Ministry of Environment of Denmark
Environmental Protection Agency

MerEFF - Environmental treatment of wastewater effluents

MiljøEffektiv Rensning af afløb fra renseanlægs EFFluenter

MUDP Report

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Preface

The aim of the MerEFF project was to develop an environmentally sustainable technology based on a strict biological approach in a semi-technical scale at Herning Water. The technology concept developed and tested at a large pilot scale, is based on the moving bed biofilm reactor principle, where it is possible to select slow-growing bacteria capable of degrading organic compounds present in effluent waters. For wastewater effluents, the organic content is low and consists primarily of medium to hardly degradable compounds, including pharmaceuticals. Based on the MBBR principle and extensive knowledge on operational regimes favouring pharmaceutical-degrading microbial consortia, the eXeno™ technology from Veolia Water Technologies was developed further and optimized for the removal of micropollutants.

Moving bed biofilm reactor (MBBR) is a biofilm-based technology where biofilms grow on specifically designed plastic carriers. MBBR systems allow for a long retention time of biomass. A hybrid solution combining biofilms growing on carriers and activated sludge (HYBAS™) is preferred when an improvement of nitrification performance is needed in municipal WWTPs. Previous studies performed on Swedish WWTPs showed that MBBR can enhance the removal of a number of pharmaceuticals compared to activated sludge (Falås et al., 2012). The increased removal efficiency of biofilm system (both as pure MBBR or hybrid solution, HYBAS™) compared to conventional activated sludge (CAS) has been associated with the increased presence of slow growing bacteria and higher biodiversity in biofilm systems (Torresi et al., 2016).

MBBR and HYBAS™ are both mature technologies used worldwide for main biological treatment and as tertiary treatment of nitrogen. While the benefit of carbon and nitrogen removal is widely recognized, little is known about their potential regarding the removal of micropollutants, also in comparison to more conventional biological systems. Full-scale sampling campaigns have been performed at several plants in Europe, where MBBR or HYBAS plants are operated in parallel to CAS systems. Through a combination of batch experiments of targeted compounds and continuous-flow sampling, it has been documented that biofilm carriers (in both pure MBBR or HYBAS) improved the degradation of more than 70% of the targeted micropollutant compounds, compared to the CAS line (Torresi in prep). This demonstrated the importance of further investigation of the capacity of micropollutant removal of biofilm systems, not only in the main line but also as polishing technology for additional removal of micropollutants in existing plants.

Following the investigation of existing WWTPs with biofilm systems, three projects were funded to study the potential of MBBR as eXeno™ technology for polishing effluent wastewater from municipal WWTP: Mermiss (Danish EPA project 2014), Bonus CLEANWATER (funded jointly by the EU and Innovation Fund Denmark, Sweden’s innovation agency VINNOVA and the German Ministry for Education and Science (BMBF) 2017), and Hepwat (Danish EPA project 2016).

The three projects were based on extensive laboratory and small pilot-scale investigations (up to 1 m^3) to remove micropollutants from effluent wastewater from municipal plants and hospital wastewater based on MBBR technology combined with additional tertiary steps such as ozonation. The results (briefly described in the Introduction section) showed the potential of MBBR in treating a wide range of micropollutants, and within the three projects the eXeno™ technology was explored under different operational conditions to optimize the removal efficiencies of targeted substances. While most of the operational conditions of eXeno™ technology were narrowed down during the three research projects, the MerEFF project was funded in order to operate eXeno™ in long-term continuous operation at a larger scale to assess the full potential of the technology.

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Glossary

AMK	recommended maximun concentration (Anbefalede maksimale Koncentration)
API	active pharmaceutical ingredients
AUH	Aarhus University Hospital
CAPEX	capital expenditure
CAS	conventional activated sludge
COD	chemical oxygen demand
DNV Gødstrup	New super hospital in western Denmark (Det nye hospital i Vest, Gødstrup)
EPJ	electronic patient journal in the danish health care system
eXeno^{TM}	effektiv, driftsøkonomisk og miljøvenlig teknologi baseret på AnoxKaldnes™ MBBR (Krüger Veolia)
FEAST	reactor inlet water comes from the primary clarifier (high carbon source)
GAC	granular activated carbon
HYBAS^{TM}	hybrid solution combining biofilms growing on carriers and activated sludge (Veolia Water Technologies)
KL	Association of Local Governments in Denmark (Kommunernes Landsforening)
MBBR	Moving Bed Biofilm Reactor
MEDSTAT	database from the Danish Health Authority with statistics on the total sales of medicines in Denmark since 1996
MUDP	The Danish Environment and Food Industry's Environmental Technology Development and Demonstration Program
NOVANA	Danish national surveillance programme for water environment and nature, The Danish Environmental Protection Agency
OPEX	operating expense
PAC	powdered activated carbon
PNEC	predicted no effect concentration
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances, a regulation from the European Comission
STARVE	inlet water for the reactor originates from the secondary clarification with very little carbon available
WFD	European Water Framework Directive
WWTP	Wastewater treatment plant

1. Introduction

1.1 Background and motivation for the MerEFF project

The increasing attention to the discharge of unwanted micropollutants from wastewater treatments plants such as pharmaceuticals pose an unwanted threat to the receiving recipients. It has been documented that wastewater treatment plants (WWTPs) based on conventional activated sludge (CAS) are not able to remove micropollutants completely, which causes a continuous discharge of micropollutants present in the wastewater. There is a need for mapping the consumption of the micropollutants, such as pharmaceuticals, and where they are discharged, e.g., point sources as hospitals or diffuse sources as private homes (all wastewater treatment plants). In addition to this, there is a need for technology development to design the most environmental and cost-effective solution for the removal of these unwanted micropollutants.

In Denmark, the consumption of pharmaceuticals has been mapped since 1996[1] in a publicly available database, and the consumption is differentiated between use in private homes or at hospitals. One of the earlier findings revealed that approx. 1-4% of the entire consumption of pharmaceuticals took place at the hospital; the remaining part was consumed in the private sector and thus discharged to municipal wastewater treatment plants (Mose-Pedersen, 2007). Based on these mappings, hospitals were identified as point source polluters due to the discharge of pharmaceuticals in wastewater, and therefore hospitals are proposed to be regulated in line with industries. A task force under the association of Local Governments Denmark (KL) proposed a list of guiding limit values for selected pharmaceuticals, according to their toxic effect on bacteria, algae, crustacean, and fish etc., and since 2015, the list has expanded to include more than 40 pharmaceuticals in total (AMK, 2015). Current regulations for discharge from Danish municipal wastewater treatment plants (WWTPs) to the environment do not include pharmaceuticals, but it is anticipated that with the construction of the new hospitals the pharmaceuticals regulation is expected to be created. The municipalities are guided by the Danish Environmental Protection Agency but decide as the competent authorities if hospitals are regarded as a point source with requirement of onsite wastewater treatment, or if the wastewater is considered as household wastewater and therefore can be handled at central WWTP facilities (Danish Environmental Protection Agency, 2019). The decision should be based on actual measurements on types of compounds and their concentrations in wastewater.

However, there are no recommendations on which compounds must be measured nor their frequency. In addition, other European countries measure different types of pharmaceuticals of concern, so it is difficult to make general screenings and conclusions on the impact of pharmaceuticals. Therefore, in the MerEFF project several lists with pharmaceuticals of environmental concern from different countries were compared, and a small comprehensive list was used for evaluating the eXeno™ technology development, see section 6.

A theoretical mapping of pharmaceuticals of environmental concern ascribed to consumption at the Aarhus University Hospital (AUH) was compared to the actual consumption by patients (noted in electronic journals), revealed that majority of these pharmaceuticals were, in fact, handed out at the hospital to patients in ambulant treatment rather than being consumed by hospitalized patients (Møller, T, 2014). However, it was unclear whether this finding was connected to only this hospital or whether this represented a trend. Therefore, more hospitals were examined in this project applying the same methodology. Furthermore, a comprehensive

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[1] https://medstat.dk/en

monitoring of pharmaceuticals in inlet and effluent samples from different wastewater treatment plants was carried out to clarify further if any common findings could be detected, see section 6.

Today, the municipalities in Denmark are in charge to regulate the hospitals effluent, but there are still discussions going on, how to address this effluent type. However, hospitals under construction are expected to identify solutions for reduction of the pharmaceutical load to the environment in Denmark, which has raised the question of decentralized or centralized treatment solutions. Decentralized solutions exist (Grundfos BioBooster 2016), but in most cases, a centralized solution seems to ensure the largest reduction of micropollutants for approx. the same costs. Many Danish R&D projects have aimed to develop new solutions or optimize conventional solutions specifically for the removal of micropollutants with a focus on pharmaceuticals. The eXeno™ technology, the Veolia solution based on Moving Bed Biofilm Technology (MBBR), has been developed as a strict biological approach for the removal of micropollutants, but also approaches using ozonation alone or in combination with activated carbon have been developed (e.g., Brædstrup, and Kalundborg WWTP). The conventional approach (generally ozonation or activated carbon) has several drawbacks, such as large environmental impact, handling activated carbon after use and/or reactivation of this, formation of unwanted toxic biproducts, and an impact on the working environment. Another challenge is how to finance the installation and operation of the technological solutions. In the EU, the cost of the removal of pharmaceuticals has been estimated to more than 6.5 billion EUR per year (OECD, 2019), and several countries have made cost-comparison for the removal at wastewater treatment plants of different size.

In Switzerland, which is the only country that has implemented regulations for 12 indicator substances, the regulated removal is dependent on wastewater treatment plant size and the receiving recipient status. For large WWTPs > 80,000 PE, a reduction of > 80% of the indicator substances is anticipated by 2040, and for medium-sized WWTP (24,000-80,000 PE) when discharging into small rivers with low dilution ration, or into water bodies used for drinking water. This has been implemented at 120 out of 700 WWTPs, and the selected technologies are activated carbon, ozonation. The centralized removal of pharmaceuticals and other micropollutants has been financed by a new nationwide tax (CHF 9 per person/year) and the remaining 25% are covered by the municipalities (OECD, 2019). In Germany, the costs are estimated between 0.14 -0.21 EUR/m^3 depending on size, and treatment is based on ozone, sand filtration and powdered activated carbon (summarized in OECD 2019). In Sweden, different technologies ensuring > 80% removal are expected to differ in price from large WWTPs (1 SEK/m^3~0.1 EUR/m^3) to smaller WWTPs (up to 5 SEK/m^3~0.49 EUR/m^3).

The cost for the eXeno™ concept was estimated based on operation at 3 L scale amounting to (0.21 DKK/m^3 ~0.03 EUR/m^3) (Kragelund et al., 2018). Since the innovative strict biological approach seemed competitive, the eXeno™ concept was investigated and developed in greater detail in this project. In addition, the technology development was combined with extensive knowledge on pharmaceutical consumption of patients at the hospitals and subsequent excretion of these micropollutants.

The current MerEFF project builds on the preceding MERMISS projects (Kragelund et al., 2015, and Kragelund et al., 2018), where both decentralized and centralized solutions for pharmaceutical removal from wastewater were investigated (Casas et al., 2015 a,b, Tang et al., 2017, 2020, and Ooi et al., 2018). The applied technology was the moving bed biofilm reactors (MBBRs) as an alternative solution to polish micropollutants from contaminated effluent, as biofilms are both more cost-effective than ozone and activated carbon and more efficient than CAS in their biodegradation of pharmaceuticals and other micropollutants. The proof-of-concept was obtained forming the basis of the new eXeno™ technology where an enhanced biological conversion of pharmaceuticals was obtained in effluent waters by optimizing the operational regimes. By operating in different periods of feast starve, it was possible to obtain sufficient and competent biomass responsible for the degradation of existing pharmaceuticals (Tang et al., 2017). This project has operated the eXeno™ technology in 3 large reactors, 5 m^3 each, and key operational parameters as time and frequency between feast and starve periods have been identified, see FIGURE 1 and section 5 and 6.

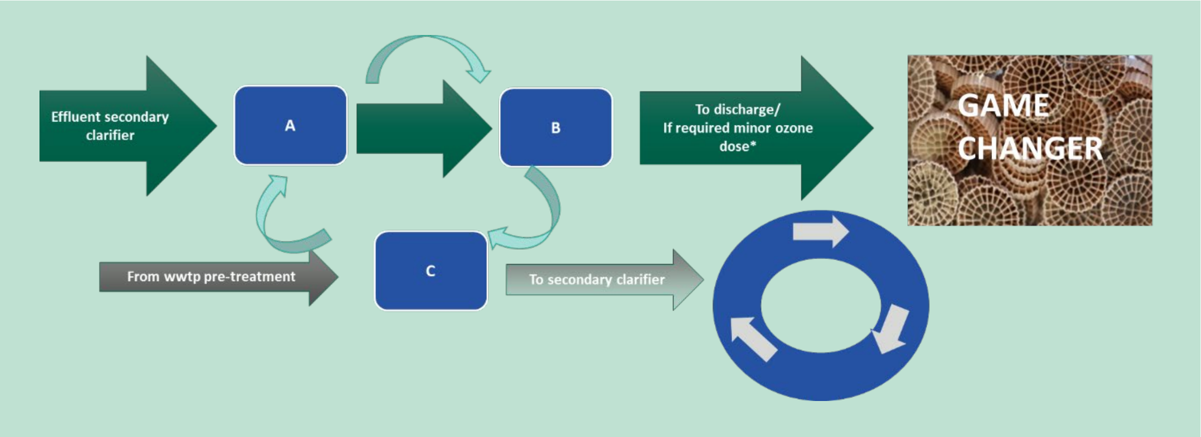

FIGURE 1. Schematic overview of the eXeno™ concept, where three MBBR-reactors (A-C) are either in the treatment line polishing the WWTP effluent or in the regeneration phase receiving pre-settled wastewater for better biofilm performance when shifted back in the treatment line (illustrated by arrows).

MERMISS project was followed by the Higher Environmental Performance in Waste Systems (HEPWAT) project (2016) granted by Environment and Food Industry's Environmental Technology Development and Demonstration Program (MUDP). The pilot consisted of 3* 1 m^3 reactors located at Assens municipality. The goal of the complete HEPWAT plant was to create an energy-efficient wastewater concept based on MBBR and MBR anammox process. By including the eXeno ™ process as the last step of the treatment plant, the goal was to additionally reduce the content of micropollutants in the water treated by MBBR and MBR. Differently from MERMISS project, the conditions applied to eXeno™ in the HEPWAT project were mainly focused on increasing the nitrifying capacity of the biofilm. Due to the high removal of organic carbon during the first steps of MBBR in the main treatment line, the conditions applied to eXeno™ during feast phase were of a load low in organic carbon but high in ammonia with a consequent increase of nitrifying capacity in the eXeno™. In addition, different media that could develop different biofilm thicknesses were also tested. The pilot showed the flexibility of operation of eXeno™ technology by sustaining the removal of micropollutants applying different types of wastewater during the feast phase. Biofilm thickness was shown to have an importance in the removal of micropollutants as well, with a medium-thick biofilm (<500 µm) preferable for the removal of a wide range of micropollutants. More information can be found on the project website (http://www.hepwat.dk/en).

The eXeno™ concept was also tested in the BONUS CLEANWATER project funded jointly by the EU and Innovation Fund Denmark, Sweden’s innovation agency VINNOVA and the German Ministry for Education and Science (BMBF) 2017. The eXeno™ pilot was operated at Landskrona, Sweden as a pure solution or in combination with ozonation. In this pilot experiment, a different operation of the eXeno™ concept was tested. The feast and starve phases were tested by exposing the carriers in different reactors to different redox conditions and by transporting the carriers between the reactors with a conveyor belt. The main goal was to perform the removal of micropollutants along with phosphorus removal. The pilot results showed that redox conditions are important in the operation of eXeno™ with aerobic conditions preferable to anoxic and anaerobic conditions. More information can be found on the project website (http://www.swedenwaterresearch.se/en/projekt/bonus-cleanwater/).

Several patent applications have been filed to protect the eXeno™ operation such as the Swedish patent application no. 1650321-A1 and European patent application no. WO 2017/153361-A1, where the Swedish patent (539304-C2) has been granted. Based on the obtained results, a 10% solution for Herning Municipality has been developed, which is benchmarked against competing technologies and described further in section 9.

2. Summary

The Municipalities are the competent authorities to decide, if the newly constructed hospitals or greatly re-constructed hospitals, will have to treat their wastewater prior to discharge into the local sewer system, or if the wastewater can be handed at the centralized wastewater facilities. The local authorities are responsible to acquire knowledge to distinguish between different pharmaceuticals present in wastewater, their concentrations and the potential impact on the environment. For this, the Danish AMK list[2] is used by the local authorities. The AMK list suggests maximum concentrations for unwanted pharmaceuticals present in wastewater and was prepared by a working group (by BIOFOS) latest actualized in 2015 as a proposal for the Association of Local Governments in Denmark. But little knowledge exists on actual concentrations present in the hospital wastewater and in the inlet and outlet of the corresponding wastewater treatment plants.

The ambition of the MerEFF project was to further investigate if hospitals were the major polluter in terms of environmentally unwanted pharmaceuticals given to committed patients. This is highly relevant as many new hospitals are under construction and the number of Bed Days are reduced. The trend for patients in ambulatory care has significantly increased for Aarhus University Hospital (AUH) with approx. 50% from 2007-2019.

Therefore, a comprehensive theoretical mapping of the pharmaceuticals associated with the hospital was implemented, using the AMK list for environmentally unwanted pharmaceuticals in hospital wastewater. It was distinguished whether the pharmaceuticals in fact were used at the hospitals or handed out to patients in ambulatory care by extracting the data from the Electronic Patient Journal, where information on treatment types, e.g., ambulatory or committed is registered. These data were compared with the publicly available data from the MedStat database, where the total sale of the different pharmaceuticals can be extracted for the primary sector and the hospital sector. The calculated environmental impact is based on the PNEC values set by the Danish authorities (Local Government Denmark (KL), 2013). A precondition in the mapping is that all prescribed pharmaceuticals are discharged directly to the toilet/sewer, which is a conservative assumption. Thus, neither metabolization of pharmaceuticals nor potential cocktail effects of pharmaceuticals and half times of pharmaceuticals are taken into consideration. The quantities of pharmaceuticals from the AMK list used at hospitals (data from MedStat or the hospitals pharmacy) was set in relation to wastewater discharge from the hospital and their respective PNEC values. By using data from the electronic patient journal, it was possible to assign the concentrations of pharmaceuticals, actually consumed at home (ambulant treatments), leading to the calculation of the hospital's actual percent contribution for the different pharmaceuticals to the local environmental impact.

The mapping documented that most of the environmental impact previously associated with the hospital originated in fact from pharmaceuticals handed out to patients in ambulatory care at Aarhus University Hospital. This investigation was extended to include all hospitals in the Central Region of Denmark consisting of 5 additional hospitals of varying size. The findings supported those from AUH and revealed that 4 pharmaceuticals were responsible for a major part of the environmental impact (mycophenolic acid, sertraline, clarithromycin, and ciprofloxacin), as well as capecitabine and sulfamethoxazole at AUH. The pharmaceuticals listed below were responsible for 88% - 97% of the environmental impact associated with the hospitals, but these pharmaceuticals were primarily handed out in ambulatory care, see TABLE 1.

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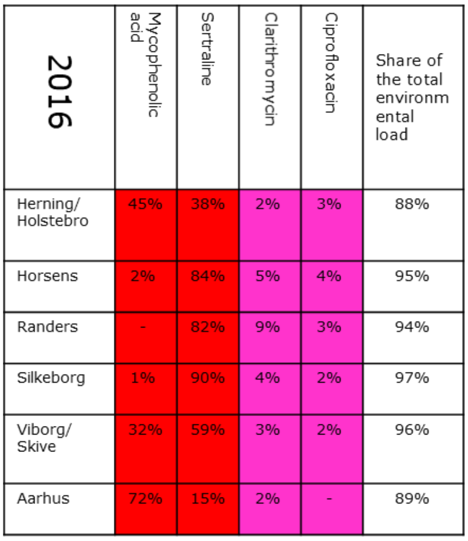
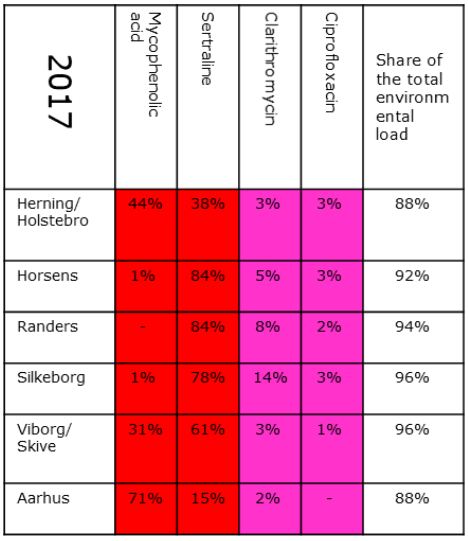
[2] https://spildevandsinfo.dk/sites/default/files/2019-06/Opdaterede%20AMK_August_2015.pdf

TABLE 1. Data from hospitals: The share of total environmental impact from both committed patients and ambulatory care patients of 4 pharmaceuticals that contribute the most are listed below by the different hospitals and with the different contributions of the local impact in percentage. Lines with two hospitals are calculated as combined data, as new super hospitals are built soon, substituting for the two hospitals. The names of the cities cover the following hospitals: Regionshospital Herning, Regionshospitalet Holstebro, Regionshospital Horsens, Regionshospitalet Randers, Regionshospitalet Silkeborg, Regionshospitalet Viborg, Regionshospitalet Skive, Aarhus Universitetshospital.



