic removal and biodegradation of pharmaceuticals. In the anaerobic abiotic removal of pharmaceuticals, the adsorption and the chemical oxidation are involved. The compounds are first adsorbed onto the Mn(IV) or Fe(III). Thereafter, these pharmaceuticals are oxidized. During the anaerobic abiotic pharmaceutical removal with Mn(IV), adsorption contributes to over 30% removal of propranolol (Chapter 6).

Adsorption is also observed in anaerobic biodegradation of pharmaceuticals coupled to dissimilatory Mn(IV) reduction. During the process, about 10% removal is observed in the first 10 days in the abiotic controls, resulting from the adsorption (Chapter 3).

(2) Chemical oxidation

Chemical oxidation is the main removal mechanism in anaerobic abiotic pharmaceutical removal with Mn(IV) or Fe(III). In this thesis, diclofenac, metoprolol, and propranolol are efficiently removed by Mn(IV) through chemical oxidation (Chapter 4 – 6), and oxidation of propranolol by Fe(III) is also observed (Chapter 6).

Interestingly, even though the chemical oxidation efficiently removes pharmaceuticals in anaerobic abiotic conditions with Mn(IV) and Fe(III) (Chapter 4 – 6), the abiotic controls in anaerobic biodegradation demonstrate insignificant pharmaceutical removal (Chapter 3). This could be due to the different matrix used in these two experiments (Table 7.1). The matrix used in anaerobic abiotic removal experiments is water or a simple buffer system while the anaerobic biodegradation experiments are performed with synthetic cultivation medium. Results in this thesis show that the presence of co-solutes such as metal ions or low concentration of phosphate could occupy the Mn(IV) surface reactive sites via interactions between negatively charged phosphate ions and positively charged oxide surfaces (Chapter 5). As a result, there are less surface reactive sites available for pharmaceuticals and their removal is inhibited. Similarly, the nutrient and trace elements in synthetic medium and the inhibitors in abiotic controls are more likely to block the surface reactive sites. Therefore, the pharmaceutical removal in abiotic controls in anaerobic biodegradation with Mn(IV) or Fe(III) is insignificant.

Similarly, the microbial inoculum in biodegradation studies could also influence the availability of the reactive sites, by bacterial adhesion using (generally negatively charged) cell surface polymers that adsorb on the positively charged metal oxide surfaces [253, 254]. This could be of effect in both biotic experimental groups and in abiotic controls with sterilized biomass. Based on previous study, the bacteria are expected to attach on the solid Mn(IV) or Fe(III) to transfer the electrons [175]. Therefore, the

pharmaceuticals are not able to directly contact to the Mn(IV), leading to insignificant removal of pharmaceuticals via chemical oxidation in abiotic controls, as well as via adsorption.

(3) Biological degradation

Biological degradation is another important removal mechanism described in this dissertation. The anaerobic biodegradation with Mn(IV) can remove caffeine, naproxen, metoprolol, and propranolol (Chapter 3). The anaerobic biodegradation with Fe(III) has been tested with metoprolol, and is found to be over 50% (Chapter 3). The biodegradation of pharmaceuticals is also tested coupled to the biological production of Mn(IV) and Fe(III). No removal is obtained with Mn(II)-oxidizing bacteria. The pharmaceutical removal efficiency during the biological production of Fe(III) is less than 20% (Chapter 6).

TABLE 7.1 Critical experimental parameters investigated and presented in this dissertation

Conditions	Anaerobic pharmaceutical biodegradation		Anaerobic abiotic pharmaceutical removal	
	Biodegradation with Mn(IV) (Chapter 3)	Biodegradation with Fe(III) (Chapter 3)	Abiotic removal with Mn(IV) (Chapter 4 – 6)	Abiotic removal with Fe(III) (Chapter 6)
T (ºC)	30	35	10 – 30	30
pH	~7	~7	4~9	~7
Redox conditions	Mn(IV)-reducing	Fe(III)-reducing	Anaerobic	Anaerobic
Matrix	Synthetic medium	Synthetic medium	Demineralized water, 10 mM MOPS buffer + 10 mM NaCl	10 mM phosphate buffer + 10 mM NaCl
Initial pharmaceutical ($mg \cdot L^{-1}$)	10	1 – 10	0.25 – 1	0.5
Experimental period (d)	40 – 70	60 – 160	1 – 1.4	5
Other conditions	Dark and static	Dark and static	Dark and static	Dark and static

7.2.2 Comparison of processes

In this thesis research, anaerobic pharmaceutical degradation is studied with Mn(IV) and Fe(III), and abiotic and biotic processes are considered and compared which one holds the most promise for successful application. When reviewing the literature on current Mn- or Fe-related pharmaceutical removal technologies, a framework is proposed and used (Chapter 2). Based on that, the anaerobic pharmaceutical degradation in Mn- or Fe-mediated systems are evaluated in the following aspects:

(1) Process performance

The primary objective of this dissertation research is to develop a mechanistic understanding and assessment on potential technologies to remove the pharmaceuticals from water using metal oxides. Therefore, important criteria are the extent and the rate of removal of different pharmaceuticals. The removal rate is for most experiments calculated for experimental time frames where the pharmaceutical removal is stable. However, in some experiments this is not possible, leading to samples taken at the beginning and at the end of the experiments, leading to rate calculations with a relatively high uncertainty range. Nevertheless these data are useful as first rough estimates.

(2) Condition

In addition to pharmaceutical removal, the environmental and operational conditions, such as pH, temperature, or presence of co-solutes, are found to affect the removal efficiency of pharmaceuticals in different processes, as well as the matrix or cultivation medium. The processes are better if they are effective under conditions which are similar or close to natural conditions, because these processes are required less cost and energy to maintain a special operational conditions.

(3) Sustainability

In the process evaluation and comparison, the sustainability of the pharmaceutical removal processes should be taken into account. In sustainability, the energy consumption of the process, and the effects of the products on environment are important factors, and the newly proposed and current water treatment processes can be compared on these aspects.

(4) Metal oxide types.

Various forms of Mn- or Fe- (hydr)oxides are the main materials consumed during the removal processes studied. The proper type of metal oxide is critical in application to achieve high enough removal efficiency. In addition, the costs and availability of the metal oxides must be considered. In case the Mn or Fe species can be used that are e.g. waste by-products such as Mn or Fe containing sludge produced at drinking water treatment plants. These could be favourable materials to be used in the application.

7.2.2.1 Process performance

The process performance is mainly evaluated by the extent and rate of removal of pharmaceuticals. The results indicated that the anaerobic Mn- or Fe-mediated removal is effective for different pharmaceuticals (Table 7.2). In general, the pharmaceutical removal rate in the anaerobic biodegradation is slower than that for abiotic removal but both of them can achieve removal efficiency over 90% of certain specific pharmaceuticals (Chapter 3 – 6).

The pharmaceutical removal technology can be applied as a tertiary treatment in wastewater treatment processes to protect the surface water and groundwater from pollution by these compounds. Also, it can be used to protect drinking water as a pre-treatment treatment unit. The pharmaceuticals, which are recalcitrant in conventional water treatment plants are the target compounds of pharmaceutical removal units. Based on literature, the seven pharmaceuticals selected in this dissertation can be classified into two groups; poorly removable pharmaceuticals (<45%), and highly removable pharmaceuticals (> 60%) (Table 7.2). Among the poorly

removable pharmaceuticals found in this study, carbamazepine, diclofenac, metoprolol, and propranolol can be listed. These compounds are also generally found to pass standard wastewater treatment plants, and are thus more likely to enter a tertiary pharmaceutical removal unit when placed in water treatment plants. Highly removable pharmaceuticals include caffeine, ibuprofen, and naproxen. Although degradable in WWTP, these compounds are also generally found in WWTP effluent due to their relatively high concentrations in the influent. For example, removal of ibuprofen in wastewater treatment plants (WWTPs) is around 72 – 100%, but the maximum concentration of ibuprofen in WWTP effluent can be 95 $\mu g \cdot L^{-1}$, much higher than other investigated pharmaceuticals in this thesis (Table 1.1). In addition, concentrations of ibuprofen observed in WWTPs effluents are found to be up to 60 times higher than the lowest predicted no effect concentrations (Table 1.1, Table 7.2). Therefore, tertiary treatment well degradable compounds like ibuprofen is also required.

The pharmaceutical removal by using metal oxides as assessed in this work varied greatly based on the pharmaceutical studied and experimental setups. Removal of carbamazepine is not observed in any of the experiments conducted in this study (Chapter 3 – 6). Complete removal of diclofenac at removal rate of 24.3 $mg \cdot L^{-1} \cdot d^{-1}$ is obtained in the anaerobic abiotic removal process using Mn(IV) (Chapter 5), whereas only 20% is removed when biodegradation is coupled to Fe(III) production at a rate of 0.0015 mg pharmaceutical$\cdot L^{-1} \cdot d^{-1}$ (Chapter 6). The fastest removal of metoprolol (0.36 mg pharmaceutical $\cdot L^{-1} \cdot d^{-1}$ achieving 33% removal, Chapter 4 & 6) is obtained in anaerobic abiotic removal with Mn(IV), followed by the anaerobic pharmaceutical biodegradation with Mn(IV) (0.17 mg pharmaceutical $\cdot L^{-1} \cdot d^{-1}$ and 96%, Chapter 3). In the anaerobic pharmaceutical biodegradation with Fe(III), 57% metoprolol is removed at 0.05 $mg \cdot L^{-1} \cdot d^{-1}$ (Chapter 3). In propranolol removal, anaerobic abiotic pharmaceutical removal with Mn(IV) is the most efficient process (51% at 0.53 $mg \cdot L^{-1} \cdot d^{-1}$, Chapter 4 & 6), while the removal rate of anaerobic

pharmaceutical biodegradation with Mn(IV), and the anaerobic abiotic pharmaceutical removal with Fe(III) is the same (0.04 $mg \cdot L^{-1} \cdot d^{-1}$, Chapter 3 & 6). For ibuprofen, only 17% is biologically degraded coupled to Fe(III) production at an extremely slow removal rate, namely 0.60×10^{-3} $mg \cdot L^{-1} \cdot d^{-1}$ (Chapter 6).

7.2.2.2 Condition

In addition to pharmaceutical removal, the conditions are found to affect the removal efficiency of pharmaceuticals, which is very relevant for the future reactor design and operation. The environmental and operational conditions applied in this thesis are quite similar to those in current water treatment technologies (Table 7.1).

The environmental conditions in both anaerobic biodegradation and abiotic removal of pharmaceuticals in Mn- or Fe-mediated systems are the same as natural conditions, namely at 10 – 40 ºC, pH ~7. These conditions are also widely used in wastewater and drinking water treatment processes such as upflow anaerobic sludge blanket reactor, or a slow sand filter [3, 363].

The specific operational conditions vary for different pharmaceutical removal processes. In the anaerobic pharmaceutical biodegradation with Mn(IV) or Fe(III), the operational parameters require further optimization and adaptation. First of all, the concentrations of pharmaceuticals in the studies in this thesis (1 -10 $mg \cdot L^{-1}$, Chapter 3 & 6) are much higher than that in the wastewater ($ng \cdot L^{-1}$ – $\mu g \cdot L^{-1}$). Thus, in the experiments in this study, pharmaceuticals can act as the only carbon and energy source supporting growth of bacteria involved in the biodegradation, giving these a selective pressure benefit. If these biological processes are applicable at lower level of concentrations of pharmaceuticals requires further study. In addition, the time needed for biodegradation of pharmaceuticals as found in our experimental work (40 – 160 days, Chapter 3 & 6) is much longer than the retention time generally considered acceptable for tertiary treatments (maximum 10 – 30 days) [290]. When the influent is stable, longer

retention times for pharmaceutical biodegradation will lead to a larger reactor, and higher cost of construction and operation. Due to the different properties of the pharmaceuticals, the time needed in biodegradation could also be different.

In the anaerobic abiotic pharmaceutical removal with Mn(IV) or Fe(III), the required operational conditions are quite similar to the slow sand filter [3]. For example, the abiotic anaerobic pharmaceutical removal with Mn(IV) usually takes 1 – 1.5 days with the most efficient removal during the first 0.2 – 0.3 days (5 – 7 hours). Generally the retention time of a slow sand filter is 0.2 – 0.5 days (5 – 13 hours). This is long enough to efficiently remove pharmaceuticals using these Mn(IV) and Fe(III) mediated processes. In addition, a typically constructed slow sand filter consists of a layer of fine sand supported by gravel. Those can be replaced by fine Mn(IV) particles supported by Mn(IV) granules, giving higher reative surface areas per unit of volume, thus reducing the filter size are allowing higher loading capacities.

The (liquid) matrix in which the pharmaceuticals are removed is also important. In the anaerobic biodegradation, the matrix is the synthetic medium containing nutrient compounds, trace elements, and vitamin solutions (Chapter 3 & 6). In the abiotic removal, however, the matrix is much simpler, namely demineralized water or phosphate buffer (Chapter 4 – 6). The presence of co-solutes is found to inhibit the anaerobic abiotic pharmaceutical removal. For example, the removal of diclofenac is observed in anaerobic abiotic removal with Mn(IV) but not in the abiotic controls of anaerobic biodegradation with Mn(IV) (Chapter 3 – 5). Thus, the anaerobic biodegradation of pharmaceuticals with Mn(IV) or Fe(III) is more suitable in wastewater treatment effluents because wastewater can supply the nutrient compounds for the bacterial activity. On the other hand, the anaerobic abiotic removal with Mn(IV) or Fe(III) is more suitable in drinking water treatment plants because the matrix is much simpler.

7.2.2.3 Sustainability

In general, the anaerobic pharmaceutical degradation with Mn(IV) or Fe(III) is a potential sustainable pharmaceutical removal technology. Firstly, pharmaceutical removal is achieved under anaerobic conditions, requiring no aeration or other high energy consumption input. For another thing, the main products in pharmaceutical removal, Mn(II) or Fe(II), can be recycled and reused (Chapter 6). This further reduces the Mn(II) or Fe(II) enter the effluent of this technology. In addition, the Mn(IV) or Fe(III) from drinking water treatment plants (DWTPs) can be used as an alternative metal oxide source (Chapter 3 & 6). These Mn or Fe species are –thus far- waste by-products in drinking water production, and which by application in water treatment for removing pharmaceuticals become a valuable product. Therefore, the anaerobic pharmaceutical removal with Mn or Fe can be considered sustainable.

However, the other products from anaerobic Mn(IV)- or Fe(III)-mediated pharmaceutical removal, such as pharmaceutical intermediates, might affect the ecosystem, human health, and the current water treatment chain. Previous studies indicate that some transformation intermediates from pharmaceuticals could be more toxic than their parent compounds [309]. Since the toxicity assessment is not included in this dissertation, it is impossible to determine if the intermediates are more toxic when they are formed in the anaerobic pharmaceutical biodegradation or in anaerobic abiotic pharmaceutical removal.

In the anaerobic biodegradation of pharmaceuticals, the growth of bacteria results in waste biomass. The waste biomass should be treated, for example, via anaerobic digestion. However, the biomass from pharmaceutical removal contains Mn or Fe, both of which will affect the anaerobic digestion. Previous studies show Mn or Fe can promote the production of butyric acid during the fermentation process [173]. The Fe(III) can also affect the removal and recovery of phosphate in anaerobic sludge digestion [37, 288]. Adsorption of phosphate onto MnO_2 has also been reported previously [227, 228]. In addition, the anaerobic biodegradation with Fe(III) is also efficient in nitrogen (ammonia) removal [232, 372].

In drinking water treatment processes, the pharmaceutical removal units are expected before sand filtration. The Mn(II) and Fe(II) generated during the anaerobic pharmaceutical degradation will enter the DWTPs. However, the Mn(II) and Fe(II) are also target compounds for drinking water production [135, 307]. Therefore, anaerobic metal-mediated pharmaceutical degradation will increase the loading of Mn and Fe removal in DWTPs. When anaerobic pharmaceutical biodegradation is applied, the loading in disinfection units in DWTPs will also be increased.

In summary, the anaerobic pharmaceutical degradation with Mn(IV) or Fe(III) is generally a sustainable process but the environmental influence of this pharmaceutical removal technology should be considered in follow-up studies. On one side, the toxic effects of pharmaceutical intermediates produced in this process require further assessment. On the other side, influence of applying this process in the current water treatment chain should be taken into account. Compared to the influence on wastewater treatment processes and in drinking water production, the anaerobic pharmaceutical degradation with Mn or Fe has less negative effects as a post-treatment unit in WWTPs.

TABLE 7.2 Removal rate ($mg \cdot L^{-1} \cdot d^{-1}$) of selected pharmaceuticals via anaerobic biodegradation or anaerobic abiotic removal in this thesis [a]

Pharmaceutical	Degree of removal	PNEC [b] ($\mu g \cdot L^{-1}$)	Removal efficiency reported (%) [c]	Pharmaceutical removal with Mn			Pharmaceutical removal with Fe		
				Biodegradation with Mn(IV) (Chapter 3)	Abiotic removal with Mn(IV) (Chapter 4 – 6)	Biodegradation coupled to Mn(IV) production (Chapter 6) [d]	Biodegradation with Fe(III) (Chapter 3)	Abiotic removal with Fe(III) (Chapter 6)	Biodegradation coupled to Fe(III) production (Chpater 6)
Caffeine	Highly removed	87 [e]	50 – 100	0.05 (26)	n.s. [f]	n.s.	n.t. [g]	n.s.	n.d.
Carbamazepine	Poorly removed	13.8	<62	n.s.	n.s.	n.s.	n.t.	n.s.	n.d.
Diclofenac	Poorly removed	9.7	<81	n.s.	24.3 (100)	n.s.	n.t.	n.s.	0.15×10^{-2} (20)
Ibuprofen	Highly removed	1.65	72 – 100	n.s.	n.s.	n.s.	n.t.	n.s.	0.60×10^{-3} (17)
Metoprolol	Poorly removed	8	3 – 56	0.17 (96)	0.36 (33)	n.s.	0.05 (57)	n.s.	n.d.
Naproxen	Highly removed	2.62	43 – 99	0.13 (52)	n.s.	n.s.	n.t.	n.s.	0.56×10^{-2} (24)
Propranolol	Poorly removed	0.244	<44 [h]	0.04 (31)	0.53 (51)	n.s.	n.t.	0.04 (43)	n.d.

[a] Numbers in the bracket is the maximum removal efficiency of pharmaceutical obtained in this thesis in percentage
[b] PNEC= Predicted No Effect Concentration Data is obtained from previous literature [317]
[c] Removal efficiency of pharmaceuticals in wastewater treatment plants. Data is obtained from previous literature [182, 258]
[d] The experiments were carried out with only Mn(II)-oxidizing bacteria without Mn added. Detailed information can be found in Chapter 6
[e] Data is obtained from previous literature [194]
[f] n.s. = not significant, the removal efficiency in the process is less than 10%
[g] n.t. = not tested
[h] Data is obtained from previous literature [351]

7.2.2.4 Metal oxide types

The Mn and Fe species are important consumable materials during the pharmaceutical removal. In this dissertation, Mn and Fe species from different sources and synthesis methods are tested (Table 7.3). The removal efficiency of pharmaceuticals with different Mn or Fe species in both biological degradation and abiotic removal are summarised in Table 7.4.

The results indicate that amorphous chemically synthesized Mn(IV) is the most suitable material in both anaerobic pharmaceutical biodegradation and anaerobic abiotic pharmaceutical removal (Chapter3 – 6). It can be used for the removal of caffeine, diclofenac, naproxen, metoprolol and propranolol. Also the Mn(IV) from drinking water treatment plants are suitable for removal of metoprolol and propranolol (Chapter 3 & 6). The pharmaceutical removal with Fe(III) is also effective, more pharmaceuticals should be tested (Chapter 3 & 6).

The reduced Mn(II) and Fe(II) species generated during the anaerobic pharmaceutical degradation with Mn(IV) or Fe(III) can be re-oxidized and reused in application (Chapter 6). Therefore, the biogenic regenerated Mn(IV) and Fe(III) oxides are the most suitable input source in stable removal process. In addition, the Mn and Fe removal process in drinking water production can also be regarded as Mn and Fe cycling. Thus, the Mn(IV) and Fe(III) from drinking water treatment plants are also suitable source in pharmaceutical removal.

TABLE 7.3 Mn and Fe species tested in different pharmaceutical removal processes in this thesis

Pharmaceutical removal processes	Mn species	Fe species
Anaerobic pharmaceutical biodegradation with Mn(IV) or Fe(III) (Chapter 3)	• Chemically synthesized Mn(IV) oxides (self-synthesized) • Mn(IV) from drinking water treatment plants	• Chemically synthesized Fe(III) hydroxides (self-synthesized) • Fe(III) from drinking water treatment plants • Fe(III)-citrate (commercial products) • Fe(III)-based sorbents (commercial products)
Anaerobic abiotic removal with Mn(IV) or Fe(III) [a] (Chapter 4 – 6)	• Chemically synthesized Mn(IV) oxides (self-synthesized) • Chemically synthesized MnO_2 (commercial products) • Mn(IV) from drinking water treatment plants	• Chemically synthesized Fe(III) hydroxides (self-synthesized) • biogenic Fe(III) hydroxides • Fe(III) from drinking water treatment plants • Fe(III)-based sorbents (commercial products)
Pharmaceutical biodegradation during biological Mn(IV) or Fe(III) production (Chapter 6)	• Not tested [b]	• $FeCl_2$

[a] In the abiotic removal of pharmaceuticals with Mn(IV), both oxic and anaerobic conditions were tested while in the removal with Fe(III), only anaerobic conditions were tested

[b] The pharmaceutical biodegradation during biological Mn(IV) production was tested with only Mn(II)-oxidizing bacteria without Mn(II). The detailed information can be found in Chapter 6

TABLE 7.4 Removal of pharmaceuticals with different types of Mn or Fe species in this dissertation [a]

Metal types	Morphology	Form used in this thesis	Pharmaceutical removed by Mn or Fe species	Removal efficiency (%)		Source
				Anaerobic pharmaceutical biodegradation	Anaerobic abiotic pharmaceutical removal	
Pharmaceutical removal with Mn species						
Chemically synthesized Mn(IV) oxides (self-synthesized, Chapter 3 – 6)	Amorphous	Solid powder (slurry)	Caffeine, Diclofenac, Metoprolol, Naproxen, Propranolol	26 – 52	33 – 100	• Chemical synthesis
Mn(IV) from drinking water treatment plants (Chapter 3 & 6)	Semi-crystalline	Granule and ground powder	Metoprolol, Propranolol	20 – 100	23 – 44	• Drinking water treatment plants
Chemically synthesized MnO_2 (commercial products, Chapter 4)	Crystalline	Solid powder	Diclofenac	n.t. [c]	21	• Commercial products
Biogenic Mn oxides (Chapter 6)	Amorphous	Solid powder with bacteria	n.a.[b]	n.t.	Not tested	• Biological production
Pharmaceutical removal with Fe species						
Chemically synthesized Fe(III) hydroxides (self-synthesized, Chapter 3 & 6)	Amorphous	Solid powder (slurry)	Metoprolol	57	n.d. [d]	• Natural source, • commercial products
Fe(III)-citrate (Chapter 3)	n.a.	Solution	Metoprolol	52	Not tested	• Commercial products

Continued Table 7.4

Metal types	Morphology	Form used in this thesis	Pharmaceutical removed by Mn or Fe species	Removal efficiency (%)		Source
				Anaerobic pharmaceutical biodegradation	Anaerobic abiotic pharmaceutical removal	
Fe(III) hydroxides from drinking water treatment plants (Chapter 3 & 6)	Semi-crystalline	Granule and ground powder	Propranolol	n.d.	43	• Drinking water treatment plants
Fe(III)-based sorbent, FerroSorp®Plus (Commercial product, Chapter 3 & 6)	Semi-crystalline	Granule and ground powder	Propranolol	n.d.	n.d.	• Commercial products
Fe(III)-based sorbent, FerroSorp®RW (Commercial product, Chapter 3 & 6)	Semi-crystalline	Granule and ground powder	Propranolol	n.d.	25	• Commercial products
Biogenic Fe (Chapter 6)	Amorphous	Solid powder with bacteria	n.a.	Not tested	n.d.	• Biological generation
$FeCl_2$ (Chapter 6)	n.a.	Solution	Diclofenac, Ibuprofen, Naproxen	17 – 24	n.d.	• Commercial products

[a] Highest removal efficiency of the pharmaceuticals under anaerobic or anaerobic conditions obtained in this dissertation is listed in the brackets

[b] n.a. = not applicable

[c] n.t. = not tested

[d] n.s. = not significant, the removal efficiency is less than 10%

7.3 Future perspective

7.3.1 From lab to application

The ultimate objective of this study is to develop anaerobic Mn- or Fe-mediated pharmaceutical degradation into an efficient, cost-effective, environmental friendly technology in water treatment. It is still a long process from the batch studies as performed in this thesis research to the full-scale application in the field. For this development, three steps are proposed.

Step 1: Exploring the limits of Mn- and Fe-mediated pharmaceutical removal

This thesis demonstrates that anaerobic Mn- or Fe-mediated pharmaceutical degradation in water is possible and it is an indication of relevant treatment conditions. To apply this process in practice, more studies are required. First of all, only seven pharmaceuticals are used in this thesis and only some of them are removed. More pharmaceuticals should be tested to further understand the pathway of pharmaceutical degradation, and to determine wider classes of compounds that can be removed. Can the anaerobic Mn- or Fe-mediated pharmaceutical degradation used as function efficient at actual concentrations? This is another important question to answer. Since the pharmaceutical concentration used in this thesis is in the range of $mg \cdot L^{-1}$, which is thousand times higher than that in often observed in waste water effluents ($ng \cdot L^{-1}$ – $\mu g \cdot L^{-1}$), it is essential to test the anaerobic biodegradation and anaerobic abiotic removal of pharmaceuticals at that level through batch experiments.

