NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTAMENT OF ENVIRONMENTAL SCIENCES AND ENGINEERING

Tiago André Eusébio Martins

Bachelor's in Environmental Engineering Sciences

# Micropollutants biotransformation under different redox conditions in *PhoRedox* conventional activated sludge systems

Master's in Environmental Engineering, Profile of Sanitary Engineering
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BSc in Environmental Engineering Sciences

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*Dedicated to all the people who have been with me in my darkest times and believed in me even when I did not. Also dedicated to the ones who left too soon and could not see this moment.*

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"*We are only as strong as we are united and as weak as we are divided*." - J. K. Rowling

"*Do not go where the path may lead, go instead where there is no path and leave a trail."* - Ralph Waldo Emerson

# ABSTRACT

The increasing consumption of pharmaceuticals and personal care products, as well as the modernization of the industrial processes, results in the production and use of different compounds that end up in wastewater treatment plants (WWTP). Even not being designed for that, WWTP biological treatment can remove part of the compounds from the wastewater, yet they release the rest into the aquatic system. These compound's degradation mechanisms and rates need to be studied to allow better design of the WWTP and assure higher removals. Therefore, this study focused on studying the removal efficiencies and biotransformation rates constant of sixteen micropollutants (4-, 5-methylbenzotriazole, azithromycin, benzotriazole, candesartan, carbamazepine, clarithromycin, diclofenac, gabapentin, hydrochlorothiazide, irbesartan, metoprolol, propranolol, sotalol, sulfamethoxazole, trimethoprim, and venlafaxine) under the three main redox conditions, throughout 48h batch test, with wastewater from Walcheren WWTP. Besides, it was also made a theoretical exercise that aim to assess the risk of different effluent discharges in different hypothetical environments. It was possible to observe that both anoxic and anaerobic redox conditions have shown higher removal rates, namely maximum removal efficiencies of 91% and 75%, respectively. However, no predominant redox condition promotes the biotransformation of all the micropollutants. Clarithromycin exhibited the highest aerobic (73%) and anaerobic removal (75%) efficiencies, while gabapentin showed the highest overall removal under anoxic conditions (91%) and all the experiments. According to the obtained results, clarithromycin is the micropollutant which it expected the greatest removal efficiency in a conventional activated sludge (CAS) system, due to the highest overall biotransformation rate constant (Aerobic: 1,46 $L.g_{SS}^{-1}.d^{-1}$; Anoxic: 0,90 $L.g_{SS}^{-1}.d^{-1}$; Anaerobic: 1,59 $L.g_{SS}^{-1}.d^{-1}$). At the same time, carbamazepine is the compound with the expected lowest removal, because it showed no removal under aerobic and anaerobic conditions and has a low anoxic biotransformation rate constant (0,07 $L.g_{SS}^{-1}.d^{-1}$). Under aerobic conditions, the biotransformation rates constant went up to 1,75 $L.g_{SS}^{-1}.d^{-1}$ in clarithromycin, while the lowest value obtained was -0,10 $L.g_{SS}^{-1}.d^{-1}$ in carbamazepine. Under anoxic conditions, the constant rates reached the highest value of 2,36 $L.g_{SS}^{-1}.d^{-1}$, in gabapentin, and the lowest value in candesartan with a constant rate of 0,03 $L.g_{SS}^{-1}.d^{-1}$. Under anaerobic conditions, the constant rate got the highest value of 1,87 $L.g_{SS}^{-1}.d^{-1}$ in clarithromycin and reached the lowest value of 0,11 $L.g_{SS}^{-1}.d^{-1}$ in 4-,5-methylbenzotriazole. The micropollutants removal in a PhoRedox CAS WWTP could be improved by optimizing the hydraulic retention time distribution in the design of the biological treatment process per redox condition. If a retrofit of the WWTP is a plan, changing the CAS for a membrane bioreactor (MBR) configuration might be the best solution. A toxicological assessment of the micropollutants with the current biological transformation and after implementing an advanced oxidation process (AOP) was conducted. It indicated that when the environmental concentrations are considerably high, the effluent discharge does not cause any significant change in the risk assessment, independently of the dilution factor, since the toxicity is already associated with the environmental concentration. Therefore, the dilution factor is more significant when the environmental concentration is low.

**Key Words:** Advanced Oxidation Process, Biological Treatment, Conventional Activated Sludge, Hydraulic retention time, Micropollutants, Pharmaceuticals, Toxicological Assessment, Wastewater

# RESUMO

O aumento do consumo de produtos farmacêuticos e de cuidados pessoais, bem como a modernização dos processos industriais, resulta na produção e utilização de diferentes compostos que acabam nas estações de tratamento de águas residuais (ETAR). Mesmo não sendo projetado para isso, o tratamento biológico da ETAR consegue remover parte dos micropoluentes das águas residuais. No entanto o remanescente acaba por ser descarregado no meio aquático. Assim, os mecanismos e taxas de degradação destes compostos precisam de ser estudados para permitir uma melhor conceção da ETAR e assegurar assim uma maior remoção. Por conseguinte, esta pesquisa centrou-se no estudo das eficiências de remoção e das taxas de biotransformação de dezasseis micropoluentes (4-, 5-methylbenzotriazole, azithromycin, benzotriazole, candesartan, carbamazepine, clarithromycin, diclofenac, gabapentin, hydrochlorothiazide, irbesartan, metoprolol, propranolol, sotalol, sulfamethoxazole, trimethoprim, and venlafaxine) sob as três principais condições redox, recorrendo a testes batch de 48h, com água residual da ETAR *Walcheren*. Além disso, foi feito um exercício teórico que visa avaliar o risco de descargas de diferentes efluentes, em diferentes ambientes hipotéticos. Foi possível observar que tanto em condições anóxicas como em anaeróbias as taxas de biotransformação são mais elevadas, nomeadamente eficiências máximas de remoção de 91% e 78%, respetivamente. No entanto, nenhuma condição redox promove predominantemente a biotransformação de todos os micropoluentes. A clarithromycin apresentou a maior eficiência de remoção aeróbia (73%) e também anaeróbia (78%), enquanto a gabapentin apresentou a maior remoção global, em condições anóxicas (91%). De acordo com os resultados obtidos, a clarithromycin é o micropoluente do qual se espera a maior eficiência de remoção num sistema CAS, devido à maior taxa global de biotransformação (Aeróbia: 1,46 $L.g_{SS}^{-1}.d^{-1}$; Anóxica: 0,90 $L.g_{SS}^{-1}.d^{-1}$; Anaeróbia: 1,59 $L.g_{SS}^{-1}.d^{-1}$). Ao mesmo tempo, prevê-se que a carbamazepine tenha a remoção mais baixa, principalmente por não apresentar remoção em condições aeróbias e anaeróbias e possuir uma baixa taxa de biotransformação anóxica (0,07$L.g_{SS}^{-1}.d^{-1}$). Em condições aeróbias, a maior taxa de biotransformação foi de 1,75 $L.g_{SS}^{-1}.d^{-1}$ na clarithromycin, e o valor mais baixo obtido foi de -0,10 $L.g_{SS}^{-1}.d^{-1}$ na carbamazepine. Em condições anóxicas, as taxas atingiram o valor mais elevado de 2,36 $L.g_{SS}^{-1}.d^{-1}$, na gabapentine, e o valor mais baixo no candesartan com uma taxa de 0,03 $L.g_{SS}^{-1}.d^{-1}$. Em condições anaeróbias, obteve-se o valor mais alto de 1,87$L.g_{SS}^{-1}.d^{-1}$ na clarithromycin e o valor mais baixo de 0,11 $L.g_{SS}^{-1}.d^{-1}$ no 4-, 5- methylbenzotriazole. A remoção de micropoluentes numa ETAR PhoRedox pode ser melhorada otimizando a distribuição dos tempos de retenção hidráulico na conceção do tratamento biológico, tendo em conta as condições redox. Contudo, se a adaptação da ETAR for o plano, mudar o tratamento biológico para uma configuração de biorreator com membrana (MBR) poderá ser a melhor solução. Os resultados da avaliação toxicológica dos micropoluentes com o cenário atual e após a implementação de um processo de oxidação avançada (AOP), indicam que quando as concentrações ambientais são consideravelmente elevadas, a descarga de efluentes não provoca alterações significativas na avaliação dos riscos, independentemente do fator de diluição, uma vez que a toxicidade já está associada à concentração ambiental. No entanto, o fator de diluição é significativo quando a concentração ambiental é baixa.

**Palavras-Chave:** Processo de Oxidação Avançado, Tratamento Biológico, Lamas Ativadas Convencionais, Tempo de Retenção Hidráulico, Micropoluentes, Medicamentos, Análise Toxicológica, Água Residual

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# ACRONYMS

| | |
|---|---|
| **AF** | Assessment Factor |
| **AGS** | Aerobic Granular Sludge |
| **AOB** | Ammonia Oxidizing Bacteria |
| **AOP** | Advanced Oxidation Process |
| **BNR** | Biological Nutrient Removal |
| **BOD** | Biological Oxygen Demand |
| **CAPEX** | Capital Expenditures |
| **CAS** | Conventional Activated Sludge |
| **COD** | Chemical Oxygen Demand |
| **DF** | Dilution Factor |
| **GAC** | Granular Activated Carbon |
| **HRT** | Hydraulic Retention Time |
| **IEX** | Ion Exchange |
| **MBBR** | Moving Bed Bioreactor |
| **MBR** | Membrane Bioreactor |
| **MEC** | Measured Environmental Concentrations |
| **MLE** | Modified Ludzack-Ettinger |
| **NOB** | Nitrite Oxidizing Bacteria |

| | |
|---|---|
| **NOEC** | No Observed Effect Concentration |
| **OMP** | Organic Micropollutant |
| **OPEX** | Operation Expenditures |
| **PAC** | Powder Activated Carbon |
| **PAO** | Phosphorus Accumulating Organisms |
| **PNEC** | Predicted No Effect Concentration |
| **RO** | Reverse Osmosis |
| **RQ** | Risk Quotient |
| **SRT** | Sludge Retention Time |
| **TC** | Total Carbon |
| **TIC** | Total inorganic Carbon |
| **TOC** | Total Organic Carbon |
| **TRL** | Technology Readiness Level |
| **TSS** | Total Suspended Solids |
| **WWTP** | Wastewater Treatment Plant |

# SYMBOLS

| | |
|---|---|
| $K_{bio}$ | Biotransformation Constant Rate |
| $K_d$ | Solid-Water Distribution Coefficient |
| $K_{OW}$ | Octanol-Water Partitioning Coefficient |
| $X_{ss}$ | Suspended Solids |

# 1

# INTRODUCTION

## 1.1 Problem Statement and Relevance

Increasing attention has been paid to the presence of micropollutants in aquatic environments and their removal in wastewater treatment plants (WWTP). This increasing attention is connected to the fact that due to global warming and water scarcity, the number of water sources will decrease during the following years, becoming a significant growth-limiting factor for some regions worldwide. Therefore it is now, more than ever, essential to take care of the surficial water resources so they can be as unpolluted as possible and easily treatable to supply (Figuiere *et al.*, 2022).

Many pharmaceuticals, personal care products, detergents, and disinfectants are used daily in households. After their use, most of these organic compounds enter the domestic sewage and go to the WWTP (Eggen *et al.*, 2014; Joss *et al.*, 2006; Kennes-Veiga *et al.*, 2022; Rogowska *et al.*, 2020). Only in Switzerland, in 2013, more than 3 000 pharmaceutical compounds were commercially available (Margot *et al.*, 2013). Most of those have a bioavailability lower than 80%, meaning that the non-absorbed part will end up in the WWTP throughout body excretions (e.g., faeces and urine). For example, the bioavailability of diclofenac is around 65%, meaning that only this percentage will enter the blood flow and the rest excreted by the body (Hinz *et al.*, 2005). However, this is not the only way that micropollutant enters the sewage, the disposal of drugs through toilet flush has also been a known problem (Fernández *et al.*, 2014; Freitas & Radis-Baptista, 2021; Shipingana *et al.*, 2022; Tong *et al.*, 2011). After these compounds arrive at the domestic sewage, they enter the WWTP, where they are partially removed. The remaining part is then discharged into the surface waters via the effluent. Micropollutant is a broad definition given to a group of compounds present in water streams at concentrations in the range of nanograms to micrograms (Eggen *et al.*, 2014; Figuiere *et al.*, 2022; Joss *et al.*, 2006; Shipingana *et al.*, 2022). These concentrations are in this range due to the dilution and biological processes (biotransformation, photodegradation, and sorption) that happen before and after the effluent is discharged (Golovko *et al.*, 2021). These compounds and their metabolites can reach the groundwater, being automatically into the drinking water system, reducing, therefore, the quality of drinking water resources, besides promoting public health problems (Fernández *et al.*, 2014).

Environmental and public health impacts in aquatic environments have already been researched and documented for some micropollutants. For example, it has been proved that diclofenac in the aquatic environment presents genotoxicity, causing growth genes dysregulation as well as reproduction and metabolic problems (Daughton & Ternes, 1999; EC, 2019; Eggen *et al.*, 2014; Heberer, 2002; Kennes-Veiga *et al.*, 2022; Ternes *et al.*, 2003; Zdarta *et al.*, 2022). These environmental impacts are a problem since most of the micropollutant discharges are not controlled, regulated, or even known, allowing therefore that some of these compounds to be discharged into the surface waters without any

treatment (Figuiere *et al.*, 2022; Martinez-Casales *et al.*, 2022; Palma *et al.*, 2022; Pathak *et al.*, 2020). Antidepressants have been shown to induce reproductive, behaviour, and feeding issues in aquatic macroinvertebrates (Shaliutina-Kolešová *et al.*, 2019). On the other hand, antibiotics may promote bacterial antibiotic resistance by developing resistance genes, resulting in a major public health problem created by multiresistant bacteria (Kraemer *et al.*, 2019; Larsson & Flach, 2021). One of the biggest problems associated with pharmaceuticals is that they are not only excreted as parent compounds but also in their metabolized and conjugated forms, as oxidation/hydrolysis products which are rarely total mineralized during cometabolic biotransformation in WWTP processes (Kennes-Veiga *et al.*, 2021; Plosz *et al.*, 2010).

Municipal wastewater treatment plants are a central disposal path for these compounds and represent a significant pollution source point in the environment (Freitas & Radis-Baptista, 2021; Tong *et al.*, 2011). Therefore, WWTPs represent an opportunity to remove micropollutants and minimize their environmental emissions, mitigating the surface water ecotoxicity promoted by micropollutants. Therefore, the fate of micropollutants during wastewater treatment and their removal mechanisms must be understood. This knowledge will allow us to update the current technologies, minimise micropollutant discharges, prevent environmental effects and reduce the costs of possible advanced treatments. Despite the high removal efficiencies of nutrients, organic matter and solids, conventional biological wastewater treatment systems are still ineffective in removing micropollutants (Boonnorat *et al.*, 2019; Castiglioni *et al.*, 2020; Liang *et al.*, 2021). The conventional WWTPs are not designed to remove OMP. However, WWTPs can be optimized, modelled, and retrofitted to reduce micropollutant concentrations as much as possible (Choi *et al.*, 2022) through different physical, chemical, and biological processes. Nevertheless, in unretrofitted WWTPs, with conventional activated sludge systems, biotransformation is the dominant removal process (Joss *et al.*, 2006; Liang *et al.*, 2021; Wei *et al.*, 2019). The biotransformation removal rate in wastewater is specific for each compound and treatment condition. Therefore, it is crucial to have a good understanding and further research on the biotransformation of micropollutants, especially in activated sludge systems, since it is the most applied technology worldwide. The best biotransformation conditions, as well as the achievable biotransformation efficiencies in conventional biological treatment, are extremely critical knowledge that is missing now for many emerging contaminants. This data will allow to design and improve the operational conditions of the WWTP and maximize the removal efficiency of these compounds (Wei *et al.*, 2019). By achieving higher micropollutant removal efficiencies in the WWTP, it will be possible to reduce the impacts on the urban water cycle (Castiglioni *et al.*, 2020) and increase the circularity of both treated wastewater and sludge (EC, 2019).

## 1.2 Research Goal

This study aims to fill the existing knowledge gap about the biotransformation rates/kinetics of sixteen targeted micropollutants (14 pharmaceuticals and 2 industrial chemicals) under three redox conditions commonly present in conventional activated sludge (CAS) wastewater treatment systems, i.e., anaerobic, anoxic, and aerobic. To complement the literature and fill this mentioned knowledge gap both removal efficiencies and removal rate constants will be determined for the sixteen compounds. It is also a study that aims to understand the different removals under different redox conditions, as well as if their removal pathway is metabolic or cometabolic. Besides, it is also a goal of this study (as a theoretical exercise) to observe the impact of the effluent wastewater treatment plant's discharge in the water streams, with and without the application of an advanced oxidation process.

# 2

# SCIENTIFIC LITERATURE REVIEW

## 2.1 General Background

Micropollutants are commonly defined as compounds (natural or synthetic) that are found in different matrixes (water, sediment, and biota) (Golovko *et al.*, 2021) and released by various sources at low concentrations (nanograms or micrograms per litre) (Chavoshani *et al.*, 2020; Kennes-Veiga *et al.*, 2022). These micropollutants can also be called organic micropollutants (OMP) if these chemical compounds have organic properties. Those compounds usually fall under one of the following categories: biocides, polyfluoroalkyl and perfluoroalkyl substances (PFAS), pharmaceuticals (PhCs), surfactants, or personal care products (PCPs) (Chavoshani *et al.*, 2020; Eggen *et al.*, 2014). The micropollutants (both organic or not) are also sometimes called emerging concern compounds due to the rise of their concentrations in the environment, mainly in the water streams, and due to the lack of legislation to monitor their environmental concentrations (Gago-Ferrero *et al.*, 2017; Rosenfeld & Feng, 2011). Whenever these compounds interfere with the normal function of the endocrine system of the human body (or other animals), they can also be called endocrine-disrupting chemicals (EDCs). These endocrine-disrupting chemicals can cause nervous and immune system malfunctions and dysregulations, as well as respiratory and metabolic problems, cancers, and many others (Gupta *et al.*, 2010; Rhomberg *et al.*, 2014).

Nowadays, wastewater treatment plants are mainly based on biological treatment processes that were not designed to remove micropollutants (Bourgin *et al.*, 2018; Kennes-Veiga *et al.*, 2021; Polesel *et al.*, 2016; Ternes *et al.*, 2003). Nevertheless, the actual biological treatments show some extent of micropollutant removal (Bourgin *et al.*, 2018; Joss *et al.*, 2006). Throughout the years, wastewater treatment plants all around the world have been adapted to society's needs: removal of the pathogen, nutrients, and now emerging micropollutants (Joss *et al.*, 2004; Joss *et al.*, 2011; Joss *et al.*, 2005; Siegrist & Joss, 2012). To achieve micropollutant removal is crucial to understand the mechanism behind their biotransformation within the most used biological processes (Hatoum *et al.*, 2019; Rogers, 1996; Shipingana *et al.*, 2022).

Due to the lack of regulation and monitoring, chemical compounds have been put on the market for several years without any environmental risk assessment as part of their authorization process (EC, 2019). Yet, some compounds were selected and analysed under the Water Framework Directive (EC, 2019). Besides, environmental assessments, biotransformation efficiencies, and similar parameters have recently become part of the acceptance process of new compounds in the EU.

Most wastewater treatment plants are designed to remove organic matter, nutrients (nitrogen and phosphorous), and solids. Usually, this removal is based on biological treatments due to their cost-

effectiveness (Jaafari *et al.*, 2019). The research developments in this field led to the appearance of biological nutrient removal (BNR) processes like A/O (Anoxic/Oxic), $A^2/O$ (Anaerobic/Anoxic/Oxic), PhoRedox, and many others, which are mainly based on activated sludge systems. The PhoRedox processes can incorporate biological and chemical removal of phosphorus (using anaerobic environments and adding ferric chlorine, as a flocculant, to precipitate the phosphorous).

The physicochemical characteristics of some micropollutants and the hydraulic retention time (HRT) applied in the wastewater treatment reactor can determine their removal (Gros *et al.*, 2010; Pomies *et al.*, 2013; Yang *et al.*, 2016). For example, diclofenac, ibuprofen, propranolol, and triclosan contain aromatic rings, making them possible recalcitrant compounds due to the energy stabilizing the ring structure (Fuchs *et al.*, 2011). This electronic density of aromatic systems reduces the bioavailability of the micropollutant since microorganisms can't use them as electron receptors (Fuchs *et al.*, 2011). Besides that, the complex molecular structures of micropollutants can be toxic to the microbial community in the reactor (Fan *et al.*, 2020). Therefore, a compound is biodegradable if, by the action of microorganisms, it can be converted into other less complex compounds. This biotransformation happens when microorganisms use these compounds as energy/carbon sources, and the compound can be called mineralized when it is converted to end carbon products such as methane (anaerobic conditions) or carbon dioxide (aerobic conditions) (Angelidaki & Sanders, 2004).

In WWTP, the micropollutant can be biologically removed either by metabolism or co-metabolism (Majewsky *et al.*, 2010; Plosz *et al.*, 2010; Suarez *et al.*, 2010). This distinction is essential to better comprehend the mechanisms involved in the biotransformation of the compounds (Kennes-Veiga *et al.*, 2022). Metabolism is the primary process for the biotransformation of the compounds when the energy generated from their catalysis is enough to promote biomass growth and maintenance. Conversely, cometabolic biotransformation is the ability of microorganisms to degrade substrates that will not provide enough energy to grow if used as the sole substrate (Fischer & Majewsky, 2014; Kennes-Veiga *et al.*, 2022; Tran *et al.*, 2013). For that reason, the presence of a more readily biodegradable substrate is necessary, at higher concentrations, to be used as an electron donor to, maintain the microbial community, and even induce the production of enzymes and cofactors capable of biotransforming the micropollutants, through their catabolic activity (Fernandez-Fontaina *et al.*, 2014; Fischer & Majewsky, 2014; Krah *et al.*, 2016). The scientific community widely assumes that cometabolism is the primary biotransformation mechanism in natural environmental conditions (Fischer & Majewsky, 2014; Kennes-Veiga *et al.*, 2022; Lema & Suarez, 2017; Tran *et al.*, 2013). However, these two mechanisms (metabolism and cometabolism) can coexist in the biotransformation process of some compounds (Mandarić, 2018).

## 2.2 Parameters Affecting Micropollutants Biotransformation

The main parameters that affect the micropollutants removal in the WWTP process are the sludge retention time (SRT), hydraulic retention time (HRT), redox condition (i.e., anaerobic, anoxic, aerobic), sludge concentration, temperature and pH (Alturki *et al.*, 2010; Falas *et al.*, 2016; Fernandez-Fontaina *et al.*, 2012; Gusmaroli *et al.*, 2020; Huang *et al.*, 2011; Joss *et al.*, 2004; Liang *et al.*, 2021; Liu & Wong, 2013; Radjenovic *et al.*, 2009; Sui *et al.*, 2011; Yu *et al.*, 2009). Furthermore, micropollutant removal is also affected by their physicochemical properties, like the compound's polarity, biodegradability, and cation-exchange properties (Fernandez-Fontaina *et al.*, 2016; Fernandez-Fontaina *et al.*, 2012; Gusmaroli *et al.*, 2020; Radjenovic *et al.*, 2009). Certainly, the treatment train that was chosen during the WWTP design, and the existent microbial community also determine the potential biotransformation of micropollutants.

The sludge concentration is one of the most critical parameters since higher removal rates are expected at high concentrations due to more bacteria, energy sources, and solids to promote sorption. Another relevant operational parameter is the redox condition since it enhances the growth of some bacteria and leads to an increased removal due to specific bacteria pathways. Also, hydraulic retention time is an important operational parameter since it regulates the contact time between the bacteria (and enzymes) with the micropollutants. Lastly, temperature and pH may be important since reaction kinetics and electron transfer depend on them (Gusmaroli *et al.*, 2020).

### 2.2.1 Design and Operational Parameters

#### 2.2.1.1 Sludge Retention Time (SRT)

The SRT is the average time the activated sludge solids stay in the system and determines the mean residence time bacteria remain inside a biological reactor. This is one of the most critical parameters in biological wastewater processes since it affects the development of microbial diversity. This parameter can vary depending on whether the system was designed or not to biologically remove nitrogen. Without nitrogen removal, the SRT usually is no longer than ten days. However, if the CAS system was designed to remove nitrogen, the SRT should vary between 10 and 20 days (Eggen & Vogelsang, 2015; Reif *et al.*, 2013), although it can go up to 80 days (Eggen & Vogelsang, 2015). This increase in nitrogen removal systems is needed to ensure the development of a diverse bacterial community capable of achieving nitrification and denitrification (Eggen & Vogelsang, 2015; Reif *et al.*, 2013).

Referring to micropollutants, Fernandez-Fontaina *et al.* (2012) found no direct correlation between SRT and removal efficiency, except for the diclofenac due to its need for specific bacteria. In the same way, both Vieno *et al.* (2007) and Falas *et al.* (2016) noted that biotransformation rates of the pharmaceutical compounds were not affected if the sludge age increased from 25 to 80 days. The increased removal observed in diclofenac could be explained through the development of a more diverse bacterial community that possesses the ability to conduct the degradation of this compound. Fernandez-Fontaina *et al.* (2012) reported that when the SRT was increased from 15 days to 150 days, the removal efficiency of diclofenac went from 15% to 70%. These results and correlations were also found by other authors (Clara *et al.*, 2005; Lishman *et al.*, 2006). Yu *et al.* (2009) studied the removal of pharmaceuticals in activated sludge with an SRT greater than two hundred days and achieved removal efficiency of around 60% - 90% for both sulfamethoxazole and trimethoprim. Those values are shown to be higher than the ones achieved by Radjenovic *et al.* (2009) with an SRT of 10 days (40% - 70%) and

also higher than the ones obtained by Ghosh *et al.* (2009) (25% - 35%) and Lin *et al.* (2009) (25% - 50%). Gobel *et al.* (2007) also observed that reducing the Food per Microorganism (F/M) parameter and increasing the SRT led to a minimization of the antibiotic biocide effect, which may be a solution to degrade some recalcitrant antibiotics.

#### 2.2.1.2 Hydraulic Retention Time (HRT)

The HRT is the average length of time that a soluble compound remains in the bioreactor and can be determined as the tank volume divided by the influent flow rate (Eggen & Vogelsang, 2015). This parameter controls the available time for sorption and biotransformation in the process, and usually, it can go up to 24h in both CAS and membrane bioreactors (MBR) (Eggen & Vogelsang, 2015; Reif *et al.*, 2013; Sari Erkan *et al.*, 2018). Yet, MBRs can be used with lower HRT because the solids concentrations are higher than in CAS processes (Sari Erkan *et al.*, 2018). However, accordingly to Guerra *et al.* (2014), for enhanced removal of micropollutants, the HRT should be longer than 16h.

Some of the few studies that correlate HRT and micropollutants degradation have reported that they have a direct proportionality (Fernandez-Fontaina *et al.*, 2012; Hatoum *et al.*, 2019). According to Boonnorat *et al.* (2019), the HRT plays an essential role in removing micropollutants because it controls the contact time between the sludge and the effluent (Pan *et al.*, 2004). It has been reported that the increase of the HRT leads to an increased micropollutant removal (Benabdallah *et al.*, 2006; Fernandez-Fontaina *et al.*, 2012; Hatoum *et al.*, 2019; Jiang *et al.*, 2018). Benabdallah *et al.* (2006) observed an increase of almost 20% when increasing the HRT from 6 to 22 days. Jiang *et al.* (2018) also observed an increase of up to 20% when the HRT increased from 6 to 24 hours. Fernandez-Fontaina *et al.* (2012) observed an increase of up to 50% in the removal of erythromycin, fluoxetine, and roxithromycin when the HRT was increased from 1 to 5 days. However, this correlation was also observed in trimethoprim to a lower extent (30%). Hatoum *et al.* (2019) noticed that even in recalcitrant compounds, an increase of the HRT from 4h to 12h led to an increase of up to 25% removal. Ghosh *et al.* (2009) observed a removal increase of almost 20% in trimethoprim when doubling the HRT from 5,5h to 11h. Yet, Lin *et al.* (2009) observed an increase of 15% when doubling the HRT, from 5h to 10h, achieving a sulfamethoxazole removal efficiency of 39%. It is possible to observe a consistent ≈20% increase when the HRT is triplicated. Benabdallah *et al.* (2006) reported that an increase of the HRT from 6 to 22 days in thermophilic anaerobic sludge digestion can also lead to a 20% removal of recalcitrant micropollutants (e.g., polycyclic aromatic hydrocarbons). Batt *et al.* (2007) observed the removal of 77% for sulfamethoxazole and 96% for trimethoprim with an HRT of 30h. Carballa *et al.* (2007a) observed removal efficiencies of 50% to sulfamethoxazole with an HRT of 24h. Besides that, according to Bo *et al.* (2009), the HRT changes had no significant increase in the biotransformation of clofibric acid and ibuprofen. The same study also concluded that ibuprofen was completely degraded while diclofenac did not show any biological degradation. However Hatoum *et al.* (2019), noticed that the simultaneous increase of the HRT and the SRT leads to an increase in the removal of carbamazepine, diclofenac and sulfamethoxazole, which could be a solution to remove some recalcitrant micropollutants. According to Falas *et al.* (2016) to achieve a venlafaxine removal of around 80%, an HRT of 14 d is needed, which is much greater than the usual HRT. That being, treatments with these HRTs cannot be accommodated in conventional WWTP due to the space, economic and technical requirements.

As shown, longer HRT increased the micropollutants removal efficiency. This increase is expected to be more significant in hydrophilic compounds, which can be found in the liquid phase. This happens because the increase of the HRT provides time for microorganisms to use the compounds as a substrate or to degrade them throughout their enzymes (Gros *et al.*, 2010). However, longer HRT also represents higher construction and operation costs (Boonnorat *et al.*, 2019; Hatoum *et al.*, 2019). Remarkably, the

theoretical HRT to achieve the goal of the Swiss Regulation of removing 80% of 80% of the compounds existent in Swiss wastewaters is 343 h for a moving bed biofilm reactor (MBBR) system, which means that base on literature, a CAS system may need more time (Liang *et al.*, 2021).

#### 2.2.1.3 Temperature

The temperature dictates the microorganism's survival, growth, and metabolic activities. Usually, higher temperatures result in higher metabolic activities unless they kill the organism (Westermann *et al.*, 1989). Three different ranges of temperature are usually applied in biological processes: psychrophilic (< 25°C), mesophilic (25°C to 45°C), and thermophilic (45°C to 60°C) (Angelidaki & Sanders, 2004).

Accordingly to Liu and Wong (2013) and Gusmaroli *et al.* (2020), the temperature may affect the degradation of some micropollutants due to the biomass increase at optimal temperatures, which may lead to higher biotransformation. Hai *et al.* (2011) observed that the biotransformation increases with temperature increase and that this correlation is more visible in hydrophobic compounds due to electron-withdrawing and donating functional groups. According to Gusmaroli *et al.* (2020), higher removals are usually obtained at mesophilic temperatures once these temperatures accelerate process kinetics and bacterial growth. Adding more energy (or heat) facilitates the micropollutant breaking reaction by providing energy to break bonds from the compounds. However, Ifelebuegu *et al.* (2010) showed that decreasing the reaction temperature from 20°C to 15°C made an increase of 20% in the sorption of endocrine-disrupting compounds (EDC) compounds like E1, E2, and EE2. According to Zeng *et al*., (2009), cited by Hörsing *et al.* (2011), high temperatures have shown lower sorption rates since they increase water solubility. The temperature affects not only the biotransformation but also the sorption, affecting, therefore, the overall removal.

#### 2.2.1.4 pH

In chemistry, pH stands for the *potential of hydrogen* and is represented as a scale to specify the acidity or basicity of a solution. In this scale, acidic solutions will have lower pH values due to their high $H^+$ concentration. On the other hand, basic solutions will have high pH values due to their low $H^+$ concentration (Covington *et al.*, 1983).

According to Gusmaroli *et al.* (2020), the pH is a parameter that has little effect on the overall micropollutant removal range between 6,5 and 7,5. However, control of the pH is important during oxidation due to the efficiency of some of the processes in acidic or neutral pH (Margot *et al.*, 2013; Ternes *et al.*, 2003). Yet, Gulde *et al.* (2014) observed that the biotransformation rate constant could be up to 10 times higher when changing the pH from 6 to 8 in cationic compounds like propranolol and phenanthrene. However, the inverse correlation was observed in anionic compounds like trimethoprim, showing ten times decrease with a pH change from 6 to 8. To neutral compounds, it was impossible to create a correlation, probably because their removal pathways are not directly dependent on the pH. Goss *et al.* (2020) also observed that the half-life of the compounds could decrease up to a factor of 3,5 when pH is increased from 7,9 to 8,9. The pH variation affects the biotransformation of the micropollutants, mainly because of the availability of electron acceptors, compounds bioavailability, and the bacterial community (Goss *et al.*, 2020; Laureni *et al.*, 2015).

Even maybe not affect the biotransformation by itself, pH affects the desorption of the pharmaceutical compounds to the sludge (Cozmuta *et al.*, 2012; Hörsing *et al.*, 2022; Hörsing *et al.*,

2011; Martin & Iwuco, 1982). At high pH values, the sorption process occurs by ion exchange (i.e., covalent bonding). In contrast, at low pH values, the sorption happens mainly by physical forces (i.e., Van der Waals forces). According to the free Gibbs energy variation, the sorption process occurs spontaneously yet at a higher rate in basic pH conditions (Cozmuta *et al.*, 2012; Martin & Iwuco, 1982). Hörsing *et al.* (2011) showed that the pH increase (from 6 to 8) could create a 20% difference in the sorption efficiency of EDC, like E1 (estrone), E2 (estradiol), and EE2 (ethynylestradiol). Moreover, the authors indicated that this pH variation affected the solid-water distribution coefficient of irbesartan and trimethoprim. However, it did not significantly influence venlafaxine, sulfamethoxazole, sotalol, or diclofenac.

#### 2.2.1.5 Redox Conditions

The redox condition is defined by the main electron acceptor available, which can oxidize other substances (Yakushev, 2016). These electron acceptors' nature and availability are significant factors that affect biotransformation (Angelidaki & Sanders, 2004; Yakushev, 2016). There are three main redox conditions in wastewater treatment: aerobic, anoxic, and anaerobic.

From a thermodynamic point of view, oxygen is the best electron acceptor (Angelidaki & Sanders, 2004; Maier & Gentry, 2015), and that's why aerobic degradation usually has the highest efficiency (Maier & Gentry, 2015). In anoxic and anaerobic conditions, the availability of electron acceptors such as nitrate, iron, sulphate, or carbon dioxide will define the biotransformation extent of the compounds (Angelidaki & Sanders, 2004). The breakdown of organic compounds by microorganisms in oxygen is called aerobic biotransformation. Aerobic bacteria have an oxygen-based metabolism, so during cellular respiration, they use oxygen to oxidize the substrate and obtain energy (Reineke, 2001). While aerobic microorganisms metabolize through oxidative reactions, in anoxic and anaerobic conditions, those metabolizations are usually reductive reactions (Reineke, 2001). The anaerobic degradation can be defined as the bioconversion process in the absence of oxygen which occurs without an external electron acceptor such as oxygen (in aerobic processes) or nitrates/phosphates/sulphates (in anoxic conditions). In anaerobic conditions, the organic carbon is converted by subsequent redox processes to its most oxidized state ($CH_4$) (Angelidaki & Sanders, 2004).

The degradation of estrone (E1) occurs in any redox condition, however, at different rates (Lishman *et al.*, 2006; Urase & Kikuta, 2005). According to Joss *et al.* (2004), the aerobic condition is the best way to remove the estrone (E1) with an increase factor of up to 10 when compared to anaerobic conditions and up to 5 when compared to anoxic conditions. On the other hand, estradiol (E2) has high removal rates in all redox conditions, with an increased factor below 3 when changing from anaerobic to aerobic conditions (Joss *et al.*, 2004). Ruas *et al.* (2022) reported that sulfamethoxazole, trimethoprim, and naproxen significantly degraded in aerobic, anoxic, and anaerobic environments. Fernandez-Fontaina *et al.* (2012) also said that trimethoprim has a higher removal efficiency (70%) in nitrifying activated sludge compared to conventional activated sludge (25%), which is in line with Ternes *et al.* (2007). In the same way, diclofenac has shown a correlation between the presence of nitrite and its removal efficiency (Arias *et al.*, 2018). According to Fernandez-Fontaina *et al.* (2012), nitrification performance significantly affects drugs, being the lowest removal efficiency in the test where the nitrification was incomplete.

Di Marcantonio *et al.* (2020) researched the effect of the aeration frequency variation (oxic/anoxic) in the micropollutant removal compared to the control (continual aeration - oxic). This research shows that aeration with a frequency of 0,6 $h^{-1}$ (higher anoxic times) improved the removal efficiency by 30% in sulfamethoxazole and 36% in carbamazepine. On the other hand, a frequency of

1,8 $h^{-1}$ (lower anoxic times) improved 28% in sulfamethoxazole and 19% in carbamazepine. These results indicated that a higher degradation rate could be established at lower anoxic conditions, which is consistent with the findings of previous works. Bains *et al.* (2019), similarly to Di Marcantonio *et al.* (2020), observed that the variation of the oxygen led to an increase in micropollutant removal (up to 40%). According to their findings, constant aeration led to a removal of 15% of sulfamethoxazole that increased up to 90% with an aeration frequency of 0,5 $h^{-1}$. However, the results did not show any correlation between aeration frequency and carbamazepine removal.

According to Falas *et al.* (2016), significant removal (> 60%) in anaerobic conditions was only found in a few micropollutants, like acetaminophen, atenolol, clarithromycin, sulfamethoxazole, and trimethoprim. Xue *et al.* (2010) also observed the removal of around 70% of anaerobic activated sludge with phosphorous removal. Falas *et al.* (2016) affirmed that anaerobic treatment of the wastewater could complement the aerobic treatment allowing the removal of persistent micropollutants like venlafaxine and its metabolites. In the same experiment with activated sludge followed by anaerobic post-treatment, Falas *et al.* (2016) analysed compounds like diatrizoate, venlafaxine, tramadol, and trimethoprim that showed a removal increase of 60% to 80% due to the anaerobic post-treatment. Therefore, Gasser *et al.* (2012) suggested that the increased removal of venlafaxine in anaerobic conditions might be promoted by anaerobic demethylation. For that reason, the anaerobic post-treatment, after anoxic/oxic conditions, enhanced (up to 70%) the removal of some pharmaceuticals like erythromycin and roxithromycin, and enhanced up to 10% the removal of compounds like sulfamethoxazole and trimethoprim (Arias *et al.*, 2018). Huang *et al.* (2011) showed that the highest removal of the diclofenac occurred in anaerobic conditions. In contrast, aerobic degradation was significant in the other pharmaceutical compounds, except salicylic acid, which showed the highest removal under an anoxic environment. Like diclofenac, indomethacin and naproxen were significantly removed in the anaerobic phase. After aerobic treatment, these three compounds were almost completely removed (>99%), and gemfibrozil, ibuprofen, and bezafibrate were largely removed. Around 40% of the clofibric acid and diclofenac remained in the wastewater.