2016	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
Herning/ Holstebro	45%	38%	2%	3%	88%
Horsens	2%	84%	5%	4%	95%
Randers	-	82%	9%	3%	94%
Silkeborg	1%	90%	4%	2%	97%
Viborg/ Skive	32%	59%	3%	2%	96%
Aarhus	72%	15%	2%	-	89%



2017	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
Herning/ Holstebro	44%	38%	3%	3%	88%
Horsens	1%	84%	5%	3%	92%
Randers	-	84%	8%	2%	94%
Silkeborg	1%	78%	14%	3%	96%
Viborg/ Skive	31%	61%	3%	1%	96%
Aarhus	71%	15%	2%	-	88%

Further investigations on pharmaceuticals present in the wastewater from the AUH hospital and inlet and outlet concentrations from different wastewater treatment plants around the country were examined. A total of 64 pharmaceuticals were measured in 24-hour samples. The untreated wastewater from AUH revealed concentrations of 16 pharmaceuticals detected above the proposed PNEC values of which 10 pharmaceuticals were present on the AMK list. Additional data from measuring campaigns from a different wastewater treatment plant showed similar results. Pharmaceuticals detected above PNEC values at the hospitals were still above PNEC values in the inlet wastewater in different wastewater treatment plants. This indicates that also other more diffuse sources such as consumption of medicine in private homes contribute significantly to the high concentrations above PNEC values detected in the wastewater treatment plants. The above data on the theoretical mapping combined with the knowledge from the measuring campaigns supports that the pharmaceuticals detected above PNEC values not only originate from the hospitals but also from the consumption in private homes. This underlines that the measures to reduce the pharmaceuticals in recipients will have a limited effect if only conducted at the hospitals.

In addition to mapping, the aim of this project was to further develop a strict biological approach with advanced operation between feast and starve periods. The technology concept is called eXeno™ and was implemented at pilot scale as a polishing step prior to wastewater discharge at Herning Water. The eXeno™ concept was upscaled to 3 tanks, 5 m^3 each, receiving a mixture of pre-settled wastewater and effluent water from secondary clarifier tanks, and the biofilm was formed on Z-carriers. The reactors were operated in a way where tank no. 1 would receive the effluent wastewater and the following tank (no. 2) would treat the effluent from tank no. 1. At the same time, tank no. 3 would operate in regeneration mode where pre-settled wastewater would be available for the bacterial community to build up.

Different operational regimes were tested during the 1-year experimental period, and the removal ability of the biofilm present on carriers was detected using spiking experiments as well as following actual wastewater concentrations throughout the tanks. In addition to the removal of pharmaceuticals, the eXeno™ technology significantly reduced both ammonia and COD present in the wastewater. Continuous measurements documented a reduction of 78-93% in ammonia and corresponding reduction of inert COD of 6-36%. These findings emphasize the efficiency of the eXeno™ technology as it liberates additional capacity in the wastewater treatment plant due to removal of ammonia and COD.

The initial period and spiking experiments confirmed biofilm development, which takes time to build up and comprise of the specialized pharmaceutical degraders. Experiments carried out from May to August show the strength and also the importance of biofilm age, which strongly correlates with the different operational modes tested in the project.

Several campaigns were conducted by following the actual concentrations of pharmaceuticals through the plant, which revealed that operational modes and corresponding biofilm age significantly increases the pharmaceutical removal. The final campaign conducted in August documented a total reduction of 93%. For some very recalcitrant compounds, the removal was less effective (e.g., Citalopram), but for most of the tested compounds very high removal rates were observed.

Interestingly, three out of four most environmentally problematic compounds connected to hospitals, based on theoretical mappings: sertraline, mycophenolic acid, clarithromycin and ciprofloxacin, were detected in the wastewater at Herning WWTP in both May and August. In August, a significantly high removal of these compounds was observed: sertraline 30-100%, clarithromycin 80-100% and mycophenolic acid 100%, which further supports the findings that the eXeno™ polishing step is an efficient polishing technology.

Based on these pilot-scale findings, dimensioning of a semi-full scale eXeno™ solution was conducted for Herning WWTP (10% of the total municipal flow was treated with the eXeno technology) and for a generic 100,000 PE treatment plant. FIGURE 2 illustrates how the eXeno™ can be implemented in existing WWTPs.

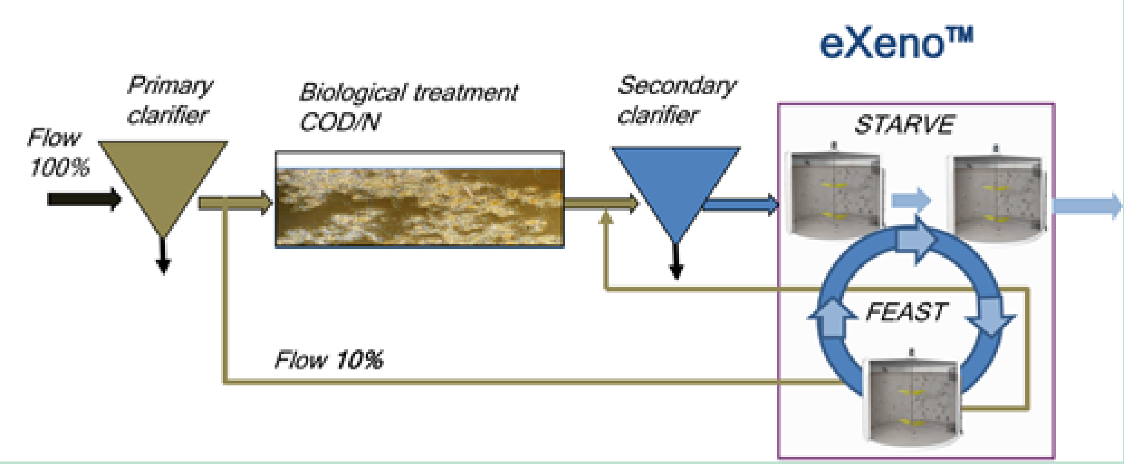


FIGURE 2. Process configuration eXeno™ post treatment.

Some of the additional advantages of the eXeno™ technology are low operational costs, low carbon footprint and environmental and sustainable technology compared to the competing ones such as ozonation, and the use of activated carbon. Also, it can be easily operated as the changing feeding modes can be completely automated. The setup of the eXeno™ technology is described below. CAPEX is estimated between 3.3-4 mio. EUR, and OPEX is estimated in TABLE 2 based on the price levels in Denmark 2020 for equipment, construction, and manpower.

TABLE 2. OPEX for the eXeno™ process.

	EUR/m^3 treated
Electricity*	0.005 - 0.006
Maintenance	0.009
Manpower	0.005
Total OPEX	~ 0.02

* Electricity prices are based on EU-27 average price, see TABLE 16 in section 7.

Regulations related to pharmaceuticals in the aquatic environment have been discussed for quite some time, but it seems that no immediate moves have been made towards changes in the existing legislation. Based on regulatory contexts such as REACH and the European Water Framework Directive (WFD), safe exposure levels for aquatic and terrestrial ecosystems have been defined, and the lowest value is typically divided by an assessment factor of 10 to 1000 to determine the PNEC (predicted no effect concentration). There are examples of PNEC values being used as treatment target for removal in WWTPs, i.e., completely disregarding the dilution occurring in the receiving aquifer and the assessment factor built into the PNEC value.

The documented removal rates for different pharmaceuticals investigated in the eXeno™ pilot tests vary from 30-90%. However, when looking at the global removal in the WWTP with an eXeno™ post treatment, the overall removal for most of the compounds reaches 70-90%. Depending on the actual compounds present in the wastewater at a specific plant, this removal rate could satisfy the requirements. It should be emphasized that the pilot plant has been operated for one year, and the progress in removal has been seen throughout the test period, and thus it cannot be ruled out that the performance will increase with time when the plant is in operation at a specific site.

In general, the eXeno™ is a flexible, easy-to-operate and robust solution with required removal efficiencies for target compounds. Furthermore, a low energy demand and producing none or a minimum of waste is also necessary when dealing with pharmaceutical pollution with pharmaceuticals on a global scale. eXeno™ post-treatment seems to be a good candidate, because together with the well-functioning main WWTP, it has shown to provide a substantial global removal of 90+%. The next step is to test the current eXeno™ setup at a full scale and validate its performance.

Specifically, for Herning Water and the hospital DNV Gødstrup, in spring (2021) a collaboration agreement will be signed which will be based on the existing legislation. The agreement will ensure a biological solution, which is considered a joint strategic focus.

3. Resumé

De lokale myndigheder er beslutningsmyndigheden for at afgøre, om nye hospitaler eller eksisterende hospitaler med ombygninger selv skal rense deres spildevand, inden det blandes med andet spildevand (punktkilde), eller om spildevandet kan renses på de centrale renseanlæg. De lokale myndigheder skal i den forbindelse konkret vurdere spildevandets stofindhold af fx lægemidler, stoffernes koncentrationsniveau og potentielle indvirkning på miljøet. I den forbindelse anvender myndighederne den danske liste over miljøproblematiske lægemidler, den såkaldte AMK-liste[3]. Listen indeholder foreslåede maksimale koncentrationer for uønskede lægemidler, der er til stede i spildevand og er udarbejdet af BIOFOS' arbejdsgruppen med den seneste aktualisering i 2015 som et forslag til Kommunernes Landsforening. Men der eksisterer kun ringe viden om de aktuelle koncentrationer i hospitalsspildevand og om de respektive ind- og udløbskoncentrationer der tilledes renseanlæg.

Ambitionen for MerEFF-projektet var at undersøge, om hospitaler kunne identificeres som værende en punktkilde, hvad angår miljøproblematiske lægemidler, der tildeles patienter i ambulant behandling. Denne viden er særdeles relevant, idet mange nye hospitaler er under opførelse, og da antallet af sengedage samtidig er reduceret. Tendensen med behandling af patienter i ambulant behandling er på Aarhus Universitets Hospital (AUH) steget med ca. 50 % fra 2007-2019.

Som følge heraf er der gennemført en omfattende teoretisk kortlægning af de lægemidler, der hidtil blev antaget indtaget på AUH. Kortlægningen skulle afklare, om disse lægemidler reelt blev indtaget på hospitalet, eller om de i højere grad blev udleveret til patienten i forbindelse med ambulant behandling. Den danske AMK-liste blev anvendt til at undersøge det faktiske forbrug af lægemidler med uønsket miljømæssig påvirkning. Ved undersøgelsen blev forbrugsdata fra AUH anvendt, som er opgjort i den elektroniske patientjournal, hvoraf det er muligt at udlede, om patienter behandles og dermed medicineres under indlæggelse eller ambulant. Disse data blev sammenholdt med data om forbrug i primær- og hospitalssektor fra den offentlig tilgængelige MedStat database. Den beregnede miljøpåvirkning er baseret på de PNEC-værdier, der er indstillet af de danske myndigheder (Kommunernes Landsforening (KL), 2013). En præmis for kortlægningen er, at alle ordinerede lægemidler udledes direkte til toilet / kloak, hvilket er en konservativ antagelse. Desuden tages hverken metabolisering af lægemidler eller potentielle cocktaileffekter af farmaceutiske lægemidler og halveringstiden af lægemidler med i betragtning. Mængderne af lægemidler fra AMK-listen anvendt på hospitaler (data fra MedStat eller hospitalets apotek) blev sat i forhold til spildevandsudledning fra hospitalet og deres respektive PNEC-værdier. Ved at bruge data fra den elektroniske patientjournal var det muligt at identificere koncentrationer af lægemidler, der faktisk forbruges derhjemme (ambulante behandlinger), hvilket gjorde det muligt at beregne hospitalernes procentvise bidrag for de forskellige lægemidler til den lokale miljøpåvirkning.

Kortlægningen og den hermed opnåede viden dokumenterede, at lægemidler med den primære miljøbelastning, som man tidligere antog forbrugt på AUH, faktisk blev udleveret til patienter i ambulant behandling. Denne undersøgelse blev udvidet til at omfatte lægemiddelforbruget på samtlige hospitaler i Region Midt, dvs. på yderligere 5 hospitaler af forskellig størrelse. Samme tendens, som blev observeret ved AUH, kunne registreres her og viste, at 4 lægemidler tegnede sig for hovedparten af miljøbelastningen (mycofenolsyre, sertralin, claritromycin, og ciprofloxacin) sammen med carpecitabin og sulfametoxazol ved AUH. De nævnte

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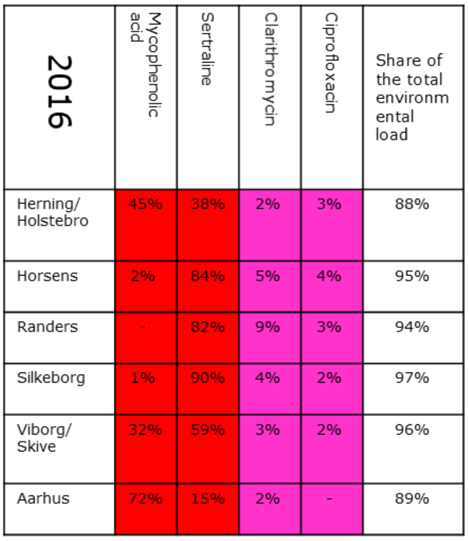
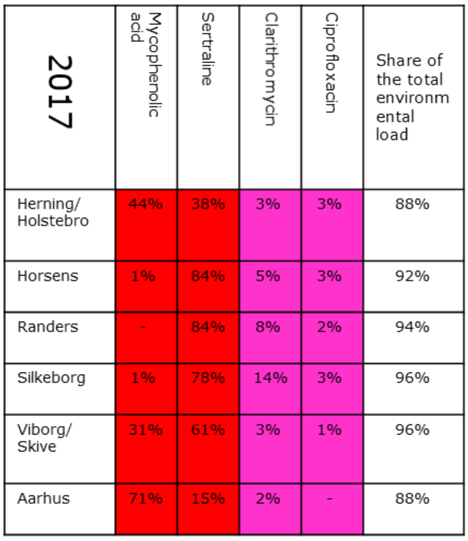
[3] https://spildevandsinfo.dk/sites/default/files/2019-06/Opdaterede%20AMK_August_2015.pdf

lægemidler udgjorde mellem 88 % og 97 % af den miljømæssige belastning, som tilskrives brug på hospitaler, men blev reelt primært udleveret under ambulant behandling, se tabel herunder.

Andelen af den totale miljømæssige påvirkning (både fra indlæggelser og fra ambulant behandling) af de 4 lægemidler, som udgør det største miljømæssige bidrag, angivet i procent for de forskellige hospitaler. Linjer med to hospitaler er beregnet som kombination af data fra begge hospitaler, da der i nær fremtid er planlagt opførsel af supersygehuse dækkende disse hospitaler. Bynavnene dækker over følgende hospitaler: Regionshospitalet Herning, Regionshospitalet Holstebro, Regionshospital Horsens, Regionshospitalet Randers, Regionshospitalet Silkeborg, Regionshospitalet Viborg og Regionshospitalet Skive og Aarhus Universitetshospital



2016	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
Herning/ Holstebro	45%	38%	2%	3%	88%
Horsens	2%	84%	5%	4%	95%
Randers	-	82%	9%	3%	94%
Silkeborg	1%	90%	4%	2%	97%
Viborg/ Skive	32%	59%	3%	2%	96%
Aarhus	72%	15%	2%	-	89%



2017	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
Herning/ Holstebro	44%	38%	3%	3%	88%
Horsens	1%	84%	5%	3%	92%
Randers	-	84%	8%	2%	94%
Silkeborg	1%	78%	14%	3%	96%
Viborg/ Skive	31%	61%	3%	1%	96%
Aarhus	71%	15%	2%	-	88%

Yderligere blev der foretaget undersøgelse af lægemidler til stede i spildevandet fra AUH samt in- og udløbskoncentrationer fra forskellige renseanlæg, der modtager hospitalsspildevand. I alt blev 64 lægemidler målt i 24-timers-prøver. Det ubehandlede spildevand fra AUH viste koncentrationer for 16 lægemidler over PNEC-værdien, hvoraf 10 lægemidler findes på AMK-listen. Tilsvarende data fra målekampagner gennemført på andre renseanlæg viste lignende resultater. Lægemidler detekteret over PNEC-værdien ved hospitalerne var stadig over PNEC-værdien i indløbet på de forskellige renseanlæg. Dette indikerer, at andre, mere diffuse kilder, som fx indtagelse af lægemidler i eget hjem, bidrager signifikant til de høje koncentrationer af lægemidler over PNEC-værdien, som blev målt i renseanlæggene. Ovenstående data fra den teoretiske kortlægning kombineret med viden fra målekampagnerne understreger, at lægemidler detekteret over PNEC-værdien ikke alene stammer fra hospitaler, men også fra privatforbrug. Dette understreger, at tiltag for at reducere mængden af lægemidler i recipienterne, kun vil have begrænset effekt, hvis de indskrænkes til alene at blive implementeret ved hospitalerne.

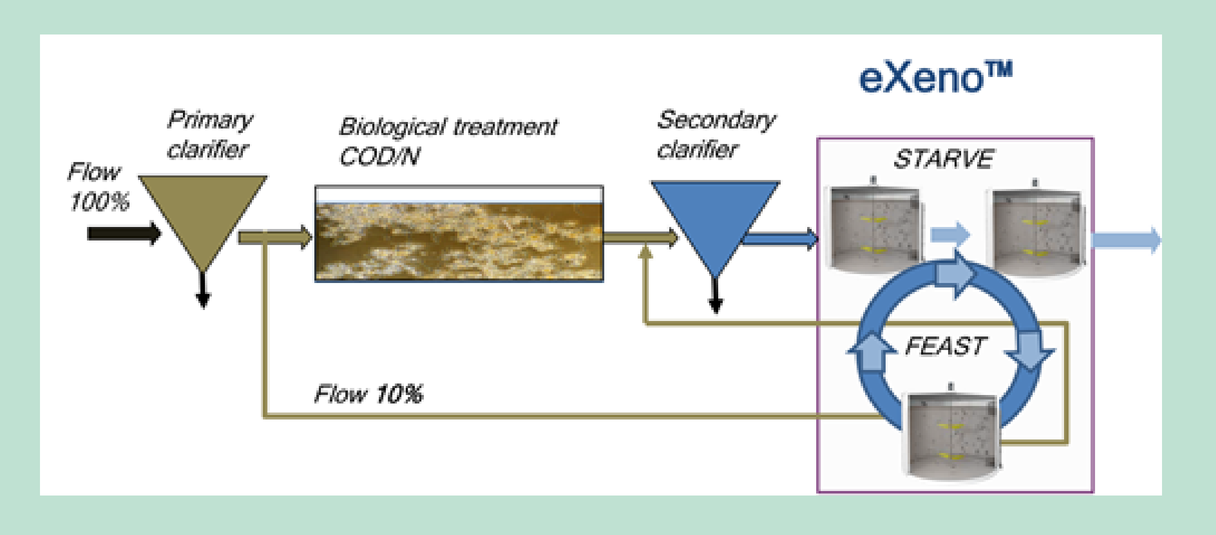
Udover kortlægningen var formålet med dette projekt at udvikle og optimere en rent biologisk tilgang med en avanceret drift vekslende mellem perioder af ”sult og fest”. Teknologikonceptet kaldes eXeno™ og blev implementeret i pilotskala som et efterpoleringstrin, inden spildevandet udledes fra Herning Vand. eXeno™-konceptet blev opskaleret til 3 tanke af hver 5 m^3, som modtog en blandet fraktion af forklaret spildevand og udløbsvand fra den sekundære efterklaringstank. Biofilmen blev dannet på Z-carriers. Reaktorerne blev opereret således, at tank 1 modtog udløbsspildevand, og tank 2 behandlede udløbsvand fra tank 1. Samtidig var tank 3 i regerenationstrinnet, hvor forklaret spildevand var tilgængeligt, så biofilmen kunne opbygges. Forskellige operationelle regimer blev testet under den 1-årige eksperimentelle periode, og fjernelsesgraden af biofilmen blev monitoreret dels vha. spiking-eksperimenter, dels ved at følge en spildevandsstrøm med aktuelle koncentrationer af lægemidler gennem tankene. Udover at fjerne lægemidler, reducerede eXeno™-teknologien også CODF og ammonium i spildevandet. Kontinuerte målinger dokumenterede en reduktion mellem 78 % og 93 % af ammonium og en tilsvarende reduktion af inert COD på 6-36 %. Dette understreger effektiviteten af eXeno™-teknologien, da den frigiver kapacitet i selve renseanlægget på grund af fjernelsen af ammonium og COD.

Den indledende periode og spikingeksperimenter bekræftede biofilmdannelsen, som det tager nogen tid at opbygge, og som består af særlige lægemiddelnedbrydende bakterier. Eksperimenter fra maj til august 2020 viste ikke alene fordelen, men også vigtigheden af biofilmens alder, som samtidig hænger nøje sammen med de driftsregimer, der blev undersøgt i projektet.

Adskillige kampagner blev gennemført ved at følge aktuelle koncentrationer af lægemidler gennem anlægget. Kampagnerne viste, at såvel forskellige operationelle regimer og biofilmens alder signifikant øger lægemiddelfjernelsen. Den sidste kampagne i august 2020 dokumenterede en total reduktion på 93 %. For nogle meget persistente lægemidler var fjernelsesgraden lavere (fx citalopram), men for de fleste testede lægemidler blev der observeret meget høje fjernelsesgrader.

Tre af de fire miljømæssigt mest problematiske lægemidler, som er forbundet med hospitaler, baseret på den teoretiske kortlægning, nemlig sertralin, mycofenolsyre, claritromycin og ciprofloxacin, blev målt i spildevandet ved Herning Vand i både maj og august. I august, blev en signifikant højere fjernelsesgrad observeret: sertralin 30-100 %, claritromycin 80-100 % og mycofenolsyre 100 %, hvilket yderligere understreger, at eXeno™-trinnet er en effektiv efterpoleringsteknologi.

Baseret på disse pilotskalaobservationer blev en eXeno™-løsning i fuldskala dimensioneret til Herning Vand (10 % af det totale kommunale spildevand blev behandlet med eXeno teknologien) og for et generisk 100.000 PE-renseanlæg. Figuren herunder illustrerer, hvordan eXeno™ kan implementeres i et eksisterende renseanlæg.



eXeno™-proces-konfiguration som efterbehandling.

Nogle af de ekstra fordele ved eXeno™-teknologien er de lavere driftsudgifter og et lavt carbon footprint, ligesom teknologien er bæredygtig og miljømæssigt forsvarlig sammenlignet med konkurrerende teknologier som ozonering og brug af aktivt kul. Den er tillige nem at styre, da de vekslende regimer mellem ”sult og fest” kan automatiseres fuldstændigt. Setuppet af eXeno™-teknologien er beskrevet nedenfor, hvor CAPEX er estimeret mellem 3,3-4 mio. EUR, og OPEX er estimeret i tabellen herunder, baseret på prisniveauet i Danmark 2020 for udstyr, konstruktion og arbejdskraft.