An advantage of anaerobic Mn- or Fe-mediated pharmaceutical degradation is the cycling and reuse of Mn and Fe species. Therefore, future studies on applying the regenerated Mn(IV) and Fe(III), namely, biologically produced Mn(IV) and Fe(III) is necessary. In this thesis, anaerobic abiotic pharmaceutical removal with these biologically produced

Mn(IV) and Fe(III) has been tested, and further investigation on applying them in anaerobic pharmaceutical biodegradation is needed.

The effects of co-solutes on anaerobic abiotic pharmaceutical removal are described in this thesis. However, they can also affect the anaerobic pharmaceutical biodegradation. For example, ammonia in the wastewater can be used by the bacteria to reduce Fe(III) [162, 267], thus competing with pharmaceuticals. In addition, the organic matter can promote biodegradation of pharmaceuticals via co-metabolism [92], or inhibit by competing with pharmaceuticals as a carbon source. The effects of organic matter on anaerobic Mn- or Fe-mediated pharmaceutical biodegradation are unknown, and worthwhile studying.

Studying the kinetics of both anaerobic biodegradation and anaerobic abiotic removal will contribute to determining the needed hydraulic retention time (HRT) and/or the solid retention time (SRT). The HRT will ensure that the pharmaceuticals are staying at a shortest time in the reactor achieving the most efficient removal. The SRT will ensure the bacteria and oxidants like MnO_2 stay in the reactor long enough to contact with pharmaceuticals and then remove these compounds. Previous studies have shown that both HRT and SRT affect the degradation of pharmaceuticals and other micropollutants in current water treatment technologies [7, 22, 39, 189]. These factors highly affect the design of the treatment reactor.

Results from previous studies have shown that the intermediates from pharmaceutical removal technologies can be more toxic than parent compounds [205]. Therefore, evaluation of the anaerobic Mn- or Fe-mediated pharmaceutical degradation should continue beyond the removal efficiency of pharmaceutical parent compounds, with a focus on the intermediates. Due to the improvement of analytical methods, it is possible to directly identify the possible intermediates of pharmaceuticals in anaerobic degradation. Furthermore, toxicity and risk assessment can be used as a new tool to evaluate the performance of the technology, which is more

comprehensive than the simple intermediates identification. In addition, the toxicity assessment could reveal the "cocktail effects" of the intermediates, which is hard to predict based on the intermediates analysis.

In this first step, most suggested studies are application-orientated. Some studies on mechanism and intermediates are also interesting to know, to better understand the pharmaceutical removal process. The results collected in this step can also be used to design a lab-scale reactor and settle the operational parameters.

Step 2: Simulating the pharmaceutical removal in a lab-scale reactor

Once the potential of Fe- and Mn- mediated pharmaceutical removal have been further explored, these conditions must be translated into process conditions for application. This thesis envisions that the anaerobic Mn- or Fe-mediated pharmaceutical degradation in water contains two units - a pharmaceutical removal unit, and a metal cycling system (also has potential to remove pharmaceuticals) (Figure 7.2). The results obtained from this dissertation and batch experiments in Step 1, together with data from literature, are sufficient for the design of a lab-scale reactor. For example, the lab-scale reactor can be designed by modifying the bed filter reactors based on the experimental results because bed filter has been used previously to remove pharmaceutical compoundss with MnO_2 under aerobic conditions [108, 109]. Similarly, the sand filter can be used as the Mn(II) or Fe(II) oxidation unit (metal cycling system), because this reaction has been used in DWTPs to remove (oxidize) Mn(II) and Fe(II) via both chemical and biological processes [239]. The lab-scale reactor can be used to test if the selected operational parameters are suitable for anaerobic Mn- or Fe-mediated pharmaceutical degradation, such as HRT, SRT, flow rate, etc. In addition, the lab-scale reactor can also be used to simulate and monitor application parameters like loading shock.

Another focus of this step is the start-up and operational strategy of the pharmaceutical removal reactor applying the anaerobic Mn- or Fe-mediated degradation, especially the bioreactor. In this dissertation, it took approximately 3 years to enrich the active culture from anaerobic sediment (Chapter 3). This is an enormous drawback for the application of the anaerobic pharmaceutical biodegradation with Mn(IV) or Fe(III). A wise start-up strategy is therefor to develop a pre-adapted inoculum and this should be studied during this step.

Biological techniques like bioaugmentation are also options in procedures for start-up. Results show that bioaugmentation can successfully improve the biodegradation of pharmaceuticals in wastewater treatment processes [210, 214, 374]. By adding adapted inocula which are efficient in anaerobic pharmaceutical biodegradation with Mn(IV) or Fe(III), it is expected to shorten the adaption period and improve the pharmaceutical removal.

The lab-scale reactor will help accumulate the experience of starting and operating the reactor in application. Combining with batch experiments, the operational parameters will be optimized to achieve the highest pharmaceutical removal efficiency.

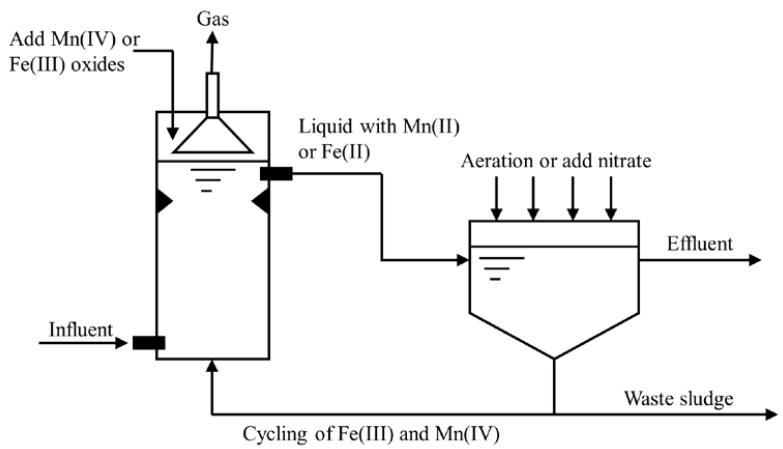


FIGURE 7.2 Proposed water treatment systems applying anaerobic pharmaceutical degradation with Mn(IV) or Fe(III)

Step 3. Translating the pharmaceutical removal to pilot-scale systems before application

The final step before real scale application is to build a pilot-scale reactor to evaluate the performance with real wastewater or groundwater, to gain operation experience, to monitor the long-term operation, and to further optimize the pharmaceutical removal. After that the anaerobic Mn- or Fe-mediated pharmaceutical degradation is ready as a market applicable technology to remove pharmaceuticals from water.

When there are enough data on the anaerobic Mn- or Fe-mediated pharmaceutical degradation, a mathematic model can be developed to describe the adsorption, chemical oxidation, as well as the biological degradation of pharmaceuticals under anaerobic conditions with Mn or Fe. This model can then be used to estimate or predict the performance of the technology in a given situation. In addition, it will promote the understanding of anaerobic degradation of pharmaceuticals with metals.

7.3.2 Other perspective

In addition to research developing the anaerobic Mn- or Fe-mediated pharmaceutical degradation into a technology, there are other research related to the future application.

A simple framework is used to partially evaluate the sustainability of pharmaceutical removal in this thesis. The investigation is missing the environmental influence of the anaerobic pharmaceutical degradation with Mn(IV) or Fe(III), such as the ecotoxicity of the (by-)products from the process. Therefore, toxicity assessment, together with pharmaceutical removal, is a good method to evaluate the process, and can be used as an indicator to compare this technology with other technologies. In addition, methods like life-cycle approach (LCA) could be more suitable. The LCA has been applied in water treatment including pharmaceutical removal technologies [249, 257]. The analysis could provide adequate assessment of

environmental changes related to the anaerobic degradation of pharmaceuticals with metal, qualification the energy consumption and intermediates emission, and their effects on human's health.

Molecular biology, such as Fluorescent *in situ* hybridization, and cloning of 16S rDNA, have often been used in environmental studies. These techniques are used to described the microbial community and biodiversity in the systems, and can be useful when it is incorporated into design and operation of water treatment processes [266]. In the anaerobic biological degradation of pharmaceuticals with Mn(IV) and Fe(III), molecular biological methods can be used to describe the microbial community in the process, contributing to better understanding which bacteria degrade the pharmaceuticals, and which genes and enzymes are involved. The structure of the microbial community, together with other conventional operational information, can also be used as an indicator to assess the stability of the system, and to diagnose the problems in operation.

In summary, the anaerobic Mn- or Fe-mediated biological degradation and abiotic removal processes can remove pharmaceuticals. The abiotic removal of pharmaceuticals is effective to remove compounds like diclofenac, metoprolol and propranolol. The anaerobic biodegradation can also remove pharmaceuticals including caffeine, naproxen, metoprolol and propranolol. The long incubation time for activating metal oxide using biomass calls for the further optimization and investigation on this promising pharmaceutical removal process. In general, the anaerobic Mn- or Fe-mediated pharmaceutical degradation process offers great potential for developing a sustainable and affordable technology to remove specific pharmaceuticals from water. With further fundamental and application-oriented research, the anaerobic pharmaceutical degradation with Mn or Fe will become an attractive technology in both WWTPs and DWTPs to remove pharmaceuticals from water. The process will contribute to the safe reuse of wastewater effluents for sustaining ecosystems and cleaner and safer water cycle.

Supplementary materials

Supplementary materials for Chapter 1

Introduction: Pharmaceuticals in the environment and pharmaceutical removal technology

TABLE S1.1 Abbreviation of countries

Abbreviation	Country	Abbreviation	Country
BRA	Brazil	JOR	Jordan
CAN	Canada	KOR	Republic of Korea
CHN	China	SGP	Singapore
CRI	Costa Rica	GBR	United Kingdom
HRV	Croatia	USA	United States
IND	India	VNM	Vietnam
JPN	Japan	SER	Yugoslavia

Supplementary materials for Chapter 3

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

TEXT S3.1 Adaption of the inoculum

The inoculums used in this study was the mixture anaerobic sediment of effluent channel at different wastewater treatment plants (WWTPs) in the Netherlands, including WWTP Bennekom, WWTP Ede, and WWTP Driebergen. The content of organic matter in the raw mixture is around 11 mg per kg dry matter. Before the inoculum showing the capacity of degrading metoprolol biologically with Mn(IV), it has been cultivated in the presence of 10 $mg \cdot L^{-1}$ metoprolol and 15 mM chemically produced Mn (IV) over 800 days.

TEXT S3.2 Theoretical calculation of Mn(IV) and Fe(III) reduction during anaerobic pharmaceutical biodegradation

The Mn(IV) and Fe(III) reduction during the anaerobic pharmaceutical biodegradation is calculated based on the balance of electron transfer. During the calculation, the O_2 is used as a bridge. The total electrons provided by complete mineralization of pharmaceuticals is calculated. Then the same amount of electrons should be accepted by Mn(IV) or Fe(III) reduction. However, the electron transferred during the pharmaceutical oxidation with Mn(IV) or Fe(III) is difficult to estimate. The calculation of pharmaceutical mineralization with O_2 is estimated based on mass balance (Table S3.3).

Table S3.1 Properties of different types of Mn(IV) and Fe(III) involved in this study

Types of (hydr)oxides [a]	Preparation process	Specification of source	Morphologies	Original form	Metal content [b]
$Mn(IV)_{chem\text{-}synthesis}$	Chemical synthesis	MnO_2 prepared based on previous studies [172].	Amorphous	Powder (slurry)	n.a. [c]
$Mn(IV)_{DWTP}$	Chemical synthesis and/or biological process	Mn(IV) oxides from drinking water production plants 'Noordbargeres' in Emmen (Waterleiding Maatschappij Drenthe, the Netherlands), provided by WLN	Unknown	Granule	10.8 $mgMn \cdot gDM^{-1}$
Fe(III)-citrate	Chemical synthesis	Commercial products from Sigma-Alrich	Not applicable	Solution	n.a.
$Fe(III)_{chem\text{-}synthesis}$	Chemical synthesis	$Fe(OH)_3$ prepared based on a previous study [153]	Amorphous	Powder (slurry)	n.a.
$Fe(III)_{DWTP}$	Chemical synthesis and/or biological process	A mixture of Fe(III) (hydr)oxides from several drinking water treatment plants, provided by Evides Waterbedrijf (the Netherlands)	Semi-crystalline	Granule	109.6 $mgFe \cdot gDM^{-1}$
$Fe(III)_{FerroSorp®Plus}$	Chemical synthesis	FeO(OH) based sorbent, FerroSorp®Plus, commercial product from HeGo Biotec	Semi-crystalline	Granule	441.2 $mgFe \cdot gDM^{-1}$
$Fe(III)_{FerroSorp®RW}$	Chemical synthesis	FeO(OH) based sorbent, FerroSorp®RW, commercial product from HeGo Biotec	Semi-crystalline	Granule	119.5 $mgFe \cdot gDM^{-1}$

[a] $Mn(IV)_{chem\text{-}synthesis}$=Chemically synthesized Mn(IV) oxides, $Mn(IV)_{DWTP}$=Mn(IV) oxides from drinking water treatment plants, $Fe(III)_{chem\text{-}synthesis}$=Chemically synthesized Fe(III) hydroxides, $Fe(III)_{DWTP}$=Fe(III) hydroxides from drinking water treatment plants, $Fe(III_{)FerroSorp®Plus}$ = Fe(III)-based sorbent, FerroSorp®Plus, $_{FerroSorp®Plus}$ = Fe(III)-based sorbent, FerroSorp®Plus

[b] DM=Dry Matter

[c] n.a.= not analysed

TABLE S3.2 Medium recipe for anaerobic biodegradation of pharmaceuticals with Mn(IV) or Fe(III)

Compounds	Final concentration ($\mu g \cdot L^{-1}$)	Compounds	Final concentration ($\mu g \cdot L^{-1}$)
$AlK(SO_4)_2$	0.1	NaCl	10
Biotin (Vitamin H)	20	$NaH_2PO_4 \cdot 2H_2O$	3.75 mM
$CaCl_2 \cdot 2H_2O$	0.75mM	$NaHCO_3$	30 mM
$CoSO_4$	1	NH_4Cl	28.0 mM
$CuSO_4 \cdot 5H_2O$	0.1	$NiCl_2 \cdot 6H_2O$	24
Cyanocobalamine (vitamin B12)	1	Nicotinamide	50
$FeSO_4 \cdot 7H_2O$	1	NTA	15
Folic acid (dihydrate)	20	p-Aminobenzoic acid (Na – salt)	50
H_3BO_3	0.1	Pantothenate (Ca – salt)	55
KCl	1.34mM	Pyridoxine (vitamin B6)	100
Lipoic acid (thioctic acid)	50	Riboflavine (vitamin B2)	50
$MnSO_4 \cdot 2H_2O$	5	Thiamine HCl (vitamin B1)	50
Na_2MoO_4	25	$ZnSO_4$	1
$Na_2S \cdot 9H_2O$	1 mM	Yeast extract	0.02% (w/w)

TABLE S3.3 Theoretical calculation of Mn(IV) and Fe(III) reduction during anaerobic pharmaceutical degradation (mol/mol pharmaceutical)

Pharmaceutical	Proposed reaction	Total electron transfer	Mn(IV) reduction	Fe(III) reduction
Caffeine ($C_8H_{10}N_4O_2$)	$C_8H_{10}N_4O_2 + 9.5O_2 \rightarrow 8CO_2 + 5H_2O + 2N_2$	38	19	38
Carbamazepine ($C_{15}H_{12}N_2O$)	$C_{15}H_{12}N_2O + 17.5O_2 \rightarrow 15CO_2 + 6H_2O + N_2$	70	35	70
Ibuprofen ($C_{13}H_{18}O_2$)	$C_{13}H_{18}O_2 + 16.2O_2 \rightarrow 13CO_2 + 9H_2O$	66	33	66
Metoprolol ($C_{15}H_{25}NO_3$)	$C_{15}H_{25}NO_3 + 21.25O_2 \rightarrow 15CO_2 + 12.5H_2O + 0.5N_2$	85	42.5	85
Naproxen ($C_{14}H_{14}O_3$)	$C_{14}H_{14}O_3 + 16O_2 \rightarrow 14CO_2 + 7H_2O$	64	32	64
Propranolol ($C_{16}H_{21}NO_2$)	$C_{16}H_{21}NO_2 + 20.25O_2 \rightarrow 16CO_2 + 10.5H_2O + 0.5N_2$	81	40.5	81
Citrate ($C_6H_8O_7$)	$C_6H_8O_7 + 4.5O_2 \rightarrow 12CO_2 + 8H_2O$	18	9	18

FIGURE S3.1 Image of (a) granule and powder of Mn(IV) from drinking water treatment plants; (b) ground powder of Fe(III) from drinking water treatment plants, Fe(III)-based sorbent, FerroSorp®Plus, and Fe(III)-based sorbent, FerroSorp®RW (from left to right)

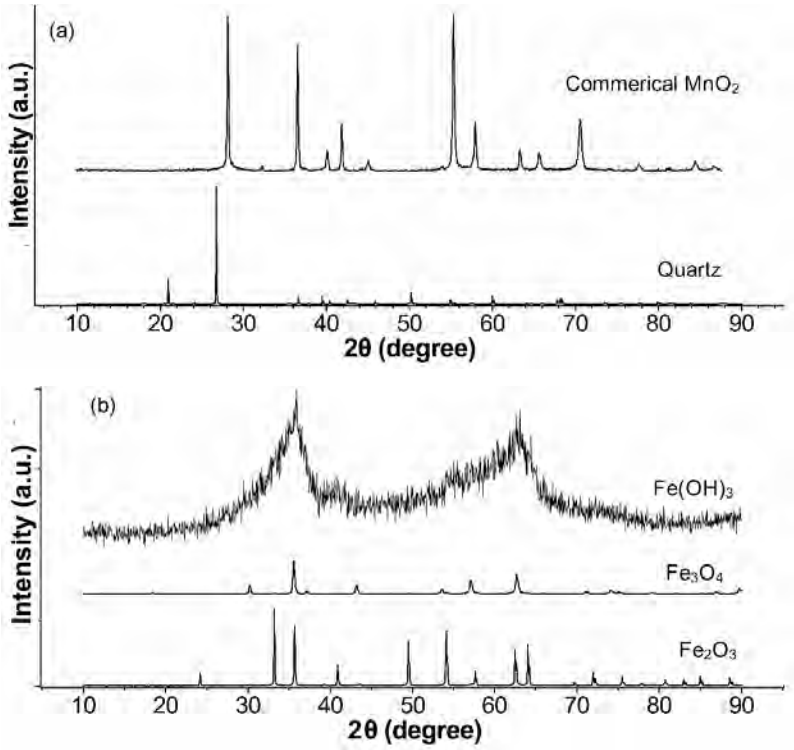


FIGURE S3.2 X-ray diffraction spectrum of reference compounds (a) chemically produced MnO_2 (commercial products) and quartz (from RRUFF database R060604); (b) $Fe(OH)_3$, Fe_3O_4, and Fe_2O_3

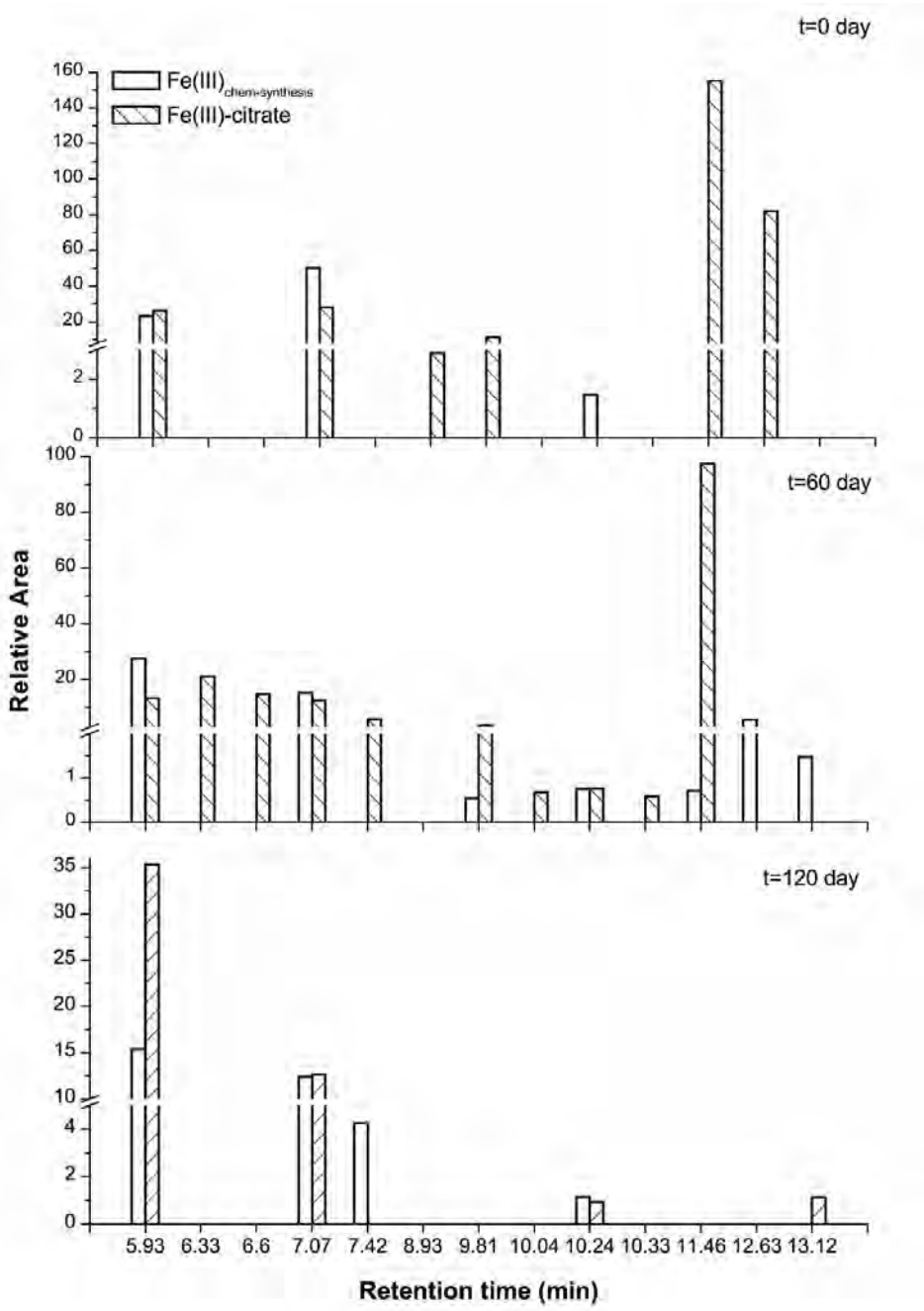


FIGURE S3.3 Presence of potential intermediates from anaerobic metoprolol biodegradation with $Fe(III)_{chem\text{-}synthesis}$ and Fe(III)-citrate at t= 0 day, t= 60 day, t= 120 day in chromatography. The relative area is the ratio between the area of the potential intermediates and the area of internal standard (fenoprofen)

Supplementary materials for Chapter 4

Anaerobic conditions promote abiotic diclofenac removal with manganese oxides

TEXT S4.1 Solution preparation

(1) Anaerobic water

The anaerobic water was prepared by boiling the demineralised water for 5 minutes. Then the boiled water was transferred to a glass bottle and bubbled with N_2 until it had cooled down to room temperature. The water was then sealed and stored at room temperature.

(2) Pharmaceutical stock

The pharmaceutical stock was included pharmaceutical mixture stock and diclofenac stock. The pharmaceutical mixture stock was prepared by dissolving 20 mg of every seven pharmaceuticals with 1 L ultrapure water. The final concentration of each pharmaceutical in pharmaceutical mixture stock is about 20mg·L^{-1}. The diclofenac stock was prepared by dissolving 125 mg diclofenac with 50 mL ultrapure water. The final diclofenac concentration in diclofenac stock is about 2500 mg·L^{-1}.

(3) Reaction solution

The reaction solution contained 7 mM MnO_2, 50 mM buffer to maintain constant pH at 7, and an appropriate amount of NaCl to maintain constant ionic strength at 0.1 M. The buffer is H_3PO_4/NaH_2PO_4 for pH ~ 4.5, NaH_2PO_4/K_2HPO_4 for pH~7.0, and K_2HPO_4 for pH ~8.5. The reaction solution was prepared with both normal demineralised water and anaerobic water.

TEXT S4.2 MnO_2 generation

Both 0.4 mM $MnCl_2$ and 0.4 mM $KMnO_4$ were prepared with demineralised water. While stirring the $KMnO_4$ solution continuously, equal volume of $MnCl_2$ was added. Then, NaOH (1N) was added into the mixture to bring the pH to 10. The MnO_2 solid then centrifuged at 5000 rpm for 15 min and then resuspended by anaerobic water. The centrifigation and suspended in anaerobic water were repeated for six times. Suspension of MnO_2 in anaerobic water were stored at 4ºC for the addition to batch experiment.

TEXT S4.3 Analysis

(1) Pharmaceutical analysis

1 mL samples were collected and centrifuged at 10000 rpm for 10 min. The supernatant then transferred to amber vials. 50 µL of internal standard (5mg L^{-1} fenoprofen) was added into the sample. The samples were stored at -20 ˚C before analysis.

The pharmaceutical analysis was performed as described [95] by a ultra-performance liquid chromatography (UPLC, ultimate 3000, Thermo, USA) with a diode array detector, and a CSH phenyl-Hexyl column (1.7µm, 130 Å, 2.1 × 150 mm). A mixture of water with 0.1% formic acid (solution A) and acetonitrile with 0.1% formic acid (solution B) was used as the mobile phase. The analysis started with 100% solution A for 0.5 min. Then it decreased to 20% at 13 min. After staying for 3 min, it went back to 100% at 17 min and stopped at 22.4 min. The flow rate is 0.3mL/min while the oven is at 40 ℃. The injection volume of samples was 10 µl. The pharmaceutical concentration was calculated based on relative area (area of pharmaceutical/area of internal standard) and the slope of the calibration curve.