Despite the positive results of the anaerobic deiodination and demethylation of specific micropollutants, it is questionable whether anaerobic treatment for enhanced deiodination and demethylation should be practically applied in WWTP due to the slow rate at which these processes occur (Falas *et al.*, 2016; Gasser *et al.*, 2012). Iron supplemented anaerobic reactors showed an increase of 10% to 30% in the removal rates compared to the sulphate-supplemented or methanogenic ones due to the increased production of sludge that may have led to higher adsorption (Falas *et al.*, 2016).

#### 2.2.1.6 Process Treatment Sequence and Treatment Levels

The removal of micropollutants depends on the existing process treatment sequence and treatment levels in the WWTP (Alturki *et al.*, 2010; Choi *et al.*, 2022; Jiang *et al.*, 2018; Pathak *et al.*, 2020). Alturki *et al.* (2010), for example, recurring to MBR coupled with low-pressure reverse osmosis (RO), observed an overall removal of 95% in the 40 tested compounds (like carbamazepine, sulfamethoxazole, trimethoprim, and diclofenac). Contrary, Choi *et al.* (2022) studied different secondary and tertiary treatments and observed their efficiencies. It was possible to observe that an anaerobic-anoxic-oxic reactor coupled with one membrane bioreactor can lead to an overall removal of 96%. At the same time, a CAS system can only remove up to 85%. Even so, the Modified Ludzack-Ettinger (MLE) system noticed a removal of up to 92%. These efficiencies can even be higher if coupled with a tertiary treatment level. The results of the mentioned study showed that adding a step with biological activated carbon (BAC) may increase up to 85% the removal of compounds like metformin. Jiang *et al.* (2018), on the other side, used a moving bed biofilm reactor (MBBR) coupled with an MBR and obtained

variable micropollutant removals (from 11,0% to 99,5%). Nevertheless, in a total of 22 compounds, it was observed an average removal was above 70%.

Lately, MBR has been coupled with reverse osmosis or nanofiltration to minimize the concentration of pollutants in the streams. MBR can remove most of the hydrophobic compounds due to their adsorption at the sludge with enhanced residence time, comparable to CAS (Alturki *et al.*, 2010). On the other hand, reverse osmosis and nanofiltration can remove the hydrophilic organic compounds, allowing, accordingly to Alturki *et al.* (2010), the removal efficiency of at least 95% for recalcitrant compounds like carbamazepine, diclofenac, sulfamethoxazole, and trimethoprim. Radjenovic *et al.* (2009) showed that MBR technologies generally outperform the CAS treatments in removing pharmaceuticals and personal care products, achieving removal rates up to 65% in recalcitrant compounds like indomethacin, diclofenac, and gemfibrozil due to the membrane existent in the MBR. This happens due to MBR operation (higher solid concentrations and SRT). The degradation of natural estrogens (E1 and E2) has shown to be higher in MBR than in CAS by a factor of 2-3, accordingly to Joss *et al.* (2004), mainly due to the age and size of the flocs in the MBR sludge.

According to Sui *et al.* (2011), the removal efficiency of the MBR system is higher than the ones in CAS or biological nutrients removal (BNR) systems. BNR systems are very temperature dependent, while CAS and MBR are maybe less, even if all biological systems are temperature dependent. Caffeine, for example, could be well degraded by all three processes. However, while MBR maintains the removal efficiency steady and greater than 99%, CAS could have fluctuations around 96%, and BNR systems could vary from 70% to 80%. However, in the case of bezafibrate, the temperature variation from winter to summer could go the removal efficiency from 30% (December to March) to 60% (May to September) (Sui *et al.*, 2011). For example, in the case of diclofenac, the MBR can remove up to 61%, CAS can only remove up to 37%, and BNR systems up to 21% (Radjenovic *et al.*, 2009; Sui *et al.*, 2011). Huang *et al.* (2011) reported that in CAS, salicylic acid, indomethacin, and naproxen were almost completely removed (>99%), while bezafibrate, ibuprofen, and gemfibrozil were substantially removed (>75%). Both clofibric acid and diclofenac have shown recalcitrant characteristics, and only 60-70% were removed.

Liang *et al.* (2021) and Tang *et al.* (2017) also refer that an MBBR is a suitable alternative to conventional activated sludge when removing micropollutants. However, Kora *et al.* (2020) as shown that benzotriazole has 0% removal efficiency with MBBR in anaerobic conditions and around 30%-60% within aerobic conditions. Torresi *et al.* (2017) reported that only positively charged compounds presented any degree of sorption, while neutral or negative charged compounds showed negligible sorption. Torresi *et al.* (2017) also compared the solid-water distribution coefficients of the micropollutants between MBR, CAS, and MBBR, and, in general, the coefficients are larger in the following sequence: MBBR > CAS > MBR. The enhanced sorption is associated with the size of the sludge flocs that are smaller in MBBR and bigger in CAS, allowing a larger accessible surface area for sorption (di Biase *et al.*, 2019; Torresi *et al.*, 2017). In addition Liang *et al.* (2021) determined the biotransformation rates in feast-famine MBBR system for benzotriazole, diclofenac, gabapentin, metoprolol, propranolol, sotalol, sulfamethoxazole and venlafaxine, which are, respectively, 0,0017 L/(g.h), 0,0110 L/(g.h), 0,0450 L/(g.h), 0,0450 L/(g.h), 0,2320 L/(g.h), 0,0088 L/(g.h), 0,0010 L/(g.h), 0,0055 L/(g.h). Those values were also compared to the ones obtained by Tang *et al.* (2017), who also used feast-famine MBBR. It is possible to notice that Liang *et al.* (2021) obtained lower biotransformation rates, mainly due to the carbonate precipitation found in the carriers. Meaning that the attached solids did not represent the actual biomass underestimating the normalized biotransformation rate ($K_{bio}$) in this study.

Once part of the micropollutants is adsorbed to the sludge is also important to understand if those compounds can be biodegraded in anaerobic digestion conditions. Gonzalez-Gil *et al.* (2016) stated that the anaerobic digestion promoted a removal above 50% in compounds like sulfamethoxazole, trimethoprim, fluoxetine, and citalopram. However, half of the compounds detected were persistent during digestion (Gonzalez-Gil *et al.*, 2016).

It is also essential to understand how the disinfection/oxidation process affects the removal of the micropollutants since this could also be a solution. For that reason, Ternes *et al.* (2003) stated that contrast compounds like iopamidol, iopromide, and iomeprol could be oxidized and removed up to 83% with the addition of a 15 mg/L dose of ozone. However, other compounds like carbamazepine, diclofenac, and sulfamethoxazole have varied efficiencies depending on the applied oxidation system, the oxidant dose, and the UV-C contact time (Rodriguez-Chueca *et al.*, 2019).

### 2.2.2 Micropollutant Biotransformation Coefficients

As mentioned earlier, the micropollutant physicochemical characteristics significantly impact their biodegradability and removal from wastewater. These characteristics are related to some of the removal coefficients of the compounds. Two of the principal specific removal coefficients that determine the bioremoval and its extension are the solid-water distribution coefficient, or partitioning coefficient ($K_d$), and the biotransformation rate ($k_{bio}$).

The solid-water distribution coefficient is specific to a particular solid phase and can only be experimentally determined. The only other coefficient that could give some information about the micropollutant tendency to be in the liquid or solid phase is the octanol-water partition coefficient ($K_{ow}$). However, this coefficient is not sufficiently accurate for prediction. The distribution coefficient needs to be performed in each WWTP because of the strong influence of matrix characteristics (Pomies *et al.*, 2013), as well as the influence of the temperature, pH, or metallic ion concentration (Pathak *et al.*, 2020).

According to Pathak *et al.* (2020) and Gusmaroli *et al.* (2020), for hydrophobic compounds ($\log K_{ow} > 3,2$), the sorption mechanism is an important removal mechanism, while hydrophilic compounds ($\log K_{ow} < 3,2$) are more prone to biotransformation as follows:

- $\log K_{ow} < 3,2$ – low sorption potential;
- $3,2 < \log K_{ow} < 4,0$ – medium sorption potential;
- $\log K_{ow} > 4,0$ – high sorption potential.

Low $K_d$ coefficients mean low sorption to the solid phase of the activated sludge. For that reason, micropollutants with low $K_d$ can be defined by negligible sorption, suggesting that the removal is mainly the result of biotransformation, as referred to previously (Pathak *et al.*, 2020; Ternes *et al.*, 2004).

Previous studies (Golovko *et al.*, 2021; Luo *et al.*, 2014) suggested that the compounds can also be classified due to their $K_d$, as follows:

- $K_d < 2,5$ – the compound is most likely to stay in the aqueous phase
- $K_d > 3,2$ – the compound is most likely to stay in the solid phase

The suspended solids are one of the parameters responsible for moderating the degradation and sorption of micropollutants in wastewater (Aminot *et al.*, 2018). This happens since part of the solid's

existence is bacteria that will be responsible for the degradation itself of the compounds, and the other part is an organic matter where the compounds can be absorbed.

The biotransformation rate data is usually modelled as pseudo-first-order kinetics to obtain the reaction rate constant ($k_{bio}$), which is used to assess and predict the degradation of the micropollutants at the WWTP. Higher degradation values mean a bigger percentage of transformation of that compound (Joss *et al.*, 2006; Wei *et al.*, 2019).

According to Joss *et al.* (2006) and Wei *et al.* (2019) micropollutants can be classified to their biotransformation rate ($k_{bio}$) as follows:

- $k_{bio} < 0{,}1$ L.gSS$^{-1}$.d$^{-1}$ – micropollutants not removed to a significant extent (<20%);
- $0{,}1 < k_{bio} < 10$ L.gSS$^{-1}$.d$^{-1}$ – moderate removal is expected;
- $k_{bio} > 10$ L.gSS$^{-1}$.d$^{-1}$ – micropollutants transformed by more than 90%.

## 2.3 Micropollutants Removal Mechanisms

It is found in the literature that the main micropollutant removal mechanisms in CAS are: (1) Sorption in suspended solids in the wastewater – to the activated sludge – and subsequent removal by sedimentation as secondary sludge; (2) Biological Transformation that might lead to mineralization of substance by existing bacteria; (3) Stripping by aeration, which is almost negligible due to the low micropollutant volatility. In Table 2-1, the methodology used to calculate each one of the removal mechanisms is shown.

According to the information found in the literature, biotransformation is the primary mechanism responsible for the removal of pharmaceuticals and personal care products (Fernandez-Fontaina *et al.*, 2012; Ruas *et al.*, 2022; Urase & Kikuta, 2005; Wick *et al.*, 2009). According to the conclusions obtained by the EU project NEPTUNE (Ternes *et al.*, 2010), even for somewhat volatile fragrances (not considered in this research), stripping will account for less than 10% of the compound removal. Therefore, stripping will not be considered in this study (Fernandez-Fontaina *et al.*, 2012; Gusmaroli *et al.*, 2020; Joss *et al.*, 2006; Ternes & Joss, 2006).

Under aerobic and heterotrophic conditions, the main reactions involved in the micropollutant's biotransformation are oxidation (hydrolysation, dehydrogenation, deamination, and demethylation), hydrolysis and conjugation routes (Kennes-Veiga *et al.*, 2021; Wei *et al.*, 2019). Those reactions are promoted by enzymes such as mono- and dioxygenases, dehydrogenases, hydrolases, and transferases (Kennes-Veiga *et al.*, 2021).

Moreover, dehalogenation deiodination and demethylation can be achieved under low redox conditions (anoxic and anaerobic) (Falas *et al.*, 2016; Kennes-Veiga *et al.*, 2021). The cometabolic oxidation cometabolic oxidation may achieve these reactions by the ammonium monooxygenase (AMO) enzyme, which is one of the main enzymes involved in micropollutant removal (Fernandez-Fontaina *et al.*, 2012; Kennes-Veiga *et al.*, 2021). Suitable nitrifying activities increase the biotransformation rates of many micropollutants once ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are responsible for the main degradation of these compounds in anoxic environments (Laureni *et al.*, 2015).

Table 2-1 Micropollutant removal mechanisms/rate/coefficients, in CAS, namely sorption, biological transformation, and stripping

Adapted from Ferreira (2022), Mazioti et al. (2015) and Joss et al. (2006)

| Mechanism | Rate/Coefficient | Equation |
|---|---|---|
| Biological Transformation | $k_{biol}$ – Reaction rate of biotransformation [$L.gSS^{-1}.d^{-1}$] | $\frac{dC}{dt} = \frac{-k_{biol} \times X_{SS} \times S}{1 + K_d \times X_{SS}}$<br>C - Total OMP concentration [$\mu g.L^{-1}$]<br>t - Time [d]<br>$k_{biol}$- Reaction rate constant [$L.gSS^{-1}.d^{-1}$]<br>$X_{SS}$- Suspended solids concentration in the reactor [$gSS.L^{-1}$]<br>S- Soluble OMP concentration [$\mu g.L^{-1}$] |
| Sorption | $K_d$ – Solid-Water distribution coefficient (Partitioning coefficient) [$L.gSS^{-1}$] | $K_d = \frac{X_{part}}{S} = \frac{X}{X_{SS} \times S}$<br>X - Concentration sorped onto sludge per unit reactor volume [$\mu g.L^{-1}$]<br>$X_{part}$ - Concentration sorped per amount of sludge dry matter [$\mu g.gSS^{-1}$]<br>$X_{SS}$ - Suspended solids concentration [$gSS.L^{-1}$]<br>S - Soluble micropollutant concentration [$\mu g.L^{-1}$] |
| Stripping | $K_H$ – Henry Coefficient (Air-water partitioning coefficient) [-] | $K_H = \frac{C_{air}}{S} = \frac{MW \times p_p}{S \times R \times T}$<br>$C_{air}$ – OMP concentration in air [$\mu g.L^{-1}.m^{-3}air$]<br>S – Soluble OMP concentration [$\mu g.L^{-1}.m^{-3}$]<br>MW – Molar weight [$\mu g.Mol^{-1}$]<br>$P_p$ – Partial pressure of OMP in the gas phase [Pa]<br>R – Universal gas constant; 8.314 [$J.Mol^{-1}.K^{-1}$]<br>T- temperature [K] |

## 2.4 Targeted Micropollutants

This research focuses on the 16 targeted micropollutants proposed by *Stichting Toegepast Onderzoek Waterbeheer* (STOWA - Foundation for Applied Water Management Research), namely: 4-, 5-Methylbenzotriazole or totyltriazole (METH), Azithromycin (AZI), Benzotriazole (BEN), Clarithromycin (CLA), Candesartan (CAN), Carbamazepine (CAR), Diclofenac (DIC), Gabapentin (GAB), Irbesartan (IRB), Metoprolol (MET), Hydrochlorothiazide (HYD), Propranolol (PRO), Sotalol (SOT), Sulfamethoxazole (SUL), Trimethoprim (TRI) and Venlafaxine (VEN). Yet, from the abovementioned, only 11 compounds are also suggested by the *Ministerie van Infrastructuur en Waterstaat* (Dutch Ministry of Infrastructure and Water Management) – METH, BEN, CLA, CAR, DIC, MET, HYD, PRO, SOT, SUL, and TRI. Some of these compounds have also been mentioned in the Watch List under the Water Framework Directive, produced by the European Commission (Cortes et al., 2020). Besides that, carbamazepine and

diclofenac are among the European top ten consumed pharmaceuticals. Due to their high consumption, personal care products (PPCPs) are highly prioritized in European assessments (Fernández et al., 2014).

The amount of information known about the abovementioned sixteen targeted micropollutants is variable. For example, there are many studies and information about pharmaceuticals like carbamazepine (Alturki *et al.*, 2010; Bertilsson, 1978; Zdarta *et al.*, 2022), diclofenac (Bonnefille *et al.*, 2018; Davies & Anderson, 1997; Kennes-Veiga *et al.*, 2021), and sulfamethoxazole (Archundia *et al.*, 2019; Masters *et al.*, 2003; Rudy & Senkowski, 1973) as well as the beta-blockers metoprolol, propranolol, and sotalol (Benfield *et al.*, 1986; Celiz *et al.*, 2009; Liang *et al.*, 2021). The antibiotics clarithromycin and trimethoprim have fewer studies even though they are well documented (Berges *et al.*, 2021; Brogden *et al.*, 1982; Castiglioni *et al.*, 2020; Polesel *et al.*, 2016). On the other hand, the information about anti-corrosion 4, 5-methylbenzotriazole, and benzotriazole is scarce (Katritzky *et al.*, 1991; Pillard *et al.*, 2001; Walker, 1970). Due to the extended use of the mentioned pharmaceuticals, it was already expected that the available information would come from distinct fields of knowledge: pharmaceutical consumption, toxicology, water and wastewater treatment, and environmental risk assessment. In the following sections, some information about the micropollutants in this study is summarized.

### 2.4.1 Azithromycin, Clarithromycin, Sulfamethoxazole, and Trimethoprim

Both Azithromycin (AZI), Clarithromycin (CLA), Sulfamethoxazole (SUL), and Trimethoprim (TRI) are antibiotics overall prescribed for the treatment of bacterial infections in the respiratory, urinary, and gastrointestinal tract (Langtry & Brogden, 1997; Masters *et al.*, 2003; Peters & Clissold, 1992; Rodvold, 1999; Smilack, 1999). Azithromycin has a molecular structure that consists of a large macrocyclic lactone ring to which sugar molecules are attached. Macrolide antibiotics are named after the macrocyclic lactone structure of the parent compound erythromycin. Usually, macrolides are biocides to a wide range of species, both Gram-positive and Gram-negative, as well as intracellular pathogens like Chlamydia and Legionella (Bakheit *et al.*, 2014). The bioavailability of azithromycin is approximately 37%, and faeces usually excrete it without any transformation (Lalak & Morris, 1993; Peters *et al.*, 1992). This pharmaceutical is a non-polar compound with a moderate hydrophobicity and low affinity to adsorption by activated carbon (Kim *et al.*, 2021; Wishart *et al.*, 2018).

Clarithromycin has a molecular structure like azithromycin once they are both macrolides. Clarithromycin has a bioavailability of around 50-55%, excreted principally by urine. The main metabolite of clarithromycin is the 14-hydroxyclarithromycin (Langtry & Brogden, 1997; Rodvold, 1999). This compound is non-polar and has moderate hydrophobic behaviour (Langtry & Brogden, 1997; Peters & Clissold, 1992; Rodvold, 1999). This antibiotic type was placed successfully on the market around the ’80s (Ternes & Joss, 2006). In some countries, clarithromycin is classified as one of the most abundant macrolides in treated wastewater and water (McArdell *et al.*, 2003). This micropollutant is hydrophilic, non-polar, and almost insoluble in water (Kim *et al.*, 2021; Wishart *et al.*, 2018). Clarithromycin also showed good removal by adsorption to activated carbon (Karelid *et al.*, 2017; Xu *et al.*, 2021).

Sulfamethoxazole is one of the most used antibiotics used in Europe as the first line against bacterial infections, being used constantly (Ryan *et al.*, 2011; Ternes & Joss, 2006; Wang & Wang, 2018). This compound belongs to the sulphonamides class and is polar, with hydrophobic properties and moderate affinity to adsorption by activated carbon (Archundia *et al.*, 2019; Rudy & Senkowski, 1973; Yang *et al.*, 2020b). The human metabolism of sulfamethoxazole leads to the transformation and release of hydroxylated, acetylated, and glucuronide metabolites. The metabolized percentage by the human

body is variable and usually eliminated through urine. Only about 10% is excreted as the unchanged compound, and about 50% is excreted as an inactive metabolite $N^4$-acetylsulfamethoxazole (Gobel *et al.*, 2004; Gobel *et al.*, 2005; Polesel *et al.*, 2016; Rudy & Senkowski, 1973) which can be retransformed to the active parent compound during wastewater treatment, as happen with carbamazepine (Brown & Wong, 2018; Celiz *et al.*, 2009; Joss *et al.*, 2006). Sulfamethoxazole has shown better removal rates at 25° C, once it is the optimal temperature for the main bacteria responsible for degrading this compound. In the same way, the optimal pH is alkaline due to the ease of the ion change and the activation of the enzymes of the nitrifying bacteria (Wang & Wang, 2018). This micropollutant is a hydrophilic and polar compound, completely insoluble in water (Kim *et al.*, 2021; Wishart *et al.*, 2018), with good removal by activated carbon (Bizi, 2020; Moral-Rodríguez *et al.*, 2016).

Trimethoprim is an antibiotic commonly used as a sulphonamide potentiator, like sulfamethoxazole, and used to treat urinary tract infections (Brogden *et al.*, 1982; Bushby & Hitchings, 1968). This compound is polar and presents a moderate hydrophobicity and a high affinity to activated carbon adsorption, principally in a basic pH (Berges *et al.*, 2021; Liu *et al.*, 2015). Accordingly to Brogden *et al.* (1982), the main metabolites of trimethoprim are 3-hydroxy trimethoprim, 4-hydroxy trimethoprim, and the l-oxidetrimethoprim, which represent almost 11% of the excreted. Like diclofenac, trimethoprim also needs specific aerobic conditions to achieve degradation (Falas *et al.*, 2016; Jewell *et al.*, 2016). This micropollutant is hydrophilic and polar with a low water solubility (Kim *et al.*, 2021; Wishart *et al.*, 2018) and good activated carbon adsorption (Karelid *et al.*, 2017).

### 2.4.2 Benzotriazole and 4-, 5-Methylbenzotriazole

Benzotriazole (BEN) is a known specific corrosion inhibitor widely used in industry to reduce the corrosion of copper and copper alloys under both atmospheric and immersed conditions (Ravichandran *et al.*, 2004; Walker, 1970). According to Törnkvist *et al.* (2019), the methylation of benzotriazole allows a 30% increase in the corrosion inhibition efficiency of the brass (copper and zinc alloy). Both Benzotriazole and 4-, 5-Methylbenzotriazole – also known as totyltriazole – (METH) are polar compounds with a mild hydrophilic behaviour (Kim *et al.*, 2021; Wishart *et al.*, 2018). Both benzotriazole and methylbenzotriazole are hydrophilic and polar compounds with high water solubility (Kim *et al.*, 2021; Wishart *et al.*, 2018) and also a good removal by adsorption by activated carbon (Abu-Dalo *et al.*, 2020; Wagner *et al.*, 2020). According to Dummer (2013), benzotriazole and its methylation are mainly degraded by biotransformation in the WWTP processes.

### 2.4.3 Candesartan and Irbesartan

Both Irbesartan (IRB) and Candesartan (CAN) are angiotensins (Koh *et al.*, 2004) prescribed to treat high blood pressure, heart failure, and diabetic kidney disease, sometimes combined with hydrochlorothiazide (Wishart *et al.*, 2018). This pharmaceutical has a bioavailability of around 40%, mostly excreted through faeces, with partial urine elimination (Gleiter *et al.*, 2004; Gleiter & Morike, 2002). This pharmaceutical is excreted (80%) as an unchanged parent compound, even though has been found a nonactive metabolite, O-Desethyl Candesartan (Easthope & Jarvis, 2002; Gleiter *et al.*, 2004). This micropollutant is polar, highly hydrophobic, and practically insoluble in water (Kim *et al.*, 2021; Wishart *et al.*, 2018).

Irbesartan just like candesartan, is a β-blocker antihypertensive agent (Easthope & Jarvis, 2002; Koh *et al.*, 2004). Its bioavailability can vary between 60% to 80%, excreted almost in the same percentage in urine and faeces in its nonmetabolized form. Like candesartan, there are no active metabolites of irbesartan. However, no active or nonactive metabolites were found (Croom *et al.*, 2004; Gillis & Markham, 1997). This micropollutant is non-polar and hydrophobic, with almost no water solubility (Kim *et al.*, 2021; Wishart *et al.*, 2018). Both irbesartan and candesartan have shown high removal efficiency using activated carbon, demonstrating their good affinity to activated carbon (Karelid *et al.*, 2017).

### 2.4.4 Carbamazepine

Carbamazepine (CAR) is an antiepileptic pharmaceutical used in treating epilepsy and neuropathic pain and is prescribed all around Europe and the USA. The consumption trends vary, over time, and sometimes even per season (Bertilsson, 1978; Crill, 1973; Leucht *et al.*, 2014; Ternes & Joss, 2006). This pharmaceutical is a neutral charge compound with a moderate hydrophilic behaviour and low affinity to adsorption by activated carbon. The metabolites patterns of carbamazepine are also well defined due to the extended use of this pharmaceutical, which is particularly important once carbamazepine metabolites are likely to retransform to the parent compound during the biological processes at the WWTP. The most critical carbamazepine metabolites are the 10,11-epoxide and the 10,11-dihydro-10-hydroxy-5H-dibenzazepine-5-carboxamide (DHDC), which are usually found in humans and animals until further metabolization and inactivation. Nevertheless, part of the carbamazepine is automatically hydroxylated and excreted as glucuronide (Bertilsson, 1978; Crill, 1973; Polesel *et al.*, 2016; Ternes & Joss, 2006).

### 2.4.5 Diclofenac

Diclofenac (DIC) is an antiphlogistic nonsteroidal pharmaceutical. In most European countries, it can be bought over the counter, being prescribed for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gouty arthritis (Davies & Anderson, 1997; Gan, 2010). As referred by Davies and Anderson (1997), diclofenac is excreted through urine mainly as the glucuronide conjugate, which is also eliminated as 4'-OH-Diclofenac and other hydroxy metabolites (3'-OH-Diclofenac, 5'-OH-Diclofenac, and 4'-5'-OH-Diclofenac). Accordingly to Siegrist and Joss (2012), referred by Ternes *et al.* (2003), besides musk fragrances, only diclofenac was quantified above the detention limit in samples of three activated sludge WWTP from Germany, with values between 0,2 and 0,45 mg/kg. Even though phototransformation has been identified as the main elimination process of diclofenac in drinking water, in wastewater, specifically in CAS configuration, this mechanism of removal is not significant (Ternes & Joss, 2006). According to Gusmaroli *et al.* (2020), diclofenac has been hardly removed in aerobic conditions, with a maximum of 45,7%, and no degradation under anoxic conditions. Diclofenac is a compound that requires some specific aerobic treatment to achieve degradation (Falas *et al.*, 2016). This micropollutant is hydrophobic and non-polar with almost no water solubility (Kim *et al.*, 2021; Wishart *et al.*, 2018) and has shown good adsorption (a removal up to 75%) by different types of activated carbon (Genc *et al.*, 2021; Larous & Meniai, 2016).

### 2.4.6 Gabapentin

The amino acid antiepileptic drug gabapentin (GAB) has an analogue structure to a natural body-produced neurotransmitter. This drug is indicated for adjunctive use in seizure treatment induced by epilepsy (Goa & Sorkin, 1993; McLean, 1995; Morris, 1999). Gabapentin's absolute bioavailability varies depending on the administrated dose. However, the maximum registered was 60%, and the minimum was 35% (Goa & Sorkin, 1993; McLean, 1995). This drug is not metabolized in humans and is usually excreted through urine (Goa & Sorkin, 1993; McLean, 1995). Gabapentin is a polar molecule with a hydrophilic behaviour and is freely soluble in water (Kim *et al.*, 2021; Wishart *et al.*, 2018). According to Wu (2022), gabapentin also has a moderate affinity to activated carbon adsorption.

### 2.4.7 Hydrochlorothiazide

Hydrochlorothiazide (HYD) is a diuretic drug widely used to treat hypertension and swelling due to fluid accumulation (Deppeler, 1981; Niemeyer *et al.*, 1983). The bioavailability of the hydrochlorothiazide is around 70%, being the rest is usually excreted as an intact substance by urine (Deppeler, 1981; Niemeyer *et al.*, 1983). This micropollutant is a non-polar, hydrophilic compound with low water solubility (Kim *et al.*, 2021; Wishart *et al.*, 2018). According to Hu *et al.* (2016) and Kopping *et al.* (2020), hydrochlorothiazide also presents good sorption to powder and granular activated carbon.

### 2.4.8 Metoprolol, Propranolol, and Sotalol

Both metoprolol (MET), propranolol (PRO), and sotalol (SOT) are beta-blockers, usually prescribed for hypertension, heart conditions (e.g., arrhythmias, myocardial infarct, angina), or anxiety disorders (Benfield *et al.*, 1986; Hayes & Schulz, 1987). Metoprolol and propranolol have very similar chemical structures and are polar compounds (Regardh & Johnsson, 1980). These compounds are metabolized mainly with less than 1% unmetabolized excreta for propranolol and with less than 5% unmetabolized excreta for metoprolol (Benfield *et al.*, 1986). On the other side, sotalol has a more hydrophilic behaviour (Anderson & Prystowsky, 1999) and possesses an unmetabolized excreta of more than 85% (Ternes & Joss, 2006).

Metoprolol is extensively metabolized by the hepatic mono-oxygenase system and has four main metabolites: HI 04/83, HI 17/04, O-demethylmetoprolol, and α-hydroxymetoprolol (Regardh & Johnsson, 1980). Alternatively, propranolol metabolization can follow side-chain oxidation to α-naphthoxylactic acid, ring oxidation to 4'-hydroxypropranolol, or glucuronidation to propranolol glucuronide. It can also be N-desisopropylated to become N-desisopropylpropranolol. However, the main metabolite of propranolol is 4'-hydroxypropranolol (Harrison *et al.*, 1985; Routledge & Shand, 1979; Walle *et al.*, 1994). On the other side, sotalol is not dependent on the liver enzymes once he does not suffer any biotransformation. Therefore, sotalol has no known active metabolites (Fitton & Sorkin, 1993; Hanyok, 1993; Singh *et al.*, 1987). Metoprolol and sotalol have shown hydrophilic behaviour, while propranolol is a hydrophobic compound. Both metoprolol, propranolol, and sotalol have shown a high removal rate using activated carbon, showing good adsorption (Karelid *et al.*, 2017).

### 2.4.9 Venlafaxine

Venlafaxine (VEN) is a first-line antidepressant from the selective serotonin reuptake inhibitors (SSRI) class (Gutierrez *et al.*, 2003). This drug in the market since the 1990s has been used to treat generalized depressive and anxiety disorders (Gutierrez *et al.*, 2003). Venlafaxine has a bioavailability of around 45% and is mainly eliminated by renal excretion as the unmetabolized substance or as its metabolites: O-demethylvenlafaxine, N,O-didemethylvenlafaxine, and N-demethylvenlafaxine (Holliday & Benfield, 1995). According to both Holliday and Benfield (1995), Gutierrez *et al.* (2003), and Wellington and Perry (2001), the most common metabolite is O-demethylvenlafaxine representing almost 30% of the excreted compound. This micropollutant is polar and hydrophobic, with high solubility in water (Kim *et al.*, 2021; Wishart *et al.*, 2018) and good adsorption by activated carbon (Karelid *et al.*, 2017).

## 2.5 General Physicochemical Characterization of the Micropollutants

Table 2-2 presents the identification information of the sixteen micropollutants under consideration, as well as their chemical group and common use. In Appendix 1 it is possible to observe the chemical structures of these compounds.

Table 2-2 Identification of the sixteen micropollutants in this study

| Micropollutant | CAS Number | Molecular Formula (1) (2) | Chemical group (1) | Use |
|---|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 29385-43-1 | $C_7H_7N_3$ | Benzotriazoles | Copper and copper alloy corrosion inhibitor |
| Benzotriazole | 95-14-7 | $C_6H_5N_3$ | Benzotriazole | Copper and copper alloy corrosion inhibitor |
| Carbamazepine | 298-46-4 | $C_{15}H_{12}N_2O_2$ | Dibenzazepines | Anticonvulsant/Antiepileptic |
| Clarithromycin | 81103-11-9 | $C_{38}H_{69}NO_{13}$ | Aminoglycosides/Macrolide | Antibiotic/Macrolide |
| Diclofenac | 15307-86-5 | $C_{14}H_{11}Cl_2NO_2$ | Dichlorobenzenes | Antiphlogistic |
| Hydrochlorothiazide | 58-93-5 | $C_7H_8ClN_3O_4S_2$ | Benzothiadiazines | Antihypertensive/Diuretic |
| Metoprolol | 37350-58-6 | $C_{15}H_{25}NO_3$ | Tyrosols and derivatives | Antihypertensive/Beta-blocker |
| Propranolol | 525-66-6 | $C_{16}H_{21}NO_2$ | Naphthalenes | Antihypertensive/Beta-blocker |
| Sotalol | 3930-20-9 | $C_{12}H_{20}N_2O_3S$ | Sulfananilide | Antihypertensive/Beta-blocker |
| Sulfamethoxazole | 723-46-6 | $C_{10}H_{11}N_3O_3S$ | Aminobenzenesulfonamides | Antibiotic |
| Trimethoprim | 738-70-5 | $C_{14}H_{18}N_4O_3$ | Anisole | Antibiotic |
| Azithromycin | 83905-01-5 | $C_{38}H_{72}N_2O_{12}$ | Aminoglycosides/Macrolide | Antibiotic/Macrolide |
| Candesartan | 139481-59-7 | $C_{24}H_{20}N_6O_3$ | Biphenyls and derivatives | Antihypertensive |
| Gabapentin | 60142-96-3 | $C_9H_{17}NO_2$ | Gamma amino acid | Anticonvulsants/Antiepileptic |
| Irbesartan | 138402-11-6 | $C_{25}H_{28}N_6O$ | Biphenyls and derivatives | Antihypertensive |
| Venlafaxine | 93413-69-5 | $C_{17}H_{27}NO_2$ | Anisole | Antidepressant/SSRI |

(1) Wishart *et al.* (2018); (2) Kim *et al.* (2021)

Table 2-3, on the other hand, summarizes the main chemical properties of the targeted micropollutants. This characterization includes molar weight, pKa (acid dissociation constant), Log $K_{ow}$, hydrophobicity, solubility, polarity, and the affinity to adsorption to powder-activated carbon (PAC).

Table 2-3 Characterization of the sixteen targeted micropollutants
Adapted from Ferreira (2022)

| Micropollutant | Molar weight [g.mol$^{-1}$] (1) (2) | $pK_a$ (1) (2) | Log $K_{OW}$ (2) | Hydrophobicity (1) (2) (4) | Solubility [mg/mL] (1) (2) (3) | Polarity (1) (2) | Adsorption to PAC |
|---|---|---|---|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 133,1 | 8,74 | 1,71 | Hydrophilic | 0,36600 | Polar | Good affinity |
| Benzotriazole | 119,1 | 8,37 | 1,44 | Hydrophilic | 1,00000 | Polar | Good affinity |
| Carbamazepine | 254,3 | 13,90 | 2,45 | Hydrophilic | 0,15200 | Non-polar | Low affinity |
| Clarithromycin | 747,9 | 8,99 | 3,16 | Hydrophilic | 0,00033 | Non-polar | Good affinity |
| Diclofenac | 296,2 | 4,15 | 4,50 | Hydrophobic | 0,00237 | Non-polar | Good affinity |
| Hydrochlorothiazide | 297,7 | 7,90<br>9,20 | 0,07 | Hydrophilic | 0,72200 | Non-polar | Good affinity |
| Metoprolol | 267,4 | 9,70 | 1,88 | Hydrophilic | 0,40200 | Polar | Good affinity |
| Propranolol | 259,3 | 9,42 | 3,48 | Hydrophobic | 0,06170 | Polar | Good affinity |
| Sotalol | 272,4 | 8,20<br>9,80 | 0,24 | Hydrophilic | 0,78200 | Polar | Good affinity |
| Sulfamethoxazole | 253,3 | 1,60<br>5,70 | 0,89 | Hydrophilic | 0,45900 | Polar | Good affinity |
| Trimethoprim | 290,3 | 7,12 | 0,91 | Hydrophilic | 0,61500 | Polar | Good affinity |
| Azithromycin | 749,0 | 9,57 | 4,02 | Hydrophobic | < 1,00000 | Non-polar | Low affinity |
| Candesartan | 440,5 | 2,45<br>6,70 | 4,79 | Hydrophobic | 0,00754 | Polar | Good affinity |
| Gabapentin | 171,2 | 3,68<br>10,70 | 1,10 | Hydrophilic | 4,34000 | Polar | Moderated affinity |
| Irbesartan | 428,5 | 4,29 | 5,31 | Hydrophobic | 0,00884 | Non-polar | Good affinity |
| Venlafaxine | 277,4 | 9,50<br>10,09 | 3,20 | Hydrophobic | 0,23000 | Polar | Good affinity |

(1) Wishart *et al.* (2018); (2) Kim *et al.* (2021); (3) Das *et al.* (2017); (4) Grandclement *et al.* (2017)

As mentioned before, the knowledge about the biotransformation rates of the compounds under consideration is scarce (see Table 2-4). It is possible to observe that no biotransformation rates were found in anaerobic conditions, and only Suarez *et al.* (2010), Plosz *et al.* (2010), Xue *et al.* (2010), and Mazioti *et al.* (2015) studied the biotransformation rates of some of the targeted micropollutants under anoxic conditions. While it is possible to observe many referenced articles on biotransformation rates under aerobic conditions, it is also possible to notice that compounds like 4-, 5-Methylbenzotriazole, hydrochlorothiazide, candesartan, and venlafaxine have no studies on biotransformation rates under any redox condition. Analysing the solid-water distribution coefficients, it is also possible to observe that both gabapentin and candesartan have no values.