OPEX for eXeno™-processen.

	EUR/m^3 behandlet
Elektricitet*	0.005 - 0.006
Vedligeholdelse	0.009
Arbejdskraft	0.005
Total OPEX	~ 0.02

* Elektricitetspriser er baseret på EU-27 gennemsnitspris pris, se TABLE 16 i sektion 7.

Regulering af lægemidler i akvatiske miljøer er blevet diskuteret i lang tid, men der forventes ikke ny lovgivning på området i den nærmeste fremtid. Baseret på andre regulativer såsom REACH og det Europæiske Vandramme Direktiv er tilladelige eksponeringsniveauer for akvatiske og terrestiske økosystemer blevet defineret, og disse divideres typisk med en sikkerhedsfaktor mellem 10-1.000 for at finde PNEC-værdier (ingen effekt-koncentration). Der findes eksempler på, at PNEC-værdier bruges som mål for fjernelsesgrader i renseanlæg, fx uden at medregne fortyndingsfaktoren i recipienten og den tilsvarende sikkerhedsfaktor indbygget i PNEC-værdien.

De dokumenterede fjernelsesgrader for de forskellige lægemidler undersøgt i eXeno™-pilotskalatesten, varierer fra 30 % til 90 %. Når fjernelsesgraden i det eksisterende renseanlæg kombineres med eXeno™-efterpolering, opnås 70-90 % fjernelse af lægemidler. Afhængigt af de aktuelle koncentrationer af lægemidler, som er til stede i renseanlægget, kan denne fjernelsesgrad opfylde behovet. Det skal dog understreges, at pilotskalaanlægget har kørt i et år, og at der er observeret stigende fjernelsesgrader i forsøgsperioden. Derfor kan det ikke udelukkes, at fjernelsesgraden forsat vil stige under kontinuert drift på en bestemt lokalitet.

Generelt er eXeno™ fleksibel og nem at drifte og udgør tillige en robust løsning med de krævede fjernelsesgrader for udvalgte lægemidler. Dertil kommer et lavt energibehov, ligesom der produceres ingen eller meget lidt slam, hvilket også er nødvendigt, når forurening forårsaget af lægemidler skal håndteres på global skala. eXeno™-efterpolering fremstår som en god kandidat, da teknologien har en dokumenteret fjernelsesgrad på over 90 %, når den kombineres med et velfungerende renseanlæg. Næste skridt er at teste eXeno™-setuppet i større skala og validere præstationen.

Specifikt for Herning Vand og DNV Gødstrup underskrives en samarbejdsaftale i foråret 2021, som baseres på den gældende lovgivning. Samarbejdsaftalen vil sikre en biologisk renseløsning, som er et strategisk fokus for de involverede partnere.

4. Pharmaceuticals in hospital and municipal wastewater

Pharmaceuticals are widely detected in surface water, groundwater, and drinking water all over the world. Their presence in the waterbodies is of increasing concern, and most OECD countries have established watch-lists and monitoring programmes for certain pharmaceuticals. However, different monitoring lists in different countries make comparative studies challenging. In Denmark, such a list encompasses approx. 40 compounds of environmental concern (also called the AMK list), and these pharmaceuticals are associated with medical treatment at hospitals (Local Government Denmark, 2013). To keep the analytical costs at a minimum in the MerEFF project and still monitor the unwanted pharmaceuticals, a comprehensive list of approx. 24 micropollutants has been selected. The monitored pharmaceuticals represent the pharmaceuticals of concern from overlapping lists from Denmark, Sweden, Germany, and Switzerland.

In Denmark, much debate has been connected to the question of where the removal of pharmaceuticals should occur, i.e., decentralized solutions at the hospitals or centralized treatment at the existing municipal wastewater treatment plants. To further investigate this, patients were identified whether they were in ambulatory care or hospitalized during treatment with pharmaceuticals included on the AMK list. The Medstat database encompasses all consumption data on pharmaceuticals in Denmark and can be differentiated based on primary consumption (private homes) or at hospitals (secondary sector).

A comparative study was conducted on pharmaceuticals ascribed to the hospital consumption from the Medstat database (2017) and the actual consumption by hospitalized patients using the electronic patient journal implemented in Denmark, to determine where the pharmaceuticals were in fact consumed. By comparing these two lists, it was possible to identify if pharmaceuticals registered to the hospital, were in fact handed out to patients in ambulatory care or if they were consumed by hospitalized patients. If the pharmaceuticals were handed out for ambulatory treatments, this indicates that the discharge of pharmaceuticals by these patients will take place to their local wastewater treatment plant and not at the hospital premises.

In addition, the actual measured concentrations of pharmaceuticals in the wastewater from Aarhus University Hospital, (AUH) were analysed and compared to inlet and effluent samples of the receiving municipal wastewater treatment plant in Egå. The purpose of these measurements was to identify whether all or some of the pharmaceuticals detected in the municipal wastewater could be ascribed solely to the discharge from the hospital, or if additional diffuse sources of pharmaceuticals were contributing to the pharmaceutical load of the wastewater treatment plant.

These findings were also compared to other similar sampling campaigns conducted at different wastewater treatment plants of varying sizes around the country.

4.1 Lists of monitored pharmaceuticals in the MerEFF project

The regulations in Denmark are differentiated depending on whether or not an industry enterprise, e.g., hospital is considered a point source polluter. The local authorities are responsible for both discharge permits and sewer connection permits and can demand improved wastewater treatment to reduce certain compounds. In relation to treatment of hospital wastewater, the discussion is whether the wastewater is significantly different from household wastewater and therefore subjected to point source status and subsequent treatment prior to discharge to public sewer. The considerations for the local authorities are several: whether the hospital wastewater contains compounds that are not detected in normal household wastewater, what are their concentrations and their environmental effect. Comprehensive investigation has documented pharmaceuticals in hospital wastewater, which resulted in the AMK list encompassing 40 compounds of environmental concern associated with the hospital wastewater.

The AMK list consists of more than 40 pharmaceuticals of environmental concern present in hospital wastewater. The pharmaceuticals included on the AMK list (2015) were selected according to their usage, i.e., predicted effluent concentrations in hospital wastewater, the stability score (persistence) and potential hazardous impact, i.e., Predicted No Effect Concentrations (PNEC). PNEC values represent the concentration of a pharmaceutical at which no pharmacological effect is expected to occur for a specific organism. The AMK list have identified limit values for maximum acceptable concentrations of these 40 pharmaceuticals in wastewater and the proposed removal in conventional activated sludge treatment plants (AMK 2015).

Although, no required regulatory frameworks of discharge limits of pharmaceuticals into environment exist in Denmark, in particular, into effluents of wastewater treatment plants (WWTP), some lists of monitored pharmaceuticals frequently found in WWTPs have been proposed by research groups in Europe.

Götz et.al (2015) proposed a watchlist of the targeted substances used as the indicators to evaluate the performance of conventional (i.e., activated sludge) and advanced wastewater treatment (powder activated carbon (PAC), ozonation) in Switzerland (‘Advanced treatment monitoring (PAC/Ozone)’in the table in Appendix 1). The list of pharmaceuticals included 12 representative substances which have the following criteria: 1) present in all large Swiss WWTPs (person equivalent > 10,000), 2) active substances (no transformation products), 3) not eliminated by biological treatment, 4) eliminated by both treatments PAC and ozonation to a similar extent, 5) detectable with a reliable and ready to use analytical method. The Ministry for Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia (MULNV) in Germany published a guideline report on the monitoring of substances in WWTPs (Klaus Alt et al., 2016). In this report, the substances suggested for classification according to different priority levels for monitoring and regulating were presented as shown in the TABLE 3, based on occurrence frequency, predicted no effect concentration and analysis costs (see table in Appendix 1). The Swedish list encompasses a selection of pharmaceuticals, plasticizers, flame retardants, and per- and polyfluoroalkyl substances. The respective pharmaceuticals are divided into antibacterial substances, anti-inflammatory substances, antidepressants, antipsychotic hypnotics, stimulants, antihypertensives and sex hormones (Baresel et al., 2014).

Several overlapping pharmaceuticals were chosen from the above lists, consisting of targeted and monitored compounds in MerEFF, which are presented in TABLE 3.

TABLE 3. Substances analysed in MerEFF.

Group	Compound	Group	Compound
Antibiotics	Azithromycin	Antidepressants	Citalopram
	Ciprofloxacin		Venlafaxine
	Clarithromycin		Sertraline
	Erythromycin	X-ray contrasts	Iohexol
	Sulfamethoxazole		Iomeprol
Blood pressure regulators and lipid-lowering agent	Atenolol	Immunosuppressant	Mycophenolic acid
	Metoprolol	Anti-corrosion	1H-Benzotriazole
	Hydrochlorothiazide		5-Chlorobenzotriazole
	Bezafibrate		5-Methyl-1H-benzotriazole
Anti-inflammatory	Ketoprofen	Herbicide	Clofibric acid
	Diclofenac	Analgesics	Carbamazepine
	Mefenamic acid		
	Bicalutamide		

4.2 Theoretical pharmaceutical consumption

In Denmark, a comprehensive data collection on the consumption of pharmaceuticals is available which differentiates between the consumption in private homes, e.g., primary sector, and the consumption at the hospitals, e.g., secondary sector. Among others, the data show whether the pharmaceuticals are procured by hospitals or by private consumers. This is of importance when deciding where to treat the wastewater, i.e. locally at the hospital or centrally at the municipal WWTP. It has been suggested that some of the pharmaceuticals procured and consumed at hospitals are highly toxic, which makes the local treatment and removal of pharmaceuticals from the wastewater very beneficial from an environmental point of view. This dialogue is the reason for completing the mapping of the consumption and discharge of pharmaceuticals at AUH and hospitals in general. The aim of this mapping was to clarify whether it is preferable to treat wastewater locally at the hospital or centrally at the WWTP based on 40 pharmaceuticals included on the AMK list. The mapping includes partly a theoretical mapping that is based on data from the Electronic Patient Journal (EPJ) in the Danish health care system, and partly on practical measurements of wastewater samples from AUH.

A previous mapping showed that between 1 - 4% of the total amount of the consumed pharmaceuticals in Denmark were consumed in the healthcare sector (Mose-Pedersen, 2007), while the remaining medicine was consumed in private homes. Based on this work and the work carried out by Local Government Denmark, 2013, the AMK list (Danish abbreviation of recommended maximum concentrations) was constructed based on pharmaceuticals used at hospitals to a large extent and having an unwanted environmental impact.

In addition, today the treatment of patients is to a large extent carried out in ambulatory care. This indicates that the patients are sent home directly after treatment, and hence the medicine that they are given at the hospital is discharged from their private homes.

Data from the AUH shows that from 2007 to 2019, the number of patients treated in ambulatory care at AUH has increased by 50% from approx. 600,000 to around 900,000, FIGURE 3 shows that it is also evident that the number of Bed Days (a day during which a person is confined to a bed and in which the patient stays overnight at a hospital) is decreasing during the same period. This development indicates that the environmental impact previously assigned to hospitals is now transferred to the patient’s private home, which decreases the environmental impact of the treatment of the wastewater locally at the hospital.

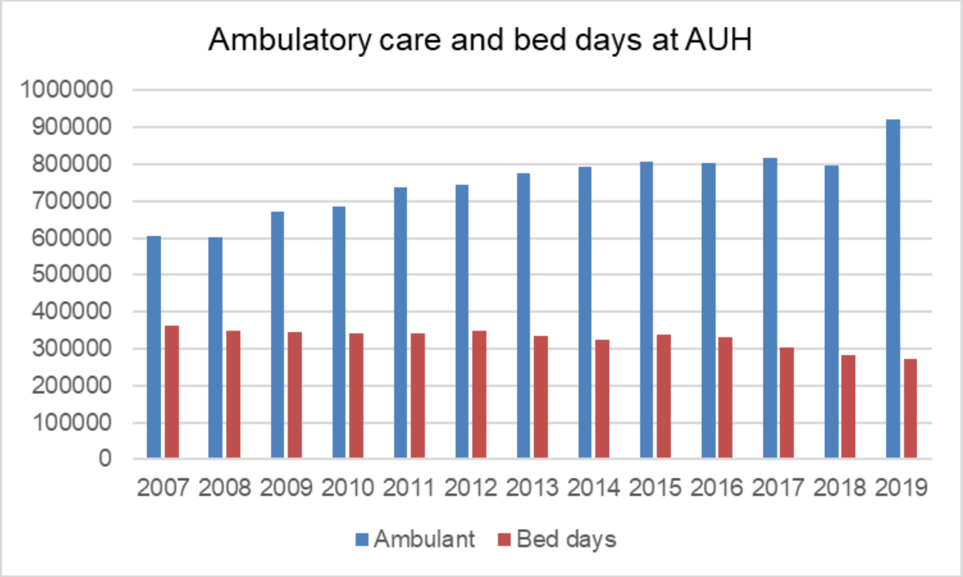


FIGURE 3. Development of ambulatory treatments at AUH from 2007-2019.

In Denmark, the Danish healthcare system has implemented EPJ (Electronic Health Record). This makes it possible to determine whether the consumption of medicine is linked to ambulatory care or hospitalized patients. The term environmental impact for the hospital is defined as the potential impact the consumption of pharmaceuticals present in the wastewater (actual grams of the 40 compounds, combined with the corresponding PNEC values) and thus potential impact on the environment due to discharge of wastewater. When we apply the term here, the previously ascribed environmental impact assigned to hospitals, is in fact related to a large extent to discharge by patients in ambulant treatment and thus not at the hospital premises due to committed patients. This distinction is highly relevant when discussing whether a decentralized treatment of the wastewater should be conducted or if a centralized treatment of the wastewater is most feasible.

As evident in FIGURE 4**,** the environmental impact of medicine consumption originates to a large extent from patients in ambulatory care. In 2017, approx. 94% of the entire environmental impact (from the 40 investigated pharmaceuticals) previously assigned to the hospital, could be traced to patients in ambulatory care. Data document that the environmental impact from AUH based on the guiding limit values for selected pharmaceuticals is much lower than previously anticipated due to ambulatory care cases.

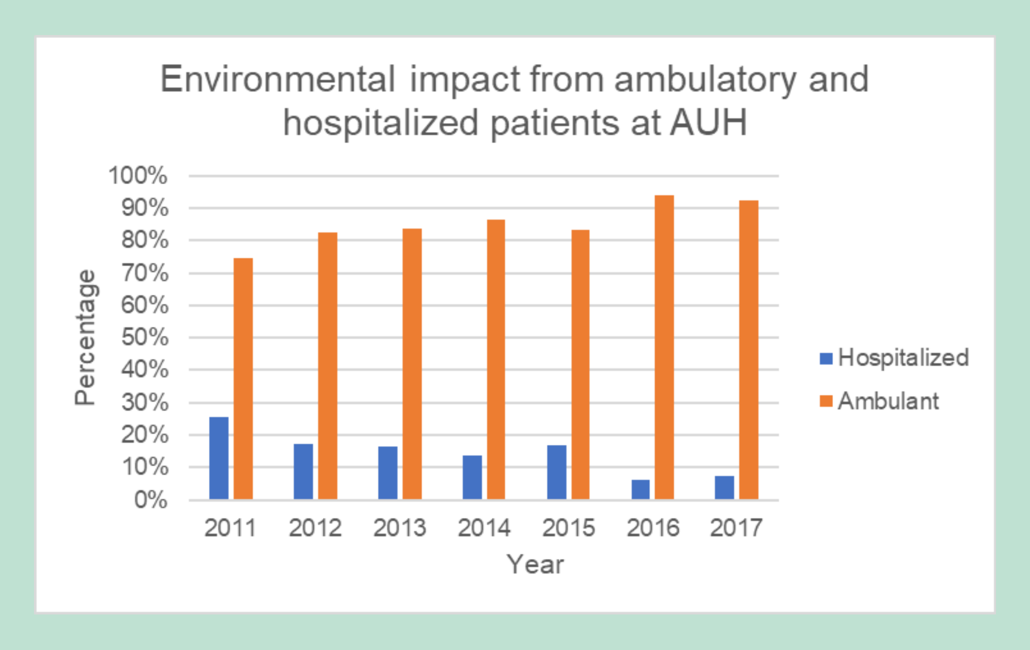


FIGURE 4. Overview of environmental impact from 2012-2017 of the medicine consumed either by hospitalized patients or patients in ambulatory care.

To validate whether the above-mentioned findings were unique for AUH, or whether they represent a general trend for hospitals, an additional mapping of the environmental impact from all other hospitals in Central Region Denmark was conducted. The size of different hospitals in the Central Region of Denmark, measured by the annual number of ambulatory patients, is listed in TABLE 4. AUH is the largest hospital in the Central Region of Denmark, being the second largest hospital in the country.

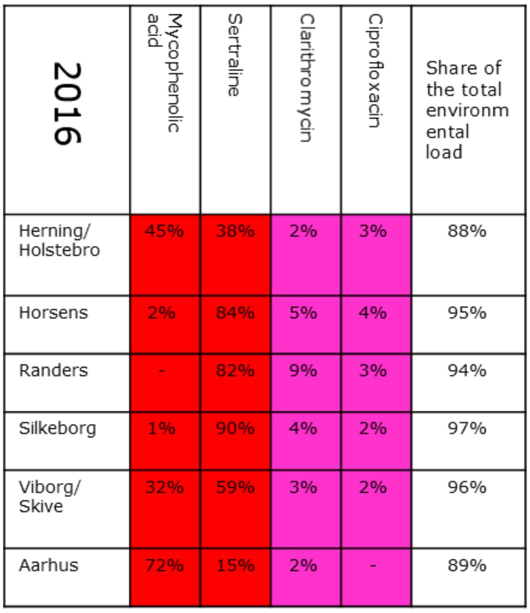
TABLE 4. The size of different hospitals in the Central Region of Denmark based on the number of ambulatories.

Hospital	Ambulatory patients per year
Aarhus University Hospital, AUH	921,091
Herning, Holstebro	414,997
Viborg, Skive	432,516
Horsens	165,481
Randers	160,873

The mapping of environmental impact from other hospitals in the Central Region of Denmark confirmed the findings from AUH. The major environmental impact due to pharmaceuticals could also be linked directly to the patients in ambulatory care at the other hospitals in the Central Region of Denmark. The mapping was based on the data from 2016 and 2017, which made it not possible to conclude whether the environmental impact from discharge of pharmaceuticals from other hospitals were increasing over time as in case of AUH. Neither was it possible to conclude whether the environmental impact linked to the discharge of pharmaceuticals from the other hospitals in the Central Region of Denmark was increasing more rapidly in the private sector (ambulatory) than directly from the hospital (hospitalized).

The pharmaceuticals identified with the greatest environmental impact were to a large extent identical, but with variations due to different specialisation areas of these hospitals. To identify the environmental impact of the pharmaceuticals, their share of the environmental impact and consumption at the different hospitals were mapped and has been indicated in TABLE 5.

TABLE 5. Contribution share of the 4 pharmaceuticals out of the total environmental impact (both hospitalized patients and ambulatory patients) in percentage.



2016	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
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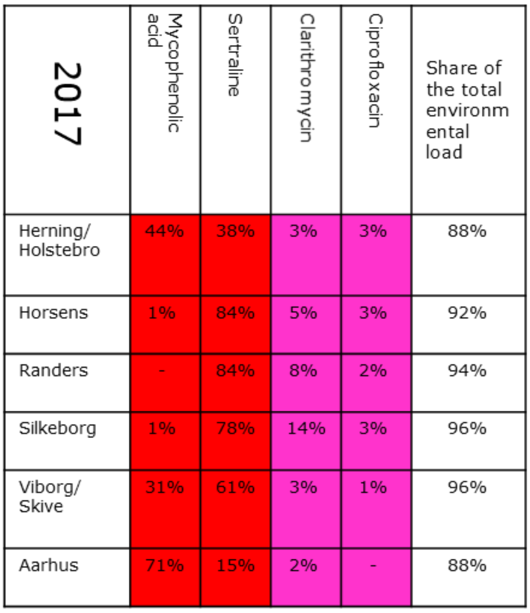
Aarhus	72%	15%	2%	-	89%



2017	Mycophenolic acid	Sertraline	Clarithromycin	Ciprofloxacin	Share of the total environmental load
Herning/ Holstebro	44%	38%	3%	3%	88%
Horsens	1%	84%	5%	3%	92%
Randers	-	84%	8%	2%	94%
Silkeborg	1%	78%	14%	3%	96%
Viborg/ Skive	31%	61%	3%	1%	96%
Aarhus	71%	15%	2%	-	88%

4.3 Measured pharmaceuticals present in wastewater samples

In addition to the theoretical mappings, it was decided to carry out campaigns on actual measurements of the content of pharmaceuticals present in wastewater at AUH. Also, campaigns have been conducted for the central municipal WWTP in Egå, Aarhus, which receives wastewater from AUH. The results from these measurements are still processed and analysed during the creation of this report, thus the conclusions from these campaigns are not included here.

The analyses of pharmaceuticals in wastewater at AUH were based on 24-hour samples taken simultaneously in each well at AUH. Nine sets of samples were taken and analysed. The measured concentrations of pharmaceuticals were evaluated and compared with PNEC values for each API (Active Pharmaceutical Ingredients). The list of analysed pharmaceuticals was comprised from AMK list, the EU Watch list 2018, the Ministerial order on wastewater permits (1625) (Danish), the NOVANA list (Danish measuring programme) and selected pharmaceuticals from the Swiss list.

The analyses results showed that concentrations above PNEC were measured at least 5 times for the pharmaceuticals included in the analyses, see FIGURE 5. To simplify the following assessment, these 15 pharmaceuticals with the greatest number of PNEC exceedances were used as a list of pharmaceuticals of concern.