(2) Mn(II) analysis

The Mn(II) generated during the removal processes was determined by an inductively coupled plasma spectrometer with optical emission spectroscopy (ICP-OES, Vista MPX Simultaneous, Varian Inc. (Part A), USA). Controls with only MnO_2 and buffers but without pharmaceuticals showed no Mn^{2+} produced.

(3) MnO_2 morphologies analysis

The two kinds of MnO_2 were characterized by X-ray diffraction (XRD, D2 PHASER, Bruker, Germany). The results showed in Figure S1. In addition, microscopes were also used to characterized the MnO_2 morphologies (Figure S4.2).

Fournier-Transform Infra-Red spectrometers

The MnO_2 solid with diclofenac or metoprolol as described in previously (section 4.2.3) were collected by centrifugation (5000rpm, 15 min) after 24h under both oxic and anaerobic conditions in deminerized water. Control sample, bare MnO_2 without pharmaceuticals, was also collected based on the same method. The samples were freeze-dried before analysis. All the samples were analyzed by a Fournier-Transform Infra-Red spectrometers (Bruker TENSOR 27). The reference spectrum of untreated diclofenac and metoprolol standard was also acquired in the same analytical condition.

TEXT S4.4 Intermediates

In the chromatography spectrum, the peaks at different retention time (RT) are representative different intermediates of diclofenac. The diclofenac is observed at RT=14.64 min. Due to the practical limits, it is impossible for us to identify the formula or structure of intermediates in diclofenac removal with MnO_2. However, the chromatography clearly shows that the intermediates formed under aerobic and anaerobic conditions are different. This may indicate that oxygen contributes to forming the intermediates.

TEXT S4.5 Size analysis

Size analysis was performed by a laser size analyzer (Mastersizer 2000, Malven, UK). The size of MnO_2 particles keep increasing during the analysis process. Therefore, it is impossible to compare the size between amorphous MnO_2 and crystalline MnO_2. Generally, the amorphous MnO_2 is smaller than crystalline MnO_2 (Figure S4.2).

TEXT S4.6 FTIR analysis

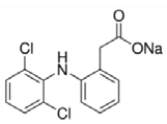
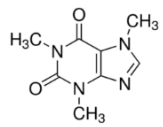
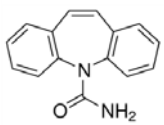
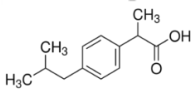
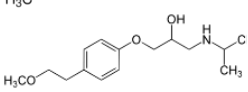
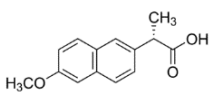
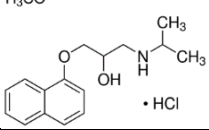
The bare MnO_2 under oxic and anaerobic conditions are the same, therefore, we only use the FTIR spectra of oxic MnO_2 in Figure S4.6.The FTIR spectra show the MnO_2 changed before and after reacting with diclofenac. The spectra of bare MnO_2 is similar to previous study [32]. The broad peak at 3380 cm^{-1} was assigned to the stretching vibration of -OH group in water and Mn-O-H. The peaks at 1634 cm^{-1} and 1056 cm^{-1} were assigned to the bending vibrations of the -OH in Mn-OH. These peaks are also the indications of the reactive sites on MnO_2 surface. The peak at 428 cm^{-1} was assigned to the bending vibration of Mn-O [32, 278].

Due to the extremely huge amount of MnO_2 added into the system comparing to diclofenac, the spectra of MnO_2 after reacting are still similar to the bare MnO_2. However, there are still some peaks appeared between 800 – 2000 cm^{-1} showing the MnO_2 is changed. Three new peaks at 1379 cm^{-1} , 1017 cm^{-1} and 828 cm^{-1} were not attributed to the pure diclofenac either (Figure S4.6 (c)). Therefore, they are probably from the intermediates. The peak at 1379 cm $^{-1}$ is assigned to alkynes, aromatics or C=O, the peak at 1017 cm-1 is assigned to C-O (2-bands) and the peak at 828 cm^{-1} is assigned to N-H wagging [278].

In addition, the peak shift and peak change were also observed. The peak at 1632 cm^{-1} decreased after reacting with diclofenac under aerobic condition while it increased under anaerobic conditions. The higher peak probably indicates that the reactive sites are activated and more available for diclofenac. Another peak shift is observed from 1272 cm^{-1} to 1287 cm^{-1}. The peak also larger. That peak is assigned to alkynes or NO_2.

All these peaks showed that under anaerobic conditions, the MnO_2 changed more than that under aerobic conditions. The change may be caused by the intermediates. The change of MnO_2 under anaerobic conditions can be the reason why anaerobic conditions promote diclofenac removal.

TABLE S4.1 Structure and properties of selected pharmaceuticals in this study

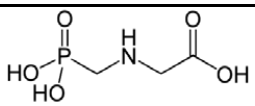
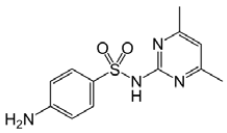
Pharmaceutical [a]	CAS No.	Chemical Structure	pK_a	$LogK_{ow}$
diclofenac (DFC)	15307-79-6		4.15	4.57
Caffeine (CAF)	58-08-2		10.4 (at 40°C)	-0.07
Carbamazepine (CBZ)	298-46-4		13.9	2.45
Ibuprofen (IBP)	15687-27-1		4.91	3.97
Metoprolol (MET)	56392-17-7		9.5	1.88
Naproxen (NAP)	22204-53-1		4.15	3.18
Propranolol (PROP)	318-98-9		9.42	3.48

[a] Text in the brackets is the abbreviation of each pharmaceutical

TABLE S4.2 Pharmaceutical concentration (C/C_0 at t= 24 h) in absence of MnO_2 in demineralised water. Experimental conditions: $[pharmaceutical]_0$=1mg·L^{-1}, pH ~8.5

Compounds	Aerobic conditions	Anaerobic conditions
caffeine	1.02	1.02
carbamazepine	1.01	1.01
diclofenac	1.00	1.09
ibuprofen	1.03	1.04
metoprolol	1.01	1.01
naproxen	1.02	1.02
propranolol	1.01	1.02

TABLE S4.3 Structure and properties of glyphosate and sulfamethazine

Compound	CAS No.	Chemical Structure	pK_a	$LogK_{ow}$
glyphosate	1071-83-6		0.8 (1st phosphonic)	-2.8
sulfamethazine	57-68-1		7.59	0.89

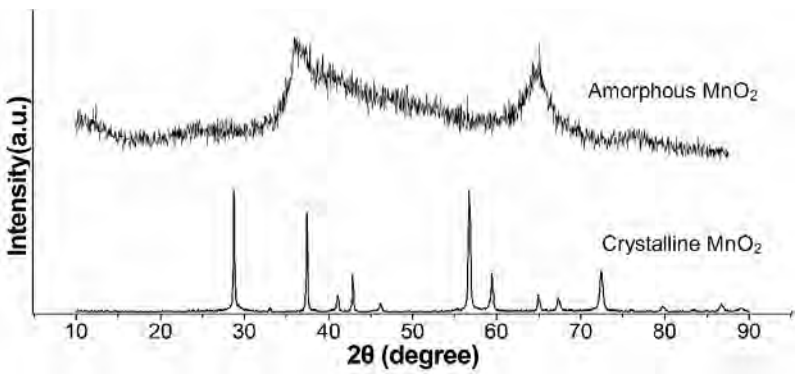


FIGURE S4.1 X-ray diffraction spectrum of amorphous MnO_2 and crystalline MnO_2

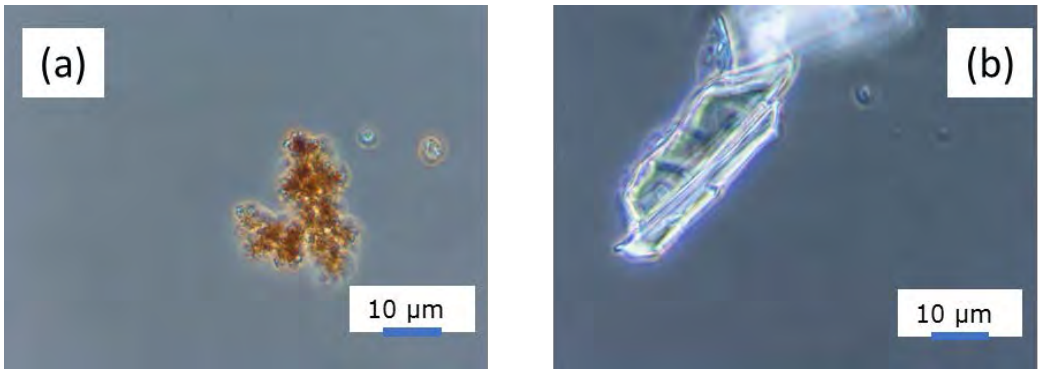


FIGURE S4.2 Microscopy of (a) amorphous MnO_2 and (b) crystalline MnO_2 at 100 times magnified

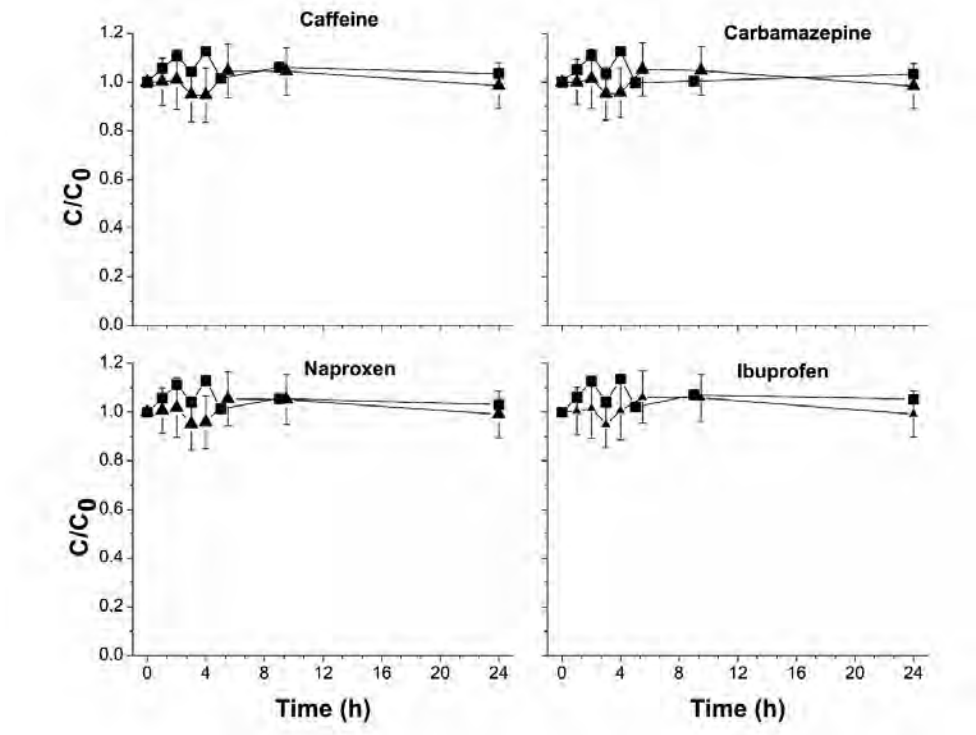


FIGURE S4.3 Pharmaceutical removal with MnO_2 in demineralised water with pharmaceutical mixture under aerobic conditions (■) and anaerobic conditions (▲). Experimental conditions: $[MnO_2]_0$=7 mM, $[pharmaceutical]_0$=1mg·L^{-1}, pH ~8.5. Error bars are standard deviations

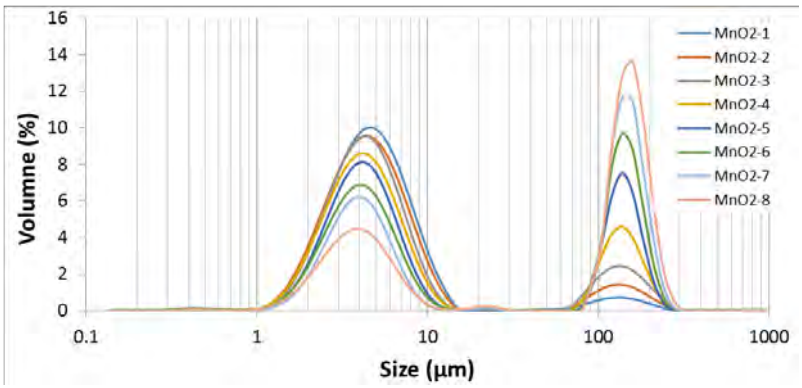


FIGURE S4.5 Size analysis of amorphous MnO_2 (eight times analysis in one single run, 40 sec/sample)

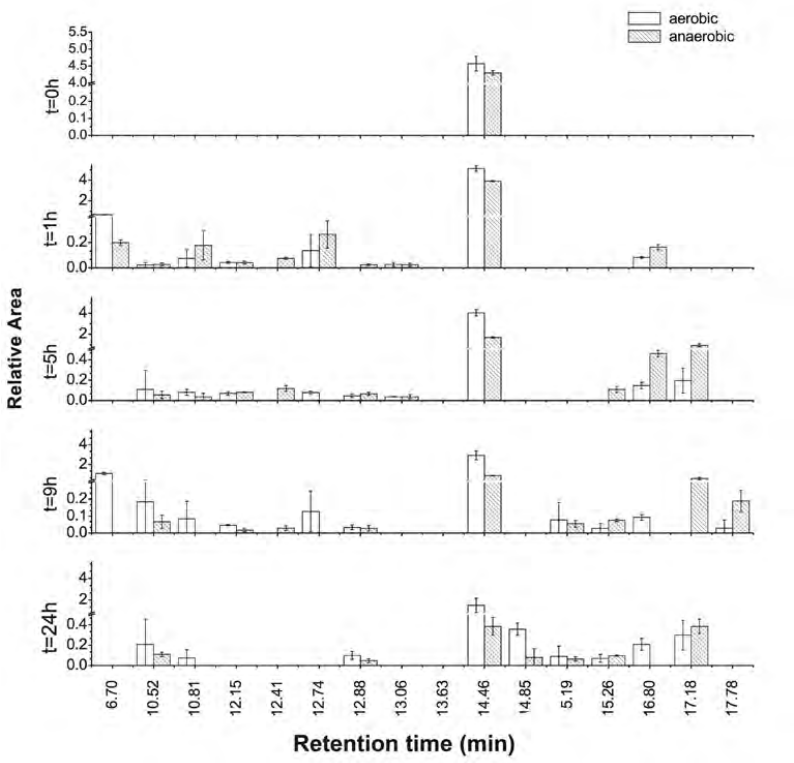


FIGURE S4.4 Presence and variation of intermediates from pharmaceuticals under aerobic and anaerobic conditions at different reaction time point showed in chromatography spectrum in demineralised water system. The relative area was the ratio between the area of the potential intermediates and the area of internal standard (fenoprofen). Error bars are standard deviations

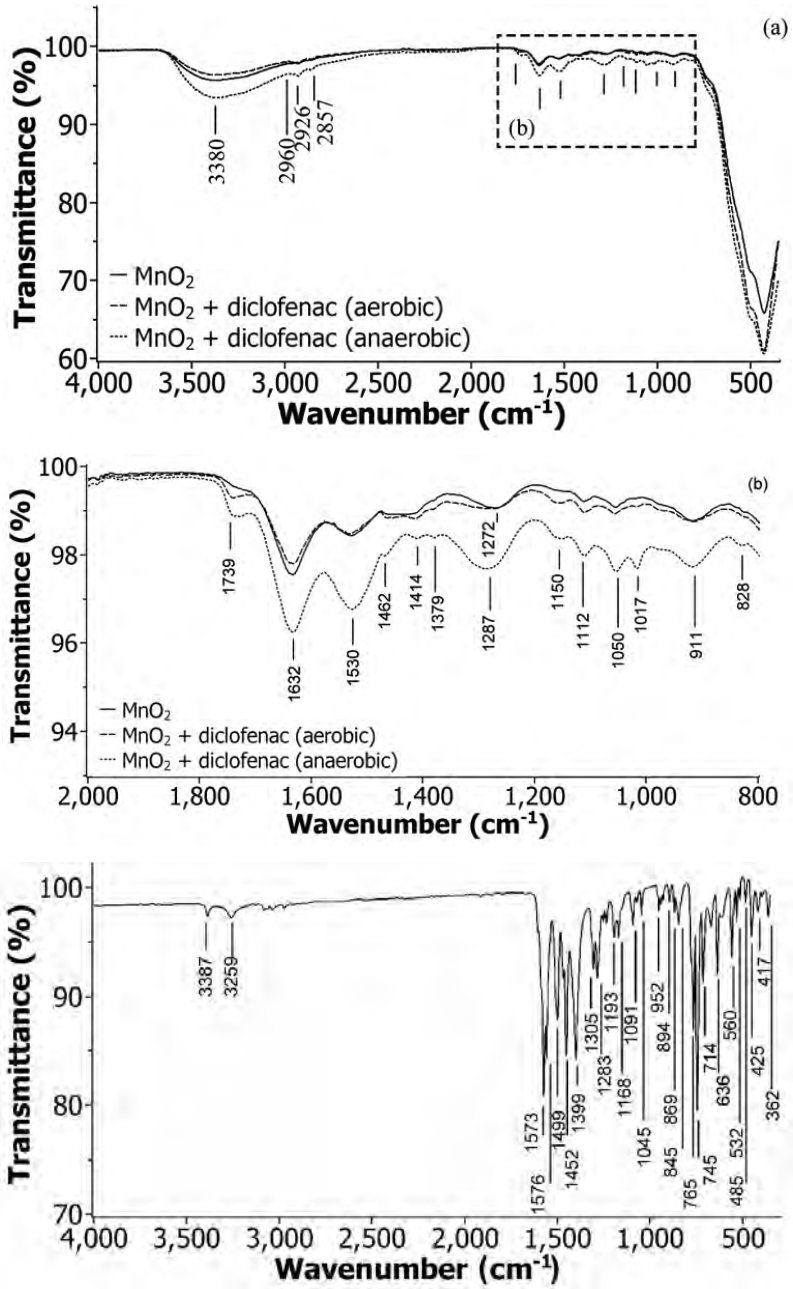


FIGURE S4.6 FTIR spectra of MnO_2 before (solid line) and after reacting with diclofenac under aerobic condition (dash line) and anaerobic condition (dot line) at (a) 350 – 4000 cm^{-1}, and (b) 800 – 2000 cm^{-1}. The pure diclofenac (c) was diclofenac sodium, used as a reference

Supplementary Material for Chapter 5

Application of manganese oxides under anaerobic conditions to remove diclofenac from water

TEXT S5.1 Kinetic model selection

Four kinetics models were used to fit the DFC removal data at 20ºC. Since the DFC removal is an obvious two-stage removal, we used both the all the data and the data from the first 9 hours to fit the pseudo-first-order model. Based on the corresponding R^2 values in Figure S1 , pseudo-first-order model with first 9 hour data, pseudo-second-order model, and mechanism-based model fitted the data similarly in general. The removal kinetics was fitted slightly better to the mechanism-based model than the other two models. All the data in the experiments were used to fit the mechanism-based model (Table S5.1). However, the plateau concentration (C_e) was not obtained in all experiments, thus will lead to inconsistent transformation trends of DFC removal. Since the other three models have similar performance, the simplest model was selected to fit all the data under different experimental conditions in this paper, which is the pseudo-first-order model with first 9 hour data.

TEXT S5.2 Results of blank experiments

The experiments without MnO_2 were carried out as the blank experiments. The results showed that no DFC was removed without MnO_2 (Table S5.2). To investigate the effects of humic acids on DFC removal under anaerobic conditions with MnO_2, the experiments without MnO_2 as controls were carried out at the lowest and highest HA concentration in this study, that is, 5mg·L^{-1} and 20 mg·L^{-1}. The results show that no DFC is removed after 33 hours (Figure S5.2).

TABLE S5.1 Summary of the observed rate constant k_{obs} simulated by a mechanism-based model ($C_t = \frac{S-C_0}{\frac{S}{C_0}e^{k_{obs}(S-C_0)t}-1}$) by Zhang, Chen and Huang [359] under different experimental conditions (pH=7, I=0.01M)

T(˚C)	Matrix	$[MnO_2]_0$ (mM)	$[DFC]_0$ (μM)	$[DFC]_e$[a]	S[b]	k	R^2
10	10 mM MOPS buffer	7	3.14	0.10	0.56	0.46 ± 0.03	0.98
20	10 mM MOPS buffer	7	3.14	0.11	0.77	0.56 ± 0.11	0.99
30	10 mM MOPS buffer	7	3.14	0.08	0.74	0.72 ± 0.05	0.98
40	10 mM MOPS buffer	7	3.14	0.59	0.36	0.14 ± 0.03	0.97
20	10 mM MOPS buffer	1.5	3.14	0.52	0.56	0.18 ± 0.06	0.98
20	10 mM MOPS buffer	3	3.14	0.38	0.71	0.20 ± 0.03	0.99
20	10 mM MOPS buffer	6	3.14	0.20	0.77	0.33 ± 0.06	0.98
20	10 mM MOPS buffer	7	0.79	0.05	0.20	1.40 ± 0.44	0.99
20	10 mM MOPS buffer	7	1.57	0.11	0.44	0.70 ± 0.12	0.99
30	0.1 mM $MnCl_2$ + 10 mM MOPS buffer	7	3.14	0.18	0.75	0.41 ± 0.03	0.99
30	0.1 mM $CaCl_2$ + 10 mM MOPS buffer	7	3.14	0.20	0.93	0.73 ± 0.05	0.94
30	0.1 mM $MgCl_2$ + 10 mM MOPS buffer	7	3.14	0.22	0.94	0.45 ± 0.26	0.94
30	0.1 mM $FeCl_3$ + 10 mM MOPS buffer	7	3.14	0.17	0.89	0.38 ± 0.04	0.97
30	1 mM $MnCl_2$ + 10 mM MOPS buffer	7	3.14	0.35	0.54	0.21 ± 0.06	0.98
30	1 mM $CaCl_2$ + 10 mM MOPS buffer	7	3.14	0.25	0.70	0.50 ± 0.03	0.95
30	1 mM $MgCl_2$ + 10 mM MOPS buffer	7	3.14	0.14	0.51	0.72 ± 0.18	0.94
30	1 mM $FeCl_3$ + 10 mM MOPS buffer	7	3.14	0.13	0.84	0.44 ± 0.06	0.99
30	5 mg·L^{-1} HA + 10 mM MOPS	7	5.57	n.a.[c]	n.a.	n.a.	n.a.
30	10 mg·L^{-1} HA + 10 mM MOPS	7	5.57	n.a.	n.a.	n.a.	n.a.
30	15 mg·L^{-1} HA + 10 mM MOPS	7	5.57	n.a.	n.a.	n.a.	n.a.
30	20 mg·L^{-1} HA + 10 mM MOPS	7	5.57	n.a.	n.a.	n.a.	n.a.

[a] $[DFC]_e$ is the plateau concentration of DFC in the experiments
[b] S=$[DFC]_0$ - $[DFC]_e$
[c] n.a.= not applicable

TABLE S5.2 DFC removal under anaerobic conditions without MnO_2 under different experimental conditions after 33 hours

T(˚C)	Matrix	$[MnO_2]_0$ (mM)	$[DFC]_0$ (μM)	C/C_0[a]	Standard deviation (%)
10	10 mM MOPS	7	3.14	0.98	5.9
20	10 mM MOPS	7	3.14	0.94	7.2
30	10 mM MOPS	7	3.14	1.05	1.4
40	10 mM MOPS	7	3.14	1.02	1.9
20	10 mM MOPS	1.5	3.14	1.13	9.2
20	10 mM MOPS	3	3.14	1.08	5.2
20	10 mM MOPS	6	3.14	1.08	9.6
20	10 mM MOPS	7	3.14	0.94	7.2
20	10 mM MOPS	7	0.79	1.06	13.8
20	10 mM MOPS	7	1.57	1.01	3.4
30	10 mM MOPS, no metal ions	7	3.14	1.05	1.4
30	0.1 mM $MnCl_2$ + 10 mM MOPS	7	3.14	1.10	1.4
30	0.1 mM $CaCl_2$ + 10 mM MOPS	7	3.14	1.00	3.8
30	0.1 mM $MgCl_2$ + 10 mM MOPS	7	3.14	0.98	3.6
30	0.1 mM $FeCl_3$ + 10 mM MOPS	7	3.14	0.99	3.6
30	1 mM $MnCl_2$ + 10 mM MOPS	7	3.14	1.05	1.7
30	1 mM $CaCl_2$ + 10 mM MOPS	7	3.14	0.98	2.2
30	1 mM $MgCl_2$ + 10 mM MOPS	7	3.14	1.02	2.8
30	1 mM $FeCl_3$ + 10 mM MOPS	7	3.14	0.95	4.1
30	10 mM MOPS, no HA	7	3.14	1.02	9.7
30	5 $mg \cdot L^{-1}$ HA +10 mM MOPS	7	5.57	1.00	4.0
30	20 $mg \cdot L^{-1}$ HA +10 mM MOPS	7	5.57	1.02	1.2

[a] C is the average DFC concentration of triplicate samples at t= 33 h in the experiment, C_0 is the average DFC concentration of triplicate samples at t=0 h

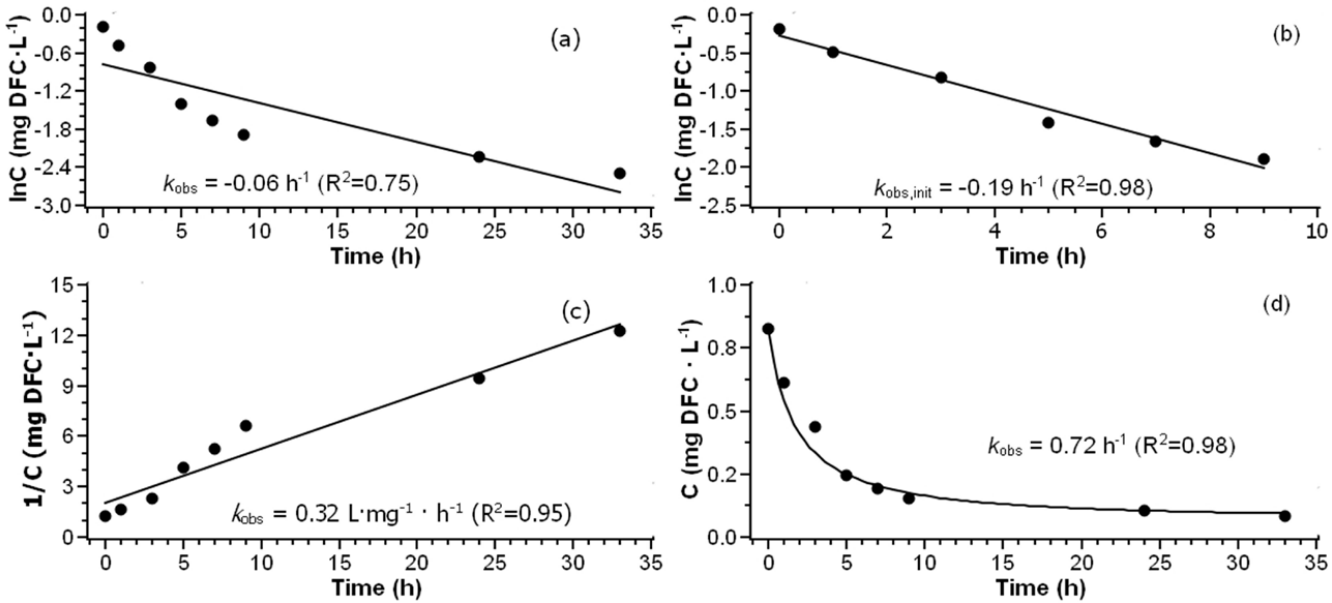


FIGURE S5.1 Fitting DFC removal under anaerobic condition at 20ºC to (a) the pseudo-first-order model; (b) the pseudo-first-order model with data from first 9 hours; (c) the pseudo-second-order model; and (d) the mechanism-based model. Reaction condition: $[DFC]_{0=}3.14\mu M$, $[MnO_2]_0=7$ mM, pH=7, I=0.01M

TEXT S5.3 Change of MnO_2 particles

Based on the microscopies, it is clear that the MnO_2 particle size at 20ºC is smaller than that at 40 ºC (Figure S5.3 (a) – (d)). In addition, when the MnO_2 particles was settled at 20ºC for a longer time (6 months in this case), the MnO_2 particle size was also increased (Figure S5.3(e), (f)). The settlement ability test also supported this indirectly (Figure S5.4). However, we failed to analyze the change of MnO_2 particles due to the practical limits as mentioned previously [172].