Table 2-4 Biotransformation rate constants and sorption (distribution) coefficients found for CAS in the bibliographic review for the targeted micropollutants

| Micropollutants | $k_{bio}$ [L.gSS$^{-1}$.d$^{-1}$] | | | $k_d$ [L.gSS$^{-1}$] |
|---|---|---|---|---|
| | **Aerobic** | **Anoxic** | **Anaerobic** | |
| 4-, 5-Methylbenzotriazole | | | | 0,122 (24)<br>0,151 (24)<br>0,165 (24)<br>0,170 (24)<br>0,179 (24)<br>0,218 (24) |
| Benzotriazole | 0,16 (24)<br>0,21 (24)<br>0,22 (24)<br>0,30 (24)<br>0,40 (24)<br>0,41 (24)<br>0,42 (24) | 0,23 (24)<br>0,24 (24)<br>0,25 (24)<br>0,32 (24)<br>0,33 (24)<br>0,34 (24) | | 0,133 (21)<br>0,211 (24)<br>0,220 (24)<br>0,229 (24) |
| Carbamazepine | 0,00 (23)(19)<br><0,01 (7)(8)<br>0,01 (19)<br><0,06 (12)<br><0,07 (18)<br><0,10 (10)<br>0,10 (3)<br>0,70 (28) | <0,03 (12) | | 0,001 (6)(18)(7)(2)<br>0,002 (10)<br>0,025 (5)<br>0,028 (6)<br>0,036 (15)<br>0,036 (17)<br>0,066 (6)<br>0,089 (16)(17)<br>0,135 (26)(9)<br>0,210 (17)<br>0,220 (17)<br>0,240 (17)<br>0,250 (17)<br>0,260 (17)<br>0,300 (17)<br>0,330 (17) |
| Clarithromycin | 0,03 (8)<br>0,20 (8)<br>≤0,40 (7)<br>0,48 (23)<br><0,50 (7) | | | 0,260 (7)(4)<br>0,262 (4)<br>1,200 (8) |

(1) McArdell *et al.* (2003); (2) Ternes *et al.* (2004); (3) Clara *et al.* (2005); (4) Gobel *et al.* (2005); (5) Jones *et al.* (2005); (6) Urase and Kikuta (2005); (7) Joss *et al.* (2006); (8) Abegglen *et al.* (2009); (9) Radjenovic *et al.* (2009); (10) Wick *et al.* (2009); (11) Plosz *et al.* (2010); (12) Suarez *et al.* (2010); (13) Xue *et al.* (2010); (14) Hörsing *et al.* (2011); (15) Stevens-Garmon *et al.* (2011); (16) Hyland *et al.* (2012); (17) Lajeunesse *et al.* (2012); (18) Suarez *et al.* (2012); (19) Fernandez-Fontaina *et al.* (2013); (20) Pomies *et al.* (2013); (21) Stasinakis *et al.* (2013); (22) Fernandez-Fontaina *et al.* (2014); (23) Blair *et al.* (2015); (24) Mazioti *et al.* (2015); (25) AstraZeneca (2017); (26) Berthod *et al.* (2017); (27) Martínez-Alcalá *et al.* (2017); (28) Nolte *et al.* (2020); (29) Tiwari *et al.* (2021)

Table 2-4 Biotransformation rate constants and sorption (distribution) coefficients found for CAS in the bibliographic review for the targeted micropollutants (Continuation)

| Micropollutants | $k_{bio}$ [L.gSS$^{-1}$.d$^{-1}$] | | | $k_d$ [L.gSS$^{-1}$] |
|---|---|---|---|---|
| | **Aerobic** | **Anoxic** | **Anaerobic** | |
| Diclofenac | <0,02 (8)<br>0,02 (19)<br>≤0,10 (7)<br>0,10 (18)(19)<br>0,30 (28)<br>0,40 (3)<br>0,50 (28)<br>0,70 (28)<br>0,80 (3)<br>0,90 (28)<br>1,20 (12) | <0,04 (12) | | 0,001 (5)<br>0,002 (2)<br>0,002 (29)<br>0,003 (29)<br>0,016 (26)(7)<br>0,030 (15)<br>0,032 (19)<br>0,118 (9)<br>0,151 (16) (26)<br>0,701 (26) |
| Hydrochlorothiazide | | | | 0,020 (9) |
| Metoprolol | 0,13 (20)<br>0,20 (28)<br>0,35 (28)<br>0,40 (10)(28)<br>0,60 (28) | 0,03 (13) | | 0,006 (10)<br>0,065 (10)<br>0,200 (13)<br>1,090 (13) |
| Propranolol | 0,36 (10)<br>0,46 (10) | | | 0,155 (27)<br>0,199 (25)<br>0,343 (10)<br>0,363 (9)<br>0,417 (25)<br>0,480 (27) |
| Sotalol | 0,40 (10)(28)<br>0,43 (10)<br>0,60 (28)<br>0,80 (28) | | | 0,018 (10)<br>0,360 (14) |

(1) McArdell *et al.* (2003); (2) Ternes *et al.* (2004); (3) Clara *et al.* (2005); (4) Gobel *et al.* (2005); (5) Jones *et al.* (2005); (6) Urase and Kikuta (2005); (7) Joss *et al.* (2006); (8) Abegglen *et al.* (2009); (9) Radjenovic *et al.* (2009); (10) Wick *et al.* (2009); (11) Plosz *et al.* (2010); (12) Suarez *et al.* (2010); (13) Xue *et al.* (2010); (14) Hörsing *et al.* (2011); (15) Stevens-Garmon *et al.* (2011); (16) Hyland *et al.* (2012); (17) Lajeunesse *et al.* (2012); (18) Suarez *et al.* (2012); (19) Fernandez-Fontaina *et al.* (2013); (20) Pomies *et al.* (2013); (21) Stasinakis *et al.* (2013); (22) Fernandez-Fontaina *et al.* (2014); (23) Blair *et al.* (2015); (24) Mazioti *et al.* (2015); (25) AstraZeneca (2017); (26) Berthod *et al.* (2017); (27) Martínez-Alcalá *et al.* (2017); (28) Nolte *et al.* (2020); (29) Tiwari *et al.* (2021)

Table 2-4 Biotransformation rate constants and sorption (distribution) coefficients found for CAS in the bibliographic review for the targeted micropollutants (Continuation)

| Micropollutants | $k_{bio}$ [L.gSS$^{-1}$.d$^{-1}$] | | | $k_d$ [L.gSS$^{-1}$] |
|---|---|---|---|---|
| | **Aerobic** | **Anoxic** | **Anaerobic** | |
| Sulfamethoxazole | <0,10 (7)<br>0,10 (18)<br>0,19 (8)<br>0,20 (8)<br>0,24 (23)<br>0,30 (12)<br>0,30 (22)<br>0,41 (11)<br>0,60 (1)(18) | 0,41 (11) | | 0,011 (19)<br>0,030 (15)<br>0,040 (8)<br>0,050 (8)<br>0,077 (9)<br>0,078 (9)<br>0,160 (7)<br>0,256 (4)<br>0,257 (4)<br>0,260 (7)<br>0,269 (16)<br>0,280 (14)<br>0,370 (14)<br>0,500 (7) |
| Trimethoprim | 0,05 (19)<br>0,09 (19)<br>0,15 (12)<br>0,22 (8)<br>0,24 (23)<br>0,65 (18) | 0,67 (13) | | 0,025 (19)<br>0,076 (26)<br>0,119 (15)<br>0,200 (16)<br>0,208 (4)<br>0,210 (13)<br>0,251 (15)(26)<br>0,253 (9)<br>0,280 (14)<br>0,300 (13)<br>0,330 (8)<br>0,420 (14) |
| Azithromycin | <0,13 (7)<br>0,17 (8)<br>0,24 (23) | | | 0,280 (7)<br>0,376 (4)<br>1,400 (8) |
| Candesartan | | | | |
| Gabapentin | 0,08 (28)<br>0,13 (28)<br>0,18 (28) | | | |

(1) McArdell *et al.* (2003); (2) Ternes *et al.* (2004); (3) Clara *et al.* (2005); (4) Gobel *et al.* (2005); (5) Jones *et al.* (2005); (6) Urase and Kikuta (2005); (7) Joss *et al.* (2006); (8) Abegglen *et al.* (2009); (9) Radjenovic *et al.* (2009); (10) Wick *et al.* (2009); (11) Plosz *et al.* (2010); (12) Suarez *et al.* (2010); (13) Xue *et al.* (2010); (14) Hörsing *et al.* (2011); (15) Stevens-Garmon *et al.* (2011); (16) Hyland *et al.* (2012); (17) Lajeunesse *et al.* (2012); (18) Suarez *et al.* (2012); (19) Fernandez-Fontaina *et al.* (2013); (20) Pomies *et al.* (2013); (21) Stasinakis *et al.* (2013); (22) Fernandez-Fontaina *et al.* (2014); (23) Blair *et al.* (2015); (24) Mazioti *et al.* (2015); (25) AstraZeneca (2017); (26) Berthod *et al.* (2017); (27) Martínez-Alcalá *et al.* (2017); (28) Nolte *et al.* (2020); (29) Tiwari *et al.* (2021)

Table 2-4 Biotransformation rate constants and sorption (distribution) coefficients found for CAS in the bibliographic review for the targeted micropollutants (Continuation)

| Micropollutants | $k_{bio}$ [L.gSS$^{-1}$.d$^{-1}$] | | | $k_d$ [L.gSS$^{-1}$] |
|---|---|---|---|---|
| | **Aerobic** | **Anoxic** | **Anaerobic** | |
| Irbesartan | 0,10 (28)<br>0,50 (28)<br>0,90 (28) | | | 0,70 (14)<br>0,94 (14) |
| Venlafaxine | | | | 0,072 (17)<br>0,100 (14)(29)<br>0,200 (17)<br>0,220 (17)<br>0,350 (17)<br>0,360 (17)<br>0,390 (17)<br>0,420 (17)<br>0,490 (17) |

(1) McArdell *et al.* (2003); (2) Ternes *et al.* (2004); (3) Clara *et al.* (2005); (4) Gobel *et al.* (2005); (5) Jones *et al.* (2005); (6) Urase and Kikuta (2005); (7) Joss *et al.* (2006); (8) Abegglen *et al.* (2009); (9) Radjenovic *et al.* (2009); (10) Wick *et al.* (2009); (11) Plosz *et al.* (2010); (12) Suarez *et al.* (2010); (13) Xue *et al.* (2010); (14) Hörsing *et al.* (2011); (15) Stevens-Garmon *et al.* (2011); (16) Hyland *et al.* (2012); (17) Lajeunesse *et al.* (2012); (18) Suarez *et al.* (2012); (19) Fernandez-Fontaina *et al.* (2013); (20) Pomies *et al.* (2013); (21) Stasinakis *et al.* (2013); (22) Fernandez-Fontaina *et al.* (2014); (23) Blair *et al.* (2015); (24) Mazioti *et al.* (2015); (25) AstraZeneca (2017); (26) Berthod *et al.* (2017); (27) Martínez-Alcalá *et al.* (2017); (28) Nolte *et al.* (2020); (29) Tiwari *et al.* (2021)

Therefore, there is a clear knowledge gap in critical data on the biotransformation and adsorption of several micropollutants. It is noticeable that both anoxic and anaerobic are much less studied than the aerobic redox condition. Yet, some compounds have not been studied for their biotransformation or sorption. This knowledge can lead engineers to better understand the biological treatment systems, allowing them to improve their overall removal.

# 3

# HYPOTHESIS AND RESEARCH QUESTIONS

## 3.1 Knowledge Gap

According to Table 2-4, it is possible to understand the lack of available data and information about the biotransformation rates for the targeted micropollutants. It is also noticeable that micropollutant biotransformation is not yet well studied and sometimes not even studied. The same situation happens with the solid-water distribution coefficients, even though those are more studied. The data available in the literature varies per compound, having some compounds present in different studies while others have not been part of any study.

Even though some biotransformation rate constants are already available in the literature, they are usually not distinguished per the redox condition applied. Which is the key to understanding and optimizing the degradation in WWTP with biological nutrient removal, where usually all these redox conditions are applied. These values allow better modelling of the HRT in each reactor, to improve the compound's biotransformation, allowing higher micropollutants removals efficiencies of the WWTP. Furthermore, most biotransformation rate constants are found under aerobic conditions, while biotransformation rate constants under anaerobic conditions are extremely limited, as observed.

## 3.2 Hypothesis

The literature review shows apparent differences in the available data. This is related to the fact that distinct locations have different wastewater characteristics, which lead to other microbial communities in the sludge and, therefore, different biotransformation rates.

Some ideas can be hypothesized as to what is expected to obtain in this research. It is expected to observe a higher removal of OMP under aerobic and anoxic redox conditions. In contrast, some compounds like sulfamethoxazole and carbamazepine are expected to remove some negative under anaerobic redox conditions due to possible parent compound retransformation.

It is also expected that compounds like clarithromycin and gabapentin present good removal. Clarithromycin has been shown in many analyses to be one of the best biotransformed compounds. At the same time, gabapentin may be more easily degraded due to its biological similarity, yet there are not enough studies to support that idea. Similarly, candesartan, hydrochlorothiazide, irbesartan, and venlafaxine are compounds that have not been extensively studied. Due to its known recalcitrant

behaviour, diclofenac is expected to have an extremely low degradation. Sulfamethoxazole is expected to also show a low to moderate biotransformation. Due to the chemical similarity between metoprolol, propranolol, and sotalol, they may also likely have similar removal mechanisms and identical biotransformation rates.

According to the physical properties of the molecules, it is expected that hydrophobic compounds present more sorption than degradation as a removal mechanism. However, the enzymes produced during cometabolism may more easily degrade hydrophilic compounds. Polar compounds have been shown in some studies to be less degraded. However, due to their increased reactivity and higher solubility, it has also been expected to show a higher removal.

## 3.3 Research Questions

### 3.3.1 Main Research Question

How do the different redox conditions, present in activated sludge systems, affect the biotransformation rate of micropollutants?

### 3.3.2 Specific Research Questions

Which redox condition or combinations of them could promote a higher removal efficiency and biotransformation rate of the targeted micropollutants (antibiotics, antiepileptic, antiphlogistic, beta-blocker, diuretic, and chemical corrosion inhibitor)?

To which extent will the targeted micropollutants be removed in a conventional activated sludge system?

Do the physicochemical characteristics of the micropollutants affect their removal?

How can a conventional activated sludge WWTP, with nutrient removal, improve the micropollutant removal?

To what extent are the achieved micropollutants concentration after biological treatment under different redox conditions environmentally relevant?

# 4

# MATERIALS AND METHODS

## 4.1 Characterization of the Wastewater Treatment Plant

The batch experiments were conducted with wastewater (influent, activated sludge, and effluent) from the WWTP Walcheren, Vlissingen in Zeeland, Netherlands (see Figure 4-1).


Figure 4-1 Satellite view of Walcheren WWTP and site identification

This WWTP has as biological treatment a PhoRedox CAS configuration (see Figure 4-2), designed for 178 700 inhabitants equivalent and a maximum flow of 8 015 $m^3$/h. This WWTP also has a 20% industrial contribution, which may bring increased variability to the influent (Ferreira, 2022).

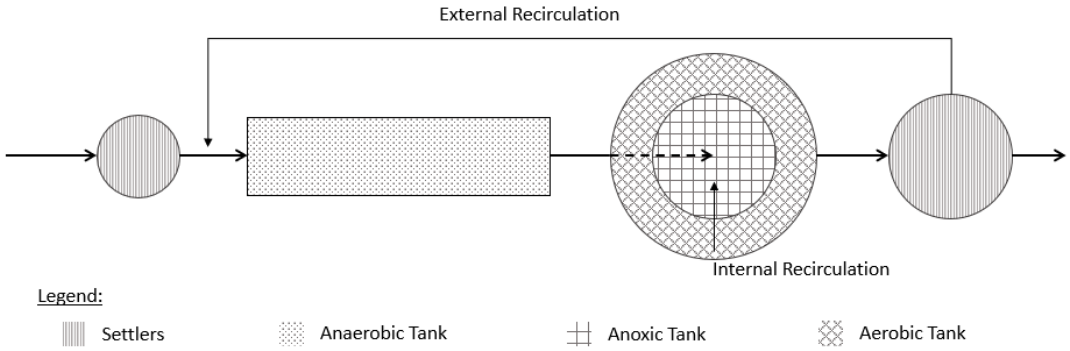


Figure 4-2 PhoRedox CAS Configuration Scheme
Adapted from Barnard (2006)

The sludge retention time (SRT) at WWTP Walcheren is 25 days, and the design hydraulic retention time (HRT) of the entire biological treatment is 6,2 hours. In Table 4-1 is possible to observe the volume of each reactor as well as its HRT by design.

Table 4-1 Design parameters of the activated sludge tanks

| Biological Process | Volume (m³) | Number of units | Total Volume (m³) | HRT (h) |
|---|---|---|---|---|
| Selector | 600 | 2 | 1 200 | 0,2 |
| Aerobic Tank | 5 320 | 2 | 10 640 | 1,3 |
| Anoxic Tank | 7 600 | 2 | 15 200 | 1,9 |
| Anaerobic Tank | 11 400 | 2 | 22 800 | 2,9 |
| Biological Reactor | 24 920 | - | 49 840 | 6,2 |

At Walcheren WWTP, the water treatment line comprises a pre-treatment (screening, grease, and sand removal), primary settler, selector, anaerobic tank, anoxic tank, aerobic tank, and secondary tank settler. This line also has an anammox process that treats the remaining water from the sludge separator before being sent to the beginning of the water line. On the other hand, the sludge treatment line comprises thickeners, a dewatering unit, anaerobic digestion, struvite production, and anammox. The remaining water from the sludge treatment line will be directed to the head of the WWTP (see Figure 4-3). In this WWTP, phosphorous removal happens through biological and chemical processes (using iron chloride for chemical precipitation). As can be seen in both Figure 4-2 and Figure 4-3 (green box), this system possesses both internal and external circulation to maximize nutrient removal and guarantee the appropriate sludge age, as well as a sludge concentration of 4,5 (± 0,5) g SS/L.

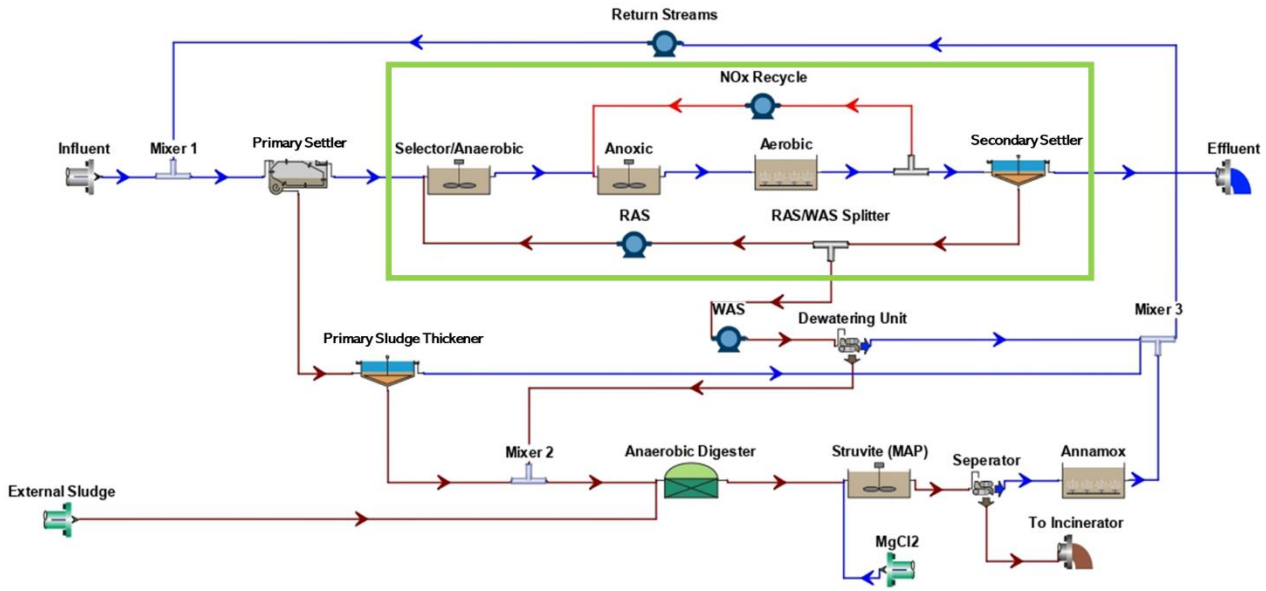


Figure 4-3 Walcheren WWTP diagram

## 4.2 Wastewater Sampling Campaign

A sample campaign at Walcheren WWTP was conducted weekly during March and April (see Figure 4-4). In Figure 4-4 is also possible to observe the type of sludge collected for characterization. The sampling campaign of the sludge was made every week to minimize as possible the storage time.

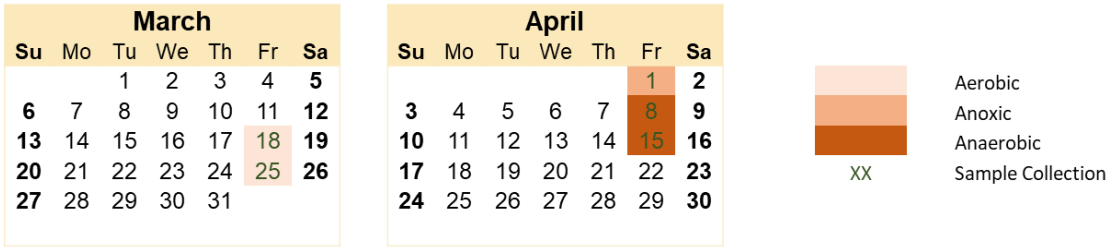


Figure 4-4 Calendar with sampling days and sludge type collected

The sampling campaign included the influent, the activated sludge (taken from the tanks under the different redox conditions), and the effluent. In Table 4-2, the samples are described, and the collection is placed on each day presented in Figure 4-4.

Table 4-2 Sampling Campaign Schedule for WWTP Walcheren

| Day of collection | Sample | Volume | Collection point |
|---|---|---|---|
| 18.03.2022 | Influent after Primary Settler | 5L | Auto-Sampler |
| | Effluent | 10L | Auto-Sampler |
| | Aerobic Activated Sludge | 5L | Aerobic Sampling Point |
| 25.03.2022 | Influent after Primary Settler | 5L | Auto-Sampler |
| | Effluent | 10L | Auto-Sampler |
| | Aerobic Activated Sludge | 5L | Aerobic Sampling Point |
| 01.04.2022 | Influent after Primary Settler | 5L | Auto-Sampler |
| | Effluent | 10L | Auto-Sampler |
| | Anoxic Activated Sludge | 5L | Anoxic Sampling Point |
| 08.04.2022 | Influent after Primary Settler | 5L | Auto-Sampler |
| | Effluent | 10L | Auto-Sampler |
| | Anaerobic Activated Sludge | 5L | Anaerobic Sampling Point |
| 19.04.2022 | Influent after Primary Settler | 5L | Auto-Sampler |
| | Effluent | 10L | Auto-Sampler |
| | Anaerobic Activated Sludge | 5L | Anaerobic Sampling Point |

After a field visit to the Walcheren WWTP, the specific process location where each of the samples of influent, activated sludge, and effluent were to be collected was defined (Figure 4-5).


Figure 4-5 Collection points of each sample from the Walcheren WWTP

In the same way, in Figure 4-6, it is possible to observe the selected collection points in more detail.


Figure 4-6 Wastewater treatment plant samples collection points

**A** – Autosampler for influent after primary settler collection; **B** – Anaerobic sludge collection point; **C** – Anoxic and aerobic sludge collection point; **D** – Anaerobic sludge collection point (detailed); **E** – Anoxic and aerobic sludge collection point (detailed)

As shown in Table 4-2 and Figure 4-6, 24-h composite samples from both influent and effluent were collected with WWTP *Efcon® OMY* autosamplers (*Efcon, Utrecht, The Netherlands*), allowing a more representative sample and minimizing possible interferences in the samples. Because there are two anaerobic reactors, it was chosen as the collection point for the anaerobic sludge in the mixing box of the anaerobic sludge of both reactors. The anoxic sludge was collected near the end part of the anoxic reactor to reduce possible oxygenation originating from the flow entrance in the reactor and guarantee it was well mixed. Lastly, the aerobic sludge was collected in the middle of the aerobic tank to ensure it was as mixed as possible. The sample volume collected was divided per reactor unit (e.g., 10L sample

of anoxic sludge equals 5L from anoxic reactor one and 5L from anoxic reactor two). After collection, these samples were transported and stored at a temperature of 4°C to stop further degradation.

The maximum and minimum temperatures and the precipitation were not too variable during April and May, as shown in Table 4-3.

Table 4-3 Weather characterization during the sampling campaign
Adapted from ClimateData (2022)

| | Maximum Temperature (°C) | Minimum Temperature (°C) | Precipitation (mm) |
|---|---|---|---|
| 18.03.2022, 8h30 | 9 | 4 | 2,3 |
| 25.03.2022, 8h30 | 10 | 5 | 1,6 |
| 01.04.2022, 8h30 | 12 | 6 | 2,3 |
| 08.04.2022, 8h30 | 10 | 5 | 1,1 |
| 19.04.2022, 8h30 | 13 | 6 | 1,0 |
| Average | 11 ± 2 | 5 ± 1 | 1,7 ± 0,6 |

## 4.3 Set-Up

Two reactors *Applikon ez-Control Bioreactor* of 2,5L each were used to conduct the experiments (see Figure 4-7 and Figure 4-8). The reactors were operated in batch mode, with a duration of 48 h, and in parallel. The reactors were equipped with a stirrer (200 rpm), a control unit (*Applikon ez-Control Bioreactor*), a sampling system, online sensors (dissolved oxygen, pH, redox, conductivity, and temperature), and an aeration control system (*Applikon ez-Control*). The bioreactors were placed against direct exposure to sunlight to minimize the possibility of photodegradation of the targeted compounds.

Figure 4-7 Applikon ez-Control bioreactor set-up and controller

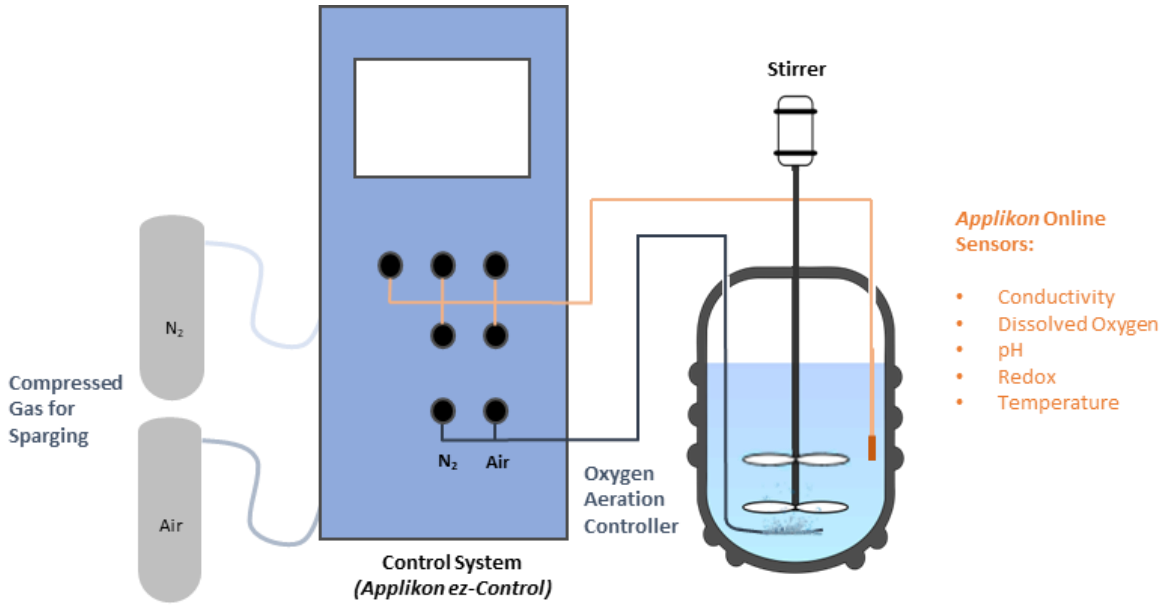


Figure 4-8 Bioreactor and controller set-up scheme

The aeration system was adapted to the redox conditions by modifying the dissolved oxygen setpoint similarly to those in a PhoRedox CAS wastewater treatment plant. This oxygen concentration was obtained by sparging air or nitrogen in the bioreactor.

### 4.3.1 Operation

The experiment aimed to determine the biotransformation rates of the targeted compounds under different redox conditions and, with and without the addition of influent, to analyse if metabolic or cometabolic processes degrade the compounds. Three batch experiments were carried out in duplicates: I) the control batch, only with effluent; II) a batch with sludge diluted in the effluent; III) a batch with sludge diluted in the effluent and fed with primary influent following a similar methodology proposed by Joss *et al.* (2006). To increase the experimental resolution of the compounds with high biotransformation rates, the sludge concentration used in both II) and III) batch tests was set to 0,5 (± 0,05) $g_{SS}$/L. The batch experiments were conducted entirely with wastewater from Walcheren WWTP, as mentioned above.

To know the correct volume of sludge in each batch, its suspended solids were measured at the beginning of each batch experiment, and then through a mass balance was possible to define the amount of sludge needed. Furthermore, in the III) batch test, the volume ratio between the activated sludge and the influent was kept at 1:1, as reported by Joss *et al.* (2006).

Since the experimental conditions should be as similar as possible to the real ones, it was aimed that the pH is maintained between 7 and 8. However, the pH was not corrected due to potential interferences in the biotransformation rate promoted by the used reagents. In the same way, during the experiment, the temperature was kept constant at 19 (± 1) ºC.

During the aerobic experiments, both reactors were intermittently aerated to keep the oxygen concentrations between 3 and 4 mg/L (Gusmaroli *et al.*, 2020; Joss *et al.*, 2006). On the other hand, the dissolved oxygen concentration determined setpoint for the anoxic batch-test experiment was 0,2 mg/L (Ruas *et al.*, 2022). Lastly, to assure the inexistence of oxygen (≈ 0,0 mg/L) in the anaerobic experiment, gaseous nitrogen was sparged with a flow rate of 1L $N_2$/h (Joss *et al.*, 2006).

The *Applikon ez-Control* measures the dissolved oxygen concentration in percentage. To assure the correct oxygen concentration, an external dissolved oxygen sensor *WTW CellOx325,* by *Xylem Analytics*® was used to guarantee the desired bioreactor dissolved oxygen concentration (Appendix 3).

To guarantee that the batch tests were working with a similar micropollutant concentration, the batches were spiked with 2 µg/L of a stock solution. This stock solution, prepared with Mili-Q® ultrapure water (MilliporeSigma, Massachusetts, USA), contain all the targeted compound in the concentrations shown in Appendix 2. Since the batch reactors had a volume of 2,5 L, the spike was made with 5 mL of the stock solution to assure the before-mentioned concentration. This concentration was defined with the detention limit of the analysis method used in this experiment, which will be explained later. It was possible to work with a stock solution with a cocktail mixture since the solid-water distribution is not affected by the presence of more compounds, whether they have equal or different distribution coefficient values (Hörsing *et al.*, 2022; Hörsing *et al.*, 2011). Each compound's biotransformation rate can vary due to the possible synergy or antagonism caused by other molecules and metabolites. Yet, in an actual wastewater treatment plant, those compounds also exist simultaneously in the influent. This methodology mimics the possible interactions between the compounds in an existing facility.

## 4.4 Batch Samples Collection

Eight samples per batch experiment were collected and two samples per control to quantify the micropollutants, for a total of 108 samples. Moreover, for the quantification of the micropollutants concentration in the wastewater (influent and effluent) additional six samples were taken, one per redox condition, for a total of 114 samples.

A sample size of 20 mL was taken at each collection time. All the batch tests were sampled at 0 min, 15 min, 40 min, 2h, 6h, 12h, 24h, and 48h. However, the controls were only sampled at the beginning and end of the experiment (0 min and 48h, respectively). After collection, the samples were filtered through an NC45 nitrocellulose membrane (Whatman, Maidstone, UK), stored in glass vials, and then preserved at -20 °C until analysis (Han *et al.*, 2021).

To ensure the solid concentration of 0,5 (± 0,05) $g_{SS}$/L in the bioreactor, the total suspended solids of the wastewater samples were quantified in triplicate. Moreover, to calculate the specific biotransformation rates ($K_{bio}$), the suspended solids of all 108 samples collected from the batch reactors were also quantified.

In addition, the wastewater samples (influent, activated sludge, and effluent) were also sampled to perform a chemical characterization (total chemical oxygen demand, dissolved chemical oxygen demand, organic carbon, total inorganic carbon, total carbon, ammonia, nitrates, nitrites, phosphates). This characterization was carried out after sampling collection. The characterization was made in triplicates to define the standard deviation of the method.

## 4.5 Physicochemical Analysis

The suspended solids were analysed following Standard Methods N° 2540 to determine the solids in wastewater (APHA, 2020). On the other hand, the chemical parameters (total chemical oxygen demand, dissolved chemical oxygen demand, total organic carbon, total inorganic carbon, total carbon, ammonia, nitrates, nitrites, and phosphates) were analysed using Cuvette Hach Test Kits. Table 4-4 indicates which Hach test kits were used, their range of detention, and to which sample.

Table 4-4 Cuvette Hach Test Kits used and respective analyse and sample

| Cuvette Hach Test Kits | Analysis | Sample |
|---|---|---|
| LCK314 (COD – 15 to 150 mg $O_2$/L) | Dissolved chemical oxygen demand and<br>Total chemical oxygen demand | Effluent |
| LCK514 (COD – 100 to 2000 mg $O_2$/L) | Dissolved chemical oxygen demand<br>Total chemical oxygen demand | Activated sludge<br>Primary effluent |
| LCK914 (COD – 5 to 60 g $O_2$/L) | Total chemical oxygen demand | Activated sludge |
| LCK381 (TOC – 60 to 735 mg TOC/L) | Total carbon<br>Total inorganic carbon<br>Total organic carbon | Activated Sludge<br>Primary effluent<br>Effluent |
| LCK303 (Ammonium – 2 to 47 mg $NH_4$-N/L) | Ammonium | Activated Sludge<br>Primary effluent<br>Effluent |
| LCK339 (Nitrate – 0,23 to 13,50 mg $NO_3$-N/L) | Nitrates | Activated Sludge<br>Primary effluent<br>Effluent |
| LCK341 (Nitrite – 0,015 to 0,600 mg $NO_2$-N/L) | Nitrites | Activated Sludge<br>Primary effluent<br>Effluent |
| LCK350 (Phosphate – 2 to 20 mg $PO_4$-P/L) | Total phosphate | Activated Sludge<br>Primary effluent<br>Effluent |

LCK 514 was used to determine the dissolved chemical oxygen demand in the activated sludge, and both total a dissolved chemical oxygen demand in the primary influent.

To quantify the micropollutants concentration in all the 114 samples collected, an analytical method based on direct injection of the sample on a C18 column in combination with mass spectrometry was used. Internal standards are first added to the wastewater sample, after which it is filtered through a 0,20 µm filter. After this, 100 µL of the sample is applied to the C18 analytical column. The analysis is performed using a Shimadzu Nexera X2 HPLC system coupled to a triple quadrupole SCIEX 6500+ mass spectrometer. The mass spectrometer is equipped with a heated electrospray ionization interface (H-ESI) and measures according to the selected reaction monitoring (SRM) principle. The analysis is performed in positive ionization mode. For the chromatographic separation, a Phenomenex Luna Omega Polar C18 column (100 mm × 2,1 mm I.D., particle size 1,6 m) is used in combination with a Phenomenex SecurityGuard Ultra precolumn. The content is calculated based on an external calibration curve, whereby correction is made for the internal standards. The method's detection limit depends on the matrix and can vary between 0,0 µg/L to 0,1 µg/L.

## 4.6 Biotransformation rates and reaction rate constants determination

The biotransformation rate determination follows the methodology applied by Joss *et al.* (2006) and by Mazioti *et al.* (2015), explained in Chapter 2.2. However, in this work, only the biological transformation will be considered. This degradation rate constant was normalized with the reactor solids concentration and determined using pseudo-first-order degradation kinetics in equation 1. All the parameters' units can be found in Table 2-1.

$$\frac{dC}{dt} = \frac{C_{t+dt} - C_t}{dt} = \frac{-k_{bio} \times X_{SS} \times C}{1 + K_d \times X_{SS}} \quad (1)$$

To simplify the determination of the biotransformation rate constant, the equation was linearized using the natural logarithmic. First, the equation was organized in order of dC/C and reduced to a constant (k) since all the affected parameters are fixed values. Equation 2 shows the process.

$$\frac{dC}{dt} = \frac{-k_{bio} \times X_{SS} \times C}{1 + K_d \times X_{SS}} \Leftrightarrow \frac{dC}{C} = \frac{-k_{bio} \times X_{SS}}{1 + K_d \times X_{SS}} dt \Leftrightarrow \frac{dC}{C} = k\, dt \quad (2)$$

Once simplified, the equation was primitivized according to equation 3, achieving the final linearized form of the equation, shown below. Once primitivized, the defined constant was replaced by the original values.

$$\frac{dC}{C} = k\, dt \Leftrightarrow \ln C = kt\, dt \Leftrightarrow \ln C = \frac{-k_{bio} \times X_{SS}}{1 + K_d \times X_{SS}} t \quad (3)$$

After linearization of the equation, it was possible to obtain the biotransformation rate constant from the slope of equation 3, according to equation 4.

$$k_{bio} = -\frac{slope}{X_{SS}} \times (1 + K_d \times X_{SS}) \quad (4)$$

If in the batch test, for a determinate compound, $K_d \times X_{SS}$ is lower than 0,1 that term can be neglected once it means that less than 10% of the compound was sorped. If this term is ignored, the determination of the biotransformation rate starts to be calculated through equation 5.

$$\frac{dC}{dt} = \frac{C_{t+dt} - C_t}{dt} = -k_{bio} \times X_{SS} \times C \quad (5)$$

To simplify the determination of the reaction rate constants, the equation was linearized using the logarithmic form, using the same process as observable in equation 6.

$$\frac{dC}{dt} = -k_{bio} \times X_{SS} \times C \Leftrightarrow \frac{dC}{C} = -k_{bio} \times X_{SS}\, dt \Leftrightarrow \frac{dC}{C} = k\, dt \quad (6)$$

Once simplified, the equation was also primitivized according to equation 7, achieving the final linearized form of the equation, shown below. Once primitivized, the defined constant was replaced by the original values.

$$\frac{dC}{C} = k\, dt \Leftrightarrow \ln C = kt\, dt \Leftrightarrow \ln C = -k_{bio} \times X_{SS} \quad (7)$$

Therefore, after linearization, it was possible to obtain the biotransformation rate constant from the slope of equation 7 according to equation 8.

$$k_{bio} = -\frac{slope}{X_{SS}} \quad (8)$$

In all the micropollutant compounds analysed in this study, seven compounds have significant sorption calculated as the solid-water distribution coefficient times the sludge biomass (Kd x Xss ≤ 0,1): azithromycin, clarithromycin, metoprolol, propranolol, irbesartan, trimethoprim, and venlafaxine. As presented in Table 2-4, for both candesartan and gabapentin, the distribution coefficient values in activated sludge were not found. However, values were found in other different matrixes, and both distribution coefficients for those were lower than the coefficient for carbamazepine (Berthod *et al.*, 2014; Berthod *et al.*, 2017; Boulard *et al.*, 2020). Therefore, in the present study, it was considered that the sorption is lower than 10% for those compounds and therefore was negligible for the $k_{bio}$ determination.