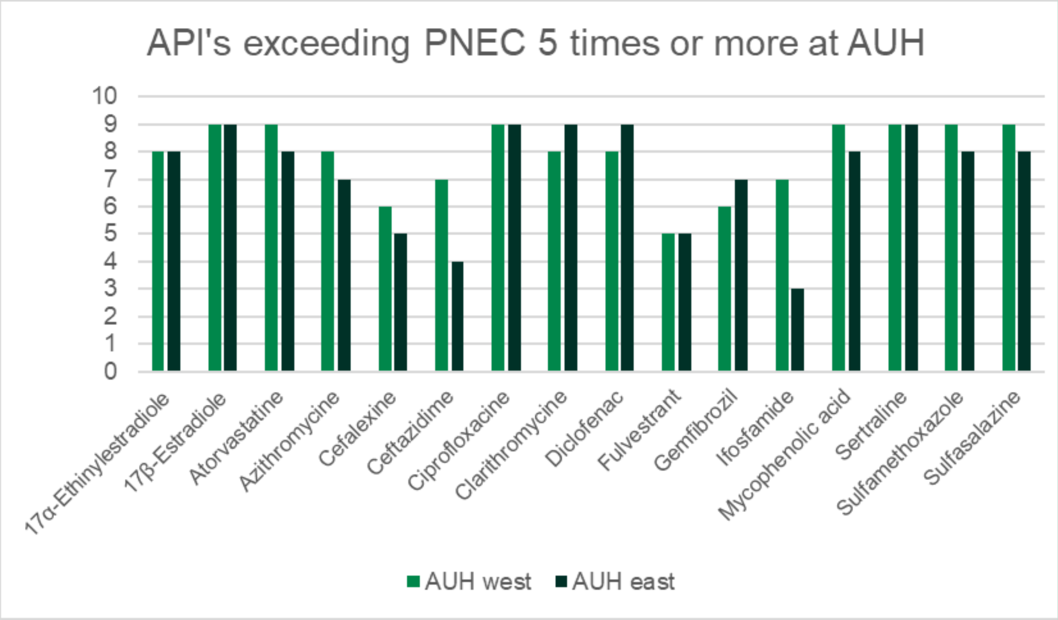


FIGURE 5. Active Pharmaceutical Ingredients (API) at AUH exceeding PNEC concentrations 5 times or more in the 9 wastewater samples.

The specific pharmaceuticals accountable for the pollution based on the measured concentrations are slightly different compared to the theoretical mapping based on EPJ (Electronic Patient Journal). This is primarily due to the theoretical mapping included on the AMK list.

In addition to the measuring campaigns at AUH, comparative analyses have been conducted on wastewater from WWTPs across Denmark: Hjørring, Brædstrup, Herning and Hillerød. The locations of these WWTPs are illustrated in FIGURE 6, which shows that these 4 WWTPs are located in different areas of Denmark.

FIGURE 6. Overview of the location of WWTPs, where measurements were carried out.

In TABLE 6, the findings of the measurement campaigns from the different WWTPs are summarized.

TABLE 6. Sizes of WWTPs included in the measurements of pharmaceuticals.

Location/city	Size of WWTP measured in PE
Egå (Aarhus)	120,000
Hjørring	120,000
Brædstrup	16,000
Herning	150,000
Hillerød	100,000

As evident inTABLE 7, 16 pharmaceuticals were detected in concentrations above PNEC in inlet samples for at least 3 out of 4 WWTPs. It is recommended to investigate this in further details. This suggests that the pharmaceuticals found in the wastewater in different parts of the country are quite similar. It also supports the conclusions from the theoretical mapping conducted for the hospitals in the Central Region of Denmark documenting a majority of the pharmaceuticals are prescribed to patients in ambulatory care, and thus discharged from private homes across the country. The 16 pharmaceuticals measured above PNEC concentrations at the 4 WWTPs are almost identical with the 15 pharmaceuticals that were measured above PNEC in AUH wastewater, at least 5 times out of the conducted 9 campaigns. This indicates that the hospital with committed patients is identified as a point source but the primary environmental impact origins from the pharmaceuticals consumed in the private homes. Ciprofloxacin was not detected in inlet samples, which indicates that this compound is degraded or adsorbed shortly after discharge into wastewater. The remaining 14 pharmaceuticals from AUH were all coinciding with this list of 16 pharmaceuticals measured above PNEC concentrations, for at least 3 of the included 4 WWTPs in the comparison of data across Denmark, see TABLE 7.

TABLE 7. Pharmaceuticals from 4 different WWTPs with exceeded PNEC values.

Pharmaceutical	Number of AUH samples above PNEC values in 9 24-hour samples	At least 5 cases above PNEC in 9 24-hour samples at AUH	Number of WWTPs with exceeded PNEC values	Pharmaceutical above PNEC values in at least 3 out of 4 WWTPs (all of the listed)
17α-ethinylestradiol	8	X	3	X
17β-estradiol	9	X	3	X
Atorvastatin	9	X	4	X
Aztihromycin	8	X	4	X
Cefalexin	6	X	4	X
Ceftazidime	7	X	3	X
Clarithromycin	8	X	4	X
Cyproterone	3		3	X
Diclofenac	8	X	4	X
Fulvestrant	5	X	4	X
Gemfibrozil	6	X	3	X
Mycophenolic acid	9	X	3	X
Sertraline	9	X	4	X
Spironolactone	4		3	X
Sulfamethoxazole	9	X	4	X

Pharmaceutical	Number of AUH samples above PNEC values in 9 24-hour samples	At least 5 cases above PNEC in 9 24-hour samples at AUH	Number of WWTPs with exceeded PNEC values	Pharmaceutical above PNEC values in at least 3 out of 4 WWTPs (all of the listed)
Sulfasalazine	9	X	3	X

Based on the campaigns conducted at the 4 WWTPs, the removal of the 16 pharmaceuticals has been calculated as an average for each WWTP based on inlet and effluent samples. For some WWTPs many samples were analysed (up to 20 samples) and for others a limited number 3-4 samples were investigated. This is the first survey conducted in Denmark representing pharmaceutical concentrations detected in in- and outlet samples from WWTPs. The results of these calculations are listed in TABLE 8. As evident in the table, some of the pharmaceuticals are completely removed at the WWTPs, as indicated by the dark green colour (70-98%), whereas the pale green denotes the removal between 40-69%. The pale yellow indicates removal rates between 25-39%, whereas the strong yellow illustrates the removal between 1-24%.

TABLE 8. Removal rates of pharmaceuticals at different activated sludge WWTPs.

Pharmaceutical	Brædstrup	Herning	Hillerød	Hjørring
17α-ethinylestradiole	NA*	97%	86%	92%
17β-estradiole	NA	97%	64%	97%
Atorvastatine	94%	79%	90%	53%
Azthromycine	59%	ND	ND	ND
Cefalexine	13%	17%	ND	ND
Ceftazidime	NA	3%	2%	ND
Clarithromycine	25%	ND	ND	ND
Cyproteron	NA	28%	ND	77%
Diclofenac	38%	27%	30%	ND
Fulvestrant	ND**	5%	ND	ND
Gemfibrozile	NA	86%	79%	28%
Mychophenolic acid	99%	73%	95%	-
Sertraline	19%	25%	ND	1%
Spironolactone	NA	24%	ND	ND
Sulfamethoxazole	67%	78%	69%	70%
Sulfasalazine	NA	17%	ND	71%

* NA, not analysed.

** ND not detected/below detection limit in inlet water of WWTP.

As evident from TABLE 8, the extent of pharmaceutical removal in the existing WWTPs varies significantly. A few pharmaceuticals were responsible for the majority of the pharmaceutical impact present in the wastewater (estradiol, mycophenolic acid, ceftazidime and sertraline), which were removed at high rates at the existing WWTPs. However, the concentrations of several pharmaceuticals in the effluent of the WWTPs were still detected above PNEC values, despite the observed removal rates.

Based on the results from the measuring campaigns at the hospital in Aarhus and the 4 WWTPs, it can be concluded that there is a strong connection between the APIs measured above PNEC at AUH and the APIs measured above PNEC at the included WWTPs. In addition to these findings, insights on hospital wastewater impact on the receiving municipality (AUH and Egå) are further investigated to determine if the hospital is responsible for exceeded PNEC values or if these can be ascribed to other diffuse sources as private homes. The conclusions here are still being processed.

However, based on the available data from AUH and other WWTPs, the same compounds were detected in both hospitals and receiving WWTPs. This indicates that the pharmaceuticals of environmental concern detected above PNEC concentrations in hospital wastewater are also detected above PNEC concentrations at the receiving municipal WWTPs. This indicates that the proposed effect of treating hospital wastewater at decentralized facilities, will only result in a partial removal of the pharmaceuticals in focus, as other diffuse sources contribute greatly to the pollution.

Conclusion:

- Significant increase in ambulatory patients has been documented at AUH from 2007-2019.
- More than 90% of the environmental impact from the mapped pharmaceuticals is ascribed to AUH is linked to ambulatory patient care in 2016 and 2017 (based on pharmaceuticals on the AMK list).
- The theoretical mapping from AUH and all other hospitals from the Central Region of Denmark from 2016 and 2017 documented that the majority of the environmental impact derives from very few different pharmaceuticals.
- The conducted theoretical mapping from 2016 and 2017 based on EPJ (Electronic Health Record) data indicate that almost the entire environmental impact originating from the pharmaceuticals from the hospitals in the Central Region of Denmark is linked to 4-6 compounds: (Mycophenolic acid (represents 71% of the environmental impact), Sertraline, Clarithromycin and Ciprofloxacin. For AUH, also Capecitabine, and Sulfamethoxazole contributed to the environmental impact from ambulatory patients.
- Measuring campaigns of pharmaceuticals from AUH wastewater document that 15 pharmaceuticals exceed the PNEC values in 5 out of 9 24-hour samples.
- There is a strong consistency between the list of 15 pharmaceuticals measured above PNEC values in AUH wastewater and the pharmaceuticals measured above PNEC at 4 WWTPs across Denmark included in this report.
    - 16 pharmaceuticals were measured above PNEC in at least 3 out of 4 of the WWTPs. 14 out of 16 pharmaceuticals are included in the mentioned pharmaceuticals from AUH.
- The capacity of pharmaceutical removal in conventional activated sludge treatment plants was shown to vary between the investigated WWTPs. These findings indicate a potential for optimizing the existing conventional activated sludge WWTPs for improved removal of, e.g., pharmaceuticals. However, several pharmaceuticals were detected in concentrations exceeding PNEC values in the effluent.
- The above-mentioned findings document that pharmaceuticals are present in the wastewater treatment plants regardless of size and number of hospitals in the catchment area. This indicates that the majority of the environmentally unwanted pharmaceuticals are discharged with household wastewater, and thereby represent a diffuse source.

The final recommendation regarding the location of treating and removing pharmaceuticals from wastewater awaits the data from the measurements conducted at the wastewater plant in Egå, Aarhus.

5. Development and construction of pilot-scale plant

5.1 Description of the eXeno™-pilot scale plant for removal of micropollutants

The pilot plant for tertiary treatment of effluent wastewater with biological removal of micropollutants was based on a fixed film technology known as Moving Bed Biofilm Reactor (MBBR) principle. The active biofilm in MBBR grows on plastic carriers enabling slow-growing microorganisms to be kept in the system giving an indefinite sludge age. The development of the biofilm and the types of microorganisms on the carriers are dependent on the organic substances present in the wastewater running into the pilot plant. Thereby, the bacteria growing on the carriers are able to grow on the organic substances present in the wastewater at a given time. The organic substances in the wastewater are therefore designing the bacterial composition of the biofilm which grows on the carriers.

The carriers used in the eXeno™-pilot were mainly AnoxKaldnes™ Z400-carriers (93%) (FIGURE 7) (AnoxKaldnes, Lund, Sweden). A small proportion of the carriers were AnoxKaldnes™ Z200-carriers (7%) added to the reactors from another pilot plant in the start-up phase for seeding and thus faster growth of biofilm. The depth of the grid on the Z400 carriers is 400 µm, providing a maximum thickness of the mature biofilm of 400 µm. The two carriers (Z400 and Z200) presented the same area for biofilm growth of 1500 m2/MP (MP, million pieces).

FIGURE 7. Z400 carriers with biofilm on the surface.

TABLE 9. General parameters of Herning Vand wastewater treatment plant (WWTP), Denmark.

Herning WWTP	
PE	150,000
Flow [m^3/y]	10,512,000
Flow [m^3/h]	1,200

The pilot-scale plant was set up at the municipal wastewater treatment plant in Herning, Denmark, with the plant parameters shown in TABLE 9. The design of the pilot plant is depicted in FIGURE 8 showing the piping for in- and outlet of each reactor, the ventilation and some instrumentation. The pilot plant consisted of three reactors with a5 m^3 volume each. The reactors contained Z400 (and Z200) carriers with a filling ratio of 23% carriers (volume), which was 63% of normal filling ratio design (37%). The results generated from the pilot testing were therefore extrapolated to normal filling degree, which will be the design of future full-scale eXeno™-plants.

$$Removal\%_{corrected} = \left(1 - \frac{C_{outlet}}{C_{inlet}}\right) \cdot \left(\frac{1}{\left(\frac{23\ \%}{35\ \%}\right)}\right)$$

$$C_{outlet,\ corrected} = (1 - C_{inlet}) \cdot Removal\%_{corrected}$$

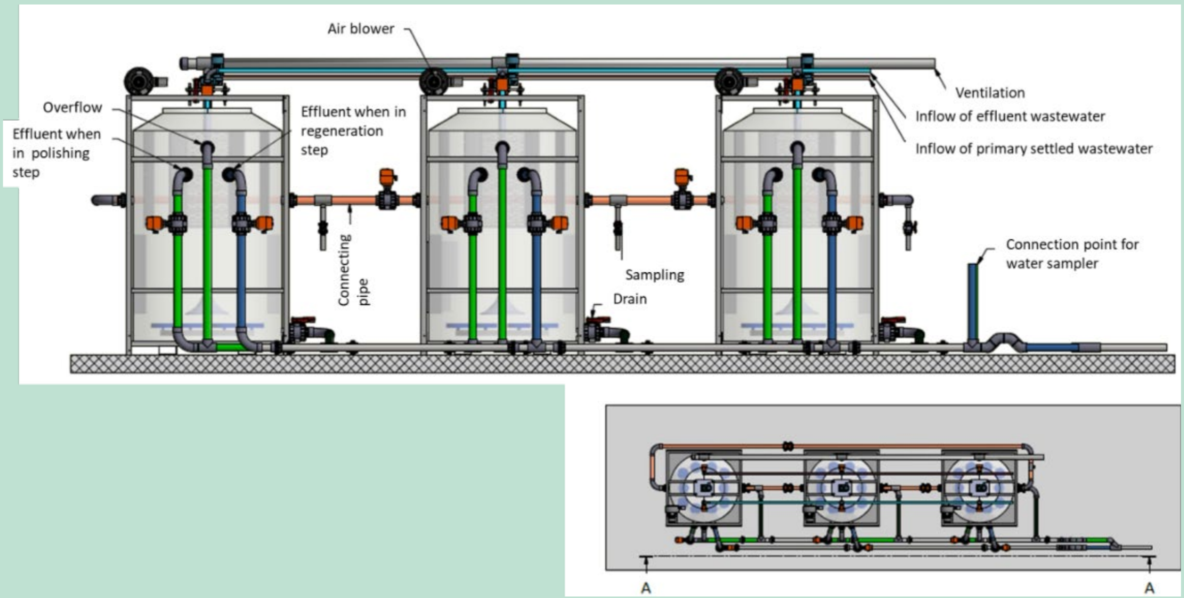


FIGURE 8. Pilot-scale eXeno™ MBBR plant constructed for the project and operated at Herning Vand, Wastewater treatment plant, front and top view, respectively.

The inlet-water to the mainline of the pilot-scale plant was effluent from Herning Vand, Wastewater treatment plant (CAS effluent). When the reactors were removing micropollutants from the effluent wastewater in the mainline, it was called the **STARVE-phase,** referring to the low concentration of organic material in the effluent wastewater (41-50 mg COD/L)**.** The inlet to the side-stream (off-line) reactor was primary settled wastewater. When the reactors were receiving this side-stream flow, it was called the **FEAST-phase,** referring to the high concentration of organic material in the primary settled wastewater (120-249 mg COD/L)**.**

A buffer tank of 1 m^3 for effluent wastewater was placed upstream in the eXeno™-pilot plant for stabilization of the feed flow to the mainline of the pilot. The buffer tank had an overflow pipe allowing a higher flow through the tank compared to the pilot plant capacity preventing anoxic conditions.

The design parameters of the pilot-scale plant are shown in TABLE 10.

TABLE 10. Design parameters of pilot-scale plant.

Pilot-design	**Main treatment line (Wastewater effluent)**	**Side-stream regeneration step (Primary settled wastewater)**
Reactor volume	2 x 5 m^3	1 x 5 m^3
Flow	2.5 - 10 m^3/h	0.1 - 2 m^3/h
Hydraulic retention time	1 - 4 h	2.5 - 50 h

The pilot plant was equipped with a PLC-control system, and the plant operation was fully automated. The operation was logged together with online measurements of oxygen, ammonia and nitrite in each reactor.

The eXeno™-pilot plant was running for 15 months in the period from June 2019 – September 2020, where the first four months were a start-up phase where biofilm growth and establishment on the bare carriers was prioritized in the pilot operation.

5.2 Description of the wastewater flow to the eXeno™-pilot plant (mainline and side-stream)

Two reactors operated in the mainline receiving effluent wastewater and working as the polishing reactors removing organic carbon including micropollutants and removing nitrogen by nitrification (Treatment process, FIGURE 9a). The third reactor (position C, FIGURE 9) was fed with primary settled wastewater (side-stream) in order to keep biofilm growth on the carriers (regeneration of biofilm). Pilot plant effluent was discharged to the municipal sewer and treated in the WWTP again.

After a defined time of operation of the reactors in either mainline or side-stream, the flow was changed, moving the second polishing reactor (position B, FIGURE 9) to the side-stream for regeneration of biofilm, and the reactor just regenerated in the side-stream (position C, FIGURE 9) was moved to first position in the main treatment line (position A, FIGURE 9). This rotation was carried out in defined time intervals in order to let all reactors get a period of regeneration (primary settled wastewater) before being placed in the mainline again for removal of micropollutants. The purpose of the rotation was to ensure that the microbial community was able to regenerate after periods with very limited organic material available. The relatively short periods in the side-stream prevented overgrowth of the carrier biomass by fast growing heterotrophic microorganisms present in the primary settled wastewater. Patent applications, Swedish patent application no 1650321-A1 and European patent application no WO 2017/153361-A1, have been filed for this operation mode, and the Swedish patent (539304-C2) has been granted.

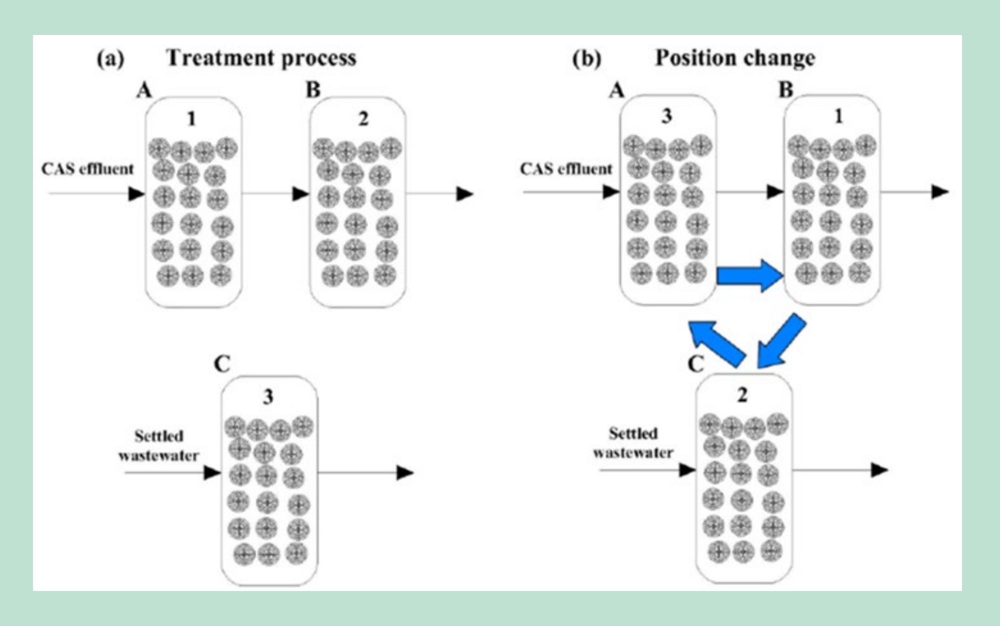


FIGURE 9. Mainline: polishing step of conventional activated sludge effluent (CAS) in two MBBR reactors (positions A and B), while the regeneration of biofilm was stimulated in the third MBBR reactor in the side-stream (position C).

5.2.1 Sampling of the pilot-scale plant

Monitoring of operational parameters

A number of parameters were monitored to ensure stable operation of the eXeno™-pilot plant. Inlet flow, pH, dissolved oxygen, ammonium, nitrate and temperature were recorded weekly or continuously with online sensors installed on each reactor. On a weekly or monthly basis, analysis was performed for COD, TOC, TN, TP, NO_3-N, NO_2-N, NH_4-N and suspended solids. Biomass amount on carriers (biofilm) was determined regularly (20 carriers per analysis).

Sampling procedures for micropollutants

Samples for analysis for micropollutant removal were sampled at the end of each experimental test-phase (see section 8). Samples for batch test analysis in the laboratory (section 6.2) consisted of 130 carriers (corresponding to filling degree at pilot scale) in one litre of wastewater from each reactor and three litres of wastewater for the experimental analysis in the laboratory. In-situ samples for measurement of micropollutant concentrations coming in and out of the pilot plant were sampled as 24-hour flow-proportional samples of the inlet and outlet of each reactor, when they were in the inlet position (Position A, FIGURE 9) and in the outlet position (Position B, FIGURE 9). Further details on sample preparation, measuring and data treatment can be found in (Tang et al., 2021).

6. Development of the eXeno™ operation

6.1 Polishing principle

The eXeno™-plant is implemented as a tertiary treatment step at municipal treatment plants, where the compounds not degraded in the existing treatment plant are polished in the eXeno™ biofilm technology. The active biofilm in the eXeno™-technology is designed by the compounds present in the effluent water, as the bacteria able to grow in the reactors are the bacteria able to degrade the compounds present in the effluent. For further description, see section 7.1.1.