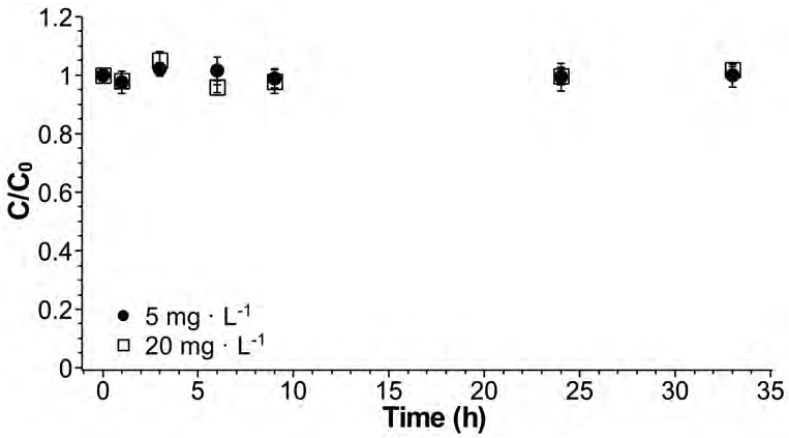


FIGURE S5.2 DFC removal without MnO_2 under anaerobic conditions at different HA concentrations. Experimental conditions: $[MnO_2]_0$= 7 mM; $[DFC]_0$= 5.57 μM, 30℃, pH~7, *I*=0.01 M. Error bars are standard deviations

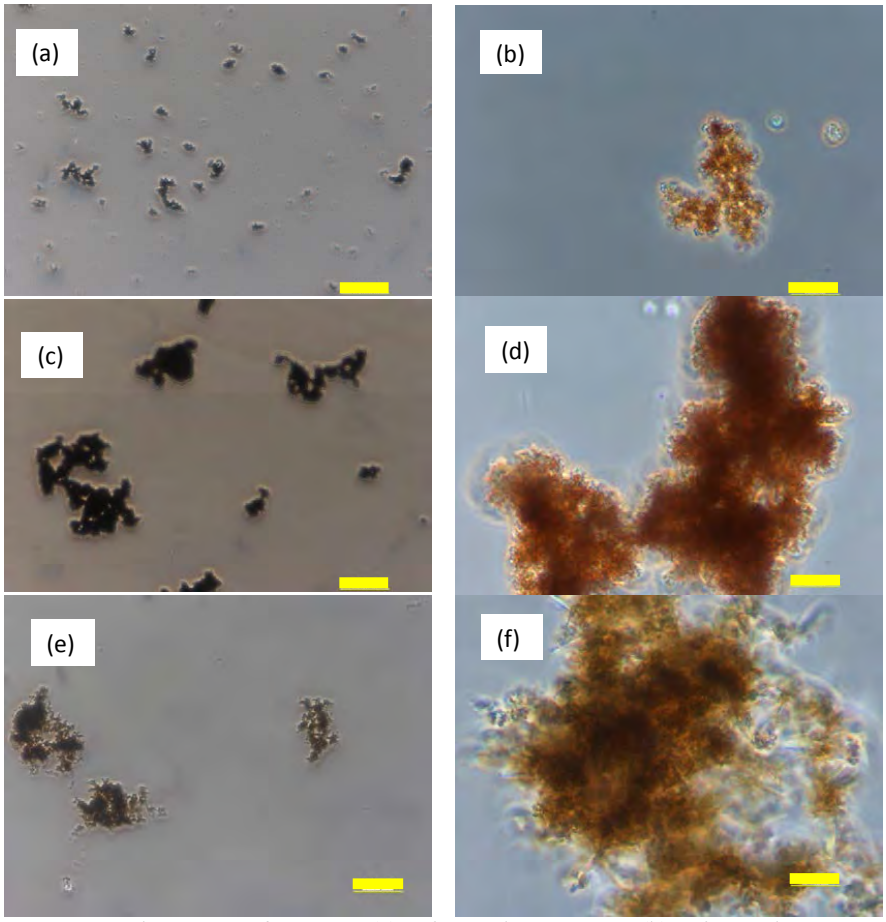


FIGURE S5.3 Microscopy of MnO_2 at (a) 20˚C, 33 hours, × 10 ; (b) 20˚C, 33 hours, × 100; (c) 40˚C, 33 hours, × 10; (b) 40˚C, 33 hours, × 100, (e) 20˚C, 6 months, × 10, (f) 20˚C, 6 months, × 100. Experimental condition: $[DFC]_0$ =3.14µM, $[MnO_2]_0$=7 mM, pH=7, *I*=0.01M. The yellow bar -represents 100 µm length in (a), (c) and (e) and 10 µm in (b), (d) and (f)

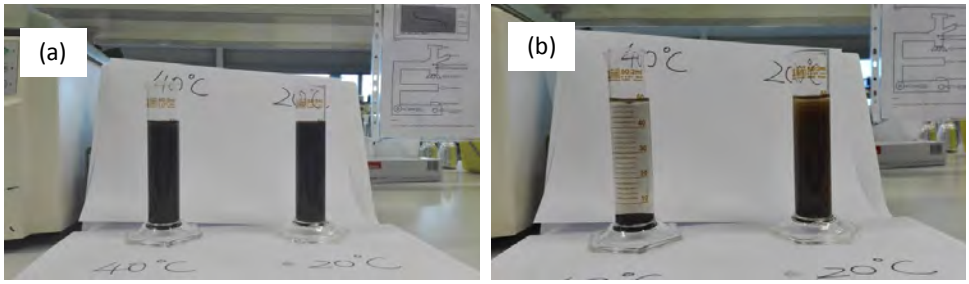


FIGURE S5.4. Settlement ability of MnO_2 treated after 33 hours at different temperatures at (a) t=0min; (b) t=30min

Supplementary materials for Chapter 6

Biological regeneration of manganese (IV) using oxygen and iron (III) using nitrate for anaerobic metal oxide mediated removal of pharmaceuticals from water

Text S6.1 Calculation of total O_2 amount

The total O_2 amount includes the O_2 in the headspace and that dissolved in the medium. The amount O_2 in the headspace is calculated according to the ideal gas law (Equation S6.1).

$$pV = nRT \quad \text{S6.1}$$

in which

p is the partial pressure of the gas (O_2).

V is the volume of the O_2, 70 mL in this experiment

n is the amount of oxygen in mole

R is gas constant, 8.314 $m^3 \cdot Pa \cdot mol^{-1} \cdot K^{-1}$

T is the absolute temperature, 303.15 K

The in O_2 dissolved is calculated according to Henry's Law (Equation S6.2).

$$p = K_H^{pc} c_{aq} \quad \text{S6.2}$$

In which

p is the partial pressure of O_2

K_H^{pc} is the Henry's Law constant. To simply the calculation, the value is O_2 in water at 30ºC, 1.18×10^{-3} $mol \cdot L^{-1} \cdot atm^{-1}$

c_{aq} is the molar concentration of dissolved O_2

TEXT S6.2 Abiotic control results

The abiotic controls show that the degradation of pharmaceutical during the anaerobic biological production of Fe(III) is insignificant without bacteria. The recovery rate of solid phase extraction (SPE) is 85 – 115%, so when the removal efficiency is less than 20%, it is hard to conclude if that is removal or that is caused by the SPE and detection deviation. In addition, propranolol was also included in the experiments. However, the recovery with the current SPE methods is poor and various from 10 – 30%. Therefore, the data is not shown here.

TABLE S6.1 Medium recipe for bioregeneration of Fe(III)

Compounds	Final concentration ($g \cdot L^{-1}$)	Compounds	Final concentration ($g \cdot L^{-1}$)
$FeCl_2$	0.11	NaHCO3	2.5
KCl	0.10	NH_4Cl	0.15
KNO_3	0.02	Vitamin	1 $mL \cdot L^{-1}$
Trace element	1 $mL \cdot L^{-1}$		
Trace element solution			
$CaCl_2.2H_2O$	1.00	$Na_2MoO_4.2H_2O$	0.10
$CoSO_4.7H_2O$	1.80	Na_2SeO_3	0.002
$CuSO_4.5H_2O$	0.10	$Na_2WO_4.2H_2O$	0.004
$FeSO_4.5H_2O$	0.87	NaCl	10.00
H_3BO_3	0.10	$NiCl_2.6H_2O$	0.30
$KAl(SO_4)_2.12H_2O$	0.20	Nitrilotriacetic acid	15.00
$MgSO_4.5H_2O$	25.61	$ZnSO_4.5H_2O$	1.57
$MnSO_4.5H_2O$	5.00		
Vitamin solution			
Biotin	0.02	Nicotinic acid	0.05
Cyanocobalamine (vitamin B12)	0.001	p-Aminobenzoic acid (Na – salt)	0.05
Pantothenate (Ca – salt)	0.05	Pyridocine-HCl	0.10
Folic acid	0.02	Riboflavine (vitamin B2)	0.05
Lipoic acid (thioctic acid)	0.05	Thiamine HCl (vitamin B1)	0.05

TABLE S6.2 Biodegradation of pharmaceuticals (C/C_0) with Mn(II)-oxidizing bacteria

Pharmaceutical	Biodegradation	Pharmaceutical	Biodegradation
Caffeine	1.05	Metoprolol	1.14
Carbamazepine	1.06	Naproxen	0.96
Diclofenac	0.95	Propranolol	1.12
Ibuprofen	0.90		

TABLE S6.3 Abiotic removal of pharmaceuticals (C/C_0) under anaerobic conditions in the blank groups

Pharmaceutical	Removal	Pharmaceutical	Removal
Caffeine	1.00	Metoprolol	1.00
Carbamazepine	1.00	Naproxen	1.00
Diclofenac	1.00	Propranolol	0.95
Ibuprofen	1.00		

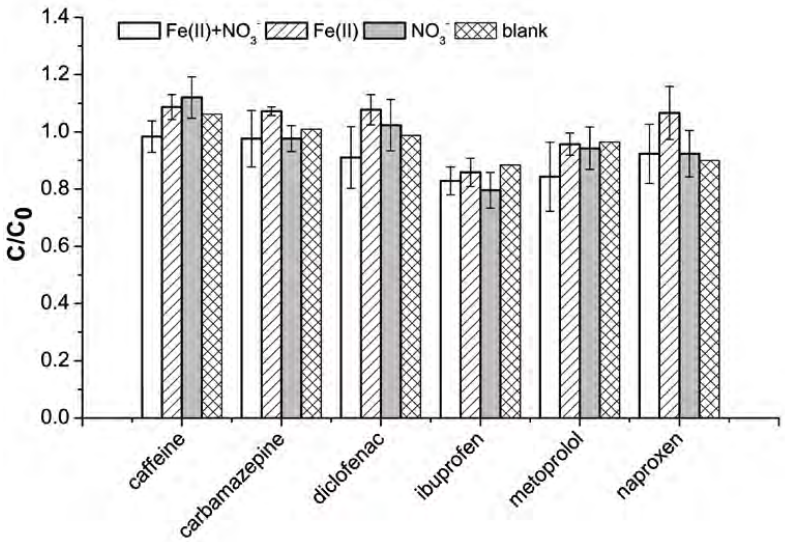


FIGURE S6.1 Degradation of pharmaceuticals in the abiotic groups during the biological Fe(III) production within 150 days. Experimental conditions: $[Fe(II)]_0$= 1.2 mM , $[NO_3^-]_0$= 0.2 mM, $[pharmaceutical]_0$=1 $mg \cdot L^{-1}$, T=30ºC, pH =7, shaking speed 120 rpm. Error bar stands for the standard deviation

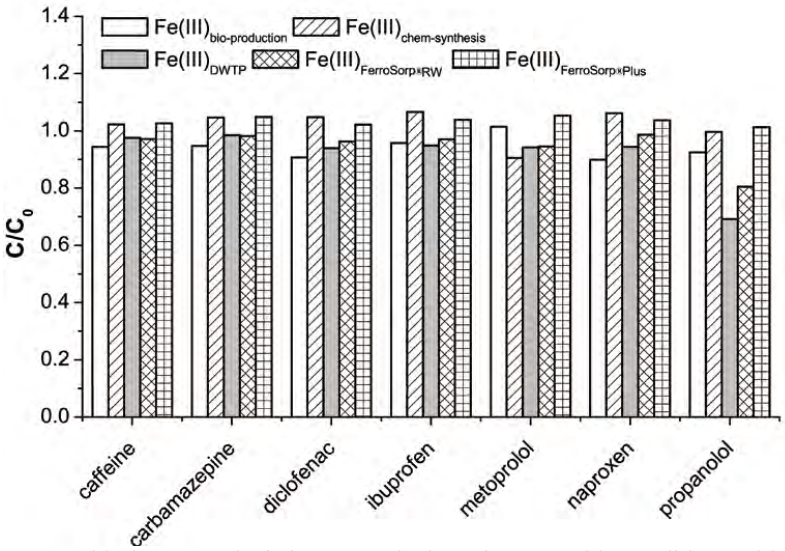


FIGURE S6.2 Abiotic removal of pharmaceuticals under anaerobic conditions with different Fe(III) powder within 5 days. $Fe(III)_{bio\text{-}production}$ = biologically produced Fe(III), $Fe(III)_{chem\text{-}synthesis}$ = chemically synthesized Fe(III), $Fe(III)_{DWTP}$ = Fe(III) from drinking water treatment plant, $Fe(III)_{FerroSorp®RW}$ = Fe(III)-based sorbent, FerroSorp®RW , $Fe(III)_{FerroSorp®Plus}$ = Fe(III)-based sorbent, FerroSorp®Plus. Experimental conditions: $[Fe(III)]_0$=20 mM. Pharmaceutical$]_0$=0.5 mg·L-1, phosphate buffer pH=7, T=30 °C

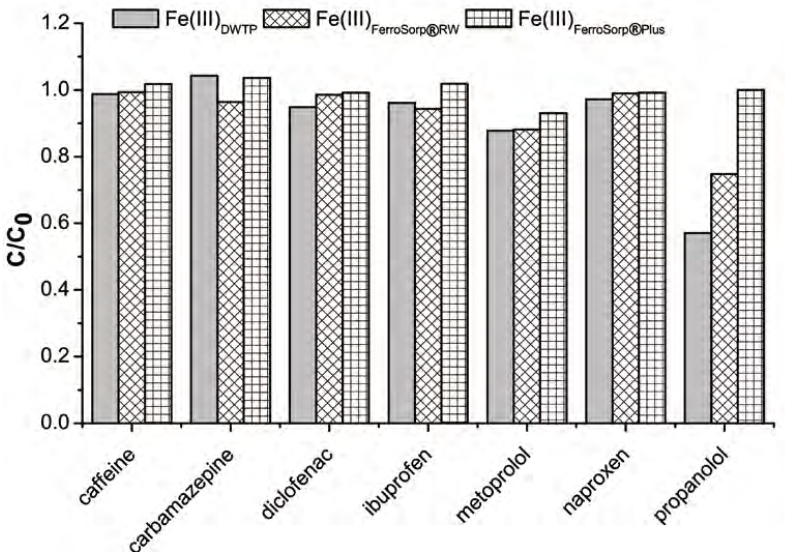


FIGURE S6.3 Abiotic removal of pharmaceuticals under anaerobic conditions with different Fe(III) granule within 5 days. $Fe(III)_{DWTP}$ = Fe(III) from drinking water treatment plant, $Fe(III)_{FerroSorp®RW}$ = Fe(III)-based sorbent, FerroSorp®RW , $Fe(III)_{FerroSorp®Plus}$ = Fe(III)-based sorbent, FerroSorp®Plus. Experimental conditions: $[Fe(III)]_0$=20 mM. Pharmaceutical$]_0$=0.5 $mg \cdot L^{-1}$, phosphate buffer pH=7, T=30 °C

Bibliography

1. Ahmed, M. M.; Chiron, S., Solar photo-Fenton like using persulphate for carbamazepine removal from domestic wastewater. *Water Research* **2014,** *48*, 229-236.

2. Aitken, M.; Kleinrock, M. *Global medicines use in 2020: Outlook and implications*; IMS Insitution for Health Informatics: 2015.

3. American Water Works Association, *Water Treatment (Fourth Edition)* American Water Works Association: USA, 2011.

4. Anderson, R. T.; Rooney-Varga, J. N.; Gaw, C. V.; Lovley, D. R., Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum contaminated aquifers. *Environmental Science & Technology* **1998,** *32*, (9), 1222-1229.

5. Andreozzi, R.; Campanella, L.; Fraysse, B.; Garric, J.; Gonnella, A.; Lo Giudice, R.; Marotta, R.; Pinto, G.; Pollio, A., Effects of advanced oxidation processes (AOPs) on the toxicity of a mixture of pharmaceuticals. *Water Science and Technology* **2004,** *50*, (5), 23-28.

6. Archer, E.; Petrie, B.; Kasprzyk-Hordern, B.; Wolfaardt, G. M., The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere* **2017,** *174*, 437-446.

7. Auvinen, H.; Gebhardt, W.; Linnemann, V.; Du Laing, G.; Rousseau, D. P. L., Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk. *Water Science and Technology* **2017**.

8. Babuponnusami, A.; Muthukumar, K., Advanced oxidation of phenol: A comparison between Fenton, electro-Fenton, sono-electro-Fenton and photo-electro-Fenton processes. *Chemical Engineering Journal* **2012,** *183*, 1-9.

9. Babuponnusami, A.; Muthukumar, K., A review on Fenton and improvements to the Fenton process for wastewater treatment. *Journal of Environmental Chemical Engineering* **2014,** *2*, (1), 557-572.

10. Badawy, M. I.; Wahaab, R. A.; El-Kalliny, A. S., Fenton-biological treatment processes for the removal of some pharmaceuticals from industrial wastewater. *Journal of Hazardous Materials* **2009,** *167*, (1-3), 567-574.

11. Bae, S.; Song, S. U.; Kim, D.; Lee, W., Oxidative degradation of pharmaceutical by Fenton-like reaction using pyrite and H_2O_2. *Abstracts of Papers of the American Chemical Society* **2012,** *244*.

12. Bao, X. L.; Qiang, Z. M.; Ling, W. C.; Chang, J. H., Sonohydrothermal synthesis of MFe_2O_4 magnetic nanoparticles for adsorptive removal of tetracyclines from water. *Separation and Purification Technology* **2013,** *117*, 104-110.

13. Barbieri, M.; Carrera, J.; Sanchez-Vila, X.; Ayora, C.; Cama, J.; Kock-Schulmeyer, M.; de Alda, M. L.; Barcelo, D.; Brunet, J. T.; Garcia, M. H., Microcosm experiments to control anaerobic redox conditions when studying the fate of organic micropollutants in aquifer material. *Journal of Contaminant Hydrology* **2011,** *126*, (3-4), 330-345.

14. Barrett, K. A.; McBride, M. B., Oxidative degradation of glyphosate and aminomethylphosphonate by manganese oxide. *Environmental Science & Technology* **2005,** *39*, (23), 9223-9228.

15. Bautitz, I. R.; Velosa, A. C.; Nogueira, R. F. P., Zero valent iron mediated degradation of the pharmaceutical diazepam. *Chemosphere* **2012,** *88*, (6), 688-692.

16. Bellusci, M.; La Barbera, A.; Padella, F.; Mancuso, M.; Pasquo, A.; Grollino, M. G.; Leter, G.; Nardi, E.; Cremisini, C.; Giardullo, P.; Pacchierotti, F., Biodistribution and acute toxicity of a nanofluid containing manganese iron oxide nanoparticles produced by a mechanochemical process. *International Journal of Nanomedicine* **2014,** *9*, 1919-1929.

17. Benitez, F. J.; Acero, J. L.; Real, F. J.; Roldan, G., Ozonation of pharmaceutical compounds: Rate constants and elimination in various water matrices. *Chemosphere* **2009,** *77*, (1), 53-59.

18. Benjamin, M. M.; Lawler, D. F., *Water Quality Engineering: Physical / Chemical Treatment Processes*. John Wiley & Sons, Inc: Hoboken, NJ, 2013.

19. Bernard, S.; Chazal, P.; Mazet, M., Removal of organic compounds by adsorption on pyrolusite (β-MnO_2). *Water Research* **1997,** *31*, (5), 1216-1222.

20. Besse, J. P.; Garric, J., Human pharmaceuticals in surface waters: Implementation of a prioritization methodology and application to the French situation. *Toxicology Letters* **2008,** *176*, (2), 104-123.

21. Betancur-Corredor, B.; Soltan, J.; Penuela, G. A., Mineralization of ibuprofen and humic acid through catalytic ozonation. *Ozone-Science & Engineering* **2016,** *38*, (3), 203-210.

22. Boonnorat, J.; Techkarnjanaruk, S.; Honda, R.; Prachanurak, P., Effects of hydraulic retention time and carbon to nitrogen ratio on micro-pollutant biodegradation in membrane bioreactor for leachate treatment. *Bioresource Technology* **2016,** *219*, (Supplement C), 53-63.

23. Bos, R., Spoelwaterbehandeling in Emmen. *H_2O* **1996,** *29*, (21), 624-628.

24. Brambilla, G.; Martelli, A., Update on genotoxicity and carcinogenicity testing of 472 marketed pharmaceuticals. *Mutation Research-Reviews* **2009,** *681*, (2-3), 209-229.

25. Bratby, J., *Coagulation and flocculation in water and wastewater treatment*. IWA Publishing: London, 2006.

26. Brausch, J. M.; Connors, K. A.; Brooks, B. W.; Rand, G. M., Human pharmaceuticals in the aquatic environment: A review of recent toxicological studies and considerations for toxicity testing. *Reviews of Environmental Contamination and Toxicology, Vol 218* **2012,** *218*, 1-99.

27. Brillas, E.; Sires, I.; Oturan, M. A., Electro-Fenton process and related electrochemical technologies based on Fenton's reaction chemistry. *Chemical Reviews* **2009,** *109*, (12), 6570-6631.

28. Budd, G. C.; Hess, A. F.; Shorney-Darby, H.; Neemann, J. J.; Spencer, C. M.; Bellamy, J. D.; Hargette, P. H. C. F. p. d. F., Coagulation applications for new treatment goals. *Journal (American Water Works Association)* **2004,** *96*, (2), 102-113.

29. Cao, L. T. T.; Kodera, H.; Abe, K.; Imachi, H.; Aoi, Y.; Kindaichi, T.; Ozaki, N.; Ohashi, A., Biological oxidation of Mn(II) coupled with nitrification for removal and recovery of minor metals by downflow hanging sponge reactor. *Water Research* **2015,** *68*, 545-553.

30. Castro, M. J. L.; Ojeda, C.; Cirelli, A. F., Advances in surfactants for agrochemicals. *Environmental Chemistry Letters* **2014,** *12*, (1), 85-95.

31. Chelliapan, S.; Sallis, P. J., Removal of organic compound from pharmaceutical wastewater using advanced oxidation processes. *Journal of Scientific & Industrial Research* **2013,** *72*, (4), 248-254.

32. Chen, G.; Zhao, L.; Dong, Y. H., Oxidative degradation kinetics and products of chlortetracycline by manganese dioxide. *Journal of Hazardous Materials* **2011,** *193*, 128-138.