## 4.7 Correlation Between Removal Efficiency and Micropollutant Physicochemical Properties

To attempt to correlate the hydrophobicity, polarity, and solubility of the targeted micropollutants and their removal efficiency, the compounds were grouped by their physicochemical properties.

After being grouped, the average removals per group were made to analyse the correlation between removal efficiency, hydrophobicity, and polarity. To investigate if the physicochemical properties were affected by the removal efficiencies under different redox conditions.

For the correlation between solubility and removal efficiency, the values were plotted to analyse whether the removal depended on the solubility values. After plotting, the suggested tendency curve was examined throughout the obtained square-R.

## 4.8 Hydraulic Retention Time Proposal

Once the biotransformation rate constants are determined, it is possible to observe the biotransformation rate of the compound based on the pseudo-first-order kinetics determined with the equation below. This equation was based on the inverse of the process used to obtain .

$$\frac{dC}{dt} = e^{-\frac{K_{bio}}{X_{SS}} \times t + \ln C_0} \quad (9)$$

With was possible to determine the existent degradation of the micropollutant in each tank based on their HRT. Thus, assuming that the initial concentration in the tank is equal to the one at the exit of the last tank (with a defined HRT), it was possible to determine the final concentration after different tank HRTs and compare the initial and final concentrations to assess the removal efficiencies.

# 5

# RESULTS AND DISCUSSION

## 5.1 Walcheren Wastewater Treatment Plant

The Walcheren WWTP is designed for 178 700 population equivalent and a maximum flow of 8 015 $m^3$/h. An assumption, based on a 2-year data series from Walcheren was made, and a peak factor of 3,8 was determined. This factor is higher than the ones usually found in Southern European countries. However, that may be linked with the rainfall in the Netherlands. Therefore, the WWTP's average design flow of 50 620 $m^3$/d was determined. Based on the same premise, currently, Walcheren is operating with an average flow of 41 600 $m^3$/d for an estimated population of 144 000 inhabitants, expecting to reach the design capacity in 25 years if a population growth rate of approximately 1% per year is maintained.

## 5.2 Wastewater Characterization

The wastewater samples collected from the Walcheren WWTP were submitted to physicochemical characterization.

Table 5-1 presents the average of each parameter's measured values and their standard deviation.

Table 5-1 WWTP Walcheren wastewater characterization

| Parameters | Units | Influent (after Primary Settler) | Anaerobic Activated Sludge | Anoxic Activated Sludge | Aerobic Activated Sludge | Effluent (after secondary settler) |
|---|---|---|---|---|---|---|
| Conductivity | mS/cm | 2,00 ± 0,59 | 1,59 ± 0,70 | 1,36 | 2,19 ± 0,05 | 1,85 ± 0,52 |
| pH | - | 7,59 ± 0,29 | 6,76 ± 0,29 | 7,12 | 7,02 ± 0,13 | 7,53 ± 0,39 |
| Redox | mV | -39,0 ± 91,1 | -107,1 ± 23,1 | -89,2 | -3,0 ± 5,2 | 148,6 ± 51,4 |
| Solids | g/L | 0,3813 ± 0,3919 | 4,9342 ± 0,5128 | 4,3717 ± 0,0797 | 4,5025 ± 0,0829 | 0,0280 ± 0,0178 |
| Total Chemical Oxygen Demand | mg $O_2$/L | 562,00 ± 216,13 | 5 885,00 ± 869,74 | 5 220,00 | 5 825,00 ± 459,62 | 69,94 ± 34,46 |
| Dissolved Chemical Oxygen Demand | mg $O_2$/L | 153,95 ± 68,28 | 138,75 ± 144,60 | 51,70 | 99,30 ± 57,06 | 41,86 ± 2,95 |
| Total Carbon | mg C/L | 200,65 ± 125,74 | 98,35 ± 30,62 | 91,20 | 132,33 ± 9,19 | 107,70 ± 25,40 |
| Total Inorganic Carbon | mg C/L | 56,29 ± 29,25 | 32,90 ± 7,92 | 30,70 | 46,40 ± 4,03 | 37,50 ± 9,10 |
| Total Organic Carbon | mg C/L | 135,02 ± 26,46 | 65,45 ± 22,70 | 60,50 | 85,83 ± 4,23 | 70,27 ± 21,71 |
| Ammonia | mg $NH_4$/L | 40,76 ± 14,82 | 26,00 ± 19,09 | 14,40 | 7,21 ± 5,36 | 4,93 ± 2,87 |
| Nitrite | mg $NO_2$/L | 0,11 ± 0,06 | 0,02 ± 0,01 | 0,02 | 0,10 ± 0,04 | 0,17 ± 0,04 |
| Nitrate | mg $NO_3$/L | 2,50 ± 0,52 | 1,76 ± 0,32 | 2,18 | 3,01 ± 0,60 | 16,63 ± 4,72 |
| Phosphorous | mg $PO_4$/L | 9,41 ± 6,65 | 19,00 ± 11,17 | 0,61 | 2,92 ± 5,15 | 0,20 ± 0,11 |

The characterization showed that the influent COD was mainly particulate with a total COD of about 562,0 ± 216,1 mg $O_2$/L and soluble COD 153,9 ± 68,3 mg $O_2$/L. It is also possible to observe that in the effluent, the nitrogen is almost all in ammonia form, while in the effluent, most of the nitrogen is found under nitrates.

The anoxic activated sludge measurements have no standard deviations since they were based on only one sample. Possible anomalies can be found in the carbon parameters (total carbon, total inorganic carbon, and total organic carbon) since, during their determination, some interferences were found. The methodology used to determine the carbon content is sensitive to the presence of sulphides. Even though inorganic parameters were not measured in the present research, the interference found associated with industries nearby (Wishart *et al.*, 2018) led to the conclusion that the interference may be connected to the chemical properties of the wastewater. Therefore, a 1/10, 1/20, and 1/50 dilution was made to analyse the samples. Only after the 1/50 dilution was it possible to observe a proper measurement. The rest are within the expected values, and the effluent parameters agree with the discharge limits (EC, 1991).

It is important to point out that the sampling campaign occurred during spring (March and April). Even though, as shown in Table 4-3, no significant precipitation may affect the wastewater concentrations, industrial effluent discharges and effluents from touristic facilities, such as the camping facilities in the surrounding areas of the wastewater treatment plant, could have influenced it.

## 5.3 Wastewater Treatment Plant Mass Balance Determination

Based on the collected data through the sampling campaign and historical data obtained from the WWTP, it was possible to estimate the actual mass balance of the CAS system of the Walcheren WWTP (Figure 5-1).

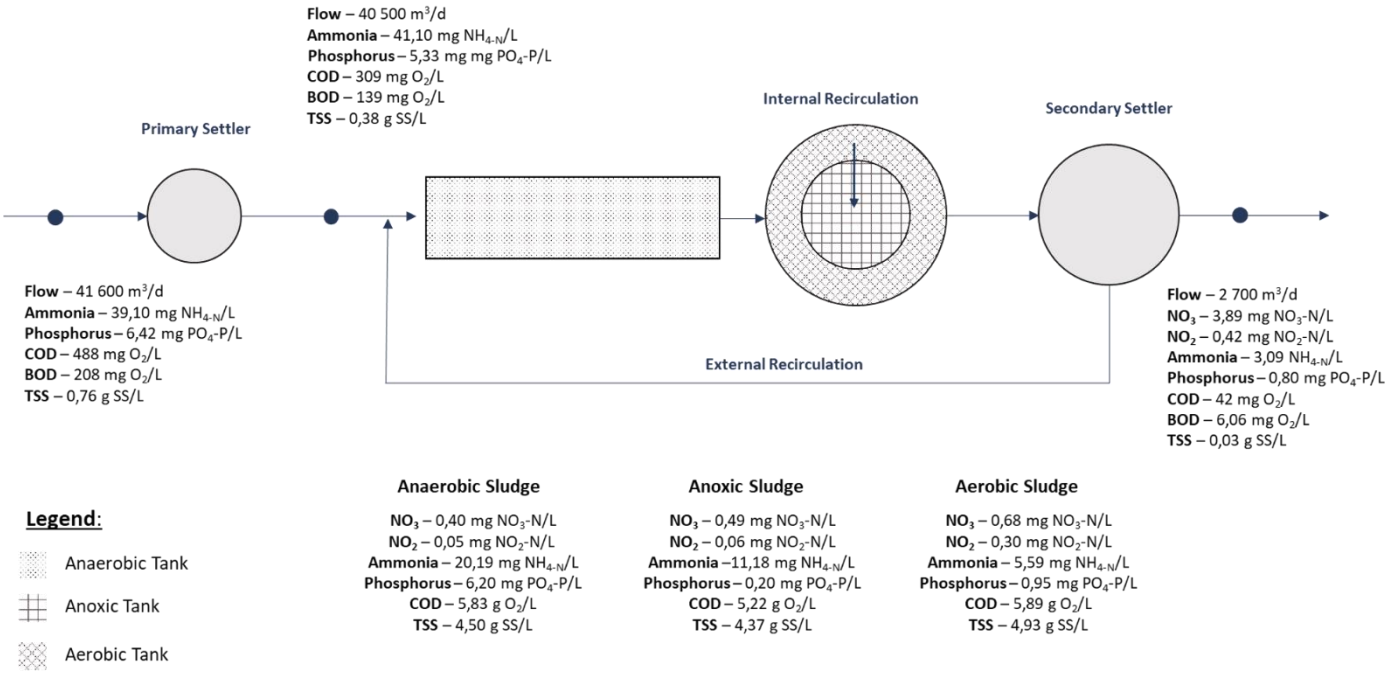


Figure 5-1 Walcheren WWTP mass balance

The mass balance indicated that the first settler showed good solids removal efficiency of around 50%. That solid removal also led to a removal of almost 40% in both chemical and biological oxygen demand (BOD and COD) and the removal of nearly 20% of phosphorus. This process produces approximately 46 $m^3$/d of sludge. As expected, from the anaerobic reactor to the anoxic reactor exists a removal of phosphorous and an ammonia removal of around 50%. At the same time, the nitrates and nitrites showed a slight increase due to the recirculation from the aerobic reactor (where the nitrification process occurs). From the anoxic reactor to the aerobic one, almost 50% of ammonia was removed, while the nitrates and nitrites increased, as expected. There is also an increase in phosphate due to phosphorous release from the phosphorous accumulator organisms (PAO).

Based on Table 5-1 values, the removal efficiencies in the Walcheren WWTP were determined (Table 5-2). These removal efficiencies are consistent with the removal rates expected in a PhoRedox system (Metcalf & Eddy, 2013).

Table 5-2 Average chemical removal efficiencies obtained at WWTP Walcheren

| Parameters | Removal Efficiency (%) |
|---|---|
| Total Chemical Oxygen Demand | 87 (±8) % |
| Dissolved Chemical Oxygen Demand | 73 (±11) % |
| Nitrogen | 52 (±17) % |
| Phosphorous | 96 (±4) % |

Phosphorus removal is higher because of biological and chemical precipitation removal in WWTP Walcheren.

The micropollutant concentrations determined in both influent (after primary settler) and effluent are presented in Table 5-3 with their corresponding average and standard deviation.

Table 5-3 Micropollutants concentration (average and standard deviation) of influent (after primary settler) and effluent

| Micropollutants | Influent after primary settler (µg/L) | Effluent (µg/L) |
|---|---|---|
| 4-, 5-Methylbenzotriazole | 0,92 ± 0,17 | 1,01 ± 0,13 |
| Benzotriazole | 5,60 ± 0,95 | 4,45 ± 1,20 |
| Carbamazepine | 0,37 ± 0,09 | 0,40 ± 0,01 |
| Clarithromycin | 0,09 ± 0,04 | 0,08 ± 0,01 |
| Diclofenac | 0,71 ± 0,29 | 0,74 ± 0,04 |
| Hydrochlorothiazide | 1,76 ± 0,59 | 1,95 ± 0,07 |
| Metoprolol | 1,60 ± 0,70 | 1,70 ± 0,07 |
| Propranolol | 0,02 ± 0,01 | 0,02 ± 0,01 |
| Sotalol | 1,61 ± 0,68 | 1,70 ± 0,07 |
| Sulfamethoxazole | 0,38 ± 0,19 | 0,18 ± 0,08 |
| Trimethoprim | 0,10 ± 0,04 | 0,10 ± 0,01 |
| Azithromycin | 0,10 ± 0,01 | 0,08 ± 0,03 |
| Candesartan | 0,29 ± 0,11 | 0,33 ± 0,04 |
| Gabapentin | 3,77 ± 1,70 | 2,85 ± 0,07 |
| Irbesartan | 0,68 ± 0,33 | 0,91 ± 0,10 |
| Venlafaxine | 0,27 ± 0,12 | 0,29 ± 0,041 |

When compared to the average concentration of the other compounds, high values of 5,60 µg/L and 3,77 µg/L for both benzotriazole and gabapentin, correspondingly, were determined by the influent of the CAS system. Besides those, hydrochlorothiazide, metoprolol, sotalol, and 4-5,-methylbenzotriazole showed moderate concentrations (> 0,9 µg/L). The high values of benzotriazole and 4-5,-methylbenzotriazole can be explained by the mentioned 20% industrial influence since part of that industry is metallurgic and therefore use anti-corrosion chemicals. On the other way, gabapentin is usually administrated at dosages of up to 4 000 mg/day, and its bioavailability is variable, depending on the administrated dose (from 80% to 20%) (Wishart *et al.*, 2018). It is also interesting to analyse that

from all the pharmaceuticals, all the ones belonging to the anti-hypertensive group showed high concentrations. This may also be associated with the fact that the average age in Vlissingen is 45 years old (AdminStat, 2020) and also with the fact that this group represents 15% of the prescribed medicines in The Netherlands, being the 3rd most sold pharmaceutical group (CBS, 2021). Besides, this study and sampling campaign was carried up in spring which may affect the concentration of the pharmaceutical compounds since, during winter, it is expected, for example, an increase in antibiotics consumption and, therefore, their presence in wastewater. Golovko *et al.* (2021) and Luo *et al.* (2014) observed a significant variability of the pharmaceutical compounds in different effluents of various locations and during separate times of the year, as expected. Yet, Fick *et al.* (2016) also observed high levels of anti-hypertensives in raw influents.

To better analyse the removal of the compounds in the Walcheren WWTP, the average removal of each compound during all sampling campaigns was determined (Table 5-4).

Table 5-4 Micropollutants removal efficiencies obtained at WWTP Walcheren

| Parameters | Removal Efficiency (%) |
|---|---|
| 4-, 5-Methylbenzotriazole | 0% |
| Benzotriazole | 28% |
| Carbamazepine | 12% |
| Clarithromycin | 19% |
| Diclofenac | 10% |
| Hydrochlorothiazide | 9% |
| Metoprolol | 15% |
| Propranolol | 17% |
| Sotalol | 17% |
| Sulfamethoxazole | 61% |
| Trimethoprim | 20% |
| Azithromycin | 21% |
| Candesartan | 5% |
| Gabapentin | 35% |
| Irbesartan | 17% |
| Venlafaxine | 15% |

Sulfamethoxazole was the analysed micropollutant with the highest removal (61%), while 4-,5-methylbenzotriazole has shown no removal. Besides sulfamethoxazole, no other micropollutant had a removal above 50%. Azithromycin, benzotriazole, gabapentin, sulfamethoxazole, and trimethoprim have shown a significant removal (>20%), while the other compounds had no substantial removal (<10%).

It is important to mention that the removals observed in Table 5-4 were obtained with an SRT of 25 days and a total HRT of 29,6 h. This HRT was determined based on the average flow that Walcheren WWTP is currently receiving (≈ 1700 $m^3$/h). Since Walcheren WWTP is not presently working at total capacity, with the increase of its flow, the HRT will start to decrease until the design values. Table 5-5

shows the HRT of the different compartments in the biological reactor and the total HRT of the biological treatment. This HRT was determined for the average current flow and complemented with the information in Table 4-1.

Table 5-5 Design and current hydraulic retention times at Walcheren WWTP

| Biological Process | Volume ($m^3$) | Design HRT (h) | Current HRT (h) |
|---|---|---|---|
| Selector | 1 200 | 0,1 | 0,7 |
| Aerobic tank | 10 640 | 1,3 | 6,3 |
| Anoxic tank | 15 200 | 1,9 | 9,0 |
| Anaerobic tank | 22 800 | 2,8 | 13,5 |
| Biological reactor | 49 840 | 6,2 | 29,6 |

Currently, the WWTP is receiving an average flow of ≈1 700 $m^3$/h (≈40 500 $m^3$/d), yet based on two years hourly dataset, it was possible to observe that the peak flow was ≈6500 $m^3$/h. With this, it was possible to determine that the tipping factor is 3,8. Using this factor was possible to determine the average flow corresponding to the design flow and reach a flow of 50 621 $m^3$/d. Therefore, assuming a population growth of 1%/year, it is possible to estimate that in 25 years, Walcheren WWTP will reach its maximum capacity.

## 5.4 Batch Test Operational Conditions

In Chapter 4, the operation conditions and the set points for different parameters were mentioned. Yet, during the experiment, some variations occurred. Therefore, Table 5-6 the average operation conditions under each redox condition.

Table 5-6 Batch operation conditions

| Parameters | Units | Anaerobic Study | Anoxic Study | Aerobic Study | Average |
|---|---|---|---|---|---|
| Conductivity | mS/cm | 1,26 (± 0,47) | 1,87 (± 0,10) | 2,05 (± 0,22) | 1,7 (± 0,5) |
| Dissolved Oxygen | g $O_2$/L | 0,0 (± 0,0) | 0,2 (± 0,1) | 3,5 (± 0,4) | - |
| pH | - | 9,1 (± 0,2) | 7,9 (± 0,5) | 7,9 (± 0,2) | 8,3 (± 0,7) |
| Solids | g SS/L | 0,46 (± 0,01) | 0,48 (± 0,1) | 0,49 (± 0,03) | 0,48 (± 0,02) |
| Temperature | °C | 18,5 (± 0,7) | 18,7 (± 0,3) | 19,1 (± 0,2) | 18,7 (± 0,5) |

The experiments were conducted under similar conditions, as pretended, to minimize possible interferences. An increase in the water conductivity of 39% between the redox conditions was observed, possibly due to the biological nutrients removal and the algae-bacteria symbiosis (Levlin, 2007) that occurred in the WWTP to the temperature increase of 0,6°C (3%). The dissolved oxygen concentration was within the stipulated in the methodology (chapter 4.3.1): 3,5 mg $O_2$/L [with a redox of 118,6 (± 23,2) mV], for aerobic condition tests; 0,2 mg $O_2$/L [with a non-defined redox due to a technical issue of

oxiredox sensor], for anoxic condition tests; 0,0 mg $O_2$/L [with a redox of -161,3 (± 84,1) mV], for anaerobic condition tests.

It is also possible to notice that the anaerobic experiment had a considerably higher pH (around 9), which is attributed to using anaerobic sludge and a different equilibrium of CO2 in the reactor. The solids concentration was also within the range defined in chapter 4.3.1.

## 5.5 Removal Efficiencies under Different Redox Conditions

As expected, all the control experiments showed no significant removal. However, gabapentin and irbesartan appear to have some degradation above the detection limit margin. It is possible to observe a slight degradation of gabapentin in both anoxic and anaerobic conditions, while irbesartan showed degradation in the three redox studied (aerobic, anoxic, and anaerobic). However, this result might be due to the chemical instability of the compounds (Jansook *et al.*, 2022).

The removal efficiencies of each compound under the different redox conditions are presented in Table 5-7.

Table 5-7 Removal efficiencies of the sixteen micropollutants in the different redox conditions, with and without influent

| Micropollutants | Aerobic | | Anoxic | | Anaerobic | |
|---|---|---|---|---|---|---|
| | Without Influent | With Influent | Without Influent | With Influent | Without Influent | With Influent |
| 4-, 5-Methylbenzotriazole | 8 (± 2) % | 18 (± 1) % | 10 (± 2) % | 13 (± 2) % | 45 (± 9) % | 10 (± 3) % |
| Benzotriazole | 25 (± 1) % | 39 (± 4) % | 40 (± 0) % | 44 (± 3) % | -8 (± 4) % | 12 (± 0) % |
| Carbamazepine | 0 (± 0) % | -10 (± 0) % | 2 (± 3) % | 6 (± 4) % | 2 (± 10) % | -7 (± 3) % |
| Clarithromycin | 67 (± 2) % | 73 (± 10) % | 62 (± 1) % | 55 (± 8) % | 72 (± 1) % | 75 (± 4) % |
| Diclofenac | 0 (± 2) % | 0 (± 0) % | 2 (± 3) % | 7 (± 2) % | 0 (± 0) % | 0 (± 0) % |
| Hydrochlorothiazide | 10 (± 6) % | 0 (± 0) % | 6 (± 4) % | 9 (± 1) % | 5 (± 9) % | -6 (± 5) % |
| Metoprolol | 51 (± 3) % | 53 (± 0) % | 33 (± 1) % | 32 (± 5) % | 2 (± 6) % | 39 (± 1) % |
| Propranolol | 78 (± 7) % | 67 (± 6) % | 62 (± 5) % | 58 (± 5) % | 54 (± 8) % | 46 (± 7) % |
| Sotalol | 34 (± 1) % | 38 (± 2) % | 26 (± 1) % | 25 (± 4) % | -12 (± 3) % | -3 (± 4) % |
| Sulfamethoxazole | 20 (± 2) % | 37 (± 2) % | 35 (± 1) % | 41 (± 3) % | 16 (± 2) % | 32 (± 1) % |
| Trimethoprim | 24 (± 6) % | 17 (± 8) % | 12 (± 2) % | 11 (± 6) % | 76 (± 1) % | 53 (± 2) % |
| Azithromycin | 61 (± 6) % | 64 (± 5) % | 21 (± 6) % | N.D. | 45 (± 9) % | 55 (± 3) % |
| Candesartan | 0 (± 0) % | 0 (± 0) % | 2 (± 2) % | 4 (± 2) % | -9 (± 7) % | -4 (± 2) % |
| Gabapentin | 38 (± 1) % | 59 (± 1) % | 77 (± 2) % | 91 (± 1) % | 3 (± 0) % | 36 (± 1) % |
| Irbesartan | 18 (± 0) % | 20 (± 6) % | 16 (± 4) % | 17 (± 4) % | 29 (± 3) % | 24 (± 3) % |
| Venlafaxine | 13 (± 1) % | 20 (± 8) % | 19 (± 2) % | 12 (± 7) % | 0 (± 0) % | -3 (± 6) % |

N.D. : Not Determined

Table 5-7 shows that both aerobic and anoxic conditions have higher overall micropollutant removal when compared to anaerobic conditions, with a respective overall average removal of 31%, 28%, and 22%. It is also possible to observe that clarithromycin is the targeted micropollutant with the highest removal, presenting removals efficiencies higher than 50% in all the redox conditions. Moreover, clarithromycin shows the highest removal under aerobic (73%) and anaerobic conditions (75%). Yet, gabapentin is the micropollutant with the highest overall removal (91%) observed under anoxic conditions.

With the results obtained (Table 5-7), it is impossible to indicate one redox condition capable of degrading all the compounds, as already expected. The best redox condition for micropollutant removal depends on the micropollutant and varies according to the targeted micropollutant. Yet, most of the compounds increase their removal efficiency, even slightly, after the addition of influent (in aerobic and anoxic conditions). This increase might not be as significant as expected, since the batch tests were carried out with effluent that has not been through an advanced oxidation process leading to a high chemical oxygen demand [69,94 (± 34,46) mg $O_2$/L] (Table 5-1), even without the addition of influent as a substrate.

Yet, the increase in the degradation of the compounds after the addition of influent might mean that for those compounds, cometabolism is the main degradation mechanism. This process happens due to the presence of autotrophic microbes (that have shown cometabolic activities in biodegrading micropollutants (Tran *et al.*, 2013), using their enzymatic processes resulting from the substrate metabolism (in this case, the influent added). However, some compounds do not follow this tendency, which may be connected to the fact that their removal happens due to metabolic mechanisms promoted by heterotrophic microbes, which are particularly important in the degradation of highly biodegradable compounds (Tran *et al.*, 2013). It is known that both AOB and NOB play a key role in the biotransformation process (by metabolization). This can be explained based on the studies on hydroxylamine and nitrate oxidoreductases (enzymes produced by AOB and NOB) (Kennes-Veiga *et al.*, 2022). These organisms play even more important roles in compounds that have shown low biodegradability (Tran *et al.*, 2013). Even though both microorganisms are important, it is also known that AOB enzymes play a more important role in biotransformation compared to NOB enzymes (Kennes-Veiga *et al.*, 2022; Kennes-Veiga *et al.*, 2021). However, a better understanding of the biotransformation mechanisms and pathways is urgently needed to design more efficient bioreactors and enhance the biotransformation of micropollutants.

Under aerobic conditions, carbamazepine, hydrochlorothiazide, propranolol, and trimethoprim had lower removal efficiencies after adding influent. Carbamazepine contrary reduced its removal after the addition of influent (in 10%), while the addition of influent led to a reduction of diclofenac removal, from low removal (of approximately 4%) to no removal. Besides, candesartan has shown no removal with or without influent addition in aerobic conditions. Clarithromycin, propranolol, and azithromycin in anoxic conditions decreased their removal after adding influent. Under anaerobic conditions, contrary to the others, most compounds reduce their removal efficiency after adding influent. Azithromycin, clarithromycin, gabapentin, metoprolol, propranolol, and sulfamethoxazole showed an increase in their removal. The reduction of the removal efficiency and negative values may be due to the retransformation of higher congeners or precursors to the parent compound (Wu *et al.*, 2017), which this redox condition may promote. There is not an actual negative removal, since that means that no removal existed, yet negative values may be associated either with retransformation or analytical errors. The different sorption and desorption of the diverse target compounds (Kotowska *et al.*, 2021; Wu *et al.*, 2017) can also explain these observed results, even to a low extent, since the targeted compounds are not expected to have high sorption (Table 2-4). Besides those possibilities, the efficiency reduction after the addition of influent (as substrate) in the anaerobic redox condition may also be due

to the release of the compound to the liquid phase after the bacteria breaking down the organic matter to each it was adsorbed (Gobel *et al.*, 2005; Grandclement *et al.*, 2017).

Moreover, azithromycin, hydrochlorothiazide, metoprolol, propranolol, and sotalol are mainly degraded under aerobic conditions, with removals of 64%, 10%, 53%, 78%, and 38% respectively; while benzotriazole, diclofenac, sulfamethoxazole, candesartan, and gabapentin are mostly removed under anoxic conditions, reaching removals of 44%, 7%, 41%, 4%, and 91%, respectively. The 4-,5-methylbenzotriazole, clarithromycin, trimethoprim, and irbesartan have shown better removal efficiencies in anaerobic conditions, of 45%, 75%, 76%, and 29%, respectively.

Carbamazepine, diclofenac, hydrochlorothiazide, and candesartan are recalcitrant compounds that have difficulty being removed by the conventional wastewater treatment processes. Therefore, it was already expected that there would be no significant removal in any redox conditions.

Under aerobic conditions, only carbamazepine has shown some negative removal after adding influent. Under anaerobic conditions, compounds like benzotriazole, candesartan, carbamazepine, hydrochlorothiazide, sotalol, and venlafaxine have shown negative removal. Benzotriazole, candesartan, and sotalol showed negative removal in the latter redox condition, with and without the addition of influent. In contrast, carbamazepine, hydrochlorothiazide, and venlafaxine showed negative removal after adding influent. As mentioned earlier, these negative removals after influent addition may also be associated with the release of the compound to the liquid phase after the bacteria breaking down the organic matter to each it was adsorbed or also due to possible retransformation of existing higher congeners or precursors, to the parent compound.

Overall, only six compounds have shown biotransformation efficiency higher than 50% in all the redox conditions (azithromycin, clarithromycin, gabapentin, metoprolol, propranolol, and trimethoprim), and only gabapentin showed a degradation efficiency higher than 80%, under anoxic conditions with influent addition, reaching 91%.

Analysing the removal efficiency per compound, it is possible to notice that 4-,5-methylbenzotriazole had a higher removal efficiency, of almost 50%, under anaerobic conditions, without adding influent. Under the other redox conditions (aerobic and anoxic), this compound has a removal rate between 10% and 20%. In aerobic and anoxic conditions, adding influent led to a removal increase of 10% under aerobic conditions and 3% under anoxic conditions. Yet, under anaerobic conditions, the removal efficiency dropped by 35% after adding the influent. This may be due to possible compound release after organic matter degradation. Another explanation may also be that the compound is mainly degraded by metabolism. Therefore, due to the influent more easily biodegradability, bacteria rather use it as a substrate instead of the compound. Kotowska *et al.* (2021), Karthikraj and Kannan (2017), and Voutsa *et al.* (2006) observed removal efficiencies from 30% to 90% in CAS, which are in the range of the ones determined in the present study. Weiss *et al.* (2006) observed values considerably lower than the ones found in the literature and shown in the present study. However, Weiss *et al.* (2006) studied the compounds separated, reaching a removal of 11% to 5-methylbenzotriazole and a -6% removal to 4-methylbenzotriazole.

In the case of benzotriazole, the anoxic redox condition showed a removal efficiency of around 44%, which was the highest for this compound after adding influent. Similarly, to the anoxic condition, under both aerobic and anaerobic conditions, there is an increase in the removal after the addition of influent. A maximum of 39% and 12% removal efficiency was reached under aerobic and anaerobic conditions, respectively. Furthermore, under anaerobic conditions was possible to observe a negative removal when no influent was added. Negative removal has been previously observed in benzotriazoles

(Kotowska *et al.*, 2021; Voutsa *et al.*, 2006; Weiss *et al.*, 2006), and it may be explained by the decomposition of the existing benzotriazoles-based compounds (e.g., antimicrobial agents, antiparasitic drugs, anticancer drugs, and others) (Kotowska *et al.*, 2021), like vorozole, fluconazole, and 4,5,6,7-tetrabromobenzotriazole (Briguglio *et al.*, 2015; Ren, 2014). Or may also be due to different sorption and desorption of the diverse target compounds (Kotowska *et al.*, 2021; Wu *et al.*, 2017). Mazioti *et al.* (2015) also observed higher removal rates in anoxic and aerobic conditions compared to anaerobic. However, the removal efficiencies obtained in this study are much higher than those of Mazioti *et al.* (2015): 18% removal in aerobic conditions and 10% in anoxic conditions. In the same way, Weiss *et al.* (2006) and Kotowska *et al.* (2021) observed a removal between 30 and 37% in activated sludge, which is close to the obtained values. Liu *et al.* (2012) observed that the biological treatment reduced benzotriazole from 7% to 27%. The higher removal efficiencies observed in the present study may be correlated with the fact that WWTP Walcheren biomass adapted its mechanisms to degrade benzotriazoles more efficiently due to the 20% industrial influent influence. Voutsa *et al.* (2006) observed a removal efficiency from -47% to 60% at different WWTP at Glatt Valley, Switzerland. In the same way, Karthikraj and Kannan (2017) found an average of 67% removal of benzotriazole in five WWTP in India (varying from 35% to 85%). The values observed by Karthikraj and Kannan (2017) match the values obtained in our study. Stasinakis *et al.* (2013) observed the removal of 60% of benzotriazole at the biological treatment. The values observed by Stasinakis *et al.* (2013) and Karthikraj and Kannan (2017) match the values obtained in our study. According to Liu *et al.* (2011), benzotriazole anaerobic removal can be inhibited due to the reduction of nitrate, sulphate, and ferric – Fe (III).

Carbamazepine is an already known recalcitrant compound, and, for that reason, it was not expected to be significant removal in any of the redox conditions. This recalcitrant behaviour is due to its heterocyclic N-containing aromatic ring that is difficult to break naturally (Margot *et al.*, 2013; Tran *et al.*, 2013). Maximum removal of 6% in the anoxic conditions after adding influent was found (see Table 5-7), while under aerobic and anaerobic conditions, the removal was 0%-2%, respectively. The addition of influent led to a removal reduction, achieving negative values of -10% under aerobic conditions and -7% under anaerobic conditions. Negative removal values were also observed in the literature (Grandclement *et al.*, 2017; Hoque *et al.*, 2014; Tiwari *et al.*, 2021; Verlicchi *et al.*, 2012; Vieno *et al.*, 2007). In the same way, removal of 0% was observed in real WWTP in different studies (Carballa *et al.*, 2007a; Carballa *et al.*, 2007b; Joss *et al.*, 2005; Lin *et al.*, 2009; Ternes *et al.*, 2007; Tiwari *et al.*, 2021). Similar removal efficiencies in the range of 0%-10%, as the ones observed in the present research, were found in several studies (Bernhard *et al.*, 2006; Clara *et al.*, 2005; Di Marcantonio *et al.*, 2020; Fan *et al.*, 2014; Fernandez-Fontaina *et al.*, 2013; Margot *et al.*, 2013; Nguyen *et al.*, 2014; Radjenovic *et al.*, 2009; Suarez *et al.*, 2010; Tiwari *et al.*, 2021). Similar to our results, Suarez *et al.* (2010) observed 1% removal under anaerobic conditions and 6% removal under anoxic. Di Marcantonio *et al.* (2020) also observed 5% removal with an aeration frequency of 0,9 1/h. Ruas *et al.* (2022) observed 60% removal using a photobioreactor, and Jelic *et al.* (2012) observed a 95% removal with activated sludge continuously fed with glucose. The latter may suggest that the main mechanism of removal of carbamazepine is cometabolism.

On the other side, under all redox conditions and removal efficiency higher than 50%, clarithromycin achieved a 75% removal under anaerobic conditions after adding influent as substrate. The addition of influent increased the removal efficiency under aerobic (67% to 73%) and anaerobic conditions (72% to 75%). However, under anoxic conditions, the identical additions lead to a decrease in the removal, from 62% to 55%. These values are consistent with the ones found by Ternes *et al.* (2007) (54%), Ghosh *et al.* (2009) (57% removal), Blair *et al.* (2015) (73% removal) and Tiwari *et al.* (2021) (76% removal). Besides, Gobel *et al.* (2007) and Margot *et al.* (2013) observed lower removal efficiency, 14%, and 37%, respectively. Lin *et al.* (2009) and Jelic *et al.* (2012) also observed a 0% removal of clarithromycin during activated sludge treatment. Besides the previous values mentioned, Lin *et al.*

(2009) also observed removal efficiencies of 99%, and Gobel *et al.* (2007) observed an increase of 80% in clarithromycin removal after increasing the SRT from 20 to 50 days.

Diclofenac is a recalcitrant compound that is difficult to remove and showed no significant removal under redox conditions. The highest removal of 7% was obtained under anoxic conditions after adding influent, while without influent, removal of 2% was achieved. No removal was observed under aerobic and anaerobic conditions. On the contrary, Arias *et al.* (2018) have only found removal under aerobic and anaerobic conditions. Quintana *et al.* (2005) and Grandclement *et al.* (2017) did not find any degradation of diclofenac over 28 days, while Suarez *et al.* (2010) observed a 0% removal of diclofenac under aerobic conditions. However, this removal increased to 74%, with SRT increasing to 170 days. Suarez *et al.* (2010) also observed the removal of around 2% under an anoxic environment which is in line with the observed results in this study. This may mean that cometabolism by nitrifying bacteria is the main degradation mechanism since Suarez *et al.* (2010) and Tran *et al.* (2009) showed that the highest removal rates were obtained in nitrifying environments. Due to the difficulty of maintaining stable anoxic conditions in batch tests, it is possible that during the experiment, both aerobic and anoxic conditions were maintained, leading to some nitrification/denitrification that might have been responsible for the observed removal under anoxic conditions. Lishman *et al.* (2006) have reported a considerable variety of removal efficiencies found in different CAS (from -143% to 77. Based on the literature review carried out (Carballa *et al.*, 2007b; Fernandez-Fontaina *et al.*, 2012; Gusmaroli *et al.*, 2020; Suarez *et al.*, 2010; Ternes *et al.*, 2007), and the differences in removal efficiencies under each redox condition, it is not possible to define a clear outline of conclusions about which is the best redox condition to remove diclofenac. According to some studies (Falas *et al.*, 2016; Gusmaroli *et al.*, 2020; Huang *et al.*, 2011), diclofenac should degrade mostly under anaerobic conditions. However, it has been observed mainly in digestion processes - with high retention time and mesophilic conditions (Falas *et al.*, 2016; Huang *et al.*, 2011). In a real WWTP higher overall removal during the treatment including sludge digestion may be expected (Falas *et al.*, 2016; Joss *et al.*, 2005; Lin *et al.*, 2009).

Hydrochlorothiazide has also shown some recalcitrant behaviour (Ratkievicius *et al.*, 2022). This compound did not show a removal higher than 10%, obtained under aerobic conditions without influent addition. For this compound, adding influent made a 10% decrease under aerobic redox conditions, leading to a 0% removal. This same decrease occurred under anaerobic conditions where adding influent led to a negative removal efficiency (from 5% to -6%). However, under anoxic conditions, this addition of influent helped increase the removal efficiency by around 3%. Radjenovic *et al.* (2009) also found the removal of hydrochlorothiazide lower than 10% in CAS, while Jelic *et al.* (2012) observed no degradation at all.

The beta-blocker metoprolol showed its higher removal efficiency under aerobic redox conditions, achieving a value of 53%In the anoxic redox condition, the removal average was 33%. Under the anaerobic redox condition, adding influent increased by more than 30% (from 2% to 39%). The values observed by Ternes *et al.* (2007) (65% removal), Lin *et al.* (2009) (67% removal), Kasprzyk-Hordern *et al.* (2009) (55% removal), and Ruel *et al.* (2011) (38% removal) are in the same range of the ones obtained in this study. Radjenovic *et al.* (2009) observed lower removals of around 25%. Besides, Lin *et al.* (2009) and Margot *et al.* (2013) also found lower values, respectively, 3-29% and 5%.