In this project, the eXeno™-technology was upscaled from proof-of-concept lab-scale reactors to large-scale operation on the effluent water flow from Herning Vand, Wastewater treatment plant, with the variations naturally occurring at full-scale treatment plants, where different seasons, precipitation variations and weather changes affect the operational parameters in the polishing step. The aim of the project was to operate the pilot plant with different operational settings and thereby define the limits of operation and optimize the removal of pharmaceuticals at different in-situ conditions, see FIGURE 10.

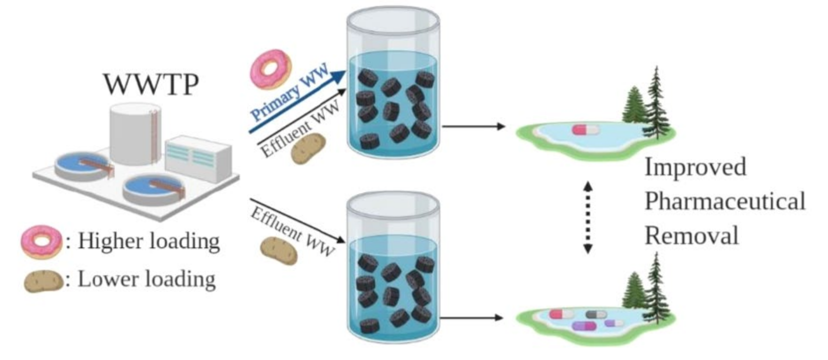


FIGURE 10. Principle in biofilm-based feast-famine technology for improving pharmaceutical removal in the polishing step, where the reactors are offline during defined periods receiving primary wastewater giving a nutrient boost of the active biofilm to be used in the subsequent polishing step of treating effluent water.

6.1.1 Laboratory-scale operation

In the MerEFF project, several experimental periods with reactor setup were carried out. The results were recently published by Tang et al., 2020, and only a selection of these data are depicted here.

In short, seven sets of MBBRs (3 L) each containing AnoxKaldnes K5 carriers (500 pieces) were placed at a municipal wastewater treatment plant (Egå, Denmark) for six months (September 2018 to March 2019). The plant has a capacity of 112,000 population equivalent (9,000 population equivalent is from a candy factory), receiving a total influent of 7,858,390 m^3/year and a total COD load of 4,100 tons/year in 2019. During this time, all reactors were constantly fed with effluent from the WWTP, while six out of the seven were also being intermittently fed with primary wastewater as an extra organic input (FIGURE 11). Effluent was fed constantly to all reactors as MBBRs in practice are intended to be used as a polishing step for WWTP effluent. Different flow rates of primary wastewater and different operating times for both the feast and famine periods were applied to the six reactors, while the seventh reactor (fed with effluent only) was used as an experimental control (See FIGURE 11). After this study period at Egå WWTP, the seven reactors were then transferred to another municipal wastewater treatment plant (Herning, Denmark) to be able to assess a broader range of feast-famine operating times. In 2017, Herning WWTP had a total influent of approximately 10,000,000 m^3/year, covering 34,000 households. This study in Herning was conducted from June 2019 until December 2019. Throughout all studies, all reactors were kept under aerobic conditions by using air pumps.

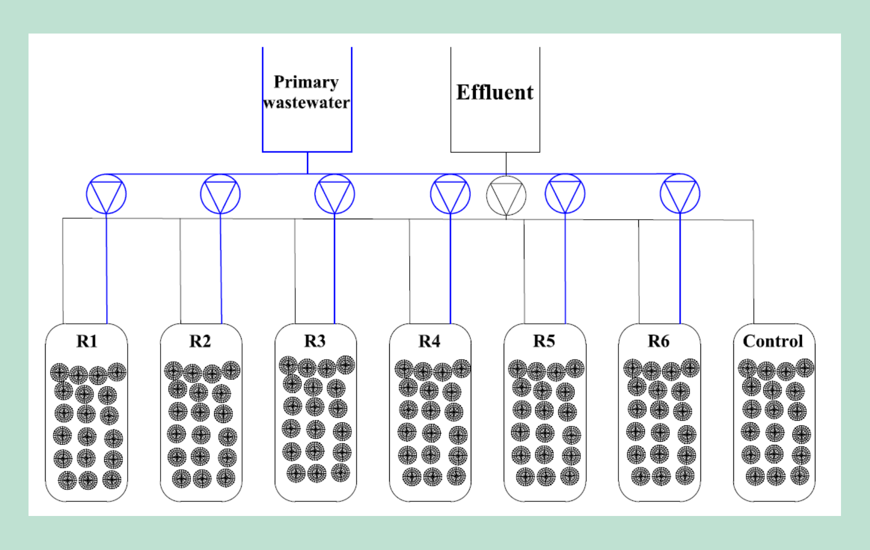


FIGURE 11. Schematic diagram of seven sets of MBBR reactors. R1-R6 were fed with different combinations of primary wastewater and effluent, while control was only fed with effluent.

On each study site (Egå and Herning), experiments were carried out during two separate time periods. For each experimental period, the reactors were operated continuously for three months in advance to ensure sufficient time for the biofilms to adapt to the differing feast-famine feeding strategies. TABLE 11 shows the differing feast-famine cycles, where the starvation period indicates how many hours the reactor was fed only with effluent wastewater, and the feast period indicates how many hours the reactor was fed with both primary wastewater and effluent.

The daily COD/NH4-N loading to each reactor was calculated from flow rates and concentrations of COD/NH4-N of primary wastewater and effluent, as well as starvation/feast period.

TABLE 11. Operational settings to all MBBRs during all experimental periods and sites. ∞ represents “constantly”, while N.A. represents “not available”.

Operational settings	Starvation period per cycle (h)				Feast period per cycle (h)				Total COD load per day (mg/d)				Total NH4-N load per day (mg/d)			
	Egå A	Egå B	Herning A	Herning B	Egå A	Egå B	Herning A	Herning B	Egå A	Egå B	Herning A	Herning B	Egå A	Egå B	Herning A	Herning B
Reactor 1	12	26	80	80	4	4	4	4	1047	1000	461	422	108	103	27	41
Reactor 2	16	32	40	40	8	4	4	4	890	860	572	490	63	73	39	48
Reactor 3	20	26	80	80	4	4	8	8	890	860	543	494	68	71	44	49
Reactor 4	20	20	60	60	4	4	8	8	1047	1000	608	567	111	101	54	56
Reactor 5	16	32	40	40	8	4	8	8	1047	1000	683	699	106	104	61	68
Reactor 6	12	20	20	20	4	4	4	4	890	860	788	779	65	70	74	76
Control	∞	∞	∞	∞	N.A.	N.A.	N.A.	N.A.	731	717	131	191	29	46	1	17

The calculated theoretical curves show the accumulated concentrations of either ammonium or COD during feast periods without any biological activity in the reactor, see FIGURE 12. These curves were compared to the actual measured concentrations of ammonium or COD along with guiding limit values for direct discharge. Measurements were taken throughout the feast period and compared to the theoretical curve without biological activity. The difference between the theoretical and the measured curves shows that the biofilm was capable to nitrify and degrade COD during feast periods, presumably in the form of organic carbon, which indicates the presence of heterotrophic organisms. In addition, the presence of nitrifiers was also evident due to the conversion of the toxic ammonia to nitrate. The reactors were only operated aerobically, and therefore a complete removal of nitrogen was not anticipated. Similar measurements were also conducted for Herning experiments (data not shown).

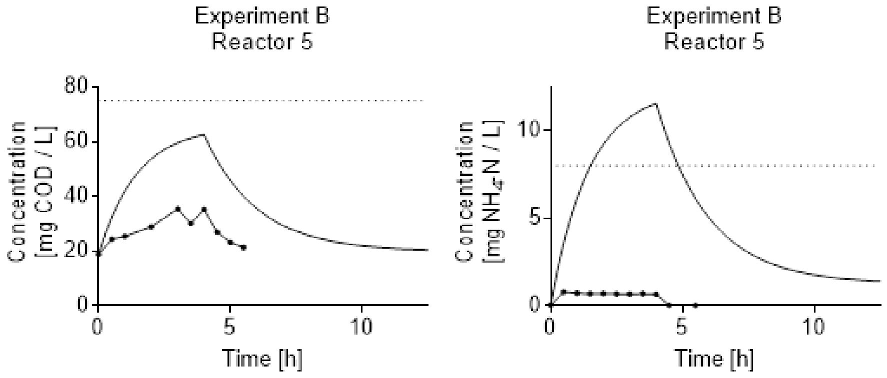


FIGURE 12. The concentration in and out of the reactor for COD and ammonium in reactor 5, in experiment B for Egå experiments. The dotted line illustrates the discharge requirements. The solid line illustrates the abiotic theoretical curve, and the black points show the measured concentrations.

Previous studies have shown a correlation between nitrification rates and the biological degradation of hardly biodegradable pharmaceuticals (Falås et al., 2012 a,b), specifically for diclofenac and sulfamethoxazole (Torresi et al., 2016). In this study, a potential correlation was also investigated, and the results are shown in FIGURE 13. During the 3 months from Experiment A to Experiment B, an increase in oxygen consumption from AOBs was observed for all reactors, and the control reactor nearly increased five times (0.003 to 0.014 mg O2 / L / min.).

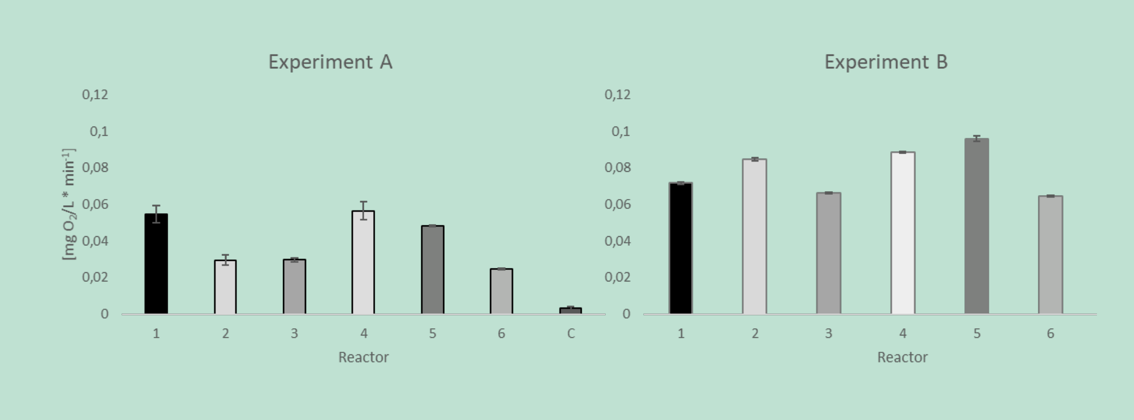


FIGURE 13. Oxygen consumption from ammonium oxidizing bacteria, AOBs in each reactor for experiment A and experiment B.

Similar increase in the ability to remove pharmaceuticals were also observed, see FIGURE 14.

To investigate the potential of micropollutant removal by MBBR reactors that were operated with different feeding settings, spiked batch experiments were conducted. Concentrations of the investigated micropollutants over time in batch experiments were plotted with first order kinetics and six selected compounds among four batches, as shown in FIGURE 14.

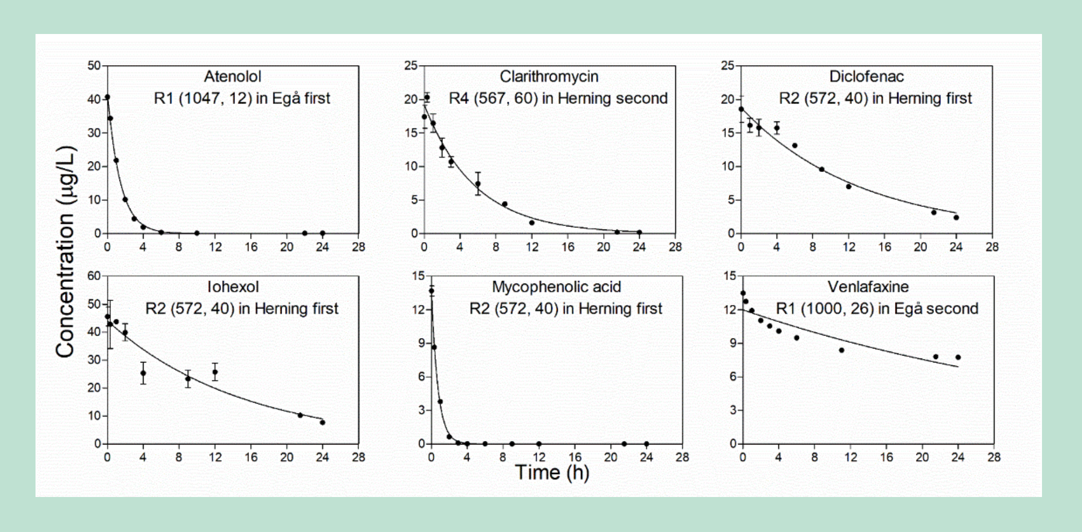


FIGURE 14. Concentrations of selected pharmaceuticals during experimental time, plotted with first order kinetics: A) atenolol; B) clarithromycin; C) diclofenac; D) iohexol; E) mycophenolic acid, and F) venlafaxine. Descriptions under each pharmaceutical name represent: Reactor no. (COD, starvation time) and study site.

FIGURE 15 represents six compounds from different groups which showed the highest kinetics under optimal operation conditions. For example, in case of atenolol, the highest kinetics were observed in Reactor 1 at Egå A, i.e., 1047 mg COD/d loading with a 12-h starvation cycle. Diclofenac showed the optimal removal kinetics with 572 mg COD/d loading and a 40-h starvation cycle.

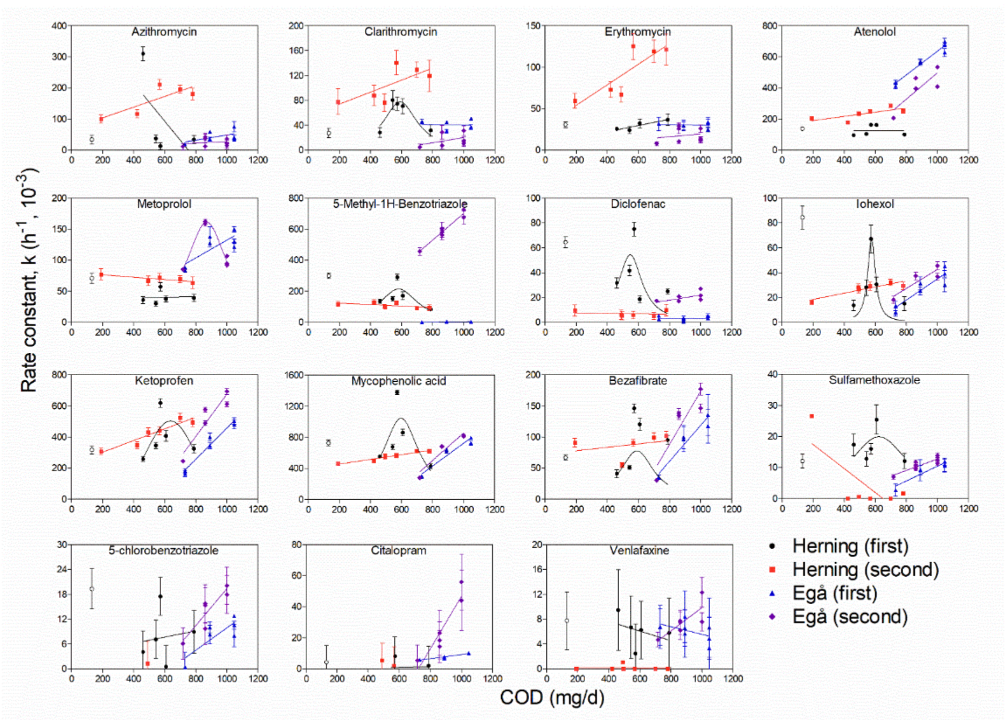


FIGURE 15. Correlations between daily COD loading (mg/d) and the rate constant of selected micropollutants.

Positive linear correlations between COD and removal rate constants were typically observed at all sites. Of the 15 micropollutants studied, the removal rate constants of 9 were observed to reach their peaks when increasing COD between 550 and 600 mg/d (except for metoprolol, where it was observed between 800 and 900 mg/d of COD), where after the removal rate constant decreased again.

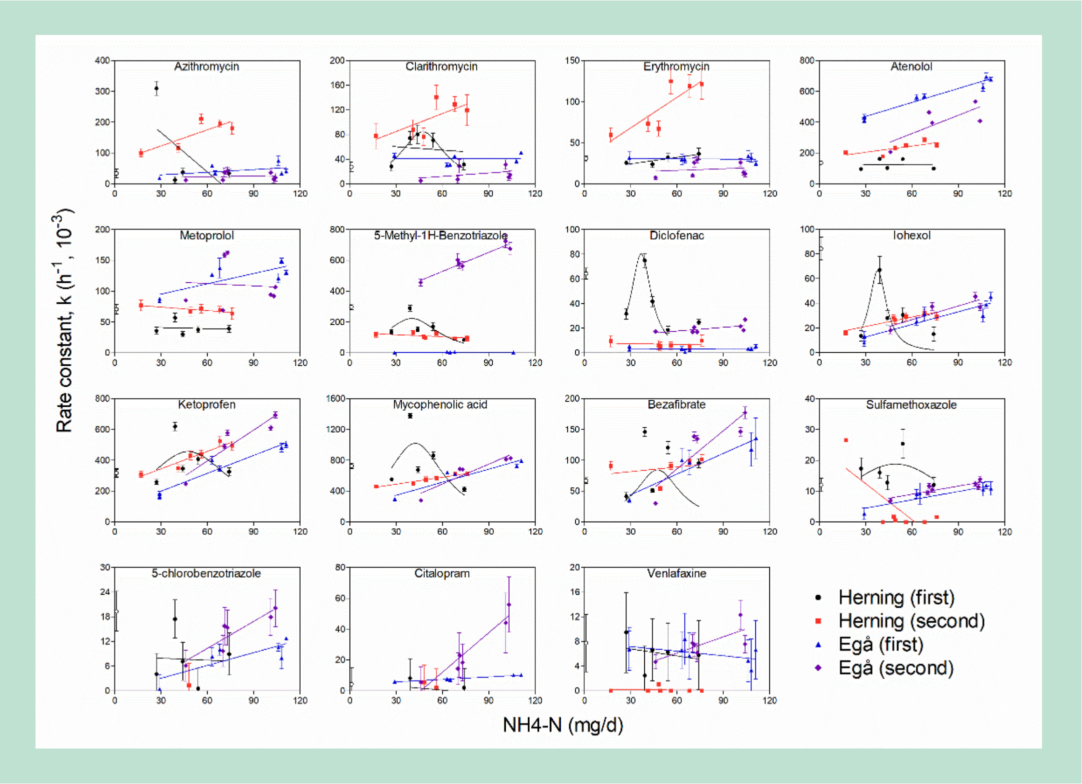


FIGURE 16. Correlations between daily NH4-N loading (mg/d) and the rate constant of selected micropollutants.

Similar relationships were also observed between rate constants and NH4-N (FIGURE 16). Typically, an increase in NH4 was linked to an increased rate constant at each site. Removal rate constants were observed to increase with NH4-N to a peak, after which the removal rate dropped again. This peak was consistent between 40-50 mg/day of NH4-N for most compounds, except for the peak for metoprolol (between 55 and 70 mg/d). Additionally, the fitting patterns of NH4-N vs. rate constant for individual compound were very similar to the fitting patterns observed for COD vs. rate constant.

6.2 Adsorption of Benzotriazoles using sludge-based biochar

Among the tertiary treatment processes, powder activated carbon (PAC) is a matured and cost-effective way to remove micropollutants, and it is already available for implementation.

In this study, biochar was provided by AquaGreen and was made from activated sludge via superheated steam drying and pyrolysis, see FIGURE 17. Due to the characteristics of activated sludge, the produced biochar had a rather soft texture making it more suitable as PAC instead of being used as filler for granular-activated carbon columns. To address the issue of uneven particle size of the biochar, it was first grounded and then filtered through a sizer to obtain particles with diameters of between 250 and 355 µm.

FIGURE 17. Image of activated carbon originating from activated sludge before grinding.

For the adsorption experiment, PAC was used on a shaker with 135 rpm for 48 hours at ambient temperature, and the effect of different doses were investigated. The effect of PAC doses (1, 5, 10, 20, 30, 50, and 100 mg/L) on the removal of micropollutants was studied using effluent water from Herning municipal wastewater treatment plant to include the realistic influence of organic matter. Benzotriazoles are a group of corrosion inhibitors that are widely detected in industrial/municipal wastewater.

To predict the required PAC dose for removal of 80% of micropollutants, a modified Freundlich model was used, and the calculated PAC doses were normalized with the effluent DOC (17 mg/L in this study) to compare with other literature studies, TABLE 12. The specific doses of PAC for removing 80% of benzotriazoles in current study were close to the doses established by other researchers. However, the applied PAC in this experiment were more cost-effective than conventionally produced PAC.

TABLE 12. The required specific doses of PAC for 80% removal.

	mg PAC/ mg DOC			
	Present study	**Altmann et al., 2014**	**Bonvin et al., 2016**	**Zietzschmann et al., 2014**
Benzotriazoles (1-H-Benzotriazole, 5-Chlorobenzotriazole, 5-methyl-1h-benzotriazole)	1.1~13	1.1	2.4	~4.0

6.2.1 Pilot-scale operation

6.2.1.1 Overview of operation conditions with defined feast/starve periods

The start-up phase lasted from June 2019 to October 2019, and in this period the biomass was established on new carriers in the pilot-plant reactors. To facilitate similar build-up of biofilm in all three reactors, the flow of wastewater to the reactors was run in the start-up phase, where each reactor concomitantly received a mixture of primary settled wastewater (3 m^3/h, 40% of total) and effluent water (4.6 m^3/h, 60% of total). A higher flow of primary settled wastewater would most likely have supported a faster build-up of biofilm, which will be the future procedure of eXeno™ start-ups. However, this was not possible with the current pilot plant design.