33. Chen, M. J.; Cao, F.; Li, F. B.; Liu, C. S.; Tong, H.; Wu, W. J.; Hu, M., Anaerobic transformation of DDT related to iron (III) reduction and microbial community structure in paddy soils. *Journal of Agricultural and Food Chemistry* **2013,** *61*, (9), 2224-2233.

34. Chen, W. R.; Ding, Y.; Johnston, C. T.; Teppen, B. J.; Boyd, S. A.; Li, H., Reaction of lincosamide antibiotics with manganese oxide in aqueous solution. *Environmental Science & Technology* **2010,** *44*, (12), 4486-4492.

35. Chen, W. R.; Huang, C. H., Transformation of tetracyclines mediated by Mn(II) and Cu(II) ions in the presence of oxygen. *Environmental Science & Technology* **2009,** *43*, (2), 401-407.

36. Chen, Z. X.; Wang, T.; Jin, X. Y.; Chen, Z. L.; Megharaj, M.; Naidu, R., Multifunctional kaolinite-supported nanoscale zero-valent iron used for the adsorption and degradation of crystal violet in aqueous solution. *Journal of Colloid and Interface Science* **2013,** *398*, 59-66.

37. Cheng, X.; Chen, B.; Cui, Y.; Sun, D.; Wang, X., Iron (III) reduction-induced phosphate precipitation during anaerobic digestion of waste activated sludge. *Separation and Purification Technology* **2015,** *143*, (Supplement C), 6-11.

38. Chieng, N.; Rades, T.; Aaltonen, J., An overview of recent studies on the analysis of pharmaceutical polymorphs. *Journal of Pharmaceutical and Biomedical Analysis* **2011,** *55*, (4), 618-644.

39. Clara, M.; Kreuzinger, N.; Strenn, B.; Gans, O.; Kroiss, H., The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research* **2005,** *39*, (1), 97-106.

40. Collado, S.; Quero, D.; Laca, A.; Diaz, M., Fe^{2+}-Catalyzed Wet Oxidation of Phenolic Acids under Different pH Values. *Industrial & Engineering Chemistry Research* **2010,** *49*, (24), 12405-12413.

41. Crane, M.; Watts, C.; Boucard, T., Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Science of the Total Environment* **2006,** *367*, (1), 23-41.

42. Crane, R. A.; Scott, T. B., Nanoscale zero-valent iron: Future prospects for an emerging water treatment technology. *Journal of Hazardous Materials* **2012,** *211*, 112-125.

43. Cruz-Morato, C.; Ferrando-Climent, L.; Rodriguez-Mozaz, S.; Barcelo, D.; Marco-Urrea, E.; Vicent, T.; Sarra, M., Degradation of pharmaceuticals in non-sterile urban wastewater by *Trametes versicolor* in a fluidized bed bioreactor. *Water Research* **2013,** *47*, (14), 5200-5210.

44. Cui, X. Y.; Zeng, P.; Qiu, G. L.; Liu, Y. Q.; Song, Y. H.; Xie, X. L.; Han, L., Pilot-scale treatment of pharmaceutical berberine wastewater by Fenton oxidation. *Environmental Earth Sciences* **2015,** *73*, (9), 4967-4977.

45. Cundy, A. B.; Hopkinson, L.; Whitby, R. L. D., Use of iron-based technologies in contaminated land and groundwater remediation: A review. *Science of the Total Environment* **2008,** *400*, (1-3), 42-51.

46. Dai, G. H.; Huang, J.; Chen, W. W.; Wang, B.; Yu, G.; Deng, S. B., Major pharmaceuticals and personal care products (PPCPs) in wastewater treatment plant and receiving water in Beijing, China, and associated ecological risks. *Bulletin of Environmental Contamination and Toxicology* **2014,** *92*, (6), 655-661.

47. De la Cruz, N.; Esquius, L.; Grandjean, D.; Magnet, A.; Tungler, A.; de Alencastro, L. F.; Pulgarin, C., Degradation of emergent contaminants by UV, UV/H_2O_2 and neutral photo-Fenton at pilot scale in a domestic wastewater treatment plant. *Water Research* **2013,** *47*, (15), 5836-5845.

48. de Velosa, A. C.; Nogueira, R. F. P., 2,4-Dichlorophenoxyacetic acid (2,4-D) degradation promoted by nanoparticulate zerovalent iron (nZVI) in aerobic suspensions. *Journal of Environmental Management* **2013,** *121*, 72-79.

49. de Wilt, A.; Butkovskyi, A.; Tuantet, K.; Leal, L. H.; Fernandes, T. V.; Langenhoff, A.; Zeeman, G., Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *Journal of Hazardous Materials* **2016,** *304*, 84-92.

50. Delgado, L. F.; Charles, P.; Glucina, K.; Morlay, C., The removal of endocrine disrupting compounds, pharmaceutically activated compounds and cyanobacterial toxins during drinking water preparation using activated carbon-A review. *Science of the Total Environment* **2012,** *435*, 509-525.

51. Deng, Y.; Ezyske, C. M., Sulfate radical-advanced oxidation process (SR-AOP) for simultaneous removal of refractory organic contaminants and ammonia in landfill leachate. *Water Research* **2011,** *45*, (18), 6189-6194.

52. Ding, L.-J.; An, X.-L.; Li, S.; Zhang, G.-L.; Zhu, Y.-G., Nitrogen loss through anaerobic ammonium oxidation coupled to iron reduction from paddy soils in a chronosequence. *Environmental Science & Technology* **2014,** *48*, (18), 10641-10647.

53. Dorer, C.; Vogt, C.; Neu, T. R.; Stryhanyuk, H.; Richnow, H. H., Characterization of toluene and ethylbenzene biodegradation under nitrate-, iron (III)- and manganese (IV)-reducing conditions by compound-specific isotope analysis. *Environmental Pollution* **2016,** *211*, 271-281.

54. Dougherty, J. A.; Swarzenski, P. W.; Dinicola, R. S.; Reinhard, M., Occurrence of herbicides and pharmaceutical and personal care products in surface water and groundwater around Liberty Bay, Puget Sound, Washington. *Journal of Environmental Quality* **2010,** *39*, (4), 1173-1180.

55. Elsner, M.; Schwarzenbach, R. P.; Haderlein, S. B., Reactivity of Fe(II)-bearing minerals toward reductive transformation of organic contaminants. *Environmental Science & Technology* **2004,** *38*, (3), 799-807.

56. European Commission, Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. In Commission, E., Ed. Brussels, **2011**.

57. European Federation of Pharmaceutical Industries and Association *The pharmaceutical industriy in figures: Edition 2016*; European Federation of Pharmaceutical Industries and Associations: Brussels, **2016**.

58. Eurpoean Union Directive 91/271/EEC of the European Parliament and of the Council. Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0271&from=EN. Accessed: 23, 09, 2017.

59. Eurpoean Union Directive 98/83/EC of the European Parliament and of the Council. Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:330:0032:0054:EN:PDF. Accessed: 23, 09, 2017.

60. Eurpoean Union Directive 2013/39/EU of the European Parliament and of the Council. Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF. Accessed: 23, 09, 2017.

61. Feitosa-Felizzola, J.; Hanna, K.; Chiron, S., Adsorption and transformation of selected human-used macrolide antibacterial agents with iron (III) and manganese (IV) oxides. *Environmental Pollution* **2009,** *157*, (4), 1317-1322.

62. Fick, J.; Soderstrom, H.; Lindberg, R. H.; Phan, C.; Tysklind, M.; Larsson, D. G. J., Contamination of surface, ground, and drinking water from pharmaceutical production. *Environmental Toxicology and Chemistry* **2009,** *28*, (12), 2522-2527.

63. Filip, J.; Zboril, R.; Schneeweiss, O.; Zeman, J.; Cernik, M.; Kvapil, P.; Otyepka, M., Environmental applications of chemically pure natural ferrihydrite. *Environmental Science & Technology* **2007,** *41*, (12), 4367-4374.

64. Forrez, I.; Carballa, M.; Fink, G.; Wick, A.; Hennebel, T.; Vanhaecke, L.; Ternes, T.; Boon, N.; Verstraete, W., Biogenic metals for the oxidative and reductive removal of pharmaceuticals, biocides and iodinated contrast media in a polishing membrane bioreactor. *Water Research* **2011,** *45*, (4), 1763-1773.

65. Forrez, I.; Carballa, M.; Verbeken, K.; Vanhaecke, L.; Schlusener, M.; Ternes, T.; Boon, N.; Verstraete, W., Diclofenac oxidation by biogenic manganese oxides. *Environmental Science & Technology* **2010,** *44*, (9), 3449-3454.

66. Froehner, S.; Piccioni, W.; Machado, K. S.; Aisse, M. M., Removal capacity of caffeine, hormones, and bisphenol by aerobic and anaerobic sewage treatment. *Water Air and Soil Pollution* **2011,** *216*, (1-4), 463-471.

67. Furgal, K. M.; Meyer, R. L.; Bester, K., Removing selected steroid hormones, biocides and pharmaceuticals from water by means of biogenic manganese oxide nanoparticles in situ at ppb levels. *Chemosphere* **2015,** *136*, 321-326.

68. Gao, A. F.; Gao, H.; Wan, J. M.; Wu, C. S., Treatment Pharmaceutical Wastewater Using Fenton Oxidation, Ultraviolet, and Ultrasound Processes. *Fresenius Environmental Bulletin* **2013,** *22*, (2), 449-454.

69. Gao, J.; Hedman, C.; Liu, C.; Guo, T.; Pedersen, J. A., Transformation of sulfamethazine by manganese oxide in aqueous solution. *Environmental Science & Technology* **2012,** *46*, (5), 2642-2651.

70. Gao, S. S.; Zhao, Z. W.; Xu, Y. P.; Tian, J. Y.; Qi, H.; Lin, W.; Cui, F. Y., Oxidation of sulfamethoxazole (SMX) by chlorine, ozone and permanganate-A comparative study. *Journal of Hazardous Materials* **2014,** *274*, 258-269.

71. Garrido-Ramirez, E. G.; Sivaiah, M. V.; Barrault, J.; Valange, S.; Theng, B. K. G.; Ureta-Zanartu, M. S.; Mora, M. D., Catalytic wet peroxide oxidation of phenol over iron or copper oxide-supported allophane clay materials: Influence of catalyst SiO_2/Al_2O_3 ratio. *Microporous and Mesoporous Materials* **2012,** *162*, 189-198.

72. Gauthier, H.; Yargeau, V.; Cooper, D. G., Biodegradation of pharmaceuticals by *Rhodococcus rhodochrous* and *Aspergillus niger* by co-metabolism. *Science of the Total Environment* **2010,** *408*, (7), 1701-1706.

73. Ghafoori, S.; Shah, K. K.; Mehrvar, M.; Chan, P. K., Pharmaceutical wastewater treatment using granular activated carbon and UV/H_2O_2 processes: Experimental analysis and modelling. *Canadian Journal of Chemical Engineering* **2014,** *92*, (7), 1163-1173.

74. Ghattas, A. K.; Fischer, F.; Wick, A.; Ternes, T. A., Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment. *Water Research* **2017,** *116*, 268-295.

75. Ghauch, A.; Ayoub, G.; Naim, S., Degradation of sulfamethoxazole by persulfate assisted micrometric Fe^0 in aqueous solution. *Chemical Engineering Journal* **2013,** *228*, (Supplement C), 1168-1181.

76. Ghauch, A.; Tuqan, A.; Assia, H. A., Antibiotic removal from water: Elimination of amoxicillin and ampicillin by microscale and nanoscale iron particles. *Environmental Pollution* **2009,** *157*, (5), 1626-1635.

77. Ghernaout, D.; Naceur, M. W., Ferrate(VI): In situ generation and water treatment - A review. *Desalination and Water Treatment* **2011,** *30*, (1-3), 319-332.

78. Gilroy, E. A. M.; Klinck, J. S.; Campbell, S. D.; McInnis, R.; Gillis, P. L.; de Solla, S. R., Toxicity and bioconcentration of the pharmaceuticals moxifloxacin, rosuvastatin, and drospirenone to the unionid mussel Lampsilis siliquoidea. *Science of the Total Environment* **2014,** *487*, 537-544.

79. Godoy, A. A.; Kummrow, F.; Pamplin, P. A. Z., Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. *Chemosphere* **2015,** *138*, (Supplement C), 281-291.

80. Golan-Rozen, N.; Chefetz, B.; Ben-Ari, J.; Geva, J.; Hadar, Y., Transformation of the recalcitrant pharmaceutical compound carbamazepine by *Pleurotus ostreatus*: Role of cytochrome P450 monooxygenase and manganese peroxidase. *Environmental Science & Technology* **2011,** *45*, (16), 6800-6805.

81. Gomez-Toribio, V.; Garcia-Martin, A. B.; Martinez, M. J.; Martinez, A. T.; Guillen, F., Induction of extracellular hydroxyl radical production by white-rot fungi through quinone redox cycling. *Applied and Environmental Microbiology* **2009,** *75*, (12), 3944-3953.

82. Gotvajn, A. Z.; Zagorc-Koncan, J.; Tisler, T., Pretreatment of highly polluted pharmaceutical waste broth by wet air oxidation. *Journal of Environmental Engineering* **2007,** *133*, (1), 89-94.

83. Gray, M. J.; Malati, M. A., Adsorption from aqueous solution by δ-manganese dioxide II. Adsorption of some heavy metal cations. *Journal of Chemical Technology and Biotechnology* **1979,** *29*, (3), 135-144.

84. Grcic, I.; Papic, S.; Zizek, K.; Koprivanac, N., Zero-valent iron (ZVI) Fenton oxidation of reactive dye wastewater under UV-C and solar irradiation. *Chemical Engineering Journal* **2012,** *195*, 77-90.

85. Grebel, J. E.; Charbonnet, J. A.; Sedlak, D. L., Oxidation of organic contaminants by manganese oxide geomedia for passive urban stormwater treatment systems. *Water Research* **2016,** *88*, 481-491.

86. Gros, M.; Cruz-Morato, C.; Marco-Urrea, E.; Longrée, P.; Singer, H.; Sarrà, M.; Hollender, J.; Vicent, T.; Rodriguez-Mozaz, S.; Barceló, D., Biodegradation of the X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin by the white rot fungus *Trametes versicolor* in hospital wastewaters and identification of degradation products. *Water Research* **2014,** *60*, (Supplement C), 228-241.

87. Guan, X. H.; He, D.; Ma, J.; Chen, G. H., Application of permanganate in the oxidation of micropollutants: A mini review. *Frontiers of Environmental Science & Engineering in China* **2010,** *4*, (4), 405-413.

88. Han, G. H.; Hur, H. G.; Kim, S. D., Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: Occurrence and toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry* **2006,** *25*, (1), 265-271.

89. Han, Z. T.; Sani, B.; Akkanen, J.; Abel, S.; Nybom, I.; Karapanagioti, H. K.; Werner, D., A critical evaluation of magnetic activated carbon's potential for the remediation of sediment impacted by polycyclic aromatic hydrocarbons. *Journal of Hazardous Materials* **2015,** *286*, 41-47.

90. Hartmann, M.; Kullmann, S.; Keller, H., Wastewater treatment with heterogeneous Fenton-type catalysts based on porous materials. *Journal of Materials Chemistry* **2010,** *20*, (41), 9002-9017.

91. Hasan, Z.; Jeon, J.; Jhung, S. H., Adsorptive removal of naproxen and clofibric acid from water using metal-organic frameworks. *Journal of Hazardous Materials* **2012,** *209*, 151-157.

92. He, K.; Yin, Q.; Liu, A.; Echigo, S.; Itoh, S.; Wu, G., Enhanced anaerobic degradation of amide pharmaceuticals by dosing ferroferric oxide or anthraquinone-2, 6-disulfonate. *Journal of Water Process Engineering* **2017,** *18*, (Supplement C), 192-197.

93. He, Y.; Xu, J.; Zhang, Y.; Guo, C.; Li, L.; Wang, Y., Oxidative transformation of carbamazepine by manganese oxides. *Environmental Science and Pollution Research* **2012,** *19*, (9), 4206-4213.

94. He, Y. J.; Langenhoff, A. A. M.; Sutton, N. B.; Rijnaarts, H. H. M.; Blokland, M. H.; Chen, F. R.; Huber, C.; Schroder, P., Metabolism of ibuprofen by *Phragmites australis*: Uptake and phytodegradation. *Environmental Science & Technology* **2017,** *51*, (8), 4576-4584.

95. He, Y. J.; Sutton, N. B.; Rijnaarts, H. H. H.; Langenhoff, A. A. M., Degradation of pharmaceuticals in wastewater using immobilized TiO_2 photocatalysis under simulated solar irradiation. *Applied Catalysis B: Environmental* **2016,** *182*, 132-141.

96. Hendratna, A. The application of MnO_2 and $KMnO_4$ for persistent organic compounds and COD removals in wastewater treatment process. KTH Royal Institute of Technology in Stockholm, Stockholm, **2011**.

97. Hennebel, T.; De Gusseme, B.; Boon, N.; Verstraete, W., Biogenic metals in advanced water treatment. *Trends in Biotechnology* **2009,** *27*, (2), 90-98.

98. Ho, L.; Lambling, P.; Bustamante, H.; Duker, P.; Newcombe, G., Application of powdered activated carbon for the adsorption of cylindrospermopsin and microcystin toxins from drinking water supplies. *Water Research* **2011,** *45*, (9), 2954-2964.

99. Hosseini, A. M.; Tungler, A.; Bakos, V., Wet oxidation properties of process waste waters of fine chemical and pharmaceutical origin. *Reaction Kinetics Mechanisms and Catalysis* **2011,** *103*, (2), 251-260.

100. Hou, M. T.; Tang, Y.; Xu, J.; Pu, Y.; Lin, A. J.; Zhang, L. L.; Xiong, J. P.; Yang, X. J.; Wan, P. Y., Nitrate reduction in water by aluminum-iron alloy particles catalyzed by copper. *Journal of Environmental Chemical Engineering* **2015,** *3*, (4), 2401-2407.

101. Hu, L.; Martin, H. M.; Arcs-Bulted, O.; Sugihara, M. N.; Keatlng, K. A.; Strathmann, T. J., Oxidation of carbamazepine by Mn(VII) and Fe(VI): Reaction kinetics and mechanism. *Environmental Science & Technology* **2009,** *43*, (2), 509-515.

102. Hu, L. H.; Martin, H. M.; Strathmann, T. J., Oxidation kinetics of antibiotics during water treatment with potassium permanganate. *Environmental Science & Technology* **2010,** *44*, (16), 6416-6422.

103. Hu, L. H.; Stemig, A. M.; Wammer, K. H.; Strathmann, T. J., Oxidation of antibiotics during water treatment with potassium permanganate: Reaction pathways and deactivation. *Environmental Science & Technology* **2011,** *45*, (8), 3635-3642.

104. Huang, Y. X.; Keller, A. A., Magnetic nanoparticle adsorbents for emerging organic contaminants. *Acs Sustainable Chemistry & Engineering* **2013,** *1*, (7), 731-736.

105. Huber, M. M.; Canonica, S.; Park, G. Y.; Von Gunten, U., Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. *Environmental Science & Technology* **2003,** *37*, (5), 1016-1024.

106. Huerta-Fontela, M.; Galceran, M. T.; Ventura, F., Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. *Water Research* **2011,** *45*, (3), 1432-1442.

107. Hug, S. J.; Leupin, O., Iron-catalyzed oxidation of arsenic (III) by oxygen and by hydrogen peroxide: pH-dependent formation of oxidants in the Fenton reaction. *Environmental Science & Technology* **2003,** *37*, (12), 2734-2742.

108. Huguet, M.; Deborde, M.; Papot, S.; Gallard, H., Oxidative decarboxylation of diclofenac by manganese oxide bed filter. *Water Research* **2013,** *47*, (14), 5400-5408.

109. Huguet, M.; Simon, V.; Gallard, H., Transformation of paracetamol into 1,4-benzoquinone by a manganese oxide bed filter. *Journal of Hazardous Materials* **2014,** *271*, 245-251.

110. Huo, S.-H.; Yan, X.-P., Metal–organic framework MIL-100 (Fe) for the adsorption of malachite green from aqueous solution. *Journal of Materials Chemistry* **2012,** *22*, (15), 7449-7455.

111. Hussain, S.; Shaikh, S.; Farooqui, M., COD reduction of waste water streams of active pharmaceutical ingredient - Atenolol manufacturing unit by advanced oxidation-Fenton process. *Journal of Saudi Chemical Society* **2013,** *17*, (2), 199-202.

112. Ikehata, K.; El-Din, M. G., Aqueous pesticide degradation by hydrogen peroxide/ultraviolet irradiation and Fenton-type advanced oxidation processes: a review. *Journal of Environmental Engineering and Science* **2006,** *5*, (2), 81-135.

113. Ikehata, K.; Naghashkar, N. J.; Ei-Din, M. G., Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: A review. *Ozone-Science & Engineering* **2006,** *28*, (6), 353-414.

114. Isarain-Chavez, E.; Arias, C.; Cabot, P. L.; Centellas, F.; Rodriguez, R. M.; Garrido, J. A.; Brillas, E., Mineralization of the drug beta-blocker atenolol by electro-Fenton and photoelectro-Fenton using an air-diffusion cathode for H_2O_2 electrogeneration combined with a carbon-felt cathode for Fe^{2+} regeneration. *Applied Catalysis B: Environmental* **2010,** *96*, (3-4), 361-369.

115. Jahn, M. K.; Haderlein, S. B.; Meckenstock, R. U., Anaerobic degradation of benzene, toluene, ethylbenzene, and o-xylene in sediment-free iron-reducing enrichment cultures. *Applied and Environmental Microbiology* **2005,** *71*, (6), 3355-3358.

116. Jain, A.; Ong, S. P.; Hautier, G.; Chen, W.; Richards, W. D.; Dacek, S.; Cholia, S.; Gunter, D.; Skinner, D.; Ceder, G.; Persson, K. A., The Materials Project: A materials genome approach to accelerating materials innovation. *APL Materials* **2013,** *1*, (1), 011002.

117. Jamalluddin, N. A.; Abdullah, A. Z., Reactive dye degradation by combined Fe(III)/TiO_2 catalyst and ultrasonic irradiation: Effect of Fe(III) loading and calcination temperature. *Ultrasonics Sonochemistry* **2011,** *18*, (2), 669-678.

118. Jelić, A.; Petrović, M.; Barceló, D., Pharmaceuticals in drinking water. In *Emerging Organic Contaminants and Human Health*, Barceló, D., Ed. Springer Berlin Heidelberg: Berlin, Heidelberg, 2012; pp 47-70.

119. Ji, Y. F.; Ferronato, C.; Salvador, A.; Yang, X.; Chovelon, J. M., Degradation of ciprofloxacin and sulfamethoxazole by ferrous-activated persulfate: Implications for remediation of groundwater contaminated by antibiotics. *Science of the Total Environment* **2014,** *472*, 800-808.

120. Jia, Y.; Aagaard, P.; Breedveld, G. D., Sorption of triazoles to soil and iron minerals. *Chemosphere* **2007,** *67*, (2), 250-258.

121. Jiang, J.-Q.; Zhou, Z.; Patibandla, S.; Shu, X., Pharmaceutical removal from wastewater by ferrate(VI) and preliminary effluent toxicity assessments by the zebrafish embryo model. *Microchemical Journal* **2013,** *110*, (Supplement C), 239-245.

122. Jiang, J. Q., Research progress in the use of ferrate (VI) for the environmental remediation. *Journal of Hazardous Materials* **2007,** *146*, (3), 617-623.

123. Jiang, J. Q., Advances in the development and application of ferrate(VI) for water and wastewater treatment. *Journal of Chemical Technology and Biotechnology* **2014,** *89*, (2), 165-177.

124. Jiang, J. Q.; Zhou, Z. W., Removal of pharmaceutical residues by ferrate (VI). *Plos One* **2013,** *8*, (2).

125. Jiang, L. Y.; Chen, J. M.; Zhu, R. Y.; Huang, C.; Ji, H., Degradation kinetics and estrogenic activity of 17β-estradiol removal by aqueous manganese dioxide. *Journal of Environmental Science and Health, Part A - Toxic/Hazardous Substances and Environmental Engineering* **2010,** *45*, (8), 938-945.

126. Jiang, S.; Kim, D.-G.; Kim, J.; Ko, S.-O., Characterization of the biogenic manganese oxides produced by *Pseudomonas putida* strain MnB1. *Environmental Engineering Research* **2010,** *15*, (4), 183-190.

127. Jin, K.; Park, J.; Lee, J.; Yang, K. D.; Pradhan, G. K.; Sim, U.; Jeong, D.; Jang, H. L.; Park, S.; Kim, D.; Sung, N.-E.; Kim, S. H.; Han, S.; Nam, K. T., Hydrated manganese (II) phosphate ($Mn_3(PO_4)_2 \cdot 3H_2O$) as a water oxidation catalyst. *Journal of the American Chemical Society* **2014,** *136*, (20), 7435-7443.

128. Johnson, A. C.; Dumont, E.; Williams, R. J.; Oldenkamp, R.; Cisowska, I.; Sumpter, J. P., Do concentrations of ethinylestradiol, estradiol, and diclofenac in European rivers exceed proposed EU environmental quality standards? *Environmental Science & Technology* **2013,** *47*, (21), 12297-12304.

129. Jones, O. A.; Lester, J. N.; Voulvoulis, N., Pharmaceuticals: a threat to drinking water? *Trends in Biotechnology* **2005,** *23*, (4), 163-167.

130. Kagle, J.; Porter, A. W.; Murdoch, R. W.; Rivera-Cancel, G.; Hay, A. G., Chapter 3 Biodegradation of Pharmaceutical and Personal Care Products. In *Advances in Applied Microbiology*, Academic Press: **2009**; Vol. 67, pp 65-108.

131. Kallel, M.; Belaid, C.; Mechichi, T.; Ksibi, M.; Elleuch, B., Removal of organic load and phenolic compounds from olive mill wastewater by Fenton oxidation with zero-valent iron. *Chemical Engineering Journal* **2009,** *150*, (2-3), 391-395.