Despite this, propranolol, a beta-blocker like metoprolol, showed completely different results. The addition of influent reduced to only 10% in removing propranolol compared to 80% for metoprolol. The same can be observed under the anoxic redox condition, where a decrease from 62% to 58% occurred. Under anaerobic conditions, removal decreased by 8% with adding influent (from 54% to 46%). Ternes *et al.* (2007) and Radjenovic *et al.* (2009) obtained the highest values in literature, respectively, 65% and 60%. These values are slightly lower than the ones obtained in this study, which

might be related to the scale-down of the process to realize the batch tests and to the variability of the influent in a real WWTP. Furthermore, Kasprzyk-Hordern *et al.* (2009), Ruel *et al.* (2011), and Margot *et al.* (2013) have obtained lower values, respectively, 35%, 20%, and 13%.

Sotalol showed higher removal under aerobic conditions, reaching 38% degradation efficiency when an influent was added and decreasing 4% when no influent was added. On the other hand, under anoxic conditions, the addition of influent did not cause any significant effect, keeping the removal efficiency around 26%. Lastly, under the anaerobic condition, a negative removal was observed, even though the addition of the influent allowed an increase in efficiency, from -12% to -3%. Vieno *et al.* (2007) and Ternes *et al.* (2007) found values of 54% and 48% correspondingly. Yet, Gurke *et al.* (2015) observed removal efficiencies from 2% to 20%, with a mean value of 10%, slightly lower than the ones obtained in the present study. At the same time, Radjenovic *et al.* (2009) and Margot *et al.* (2013) found values around 20%.

In the case of sulfamethoxazole, the best removal efficiency occurred under the anoxic redox condition reaching a value of 41% after adding influent, which led to an increase of 6%, reaching 47%. Under the aerobic redox condition, adding influent increased the removal efficiency again, this time by 17%, reaching 37%. The anaerobic redox condition yielded a removal of 16%, which increased to 32% after adding influent. The values obtained in this study were lower than the ones obtained by Yu *et al.* (2009) (65% to 96% removal), Radjenovic *et al.* (2009) (74% removal), and Ruas *et al.* (2022) (95% removal). However, Yu *et al.* (2009) conducted the experiment with an SRT greater than 200 days, and both Yu *et al.* (2009) and Radjenovic *et al.* (2009) experimental HRT was twice as high as the one used in the present study. On the other side, Ryan *et al.* (2011) and Ruas *et al.* (2022) used a photobioreactor to degrade sulfamethoxazole which is prone to photodegradation. The obtained results were consistent with the ones obtained by Ghosh *et al.* (2009) (39% removal), Lin *et al.* (2009) (45% removal), Carballa *et al.* (2007a) (50% removal), and Xiong *et al.* (2019) (47% removal), Di Marcantonio *et al.* (2020) (50% removal), Joss *et al.* (2005) (45% removal). Both Batt *et al.* (2007), Ternes *et al.* (2007), and Ghosh *et al.* (2009) observed lower removals, respectively, 36%, 24%, and 26%, which may be associated with the type of sludge and the different microbial community. Besides, both Falas *et al.* (2016) and Arias *et al.* (2018) observed an increase in sulfamethoxazole removal when an anaerobic stage is added to the oxic/anoxic activated sludge system, increasing the removal up to 60%. Both Gonzalez-Gil *et al.* (2016) and Falas *et al.* (2016) found that sulfamethoxazole is mainly removed under anaerobic conditions. However, the present study observed higher removal under anoxic conditions, similar to Arias *et al.* (2018). The anoxic/oxic sequences may have led to higher removals than the anaerobic standalone. However, Di Marcantonio *et al.* (2020) showed that low-frequency aeration resulted in the highest removal, supporting the idea that anoxic conditions can achieve higher degradation.

Trimethoprim decreased its removal efficiency under all redox conditions when influent was added. Therefore, cometabolism seems to be the main path to the removal of trimethoprim. However, the process needs high carbon sources (Yang *et al.*, 2020a). The anaerobic redox condition showed the highest efficiency of 76% to remove this pharmaceutical without influent added. Secondly, the aerobic redox condition showed 24%, and thirdly the anoxic condition with 12%. These results follow the ones found by Ghosh *et al.* (2009) (74%), Yu *et al.* (2009) (74%), Batt *et al.* (2007) (75%), and Ruas *et al.* (2022) (78%). However, it showed higher results than the ones found by Radjenovic *et al.* (2009), Ghosh *et al.* (2009), Lin *et al.* (2009), and Grandclement *et al.* (2017). This variation might be explained by the different existent bacteria communities and their different conversion capacities. Radjenovic *et al.* (2009) and Grandclement *et al.* (2017) found the lowest removal in trimethoprim (40 ± 25% removal), using synthetic wastewater. Falas *et al.* (2016) and Gonzalez-Gil *et al.* (2016) also studied the trimethoprim degradation under anaerobic conditions and found that this compound has a susceptibility to anaerobic biotransformation since it shown 60% removal on anaerobic stand-alone

reactor and approximately 80% removal after coupling an anaerobic post-treatment. Arias *et al.* (2018) also found the highest removal (around 80%) under anaerobic conditions. This can be explained due to the existence of a substituted pyrimidine ring functional group, which is substituted by a carboxyl group during anaerobic conditions (Adrian & Suflita, 1994; Arias *et al.*, 2018). Besides the similarity in the anaerobic results, Fernandez-Fontaina *et al.* (2012) also observed aerobic removals around 20% which were increased up to 70% when they were in anoxic/oxic activated sludges that allowed nitrification. This implied that nitrification improved the degradation of trimethoprim.

The macrolide azithromycin showed its higher removal under the aerobic condition achieving removal of 64% after adding influent. Yet, the addition of influent did not significantly increase the removal. The anaerobic condition resulted in a removal efficiency of 55% when the influent was added, which allowed an increase of 10%. The lowest removal was obtained under anoxic conditions of about 21%. Ghosh et al. (2009) and Yan et al. (2014) observed removal of 76%, which is the expected value in this study. That observed removal of 64%. Pan and Yau (2021) observed the removal of 64% closer to the obtained removal efficiencies. On the other side Gobel *et al.* (2007), Margot *et al.* (2013), and Blair *et al.* (2015) observed removals of around 40-55%, which are lower than the obtained values.

The candesartan, similarly, to diclofenac and carbamazepine, had low removal efficiencies, as expected, due to its recalcitrancy. The maximum removal obtained was 4% under the anoxic condition after adding influent, which led to an increase of 2%, considered insignificant. Under aerobic conditions, candesartan has shown no removal with or without adding influent. In contrast, under anaerobic conditions, candesartan showed a negative removal of -9% before adding influent and removal of -4% after. This negative removal might be due to possible retransformation of the compound or the lack of dissolution at time zero due to its low solubility. Jansook *et al.* (2022) observed that candesartan is also unstable, allowing its degradation and retransformation. As showed by the present study, Gurke *et al.* (2015) also observed a removal from -10% to 10%, having a mean value of 0%. Yet, Bayer *et al.* (2014) observed higher values, reaching 22%, probably due to the existence of biomass with better removal mechanisms, since Bayer *et al.* (2014) environmental concentrations of candesartan are three times as high as the ones observed in the present study (See Table 5-3).

Gabapentin was the compound that showed higher removal efficiency in this experiment, reaching a 91% removal in the anoxic condition after adding influent and a 77% removal without influent. After an anoxic condition, this compound also showed a good removal in aerobic condition (59%, with influent), and a lower removal in anaerobic condition was achieved with a 36% removal with added influent. Kasprzyk-Hordern *et al.* (2009) observed removal efficiencies around 80 to 90% in a complete CAS, which matches the ones obtained in this study. However, on the other side, Margot *et al.* (2013) observed a removal of 9%. This might be associated with the fact that Margot *et al.* (2013) conducted the studies with Swiss influent, where gabapentinoids (gabapentin and pregabalin) are only prescribed to treat epilepsy and neuropathic pain. Yet, in European Countries, which is the case in The Netherlands but not Switzerland, gabapentinoids are also prescribed for anxiety and substance use disorders (Chiappini & Schifano, 2016; Smith *et al.*, 2016; Van Baelen *et al.*, 2018). Apart from that, Margot *et al.* (2013) experiment was conducted approximately ten years after the European Medicines Agency and the Swiss Agency for Therapeutic Products licensed gabapentin, while the present study was conducted almost 20 years after gabapentin approval. According to Van Baelen *et al.* (2018) and Vickers-Smith *et al.* (2020), the recreational use of gabapentinoids has increased worldwide during the last decade, allowing bacteria to create new mechanisms for the existence of higher concentrations.

Irbesartan has shown its higher removal efficiency in the anaerobic condition, reaching a 29% removal with influent, with a 24% removal when no influent is added. Besides, the removal efficiency was similar under aerobic and anoxic conditions, rounding 20%, without big significance with the

influent addition, as seen in the anaerobic condition. Those values are consistent with the ones observed by Bayer *et al.* (2014) and Gurke *et al.* (2015), respectively 16-40% and 10-20%., however, However, Margot *et al.* (2013) observed higher removals (79% removal). This increased removal may be connected with Irbesartan being an angiotensin-converting enzyme \(ACE), the main anti-hypertensive administered in Switzerland (Palmer *et al.*, 2006).

Lastly, venlafaxine has shown its higher removal under aerobic conditions and with influent reaching 20% degradation, followed by the anoxic condition where it achieved a 19% degradation without influent. In the anaerobic condition, it is possible to notice a minor negative degradation (-3% and 0% removal, with and without influent, respectively). These values match the ones Castaño-Trias *et al.* (2020) observed, which obtained 27% removal and 19% removal under aerobic and anoxic conditions, respectively. In the same way, Margot *et al.* (2013) and Tiwari *et al.* (2021) observed the removal of 40% in a complete CAS. Venlafaxine is removed by demethylation (Falas *et al.*, 2016), which occurs easily at higher temperatures (Hudelson *et al.*, 2020; Li *et al.*, 2020). Tiwari *et al.* (2021) also observed that venlafaxine metabolite – desvenlafaxine – has the removal of 1,5 times higher than the parent compound. Besides that, Gurke *et al.* (2015) and Subedi and Kannan (2015) showed a removal efficiency lower than 15%. This might be attributed to Gurke *et al.* (2015) conducted their experiment in the USA with an entirely different physicochemical composition.

Figure 5-3 shows the overall comparison between the biological removal efficiency of the targeted compounds under the different redox conditions.

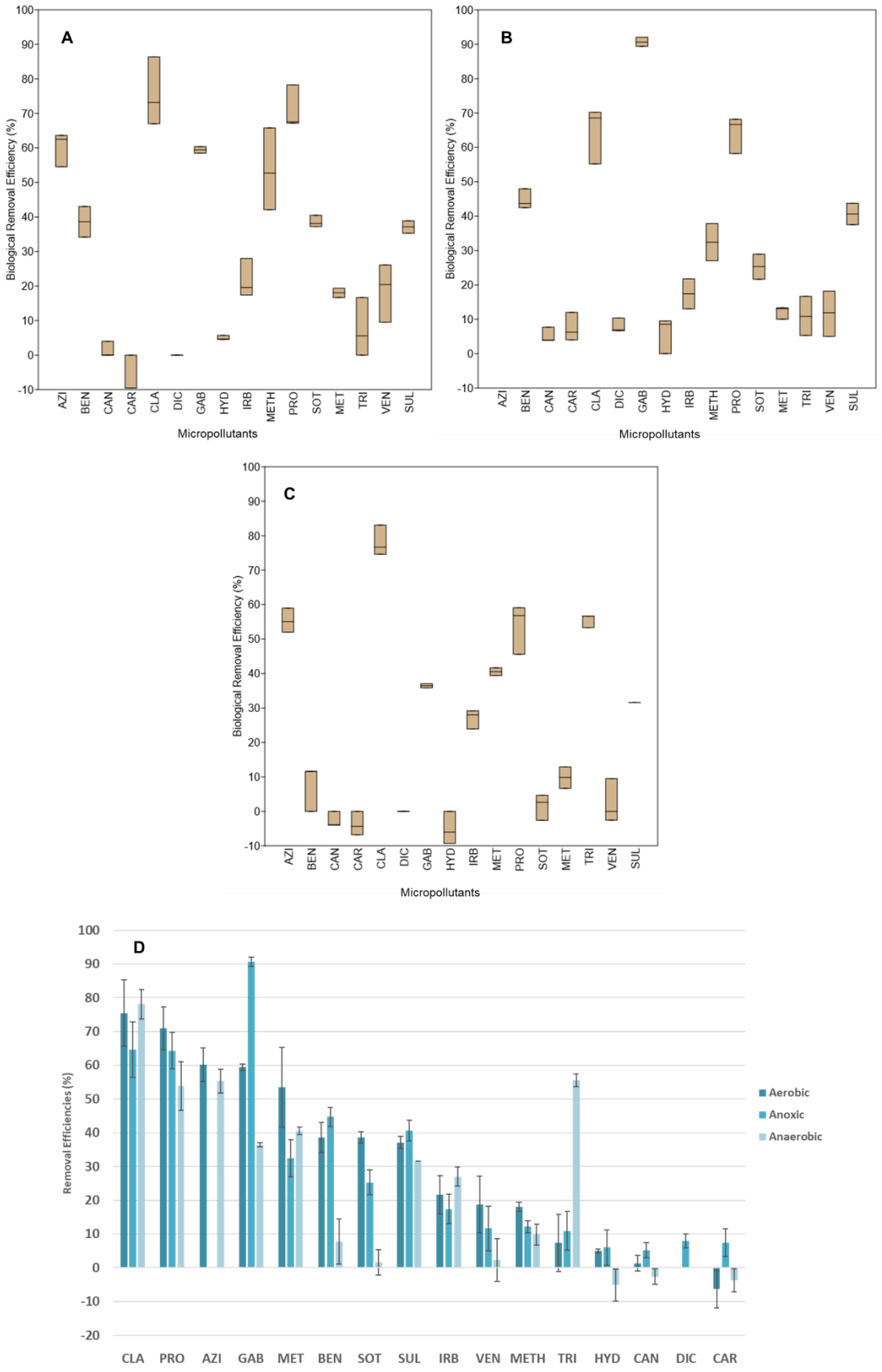


Figure 5-2 Removal efficiencies after 48h under the different redox conditions studied, after influent addition

**A** – Box plot of the aerobic removal efficiencies; **B** – Box plot of the anoxic removal efficiencies; **C** – Box plot of the anaerobic removal efficiencies; **D** – Bar graph with all removal efficiencies in the studied redox conditions, with standard deviation

Figure 5-3.D shows that in aerobic conditions 8 out of 16 compounds are being removed more than 30%, while in anoxic conditions 6 out of 16 reached that efficiency and in anaerobic conditions 7 out of 16 had removals higher than 30%. This goes against the state made by Fick *et al.* (2016), that activated sludge systems remove usually around 30% of the pharmaceutical compounds. Yet, in the mentioned study the removal was determined with concentrations ten times higher than the ones used by us. It is also possible to observe that redox has a massive impact on the removal of gabapentin, benzotriazole, sotalol, trimethoprim, diclofenac, and carbamazepine. Gabapentin, trimethoprim, and diclofenac are the compounds where this connection between removal and efficiency is more noticeable. Gabapentin has presented a good removal efficiency in all redox conditions, yet, in anoxic conditions, it reaches 91% removal, compared with 59% (aerobic) and 36% (anaerobic). Contrarily, trimethoprim has shown overall low removal (aerobic – 17%; anoxic – 11%), yet in anaerobic conditions, it increased up to 53%. This may be associated with the microorganism community present in each type of redox condition, as well as their removal mechanisms and symbiosis. Diclofenac on the other side presented no removal in both aerobic and anaerobic conditions yet presented a low extent of removal in anoxic conditions (7%).

There was an attempt to correlate the removal efficiency of the micropollutants with their physicochemical properties (hydrophobicity, polarity, and solubility).

Regarding hydrophobicity properties, a higher removal of the hydrophilic compounds (like clarithromycin, gabapentin, metoprolol, and sulfamethoxazole) than hydrophobic compounds diclofenac and candesartan was found. Being hydrophilic showed an almost 16% increase in the overall removal compared to hydrophobic compounds. This increase could be connected to the fact that hydrophilic compounds are less prone to sorption, allowing them to be more easily degraded by bacteria. This is in agreement with the correlation observed by Niaounakis (2015). Under aerobic conditions, this is more noticeable since hydrophilic compounds have more than twice the removal efficiency (up to 44%). In comparison, under the anoxic and the anaerobic conditions, this removal increased by around 10% (up to 36% and 28%, respectively). The observed high removal of hydrophilic compounds under aerobic conditions is associated with compounds being mostly degraded co-metabolically, and enzymes degrade hydrophilic compounds more easily (Ratner *et al.*, 2013; Saravanan *et al.*, 2021).

Polarity allowed an overall increase of the removal of almost 15%. This correlation was expected since polar compounds are more reactive, being, therefore, more prone to degrade (Niaounakis, 2015). Under aerobic and anoxic conditions, polar compounds removal increase by less than 10%, while under anaerobic conditions, this increase goes up to 25%.

Lastly, the solubility and removal of the compound showed no significant correlation, being the maximum squared-R obtained of 0,06. Table 5-8 presents the obtained correlation values.

Table 5-8 Correlation between the removal efficiency of the micropollutants and their physicochemical properties

| | Polarity | Hydrophobicity | Solubility |
|---|---|---|---|
| | Percentual Difference | | Square-R |
| General | 45% | 75% | 0,044 |
| Aerobic conditions | 19% | 108% | 0,000 |
| Anoxic conditions | 16% | 52% | 0,019 |
| Anaerobic Conditions | 79% | 64% | 0,064 |

## 5.6 Biotransformation Rate Constants under Different Redox Conditions

To determine the biotransformation rates constant, curves with the logarithmic concentration variation throughout time were plotted. Table 5-9 presents the biotransformation rates constant for all the compounds, assuming that sorption is negligible (Kd x Xss ≤ 0,1).

Table 5-9 Biotransformation rates for the sixteen targeted compounds in the three main redox conditions, and with and without the addition of an influent when Kd x Xss ≤ 0,1

| $K_d \times X_{ss} \leq 0,1$ | | | | | | |
|---|---|---|---|---|---|---|
| Micropollutants | Aerobic | | Anoxic | | Anaerobic | |
| | Without Influent | With Influent | Without Influent | With Influent | Without Influent | With Influent |
| | $L.g_{SS}^{-1}.d^{-1}$ | | | | | |
| 4-, 5-Methylbenzotriazole | 0,09 ± 0,02 | 0,18 ± 0,01 | 0,13 ± 0,03 | 0,06 ± 0,02 | 0,20 ± 0,03 | 0,11 ± 0,05 |
| Benzotriazole | 0,30 ± 0,01 | 0,47 ± 0,06 | 0,52 ± 0,01 | 0,58 ± 0,10 | -0,09 ± 0,05 | 0,14 ± 0,18 |
| Carbamazepine | 0,00 ± 0,00 | -0,10 ± 0,14 | 0,03 ± 0,03 | 0,07 ± 0,05 | 0,03 ± 0,08 | -0,07 ± 0,03 |
| Clarithromycin | 1,23 ± 0,11 | 1,46 ± 0,50 | 1,06 ± 0,01 | 0,90 ± 0,20 | 1,20 ± 0,15 | 1,59 ± 0,12 |
| Diclofenac | 0,00 ± 0,00 | 0,00 ± 0,00 | 0,10 ± 0,06 | 0,07 ± 0,04 | 0,00 ± 0,00 | 0,00 ± 0,00 |
| Hydrochlorothiazide | 0,10 ± 0,08 | 0,05 ± 0,01 | 0,08 ± 0,04 | 0,09 ± 0,05 | 0,06 ± 0,07 | -0,06 ± 0,05 |
| Metoprolol | 0,78 ± 0,09 | 0,79 ± 0,33 | 0,45 ± 0,02 | 0,36 ± 0,07 | 0,02 ± 0,06 | 0,56 ± 0,01 |
| Propranolol | 1,55 ± 0,34 | 1,29 ± 0,19 | 1,22 ± 0,08 | 0,87 ± 0,08 | 0,72 ± 0,19 | 0,66 ± 0,14 |
| Sotalol | 0,46 ± 0,02 | 0,46 ± 0,05 | 0,32 ± 0,01 | 0,25 ± 0,02 | -0,11 ± 0,01 | -0,03 ± 0,22 |
| Sulfamethoxazole | 0,27 ± 0,04 | 0,42 ± 0,00 | 1,63 ± 0,32 | 2,02 ± 0,33 | 0,09 ± 0,02 | 0,42 ± 0,03 |
| Trimethoprim | 0,36 ± 0,10 | 0,21 ± 1,09 | 0,15 ± 0,06 | 0,10 ± 0,04 | 1,59 ± 0,03 | 0,97 ± 0,03 |
| Azithromycin | 0,72 ± 0,39 | 1,10 ± 0,24 | 0,24 ± 0,15 | N.D. | 0,75 ± 0,09 | 0,97 ± 0,13 |
| Candesartan | 0,04 ± 0,03 | 0,05 ± 0,03 | 0,03 ± 0,03 | 0,03 ± 0,04 | -0,17 ± 0,11 | -0,05 ± 0,03 |
| Gabapentin | 0,51 ± 0,02 | 0,86 ± 0,01 | 1,52 ± 0,14 | 2,36 ± 0,30 | 0,04 ± 0,01 | 0,49 ± 019 |
| Irbesartan | 0,09 ± 0,00 | 0,23 ± 0,04 | 0,14 ± 0,04 | 0,18 ± 0,06 | 0,35 ± 0,01 | 0,33 ± 0,03 |
| Venlafaxine | 0,07 ± 0,01 | 0,42 ± 0,16 | 0,27 ± 0,05 | 0,12 ± 0,07 | 0,00 ± 0,00 | -0,03 ± 0,13 |

N.D. : Not Determined

Yet, as explained in chapter 4.6, some compounds have Kd x Xss > 0,1, which means that their sorption has a considerable influence (>10%) on the overall degradation rate and, therefore, sorption is not negligible. According to our determination, sorption was significant for azithromycin, clarithromycin, irbesartan, metoprolol, propranolol, trimethoprim, and venlafaxine since their Kd x Xss was greater than 0,1. Table 5-10 shows the determined biotransformation constant rates for the mentioned compounds when Kd x Xss > 0,1– accounting sorption.

Table 5-10 Biotransformation rates constants for micropollutants under all redox conditions, and with and without the addition of Influent when Kd x Xss > 0,1

| $K_d \times X_{ss} > 0,1$ | | | | | | |
|---|---|---|---|---|---|---|
| Micropollutants | Aerobic | | Anoxic | | Anaerobic | |
| | Without Influent | With Influent | Without Influent | With Influent | Without Influent | With Influent |
| | $L.g_{SS}^{-1}.d^{-1}$ | | | | | |
| 4-, 5-Methylbenzotriazole | | | | | | |
| Benzotriazole | | | | | | |
| Carbamazepine | | | | | | |
| Clarithromycin | 1,45 ± 0,12 | 1,75 ± 0,59 | 1,26 ± 0,02 | 1,08 ± 0,23 | 1,42 ± 0,17 | 1,87 ± 0,14 |
| Diclofenac | | | | | | |
| Hydrochlorothiazide | | | | | | |
| Metoprolol | 0,90 ± 0,11 | 0,92 ± 0,39 | 0,52 ± 0,02 | 0,42 ± 0,08 | 0,02 ± 0,07 | 0,65 ± 0,01 |
| Propranolol | 1,79 ± 0,39 | 1,51 ± 0,21 | 1,42 ± 0,09 | 1,02 ± 0,09 | 0,83 ± 0,22 | 0,76 ± 0,16 |
| Sotalol | | | | | | |
| Sulfamethoxazole | | | | | | |
| Trimethoprim | 0,40 ± 0,12 | 0,23 ± 1,21 | 0,17 ± 0,06 | 0,12 ± 0,05 | 1,75 ± 0,03 | 1,07 ± 0,03 |
| Azithromycin | 0,95 ± 0,34 | 1,48 ± 0,31 | 0,32 ± 0,20 | N.D. | 0,99 ± 0,12 | 1,27 ± 0,17 |
| Candesartan | | | | | | |
| Gabapentin | | | | | | |
| Irbesartan | 0,29 ± 0,00 | 0,33 ± 0,06 | 0,20 ± 0,06 | 0,25 ± 0,08 | 0,49 ± 0,02 | 0,45 ± 0,04 |
| Venlafaxine | 0,17 ± 0,02 | 0,48 ± 0,18 | 0,30 ± 0,06 | 0,13 ± 0,08 | 0,00 ± 0,07 | -0,04 ± 0,15 |

N.D. : Not Determined

Remarkably, when sorption is considered for the same compound, the increase of the biotransformation rate constant when influent is added is higher than when no substrate is added. It is possible to observe that this correlation is directly proportional to the solid-water distribution coefficient, that being, higher solid-water distribution coefficient values have shown higher increases.

Once again, and as mentioned before, it is possible to analyse that no redox condition shows greater biotransformation rates since the degradation mechanisms are different from compound to compound. Table 5-11 presents a heatmap with the removals ranging from -0,17 to 2,36 $L.g_{SS}^{-1}.d^{-1}$.

Table 5-11 Heatmap of the biotransformation rate constants of micropollutants ranging from -0,17 to 2,36 L.gSS-1.d-1

| Micropollutants | Aerobic | | Anoxic | | Anaerobic | |
|---|---|---|---|---|---|---|
| | Without Influent | With Influent | Without Influent | With Influent | Without Influent | With Influent |
| 4-, 5-Methylbenzotriazole | | | | | | |
| Benzotriazole | | | | | | |
| Carbamazepine | | | | | | |
| Clarithromycin | | | | | | |
| Diclofenac | | | | | | |
| Hydrochlorothiazide | | | | | | |
| Metoprolol | | | | | | |
| Propranolol | | | | | | |
| Sotalol | | | | | | |
| Sulfamethoxazole | | | | | | |
| Trimethoprim | | | | | | |
| Azithromycin | | | | | | |
| Candesartan | | | | | | |
| Gabapentin | | | | | | |
| Irbesartan | | | | | | |
| Venlafaxine | | | | | | |


Based on Table 5-11 and applying the categorization suggested by Joss *et al.* (2006), it is possible to infer that candesartan, carbamazepine, diclofenac, and hydrochlorothiazide have low biotransformation constant rates ($K_{bio}$ < 0,1 L/$g_{ss}$.$d^{-1}$; <20% of bioremoval) under all the redox conditions. On the contrary, 4-, 5-methylbenzotriazole, azithromycin, benzotriazole, clarithromycin, gabapentin, irbesartan, metoprolol, propranolol, trimethoprim, and sulfamethoxazole showed moderate values of biotransformation constant rates under all the redox conditions (0,1<$K_{bio}$<10 L/$g_{ss}$.$d^{-1}$; bioremoval between 20% and 90%). Furthermore, 4-, 5-methylbenzotriazole showed a moderate removal under all the redox conditions when influent was added, but the obtained value is in the lower limit of this class. It is also possible to observe that both sotalol and venlafaxine observed moderate removal under all the redox conditions unless under anaerobic conditions in which negative values were obtained. Moreover, any of the compounds exhibited high removal since all the biotransformation rate constants determined are below 10 L/$g_{ss}$.$d^{-1}$.

In order to quickly compare the obtained biotransformation rate constants and the ones observed in the literature, the averages and respective standard deviations of the values mentioned in Table 2-4 were calculated and summarized in Table 5-12.

Table 5-12 Average and standard deviation of literature biotransformation constant rates for all micropollutants and respective percentual difference with the determined values in this study.

| | Aerobic | Anoxic | Anaerobic |
|---|---|---|---|
| | $L.g_{SS}^{-1}.d^{-1}$ | | |
| 4-, 5-Methylbenzotriazole | | | |
| Benzotriazole | 0,30 ± 0,11 (0%) | 0,28 ± 0,05 (86%) | |
| Carbamazepine | 0,04 ± 0,04 (100%) | 0,03 (0%) | |
| Clarithromycin | 0,40 ± 0,14 (208%) | | |
| Diclofenac | 0,38 ± 0,33 (100%) | 0,04 (75%) | |
| Hydrochlorothiazide | | | |
| Metoprolol | 0,35 ± 0,17 (123%) | 0,03 (1 100%) | |
| Propranolol | 0,41 ± 0,07 (215%) | | |
| Sotalol | 0,46 ± 0,10 (0%) | | |
| Sulfamethoxazole | 0,27 ± 0,16 (0%) | 0,41 (298%) | |
| Trimethoprim | 0,23 ± 0,22 (0%) | 0,67 (75%) | |
| Azithromycin | 0,18 ± 0,06 (300%) | | |
| Candesartan | | | |
| Gabapentin | 0,13 ± 0,05 (292%) | | |
| Irbesartan | 0,50 ± 0,40 (34%) | | |
| Venlafaxine | | | |

The average values and their standard deviation shown in this table were calculated based on the literature review values presented in Table 2-4. The percentual difference between our results (Table 5-8 and Table 5-9) and the average values mentioned in this table can be observed inside the brackets.

Comparing biotransformation rates constant is difficult since they are intrinsically connected to the wastewater matrix and biomass.

Under aerobic conditions, four compounds (benzotriazole, sotalol, sulfamethoxazole, and trimethoprim) have observed no error (See Table 5-12) when comparing the obtained values with the average observed in the literature (See Table 5-12). On the contrary, Irbesartan showed a difference of 34%. Moreover, carbamazepine and diclofenac observed an error of 100% because the present study did not show any removal under aerobic conditions. In contrast, some studies in the literature had. Since these compounds are highly recalcitrant, this difference was expected. Some studies showed removal due to higher SRT that allowed the growth of bacteria and a new removal mechanism that can degrade these compounds. Compounds like azithromycin, clarithromycin, gabapentin, metoprolol, and propranolol showed a difference higher than 100%. As mentioned previously, these differences may be attributed to the different operating conditions and biomass, since bacteria can have developed removal mechanisms for some compounds and not others. Under anoxic conditions, however, only carbamazepine showed a percentual error of 0%, while benzotriazole, diclofenac, and trimethoprim showed a difference lower than 100% when compared to the literature values. Sulfamethoxazole and metoprolol showed differences higher than 100%, particularly metoprolol which showed an error of 1 100%. Yet, like the other micropollutants, the error of metoprolol was determined based only on one value found in the literature. The anoxic errors need to be interpreted as the difference between the two studies and not as the study's accuracy compared with the literature.

## 5.7 Micropollutants Removal under Different Activated Sludge Systems

This research was conducted from a PhoRedox configuration of a conventional activated sludge system. In a PhoRedox CAS system, the process includes anoxic and anaerobic conditions to remove nitrogen and phosphorous. Usually, these systems work with conventional aeration, for which the aimed operation of MLSS can vary from 2,5 $g_{SS}$/L up to 5 $g_{SS}$/L (Keller & Giesen, 2010; Metcalf & Eddy, 2013; Rosa-Masegosa *et al.*, 2021). In this research, the PhoRedox was the selected system that operates with 4,5 $g_{SS}$/L (see Table 5-1). However, different treatment lines can yield different removal rates. Activated sludge systems can have different design characteristics (Metcalf & Eddy, 2013).

Wastewater treatment configurations, such as membrane bioreactors (MBR) and aerobic granular sludge (AGS) systems, with a technological readiness level (TRL) of 8-9 have been applied for new (greenfield) or retrofitted (brownfield) activated sludge-based wastewater treatment systems.

The aerobic granular sludge system is an emergent technology with over 90 installations in 20 countries worldwide. This technology works in a reactor with a three-step fill-and-draw sludge system(Pronk, 2016; van der Roest *et al.*, 2011). AGS has a bigger particle size (up to 10 mm) when compared to the CAS particle size (≤ 100 µm) (Keller & Giesen, 2010; Rosa-Masegosa *et al.*, 2021). This treatment process has a lower carbon footprint since it needs less space and energy and has lower operation costs (Rosa-Masegosa *et al.*, 2021). The granular structure of the biomass exhibited all redox conditions (aerobic, anoxic, and anaerobic), allowing the conversion of carbon and nutrients (nitrogen and phosphorous) inside the granule and in the same reactor. In the case of AGS, the aimed MLSS rounds the 10 $g_{SS}$/L (Keller & Giesen, 2010; Rosa-Masegosa *et al.*, 2021).

Membrane bioreactors (MBR) have been increasingly applied during the last few years. MBR is a wastewater treatment process with the same principle as CAS but where the clarifier is substituted by membranes, allowing full biomass retention. In this process, ultrafiltration or microfiltration membranes are after the activated sludge system, avoiding the need for a secondary clarifier and allowing a higher effluent quality (Lousada-Ferreira *et al.*, 2010). The aimed MLSS can go up to 18 gSS/L in this process. However, they usually work between 10 and 12 $g_{SS}$/L (Lousada-Ferreira *et al.*, 2010; Metcalf & Eddy, 2013).

The variation of the biomass concentration in the activated sludge follows CAS < AGS < MBR. This might lead to the hypothesis that CAS will have the lower micropollutants removal while MBR can guarantee higher removals. Higher biomass concentrations will lead to a higher number of bacteria and therefore it is expected higher conversion capacity per reactor volume, as well. This increase in biomass could also mean an increase in sorption; however, the targeted compounds do not have significant $K_d$ values. Yet, for compounds with high $K_d$, this may be something to have in mind.

Table 5-14 presents some of the observed removal efficiencies for CAS, AGS and MBR. It is important to mention that this comparison is limited since the values were not obtained in the same operational conditions, yet it allows to have some degree of sensitivity.

Table 5-13 Comparison of removal efficiencies from CAS vs AGS vs MBR (values taken from both batch and continuous studies)

| Micropollutants | CAS [1] | AGS | MBR |
|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 35 (± 4)% | 25% [7] | |
| Benzotriazole | 69 (± 9)% | 40% [7] | 97% [8] |
| Carbamazepine | -2 (± 8)% | 2% [7] | 11% [3]<br><10% [5] |
| Clarithromycin | 98 (± 14)% | 98% [7] | 57% [4] |
| Diclofenac | 8 (± 2)% | 15% [7]<br>17% [9] | 60% [2]<br>87% [5] |
| Hydrochlorothiazide | 6 (± 7)% | | 66% [5] |
| Metoprolol | 81 (± 13)% | 16% [7] | 59% [5] |
| Propranolol | 95 (± 11)% | -5% [9] | 78% [6] |
| Sotalol | 55 (± 6)% | 10% [7] | 53% [6] |
| Sulfamethoxazole | 74 (± 4)% | 95% [7]<br>67% [9] | 57% [3]<br>61% [5] |
| Trimethoprim | 63 (± 10)% | 12% [7]<br>19% [9] | 87% [4] |
| Azithromycin | 82 (± 6)% | 92% [7]<br>45% [9] | 24% [4] |
| Candesartan | 4 (± 4)% | | |
| Gabapentin | 98 (± 2)% | 70% [7] | |
| Irbesartan | 53 (± 8)% | | |
| Venlafaxine | 30 (± 12)% | -5% [9] | |

(1) Results obtained in this experiment; (2) Clara *et al.* (2004); (3) Kreuzinger *et al.* (2004); (4) Gobel *et al.* (2007); (5) Radjenovic *et al.* (2007); (6) Radjenovic *et al.* (2009); (7) Margot *et al.* (2016); (8) Kowalska *et al.* (2019); (9) Burzio *et al.* (2022)

Table 5-13 presents an overview and comparison of removal efficiencies with different activated sludge systems, including the results of our batch tests using PhoRedox CAS sludge. 4-5-methylbenzotriazole showed higher removal efficiency in the CAS system compared to AGS, like benzotriazole, metoprolol, propranolol, and sotalol sulfamethoxazole, trimethoprim, gabapentin, and venlafaxine. The MBR system found the best removals for benzotriazole, carbamazepine, diclofenac, hydrochlorothiazide, and trimethoprim. When analysing diclofenac, it is possible to observe that AGS exhibited higher efficiency than CAS, but the highest is still the MBR.

The removal of the micropollutants varies between the different systems, but still, much research is going on. Since AGS is still a recent technology, limited research has yet been published about its capacity to remove our targeted micropollutants.

Burzio *et al.* (2022) have analysed the removal of some micropollutants in granular activated sludge and compared it with the conventional activated sludge. Results showed that AGS seems to be less effective (a difference of up to 50%) in removing the targeted micropollutants when compared to the conventional activated sludge. Margot *et al.* (2016) have also observed better removal of most micropollutants with CAS. However, some compounds are more easily removed using AGS technology than CAS or MBR. Margot *et al.* (2016) reported high removal (> 70%) of both azithromycin,

clarithromycin, and sulfamethoxazole when using AGS, compared to a 70%, 40%, and 20% removal obtained with CAS, respectively. These values are not aligned with the ones observed by Burzio *et al.* (2022) since it was mentioned that azithromycin, carbamazepine, clarithromycin, diclofenac, metoprolol, propranolol, and sulfamethoxazole the CAS technology obtained better results (with differences of 25%, 50%, 55%, 20%, 25%, 25%, and 5%, respectively). Margot *et al.* (2016) have also pointed out that AGS achieved higher removals of 40%, 15%, 75%, and 20% for compounds like benzotriazole, diclofenac, gabapentin, and metoprolol, correspondingly. On the contrary, it showed less effectiveness in removing 4-,5-methylbenzotriazole, carbamazepine, sotalol, and trimethoprim, respectively, 15%, <5%, 10%, and 10%.

Radjenovic *et al.* (2009) studied the removal of some micropollutants in both CAS and MBR, showing that MBR exhibited a higher removal efficiency than CAS systems in removing micropollutants (up to 60%). However, this was not observed for both carbamazepine and hydrochlorothiazide. This might be associated with their known recalcitrancy. Yet, diclofenac has shown an increased removal of 45%. According to Verlicchi *et al.* (2012), MBR generally shows a higher removal efficiency than CAS. Compounds like carbamazepine, diclofenac, and trimethoprim that have exhibited low removal in CAS have shown higher removal when the CAS is upgraded to an MBR. Sulfamethoxazole, however, has shown similar results with both MBR and CAS (Verlicchi *et al.*, 2012).

## 5.8 Hydraulic Retention Time Improvement

Hydraulic Retention Time influence on micropollutants removal- lessons for future CAS designs Walcheren WWTP is not yet working at total capacity, as shown in Table 5-5. Therefore, the actual HRT of the CAS system is around 29,6h, even though the design is around 6,2h. According to the information found in the literature, the HRT of a CAS should not be greater than 24h, yet the current WWTP is working with a higher HRT. Thus, when the served population of this wastewater treatment plant increases, the biological micropollutants removal efficiency may decrease.