After the start-up phase, the pilot plant was running in polishing mode, where the reactors were run in series shifting between the polishing and regeneration mode at defined time intervals (TABLE 13) (for further description, see section 5.1, FIGURE 9). The continuous operation of polishing mode lasted 10 months (from October 29, 2019 to August 31, 2020), where the operational settings were changed during the experiment with seven different operational phases. At the end of each operational phase, samples were analysed for micropollutant removal ability through spiking experiments. Moreover, the actual micropollutant removal in the pilot plant was measured in April, May and August (in situ sampling, TABLE 13). The operational parameters of the pilot plant were logged during the entire experiment either with online sensors or through lab-analysis (for detailed description, see section 7.1.3). From these measurements, the removal of TOC, COD and ammonia was monitored.

The seven experimental phases of the project differed in the length of feast-starve periods and the feast-flow (TABLE 13), where the starve phase ranged from 36-72 hours and the feast phase ranged from 18-36 hours, while the feast flow ranged from 10-14% of the total flow. The different feast-starve conditions were investigated to understand the connection of the feast-conditions and the micropollutant removal efficiency even further.

TABLE 13. Operational parameters of the eXeno™ pilot plant during the MerEFF project. The operational parameters were selected to test the limits of the performance of the pilot plant and not to gain optimal performance. During the project, seven different operational phases were examined in different settings. After each phase, the removal of pharmaceuticals was evaluated. Average concentrations (+/- std.dev) of COD, ammonia and biomass are shown.

	December	February	April	May	June	July	August
Period	29 Oct 19- 17 Dec 19	17 Dec 19- 10 Feb 20	19 Feb 20- 15. Apr 20	15 Apr 20- 15 May 20	15 May 20- 19 Jun 20	19 Jun 20- 3 Aug 20	3 Aug 20- 31 Aug 20
Samples (date, name)	041220 Batch 1 (no in situ)	030220 Batch 2 (no in situ)	3003-020420 Batch 3 In situ April	12-150520 Batch 4 In situ May	190620 Batch 5 (no in situ)	210720 Batch 6 (no in situ)	21-280820 Batch 7 In situ August
Polishing (starve) [h]	48	36	48	48	48	48	72
COD [mg/L]	50 (±11)	45 (±14)	41 (±12)	43 (±6)	45 (±14)	42 (±10)	43 (±20)
NH4-N [mg/L]	3.3 (±1.2)	4.1 (±0.7)	5.5 (±1.7)	3.0 (±0.7)	1.8 (±0.6)	1.3 (±0.3)	2.7 (±1.7)
Regeneration (feast) [h]	24	18	24	24	24	24	36
COD [mg/L]	182 (±67)	154 (±51)	120 (±46)	209 (±52)	249 (±89)	183 (±70)	195 (±90)
NH4-N [mg/L]	14 (±5)	12 (±7)	10 (±9)	23 (±5)	27 (±5)	27 (±15)	35 (±39)
Flow - Feast /Starve [%]	10	10	14	14	14	10	10

	December	February	April	May	June	July	August
Temperature [°C]	13	11	10	12	15	17	17
Hydraulic Retention Time [h]	2	2	2	2	2	2	2
Biomass per carrier [g]	0.90 ±0.11)	1.23 (±0.25)	1.45 (±0.09)	2.05 (±0.35)	1.62 (±0.41)	1.42 (±0.12)	0.99 (±0.01)

As a consequence of the different seasons and precipitation during the experimental period (October to September), the average COD and ammonia-concentrations in the pilot plant differed in each experimental phase (following the incoming wastewater concentrations to Herning Vand WWTP). In the effluent water, the COD concentrations were between 41 and 50 mg/L, and ammonia concentrations varied with a factor 4 from 1.3 to 5.5 mg/L (TABLE 13). In the primary settled water, the COD concentrations were varying with a factor 2 between 120 and 249 mg/L, and the ammonia concentrations were varying with a factor 3, between 10 and 35 mg/L. The average water temperature ranged from 10-17°C (TABLE 13). Altogether, these variations followed the annual variation of the wastewater treatment plant and resulted in very different operational conditions during the pilot experiment.

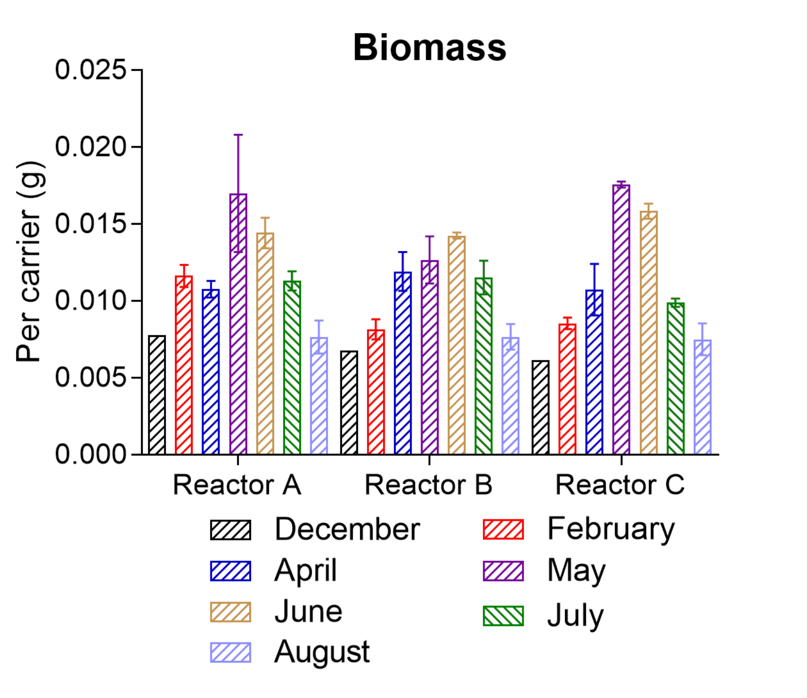


FIGURE 18. Overview of biofilm content on carriers during the different batch experiments.

To follow the development of the biofilm in the reactors, the biofilm mass per carrier was evaluated during the experiment together with each sampling event. As seen from FIGURE 18, the biomass abundance between the three reactors was very similar and only minor differences were detected, which suggests that the patented shifting flow operation between the three reactors was able to obtain a homogenous biomass concentration. However, the amount of biomass varied during the experiment with the highest mass detected in May and June, where the reactors had the best FEAST conditions with the highest flow of primary settled wastewater (14% of effluent flow, TABLE 13) together with the highest measured concentrations of COD and ammonia in the feast-phase (TABLE 13), which most probably explains the higher biomass detected in these months.

The biofilm amount in the reactors decreased from May to August corresponding to the reduction in the flow of primary settled wastewater in the feast-phase (from 14% to 10% of effluent flow) and prolonged starve-phases with the longest duration of 72 hours (TABLE 13) in August. This highlights the importance of the organic load conditions in the feast-phase for the biofilm growth and proliferation and that the feast-phase is an important design parameter in the optimization of the best biofilm for removal of pharmaceuticals.

6.2.1.2 Nutrient removal efficiency of the eXeno™ pilot plant (COD, N)

During the pilot experiments, measurements of key parameters were followed to monitor the concentrations and removal of dissolved organics and nitrogen, analysed as COD and ammonia, see FIGURE 19 and FIGURE 20.

The results showed that the eXeno™-pilot supplements the WWTP in both COD and ammonia removal, providing increased capacity of the treatment plant when implementing the eXeno™ polishing technology.

COD-removal in the pilot plant

The COD concentrations in both the inlet (33-73 mg/L) and outlet (26-56 mg/L) wastewater of the pilot plant during the one-year experiment were constantly below the discharge limit of 75 mg/L (FIGURE 19). Moreover, the outlet of mixed effluents (combined with effluent from regeneration reactors and starving reactors) in terms of COD concentrations, with an average of 44 mg/L, were also well below the discharge limit allowing even further expansion of the WWTP capacity when implementing the eXeno™ polishing step (outlet mixed in FIGURE 19). However, during the pilot-scale experiments, the regeneration effluents were instead directed back to the secondary clarifier of the WWTP for full treatment.

The average COD removal in the pilot plant was 14% during the entire experimental period with maximum removals of up to 46% (FIGURE 19). When considering only the last two experimental phases (phase 6+7) where the biofilm was fully established, the average COD removal was 17% (6-36%). The COD species out of the pilot were mostly inert soluble COD, and therefore higher COD reductions are not expected. In this study, the COD measurements were based on grab samples, which most probably explains the observed variations in the COD removal over time. These variations will most likely be levelled out if 24h continuous flow samples were analysed instead.

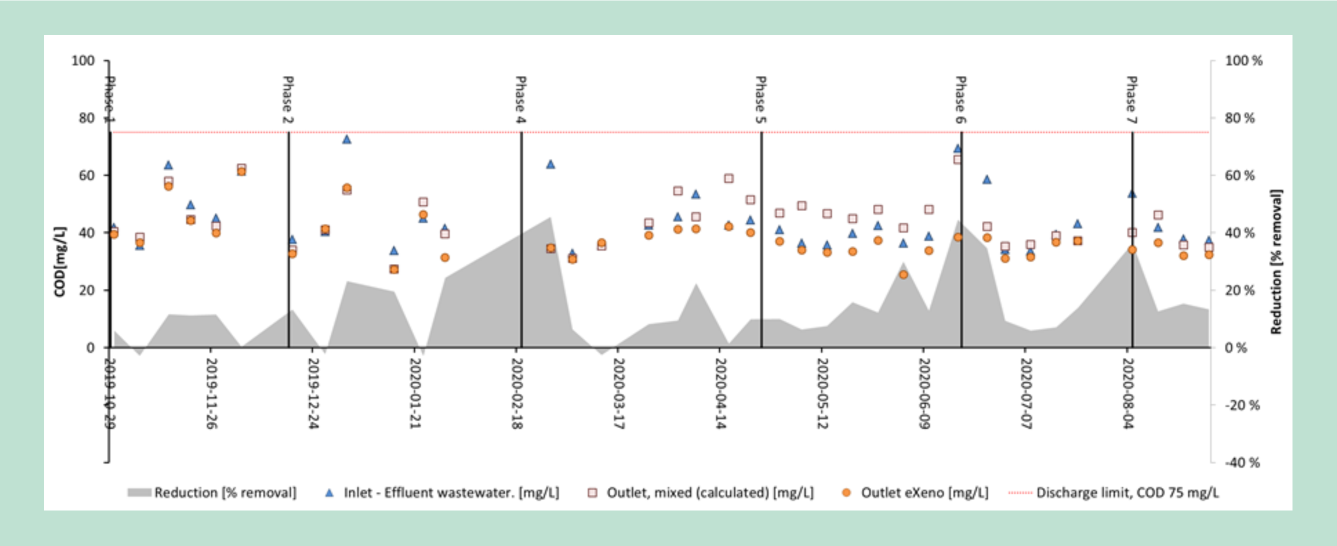


FIGURE 19. Removal of COD in the eXeno™ pilot [grey area]. Inlet- and outlet-concentrations of COD [mg/l, filled symbols] and the calculated outlet COD concentrations, when including the outlet from the reactor in regeneration phase (primary settled water) [mg/l, red square]. The discharge limit at Herning WWTP is shown as a dotted red line. Shift in operational phases is illustrated with vertical lines.

Ammonia removal in the pilot plant

The ammonia concentrations in the inlet wastewater to the eXeno™ pilot plant varied significantly during the year (0.9-8.7 mg/L) with an average concentration of 3.3 mg/L (FIGURE 20). The highest observed ammonia concentrations in the inlet wastewater were a result of periods with high rainfalls in November to March. Importantly, these high inlet ammonia concentrations did not affect the ammonia removal in the pilot plant, and the outlet concentrations (0.08-1.9 mg/L) of the pilot were always significantly below the discharge limit (3 mg/L) in spite of the inlet variations.

The observed constant high ammonia removal with an average of 93% (78-97%) in the pilot plant (FIGURE 20) expands the total ammonia removal capacity of the entire treatment plant when implementing the eXeno™ polishing step. The amount to which the eXeno™ technology exactly adds to the total ammonia-removal design-capacity must be established further under different operational parameters and plant designs.

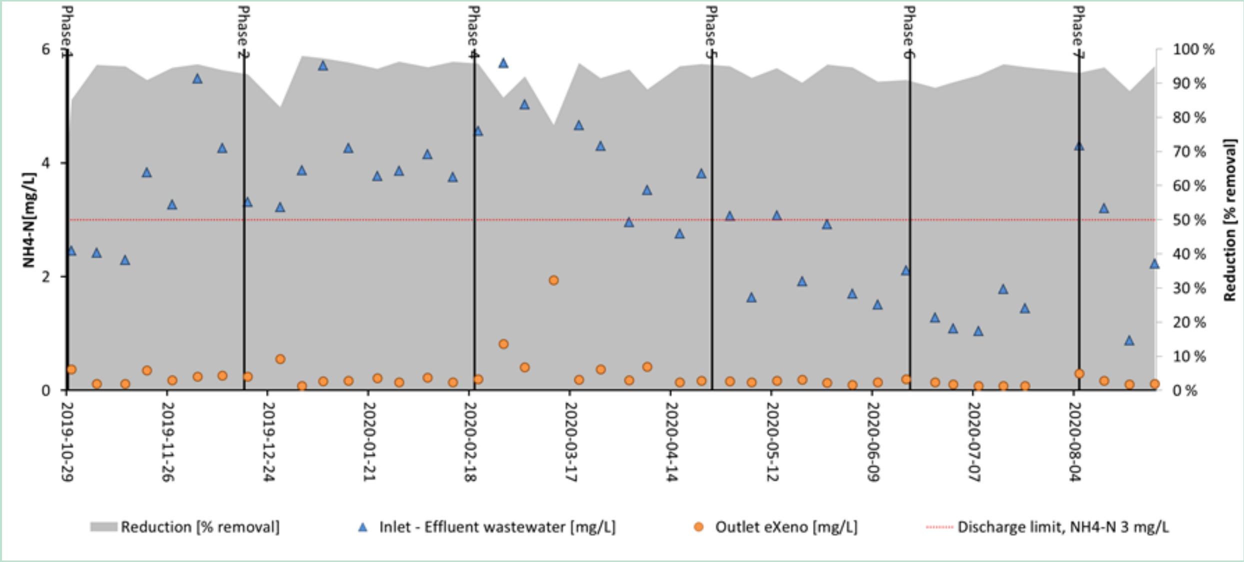


FIGURE 20. Removal of ammonia in the eXeno™ pilot [grey area]. Inlet- and outlet-concentrations of ammonia [mg/l, filled symbols]. The discharge limit at Herning WWTP is shown as a red line. Shift in operational phases is illustrated with vertical lines.

<u>Removal of total nitrogen</u>

In the pilot experiment, anoxic regimes favouring denitrifying conditions were not prioritized, as focus was exclusively on the removal of pharmaceuticals under oxic conditions. Therefore, the reduction of nitrate generated from ammonia was not evaluated further, but this would be possible by further optimization and prioritizing of the length of the anoxic phases in the reactor in the regeneration phase to increase the removal of total nitrogen byurther adding capacity to the wastewater treatment plant.

6.2.1.3 Removal potential for micropollutants in the pilot-scale eXeno™

To evaluate the potential of the removal of micropollutants by the biofilm in each reactor, batch experiments were conducted at the end of each operational phase (May, June, July and August). The carriers from each reactor (A, B and C) in pilot were taken to the laboratory and were added into 3 L of glass reactors which were used to mimic the operational conditions in the pilot. The same filling ratio was applied as in the pilot. A solution of the investigated micropollutants were spiked into each glass reactor. Samples were taken over a period of 24 hours. The concentrations of the micropollutants were fitted with first-order kinetics to give first-order rate constants of the compounds, as shown in FIGURE 21.

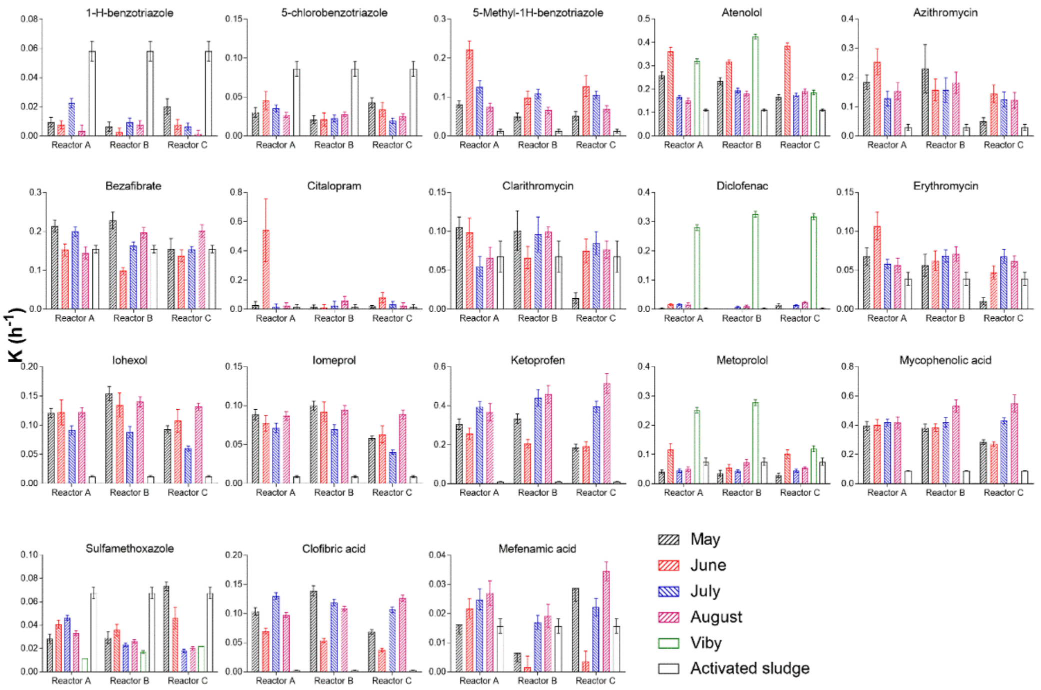


FIGURE 21. Comparison of the first-order rate constants of the selected compounds among different batches including the activated sludge. Corresponding values from Viby (Tang et al., 2017) are included for comparison.

Based on the results in FIGURE 21, higher rate constants for half of the compounds were observed for the batch in June (5-chlorobenzotriazole, 5-methyl-1H-benzotriazole, atenolol, azithromycin, citalopram, erythromycin and metoprolol). The batch in June was operated with 14% of flow ratio between primary wastewater and effluent water under the feeding strategy of a 48-hour starvation cycle followed by a 24-hour feast. In the batch experiments, higher rate constants for the remaining compounds were found in MBBR, compared to activated sludge from Herning (2.7 g/L of total suspended solids), except for 1-H-benzotriazole, 5-chlorobenzotriazole and sulfamethoxazole. This is in line with the previous work, where MBBR has been proven to have a much higher capacity of micropollutant removal than CAS (Escolà Casas et al., 2015; Ooi et al., 2018; Tang et al., 2021). Interestingly, the rate constants of iohexol and iomeprol from the X-ray contrast media group, which are commonly detected in hospital wastewater, are 10 times higher in MBBR than in activated sludge. However, the rate constants of diclofenac in this project were still significantly lower than the rate constant rate identified in the MERMISS project, where two 3 L of staged MBBR were used for polishing the pharmaceuticals in municipal effluent in Viby and were intermittently fed with raw wastewater. This feeding strategy has been patented and was also applied in this project. The removal differences, even under the same feeding solution, could be attributed to the much older biofilm existing in the Viby WWTP with abundant bio-degraders which can remove diclofenac efficiently. Therefore, the microbiological communities are continuously examined on different biomasses from the MBBR carriers in the studies within MERMISS project and in this project with an aim to find that key bacteria play a role in the removal of diclofenac, which is a hardly biodegradable compound.

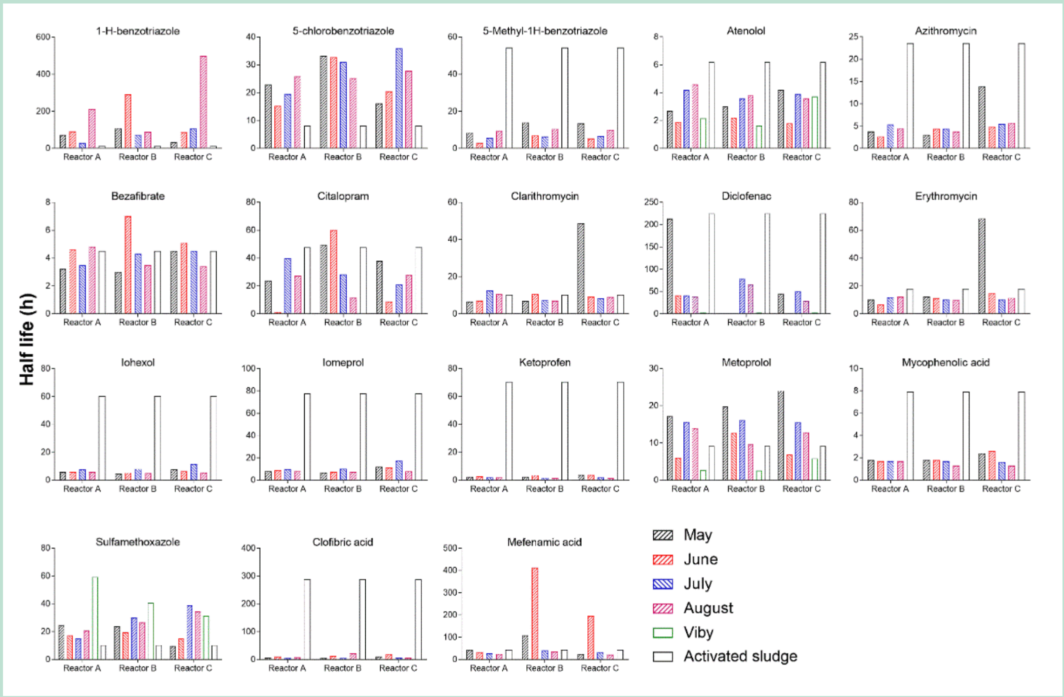


FIGURE 22. A comparison of the half-life time of the selected compounds among different batches in this project including the activated sludge from Herning WWTP and the previous study in Viby WWTP.

In contrast to the results in FIGURE 21 where the higher number signifies a higher removal performance, here the shorter half-life time the better removal of the compound. According to the results in FIGURE 22, atenolol, bezafibrate have a relatively shorter half-life time compared to other compounds in both MBBR and activated sludge. For 5-methyl-1H-benzotriazole, azithromycin, diclofenac, iohexol, iomeprol, ketoprofen, mycophenolic acid, and clofibric acid, their half-life times are approximately 4 to 60 times shorter in MBBR than in activated sludge, while the shorter or similar half-life time was observed in activated sludge compared to MBBR, for citalopram, metoprolol, sulfamethoxazole, and mefenamic acid.