132. Kang, K. H.; Dec, J.; Park, H.; Bollag, J. M., Effect of phenolic mediators and humic acid on cyprodinil transformation in presence of birnessite. *Water Research* **2004,** *38*, (11), 2737-2745.

133. Keenan, C. R.; Sedlak, D. L., Ligand-enhanced reactive oxidant generation by nanoparticulate zero-valent iron and oxygen. *Environmental Science & Technology* **2008,** *42*, (18), 6936-6941.

134. Keller, A. A.; Garner, K.; Miller, R. J.; Lenihan, H. S., Toxicity of nano-zero valent iron to freshwater and marine organisms. *Plos One* **2012,** *7*, (8).

135. Khadse, G. K.; Patni, P. M.; Labhasetwar, P. K., Removal of iron and manganese from drinking water supply. *Sustainable Water Resources Management* **2015,** *1*, (2), 157-165.

136. Khan, N. A.; Hasan, Z.; Jhung, S. H., Adsorptive removal of hazardous materials using metal-organic frameworks (MOFs): A review. *Journal of Hazardous Materials* **2013,** *244*, 444-456.

137. Kharisov, B. I.; Dias, H. V. R.; Kharissova, O. V.; Jimenez-Perez, V. M.; Perez, B. O.; Flores, B. M., Iron-containing nanomaterials: synthesis, properties, and environmental applications. *RSC Advances* **2012,** *2*, (25), 9325-9358.

138. Khin, M. M.; Nair, A. S.; Babu, V. J.; Murugan, R.; Ramakrishna, S., A review on nanomaterials for environmental remediation. *Energy & Environmental Science* **2012,** *5*, (8), 8075-8109.

139. Klavarioti, M.; Mantzavinos, D.; Kassinos, D., Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environment International* **2009,** *35*, (2), 402-417.

140. Kochi, J. K., Mechanisms of Organic Oxidation and Reduction by Metal Complexes. *Science* **1967,** *155*, (3761), 415-&.

141. Komlos, J.; Kukkadapu, R. K.; Zachara, J. M.; Jaffe, P. R., Biostimulation of iron reduction and subsequent oxidation of sediment containing Fe-silicates and Fe-oxides: Effect of redox cycling on Fe(III) bioreduction. *Water Research* **2007,** *41*, (13), 2996-3004.

142. Kong, L. N.; Wei, W.; Zhao, Q. F.; Wang, J. Q.; Wan, Y., Active coordinatively unsaturated manganese monoxide-containing mesoporous carbon catalyst in wet peroxide oxidation. *Acs Catalysis* **2012,** *2*, (12), 2577-2586.

143. Kouraichi, R.; Delgado, J. J.; Lopez-Castro, J. D.; Stitou, M.; Rodriguez-Izquierdo, J. M.; Cauqui, M. A., Deactivation of Pt/MnOx-CeO_2 catalysts for the catalytic wet oxidation of phenol: Formation of carbonaceous deposits and leaching of manganese. *Catalysis Today* **2010,** *154*, (3-4), 195-201.

144. Kouzbour, S.; El Azher, N.; Gourich, B.; Gros, F.; Vial, C.; Stiriba, Y., Removal of manganese (II) from drinking water by aeration process using an airlift reactor. *Journal of Water Process Engineering* **2017,** *16*, (Supplement C), 233-239.

145. Kowalski, K. P.; Sogaard, E. G., Implementation of zero-valent iron (ZVI) into drinking water supply - Role of the ZVI and biological processes. *Chemosphere* **2014,** *117*, 108-114.

146. Kuan, W. H.; Hu, C. Y.; Liu, B. S.; Tzou, Y. M., Degradation of antibiotic amoxicillin using 1 x 1 molecular sieve-structured manganese oxide. *Environmental Technology* **2013,** *34*, (16), 2443-2451.

147. Kümmerer, K., *Pharmaceuticals in the environment: Sources, fate, effects and risks*. Springer: Berlin, **2004**.

148. Küster, A.; Adler, N., Pharmaceuticals in the environment: scientific evidence of risks and its regulation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2014,** *369*, (1656).

149. la Farre, M.; Perez, S.; Kantiani, L.; Barcelo, D., Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *Trac-Trends in Analytical Chemistry* **2008,** *27*, (11), 991-1007.

150. Laine, D. F.; Blumenfeld, A.; Cheng, I. F., Mechanistic study of the ZEA organic pollutant degradation system: Evidence for H_2O_2, HO• center dot, and the homogeneous activation of O_2 by Fe^{II}EDTA. *Industrial & Engineering Chemistry Research* **2008,** *47*, (17), 6502-6508.

151. Langenhoff, A.; Inderfurth, N.; Veuskens, T.; Schraa, G.; Blokland, M.; Kujawa-Roeleveld, K.; Rijnaarts, H., Microbial removal of the pharmaceutical compounds ibuprofen and diclofenac from wastewater. *Biomed Research International* **2013**.

152. Langenhoff, A. A. M.; BrouwersCeiler, D. L.; Engelberting, J. H. L.; Quist, J. J.; Wolkenfelt, J. G. P. N.; Zehnder, A. J. B.; Schraa, G., Microbial reduction of manganese coupled to toluene oxidation. *Fems Microbiology Ecology* **1997,** *22*, (2), 119-127.

153. Langenhoff, A. A. M.; Zehnder, A. J. B.; Schraa, G., Behaviour of toluene, benzene and naphthalene under anaerobic conditions in sediment columns. *Biodegradation* **1996,** *7*, (3), 267-274.

154. Lee, C.; Kim, J. Y.; Lee, W. I.; Nelson, K. L.; Yoon, J.; Sedlak, D. L., Bactericidal effect of zero-valent iron nanoparticles on *Escherichia coli*. *Environmental Science & Technology* **2008,** *42*, (13), 4927-4933.

155. Lee, Y.; Zimmermann, S. G.; Kieu, A. T.; von Gunten, U., Ferrate (Fe(VI)) application for municipal wastewater treatment: A novel process for simultaneous micropollutant oxidation and phosphate removal. *Environmental Science & Technology* **2009,** *43*, (10), 3831-3838.

156. Leung, H. W.; Jin, L.; Wei, S.; Tsui, M. M. P.; Zhou, B.; Jiao, L.; Cheung, P. C.; Chun, Y. K.; Murphy, M. B.; Lam, P. K. S., Pharmaceuticals in tap water: human health risk assessment and proposed monitoring framework in China. *Environmental Health Perspectives* **2013,** *121*, (7), 839.

157. Levec, J.; Pintar, A., Catalytic wet-air oxidation processes: A review. *Catalysis Today* **2007,** *124*, (3-4), 172-184.

158. Li, L.; Fan, M. H.; Brown, R. C.; Van Leeuwen, J. H.; Wang, J. J.; Wang, W. H.; Song, Y. H.; Zhang, P. Y., Synthesis, properties, and environmental applications of nanoscale iron-based materials: A review. *Critical Reviews in Environmental Science and Technology* **2006,** *36*, (5), 405-431.

159. Li, S. L.; Wang, W.; Liu, Y. Y.; Zhang, W. X., Zero-valent iron nanoparticles (nZVI) for the treatment of smelting wastewater: A pilot-scale demonstration. *Chemical Engineering Journal* **2014,** *254*, 115-123.

160. Li, W.; Nanaboina, V.; Zhou, Q. X.; Korshin, G. V., Effects of Fenton treatment on the properties of effluent organic matter and their relationships with the degradation of pharmaceuticals and personal care products. *Water Research* **2012,** *46*, (2), 403-412.

161. Li, W. C., Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental Pollution* **2014,** *187*, (Supplement C), 193-201.

162. Li, X.; Hou, L.; Liu, M.; Zheng, Y.; Yin, G.; Lin, X.; Cheng, L.; Li, Y.; Hu, X., Evidence of nitrogen loss from anaerobic ammonium oxidation coupled with ferric iron reduction in an intertidal wetland. *Environmental Science & Technology* **2015,** *49*, (19), 11560-11568.

163. Li, Y.; Wei, D.; Du, Y., Oxidative transformation of levofloxacin by δ-MnO_2: Products, pathways and toxicity assessment. *Chemosphere* **2015,** *119*, (Supplement C), 282-288.

164. Li, Z.; Sobek, A.; Radke, M., Fate of pharmaceuticals and their transformation products in four small European rivers receiving treated wastewater. *Environmental Science & Technology* **2016,** *50*, (11), 5614-5621.

165. Li, Z. H.; Fitzgerald, N. M.; Albert, Z.; Schnabl, A.; Jiang, W. T., Contrasting mechanisms of metoprolol uptake on kaolinite and talc. *Chemical Engineering Journal* **2015,** *272*, 48-57.

166. Lima, D. R. S.; Baêta, B. E. L.; Aquino, S. F.; Libânio, M.; Afonso, R. J. C. F., Removal of pharmaceuticals and endocrine disruptor compounds from natural waters by clarification associated with powdered activated carbon. *Water, Air, & Soil Pollution* **2014,** *225*, (11), 2170.

167. Lin, K.; Liu, W.; Gan, J., Oxidative removal of bisphenol A by manganese dioxide: Efficacy, products, and pathways. *Environmental Science & Technology* **2009,** *43*, (10), 3860-3864.

168. Liu, C.; He, Y.; Li, F.; Wang, H., Preparation of poly ferric sulfate and the application in micro-polluted raw water treatment. *Journal of the Chinese Advanced Materials Society* **2013,** *1*, (3), 210-218.

169. Liu, W.; Langenhoff, A. A. M.; Sutton, N. B.; Rijnaarts, H. H. M., Application of manganese oxides under anoxic conditions to remove diclofenac from water. **Submitted**.

170. Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Pharmaceutical removal from water with iron- or manganese-based technologies: A review. *Critical Reviews in Environmental Science and Technology* **2016,** *46*, (19-20), 1584-1621.

171. Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anaerobic biodegradation of pharmaceutical compounds coupled to dissimilatory manganese (IV) or iron (III) reduction. **In preparation**.

172. Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anoxic conditions are favourable for abiotic diclofenac removal from water with manganese oxides. **Submitted**.

173. Liu, X.; Wang, J.; Duan, L.; Song, Y.; Hu, X.; Wei, J., Enhancing the production of butyric acid from sludge fermentation with an emphasis on zinc, cobalt, cuprum, ferrum and manganese. *Environmental Earth Sciences* **2015,** *73*, (9), 5057-5066.

174. Lonappan, L.; Brar, S. K.; Das, R. K.; Verma, M.; Surampalli, R. Y., Diclofenac and its transformation products: Environmental occurrence and toxicity - A review. *Environment International* **2016,** *96*, 127-138.

175. Lovley, D., Dissimilatory Fe(III)- and Mn(IV)-reducing prokaryotes. In *The Prokaryotes: Prokaryotic Physiology and Biochemistry*, Rosenberg, E.; DeLong, E. F.; Lory, S.; Stackebrandt, E.; Thompson, F., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, **2013**; pp 287-308.

176. Lovley, D. R., Dissimilatory Fe(III) and Mn(IV) Reduction. *Microbiological Reviews* **1991,** *55*, (2), 259-287.

177. Lovley, D. R.; Holmes, D. E.; Nevin, K. P., Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology* **2004,** *49*, 219-286.

178. Lovley, D. R.; Phillips, E. J. P., Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Microbiology* **1986,** *51*, (4), 683-689.

179. Lovley, D. R.; Phillips, E. J. P., Novel mode of microbial energymetabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology* **1988,** *54*, (6), 1472-1480.

180. Lu, L.; Li, J.; Yu, J.; Song, P.; Ng, D. H. L., A hierarchically porous $MgFe_2O_4$/γ-Fe_2O_3 magnetic microspheres for efficient removals of dye and pharmaceutical from water. *Chemical Engineering Journal* **2016,** *283*, (Supplement C), 524-534.

181. Lu, Y.; Hernandez, P.; Abegunde, D.; Edejer, T., The world medicines situation 2011. *Medicine expenditures. World Health Organization, Geneva* **2011**.

182. Luo, Y. L.; Guo, W. S.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang,

X. C. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment* **2014,** *473*, 619-641.

183. Lv, A. H.; Hu, C.; Nie, Y. L.; Qu, J. H., Catalytic ozonation of toxic pollutants over magnetic cobalt and manganese co-doped gamma-Fe_2O_3. *Applied Catalysis B: Environmental* **2010,** *100*, (1-2), 62-67.

184. Lv, A. H.; Hu, C.; Nie, Y. L.; Qu, J. H., Catalytic ozonation of toxic pollutants over magnetic cobalt-doped Fe_3O_4 suspensions. *Applied Catalysis B: Environmental* **2012,** *117*, 246-252.

185. Ma, J.; Sui, M. H.; Chen, Z. L.; Wang, L. N., Degradation of refractory organic pollutants by catalytic ozonation - Activated carbon and Mn-loaded activated carbon as catalysts. *Ozone-Science & Engineering* **2004,** *26*, (1), 3-10.

186. Mackuľak, T.; Mosný, M.; Grabic, R.; Golovko, O.; Koba, O.; Birošová, L., Fenton-like reaction: A possible way to efficiently remove illicit drugs and pharmaceuticals from wastewater. *Environmental Toxicology and Pharmacology* **2015,** *39*, (2), 483-488.

187. Madhavan, J.; Panneerselvam, S.; Anandan, S.; Zhou, M. F.; Grieser, F.; Ashokkumar, M., Ultrasound assisted photocatalytic degradation of diclofenac in an aqueous environment. *Chemosphere* **2010,** *80*, (7), 747-752.

188. Madras, G.; McCoy, B. J., Temperature effects during Ostwald ripening. *Journal of Chemical Physics* **2003,** *119*, (3), 1683-1693.

189. Maeng, S. K.; Choi, B. G.; Lee, K. T.; Song, K. G., Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors. *Water Research* **2013,** *47*, (9), 3151-3162.

190. Mahmoodi, N. M., Manganese ferrite nanoparticle: Synthesis, characterization, and photocatalytic dye degradation ability. *Desalination and Water Treatment* **2015,** *53*, (1), 84-90.

191. Mailler, R.; Gasperi, J.; Coquet, Y.; Deshayes, S.; Zedek, S.; Cren-Olive, C.; Cartiser, N.; Eudes, V.; Bressy, A.; Caupos, E.; Moilleron, R.; Chebbo, G.; Rocher, V., Study of a large scale powdered activated carbon pilot: Removals of a wide range of emerging and priority micropollutants from wastewater treatment plant effluents. *Water Research* **2015,** *72*, 315-330.

192. Marco-Urrea, E.; Perez-Trujillo, M.; Vicent, T.; Caminal, G., Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by *Trametes versicolor*. *Chemosphere* **2009,** *74*, (6), 765-772.

193. Marco-Urrea, E.; Radjenovic, J.; Caminal, G.; Petrovic, M.; Vicent, T.; Barcelo, D., Oxidation of atenolol, propranolol, carbamazepine and clofibric acid by a biological Fenton-like system mediated by the white-rot fungus *Trametes versicolor*. *Water Research* **2010,** *44*, (2), 521-532.

194. Martin, J.; Camacho-Munoz, D.; Santos, J. L.; Aparicio, I.; Alonso, E., Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials* **2012,** *239*, 40-47.

195. Martins, R. C.; Cardoso, M.; Dantas, R. F.; Sans, C.; Esplugas, S.; Quinta-Ferreira, R. M., Catalytic studies for the abatement of emerging contaminants by ozonation. *Journal of Chemical Technology and Biotechnology* **2015,** *90*, (9), 1611-1618.

196. Maszkowska, J.; Stolte, S.; Kumirska, J.; Lukaszewicz, P.; Mioduszewska, K.; Puckowski, A.; Caban, M.; Wagil, M.; Stepnowski, P.; Bialk-Bielinska, A., Beta-blockers in the environment: Part I. Mobility and hydrolysis study. *Science of the Total Environment* **2014,** *493*, 1112-1121.

197. Maszkowska, J.; Stolte, S.; Kumirska, J.; Lukaszewicz, P.; Mioduszewska, K.; Puckowski, A.; Caban, M.; Wagil, M.; Stepnowski, P.; Bialk-Bielinska, A., Beta-blockers in the environment: Part II. Ecotoxicity study. *Science of the Total Environment* **2014,** *493*, 1122-1126.

198. Matamoros, V.; Hijosa, M.; Bayona, J. M., Assessment of the pharmaceutical active compounds removal in wastewater treatment systems at enantiomeric level. Ibuprofen and naproxen. *Chemosphere* **2009,** *75*, (2), 200-205.

199. Matilainen, A.; Vepsäläinen, M.; Sillanpää, M., Natural organic matter removal by coagulation during drinking water treatment: A review. *Advances in Colloid and Interface Science* **2010,** *159*, (2), 189-197.

200. Meerburg, F.; Hennebel, T.; Vanhaecke, L.; Verstraete, W.; Boon, N., Diclofenac and 2-anilinophenylacetate degradation by combined activity of biogenic manganese oxides and silver. *Microbial Biotechnology* **2012,** *5*, (3), 388-395.

201. Melero, J. A.; Martinez, F.; Botas, J. A.; Molina, R.; Pariente, M. I., Heterogeneous catalytic wet peroxide oxidation systems for the treatment of an industrial pharmaceutical wastewater. *Water Research* **2009,** *43*, (16), 4010-4018.

202. Meng, Y. T.; Zheng, Y. M.; Zhang, L. M.; He, J. Z., Biogenic Mn oxides for effective adsorption of Cd from aquatic environment. *Environmental Pollution* **2009,** *157*, (8-9), 2577-2583.

203. Metcalf, L.; Eddy, H. P.; Tchobanoglous, G., *Wastewater engineering: Treatment, disposal, and reuse*. McGraw-Hill: New York, **2004**.

204. Michael, I.; Hapeshi, E.; Michael, C.; Varela, A. R.; Kyriakou, S.; Manaia, C. M.; Fatta-Kassinos, D., Solar photo-Fenton process on the abatement of antibiotics at a pilot scale: Degradation kinetics, ecotoxicity and phytotoxicity assessment and removal of antibiotic resistant enterococci. *Water Research* **2012,** *46*, (17), 5621-5634.

205. Mohapatra, D. P.; Brar, S. K.; Tyagi, R. D.; Picard, P.; Surampalli, R. Y., Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine. *Science of the Total Environment* **2014,** *470*, (Supplement C), 58-75.

206. Mohatt, J. L.; Hu, L. H.; Finneran, K. T.; Strathmann, T. J., Microbially mediated abiotic transformation of the antimicrobial agent sulfamethoxazole under iron-reducing soil conditions. *Environmental Science & Technology* **2011,** *45*, (11), 4793-4801.

207. Monteiro, S. C.; Boxall, A. B., Occurrence and fate of human pharmaceuticals in the environment. *Rev Environ Contam Toxicol* **2010,** *202*, 53-154.

208. Murray, J. W., The surface chemistry of hydrous manganese dioxide. *Journal of Colloid and Interface Science* **1974,** *46*, (3), 357-371.

209. Murray, J. W., The interaction of metal ions at the manganese dioxide-solution interface. *Geochimica et Cosmochimica Acta* **1975,** *39*, (4), 505-519.

210. Muter, O.; P\erkons, I.; Selga, T.; Berzins, A.; Gudra, D.; Radovica-Spalvina, I.; Fridmanis, D.; Bartkevics, V., Removal of pharmaceuticals from municipal wastewaters at laboratory scale by treatment with activated sludge and biostimulation. *Science of the Total Environment* **2017,** *584*, (Supplement C), 402-413.

211. Neta, P.; Huie, R. E.; Ross, A. B., Rate constants for reactions of inorganic radicals in aqueous solution. *Journal of Physical and Chemical Reference Data* **1988,** *17*, (3), 1027-1284.

212. Neyens, E.; Baeyens, J., A review of classic Fenton's peroxidation as an advanced oxidation technique. *Journal of Hazardous Materials* **2003,** *98*, (1-3), 33-50.

213. Nfodzo, P.; Choi, H., Sulfate radicals destroy pharmaceuticals and personal care products. *Environmental Engineering Science* **2011,** *28*, (8), 605-609.

214. Ng, K. K.; Shi, X.; Ng, H. Y., Evaluation of system performance and microbial communities of a bioaugmented anaerobic membrane bioreactor treating pharmaceutical wastewater. *Water Research* **2015,** *81*, (Supplement C), 311-324.

215. Nielsen, J. L.; Nielsen, P. H., Microbial nitrate-dependent oxidation of ferrous iron in activated sludge. *Environmental Science & Technology* **1998,** *32*, (22), 3556-3561.

216. Noubactep, C., Comments on “Sorption of triazoles to soil and iron minerals” by Y. Jia et al. [Chemosphere 67 (2007) 250–258]. *Chemosphere* **2008,** *71*, (4), 802-806.

217. Noubactep, C., A critical review on the process of contaminant removal in Fe^0–H_2O systems. *Environmental Technology* **2008,** *29*, (8), 909-920.

218. Noubactep, C., The fundamental mechanism of aqueous contaminant removal by metallic iron. *Water Sa* **2010,** *36*, (5), 663-670.

219. Oaks, J. L.; Gilbert, M.; Virani, M. Z.; Watson, R. T.; Meteyer, C. U.; Rideout, B. A.; Shivaprasad, H. L.; Ahmed, S.; Chaudhry, M. J. I.; Arshad, M.; Mahmood, S.; Ali, A.; Khan, A. A., Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature* **2004,** *427*, (6975), 630-633.

220. Ociński, D.; Jacukowicz-Sobala, I.; Mazur, P.; Raczyk, J.; Kociołek-Balawejder, E., Water treatment residuals containing iron and manganese oxides for arsenic removal from water – Characterization of physicochemical properties and adsorption studies. *Chemical Engineering Journal* **2016,** *294*, (Supplement C), 210-221.

221. Ogier, J., *Fenton process for contaminant control: Investigation of OH radical formation with two water types*. KWR Watercycle Research Institute: Nieuwegein, **2008**.

222. Olmez-Hanci, T.; Arslan-Alaton, I.; Dursun, D., Investigation of the toxicity of common oxidants used in advanced oxidation processes and their quenching agents. *Journal of Hazardous Materials* **2014,** *278*, 330-335.

223. Olvera-Vargas, H.; Oturan, N.; Brillas, E.; Buisson, D.; Esposito, G.; Oturan, M. A., Electrochemical advanced oxidation for cold incineration of the pharmaceutical ranitidine: Mineralization pathway and toxicity evolution. *Chemosphere* **2014,** *117*, 644-651.

224. Olvera-Vargas, H.; Oturan, N.; Oturan, M. A.; Brillas, E., Electro-Fenton and solar photoelectro-Fenton treatments of the pharmaceutical ranitidine in pre-pilot flow plant scale. *Separation and Purification Technology* **2015,** *146*, 127-135.

225. Onesios, K. M.; Yu, J. T.; Bouwer, E. J., Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: A review. *Biodegradation* **2009,** *20*, (4), 441-466.

226. Oulhote, Y.; Mergler, D.; Barbeau, B.; Bellinger, D. C.; Bouffard, T.; Brodeur, M. E.; Saint-Amour, D.; Legrand, M.; Sauve, S.; Bouchard, M. F., Neurobehavioral function in school-age children exposed to manganese in drinking water. *Environmental Health Perspectives* **2014,** *122*, (12), 1343-1350.

227. Ouvrard, S.; Simonnot, M.-O.; Sardin, M., Reactive behavior of natural manganese oxides toward the adsorption of phosphate and arsenate. *Industrial & Engineering Chemistry Research* **2002,** *41*, (11), 2785-2791.

228. Pan, B. C.; Han, F. C.; Nie, G. Z.; Wu, B.; He, K.; Lu, L., New strategy to enhance phosphate removal from water by hydrous manganese oxide. *Environmental Science & Technology* **2014,** *48*, (9), 5101-5107.

229. Pankow, J. F., *Aquatic Chemistry Concepts*. Taylor & Francis: USA, **1991**.

230. Pantelidou, N. A.; Theologides, C. P.; Olympiou, G. G.; Savva, P. G.; Vasquez, M. I.; Costa, C. N., Catalytic removal of pharmaceutical compounds in water medium under an H_2 stream over various metal-supported catalysts: A promising process. *Desalination and Water Treatment* **2015,** *53*, (12), 3363-3370.

231. Park, H. S.; Koduru, J. R.; Choo, K. H.; Lee, B., Activated carbons impregnated with iron oxide nanoparticles for enhanced removal of bisphenol A and natural organic matter. *Journal of Hazardous Materials* **2015,** *286*, 315-324.

232. Park, W.; Nam, Y.-K.; Lee, M.-J.; Kim, T.-H., Anaerobic ammonia-oxidation coupled with Fe^{3+} reduction by an anaerobic culture from a piggery wastewater acclimated to NH_4^+/Fe^{3+} medium. *Biotechnology and Bioprocess Engineering* **2009,** *14*, (5), 680-685.

233. Parrella, A.; Lavorgna, M.; Criscuolo, E.; Russo, C.; Fiumano, V.; Isidori, M., Acute and chronic toxicity of six anticancer drugs on rotifers and crustaceans. *Chemosphere* **2014,** *115*, 59-66.

234. Paul, D.; Paul, K., Genotoxicity: A real concern of the pharmaceutical industry. *World Journal of Pharmacy and Pharmaceutical Sciences* **2014,** *4*, (2), 1124-1153.

235. Phenrat, T.; Long, T. C.; Lowry, G. V.; Veronesi, B., Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. *Environmental Science & Technology* **2009,** *43*, (1), 195-200.

236. Phillips, D. H.; Arlt, V. M., Genotoxicity: damage to DNA and its consequences. In *Molecular, Clinical and Environmental Toxicology: Volume 1: Molecular Toxicology*, Birkhäuser Basel: Basel, **2009**; pp 87-110.