A simple correlation between the biotransformation rate constants and the hydraulic retention time (HRT) was made to suggest an HRT that allows a higher removal of the micropollutants. All redox conditions, as well as the actual HRT, were taken into consideration.

Using the obtained biotransformation rate constants (Table 5-9 and Table 5-10), the expected degradation of micropollutants through time was plotted using (See Figure 5-3). It was assumed that the initial concentration was the average obtained during the sampling campaign presented in Table 5-2. The figures for 4-, 5-methylbenzotriazole, diclofenac, gabapentin, irbesartan, metoprolol, and trimethoprim were chosen as representative as possible. Yet, the rest of the compounds were also plotted and can be found in the Appendix. The compounds presented in the figure below have different total removal times ranging from 10 days (gabapentin) to 300 days (diclofenac).

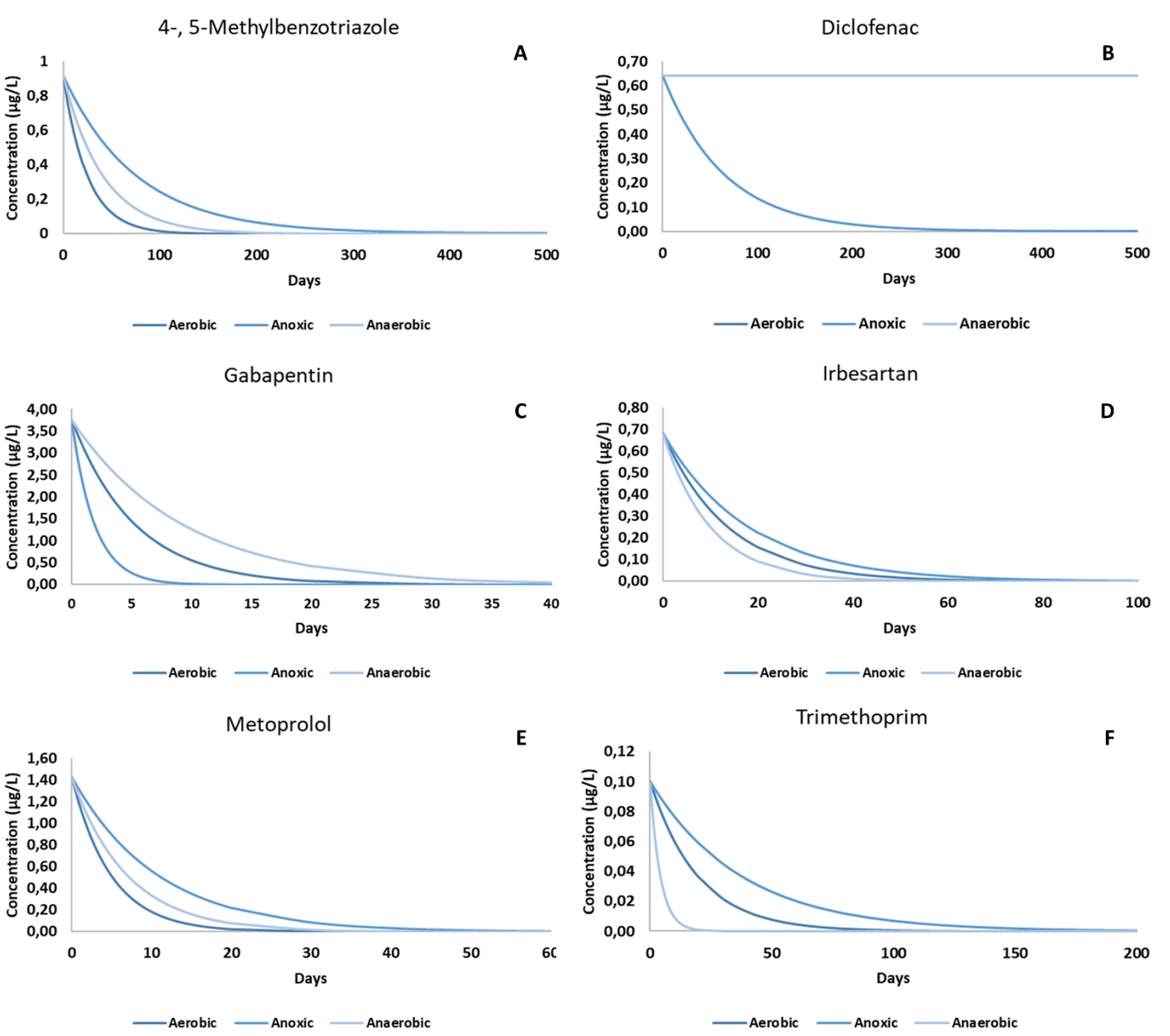


Figure 5-3 Theoretical degradation of six of the sixteen targeted micropollutants under aerobic, anoxic, and anaerobic conditions (**A**: 4-,5-methylbenzotriazole; **B**: diclofenac; **C**: gabapentin; **D**:irbesartan; **E**: metoprolol; **F**: trimethoprim)

Gabapentin, for example, can be removed in less than 10 days under anoxic conditions or almost 40 days under anaerobic conditions. Irbesartan will degrade in around 60 days under redox conditions. At the same time, diclofenac will take more than 300 days to be degraded under anoxic conditions biologically, and no removal will occur under aerobic and anaerobic conditions.

The time needed to observe an 80% removal of 80% of the micropollutants, as suggested by the Swiss law (Swiss Federal Council, 2021) and as pretended to implement by the European Parliament about 90 days will be needed, which is more than the 29,6h – actual HRT (See Table 5-5). As mentioned in the literature review, Liang *et al.* (2021) observed a need of 14 days to follow the Swiss law, which means the CAS system, according to our results, needs almost seven times more time.

Yet, a CAS with an HRT of 190 days, or even 15 days, is unfeasible due to technical and operational impossibilities, capital (CAPEX) and operating expenses (OPEX), and the required area footprint. This HRT would prevent the system to work properly, keeping the wastewater retained for months.

Using the biotransformation rates determined in this research and assuming that the ratio between the aerobic, anoxic, and anaerobic tank was the same (see Table 5-5), the expected removals per compound at different HRTs (design -6,2h-, actual -29,6h-, 190 and 300 days) were calculated, according (see Table 5-14).

Table 5-14 Comparison of the estimated micropollutants removal efficiencies with different HRT

| Micropollutants | Design HRT (6,2 h) | Actual HRT (29,6 h) | 190 days HRT | 300 days HRT |
|---|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 1% | 3% | 99% | 100% |
| Benzotriazole | 2% | 9% | 100% | 100% |
| Carbamazepine | 0% | -1% | -275% | -706% |
| Clarithromycin | 8% | 35% | 100% | 100% |
| Diclofenac | 0% | 1% | 60% | 76% |
| Hydrochlorothiazide | 0% | 0% | 37% | 52% |
| Metoprolol | 3% | 16% | 100% | 100% |
| Propranolol | 5% | 24% | 100% | 100% |
| Sotalol | 1% | 4% | 100% | 100% |
| Sulfamethoxazole | 5% | 22% | 100% | 100% |
| Trimethoprim | 3% | 14% | 100% | 100% |
| Azithromycin | 5% | 24% | 100% | 100% |
| Candesartan | 0% | 0% | -13% | -21% |
| Gabapentin | 6% | 27% | 100% | 100% |
| Irbesartan | 2% | 9% | 100% | 100% |
| Venlafaxine | 1% | 3% | 99% | 100% |
| **Average** | **3%** | **12%** | **63%** | **38%** |

Comparing the results observed in Table 5-14 (see Actual HRT column) with the obtained removal efficiencies of the Walcheren WWTP CAS system (see Table 5-4), it is noticeable that the observed removals at Walcheren WWTP, during the sampling campaign, are slightly higher than the ones estimated from the biotransformation rate constant. The overall removal observed in the sampling campaigns was 19%, while the general removal in Table 5-14 is 12%. This might be associated with some compounds degrading better when exposed to different redox conditions.

Comparing the actual micropollutants removal with the expected one when the WWTP is working at its maximum, it is possible to observe a reduction of the removal, if the influent concentrations will not change, and the microbial community composition of the reactor will be similar. This means that the micropollutants will start being released in higher concentrations in the *Kanaal door* Walcheren if only biological treatment is applied in the current situation.

The increase of the HRT leads to an increase in the compound's removal; however, some compounds do not degrade, and some metabolites also retransform to their parent compound (e.g., clarithromycin, candesartan, and sulfamethoxazole).

The anaerobic reactor showed most of the compound's retransformation and is also the bigger reactor. Moreover, higher removals were observed under aerobic and anoxic conditions, being, for that reason, the compartments of the biological reactor that should have bigger volumes and, therefore, longer HRTs. Thus, to optimize the micropollutants removal for a future design, the aerobic reactor volume should be bigger, followed by the anoxic and the anaerobic (aerobic > anoxic > anaerobic). Nevertheless, this change will not create substantial changes since these compounds have low biotransformation rates. For example, by changing the HRT of the aerobic and the anaerobic tank, it is possible to observe an increase of around 0,5% (anaerobic tank: 6,3h, aerobic tank: 13,5h; anoxic: 9h;). However, these changes may affect the biological process and nutrient removal, therefore, this nutrient removal need also to be accounted for and not only the micropollutant biotransformation.

The biological reactor in a wastewater treatment plant works as a continuous reactor with different inflows and outflows. Therefore, this is only a simple analysis of the redox conditions and the HRT distribution through the three compartments of the bioreactor since it should be modelled with all the associated variability and accounting not only the nutrient removal but also the biotransformation rates of the micropollutants. Once the system is modelled and the biotransformation is improved, the next step will be to apply tertiary treatment as a post-treatment of the biological process to assure an optimal removal of the micropollutants. This tertiary treatment for micropollutant removal is mainly advanced oxidation processes to allow the oxidation of the micropollutants that are not biotransformed during the CAS system. This process is going to be further developed in the next chapter.

# 6

# TOXICOLOGICAL ASSESSMENT

## 6.1 General Introduction

Due to the unavailability of information about the targeted micropollutants concentrations in the canal where Walcheren WWTP discharged the effluent, a theoretical toxicological assessment was carried out in a hypothetical localization, with hypothetical environmental concentrations. Therefore, literature values will be used in this theoretical exercise to fulfil the hypothetical values.

Since the removal of micropollutants is rarely 100%, whether relying on biological treatment or in an advanced oxidation process. The discharge of the effluent will always make some impact on the receiving environment. These impacts can go from problems in the aquatic ecosystem to public health problems. The present chapter will be developed an exercise on how to perform a simple analysis of the toxicity and risk of the discharged effluent with the micropollutants at different concentrations. It will also be compared with PNEC values observed in the literature, using the *European Commission Technical Guidelines for Risk Assessment* (EC, 2003) to determine potential risk. The referred analysis will use the micropollutants concentrations shown in see Table 5-3, and the HRT in chapter 5.4, for the sake of the exercise. A complete full set of data would require environmental measurements upstream and knowledge of discharged water body, namely the correct dilution factor, which was not measured. Tweaking of the HRT as suggested in the previous chapter will also be discussed. Advanced treatment with an overall 90% removal, will also be considered.

A toxicological assessment evaluates the safety of a determinate product based on its composition and intended uses. It is part of the Environmental Risk Assessment (ERA) that pharmaceutical and chemical companies must consider when registering a drug or chemical in the European Union. The ecotoxicity tests of these compounds used in humans and animals follow strict requirements defined by the EMEA (European Medicines Agency) guidelines as well as by the Directives 2019/6/EC and 2001/83/EC (Załęska-Radziwiłł, 2011). These studies are complex and include many chemical, biological and toxicology disciplines. The required assessments are divided into hazardous and risk assessments (Christiani *et al.*, 2007). The difference between them is that a dangerous assessment is linked to the ability of a potentially harmful event in case of exposure. On the contrary, risk assessments are already connected to the probability and extent of the damage caused by the harmful event (Scheer *et al.*, 2014). This means that hazardous situations are only risky if the subject is exposed to them.

Usually, these studies have qualitative and quantitative components based on four main steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization (Christiani *et al.*, 2007). To accomplish the second and third steps, bioassays are usually used, aiming to

determine the relative strength of a compound on test subjects (organism or cells) with a standard preparation and then comparing its effects (Kienle *et al.*, 2022). Nowadays, bioassays can be in vitro or in vivo and silico, increasing the data's reliability and allowing for more complex analysis. In silico bioassays also permit knowledge development instead of animals since they are based on known data and independent models, like QSAR (Quantitative structure-activity relationship) (Benfenati *et al.*, 2019).

These bioassays can determine different endpoints (No Observed Effect Concentration – NOEC; Lethal Concentration – LC; Effective Concentration – EC; Genotoxicity; Mutagenicity and others), as well as observed in different reference organisms (Kienle *et al.*, 2022). These endpoint values are critical to toxicological assessments once they allow the determination of parameters like the predicted no-effects concentrations (PNEC) that are widely used in environmental risk assessments (ERA) of chemicals in aquatic ecosystems (Figuiere *et al.*, 2022).

## 6.2 Predicted No-Effect Concentration Determination

According to the European Union Directive 93/67 (EC, 2003), as well as executed by Figuiere *et al.* (2022) and Załęska-Radziwiłł (2011), the PNEC values are calculated based on the minimal concentration that has shown no effect to the most sensitive organism and then divided by an assessment factor (AF), since it dictates that long-term toxicity tests made on standard species of algae, invertebrates, and fishes are sufficient to determine the PNEC value for the aquatic ecosystems (EC, 2003; Figuiere *et al.*, 2022; Załęska-Radziwiłł, 2011). Table 6-1 presents the literature endpoints used to determine the PNEC value for the targeted micropollutants.

Table 6-1 Endpoints used in the PNEC calculation for the targeted micropollutants

| Micropollutant | Algae | | Invertebrates | | Fish | |
|---|---|---|---|---|---|---|
| | Effect Concentration (µg/L) | Toxicological Endpoint | Effect Concentration (µg/L) | Toxicological Endpoint | Effect Concentration (µg/L) | Toxicological Endpoint |
| 4-, 5-Methylbenzotriazole | 2 500 | NOEC (3) | 1 000 | NOEC (3) | | |
| Benzotriazole | 75 000 | ErC50 (6) | 15 800 | EC50 (6) | 240 000 | LC50 (6) |
| Carbamazepine | 500 | NOEC (4) | 100 | NOEC (4) | 19 900 | LC50 (4) |
| | 100 000 | NOEC (1) | 377 | NOEC (1) | 25 500 | NOEC (1) |
| Clarithromycin | 2 | EC 50 (8) | 4 600 | NOEC (8) | 680 000 | NOEC (8) |
| Diclofenac | 10 000 | NOEC (8) | 45 000 | NOEC (8) | 1 | NOEC (8) |
| | 10 000 | NOEC (2) | 30 700 | LC50 (2) | 82 000 | LC50 (2) |
| | 10 000 | NOEC (1) | 12 500 | NOEC (1) | 4 000 | NOEC (1) |
| Hydrochlorothiazide | | | | | | |
| Metoprolol (RIVM) | 7 600 | LC50 (8) | 133 000 | LC50 (8) | 106 000 | LC50 (8) |
| Propranolol | 160 | NOEC (8) | 9 | NOEC (8) | 130 | LOEC (8) |
| | 100 | NOEC (5) | 50 | NOEC (5) | 130 | NOEC (5) |
| Sotalol | | | | | | |
| Sulfamethoxazole | 5,9 | NOEC (8) | 5,9 | NOEC (8) | 10 | NOEC (8) |
| | 22 | NOEC (2) | 5,9 | NOEC (2) | 10 | NOEC (2) |
| Trimethoprim | 16 000 | LC50 (2) | 123 000 | LC50 (2) | 100 000 | LC50 (2) |
| Azithromycin | 5,2 | NOEC (8) | 100 000 | EC 50 (8) | 4 600 | NOEC (8) |
| Candesartan | | | | | | |
| Gabapentin | | | | | | |
| Irbesartan | 130 | EC 50 (8) | 5 600 | EC50 (8) | 5 300 | LC 50 (8) |
| | 23 000 | NOEC (2) | 7 400 | NOEC (2) | 10 400 | NOEC (2) |
| Venlafaxine | 480 000 | EC 50 (8) | 14 000 | EC 50 (8) | 5,2 | LOEC (8) |

(1) Ferrari et al. (2003); (2) van der Aa et al. (2011); (3) Seeland et al. (2012); (4) Moermond (2014); (5) AstraZeneca (2017); (6) Carl Roth (2018); (7) Sodré et al. (2018); (8) Figuiere et al. (2022)

Once having the needed endpoints for each compound, it is possible to define the AF parameter based on the type and number of toxicological studies available according to Table 6-2 as mentioned in the risk assessment European technical guidelines (EC, 2003).

Table 6-2 Calculation method of the assessment factor (AF) for the PNEC calculation
Adapted from EC (2003); Figuiere et al. (2022); Załęska-Radziwiłł (2011)

| Data Available | Assessment Factor |
|---|---|
| Long-term no observed effect concentration (NOEC) data were available for each of the three taxonomic groups. | 10 |
| Long-term no observed effect concentration (NOEC) data were available for two of the three taxonomic groups. | 50 |
| Long-term no observed effect concentration (NOEC) data were available for one of the three taxonomic groups. | 100 |
| Only short-term $L_{50}$ data were available | 1000 |
| In case of only available the lowest observed effect concentration (LOEC) | X 2 |

Once defined, the endpoint is used, and the correct AF, it is possible to determine the PNEC value based on the following equation.

$$PNEC_i = \frac{\min(NOEC_{algae,i}\ , NOEC_{invertebrates,i}\ , NOEC_{fish,i}\ , EC_{50,algae,i}\ , EC_{50,invertebrates,i}\ , EC_{50,fish,i})}{AF} \quad (10)$$

To define the PNEC values for this study, a literature review was done, and values were summarized in Table 6-3, with the respective AF value considered. It is, however, important to refer that both the endpoints and the PNEC values are very variable due to the used organisms and experiment conditions, even following the standard methods. Besides, some PNEC values were obtained *in* silico modelling processes, increasing the variability.

Table 6-3 PNEC values and AF values for the targeted micropollutants

| Micropollutant | PNEC (µg/L) | Assessment Factor (AF) |
|---|---|---|
| 4-, 5-Methylbenzotriazole | 8 [8][6] | 50 [8][6] |
| Benzotriazole | 15,8 [6][4] | 1000 [6][4] |
| | 776 522 [8] | 1000 [8] |
| Carbamazepine | 0,25 [5] | 10 [5] |
| | 5 [8] | 10 [8] |
| | 5 [2] | 100 [2] |
| Clarithromycin | 0,04 [7] | 50 [7] |
| | 12 [8] | 20 [8] |
| Diclofenac | 0,1 [7] | 10 [7] |
| | 1 [5] | 100 [5] |
| | 5 [8] | 10 [8] |
| | 31 [2] | 1000 [2] |
| Hydrochlorothiazide | 838 089 [8] | 1 000 [8] |
| Metoprolol | 7,6 [2] | 1 000 [2] |
| | 7,9 [7] | 1000 [7] |
| | 86 [8] | 50 [8] |
| Propranolol | 411 [8] | 10 [8] |
| | 0,01 [5] | 50 [5] |
| | 0,9 [7] | 10 [7] |
| | 0,23 [3] | 10 [3] |
| Sotalol | 0,65 [8] | 1000 [8] |
| Sulfamethoxazole | 0,01 [5] | 100 [5] |
| | 0,59 [2] | 10 [2] |
| | 0,59 [7] | 10 [7] |
| | 6 [8] | 10 [8] |
| Trimethoprim | 0,016 [5] | 100 [5] |
| | 10 [8] | 1000 [8] |
| | 16 [2] | 1000 [2] |
| Azithromycin | 0,019 [5] | 1 000 [5] |
| | 0,1 [7] | 50 [7] |
| | 19 [8] | 10 [8] |
| Candesartan | 306 [8] | 1000 [8] |
| Gabapentin | 1 [8] | 100 [8] |
| Irbesartan | 0,13 [7] | 1 000 [7] |
| | 704 [8] | 1000 [8] |
| | 704 [2] | 10 [2] |
| Venlafaxine | 0,026 [7] | 200 [7] |
| | 38 [8] | 100 [8] |

(1) van der Aa et al. (2011); (2) Moermond (2014); (3) AstraZeneca (2017); (4) Carl Roth (2018);

(5) Sodré et al. (2018); (6) ECHA (2022); (7) Figuiere et al. (2022); (8) NORMAN et al. (2022)

Due to the meaning of the PNEC value, the lower this parameter is, the more that compound is dangerous in aquatic environments once lower concentrations are needed to create an effect in the aquatic biota. Therefore, venlafaxine and clarithromycin are the most concerning compounds on the list. Figuiere *et al.* (2022) show that venlafaxine and clarithromycin are the most concerning compounds in Swedish aquatic ecosystems. Together with those compounds, azithromycin, diclofenac, and irbesartan are listed.

The most common metabolite of venlafaxine is O-Desmethylvenlafaxine, which is responsible for the compound's higher toxicity (and risk) for Sweden ecosystems (Figuiere *et al.*, 2022). Clarithromycin has a low PNEC and, according to Figuiere *et al.* (2022), it does not pose a risk to the aquatic ecosystem nor does irbesartan. On the other hand, diclofenac presents a moderate risk due to the quantity of consumption and the difficulty of removal.

## 6.3 Estimation of the Upstream Concentration

Once the PNEC value is determined, it is necessary to determine both the micropollutants concentrations before and after the effluent discharge. The concentrations before discharge (upstream) were defined based on the observed values in the literature for European countries, Asia, and the USA. The need to use other continent information is based on the lack of studies on the European rivers about the targeted micropollutants of emerging concern.

In this exercise, no values upstream of the discharge point are known. Therefore, to produce an estimation with values that could be found at one location, literature values were used (Table 7.4). To assess as cautiously as possible, an average of the maximum observed values was used due to their variability per compound. Unfortunately, not all compounds have values reported to the best of our knowledge.

Table 6-4 Upstream environmental concentration (MEC) found in the literature for the sixteen targeted micropollutants

| Micropollutants | Environmental concentration (ng/L) | |
|---|---|---|
| | Maximum | Minimum |
| 4-, 5-Methylbenzotriazole | | |
| Benzotriazole | | |
| Carbamazepine | 31,6 [5][2] | 0,5 [2] |
| | 65 [9] | 1 [2] |
| | 174 [3] | 102 [1][6] |
| | 1 194 [1][6] | 2,4 [9] |
| | 495 [2] | 4 [3] |
| | 595 [3] | ND [5] |
| Clarithromycin | 115 [3] | 0,87 [9] |
| | 2,8 [9] | |
| | 443 [3] | |
| | 50,2 [2] | |
| Diclofenac | 1 043 [8] | 0,5 [2] |
| | 261 [2] | 0,5 [2] |
| | 35 [5] | 0,8 [8] |
| | 40 [9] | 2,4 [9] |
| | 85 [2] | ND [5] |
| Hydrochlorothiazide | | |
| Metoprolol | 8 [2] | 0,5 [2] |
| | 93 [9] | 3 [9] |
| Propranolol | 3 [9] | 0,5 [2] |
| | 9 [2] | 1,4 [9] |
| | 27 [3][2] | 21 [3] |
| | 43 [3] | |
| Sotalol | 7,7 [9] | 1,8 [9] |
| Sulfamethoxazole | 7,7 [9] | 0,5 [2][4] |
| | 1 [2] | 3,1 [9] |
| | 38 [7] | ND [5] |
| | 5,1 [5] | ND [7] |
| | 60 [4] | |
| Trimethoprim | 3,5 [9][4] | 0,5 [2][4] |
| | 7 [2] | 1,9 [9] |
| | 9,1 [7] | 50 [2] |
| | 90 [2] | ND [7] |
| Azithromycin | 5,1 [9] | 3,1 [9] |
| Candesartan | | |
| Gabapentin | 210 [2] | 27 [2] |
| | 565 [2] | 52 [2] |
| Irbesartan | 42 [9] | 1,3 [9] |
| Venlafaxine | 83 [9] | 3,9 [9] |

ND: Non-detected

*(1) Reinstorf* et al. *(2008); (2) Kasprzyk-Hordern* et al. *(2009); (3) Kim* et al. *(2009); (4) Lin* et al. *(2011); (5) Vulliet* et al. *(2009); (6) Regnery and Puttmann (2010); (7) Wang* et al. *(2011); (8) Stasinakis* et al. *(2012); (9) Golovko* et al. *(2021);*

## 6.4 Dilution Factor

As said before, after having the upstream measured environment concentrations (MEC), it is necessary to have the discharged effluent concentrations, to have the downstream MEC. However, to do that was needed to know the flow of the stream where the effluent is discharged since the dilution factor (DF) is determined based on both flows, as shown below (Colman *et al.*, 2016).

$$DF = \frac{Q_p + Q_e}{Q_e} \tag{11}$$

The author's Morgan and Corominas (2021), presented by Abily *et al.* (2021) analysed DF values. For the case of the continuation of the exercise, we will use those values. Abily *et al.* (2021) referred that due to climate change and the increase in temperature, during the following years, the dilution factor may change. That study was made for different global warming scenarios, as presented in the IPCC report Global Warming of 1.5 ºC (IPCC, 2018). According to the referred dataset, it is possible to analyse that the dilution factors in the Netherlands will mainly increase until 2040, independently of the global warming scenario considered. In Table 6-5 is possible to observe the values mentioned by Morgan and Corominas (2021).

Table 6-5 Dilution factors for The Netherlands in the different global warming scenarios
Adapted from Abily *et al.* (2021); Morgan and Corominas (2021)

| **Statistical analysis** | **Current dilution factor** | **Dilution factor forecast for 2040** (Worst global warming scenario) | **Dilution factor forecast for 2040** (Best global warming scenario) |
|---|---|---|---|
| Maximum | 263 202,31 | 267 808,34 | 280 310,41 |
| Average | 3 225,25 | 3 265,06 | 3 447,41 |
| Minimum | 2,10 | 2,07 | 2,12 |

The Noord-Brabant area's dilution factor varies between 300 and 400, not significantly affected by global warming (Morgan & Corominas, 2021). This variation is connected to the size of the rivers/canals and their hydrodynamic and hydrogeological characteristics. Therefore, was analysed different DF was to better understand the effect of the effluent dilution as best as possible.

## 6.5 Estimation of the Downstream Concentration

Table 6-6 shows the downstream measured environmental concentration after an effluent discharge, considering different dilution factors, and assuming

Table 6-6 Dilution factor effect on the downstream MEC, with high upstream concentrations

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effluent Concentration (µg/L) | 0,40 | 0,08 | 0,74 | 1,70 | 0,02 | 1,70 | 0,18 | 0,10 | 0,08 | 2,85 | 0,91 | 0,29 |
| Upstream MEC* (µg/L) | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| **Dilution Factors** | | | | | | | | | | | | |
| 100 | 0,430 | 0,154 | 0,300 | 0,068 | **0,021** | 0,025 | 0,024 | 0,029 | 0,006 | 0,416 | 0,051 | 0,086 |
| 200 | 0,428 | **0,153** | 0,297 | 0,059 | 0,021 | 0,017 | 0,023 | **0,028** | 0,006 | 0,402 | 0,047 | 0,084 |
| 300 | 0,427 | 0,153 | 0,295 | 0,056 | 0,021 | 0,014 | 0,023 | 0,028 | **0,005** | 0,397 | 0,045 | 0,084 |
| 400 | 0,427 | 0,153 | 0,295 | 0,055 | 0,021 | 0,012 | 0,023 | 0,028 | 0,005 | 0,395 | 0,044 | 0,084 |
| 500 | 0,427 | 0,153 | 0,294 | 0,054 | 0,021 | 0,011 | 0,023 | 0,028 | 0,005 | 0,393 | 0,044 | 0,084 |
| 1 000 | **0,426** | 0,153 | 0,294 | 0,052 | 0,021 | 0,010 | 0,023 | 0,028 | 0,005 | 0,390 | 0,043 | **0,083** |
| 2 000 | 0,426 | 0,153 | **0,293** | **0,051** | 0,021 | 0,009 | 0,022 | 0,028 | 0,005 | 0,389 | **0,042** | 0,083 |
| 3 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,009 | 0,022 | 0,028 | 0,005 | **0,388** | 0,042 | 0,083 |
| 4 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | **0,008** | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 5 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 10 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 20 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 30 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 40 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 50 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 100 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 200 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |
| 300 000 | 0,426 | 0,153 | 0,293 | 0,051 | 0,021 | 0,008 | 0,022 | 0,028 | 0,005 | 0,388 | 0,042 | 0,083 |

*Upstream MEC was determined based on the average literature values mentioned in Table 6-4.

From Table 6-6 it is observed that each compound needs different dilution factors to stop having some significance in the MEC. While for clarithromycin, a DF of 200 is enough. Carbamazepine needs a DF of 1000, while diclofenac, metoprolol, sulfamethoxazole, and irbesartan need a DF of 2 000 to be in the same concentration of the upstream MEC. Sotalol is the compound that needs higher DF to equalize the environmental concentration, requiring a DF of 4 000.

## 6.6 Risk Quotient Determination

After having the PNEC and the downstream MEC values, it is possible to determine the risk quotient (RQ) following the equation shown below, which correlates the MEC and the PNEC of a determined compound in a determined location.

$$RQ_{i,j} = \frac{MEC_{i,j}}{PNEC_i} \quad (12)$$

The risk quotient allows differentiating compounds with negligible, low, moderate, and high risk to the environment in the location assessed. The range of values of each risk category, following the risk assessment European technical guidelines (EC, 2003), can be found in Table 6-7.

Table 6-7 Risk quotient and their range of values
Adapted from EC (2003); Figuiere *et al.* (2022); Załęska-Radziwiłł (2011)

| Risk Quotient | Range of Values |
|---|---|
| Negligible risk to the environment | 0,01 < RQ |
| Low risk for the environment | 0,01 < RQ < 0,1 |
| Moderate risk to the environment | 0,1 < RQ < 1 |
| High risk for the environment | 1 < RQ |

Based on and Table 6-7 it is possible to determine the environmental risk for each compound at the current WWTP status, assuming different DF. Table 6-8 presents the RQ for each analysed compound with the dilution factors of 300, 400, 3 000, 4 000, 200 000, and 300 000, since they are, respectively, the average values of the closes area of Vlissingen (Noord-Brabant), the average values for The Netherlands and the highest dilution factor values for The Netherlands.

Table 6-8 Risk quotient for the targeted compounds, assuming the highest upstream MEC concentration and current WWTP status

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PNEC (µg/L) | 0,250 | 0,040 | 0,100 | 7,900 | 0,010 | 651 849 | 0,010 | 0,016 | 0,019 | 1,000 | 0,130 | 0,026 |
| RQ at DF 300 | 1,709 | 3,832 | 2,953 | 0,007 | 2,057 | 0,000 | 2,300 | 1,740 | 0,282 | 0,397 | 0,346 | 3,229 |
| RQ at DF 400 | 1,707 | 3,830 | 2,947 | 0,007 | 2,055 | 0,000 | 2,285 | 1,734 | 0,279 | 0,395 | 0,341 | 3,220 |
| RQ at DF 3 000 | 1,704 | 3,826 | 2,930 | 0,006 | 2,051 | 0,000 | 2,246 | 1,721 | 0,270 | 0,388 | 0,325 | 3,196 |
| RQ at DF 4 000 | 1,704 | 3,826 | 2,930 | 0,006 | 2,051 | 0,000 | 2,245 | 1,720 | 0,269 | 0,388 | 0,325 | 3,195 |
| RQ at DF 200 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,240 | 1,719 | 0,268 | 0,388 | 0,323 | 3,192 |
| RQ at DF 300 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,240 | 1,719 | 0,268 | 0,388 | 0,323 | 3,192 |

The results presented in Table 6-8 indicate that carbamazepine, clarithromycin, diclofenac, propranolol, sulfamethoxazole, trimethoprim, and venlafaxine present high environmental risk.

However, coupled with the information shown in Table 6-6, it is possible to notice that this risk is not due to the discharge itself but to the environmental concentration considered. Even though the increase in dilution leads to a decrease in the risk quotient, this decrease is not substantial. Both azithromycin, gabapentin, and irbesartan observed moderate environmental risk and only metoprolol and sotalol observed negligible environmental risk, mainly due to their low PNEC value.

The risk assessment of the micropollutants was also analysed for the situation where the WWTP was improved with the AOP implementation, as suggested in Appendix 7. This analysis assumed an AOP removal efficiency of 90%. Therefore, only 10% of the micropollutants concentration was used to determine the RQ. The risk quotient results are shown in Table 6-9 for the micropollutants, assuming the highest upstream MEC concentration and WWTP upgraded with the suggested AOP (Appendix 7).

Table 6-9 Risk quotient for the targeted micropollutants, assuming the highest upstream MEC concentration and WWPT upgraded with AOP ($O_3$/GAC)

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PNEC (µg/L) | 0,250 | 0,040 | 0,100 | 7,900 | 0,010 | 651 849 | 0,010 | 0,016 | 0,019 | 1,000 | 0,130 | 0,026 |
| RQ at DF 300 | 1,704 | 3,826 | 2,930 | 0,006 | 2,051 | 0,000 | 2,246 | 1,721 | 0,270 | 0,388 | 0,325 | 3,196 |
| RQ at DF 400 | 1,704 | 3,826 | 2,930 | 0,006 | 2,051 | 0,000 | 2,245 | 1,720 | 0,269 | 0,388 | 0,325 | 3,195 |
| RQ at DF 3 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,241 | 1,719 | 0,269 | 0,388 | 0,323 | 3,193 |
| RQ at DF 4 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,240 | 1,719 | 0,269 | 0,388 | 0,323 | 3,193 |
| RQ at DF 200 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,240 | 1,719 | 0,268 | 0,388 | 0,323 | 3,192 |
| RQ at DF 300 000 | 1,703 | 3,825 | 2,928 | 0,006 | 2,050 | 0,000 | 2,240 | 1,719 | 0,268 | 0,388 | 0,323 | 3,192 |

As expected, this decrease in the effluent micropollutants concentrations is not highly significant in the risk determination since it is connected to the existing environmental concentrations. Therefore, the possible biotransformation associated with optimizing the biological treatment will not also have an impact since it will be smaller than the 90% removal efficiency obtained with the proposed AOP.

To observe the impact of the discharge of the effluent on the environment, assuming lower upstream measured concentrations, the same methodology was applied, considering now the average values of the minimal concentrations observed in the literature, presented in Table 6-4. The dilution factor effect on the downstream MEC with low upstream concentrations is shown in Table 6-10.

Table 6-10 Dilution factor effect on the downstream MEC, with low upstream concentrations

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effluent Concentration (µg/L) | 0,40 | 0,08 | 0,74 | 1,70 | 0,02 | 1,70 | 0,18 | 0,10 | 0,08 | 2,85 | 0,91 | 0,29 |
| Upstream MEC* (µg/L) | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| **Dilution Factors** | | | | | | | | | | | | |
| 100 | 0,022 | 0,002 | 0,008 | 0,019 | **0,008** | 0,019 | 0,003 | 0,014 | 0,004 | 0,068 | 0,010 | 0,007 |
| 200 | 0,020 | **0,001** | 0,005 | 0,010 | 0,008 | 0,010 | 0,002 | 0,014 | 0,004 | 0,054 | 0,006 | 0,005 |
| 300 | 0,020 | 0,001 | 0,003 | 0,007 | 0,008 | 0,007 | 0,002 | **0,013** | **0,003** | 0,049 | 0,004 | 0,005 |
| 400 | 0,019 | 0,001 | 0,003 | 0,006 | 0,008 | 0,006 | **0,001** | 0,013 | 0,003 | 0,047 | 0,004 | 0,005 |
| 500 | 0,019 | 0,001 | 0,002 | 0,005 | 0,008 | 0,005 | 0,001 | 0,013 | 0,003 | 0,045 | 0,003 | **0,004** |
| 1 000 | 0,019 | 0,001 | 0,002 | 0,003 | 0,008 | 0,004 | 0,001 | 0,013 | 0,003 | 0,042 | 0,002 | 0,004 |
| 2 000 | 0,019 | 0,001 | **0,001** | 0,003 | 0,008 | 0,003 | 0,001 | 0,013 | 0,003 | 0,041 | 0,002 | 0,004 |
| 3 000 | **0,018** | 0,001 | 0,001 | **0,002** | 0,008 | **0,002** | 0,001 | 0,013 | 0,003 | **0,040** | 0,002 | 0,004 |
| 4 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,002 | 0,004 |
| 5 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | **0,001** | 0,004 |
| 10 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 20 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 30 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 40 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 50 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 100 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 200 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |
| 300 000 | 0,018 | 0,001 | 0,001 | 0,002 | 0,008 | 0,002 | 0,001 | 0,013 | 0,003 | 0,040 | 0,001 | 0,004 |

*Upstream MEC is determined based on the average literature values mentioned in Table 6-2.

The first downstream micropollutant concentration equal to the upstream concentration is highlighted in bold.

From Table 6-10, it is possible to observe that clarithromycin needs a DF of 200 to reach the environmental concentrations. In contrast, diclofenac needs a DF of 2 000, and carbamazepine and metoprolol, sotalol, and gabapentin need a DF of 3 000. On the other hand, sulfamethoxazole starts needing only a DF of 400, and irbesartan goes for a DF of 5 000, being the compound that requires a higher DF to equalize the environmental concentration.

Table 6-11 shows the RQ values calculated assuming the lowest upstream MEC concentration and current WWTP status.