6.2.1.4 In situ removal efficiency of pharmaceuticals in the eXeno™ pilot plant

To investigate the removal efficiency of the compounds present in the wastewater at Herning Wastewater Treatment Plant, wastewater from the inlet and outlet of the pilot plant were sampled in May and August. These were24h continuous flow samples of the inlet and outlet water from the mainline of the pilot plant (section 7.1.1). The main treatment line is two of the pilot reactors treating the wastewater in series. In this sampling campaign, this meant sampling reactor C and reactor A together (Reactor C+A), sampling reactor A and reactor B together (Reactor A+B), and sampling reactor B and reactor C together (Reactor B+C). In total, 15 different pharmaceutical compounds were detected in the inlet (corresponds to WWTP effluent) at concentrations above the detection limit. Three compounds (diclofenac, sertraline and venlafaxine) were above the PNEC values in both May and August (FIGURE 23). Six of the detected compounds do not have any recommended PNEC values yet. The inlet concentrations of each compound varied significantly from day to day, especially in the August samples, resulting in different load conditions (and subsequent micropollutant concentrations) in the reactors when polishing the effluent wastewater.

In May, no significant removal of the compounds was observed, except from mycophenolic acid, which was removed completely in reactor C+A (FIGURE 23). The poor removal observed in May was most likely a consequence of a long period of rain, which had resulted in low concentrations of ammonia and COD in the feast-phase, and therefore a biofilm less fitted for efficient removal. In August, however, a high removal of all compounds was observed in one or several reactors, which was evident after one year of different operational conditions. It was indeed possible to achieve a competent biofilm with a generally high removal of the pharmaceuticals. Moreover, as a consequence of the polishing with the pilot plant, the concentrations of effluent water with the pilot plant, 5-chlorobenzotriazole, bezafibrate, diclofenac, iohexol, mycophenolic acid, sertraline and venlafaxine were reduced to concentrations below PNEC in at least one of the reactors. Interestingly, three of the four most environmentally problematic compounds from the hospitals (sertraline, mycophenolic acid, clarithromycin and ciprofloxacin), as described in section 6.2, were detected in the wastewater in both May and August. Here, a significantly high removal was detected in August, where 30-100% removal of sertraline, 80-100% removal of clarithromycin and 100% removal of mycophenolic acid was observed (FIGURE 23).

FIGURE 23. In- and outlet concentrations of the pilot plant in May (top) and August (Bottom). The concentrations are shown for each reactor combination of the pilot plant. In case of compounds where PNEC can be found in literature, these are included in the graphs (EU list, the AMK list 2015, ECHA). Note that these results are from 2020, while the results from the WWTP in chapter 6 are from 2019 and therefore not the same.

Generally, the combination of B+C reactors showed a better removal than the C+A and A+B reactors (FIGURE 24). We have no obvious explanation to this discrepancy except that the different reactors were exposed to different concentrations during the sampling campaign of three following days, with 24-hour sampling of each reactor combination. For further understanding of the detected differences in reactor performance, we are looking further into the bacterial composition of the reactor biofilm to evaluate which bacterial species are responsible for the removal of pharmaceuticals, making it possible to select these species and thereby design a proper biofilm with the highest removal of the pharmaceuticals, which in this study was 79% of the B+C reactors, and which on top of the upstream removal in the WWTP gave an average total removal of 93% for different pharmaceuticals (FIGURE 24).

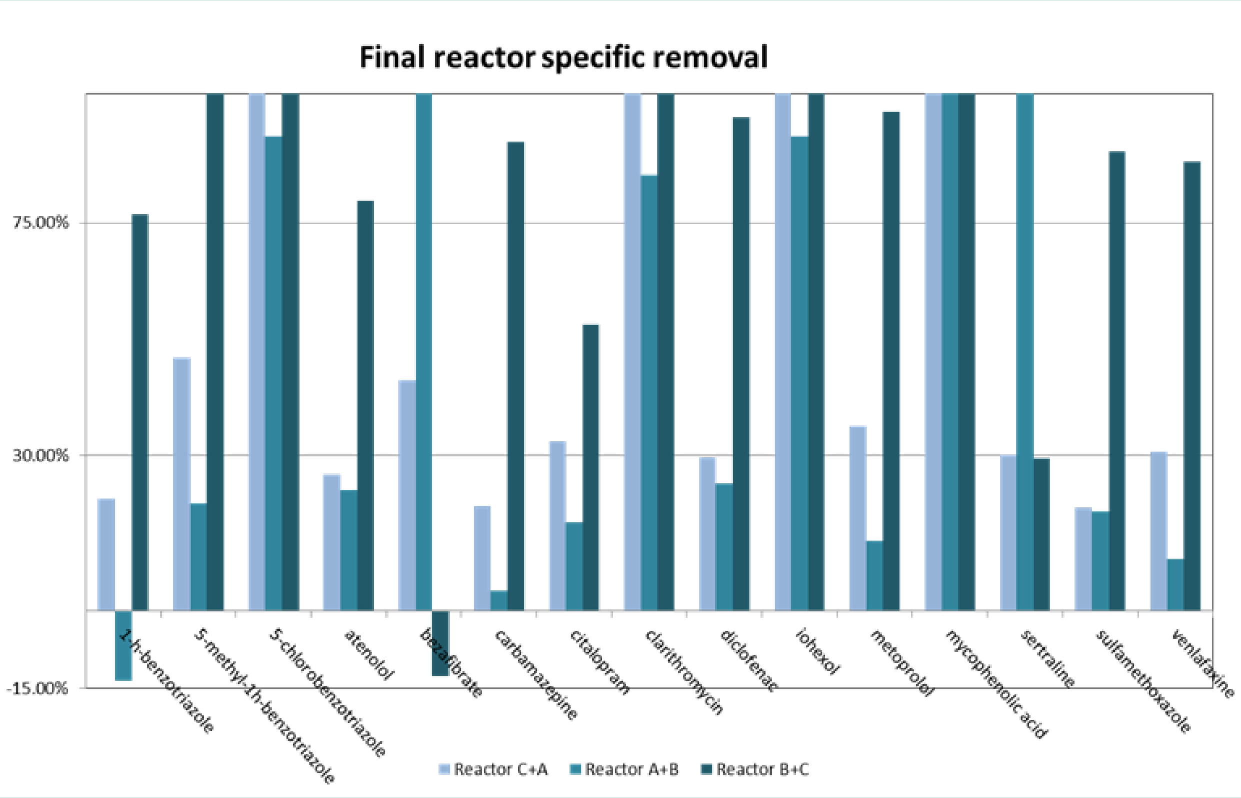


FIGURE 24. In-situ removal of pharmaceuticals present in the effluent water of Herning WWTP. The removal is shown for each reactor combination of the pilot plant.

A negative removal was observed for some compounds. This phenomenon can be ascribed to the fact that some compounds are excreted from the human body as metabolites (conjugated pharmaceuticals). This means that during metabolization, hydrophilic groups, such as glucuronic acid and sulphate groups, are added to the molecule in order to aid excretion from the human body. During biodegradation in the pilot plant, these conjugates can be broken down to the original compound, which is therefore detected in a higher concentration in the outlet, relative to the inlet (Nguyen *et al.* 2021).

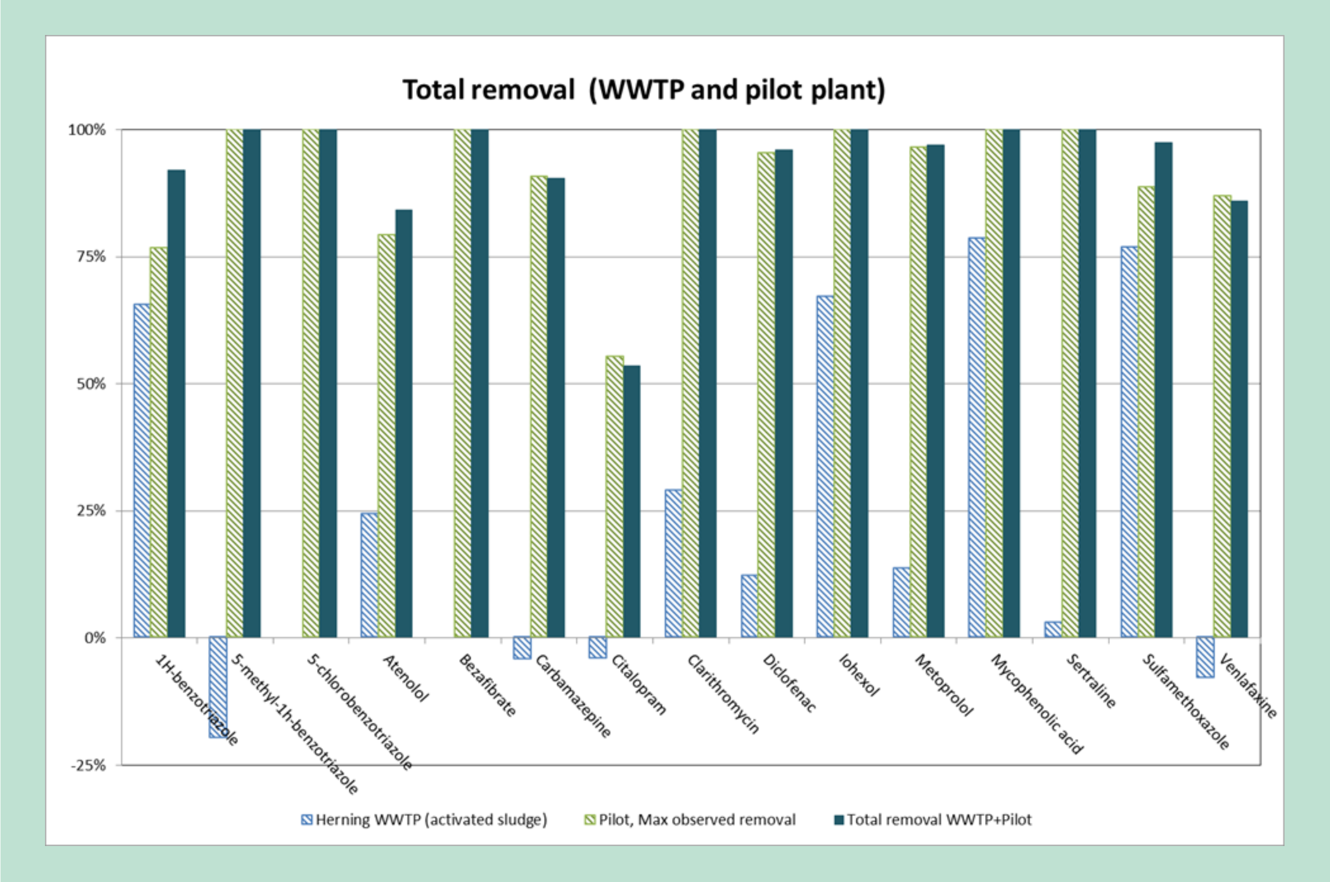


FIGURE 25. The highest observed removals of detected pharmaceuticals present in the inlet of the pilot plant. Blue-shaded columns show the removal in the Herning wastewater treatment plant in 2019, green-shaded columns show the removal in the eXeno™ pilot plant in 2020, and the filled columns show the calculated removal when considering removal in both the WWTP and the eXeno™ pilot plant. 5-chlorobenzotriazole and bezafibrate were not detected in the WWTP in 2019, but in the wastewater in 2020.

The highest observed removal in the eXeno™ pilot plant during the experiment is depicted in FIGURE 25 together with the observed removal in the Herning WWTP. Generally, the removal was significantly higher in the pilot plant as compared to the WWTP, and a high average total removal of 93% of all the detected compounds was observed (FIGURE 24). The compounds 5-methyl-1h-benzotriazole, carbamazepine, citalopram and venlafaxine had a negative removal in the WWTP but had significant high removals in the eXeno™ pilot plant (55-100%), altogether ensuring a high removal of these compounds from the wastewater due to the eXeno™ polishing step (FIGURE 25).

6.3 Conclusions of biological removal of pharmaceuticals

According to the present study, with an average removal of 93% of the pharmaceuticals detected in the wastewater, it is evident that the biological removal of pharmaceuticals is indeed possible. Considering the fact that the high removals observed in the present study were in the final sampling campaign of the pilot experiment (August), where the specialized biofilm had evolved for one year. This is a strong indication that it will be possible to optimize the biological removal rates even more by allowing the biofilm to evolve further and adapt to the specific conditions at the WWTP and the specific effluent water to be treated. A faster start-up phase at full-scale can be reached by inoculating eXeno™ with media (e.g., 20-30% of total volume of carriers) from existing nitrifying plants (pure MBBR or HYBAS ™ systems). This is certainly feasible, as it is already realized for other biofilm processes, such as anammox (Sjölunda biofarm, Sweden).

Compounds which have previously been presumed biologically recalcitrant, e.g., the contrast media iohexol, were in this study removed biologically (90-100%), which proves that adaptation of the naturally occurring biology is possible. This makes a biological and thereby environmentally friendly removal of micropollutants a promising technology and worth the effort for further optimization. This experiment clearly showed that the feast conditions were crucial for the growth of the biomass and that a high amount of biomass does not necessary correspond to a specialized and well-adapted biomass for pharmaceutical removal. Therefore, the control of the feast-starve phases is highly important in obtaining the highest removal rates.

Interestingly, three out of the four most environmentally problematic compounds identified from the hospitals (sertraline, mycophenolic acid, clarithromycin and ciprofloxacin; section 6.2) were detected in the wastewater at Herning WWTP in both May and August. In August, a significantly high removal of these compounds was observed: sertraline 30-100%, clarithromycin 80-100% and mycophenolic acid 100%, which further supports the findings that the eXeno™ polishing step is an efficient technology.

7. Dimensioning of the eXeno™ solution

7.1 The eXeno™ technology and its relevance for full-scale plants

The eXeno™ pilot plant, which has been operated for a year according to the eXeno™ principle, has proven to be a viable and environmentally friendly alternative for municipal wastewater treatment plants in the reduction of pharmaceuticals and other priority pollutants to the environment. Removal rates for target compounds vary between 30-90%, which for some compounds are lower than what is achieved with traditional technologies with ozone and activated carbon, while the biological eXeno™ process is more effective for other compounds.

Considering the characteristics of the eXeno™ technology, i.e., uncomplicated, robust, low operational cost and environmentally friendly, the eXeno™ technology can be implemented not only at large highly automated WWTPs, but also at smaller and at low-tech WWTPs. The eXeno™ post treatment process is a new innovation, and it is patented.

7.1.1 Design of 10% eXeno™ solution for Herning Water Authority

A design and cost estimate has been prepared for the implementation of a reduced capacity solution for Herning Water Authority, i.e., treating estimated ~10% of the total flow with eXeno™ technology at Herning Water Authority WWTP. The background for focusing on a reduced capacity eXeno™ solution were the discussion on whether the new hospital should have its own WWTP or it should be connected to the Herning Water WWTP. Both environmental and financial conditions were to be assessed. Connecting the WW discharge from the new hospital to Herning Water WWTP should not lead to an increase of the amount of discharged pharmaceuticals to the environment compared to a solution where the hospital established its own decentralized WWTP with discharge to the environment. The hydraulic load from the hospital is expected to be approx. 116,000 m^3/year, which is approx. 10% of the current annual load to Herning WWTP of approx. 10 million m^3/year.

It was decided to design the eXeno™ post-treatment for a capacity of 200 m^3/h, corresponding to approx. 17% of the total daily flow to Herning Water WWTP. The capacity larger than the 10% (flow hospital wastewater/flow Herning WWTP) for the eXeno™ post-treatment was chosen to ensure that even with lower removal rates for some compounds compared to treatment with ozone and activated carbon the load to the environment with the centralized eXeno™ solution would be equal or less than with the decentralized solution at the hospital. To illustrate the effect of a centralized solution, a separate WWTP for the hospital with an estimated 95% removal of pharmaceuticals would reduce the discharge of pharmaceuticals by 1/3 of the amount that the "10% eXeno™ solution" would reduce at Herning WWTP, even when assuming only 50% removal of the pharmaceuticals in this plant.

No additional modification of Herning WWTP, except for the construction of the eXeno™ reactors, would be required, as the additional organic and nitrogen load by the hospital wastewater is entirely handled by the capacity of the side-stream process tank of eXeno™ post-treatment.

The cost estimates have been prepared specifically for Herning Water Authority as part of the MerEFF project and do not have a general relevance due to the specific circumstances. The estimated CAPEX for the reduced capacity eXeno™ solution (17% of total influent or in total 1.5

million m^3/y) was below 50% of the CAPEX for a recently tendered WWTP dedicated for a hospital in Denmark.

OPEX has also been calculated for the “10% solution” and is presented in TABLE 14. For eXeno™, the following operating costs have been included:

Energy:

- eXeno™ uses energy to mix the content (water and carriers) in the reactors.
- eXeno™ uses energy to oxygenate the wastewater mainly in FEAST phase to obtain COD and nitrogen removal. Note that this energy demand is basically moved from the main treatment line to the FEAST reactor, freeing capacity in the main treatment. In the STARVE period, oxygen demand is very limited and consists mainly of nitrification of residual ammonia and limited conversion of COD.

Manpower:

- Supervision of the eXeno™ system is mainly to control that the equipment is running and to calibrate the online instruments.
- Maintenance is limited as there are only a few mechanical parts (valves, instruments and feed pump).

TABLE 14. Calculated OPEX for ”10% solution” at Herning WWTP.

Cost	Unit price	Cost per m^3
Electricity	0.07-0.1 kWh/m^3 (0.10 €/kWh)	0.007 – 0.01 €/m^3
Manpower	0.5 h/d at 80 €/h	0.008 €/m^3
Maintenance	1.5% of Mechanical and Electrical equipment.	0.011 €/m^3
Total OPEX	**Excl. analysis and capital cost)**	**~ 0.028 €/m^3; ~ 42,000 €/year**

For comparison, OPEX including the same categories of expenses for a recently tendered WWTP for a Danish hospital was in the range of 2.1 to 2.9 €/m^3 or would for a separate hospital WWTP for Herning hospital have been in the order of 250,000 €/year.

7.1.2 Design of an eXeno™ solution for 100,000 PE WWTP

To illustrate the economy (CAPEX and OPEX) of the eXeno™ polishing technology more generally, a cost estimate has been prepared for establishing this treatment step at a municipal plant with approx.100,000 PE connected.

The polishing MBBR reactors operate in a rotating mode allowing bacteria at intervals to be `off line´ in order to get access to an easily degradable carbon source to cover their demand for cell growth. When `in line´ and treating the main flow coming from the secondary clarification (STARVE period), the availability of carbon source is extremely limited (as mostly removed in the WWTP upstream to eXeno™) and not sufficient for maintaining a healthy and active biofilm. In the `off line´ period (FEAST), carbon source is provided to ensure cell growth in order to compensate for cell decay during `in line´ operation. However, it is important to avoid excessive bacteria growth in the FEAST period as this could result in overgrowth of essential bacteria for the degradation of pharmaceuticals.

A part of the flow from the primary clarifier is by-passed in the existing biological treatment step and directed to the eXeno™ reactor in FEAST mode, see FIGURE 26. The by-passed flow corresponds to approx. 10% of the total flow to the WWTP, and this by-passed flow is fully biologically treated (COD removal and nitrification/denitrification) in the eXeno™ reactor in FEAST mode. In practice, this means that the process also provides the treatment plant with a 10% increase of treatment capacity for COD and N removal and solves, for example, issues related to limited aeration capacity or biological treatment volume in the existing plant.

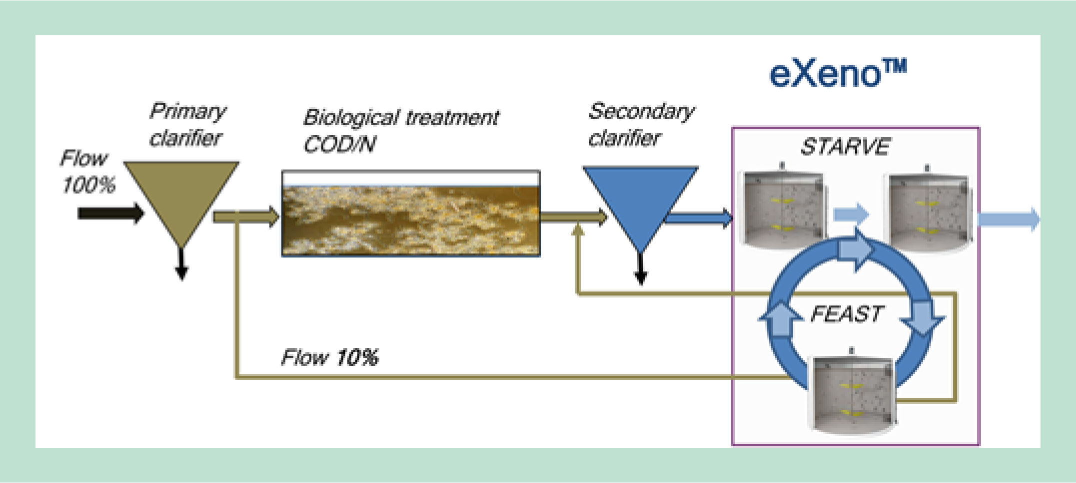


FIGURE 26. Process configuration eXeno™ post-treatment.

7.1.2.1 Design basis

For CAPEX and OPEX estimates for a full-scale eXeno™ installation, a wastewater treatment plant of middle capacity, i.e., with connected 100,000 PE has been used as design basis. It has been assumed that the main biological treatment step is designed with biological nitrogen removal, see TABLE 15.

TABLE 15. Key parameters used for CAPEX/OPEX estimates.

Wastewater treatment plant assumptions for design	
Annual influent flow:	7 Mm^3
Daily flow:	19,000 m^3/d
Hourly average flow:	792 m^3/h
Design flow eXeno™:	1200 m^3/h
Peak hydraulic flow:	2400 m^3/h

7.1.2.2 Design

The eXeno™ plant is designed with three equally sized MBBR reactors. All reactors are equipped with aeration system and mixers. The operation of the plant is fully automated with the process controlled by online measurements of oxygen, ammonia and nitrate in all reactors.