237. Piepenbrock, A.; Schröder, C.; Kappler, A., Electron transfer from humic substances to biogenic and abiogenic Fe(III) oxyhydroxide minerals. *Environmental Science & Technology* **2014,** *48*, (3), 1656-1664.

238. Pignatello, J. J.; Oliveros, E.; MacKay, A., Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry. *Critical Reviews in Environmental Science and Technology* **2006,** *36*, (1), 1-84.

239. Postawa, A.; Hayes, C.; Criscuoli, A.; Macedonio, F.; Angelakis, A. N.; Rose, J. B.; Maier, A.; McAvoy, D. C., *Best practice guide on the control of iron and manganese in water supply*. IWA publishing: London, UK, **2013**.

240. Pradyot, P., Preliminary separation methods. In *Dean's analytical chemistry handbook, Second Edition*, McGraw Hill: **2004**.

241. Pruden, A.; Pei, R. T.; Storteboom, H.; Carlson, K. H., Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environmental Science & Technology* **2006,** *40*, (23), 7445-7450.

242. Qin, F.-X.; Jia, S.-Y.; Liu, Y.; Li, H.-Y.; Wu, S.-H., Adsorptive removal of bisphenol A from aqueous solution using metal-organic frameworks. *Desalination and Water Treatment* **2015,** *54*, (1), 93-102.

243. Quadra, G. R.; de Souza, H. O.; Costa, R. D.; Fernandez, M. A. D., Do pharmaceuticals reach and affect the aquatic ecosystems in Brazil? A critical review of current studies in a developing country. *Environmental Science and Pollution Research* **2017,** *24*, (2), 1200-1218.

244. Quinlivan, P. A.; Li, L.; Knappe, D. R. U., Effects of activated carbon characteristics on the simultaneous adsorption of aqueous organic micropollutants and natural organic matter. *Water Research* **2005,** *39*, (8), 1663-1673.

245. Rao, Y. F.; Qu, L.; Yang, H. S.; Chu, W., Degradation of carbamazepine by Fe(II)-activated persulfate process. *Journal of Hazardous Materials* **2014,** *268*, 23-32.

246. Ratke, L.; Voorhees, P. W., *Growth and coarsening: Ostwald ripening in material processing*. Springer Science & Business Media: Berlin, **2013**.

247. Raychoudhury, T.; Scheytt, T., Potential of zerovalent iron nanoparticles for remediation of environmental organic contaminants in water: A review. *Water Science and Technology* **2013,** *68*, (7), 1425-1439.

248. Remucal, C. K.; Ginder-Vogel, M., A critical review of the reactivity of manganese oxides with organic contaminants. *Environmental Science-Processes & Impacts* **2014,** *16*, (6), 1247-1266.

249. Renou, S.; Thomas, J. S.; Aoustin, E.; Pons, M. N., Influence of impact assessment methods in wastewater treatment LCA. *Journal of Cleaner Production* **2008,** *16*, (10), 1098-1105.

250. Rentz, J. A.; Turner, I. P.; Ullman, J. L., Removal of phosphorus from solution using biogenic iron oxides. *Water Research* **2009,** *43*, (7), 2029-2035.

251. Rey, A.; Faraldos, M.; Casas, J. A.; Zazo, J. A.; Bahamonde, A.; Rodriguez, J. J., Catalytic wet peroxide oxidation of phenol over Fe/AC catalysts: Influence of iron precursor and activated carbon surface. *Applied Catalysis B: Environmental* **2009,** *86*, (1-2), 69-77.

252. Rice, E. W.; Bridgewater, L.; American Public Health Association; American Water Works Association; Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater, 22nd Edition*. American Public Health Association: **2012**.

253. Rijnaarts, H. H. M.; Norde, W.; Bouwer, E. J.; Lyklema, J.; Zehnder, A. J. B., Reversibility and mechanism of bacterial adhesion. *Colloids and Surfaces B: Biointerfaces* **1995,** *4*, (1), 5-22.

254. Rijnaarts, H. H. M.; Norde, W.; Lyklema, J.; Zehnder, A. J. B., DLVO and steric contributions to bacterial deposition in media of different ionic strengths. *Colloids and Surfaces B: Biointerfaces* **1999,** *14*, (1-4), 179-195.

255. Rivera-Utrilla, J.; Sanchez-Polo, M.; Ferro-Garcia, M. A.; Prados-Joya, G.; Ocampo-Perez, R., Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere* **2013,** *93*, (7), 1268-1287.

256. Rodriguez-Alvarez, T.; Rodil, R.; Quintana, J. B.; Trinanes, S.; Cela, R., Oxidation of non-steroidal anti-inflammatory drugs with aqueous permanganate. *Water Research* **2013,** *47*, (9), 3220-3230.

257. Rodríguez, R.; Espada, J. J.; Pariente, M. I.; Melero, J. A.; Martínez, F.; Molina, R., Comparative life cycle assessment (LCA) study of heterogeneous and homogenous Fenton processes for the treatment of pharmaceutical wastewater. *Journal of Cleaner Production* **2016,** *124*, (Supplement C), 21-29.

258. Roig, B., *Pharmaceuticals in the environment: Current Knowledge and need assessment to reduce presence and impact*. IWA Publishing: USA, **2010**.

259. Ruamchat, T.; Hayashi, R.; Ngamprasertsith, S.; Oshima, Y., A novel on-site system for the treatment of pharmaceutical laboratory wastewater by supercritical water oxidation. *Environmental sciences: an international journal of environmental physiology and toxicology* **2005,** *13*, (5), 297-304.

260. Rubert, K. F.; Pedersen, J. A., Kinetics of oxytetracycline reaction with a hydrous manganese oxide. *Environmental Science & Technology* **2006,** *40*, (23), 7216-7221.

261. Salas, E. C.; Berelson, W. M.; Hammond, D. E.; Kampf, A. R.; Nealson, K. H., The influence of carbon source on the products of dissimilatory iron reduction. *Geomicrobiology Journal* **2009,** *26*, (7), 451-462.

262. Samaras, V. G.; Stasinakis, A. S.; Thomaidis, N. S.; Mamais, D.; Lekkas, T. D., Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. *Bioresource Technology* **2014,** *162*, 365-372.

263. Sanderson, H.; Brain, R. A.; Johnson, D. J.; Wilson, C. J.; Solomon, K. R., Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones. *Toxicology* **2004,** *203*, (1-3), 27-40.

264. Santiago, A. F. J.; Sousa, J. F.; Guedes, R. C.; Jeronimo, C. E. M.; Benachour, M., Kinetic and wet oxidation of phenol catalyzed by non-promoted and potassium-promoted manganese/cerium oxide. *Journal of Hazardous Materials* **2006,** *138*, (2), 325-330.

265. Santosa, I.; Grossmana, M. J.; Sartorattob, A.; Ponezib, A. N.; Durranta, L. R., Degradation of the recalcitrant pharmaceuticals carbamazepine and 17α-ethinylestradiol by ligninolytic fungi. *Chemical Engineering* **2012,** *27*, 169-174.

266. Sanz, J. L.; Köchling, T., Molecular biology techniques used in wastewater treatment: An overview. *Process Biochemistry* **2007,** *42*, (2), 119-133.

267. Sawayama, S., Possibility of anoxic ferric ammonium oxidation. *Journal of Bioscience and Bioengineering* **2006,** *101*, (1), 70-72.

268. Schmidt, N.; Page, D.; Tiehm, A., Biodegradation of pharmaceuticals and endocrine disruptors with oxygen, nitrate, manganese (IV), iron (III) and sulfate as electron acceptors. *Journal of Contaminant Hydrology* **2017,** *203*, 62-69.

269. Schmoll, O.; Howard, G.; Chilton, J.; Chorus, I., *Protecting groundwater for health: Managing the quality of drinking-water sources*. IWA Publishing: UK, **2006**.

270. Segura, Y.; Martinez, F.; Melero, J. A., Effective pharmaceutical wastewater degradation by Fenton oxidation with zero-valent iron. *Applied Catalysis B: Environmental* **2013,** *136*, 64-69.

271. Segura, Y.; Martinez, F.; Melero, J. A.; Fierro, J. L. G., Zero valent iron (ZVI) mediated Fenton degradation of industrial wastewater: Treatment performance and characterization of final composites. *Chemical Engineering Journal* **2015,** *269*, 298-305.

272. Senko, J. M.; Dewers, T. A.; Krumholz, L. R., Effect of oxidation rate and Fe(II) state on microbial nitrate-dependent Fe(III) mineral formation. *Applied and Environmental Microbiology* **2005,** *71*, (11), 7172-7177.

273. Shah, N. S.; He, X. X.; Khan, H. M.; Khan, J. A.; O'Shea, K. E.; Boccelli, D. L.; Dionysiou, D. D., Efficient removal of endosulfan from aqueous solution by UV-C/peroxides: A comparative study. *Journal of Hazardous Materials* **2013,** *263*, 584-592.

274. Sharma, V. K.; Kazama, F.; Jiangyong, H.; Ray, A. K., Ferrates (iron (VI) and iron (V)): environmentally friendly oxidants and disinfectants. *Journal of water and health* **2005,** *3*, (1), 45-58.

275. Shemer, H.; Kunukcu, Y. K.; Linden, K. G., Degradation of the pharmaceutical metronidazole via UV, Fenton and photo-Fenton processes. *Chemosphere* **2006,** *63*, (2), 269-276.

276. Shin, J. Y.; Cheney, M. A., Abiotic transformation of atrazine in aqueous suspension of four synthetic manganese oxides. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2004,** *242*, (1-3), 85-92.

277. Shirazi, E.; Torabian, A.; Nabi-Bidhendi, G., Carbamazepine removal from groundwater: Effectiveness of the TiO_2/UV, nanoparticulate zero-valent iron, and fenton (nZVI/H_2O_2) processes. *Clean-Soil Air Water* **2013,** *41*, (11), 1062-1072.

278. Silverstein, R. M.; Webster, F. X.; Kiemle, D. J.; Bryce, D. L., *Spectrometric identification of organic compounds*. John wiley & sons: USA, **2014**.

279. Silvy, N. J., *The Wildlife Techniques Manual*. Johns Hopkins University Press: USA, **2012**; Vol. 1.

280. Simazaki, D.; Kubota, R.; Suzuki, T.; Akiba, M.; Nishimura, T.; Kunikane, S., Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Research* **2015,** *76*, 187-200.

281. Singh, K. K.; Senapati, K. K.; Borgohain, C.; Sarma, K. C., Newly developed Fe_3O_4-Cr_2O_3 magnetic nanocomposite for photocatalytic decomposition of 4-chlorophenol in water. *Journal of Environmental Sciences* **2017,** *52*, 333-340.

282. Sires, I.; Garrido, J. A.; Rodriguez, R. M.; Brillas, E.; Oturan, N.; Oturan, M. A., Catalytic behavior of the Fe^{3+}/Fe^{2+} system in the electro-Fenton degradation of the antimicrobial chlorophene. *Applied Catalysis B: Environmental* **2007,** *72*, (3-4), 382-394.

283. Snyder, R. D.; Green, J. W., A review of the genotoxicity of marketed pharmaceuticals. *Mutation Research-Reviews* **2001,** *488*, (2), 151-169.

284. Snyder, V. A.; Akaiwa, N.; Alkemper, J.; Voorhees, P. W., The influence of temperature gradients on Ostwald ripening. *Metallurgical and Materials Transactions a-Physical Metallurgy and Materials Science* **1999,** *30*, (9), 2341-2348.

285. Sousa, J. M.; Macedo, G.; Pedrosa, M.; Becerra-Castro, C.; Castro-Silva, S.; Pereira, M. F. R.; Silva, A. M. T.; Nunes, O. C.; Manaia, C. M., Ozonation and UV_{254nm} radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. *Journal of Hazardous Materials* **2017,** *323*, (Part A), 434-441.

286. Spasiano, D.; Marotta, R.; Di Somma, I.; Andreozzi, R.; Caprio, V., Fe(III)-photocatalytic partial oxidation of benzyl alcohol to benzaldehyde under UV-solar simulated radiation. *Photochemical & Photobiological Sciences* **2013,** *12*, (11), 1991-2000.

287. Srinivasan, R.; Sorial, G. A., Treatment of taste and odor causing compounds 2-methyl isoborneol and geosmin in drinking water: A critical review. *Journal of Environmental Sciences* **2011,** *23*, (1), 1-13.

288. Stabnikov, V. P.; Tay, S. T. L.; Tay, D. K.; Ivanov, V. N., Effect of iron hydroxide on phosphate removal during anaerobic digestion of activated sludge. *Applied Biochemistry and Microbiology* **2004,** *40*, (4), 376-380.

289. Stadler, L. B.; Su, L. J.; Moline, C. J.; Ernstoff, A. S.; Aga, D. S.; Love, N. G., Effect of redox conditions on pharmaceutical loss during biological wastewater treatment using sequencing batch reactors. *Journal of Hazardous Materials* **2015,** *282*, 106-115.

290. Stasinakis, A. S., Review on the fate of emerging contaminants during sludge anaerobic digestion. *Bioresource Technology* **2012,** *121*, 432-440.

291. Straub, K. L.; Benz, M.; Schink, B., Iron metabolism in anoxic environments at near neutral pH. *Fems Microbiology Ecology* **2001,** *34*, (3), 181-186.

292. Straub, K. L.; Benz, M.; Schink, B.; Widdel, F., Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology* **1996,** *62*, (4), 1458-1460.

293. Stroes-Gascoyne, S.; Kramer, J. R.; Snodgrass, W. J., Preparation, characterization and aging of δ-MnO_2, for use in trace metal speciation studies. *Applied Geochemistry* **1987,** *2*, (2), 217-226.

294. Stumm, W.; Morgan, J. J., *Aquatic chemistry: Chemical equilibria and rates in natural waters*. John Wiley & Sons: Hoboken, NJ, 2013; Vol. 105.

295. Su, J.; Deng, L.; Huang, L.; Guo, S.; Liu, F.; He, J., Catalytic oxidation of manganese (II) by multicopper oxidase CueO and characterization of the biogenic Mn oxide. *Water Research* **2014,** *56*, (Supplement C), 304-313.

296. Sui, M. H.; Xing, S. C.; Sheng, L.; Huang, S. H.; Guo, H. G., Heterogeneous catalytic ozonation of ciprofloxacin in water with carbon nanotube supported manganese oxides as catalyst. *Journal of Hazardous Materials* **2012,** *227*, 227-236.

297. Sui, Q.; Cao, X.; Lu, S.; Zhao, W.; Qiu, Z.; Yu, G., Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. *Emerging Contaminants* **2015,** *1*, (1), 14-24.

298. Sun, J.; Luo, Q.; Wang, D. H.; Wang, Z. J., Occurrences of pharmaceuticals in drinking water sources of major river watersheds, China. *Ecotoxicology and Environmental Safety* **2015,** *117*, 132-140.

299. Tan, W. F.; Lu, S. J.; Liu, F.; Feng, X. H.; He, J. Z.; Koopall, L. K., Determination of the point-of-zero, charge of manganese oxides with different methods including an improved salt titration method. *Soil Science* **2008,** *173*, (4), 277-286.

300. Taylor, K. M. L.; Rieter, W. J.; Lin, W. B., Manganese-based nanoscale metal-organic frameworks for magnetic resonance imaging. *Journal of the American Chemical Society* **2008,** *130*, (44), 14358-+.

301. Tebo, B. M.; Bargar, J. R.; Clement, B. G.; Dick, G. J.; Murray, K. J.; Parker, D.; Verity, R.; Webb, S. M., Biogenic manganese oxides: Properties and mechanisms of formation. *Annual Review of Earth and Planetary Sciences* **2004,** *32*, 287-328.

302. Tekin, H.; Bilkay, O.; Ataberk, S. S.; Balta, T. H.; Ceribasi, I. H.; Sanin, F. D.; Dilek, F. B.; Yetis, U., Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater. *Journal of Hazardous Materials* **2006,** *136*, (2), 258-265.

303. Ternes, T.; Joss, A.; Oehlmann, J., Occurrence, fate, removal and assessment of emerging contaminants in water in the water cycle (from wastewater to drinking water). *Water Research* **2015,** *72*, 1-2.

304. Tiedeken, E. J.; Tahar, A.; McHugh, B.; Rowan, N. J., Monitoring, sources, receptors, and control measures for three European Union watch list substances of emerging concern in receiving waters - A 20 year systematic review. *Science of the Total Environment* **2017,** *574*, 1140-1163.

305. Tiwari, D.; Lee, S.-M., Ferrate (VI) in the treatment of wastewaters: A new generation green chemical. In *Waste Water-Treatment and Reutilization*, Einschlag, F. S. n. G., Ed. InTech: Available from: http://www.intechopen.com/books/waste-watertreatment-and-reutilization/ferrate-vi-in-the-treatment-of-wastewaters-a-new-generation-green-chemical, **2011**.

306. Tixier, C.; Singer, H. P.; Oellers, S.; Muller, S. R., Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environmental Science & Technology* **2003,** *37*, (6), 1061-1068.

307. Tobiason, J. E.; Bazilio, A.; Goodwill, J.; Mai, X.; Nguyen, C., Manganese removal from drinking water sources. *Current Pollution Reports* **2016,** *2*, (3), 168-177.

308. Todd, P. A.; Sorkin, E. M., Diclofenac Sodium - a Reappraisal of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Efficacy. *Drugs* **1988,** *35*, (3), 244-285.

309. Tong, A. Y. C.; Braund, R.; Warren, D. S.; Peake, B. M., TiO_2-assisted photodegradation of pharmaceuticals — A review. *Central European Journal of Chemistry* **2012,** *10*, (4), 989-1027.

310. Tratnyek, P. G.; Johnson, R. L., Nanotechnologies for environmental cleanup. *Nano Today* **2006,** *1*, (2), 44-48.

311. Trovo, A. G.; Nogueira, R. F. P.; Aguera, A.; Fernandez-Alba, A. R.; Sirtori, C.; Malato, S., Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation. *Water Research* **2009,** *43*, (16), 3922-3931.

312. Tu, J. J.; Yang, Z. D.; Hu, C.; Qu, J. H., Characterization and reactivity of biogenic manganese oxides for ciprofloxacin oxidation. *Journal of Environmental Sciences* **2014,** *26*, (5), 1154-1161.

313. Ukrainczyk, L.; Mcbride, M. B., Oxidation of phenol in acidic aqueous suspensions of manganese oxides. *Clays and Clay Minerals* **1992,** *40*, (2), 157-166.

314. US Environmental Protection Agency Contaminant Candidate List 3 - CCL 3. Retrieved from http://www2.epa.gov/ccl/contaminant-candidate-list-3-ccl-3#main-content (November 10),

315. Üzüm, Ç.; Shahwan, T.; Eroğlu, A. E.; Hallam, K. R.; Scott, T. B.; Lieberwirth, I., Synthesis and characterization of kaolinite-supported zero-valent iron nanoparticles and their application for the removal of aqueous Cu^{2+} and Co^{2+} ions. *Applied Clay Science* **2009,** *43*, (2), 172-181.

316. Veloutsou, S.; Bizani, E.; Fytianos, K., Photo-Fenton decomposition of beta-blockers atenolol and metoprolol; study and optimization of system parameters and identification of intermediates. *Chemosphere* **2014,** *107*, 180-186.

317. Verlicchi, P.; Al Aukidy, M.; Zambello, E., Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment* **2012,** *429*, (Supplement C), 123-155.

318. Verma, A. K.; Dash, R. R.; Bhunia, P., A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Journal of Environmental Management* **2012,** *93*, (1), 154-168.

319. Vieno, N.; Sillanpaa, M., Fate of diclofenac in municipal wastewater treatment plant - A review. *Environment International* **2014,** *69*, 28-39.

320. Villacis-Garcia, M.; Villalobos, M.; Gutierrez-Ruiz, M., Optimizing the use of natural and synthetic magnetites with very small amounts of coarse Fe(0) particles for reduction of aqueous Cr(VI). *Journal of Hazardous Materials* **2015,** *281*, 77-86.

321. Villalobos, M.; Sposito, G.; Bargar, J. R., Pb(II) reactivity on a biogenic Mn oxide: Evidence of inner-sphere bonding at internal and external sites. *Abstracts of Papers of the American Chemical Society* **2004,** *227*, U1217-U1217.

322. Villalobos, M.; Toner, B.; Bargar, J.; Sposito, G., Characterization of the manganese oxide produced by *Pseudomonas putida* strain MnB1. *Geochimica et Cosmochimica Acta* **2003,** *67*, (14), 2649-2662.

323. Villatoro-Monzon, W. R.; Mesta-Howard, A. M.; Razo-Flores, E., Anaerobic biodegradation of BTEX using Mn(IV) and Fe(III) as alternative electron acceptors. *Water Science and Technology* **2003,** *48*, (6), 125-131.

324. Vogel, T. M.; Criddle, C. S.; Mccarty, P. L., Transformations of halogenated aliphatic compounds. *Environmental Science & Technology* **1987,** *21*, (8), 722-736.

325. Wan, J.; Deng, H.; Shi, J.; Zhou, L.; Su, T., Synthesized magnetic manganese ferrite nanoparticles on activated carbon for sulfamethoxazole removal. *Clean – Soil, Air, Water* **2014,** *42*, (9), 1199-1207.

326. Wang, C. C.; Li, J. R.; Lv, X. L.; Zhang, Y. Q.; Guo, G. S., Photocatalytic organic pollutants degradation in metal-organic frameworks. *Energy & Environmental Science* **2014,** *7*, (9), 2831-2867.

327. Wang, H.; Yao, H.; Sun, P. Z.; Li, D. S.; Huang, C. H., Transformation of tetracycline antibiotics and Fe(II) and Fe(III) species induced by their complexation. *Environmental Science & Technology* **2016,** *50*, (1), 145-153.

328. Wang, H.; Yao, H.; Sun, P. Z.; Pei, J.; Li, D. S.; Huang, C. H., Oxidation of tetracycline antibiotics induced by Fe(III) ions without light irradiation. *Chemosphere* **2015,** *119*, 1255-1261.

329. Wang, J.; Sun, W.; Zhang, Z. H.; Jiang, Z.; Wang, X. F.; Xu, R.; Li, R. H.; Zhang, X. D., Preparation of Fe-doped mixed crystal TiO_2 catalyst and investigation of its sonocatalytic activity during degradation of azo fuchsine under ultrasonic irradiation. *Journal of Colloid and Interface Science* **2008,** *320*, (1), 202-209.

330. Wang, J.; Wang, S., Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. *Journal of Environmental Management* **2016,** *182*, (Supplement C), 620-640.

331. Wang, P.; Shi, Q. H.; Shi, Y. F.; Clark, K. K.; Stucky, G. D.; Keller, A. A., Magnetic permanently confined micelle arrays for treating hydrophobic organic compound contamination. *Journal of the American Chemical Society* **2009,** *131*, (1), 182-188.

332. Wang, S. B.; Wang, X. C., Multifunctional metal-organic frameworks for photocatalysis. *Small* **2015,** *11*, (26), 3097-3112.

333. Wang, Y. Q.; Cheng, R. M.; Wen, Z.; Zhao, L. J., Synthesis and characterization of single-crystalline $MnFe_2O_4$ ferrite nanocrystals and their possible application in water treatment. *European Journal of Inorganic Chemistry* **2011**, (19), 2942-2947.

334. Watts, R. J.; Sarasa, J.; Loge, F. J.; Teel, A. L., Oxidative and reductive pathways in manganese-catalyzed Fenton's reactions. *Journal of Environmental Engineering* **2005,** *131*, (1), 158-164.

335. Weelink, S. A. B.; van Doesburg, W.; Saia, F. T.; Rijpstra, W. I. C.; Roling, W. F. M.; Smidt, H.; Stams, A. J. M., A strictly anaerobic betaproteobacterium *Georgfuchsia toluolica* gen. nov., sp nov degrades aromatic compounds with Fe(III), Mn(IV) or nitrate as an electron acceptor. *Fems Microbiology Ecology* **2009,** *70*, (3), 575-585.

336. Wei, H.; Hu, D.; Su, J.; Li, K. B., Intensification of levofloxacin sono-degradation in a US/H_2O_2 system with Fe_3O_4 magnetic nanoparticles. *Chinese Journal of Chemical Engineering* **2015,** *23*, (1), 296-302.

337. Wen, X. H.; Jia, Y. N.; Li, J. X., Enzymatic degradation of tetracycline and oxytetracycline by crude manganese peroxidase prepared from *Phanerochaete chrysosporium*. *Journal of Hazardous Materials* **2010,** *177*, (1-3), 924-928.

338. Wilde, M. L.; Mahmoud, W. M. M.; Kümmerer, K.; Martins, A. F., Oxidation–coagulation of β-blockers by $K_2Fe^{VI}O_4$ in hospital wastewater: Assessment of degradation products and biodegradability. *Science of the Total Environment* **2013,** *452*, (Supplement C), 137-147.

339. Wilt, A. d.; He, Y.; Sutton, N. B.; Langenhoff, A. A. M.; Rijnaarts, H. H. M., Sorption and biodegradation of six pharmaceutically active compounds under four different redox conditions. **Submitted**.

340. World Health Organization, *Calcium and magnesium in drinking-water: Public health significance*. World Health Organization: Spain, **2009**.

341. World Health Organization, *Guidelines for drinking-water quality - 4th Edition*. IWA Publishing: Malta, **2011**.

342. World Health Organization, *Pharmaceuticals in drinking-water*. WHO Press: Switzerland, **2012**.

343. World Health Organization, *Antimicrobial resistance: global report on surveillance*. World Health Organization: France, **2014**.

344. Xia, S. Q.; Gu, Z. L.; Zhang, Z. Q.; Zhang, J.; Hermanowicz, S. W., Removal of chloramphenicol from aqueous solution by nanoscale zero-valent iron particles. *Chemical Engineering Journal* **2014,** *257*, 98-104.

345. Xing, Z. P.; Sun, D. Z., Treatment of antibiotic fermentation wastewater by combined polyferric sulfate coagulation, Fenton and sedimentation process. *Journal of Hazardous Materials* **2009,** *168*, (2-3), 1264-1268.