Table 6-11 Risk quotient for the targeted compounds, assuming the lowest upstream MEC concentration and current WWTP status

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PNEC (µg/L) | 0,250 | 0,040 | 0,100 | 7,900 | 0,010 | 651 849 | 0,010 | 0,016 | 0,019 | 1,000 | 0,130 | 0,026 |
| RQ at DF 300 | 0,079 | 0,028 | 0,033 | 0,001 | 0,770 | 0,000 | 0,150 | 0,840 | 0,177 | 0,049 | 0,033 | 0,187 |
| RQ at DF 400 | 0,077 | 0,027 | 0,027 | 0,001 | 0,768 | 0,000 | 0,135 | 0,834 | 0,174 | 0,047 | 0,028 | 0,178 |
| RQ at DF 3 000 | 0,074 | 0,022 | 0,011 | 0,000 | 0,764 | 0,000 | 0,096 | 0,821 | 0,165 | 0,040 | 0,012 | 0,154 |
| RQ at DF 4 000 | 0,074 | 0,022 | 0,010 | 0,000 | 0,764 | 0,000 | 0,095 | 0,820 | 0,164 | 0,040 | 0,012 | 0,153 |
| RQ at DF 200 000 | 0,073 | 0,022 | 0,008 | 0,000 | 0,763 | 0,000 | 0,090 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |
| RQ at DF 300 000 | 0,073 | 0,022 | 0,008 | 0,000 | 0,763 | 0,000 | 0,090 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |

Looking at the values in Table 6-11, the RQ values are much lower than the ones observed previously in both Table 6-8 and Table 6-9. This may prove that in an environment with a high concentration of micropollutants, the discharge of the WWTP effluent will not have a significant impact. However, suppose the discharge of micropollutants is reduced in all the WWTP. In that case, those compounds will not arrive in the environment, making, therefore, lowering the upstream environmental concentrations and reducing the environmental risk. None of the compounds presents a high environmental risk, being trimethoprim, the analysed micropollutant with a higher risk, and still only presents a moderate environmental risk. In this case, it is possible to observe the effect of the dilution of the risk reduction, mainly in diclofenac and sulfamethoxazole. A dilution factor of 4 000 allowed a decrease from low environmental risk to a negligible risk in diclofenac. A dilution of 3 000 allowed a reduction from moderate environmental risk to low risk in sulfamethoxazole. Sotalol continues to have an RQ of 0 (negligible environmental risk) due to its high PNEC value.

To conclude the analysis, Table 6-12 shows the RQ values assuming the lowest upstream MEC concentration and WWTP upgraded with the suggested AOP (Appendix 7).

Table 6-12 Risk quotient for the targeted compounds, assuming the lowest upstream MEC concentration and WWPT upgraded with AOP ($O_3$/GAC)

| | CAR | CLA | DIC | MET | PRO | SOT | SUL | TRI | AZI | GAB | IRB | VEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PNEC (µg/L) | 0,250 | 0,040 | 0,100 | 7,900 | 0,010 | 651 849 | 0,010 | 0,016 | 0,019 | 1,000 | 0,130 | 0,026 |
| RQ at DF 300 | 0,074 | 0,022 | 0,011 | 0,000 | 0,764 | 0,000 | 0,096 | 0,821 | 0,165 | 0,040 | 0,012 | 0,154 |
| RQ at DF 400 | 0,074 | 0,022 | 0,010 | 0,000 | 0,764 | 0,000 | 0,095 | 0,820 | 0,164 | 0,040 | 0,012 | 0,153 |
| RQ at DF 3 000 | 0,073 | 0,022 | 0,009 | 0,000 | 0,763 | 0,000 | 0,091 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |
| RQ at DF 4 000 | 0,073 | 0,022 | 0,009 | 0,000 | 0,763 | 0,000 | 0,090 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |
| RQ at DF 200 000 | 0,073 | 0,022 | 0,008 | 0,000 | 0,763 | 0,000 | 0,090 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |
| RQ at DF 300 000 | 0,073 | 0,022 | 0,008 | 0,000 | 0,763 | 0,000 | 0,090 | 0,819 | 0,163 | 0,040 | 0,010 | 0,150 |

Once again, the AOP-associated removal did not substantially impact the determined RQ in Table 6-11. Nevertheless, higher variations are found than those obtained when analysing the RQ (with and without AOP) at the highest upstream concentrations. Besides the ecotoxicology risk, as mentioned, the

removal of micropollutants by AOP allows for lower upstream and downstream concentrations, reducing, therefore, the harmful potential of the compounds in the environment.

The obtained results in Table 6-11 comply with the ones published by the *Waterschap Scheldestromen,* where it is mentioned that the ecological status of the canal is good and that none of the species is threatened.

Besides all the analyses, this approach assumes a determined influent concentration removal efficiency and environmental concentration. All those factors are variable. During winter, due to the lower temperature, the removal efficiencies are lower than the ones in summer (Figuiere *et al.*, 2022; Gago-Ferrero *et al.*, 2017). Besides that, as mentioned, it is also expected that the micropollutants concentration in the WWTP influent may be higher during winter. These changes may create an entirely different ecotoxicology assessment scenario.

# 7

# CONCLUSIONS AND FUTURE OUTLOOK

## 7.1 Conclusions

With the obtained results, it was possible to observe that both aerobic and anoxic redox conditions have shown higher removal rates. However, no predominant redox condition promotes the biotransformation of all the micropollutants. Clarithromycin exhibited the highest aerobic (76%) and anaerobic removal (78%) efficiencies, while gabapentin showed the highest removal under anoxic conditions (91%) and all the experiments. Clarithromycin is the micropollutant with the highest expected removal in a PhoRedox CAS system, according to the experiment results, while carbamazepine is the one with the lowest expected removal.

Under aerobic conditions, the biotransformation constant rate goes up to 1,75 $L.g_{SS}^{-1}.d^{-1}$ in clarithromycin, while the lowest value observed is -0,10 $L.g_{SS}^{-1}.d^{-1}$ in carbamazepine. Both candesartan and hydrochlorothiazide presented a low removal constant rate (<0,1 $L.g_{SS}^{-1}.d^{-1}$), while diclofenac showed a value of 0,00 $L.g_{SS}^{-1}.d^{-1}$. In this redox condition, adding influent led to a maximum increase of up to 64% in venlafaxine. At anoxic conditions, the constant rates go up to 2,36 $L.g_{SS}^{-1}.d^{-1}$ in gabapentin and reach the lowest value in candesartan with a constant rate of 0,03 $L.g_{SS}^{-1}.d^{-1}$. Candesartan, carbamazepine, diclofenac, and hydrochlorothiazide presented a low removal constant rate (<0,1 $L.g_{SS}^{-1}.d^{-1}$). In this redox, the condition is possible to observe that the addition of influent led to a maximum increase of 56% in carbamazepine. Under anaerobic conditions, the constant rate got the highest value of 1,87 $L.g_{SS}^{-1}.d^{-1}$ in clarithromycin and reached the lowest value of 0,11 $L.g_{SS}^{-1}.d^{-1}$ in 4-,5-methylbenzotriazole. Candesartan, carbamazepine, hydrochlorothiazide, sotalol and venlafaxine had shown negative $K_{bio}$ (between - 0,07 and - 0,03 $L.g_{SS}^{-1}.d^{-1}$) under anaerobic conditions, while diclofenac observed no removal (0,00 $L.g_{SS}^{-1}.d^{-1}$). In this redox condition, the addition of influent led to a maximum removal improvement of 164% in benzotriazole. Moreover, it is possible to observe that aerobic and anoxic conditions are the ones that show higher overall removals when compared with the anaerobic conditions.

Regarding hydrophobicity properties, a higher removal of the hydrophilic compounds (like clarithromycin, gabapentin, metoprolol, and sulfamethoxazole) than hydrophobic compounds (like diclofenac and candesartan) was found. Being hydrophilic showed an almost 16% increase in the overall removal compared to hydrophobic compounds. Under aerobic conditions, this is more noticeable since hydrophilic compounds have more than twice the removal efficiency (up to 44%). In comparison, under the anoxic and the anaerobic conditions, this removal increased by around 10% (up to 36% and 28%, respectively). Polarity allowed an overall increase of the removal of almost 15%. Under aerobic and anoxic conditions, polar compounds removal increase by less than 10%, while under anaerobic conditions, this increase goes up to 25%.

The micropollutants removal in a PhoRedox CAS WWTP could be improved by optimizing the HRT distribution in the design of the biological treatment process per redox condition, complying with the carbon, nutrient and micropollutant removal, and implementing an advanced treatment for micropollutant removal. If a retrofit of the WWTP is a plan, changing the CAS for an MBR configuration might be the best solution since this system has shown the best capacity to remove the targeted micropollutants based on its higher biomass concentration and, therefore higher conversion capacity. An advanced oxidation process could be applied as advanced treatment in the WWTP to maximize the removal.

An exercise of a toxicological assessment of the micropollutants, where the current biological treatment and a determined AOP would be implemented was demonstrated. Through our exercise, which does not reflect the existing environmental concentrations, because they were not measured, the effect of the effluent discharge was assessed. In this exercise, when the environmental concentrations are considerably high, the effluent discharge does not cause any significant change in the risk assessment, independently of the dilution factor since the toxicity is already associated with the environmental concentration. However, when the environmental concentration is low, the dilution factor can change some micropollutant concentrations from moderate to negligible environmental risk. For a full toxicological assessment, measurements of the environmental concentrations upstream and reliable estimations of the dilution factor upon discharge are required.

## 7.2 Outlook and recommendations

Even though this study is one of the first of its kind, mainly due to analysing the three redox conditions, more similar studies should be carried out. To do better analysis, the micropollutant mass balance should be closed by determining the sorped part of the micropollutants. After that, the same methodology can and should be applied to different WWTP in The Netherlands to analyse the variations in the biotransformation rates, with the proper solid-water partitioning coefficients. Also, to do a more robust analysis between technologies, the same methodology should be applied to sludges from CAS, MBR, and AGS with the same influent to properly compare the micropollutant's removal efficiency. Continuous lab experiments with similar conditions and different configurations will be of interest. With the micropollutant biotransformation rate constants and a CAS model of the WWTP design, the whole biological treatment process can be modelled, aiming to maximize the biological degradation of the micropollutants, and couple it to a possible advanced treatment to determine the overall removal of micropollutants in a future scenario.

To increase the robustness of the methodology, all experiments and sampling campaign analyses should be done with more replicates to guarantee that the obtained concentrations and biotransformation rates are as accurate as possible. Moreover, seasonal variation must be studied since temperature and flow change the water characteristics as well as the micropollutants concentrations. Besides, due to the limit of detention of the applied analytical method, a higher spike dose should be added to the batch test to guarantee that the concentration differences are associated with the degradation and not analytical errors. However, this will imply validating the method for much higher concentrations and calibration curves to avoid dilution errors. And lastly, to better quantify whether metabolism or cometabolism is the primary degradation mechanism of the compounds, an effluent after advanced treatment should be used (to dilute the sludge) to guarantee a significantly low (<5%) COD concentration compared to the influent.

# 8

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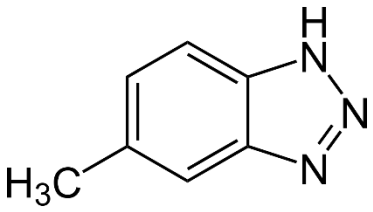
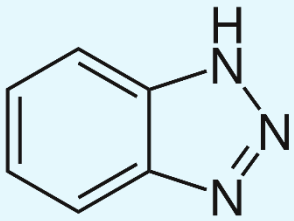
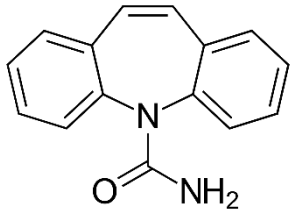
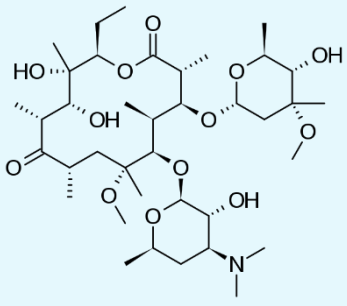
# A

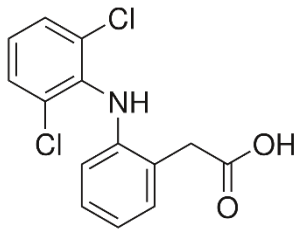
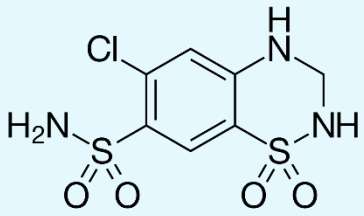
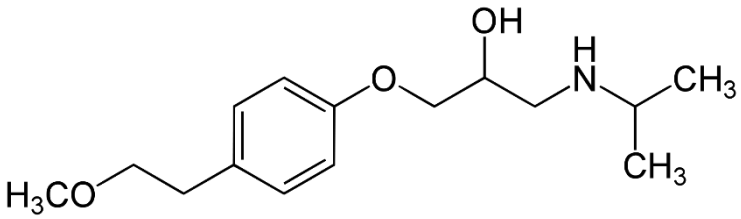
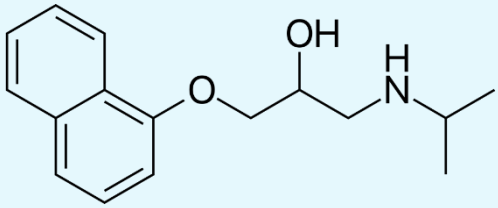
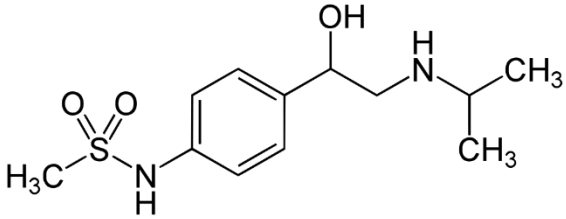
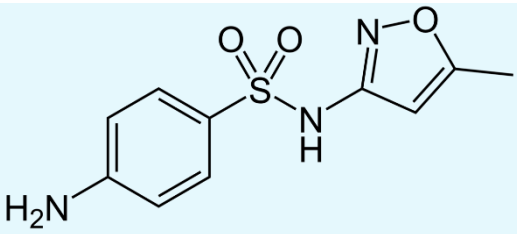
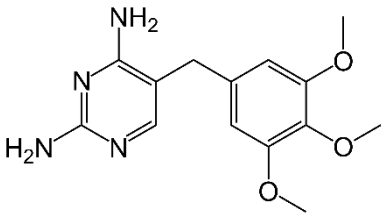
# APPENDIXES

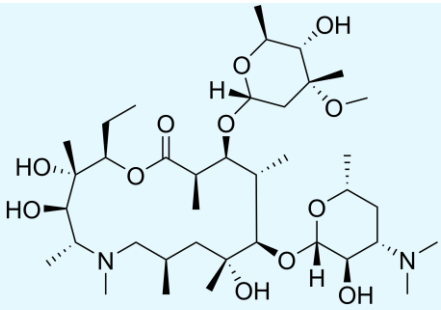
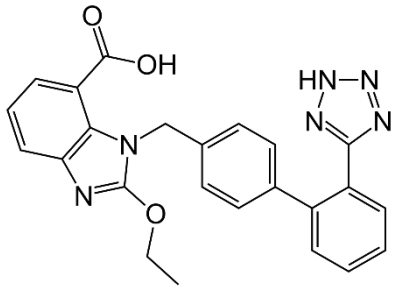
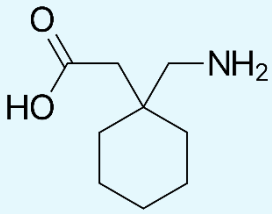
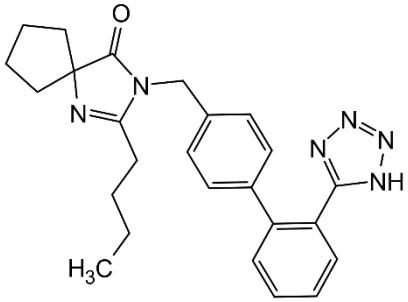
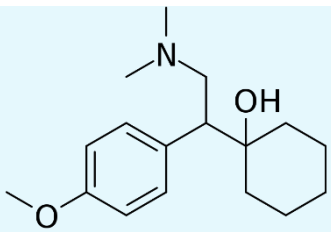
## A.1 Targeted Micropollutants Chemical Structure

The chemical structure of the targeted compounds is presented in Table A-1.

Table A-1 Chemical structure of the micropollutants

| Micropollutants | Chemical Structure |
|---|---|
| 4-, 5-Methylbenzotriazole |  |
| Benzotriazole |  |
| Carbamazepine |  |
| Clarithromycin |  |

| | |
|---|---|
| Diclofenac |  |
| Hydrochlorothiazide |  |
| Metoprolol |  |
| Propranolol |  |
| Sotalol |  |
| Sulfamethoxazole |  |
| Trimethoprim |  |

| | |
|---|---|
| Azithromycin |  |
| Candesartan |  |
| Gabapentin |  |
| Irbesartan |  |
| Venlafaxine |  |

# A.2 Spike Solution Concentrations and Correspondent Theoretical Oxygen Demand

The theoretical oxygen demand (ThOD) is the calculated oxygen needed to oxidize a compound and its final oxidation products. However, different standard methods influence the obtained results. For example, this determination can assume that the generated nitrogen from the degraded compounds is released as ammonia, while others consider the oxidation of ammonia to nitrate. To know the ThOD associated with the degradation of the compounds spiked, it was determined using the OECD methodology (Test No. 301: Ready Biodegradability Test) (OECD, 1992). According to OECD (1992) and Metcalf and Eddy (2013), the calculation of the ThOD can consider, or not, the oxygen requirements for the nitrification process, as explained before. Therefore, in this study, both equations were applied. The determinations of the ThOD are based on the hypothetical compound $C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ and are shown below (Equations A1 and A2).

$$ThOD_{Without\ nitrification} = \frac{16\left[2c + \frac{1}{2}(h - cl) + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right] mg/mg}{Molar\ Weight} \quad \text{(A1)}$$

$$ThOD_{With\ nitrification} = \frac{16\left[2c + \frac{1}{2}(h - cl - 3n) + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right] mg/mg}{Molar\ Weight} \quad \text{(A2)}$$

The obtained ThOD per compound and the total ThOD requirements for the spike solution can be observed in Table A-2, as well as the concentrations of each micropollutant in the spike solution used.

Table A-2 Organic micropollutants concentration in the stock solution and respective ThOD

| **Micropollutant** | **Concentration (mg/L)** | **ThOD (mg $O_2$/L) without nitrification** | **ThOD (mg $O_2$/L) with nitrification** |
|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 1,03 | 1,61 | 3,09 |
| Benzotriazole | 0,97 | 1,30 | 2,87 |
| Carbamazepine | 0,95 | 1,87 | 2,35 |
| Clarithromycin | 0,89 | 1,83 | 1,90 |
| Diclofenac | 0,95 | 1,63 | 1,84 |
| Hydrochlorothiazide | 0,89 | 0,78 | 1,19 |
| Metoprolol | 0,92 | 2,09 | 2,31 |
| Propranolol | 0,92 | 2,21 | 2,44 |
| Sotalol | 0,96 | 1,75 | 2,20 |
| Sulfamethoxazole | 0,91 | 1,21 | 1,90 |
| Trimethoprim | 0,88 | 1,36 | 2,13 |
| Azithromycin | 0,90 | 1,86 | 2,02 |
| Candesartan | 0,95 | 1,59 | 2,42 |
| Gabapentin | 0,97 | 2,08 | 2,45 |
| Irbesartan | 0,77 | 1,54 | 2,25 |
| Venlafaxine | 0,87 | 2,61 | 2,84 |
| | **Total** | 27,32 | 36,19 |

## A.3 Dissolved Oxygen Concentration and Percentage Correlation Curve

As previously mentioned, a correlation between the concentration and percentage of dissolved oxygen was needed. Figures A-1 and A-2 show the correlation curves, at a temperature of 18,5 ºC, between the percentage and the concentration of dissolved oxygen in each of the bioreactors used.

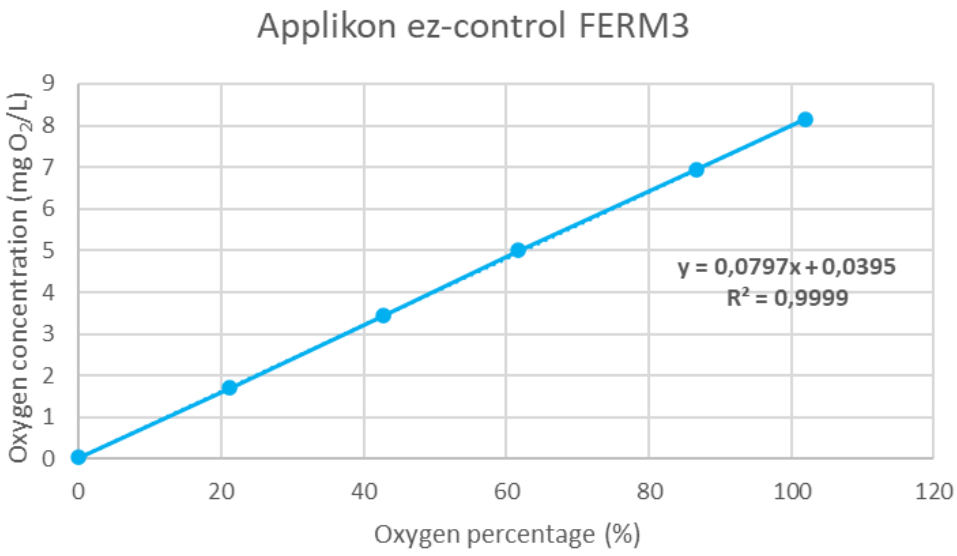



Figure A-1 Concentration and percentage of OD correlation curve of the DO of FERM3 controller

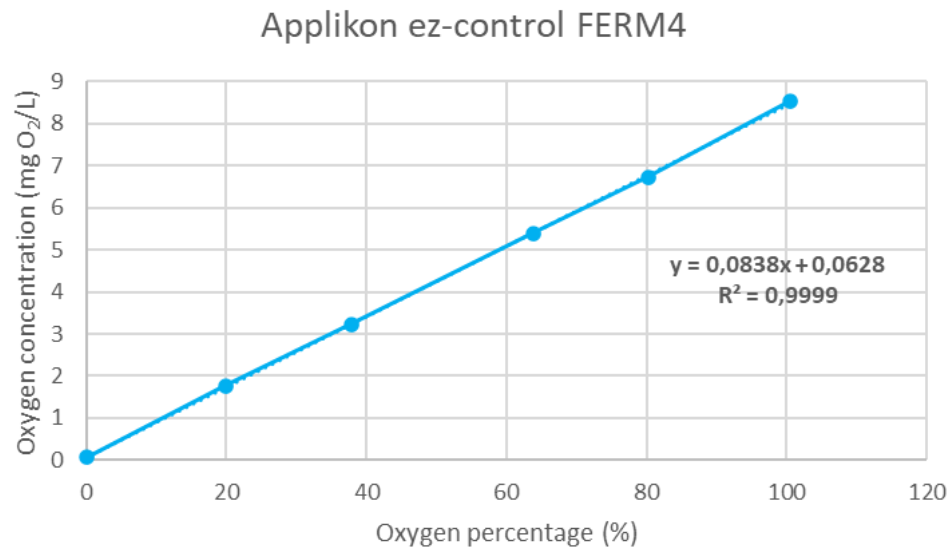



Figure A-2 Concentration and percentage of OD correlation curve of the DO of FERM3 controller

## A.4 Influent and Effluent Micropollutants Concentrations in each WWTP Sample Collection

The micropollutants concentrations in both influent and effluent were analysed three times (one per each redox condition experiment). Tables A-3, A-4 and A-5 present the concentrations in the influent and effluent, and their removal efficiencies for the three analyses.

Table A-3 Influent and effluent concentrations and respective removal efficiencies (sampling day 18/03/2022)

| Micropollutant | Influent Concentration (µg/L) | Effluent Concentration (µg/L) | Removal Efficiency (%) |
|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 0,89 | 0,89 | 0% |
| Benzotriazole | 6,10 | 3,60 | 41% |
| Carbamazepine | 0,44 | 0,44 | 0% |
| Clarithromycin | 0,11 | 0,08 | 27% |
| Diclofenac | 0,91 | 0,82 | 10% |
| Hydrochlorothiazide | 2,10 | 2,00 | 5% |
| Metoprolol | 2,00 | 1,90 | 5% |
| Propranolol | 0,02 | 0,02 | 0% |
| Sotalol | 2,10 | 1,90 | 10% |
| Sulfamethoxazole | 0,56 | 0,12 | 79% |
| Trimethoprim | 0,14 | 0,11 | 21% |
| Azithromycin | 0,09 | 0,06 | 33% |
| Candesartan | 0,30 | 0,30 | 0% |
| Gabapentin | 5,50 | 2,80 | 49% |
| Irbesartan | 0,96 | 0,84 | 13% |
| Venlafaxine | 0,35 | 0,32 | 9% |

Table A-4 Influent and effluent concentrations and respective removal efficiencies (sampling day 01/04/2022)

| Micropollutant | Influent Concentration (µg/L) | Effluent Concentration (µg/L) | Removal Efficiency (%) |
|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 1,10 | 1,10 | 0% |
| Benzotriazole | 6,20 | 5,30 | 15% |
| Carbamazepine | 0,47 | 0,36 | 23% |
| Clarithromycin | 0,09 | 0,08 | 11% |
| Diclofenac | 0,88 | 0,66 | 25% |
| Hydrochlorothiazide | 2,20 | 1,90 | 14% |
| Metoprolol | 2,00 | 1,50 | 25% |
| Propranolol | 0,03 | 0,02 | 33% |
| Sotalol | 2,00 | 1,50 | 25% |
| Sulfamethoxazole | 0,40 | 0,23 | 43% |
| Trimethoprim | 0,11 | 0,09 | 18% |
| Azithromycin | 0,11 | 0,10 | 9% |
| Candesartan | 0,39 | 0,35 | 10% |
| Gabapentin | 3,70 | 2,90 | 22% |
| Irbesartan | 0,98 | 0,76 | 22% |
| Venlafaxine | 0,33 | 0,26 | 21% |

Table A-5 Influent and effluent concentrations and respective removal efficiencies (sampling day 08/04/2022)

| Micropollutant | Influent Concentration (µg/L) | Effluent Concentration (µg/L) | Removal Efficiency (%) |
|---|---|---|---|
| 4-, 5-Methylbenzotriazole | 1,10 | | |
| Benzotriazole | 6,20 | | |
| Carbamazepine | 0,47 | | |
| Clarithromycin | 0,09 | | |
| Diclofenac | 0,88 | | |
| Hydrochlorothiazide | 2,20 | | |
| Metoprolol | 2,00 | | |
| Propranolol | 0,03 | | |
| Sotalol | 2,00 | | |
| Sulfamethoxazole | 0,40 | | |
| Trimethoprim | 0,11 | | |
| Azithromycin | 0,11 | | |
| Candesartan | 0,39 | | |
| Gabapentin | 3,70 | | |
| Irbesartan | 0,98 | | |
| Venlafaxine | 0,33 | | |

There are no values for the effluent concentrations due to a technical problem.

# A.5 Experimental Biotransformation Rates Curves, with Influent Addition

In this appendix, the experimental biotransformation rate curves, with influent addition, are presented.

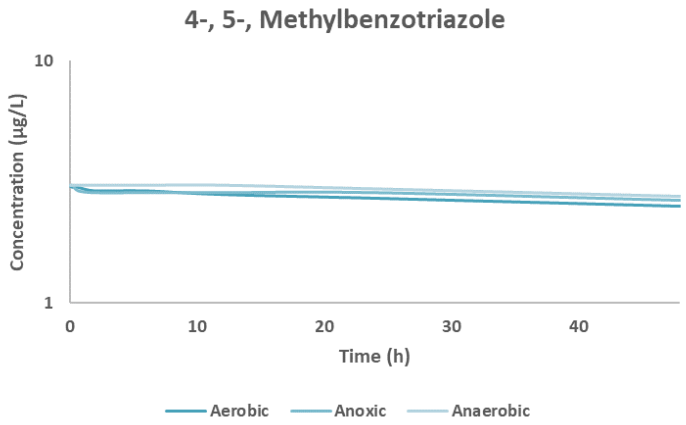



Figure A-3 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

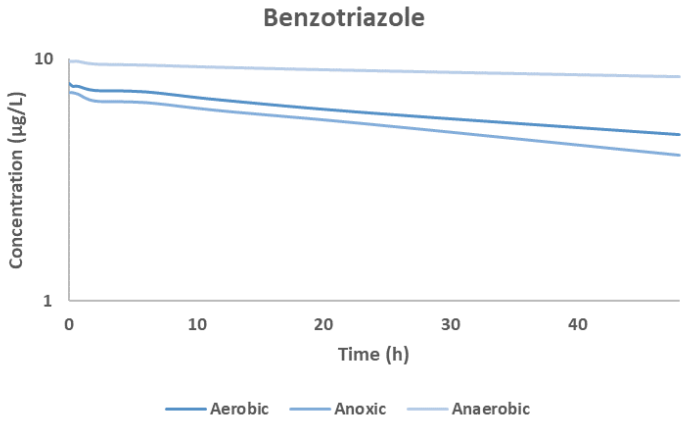



Figure A-4 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

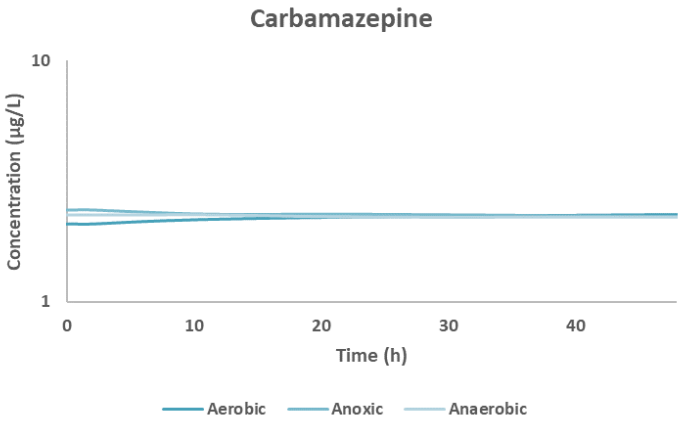



Figure A-5 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

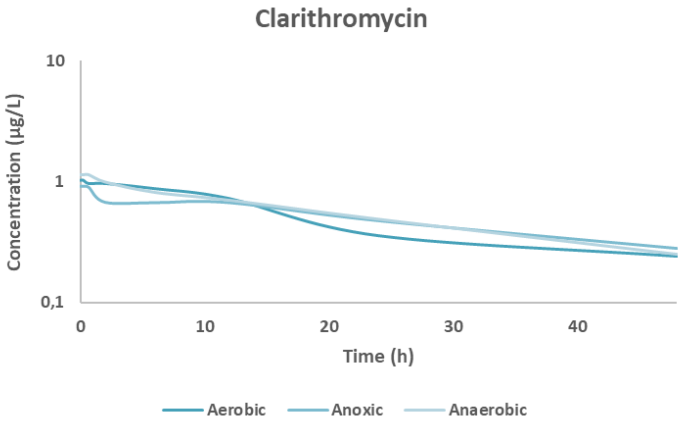



Figure A-6 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

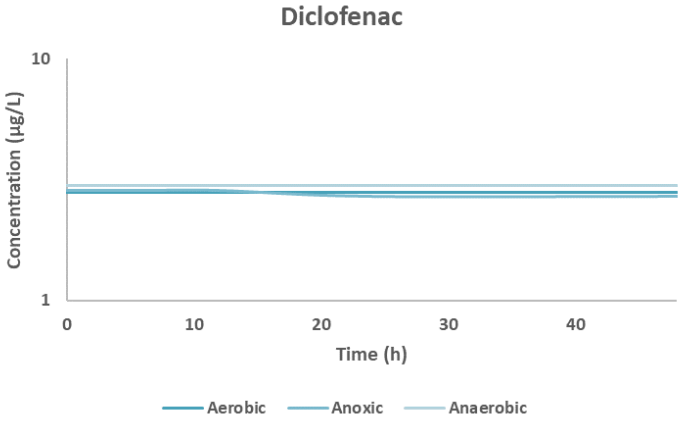



Figure A-7 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

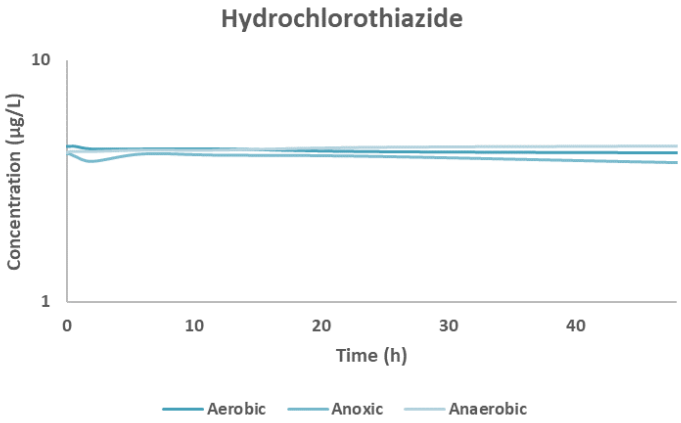



Figure A-8 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

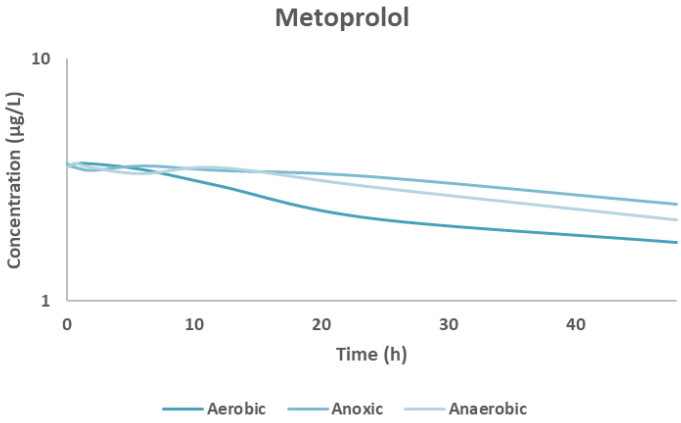



Figure A-9 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

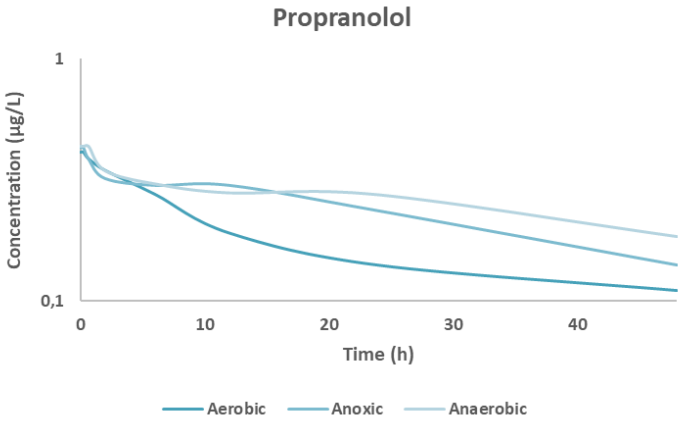



Figure A-10 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

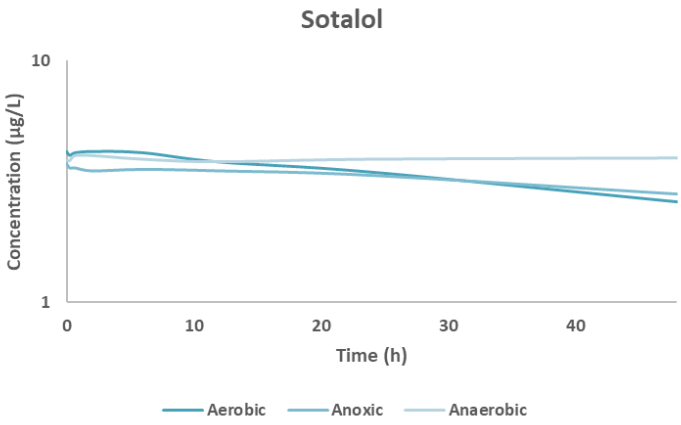



Figure A-11 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

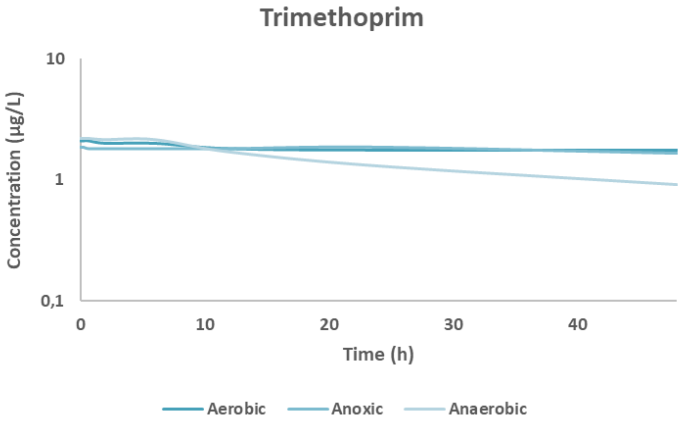



Figure A-12 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

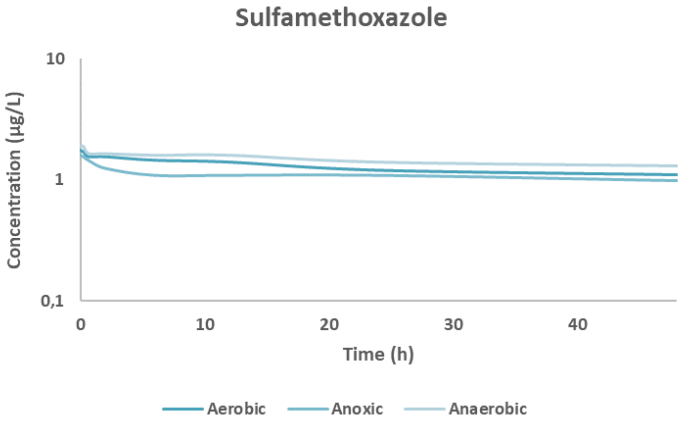



Figure A-13 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

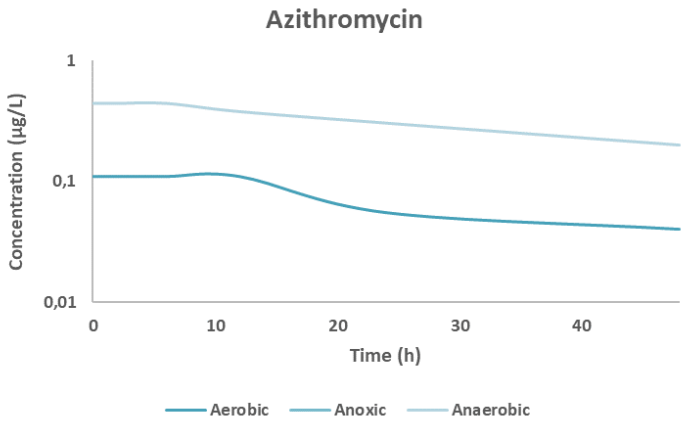



Figure A-14 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

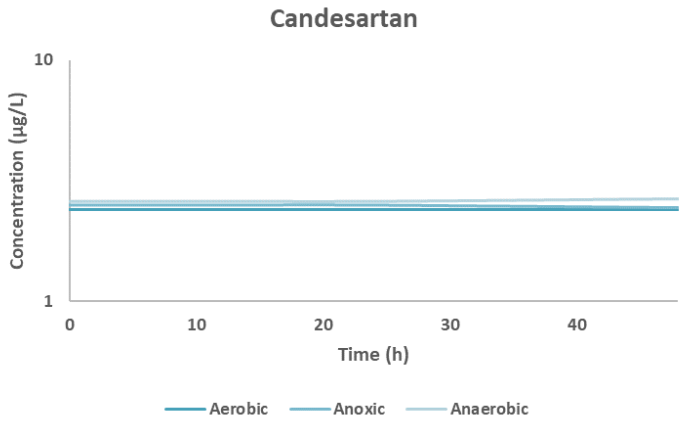



Figure A-15 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

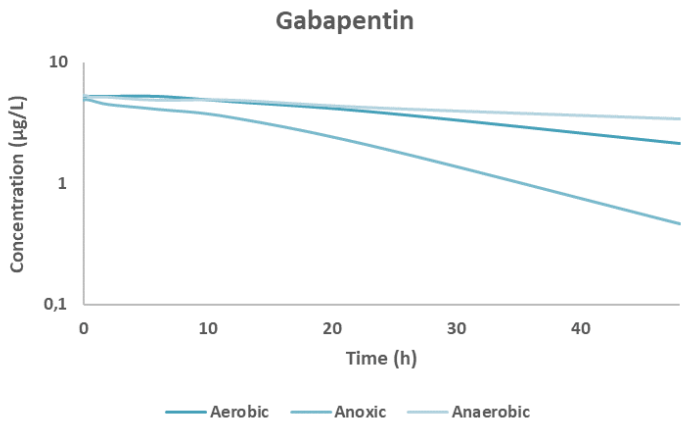



Figure A-16 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

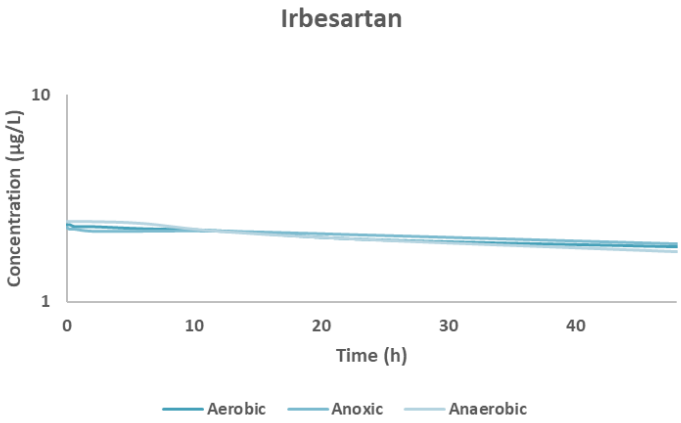



Figure A-17 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

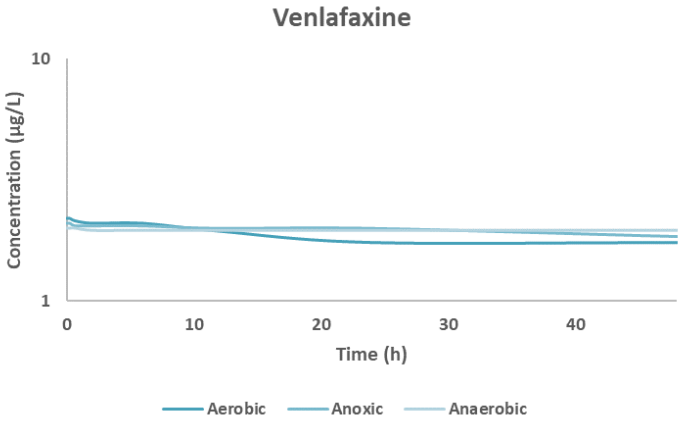



Figure A-18 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

# A.6 Square-R of the Determined Biotransformation Rate Constants

Table A-6 presents the square-R values of the obtained biotransformation rate constants.

Table A-6 Square-R values of the determined biotransformation rate constants

| Micropollutants | Aerobic | | Anoxic | | Anaerobic | |
|---|---|---|---|---|---|---|
| | Without Influent | With Influent | Without Influent | With Influent | Without Influent | With Influent |
| | $L.g_{SS}^{-1}.d^{-1}$ | | | | | |
| 4-, 5-Methylbenzotriazole | 0,821 | 0,946 | 0,850 | 0,985 | 0,931 | 0,927 |
| Benzotriazole | 0,956 | 0,990 | 0,908 | 0,993 | 0,900 | 0,996 |
| Carbamazepine | * | 0,904 | 0,716 | 0,951 | 0,794 | 0,941 |
| Clarithromycin | 0,985 | 0,950 | 0,967 | 0,903 | 0,909 | 0,996 |
| Diclofenac | * | * | 0,761 | 0,897 | * | * |
| Hydrochlorothiazide | 0,978 | 0,868 | 0,882 | 0,945 | 0,957 | 0,896 |
| Metoprolol | 0,969 | 0,965 | 0,961 | 0,928 | 0,759 | 0,943 |
| Propranolol | 0,738 | 0,874 | 0,928 | 0,953 | 0,658 | 0,954 |
| Sotalol | 0,961 | 0,974 | 0,884 | 0,927 | 0,897 | 0,847 |
| Sulfamethoxazole | 0,931 | 0,868 | 0,956 | 0,898 | 0,633 | 0,848 |
| Trimethoprim | 0,962 | 0,838 | 0,822 | 0,961 | 0,991 | 0,956 |
| Azithromycin | 0,695 | 0,889 | 0,908 | N.D. | 0,849 | 0,994 |
| Candesartan | 0,768 | 0,714 | 0,751 | 0,258 | 0,880 | 0,967 |
| Gabapentin | 0,986 | 0,965 | 0,964 | 0,981 | 0,783 | 0,965 |
| Irbesartan | 0,987 | 0,973 | 0,802 | 0,977 | 0,985 | 0,895 |
| Venlafaxine | 0,926 | 0,976 | 0,843 | 0,880 | * | 0,838 |

N.D. : Not Determined

* $K_{bio}$ = 0 $L.gSS^{-1}.d^{-1}$, meaning slope also equal to 0, therefore impossible to determine the square-R.

# A.7 Advanced Oxidation Processes

The advanced oxidation processes (AOP) should be seen as a posttreatment after the biological degradation of the micropollutants. There is nowadays a multitude of different technologies considered AOP. The most used and known are activated carbon (granular and powder), Fenton-based processes, ozone $O_3$-based processes, hydrogen peroxide $H_2O_2$-based processes, photocatalysis, and catalytic wet peroxide oxidation, among others. These processes are also coupled in series to maximize oxidation (Sillanpaa, 2020). The application of AOPs can create subproducts, usually called oxidation byproducts (OBP) (Ike *et al.*, 2019). This OBP are generally formed in the presence of high organic matter concentrations due to the presence of halogenated compounds like iodine, bromine, and chlorine (Jasper *et al.*, 2017), which can form trihalomethanes that are usually related to cancer, reproductive problems and congenital disabilities (Medeiros *et al.*, 2019).

Miklos *et al.* (2018) have categorized different AOP faces to their energy consumption. Processes with less than 1kWh/$m^3$, like $O_3$, $O_3/H_2O_2$, $O_3$/UV, UV/$H_2O_2$, UV/persulfate, and UV/chlorine; Processes that need between 1 and 100 kWh/$m^3$, like photo-Fenton, plasma, and electrolytic AOPs; and processes that require more than 100 kWh/$m^3$, like photocatalytic-based AOPs, ultrasound, and microwave-based AOPs. As expected, the most used technologies are the ones that have less energy consumption since this factor influence the price of the technology, mainly due to the actual global energy crisis (Singh, 2021). Since these technologies are energy-dependent, their price is variable, depending on the country and the energy production type. To reduce these as much as possible, these costs sometimes pre-treatments are applied before the advanced oxidation process to minimize the oxidant dose and improve the process efficiency (Hofman-Caris *et al.*, 2016).

It has also studied the use of reagents like permanganate (Yang *et al.*, 2018) and peracetic acid (Mauricio *et al.*, 2020) to oxidize micropollutants due to their less probability of creating toxic oxidation byproducts. Peracetic acid-based wastewater treatments have been showing good EDCs removals by oxidation. Yet, their application increases the COD of the wastewater to high levels, not complying with the discharge limits (Leite *et al.*, 2021). Due to its intense colour, permanganate is also not commonly used as an oxidant in wastewater treatment. However, it is used in water supply treatment due to its oxidant power, followed by activated carbon (Dugan *et al.*, 2018).

All the technologies have advantages and points of concern, since energy consumption, price, and technical needs. This chapter will only be approached the technologies considered applicable in a real WWTP of the Walcheren size. Therefore, the processes like Fenton, photo-Fenton, ultrasound, or microwave oxidation will not be approached.

Ion exchange (IEX), which is sometimes used as a pre-treatment for AOPs, allows the effective removal of organic matter and an efficiency increase in the oxidation process, as mentioned previously. However, usually, to regenerate the resin chemicals like sodium chlorine are needed. This regeneration process produces a concentrate rich in salts, humic acids, and micropollutants (Guida *et al.*, 2021; Huang *et al.*, 2020).

The activated carbon process, on the other way, allows good retention of micropollutants and toxic compounds. The activated carbon after saturation needs regeneration, which leads to high energy demands due to the thermal process. Yet, an advantage of granular activated carbon (GAC) and powder activated carbon (PAC) is that they do not create any form of products or by-products, which means no other harmful compounds are added to the effluent. On the contrary, it adsorbs them, removing any other existing compounds (Hörsing *et al.*, 2022)

The UV/$H_2O_2$ coupled has shown to be highly effective for converting several contaminants. Still, the UV process has high energy demands (0,4 kWh/$m^3$) and constant maintenance (Hofman-Caris *et al.*, 2016). The $O_3$/$H_2O_2$ coupled has also shown to be highly effective for the conversion of several contaminants, and since it does not have UV, it has a low energy demand (0,05 kWh/$m^3$). Besides, both processes produce oxidation by-products that, as mentioned, may be harmful and need to be removed through filtration. Both approaches use $H_2O_2$ which needs to be stored and dosed. Because of the use of ozone, the latter also requires a separate room for ozone generation from oxygen, which needs to be bought. In this case, the energy used is to convert oxygen into ozone. The last one is UV/$O_3$ coupled, a mix of technologies that have been already described. It has also shown highly effective for the conversion of several contaminants yet has high demand energy for UV light, can also create oxidation by-products, needs ozone production and therefore a separate room for oxygen storage and production, and power to transform $O_2$ into $O_3$.

The price associated with the application of the different AOP technologies depends on the country, and even region in the country, due to the different environmental and water quality composition, access/costs of chemical reagents, and energy costs, among others. Besides, the price also depends on the size of the WWTP. In the case of this study, Walcheren WWTP, located in The Netherlands, is considered a medium-size WWTP (178 000 p.e.).

STOWA quantified different advanced oxidation process costs per population equivalent and concluded that in The Netherlands, an advanced oxidation process can go from 5€/(p.e . year) to 34€/(p.e . year), depending on the process complexity (EurEau, 2019). This study also concludes that the price of these advanced technologies is indirectly proportional to the population equivalent treated by the WWTP, no matter the chosen one (EurEau, 2019), meaning that a larger WWTP will have less economic burden per cubic meter or population equivalent.

Under the NEPTUNE project, O3/sand filtration and PAC/sand filtration costs were studied and compared for different WWTP sizes (EurEau, 2019; Siegrist *et al.*, 2021). Even though powder-activated carbon (PAC) has fewer electricity needs, it requires much energy for the regeneration process (0,5 to 0,8 kWh/$m^3$) (EurEau, 2019; Siegrist *et al.*, 2021). The estimated cost of the mentioned AOP for a WWTP like Walcheren is around 0,20 €/$m^3$ for the PAC and 0,12 €/$m^3$ for the ozonation processes. However, the ozonation price may vary in agreement with the dose used. Hofman-Caris *et al.* (2016) mentioned the costs reported by PureBlue Water that go from 0,03€/$m^3$ to 0,16€/$m^3$, according to the ozone dose, respectively, 12,5 mg $O_3$/L and 62,4 mg $O_3$/L. Usually, an ozone dose of 31 mg $O_3$/L is enough to remove the pharmaceutical compounds, which has an associated cost of 0,08 €/$m^3$. Besides the reagents needed, it also needs to account for the technical needs for the treatment to work correctly (e.g., pump and pumping reservoir), which will have an associated cost of more or less 0,04 €/$m^3$ (Hofman-Caris *et al.*, 2016). Recently a new bromate norm of < 1 µg/l) has been issued by the Dutch Government for discharge of surface water, i.e., for ozonation of municipal wastewater (Knol *et al.*, 2015). In comparison to, for example, the regulations in Germany (< 10 µg/l), this is much lower. Bromate is an oxidation product of bromide that can be formed by ozone under certain conditions. The timing of this new regulation is precarious since it may directly affect the technology selection for micropollutants removal (Nijhuis, 2022).

The EurEau (2019) report mentioned a Finnish study where the $O_3$/GAC system costs were analysed. For a WWTP with more than 100 000 p.e., this system can cost between 0,40 €/$m^3$ and 0,60 €/$m^3$. The coupling of the GAC after the ozonation process is to adsorb the oxidation byproducts that may cause harm to the ecosystem. This is so far the most promising and high technology readiness level combination of technology for micropollutant removal at the moment.

According to Mousset *et al.* (2021), the Fenton-based processes are more cost-effective and have also proven to be competitive in micropollutant transformation compared with ozonation or UV/$H_2O_2$. However, this analysis was made in a lab environment and therefore needed to be applied to pilots and real-scale WWTP to verify the results.

The discharged effluent of a WWTP contains many micropollutants, as observed previously. These compounds may be harmful to ecosystems and public health. Biological treatments are not enough to remove these compounds, as has been proved in the previous chapters. Therefore, the biological treatment should be coupled with other processes, like AOP, to transform the compounds as much as possible and reduce their toxicity. Kienle *et al.* (2022) observed that post-treatment could reduce the toxicity to half the one obtained after the secondary treatment (biological reactor plus secondary settler).

Thus far, also accordingly to Kienle *et al.* (2022), a study realized at WWTP Neugut showed that ozonisation produced mutagenic compounds. However, those were removed by the fixed and moving bed and granular activated carbon. These results support the previous study by Volker *et al.* (2019) that showed mutagenicity after ozonisation and others post-treatment. Besides that, results show that ozonisation as an advanced post-treatment significantly reduces the general ecotoxicity of the effluent.

Other studies (Magdeburg *et al.*, 2014; Mestankova *et al.*, 2014; Schindler Wildhaber *et al.*, 2015) showed that the mutagenicity of the ozonated wastewater is dependent on its type and that ozonated wastewater with strong industrial wastewater influence has higher mutagenicity than domestic ones, which could be explained by the presence of organochlorine compounds like trihalomethanes. Mestankova *et al.* (2014) and Magdeburg *et al.* (2014) also showed that other post-treatments coupled with ozonisation reduce the mutagenicity effects.

The UV genotoxicity has also been studied (Hofman-Caris *et al.*, 2013), and have been found that doses until 70 mJ/cm$^2$ do not show any significant positive response in the Ames Fluctuation Assay, no matter the water type treated or the UV-lamp (LP or MP). Still, if a substantial UV dose increases, the Ames Fluctuation Assay also shows an increase in its positive response. The $H_2O_2$ addition gives a lower response, indicating that the mutagenic by-products are formed during photolysis and not the oxidation process itself. Using an LP UV lamp showed a decrease in the mutagenicity, supporting the thesis that photolysis is the process responsible for the formation of mutagenic by-products.

As observed in this chapter, there is significant availability of different AOP processes; however, not all of them have reached the technological maturity to be implemented at full-scale as AOP, but sometimes are used as disinfection processes. Ozonation has been applied in most cases due to its efficiency and practicality. Siegrist *et al.* (2021) observed that beta-blockers (e.g., metoprolol, propranolol, and sotalol) showed high reactivity towards ozone, while 4,-5-methylbenzotriazole has not shown high removal. Ozone can selectively oxidize and remove estrogens and other toxic micropollutants, yet chlorine dioxide is better for pharmaceutical oxidation. However, the latter has been associated with a higher formation of oxidation byproducts (Hörsing *et al.*, 2022). To absorb these compounds, activated carbon should be used as a follow-up step. However, the more oxidation byproducts produced, the more activated carbon needs to be dosed (PAC), or in the case of using GAC, it will need to be regenerated more often.

The PAC efficiency decreases with the dissolved organic carbon (DOC) increase. This removal is also needed to guarantee a better biological removal process and minimize the costs of the AOP. Siegrist *et al.* (2021) observed that 10 mg/L of PAC is enough to remove up to 90% of the pharmaceuticals in the effluent. However, this process also leads to increased sludge production (up to 10%) that will need

to be treated, which means more sludge management costs. Both PAC and GAC are the most efficient sorbent. However, PAC has a particular efficiency increase for refractory non-biodegradable compounds (Hörsing *et al.*, 2022). For example, carbamazepine and metoprolol can be easily removed with a dose of 1 g PAC/$m^3$. However, diclofenac may need around 15 g PAC/$m^3$, according to studies made with Swedish effluent (Hörsing *et al.*, 2022).

Ozone coupled with biofiltration is a good pre-treatment for hydrophobic compounds, but Ion exchange (IEX) has received lots of attention lately. Studies have shown that an IEX pre-treatment with UV/$H_2O_2$ technology is a robust wastewater treatment. However, the IEX disadvantage is with the concentrate after regeneration (Hofman-Caris *et al.*, 2016).

Because the Walcheren WWTP effluent does not have any particular big problem (see Table 5-3), a simple pre-treatment as a biofilter will be proposed, to reduce the costs. Based on the current state of the art, and in order not to increase the toxicity of the effluent with oxidation byproducts, the solution proposed is ozone followed by granular activated carbon $O_3$/GAC. This technology has been applied all around The Netherlands and will allow the oxidation of the compounds throughout the ozone, and the GAC will adsorb any possible byproduct originating in the oxidation. Kienle *et al.* (2022) also observed that this technology coupling achieved the highest decrease of micropollutants and ecotoxicity in the treated effluent.

Therefore, the technology implementation cost (CAPEX and OPEX) was roughly estimated. This estimation was made for the actual scenario (the year 2022) and as a forecast for 2035 and 2050. This calculation was conducted using the range of values mentioned previously. To be conservative, an average of 0,30 €/$m^3$ was used, assuming already the accounting of the initial investment (CAPEX) needed (around 5 M€) (EurEau, 2019; Hofman-Caris *et al.*, 2016). Table A-7 shows the yearly estimation costs for the post-treatment of the Walcheren WWTP.

Table A-7 Advanced oxidation process rough cost estimation in 2022, 2035 and 2050

| | Year 2022 | Year 2035 | Year 2050 |
|---|---|---|---|
| Estimated Population (p.e.) | 144 000 | 161 800 | 185 100 |
| Average flow ($m^3$/h) | 113 | 126 | 145 |
| Average flow ($m^3$/d) | 2 700 | 3 030 | 3 470 |
| Average flow ($m^3$/y) | 985 500 | 1 107 240 | 1 266 500 |
| Annual AOP Cost (€) | 295 650 | 332 170 | 379 950 |
| Updated annual AOP Cost (€) | 295 650 | 226 190 | 166 100 |

The estimated costs in Table A-7 corresponded to the operational costs per year. They were determined assuming that the population will increase around 1%/year and that the wastewater production per person is the same during the considered time, even with the population increase. The annual cost was also updated based on a 3% update rate (WorldData, 2022). This calculation indicates that implementing AOP for micropollutants removal costs in 2022 is about 300 000€, in 2035, around 230 000€ in 2050 around 170 000 €. Moreover, this AOP operational cost is estimated to be about 7,5 M€ by 2050. Determining the price per person, this technology costs around 2€ per person and per year, staying below the STOWA suggested range of values (EurEau, 2019). Even though a substantial investment is needed, this technology will guarantee the removal of around 90% of the micropollutants,

which means a decrease in ecological toxicity issues and also mitigation of future public health problems. This was more explored in Chapter 6.

# A.8 Obtained Theoretical Biotransformation Rates

The biotransformation rate was plotted through time based on and the obtained biotransformation rate constants. Figures A-19 to A-34 present the expected biotransformation rate per redox condition for the sixteen targeted compounds. For that, the starting concentration used is the average influent concentration (See Table 5-3).

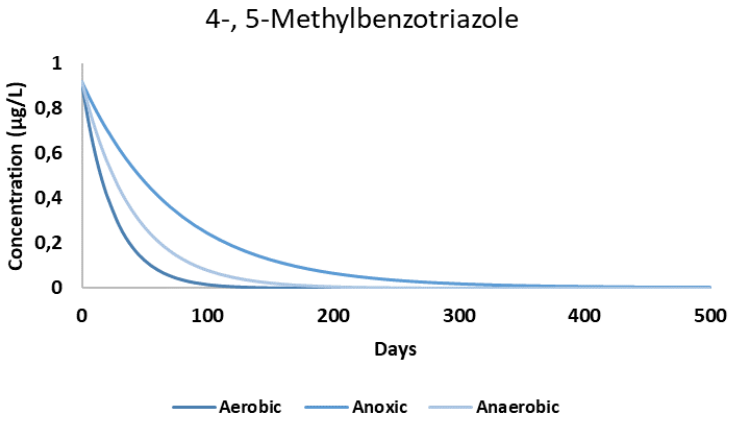



Figure A-19 Theoretical biotransformation rates, under the different redox conditions (4-,5-Methylbenzotriazole)

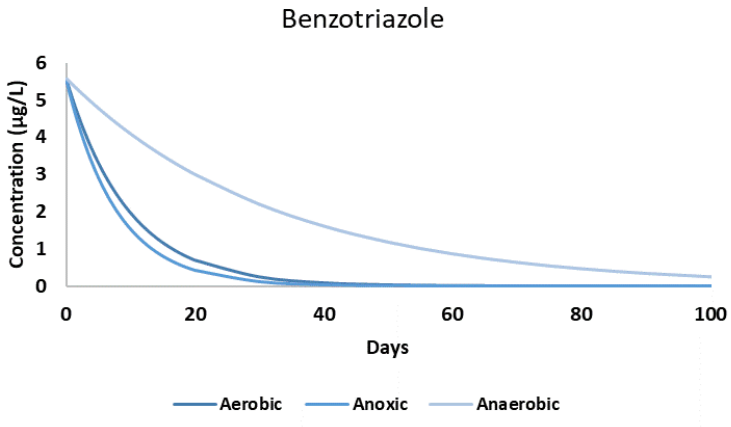



Figure A-20 Theoretical biotransformation rates, under the different redox conditions (Benzotriazole)

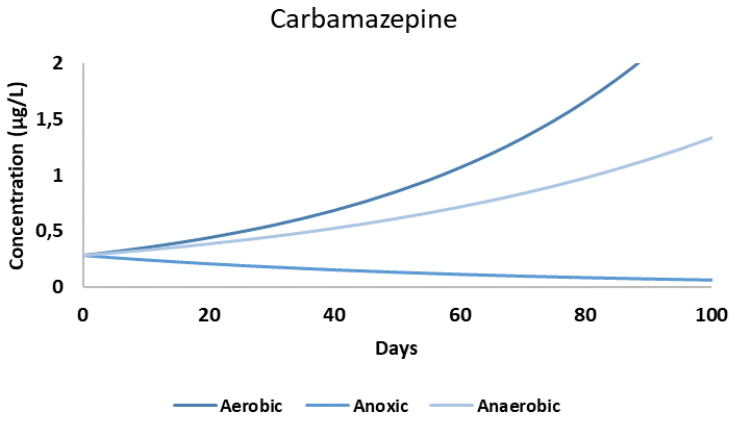



Figure A-21 Theoretical biotransformation rates, under the different redox conditions (Carbamazepine)

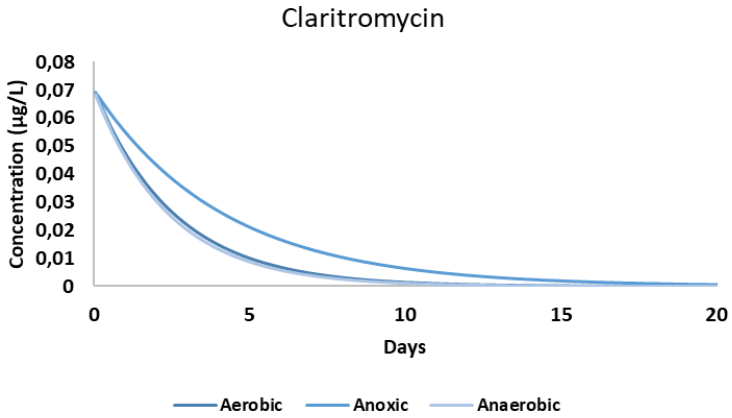



Figure A-22 Theoretical biotransformation rates, under the different redox conditions (Clarithromycin)

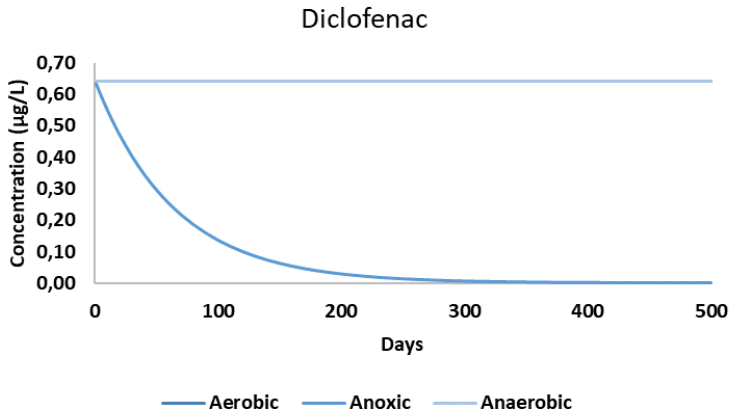



Figure A-23 Theoretical biotransformation rates, under the different redox conditions (Diclofenac)

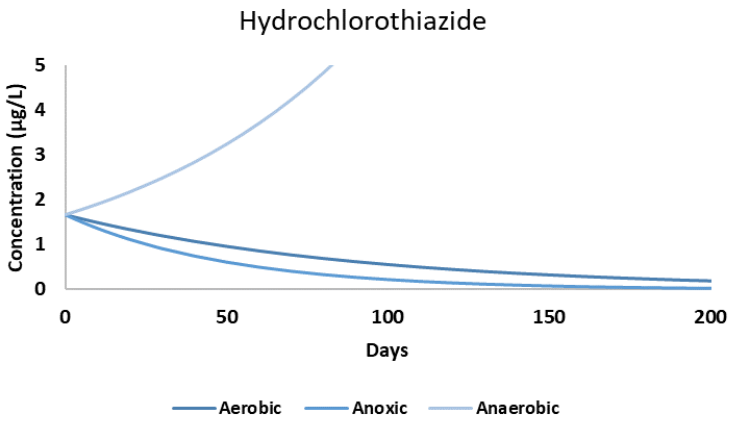



Figure A-24 Theoretical biotransformation rates, under the different redox conditions (Hydrochlorothiazide)

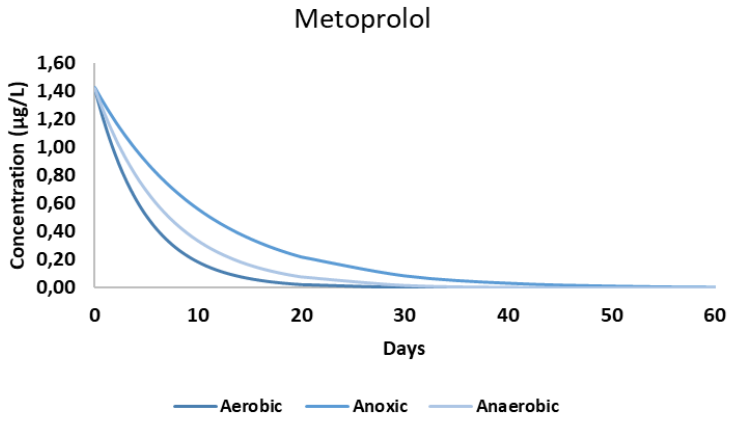



Figure A-25 Theoretical biotransformation rates, under the different redox conditions (Metoprolol)

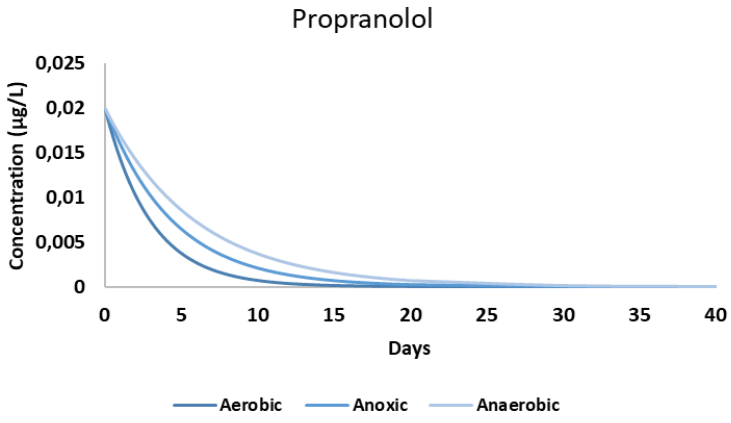



Figure A-26 Theoretical biotransformation rates, under the different redox conditions (Propranolol)

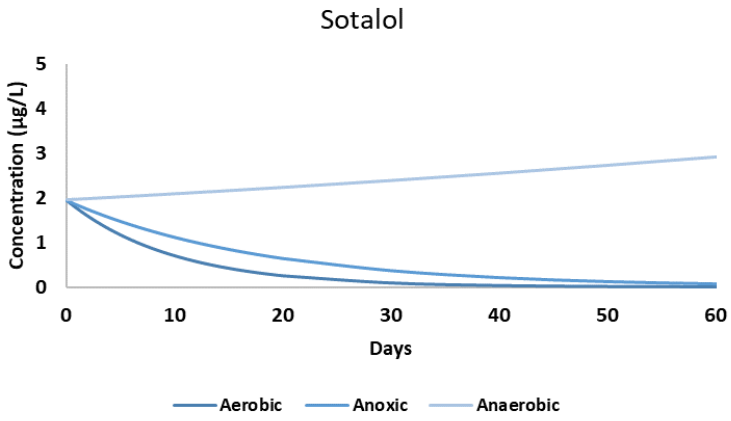



Figure A-27 Theoretical biotransformation rates, under the different redox conditions (Sotalol)

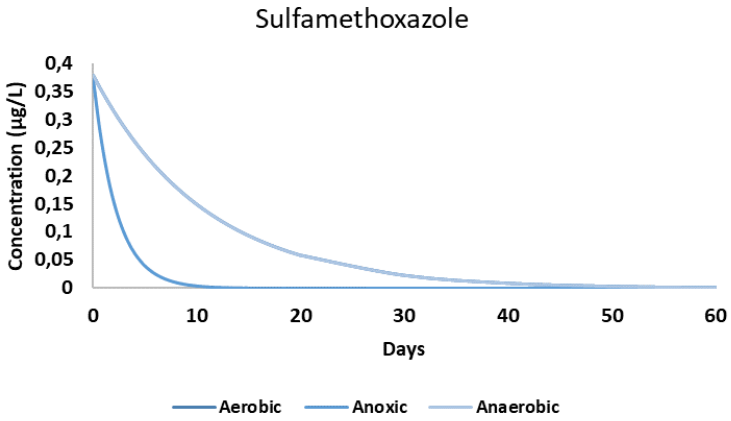



Figure A-28 Theoretical biotransformation rates, under the different redox conditions (Sulfamethoxazole)

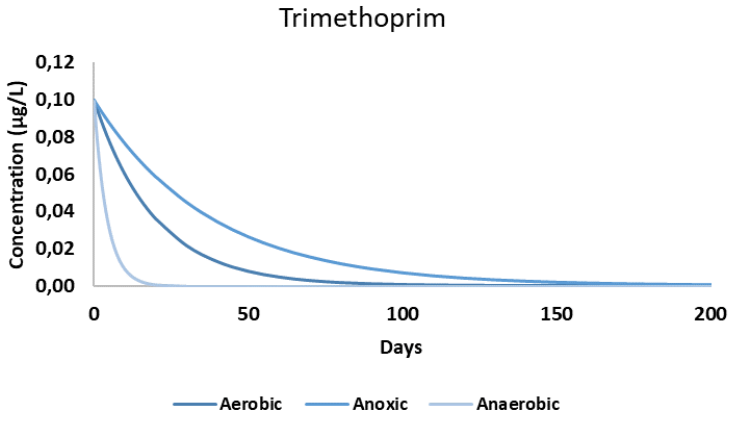



Figure A-29 Theoretical biotransformation rates, under the different redox conditions (Trimethoprim)

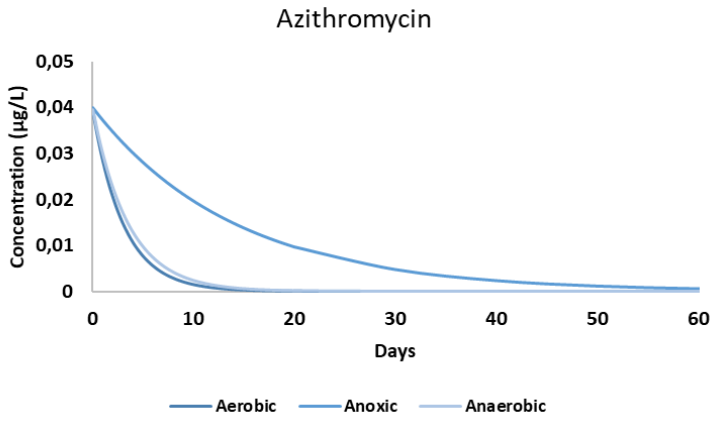



Figure A-30 Theoretical biotransformation rates, under the different redox conditions (Azithromycin)

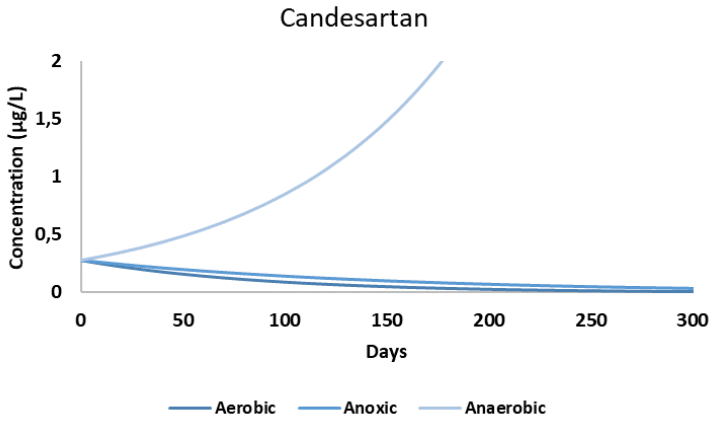



Figure A-31 Theoretical biotransformation rates, under the different redox conditions (Candesartan)

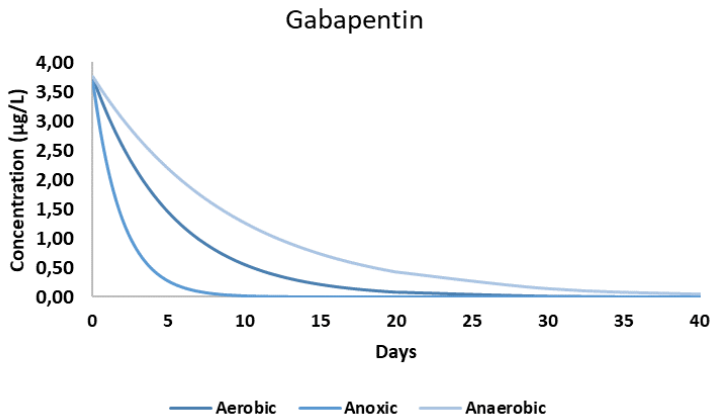



Figure A-32 Theoretical biotransformation rates, under the different redox conditions (Gabapentin)

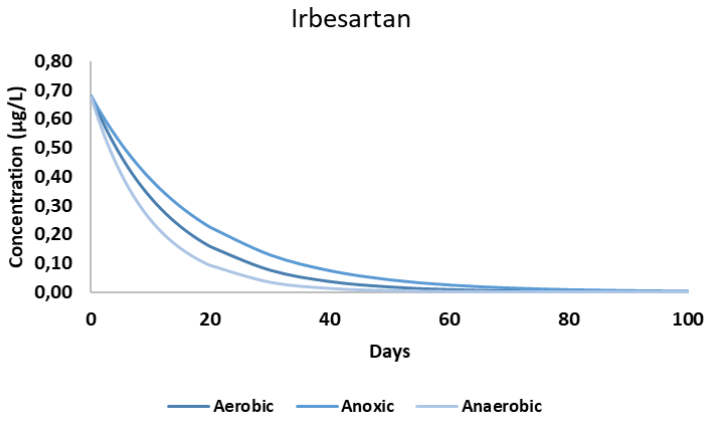



Figure A-33 Theoretical biotransformation rates, under the different redox conditions (Irbesartan)

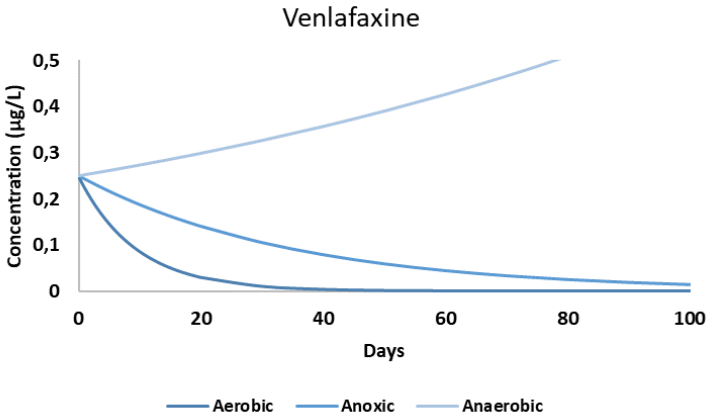



Figure A-34 Theoretical biotransformation rates, under the different redox conditions (Venlafaxine)

With the Support of:

KWR