346. Xu, L.; Xu, C.; Zhao, M. R.; Qiu, Y. P.; Sheng, G. D., Oxidative removal of aqueous steroid estrogens by manganese oxides. *Water Research* **2008,** *42*, (20), 5038-5044.

347. Yan, J. C.; Lei, M.; Zhu, L. H.; Anjum, M. N.; Zou, J.; Tang, H. Q., Degradation of sulfamonomethoxine with Fe_3O_4 magnetic nanoparticles as heterogeneous activator of persulfate. *Journal of Hazardous Materials* **2011,** *186*, (2-3), 1398-1404.

348. Yan, Q.; Gao, X.; Chen, Y. P.; Peng, X. Y.; Zhang, Y. X.; Gan, X. M.; Zi, C. F.; Guo, J. S., Occurrence, fate and ecotoxicological assessment of pharmaceutically active compounds in wastewater and sludge from wastewater treatment plants in Chongqing, the Three Gorges Reservoir Area. *Science of the Total Environment* **2014,** *470*, 618-630.

349. Yang, B.; Ying, G. G.; Zhao, J. L.; Liu, S.; Zhou, L. J.; Chen, F., Removal of selected endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) during ferrate(VI) treatment of secondary wastewater effluents. *Water Research* **2012,** *46*, (7), 2194-2204.

350. Yang, L.; Hu, C.; Nie, Y. L.; Qu, J. H., Catalytic ozonation of selected pharmaceuticals over mesoporous alumina-supported manganese oxide. *Environmental Science & Technology* **2009,** *43*, (7), 2525-2529.

351. Yang, Y.; Ok, Y. S.; Kim, K.-H.; Kwon, E. E.; Tsang, Y. F., Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Science of the Total Environment* **2017,** *596*, (Supplement C), 303-320.

352. Yao, W. S.; Millero, F. J., Adsorption of phosphate on manganese dioxide in seawater. *Environmental Science & Technology* **1996,** *30*, (2), 536-541.

353. Yao, Y. J.; Cai, Y. M.; Lu, F.; Wei, F. Y.; Wang, X. Y.; Wang, S. B., Magnetic recoverable $MnFe_2O_4$ and $MnFe_2O_4$-graphene hybrid as heterogeneous catalysts of peroxymonosulfate activation for efficient degradation of aqueous organic pollutants. *Journal of Hazardous Materials* **2014,** *270*, 61-70.

354. Ye, Y.; Feng, Y.; Bruning, H.; Yntema, D.; Rijnaarts, H. H. M., Photocatalytic degradation of metoprolol by TiO_2 nanotube arrays and UV-LED: Effects of catalyst properties, operational parameters, commonly present water constituents, and photo-induced reactive species. *Applied Catalysis B: Environmental* **2018,** *220*, (Supplement C), 171-181.

355. Zelmanov, G.; Semiat, R., Iron (3) oxide-based nanoparticles as catalysts in advanced organic aqueous oxidation. *Water Research* **2008,** *42*, (1), 492-498.

356. Zelmanov, G.; Semiat, R., Iron (2,3) oxides based nano-particles as catalysts in advanced organic aqueous oxidation. *Desalination and Water Treatment* **2009,** *6*, (1-3), 190-191.

357. Zhan, W.; Wang, X. C.; Li, D. S.; Ren, Y. Z.; Liu, D. Q.; Kang, J. X., Catalytic wet air oxidation of high concentration pharmaceutical wastewater. *Water Science and Technology* **2013,** *67*, (10), 2281-2286.

358. Zhang, H.; Huang, C. H., Adsorption and oxidation of fluoroquinolone antibacterial agents and structurally related amines with goethite. *Chemosphere* **2007,** *66*, (8), 1502-1512.

359. Zhang, H. C.; Chen, W. R.; Huang, C. H., Kinetic modeling of oxidation of antibacterial agents by manganese oxide. *Environmental Science & Technology* **2008,** *42*, (15), 5548-5554.

360. Zhang, H. C.; Huang, C. H., Oxidative transformation of triclosan and chlorophene by manganese oxides. *Environmental Science & Technology* **2003,** *37*, (11), 2421-2430.

361. Zhang, H. C.; Huang, C. H., Oxidative transformation of fluoroquinolone antibacterial agents and structurally related amines by manganese oxide. *Environmental Science & Technology* **2005,** *39*, (12), 4474-4483.

362. Zhang, H. C.; Huang, C. H., Reactivity and transformation of antibacterial N-oxides in the presence of manganese oxide. *Environmental Science & Technology* **2005,** *39*, (2), 593-601.

363. Zhang, L.; Hendrickx, T. L. G.; Kampman, C.; Temmink, H.; Zeeman, G., Co-digestion to support low temperature anaerobic pretreatment of municipal sewage in a UASB–digester. *Bioresource Technology* **2013,** *148*, (Supplement C), 560-566.

364. Zhang, L.; Hu, J.; Zhu, R.; Zhou, Q.; Chen, J., Degradation of paracetamol by pure bacterial cultures and their microbial consortium. *Applied Microbiology and Biotechnology* **2013,** *97*, (8), 3687-3698.

365. Zhang, L. L.; Tu, J. J.; Lyu, L.; Hu, C., Enhanced catalytic degradation of ciprofloxacin over Ce-doped OMS-2 microspheres. *Applied Catalysis B: Environmental* **2016,** *181*, 561-569.

366. Zhang, M.; Zheng, P.; Li, W.; Wang, R.; Ding, S.; Abbas, G., Performance of nitrate-dependent anaerobic ferrous oxidizing (NAFO) process: A novel prospective technology for autotrophic denitrification. *Bioresource Technology* **2015,** *179*, 543-548.

367. Zhang, W.; Huang, M.-h.; Qi, F.-f.; Sun, P.-z.; Van Ginkel, S. W., Effect of trace tetracycline concentrations on the structure of a microbial community and the development of tetracycline resistance genes in sequencing batch reactors. *Bioresource Technology* **2013,** *150*, (Supplement C), 9-14.

368. Zhang, Y.; Yang, Y.; Zhang, Y.; Zhang, T.; Ye, M., Heterogeneous oxidation of naproxen in the presence of α-MnO_2 nanostructures with different morphologies. *Applied Catalysis B: Environmental* **2012,** *127*, (Supplement C), 182-189.

369. Zhang, Y. J.; Geissen, S. U.; Gal, C., Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere* **2008,** *73*, (8), 1151-1161.

370. Zhao, H.; Cui, H.-J.; Fu, M.-L., Synthesis of core-shell structured Fe_3O_4@α-MnO_2 microspheres for efficient catalytic degradation of ciprofloxacin. *RSC Advances* **2014,** *4*, (74), 39472-39475.

371. Zheng, Y. In *Pretreatment of pharmaceutical wastewater by catalytic wet air oxidation (CWAO)*, Water Resource and Environmental Protection (ISWREP), 2011 International Symposium, IEEE: **2011**; pp 1316-1318.

372. Zhou, G.-W.; Yang, X.-R.; Li, H.; Marshall, C. W.; Zheng, B.-X.; Yan, Y.; Su, J.-Q.; Zhu, Y.-G., Electron shuttles enhance anaerobic ammonium oxidation coupled to iron (III) reduction. *Environmental Science & Technology* **2016,** *50*, (17), 9298-9307.

373. Zhou, M. H.; Yu, J. G.; Cheng, B., Effects of Fe-doping on the photocatalytic activity of mesoporous TiO_2 powders prepared by an ultrasonic method. *Journal of Hazardous Materials* **2006,** *137*, (3), 1838-1847.

374. Zhou, N. A.; Lutovsky, A. C.; Andaker, G. L.; Ferguson, J. F.; Gough, H. L., Kinetics modeling predicts bioaugmentation with Sphingomonad cultures as a viable technology for enhanced pharmaceutical and personal care products removal during wastewater treatment. *Bioresource Technology* **2014,** *166*, (Supplement C), 158-167.

375. Zhou, T.; Lim, T. T.; Li, Y. Z.; Lu, X. H.; Wong, F. S., The role and fate of EDTA in ultrasound-enhanced zero-valent iron/air system. *Chemosphere* **2010,** *78*, (5), 576-582.

376. Zhou, Y. M.; Gao, B.; Zimmerman, A. R.; Chen, H.; Zhang, M.; Cao, X. D., Biochar-supported zerovalent iron for removal of various contaminants from aqueous solutions. *Bioresource Technology* **2014,** *152*, 538-542.

377. Zhu, W.; Nan, Y.; Huang, T.; Wu, F., The mechanism, thermodynamic and kinetic characteristics of the microbial reduction of goethite mediated by anthraquinone-2-sulfonate. *Geomicrobiology Journal* **2013,** *30*, (10), 928-940.

378. Zou, L. Y.; Li, Y.; Hung, Y.-T., Wet air oxidation for waste treatment. In *Advanced Physicochemical Treatment Technologies*, Humana Press: Totowa, NJ, **2007**; pp 575-610.

379. Zouboulis, A. I.; Moussas, P. A.; Vasilakou, E., Polyferric sulphate: Preparation, characterisation and application in coagulation experiments. *Journal of Hazardous Materials* **2008,** *155*, (3), 459-468.

Appendices

摘要

水体中的药品残留物主要来自于制药工业和公众的日常使用。这种污染物在地表水，地下水，城市污水和饮用水中以极低的浓度存在。目前，已经有水中药品残留物及其代谢产物不利影响的研究，包括对人类和生态系统的毒性，以及耐药性。为了防止这些不利的影响，需要通过各种技术手段去除水体中的药品残留物，包括使用在有氧气存在（好氧）和没有氧气存在（厌氧）环境下的非生物去除和生物降解技术。厌氧技术通常更具有可持续性和吸引力，因为这些技术与好氧技术相比能耗更低，产生的诸如温室气体等的污染物更少。利用锰和铁的厌氧降解技术在饮用水处理和污水处理过程中具有明显的优势。研究利用锰和铁的厌氧技术去除水中的药品残留物，并将其发展成一项水处理工艺很有前景。本论文研究了利用锰和铁处理水中药品残留物的厌氧技术，包括生物降解和非生物去除。论文第一章主要阐述了本论文研究的必要性和动机。

论文第二章对能够去除水中药品残留物的与锰和铁相关的技术进行了回顾。根据出去机理，这些技术被分为了 3 类：物理化学技术，化学技术，以及生物相关技术。之前的研究表明，利用锰和铁的相关技术能够高效的除去水中的药品残留物，去除效率因技术而异。之后我对各项技术的非特异性、处理条件、产生的中间产物和副产物以及含锰和铁的化合物的影响进行比较，对于这些技术的优缺点进行了评估。第二章还介绍了一些能够在将来用于药品残留物去除的新兴锰和铁相关技术。其中异化锰铁还原技术因其的运行条件接近自然条件，并且最终能够将污染物完全矿化等优势，而成为极具吸引力，可持续性，以及低成本的药品残留物处理技术。

论文第三章主要研究了利用异化金属还原出去药品的厌氧生物降解，以及不同类型的锰和铁对药品生物降解的影响。此研究中使用的微生物是经过美特普洛和化学方法制备的四价锰驯化的底泥混合物。这些微生物能够在 42 天内利用化学制备的无定形态的四价锰去除 26%的咖啡因和 52%的萘普生。此外，这些微生物还能利用给水厂产生的四价锰。经过 196 天的培养，这些微生物能够去除 96%的美特普

洛和 31%的普萘洛尔。这些微生物同样能够利用三价铁作为电子受体去除美特普洛。实验结果表明这些微生物利用不可溶性的化学制备的三价铁和可溶性的柠檬酸铁取得 57%和 52%的去除率。在整个研究过程中，所有实验的非生物对照组都没有明显的药品残留物的去除，这表明生物降解是利用异化金属还原出去药品厌氧技术的主要机理。

论文第四章比较了好氧环境和厌氧环境对于利用二氧化锰出去药品残留物的影响。结果表明厌氧环境能够促进双氯芬酸的去除，但却会抑制美特普洛和普萘洛尔的去除。在纯水中，厌氧环境下二氧化锰对双氯芬酸的去除率为 78%，高于好氧环境下的 59%。在 50mM 的磷酸盐缓冲液中，二氧化锰能够在好氧环境下将双氯芬酸完全去除，而其在厌氧环境下的去除率与在纯水中没有区别。初步研究表明，酸性条件（pH 4-5）对二氧化锰厌氧去除双氯芬酸有利。同时，相比于二氧化锰晶体，无定形态的二氧化锰更适合用于双氯芬酸的去除。有上述结果得知，药品的化学结构和性质，二氧化锰的性质以及二氧化锰的表面活性位是决定厌氧环境下利用二氧化锰对药品的出去了程度。

论文第五章进一步研究了厌氧环境下利用二氧化锰去除双氯芬酸的过程。实验结果表明，将实验温度从 10°C 提高到 30°C 能够提高双氯芬酸的去除。然而当温度再次提升到 40°C 时，双氯芬酸的出去收到了抑制。这可能是由奥氏熟化，或者老化，或者两者共同造成的。增加二氧化锰的相对量能够提高双氯芬酸的去除。但是当二氧化锰与双氯芬酸的物质的量的比从 2200：1 提高到 8900：1 时，双氯芬酸的去除没有进一步增加。这可能是由于二氧化锰氧化双氯芬酸的能力有限。金属离子对双氯芬酸的去除有强烈的抑制作用，并遵循以下顺序：$Mn^{2+} > Ca^{2+} \approx Mg^{2+} > Fe^{3+}$。 金属离子能够吸附在二氧化锰的表面，并和双氯芬酸竞争二氧化锰表面的活性位。磷酸盐对双氯芬酸的去除有不同的作用：在低浓度时，磷酸盐回抑制双氯芬酸的去除，而在高浓度时回促进去除。腐殖酸能够显著的提高二氧化锰对双氯芬酸的去除。只是由于腐殖酸能够释放二氧化锰表面被其他物质占据的活性位，并激活新的活性位。

为了能够将药品去除过程中使用过的锰和铁再次使用，论文第六章研究了限制氧气浓度环境下四价锰的生物产生过程，以及硝酸盐还原条件下三价铁的生物产生过程。在限制氧气浓度的条件下，二价锰氧化菌成功产生出四价锰，并且产生的四价锰固体为无定形态的。二价锰氧化菌不能降解药品物质。在非生物去除部分，厌氧环境中，给水厂产生的四价锰能够 有效的去除美特普洛和普萘洛尔。通过生物过程，三价铁也曾成功的在硝酸盐还原条件下制备。这种三价铁固体也是无定形态的。在此生物铁氧化过程中，没有发现药品的去除。对比研究中使用的各种三价铁化合物，只有给水厂生产的三价铁和一种三价铁吸附剂能够去除普萘洛尔。

论文的最后一章对本论文的实验结果进行了讨论，并且对利用锰和铁处理水中药品残留物的厌氧技术的实际应用提供了见解（第七章）。利用锰和铁处理水中药品残留物的机理主要包括吸附，化学氧化和生物降解。在不通的处理技术中，各种机理对药品残留物去除的贡献不同。对于不同去除过程的评估比较主要基于对药品残留物的去除效果，环境状态和运行条件，去除过程的可持续性，以及不同锰和铁化合物的应用。本论文的实验结果证明，利用锰和铁处理水中的厌氧技术可以去除水中的药品残留物。为了将这一过程转化为实际的水处理单元，未来的研究需要经过三个步骤：（1）探索利用锰和铁处理水中药品残留物的厌氧技术的极限；（2）在可控范围内进行小规模的实验模拟；（3）中试实验。此外，论文中也提出了一些其他相关的研究课题。总的来说，利用锰和铁的厌氧技术可以通过生物和非生物的过程去除水体中的药品残留物。这一过程有希望发展成高效，经济，环境友好并且可持续发展的水中药品残留物去除技术。

Publications

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Pharmaceutical removal from water with iron- or manganese-based technologies: A review. *Critical Reviews in Environmental Science and Technology* 2016, *46*, (19-20), 1584-1621.

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anoxic conditions are favourable for abiotic diclofenac removal from water with manganese oxides. Submitted.

Liu, W.; Langenhoff, A. A. M.; Sutton, N. B.; Rijnaarts, H. H. M., Application of manganese oxides under anoxic conditions to remove diclofenac from water. Submitted.

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M., Anaerobic biodegradation of pharmaceutical compounds coupled to dissimilatory manganese (IV) or iron (III) reduction. In preparation.

Li, H.; Peng, D.; Liu, W.; Wei, J.; Wang, Z.; Wang, B., N_2O generation and emission from two biological nitrogen removal plants in China. *Desalination and Water Treatment* 2016, 57, (25), 11800-11806

Presentations

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M. (2017) "Diclfoenac removal under anoxic condition with manganese oxides" 10th Micropol and Ecohazard Conference 2017, September 17-20, 2017, Vienna, Austria (Oral)

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M. (2016), "Under anoxic condition, abiotic removal of pharmaceuticals with MnO_2" The China-Netherlands Environmental Technology Symposium & Doctoral Forum, June 12-13, 2016, Xi'an, China (Oral)

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M. (2016) "Pharmaceutical removal from water with Fe(III) or Mn(IV)", The CHINED-4D Environmental Solutions Symposium, June 6-7, 2016, Beijing, China (Oral and Poster)

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M. (2015) "Pharmaceutical removal from water with Fe(III) or Mn(IV)" 9th Micropol and Ecohazard Conference 2015, November 22-25, 2017, Singapore, Singapore (Poster)

Liu, W.; Sutton, N. B.; Rijnaarts, H. H. M.; Langenhoff, A. A. M. (2015) "Pharmaceutical removal from water with Fe(III) or Mn(IV)", Environmental Technology for Impact 2015, April 29-30, 2015, Wageningen, the Netherlands (Oral)

Acknowledgement

At this time when I am finishing my PhD, I can't help to recalling my life in the Netherlands. I am not able to complete the whole work myself without help from my promoter and supervisors, my colleagues, my friends, and my family.

My first appreciation goes to my supervision team, the “Golden Triangel” – Huub, Alette, and Nora. Huub, you are the person who shows me what a scientist is like and teaches me how to think and work as an independent scientist. Your unique capacity to identify critical information about my research, and sharp view of the top science have contributed significantly to my development over the last four years. Thank you for your tremendous support and your guidance to my career. Alette, from the start of my PhD work you gave me space to plan and run the project independently, supporting my practical work as well as my life in the Netherlands, believing in my ability to take responsibilities on managing my project. Thank you for helping me develop as a qualified researcher. I also thank you for starting the amazing group dinner, in which I always had delicious food and tons of fun. Nora, thank you for your supporting me in my experiments and my research work when Alette is absent. After you officially became me co-promoter, your knowledge and intellect impressed me. I have learned a lot from you not only on research but also on how to enjoy the life. I would also like to thank Thomas ter Laak from KWR for his supervision and support at the beginning of my PhD.

This thesis would not be possible without a strong support from the ETE laboratory team. I appreciate the help from Hans, Jan Kubiak, Livio, Jean, Ilse, Katja, Vinnie, and Bert. All of you have helped to brainstorm the solutions to all of challenges in my experiments. I would like to thank all my and students, Yanbo, Menghan, Lydia, Yiyuan, Marijn, Rebecca, and Julia, for their work in the laboratory which contribute directly or indirectly to the content of this thesis. Thank you very much!

Without people in ETE, it is impossible for me to complete the thesis and propositions so smoothly. First of all, I would like to thank Liesbeth, Gea, and other members of secretary team for their support of my PhD project from the administration side. You helped me organize all the meetings, rooms, and most important, all the money.

The atmosphere of "togetherness" at ETE is important to the success of my PhD. I would like to first thank the people in the micro-pollutant team: Arnoud, Thomas, Yujie, Yin, Yujia, Andrii, AndreaBrunsch, Luara, Andrea Aldas Vargas, and Elackiya for their irreplacable support and conversations in the lab and in the meeting. I would also like to acknowledge all of my great office mates, lunch friends, borrel buddies, and other ETEers. I enjoy the food, the beer, the talk, as well as the department trip because of you: Aken, Andrii, Annemerel, Annemiek, Bao Park, Bruna, Carlos, Casper, Celia, Chunjing, Daria, David, Delaram, Els, Emilius, Grietije, Gunther, Hardy, Indra, Jan Vreeburg, Jan Weijma, Jinye, Joeri Willet, Justine, Kanjana, Kasia, kasper, Koen, Laima, Lei, Leire, Lucia, Ludo, Miriam, Momo, Pim, Pradip, Renata, Rosaane, Rosaane, Roxani, Shenao, Shengle, Shiyang, Shoukou, Silivi, Tania, Tim, Viola, Yifan, Yingdi, Yvonne, Zhimou, Zhuobiao and other people I don’t mention here. Special appreciation goes to Danis, Sanne, Livio and Harry, without your inspiration, I am not able to submit my proposition and my thesis on time. My “paranimfen”, Dandan and Azie, thank you for willing to support me during my public defence.

I would like to appreciate my Chinese friend in WUR: Yifan Zhu, Shijiang, Ying, Huicui, Wenbiao, Tao, and Xiaoshen. Even we don’t meet very often, I always know that I can find you when I need. Thank you very much. I would like to appreciate my friends outside of Wageningen: Bao Li, Deqing, Heng, Lunqi, Yuexin, and Zhiyi. You distract me from the stress of continuous lab work and endless manuscript preparation and revisions. You help me find my true heart inside.

I would like to appreciate my friends in China. We are sharing the sweet and sad moments during the PhD. Special thanks go to Huijuan who helped me on the Chinese summary.

My family have given me huge support to help me survive the grey winter in the Netherlands, and every challenge in my life even though you don't understand my work at all. You always support me to chase my own goal, follow my own path, and being myself. You cares about me more than anything else in the world and that is the most valuable treasure in my life.

I would also like to thank Guolin Yang for the providing the cover of this thesis.

我要感谢我的家人。虽然你们不懂我的研究，但是你们仍旧全心全意的支持我。你们的支持让我成功度过荷兰寒冷灰暗的冬天，和生命中的每一个挑战。是你们支持我追求自己的目标，追寻自己的道路，最终实现自我价值。你们把我当成世界上最宝贵的财富，你们也是我生命中最宝贵的东西。

感谢杨国磷为本论文提供封面图片。

About the author

Wenbo Liu was born on September 11, 1987, in Qingdao, China. In 2006, he started to study *Environmental Engineering* at the Wuhan University of Technology. In 2010, he finished his thesis about "*Pollution loading evaluation from both Urban life and industrial sources and its forecast in the Hubei region of Three Gorges Reservoir area*." The work belonged to Major Science and Technology Program for Water Pollution Control and Treatment (2009ZX07104-001). Based on the methods provided in this work, two peer-reviewed papers were published in Chinese. In 2010, he started his MSc program in Xi'an University of Architecture and Technology, supervised by Professor Dangcong Peng. He studied the project about non-CO_2 greenhouse gas emission from wastewater treatment processes. Also, he worked on the topics of ammonia removal under anaerobic conditions. During his MSc period, he attended the "3rd International Symposium of the Environmental Leaders" and presented part of his work about using the iron to remove ammonia via an oral presentation. In 2013, his submitted his thesis titled "*Non-carbon dioxide greenhouse gas emission from different municipal wastewater treatment processes*". After successfully applied the scholarship from China Scholarship Council (CSC, File No. 201308610161), he started his PhD program in Environmental Technology, Wageningen University and Research in September 2013. In 2017, he finished his thesis about pharmaceutical degradation in manganese or iron-mediate systems.

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

D I P L O M A

For specialised PhD training

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Wenbo Liu

born on 11 September 1987 in Shandong, China

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Wageningen, 26 January 2017

the Chairman of the SENSE board	the SENSE Director of Education
Prof. dr. Huub Rijnaarts	Dr. Ad van Dommelen

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

The SENSE Research School declares that **Mr Wenbo Liu** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 38 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2014)
- SENSE writing week (2015)
- Research in context activity: 'Co-organizing the CHI-NED 4D Dialogue Seminar and a PhD study trip to China' (2016)

Other PhD and Advanced MSc Courses

- Project and time management, Wageningen University (2013)
- Teaching and supervising thesis students, Wageningen University (2014)
- Techniques for writing and presenting a scientific paper, Wageningen University (2014)
- Francqui inaugural and further lectures by David Sedlak, Ghent University (2015)
- Toxicant identification in water, sediment and biota, Helmholtz Interdisciplinary Graduate School for Environmental Research (2016)
- Presenting with impact, Wageningen University (2016)

Management and Didactic Skills Training

- Assisting practical of the BSc course 'Introduction Environmental Technology' (2015-2016)
- Supervising five MSc students with theses entitled:
  - 'Pharmaceutical Removal with Fe(III) or Mn(IV) from water' (2014)
  - 'Abiotic removal of pharmaceuticals with MnO_2'(2015)
  - 'Pharmaceutical removal with manganese oxides' (2015)
  - 'Bio-regeneration of Mn(IV) and Fe(III)' (2016)
  - 'Biodegradation of selected β-blockers by dissimilatory reduction of Mn(IV)' (2017)
- Supervising two BSc students with theses entitled:
  - 'Abiotic removal of pharmaceuticals with manganese as reactive compound' (2015)
  - 'The effects of co-solutes on pharmaceutical biodegradation with MnO_2' (2017)

Oral Presentations

- *Pharmaceutical removal from water with Fe(III) or Mn(IV)*. Environmental Technology for Impact, 29-30 April 2015, Wageningen, The Netherlands
- *Diclofenac removal with manganese oxides under anoxic conditions.* 10th Micropol and Ecohazard Conference, 17-20 September 2017, Vienna, Austria

SENSE Coordinator PhD Education

Dr. Monique Gulickx

The support provided by China Scholarship Council (CSC) for the research of Wenbo Liu at Wageninge University is kindly acknowledged.

Final support from Wageningen University for printing this thesis is gratefully acknowledged.

Photoes of cover and invitation is taken by Guolin Yang