It is assumed that the effluent from the final clarification step in main biological treatment is of good quality. This is the feed to the eXeno™ plant (STARVE reactors). There is very limited or no net sludge production in the mainstream eXeno™, i.e., no special separation step for solids is required with eXeno™.

Approx. 10% of the effluent from the primary treatment step are diverted to the eXeno™ FEAST reactor. The FEAST reactor is operated under intermitted aeration conditions ensuring full nitrification and denitrification. The effluent from the reactor in FEAST mode is diverted to the secondary clarifier for separation of solids. The quality of the stream resembles the quality from the main WWTP´s biological treatment reactors.

The eXeno™ process does not increase sludge production, hence no additional sludge treatment capacity is required. The biological treatment capacity freed in the main treatment plant opens up for additional organic and nitrogen load of the WWTP, which, if utilized, will increase the sludge production accordingly. The freed capacity can also be used to handle potential overload situations or ensure nitrification during, for example, low temperature conditions (as showed in the results from the pilot under winter conditions (FIGURE 19, FIGURE 20)).

7.1.2.3 eXeno™ CAPEX estimate

CAPEX estimate is based on the price levels in Denmark in2020 for equipment, construction, and manpower.

It has been assumed that the three main reactors are combined in a concrete structure located outdoors with winter-protected equipment according to Danish climate conditions.

The only equipment outside of the reactors are compressors supplying air to the process and pumps feeding the system. The compressors small in size and are assumed to be the standard containerized type.

No additional building is included in the CAPEX estimate. Manpower requirements to operate and maintain the system are very limited, i.e., existing on-site staff facilities have been assumed to cover any demand. Likewise, it has been assumed that the existing control room and electrical room can provide the limited space required for the additional equipment and its control.

CAPEX estimate: 3.3 – 4 million EUR for a 100,000 PE WWTP.

7.1.2.4 eXeno™ OPEX estimate

The only consumable required for the eXeno™ process is electric energy for pumps, blowers and mixers, as shown in TABLE 16.

TABLE 16. Electricity consumption for the eXeno™ process.

	kWh per m^3 treated water	Price EUR per kWh*	OPEX eXeno™ Electricity EUR/m^3
Total electricity consumption	0.07 – 0.10	0.125	0.0085 - 0.012
Net electricity consumption, i.e., excluding aeration in FEAST reactor**	0.04 – 0.05	0.125	0.005 - 0.006

* The EU-27 average price in the first semester of 2020 - a weighted average using the most recent data for electricity consumption by non-household consumers (2020).

** The electricity consumption for biological treatment (COD and nitrogen removal) in the side-stream FEAST reactor is replacing the corresponding electricity consumption in the mainstream and can therefore in principle be detracted from the eXeno™ OPEX for electricity.

Other expenses related to the operation of the eXeno™ process are installation maintenance and manpower. TABLE 17 summarizes the operational expenses.

TABLE 17. OPEX for the eXeno™ process.

	EUR/m^3 treated
Electricity (net Table 16)	0.005 - 0.006
Maintenance	0.009
Manpower	0.005
Total OPEX	~ 0.02

7.1.2.5 Ozonation as post-treatment after eXeno™

With eXeno™, approx. 30-90% of the pharmaceuticals in the wastewater are removed. If higher removal is required, an additional polishing can be performed by ozonation. In this connection, it should be noted that the required dosing level of ozone will be considerably lower compared to cases if effluent from the secondary clarifier must be treated by ozone for removal of pharmaceuticals. The lower ozone requirement is a result of the biological removal in eXeno™ of COD including pharmaceuticals and the improved ammonia removal. The lower ozone dosage means that less potentially toxic ozone degradation products are formed and the better nitrification obtained by eXeno™ combined with the lower ozone dose curb the formation of carcinogenic nitrosamines. It should be noted that after ozonation, a post-treatment with a compact MBBR technology is required to remove most of the metabolites and degradation products produced by ozonation. This additional polishing step after the ozonation is also included in the cost estimate, and it consists of a small MBBR with only a mixer (no aeration is installed) in order to mix the carrier in the tank.

The concept of post-treatment with ozone is illustrated in FIGURE 27.

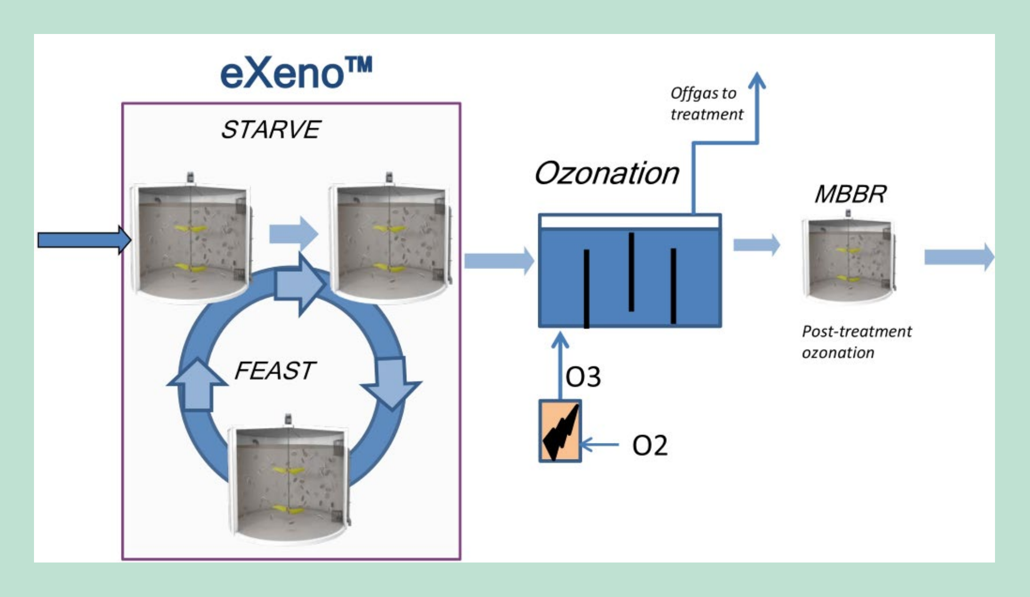


FIGURE 27. Polishing with ozone after eXeno™.

It is estimated that a dosage of 5 mg ozone per litre would be required to obtain approx. 80% global removal with the combination eXeno™ followed by ozone.

In the CAPEX estimate for the ozone system, a building has been included as the generator and control system require a dedicated space. Additional power supply is also required. The estimate is based on WWTP with a annual load of 7 Mm^3 (i.e., same basis as for eXeno™). CAPEX estimate is 2.5 - 3 million EUR.

TABLE 18 shows the calculated OPEX for ozonation and MBBR system, and the comparison and comparison with eXeno™.

TABLE 18. OPEX for post-ozonation after eXeno™.

	Unit price	Post-treatment 5 mg O3/l after eXeno™ EUR /m^3 treated water	eXeno™	eXeno™ incl. post-treatment with O3
Oxygen	0.70 EUR/kg*	0.035**		
Electricity	0.125 EUR/kWh	0.0075***	0.005 -0.006	
Maintenance		0.0142	0.006	
Manpower		0.0050	0.005	
Total OPEX/m^3 excl. capital costs		0.0617	0.017	0.08
Capital costs/m^3 ****		0.043	0.055	0.1
OPEX		0.11	0.072	0.18

* Delivered to site (Denmark), including transport, lease of storage tank and evaporator.

** Based on 10 kg O2/kg O3

*** Based on 10 kW/kg O3

**** Depreciation 10 years and interest rate 2%.

7.1.3 Conclusion

eXeno™ is a robust and affordable process for the reduction of discharge of pharmaceuticals from municipal wastewater treatment plants to the environment. The process is easy to operate, requires very little attendance and can easily be implemented at both small and big plants and at both high-tech and low-tech installations. Furthermore, its environmental footprint compared to the alternatives, ozone and activated carbon, makes it an attractive option in a world where CO_2 emissions must be kept as low as possible.

A shortcoming of the process is that in the eXeno™ pilot tests the removal rates for different pharmaceuticals have been observed to vary from 30-90%. However, when looking at the global removal in the WWTP with an eXeno™ post-treatment, the overall removal for most of the compounds reaches 70 - 90%. Depending on the actual compounds present in the wastewater at a specific plant, this removal rate could satisfy the requirements. It should be emphasized that the pilot plant has been operated for one year, and the progress in removal has been observed throughout the test period. Therefore, it cannot be ruled out that the performance will increase with time when the plant is in operation at a specific site.

If no sufficient removal is obtained with eXeno™, an additional polishing step with ozone plus a compact MBBR is an option. With eXeno™ as pre-treatment for removal of pharmaceuticals, the expected requirement of ozone to reach a global 80% removal will be reduced compared to ozonation directly on the effluent from the secondary clarifier. With the combination eXeno™ and ozone + MBBR, it is estimated that the ozone dose can be reduced by approx. 50%.

The reduction of the ozone requirement by 50% might not sound convincing for having a two-stage process. However, considering the high cost of generating ozone and the environmental concerns related to the formed reaction products, a two-stage treatment where a considerable part of the micropollutants have been removed in an environmentally friendly way should not be disregarded. Furthermore, ozone production is extremely energy intensive, and energy prices can only be expected to increase in the future making alternatives for reducing ozone consumption even more attractive in the future than today.

7.1.4 Benchmarking with other technologies

The traditional methods applied to reduce the content of complex organic pollutants from wastewater are ozone or activated carbon in the form of either PAC or GAC. Switzerland and Germany are currently implementing these technologies at some of their WWTPs in order to reduce the discharge of pharmaceuticals from municipal WWTPs into rivers and lakes.

Each method has its advantages and drawbacks. It should be noted that no single method will provide a complete removal of all pharmaceuticals. For example, some pharmaceuticals require a considerably higher ozone dose for oxidation than normally applied (Hansen et al. 2016), and adsorption on activated carbon vary as does the adsorption capacity for different compounds. In TABLE 19, traditional technologies such as ozone and activated carbon are compared to the eXeno™.

TABLE 19. Advantages and drawbacks for ozone, activated carbon (PAC and GAC) and eXeno™.

Technology	Advantages	Drawbacks
Ozone	-High removal of many pharmaceuticals -Partial disinfection -Compact	-Formation of metabolites with unknown environmental effect -Need for post-treatment to remove organic by-products -Formation of bromates -Formation of nitrosamines -High energy demand -Maintenance critical
PAC	-High adsorption of many pharmaceuticals -No formation of by-products -Additional COD removal	-Energy-consuming to produce -Increased waste to disposal as PAC cannot be regenerated -Expensive -Adsorption capacity varies between different compounds -Attention must be given to avoiding dust problems (explosion risk). Handling issues
GAC	-Good adsorption of many pharmaceuticals -No formation of by-products -Additional COD removal -GAC can be regenerated, i.e. less waste than PAC -In columns, i.e. closed system	-Energy-consuming to produce -Energy-consuming regeneration (steam and transport) -Adsorption capacity drops after each regeneration -Adsorption capacity varies between different compounds. Breakthrough faster for some compounds -GAC to be disposed of after 10 regenerations -GAC exchange in column not uncomplicated -Expensive
eXeno™	-Low energy consumption -Low carbon footprint -No residual waste -Improved ammonia removal (reduced toxicity) -Additional COD removal -Easy to operate -Low maintenance	-Removal of compound dependent and removal pharmaceuticals on average 50% (30 – 90%) -Needs space for reactors

For comparison, CAPEX and OPEX estimates for the different post-treatment methods are illustrated in TABLE 20 for:

- Ozone (dose 10 mg O3/l) followed by a biological scrubber (MBBR). Ozone from LOX.
- GAC columns, based on retention time 18 minutes and GAC consumption 50 mg/l.
- Design capacity as described in chapter 9.1.2.1.

TABLE 20. Comparison of OPEX for eXeno™ with OPEX for Ozone and GAC.

	Unit price	**Ozone***** 10 mg O3/l EUR /m^3**	**GAC Filter 50 mg GAC/l**	**eXeno™**	**eXeno™ incl. post treatment with 5 O3/m^3**
Oxygen	0.70 EUR/kg*	0.070**			
GAC	2.70 EUR/kg*		0.135		
GAC disposal /transport (estimate)	0.4 EUR/kg		0.02		
Electricity	0.125 EUR/kWh	0,0138***	0.014	0.005 - 0.006	
Maintenance		0,0214	0.0154	0.006	
Manpower		0.0050	0.010	0.005	
Total OPEX/m^3 excl. capital costs		0.11	0.195	0.017	0.08
Capital costsMEUR		4 – 4.5	4 - 4.5	3.3 – 3.8	6 - 6.5
Capital costs/m^3 ****		0.067	0.067	0.055	0.1
OPEX EUR/m^3		0.18	0.26	0.075	0.18

* Delivered to site (Denmark), including transport. For O2 lease of storage tank and evaporator incl.

** Based on 10 kg O2/kg O3

*** Based on 10 kW/kg O3

**** Depreciation 10 years and interest rate 2%

****** Bioscrubber after Ozone included

The difference in OPEX is illustrated in FIGURE 28.

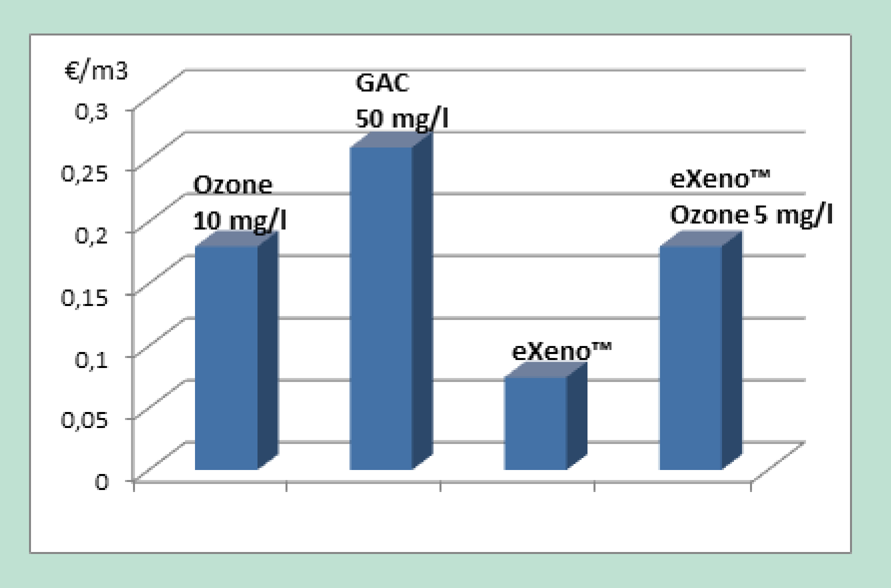


FIGURE 28. Cost comparison OPEX for Ozone, GAC and eXeno™.

7.1.5 Future perspectives

Regulations related to pharmaceuticals in the aquatic environment have been discussed for quite some time, but it seems that no immediate moves have been made towards legislation. Based on regulatory contexts such as REACH and the European Water Framework Directive (WFD), safe exposure levels for aquatic and terrestrial ecosystems have been defined, and the lowest value is typically divided by an assessment factor of 10 to 1000 to arrive at the PNEC. There are examples of PNEC values being used as treatment target for removal in WWTPs, i.e., completely disregarding the dilution occurring in the receiving aquifer and the assessment factor built into the PNEC value.

There is no doubt about the importance of reducing discharge of pharmaceuticals and other priority pollutants to the aquatic environment. However, the introduction of treatment technologies producing by-products in concentrations potentially harmful to the environment, must ns be avoided. Furthermore, in recent years the WWTPs have been challenged in their energy consumption. To be energy neutral or even to become an energy producer by fully utilizing the energy content in wastewater, the WWTPs could contribute to the reduction of CO_2 emissions only by reducing the energy demands. Energy consumption is thus also of concern when selecting sustainable technologies to handle emissions of pharmaceuticals.

As mentioned before, there is uncertainty regarding the regulations and the expected different ambition levels related to the removal of pharmaceuticals depending on whether it is a highly industrialized country or a developing country, and whether WWTPs are large or small. Furthermore, there will a continuous introduction of new pharmaceuticals, and it can be expected that new methods will be developed to improve the handling, for example, of urine from patients receiving APIs of concern. The demand for reduction of CO_2 emissions also defines the standards for the introduction of new technologies for large-scale implementation.

To sum up, flexible, easily operated and robust solutions with required removal efficiencies for target compounds will be required. Furthermore, a low energy demand and the production of no or minimum waste will be also required when combating pollution with pharmaceuticals at a global scale. eXeno™ post-treatment seems to be a good candidate as it, together with the well-functioning main WWTP, has shown to provide a substantial global removal of pharmaceuticals of 90+%. The next step is to test this eXeno™ setup at a bigger scale and to validate its performance.

Specifically, for Herning Water and the hospital DNV Goedstrup, in spring (2021) a collaboration agreement will be signed which will be based on the existing legislation. The agreement will ensure a biological solution, which is considered a joint strategic focus.

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9. Appendix 1

Table: Overview of measured pharmaceuticals in MerEFF and overlap with the Danish AMK list, the list from Germany and Switzerland.

All substances for treatment characterisation									
	DTU	Denmark)	Sweden X)	Germany)	Veolia & Tc)	Switzerland)	Germany)		
Name		33+3		Expanded monitoring in WWTP		Advanced treatment monitoring (PAC/Ozone)	Minimum required monitoring in WWTP (5 day sampling)	Advanced treatment monitoring (24-hour sampling)	
								Ozone	PAC
Carbamazepine	🟩 •	A	D	C	X	B2	>80%	High (>80%)	High (>80%)
Citalopram	🟩 •	A	D		X	B2			
Clarithromycin	🟩 •	A	D	C	X/T	B2	>80%	High (>80%)	Medium (50%-80%)
Diclofenac	🟩 •	A	D	C	X	B2	>80%	High (>80%)	High (>80%)
Metoprolol	🟩 •		D	C	X	B2	>80%	Medium (50%-80%)	High (>80%)
Venlafaxine	🟩 •				X	B2			
Amisulpride	🟩					B2			
Hydrochlorothiazide	🟩 •					B2			
Candesartan		A		C		B1			
5-Methyl-1H-benzotriazole	🟩 •					B1			
1H-Benzothiazole	🟩 •			C		B1	>80%	Medium (50%-80%)	High (>80%)
Irbesartan						B1			
Azithromycin	🟩 •	A			X				
Ciprofloxacin	🟩 •	A	D	C	X/T				
Erythromycin	🟩 •	A			X				
Ibuprofen		A		C	X				
Sulfamethoxazole	🟩 •	A	D	C	X		>80%	High (>80%)	Medium (50%-80%)
Amlodipine		A							
Bicalutamide	🟩 •	A							
Buprenorphine		A							
Capecitabine		A							

All substances for treatment characterisation									
	DTU	**Denmark)**	**Sweden X)**	**Germany)**	**Veolia & T$^{c)}$**	**Switzerland)**	**Germany)**		
Name		33+3		Expanded monitoring in WWTP		Advanced treatment monitoring (PAC/Ozone)	Minimum required monitoring in WWTP (5 day sampling)	Advanced treatment monitoring (24-hour sampling)	
								Ozone	PAC
Ceftazidime		A							
Cyproterone		A							
Deferasirox		A							
Disulfiram		A							
Dronedarone		A							
Duloxetine		A							
Efavirenz		A							
Fluoxetine		A							
Fulvestrant		A							
Furosemide		A							
Lanthanum		A							
Mycophenolic acid	•	A			T				
Naproxen		A							
Nilotinib		A							
Ofloxacin		A							
Olanzapine		A							
Paracetamol		A							
Prednisolone		A							
Propofol		A							
Propranolol		A							
Quetiapine		A							
Tramadol		A							
Atenolol	•		D		X				
Clofibric acid	•				X				
Estradiol				C	X				
Estrone				C	X				
Iomeprol	•			C	X				
Iohexol	•			C	X				
Ketoprofen	•		D		X				
Terbutryn				C			>80%		
Valsartan				C					
Losartan				C					
Gabapentin				C					
Guanylurea				C					

All substances for treatment characterisation									
	DTU	Denmark)	Sweden X)	Germany)	Veolia & Tc)	Switzerland)	Germany)		
Name		33+3		Expanded monitoring in WWTP		Advanced treatment monitoring (PAC/Ozone)	Minimum required monitoring in WWTP (5 day sampling)	Advanced treatment monitoring (24-hour sampling)	
								Ozone	PAC
17α-Ethynylestradiol				C					
Mecoprop				C					
Isoproturon				C					
Flufenacet				C					
Tebuconazole				C					
Propiconazol				C					
Galaxolide				C					
Perfluorooctanoic acid (PFOA)				C					
Perfluorooctanesulfonic acid (PFOS)				C					
H 4-PFOS				C					
Acesulfame K				C					
Sertraline	🟩 •		D		T				
Bezafibrate	🟩 •								
Mefenamic acid	🟩 •								
5-Chlorobenzotriazole	🟩 •								

A: Local Government Denmark (KL): Hospital wastewater – Tool for sewer connection permits. December 2013, (Full scale advanced wastewater treatment at Herlev Hospital, Grundfos BioBooster A/S).
T:Critical substances in hospital wastewater from Region Midt, Thomas Møller

a): Local Government Denmark (KL): Full scale advanced wastewater treatment at Herlev Hospital, 2016 (BioBooster, 2016).
b): German list for lead substances and evaluation criteria for advanced monitoring (Arge, 2014; Klaus Alt et al., 2016)
c): Veolia: the monitoring list of the substances required by Veolia; T: Environmental report from Aarhus University Hospital, technical department, Region Midt. (in danish), Thomas Møller, (2014, 2017)
d): A watch list of the targeted substances used as the indicators to evaluate the performance of conventional (i.e. activated sludge) and advanced wastewater treatment (powder activated carbon (PAC), ozonation) in Switzerland, (Götz et al., 2015)
X) Barasel et al., 2014)

🟥	🟥		B2: 4 out of 12 substances, which were removed by ozone/PAC (>80%).
🟦	🟦		B1: 2 out of 12 substances, which were removed by ozone/PAC (50%-80%).

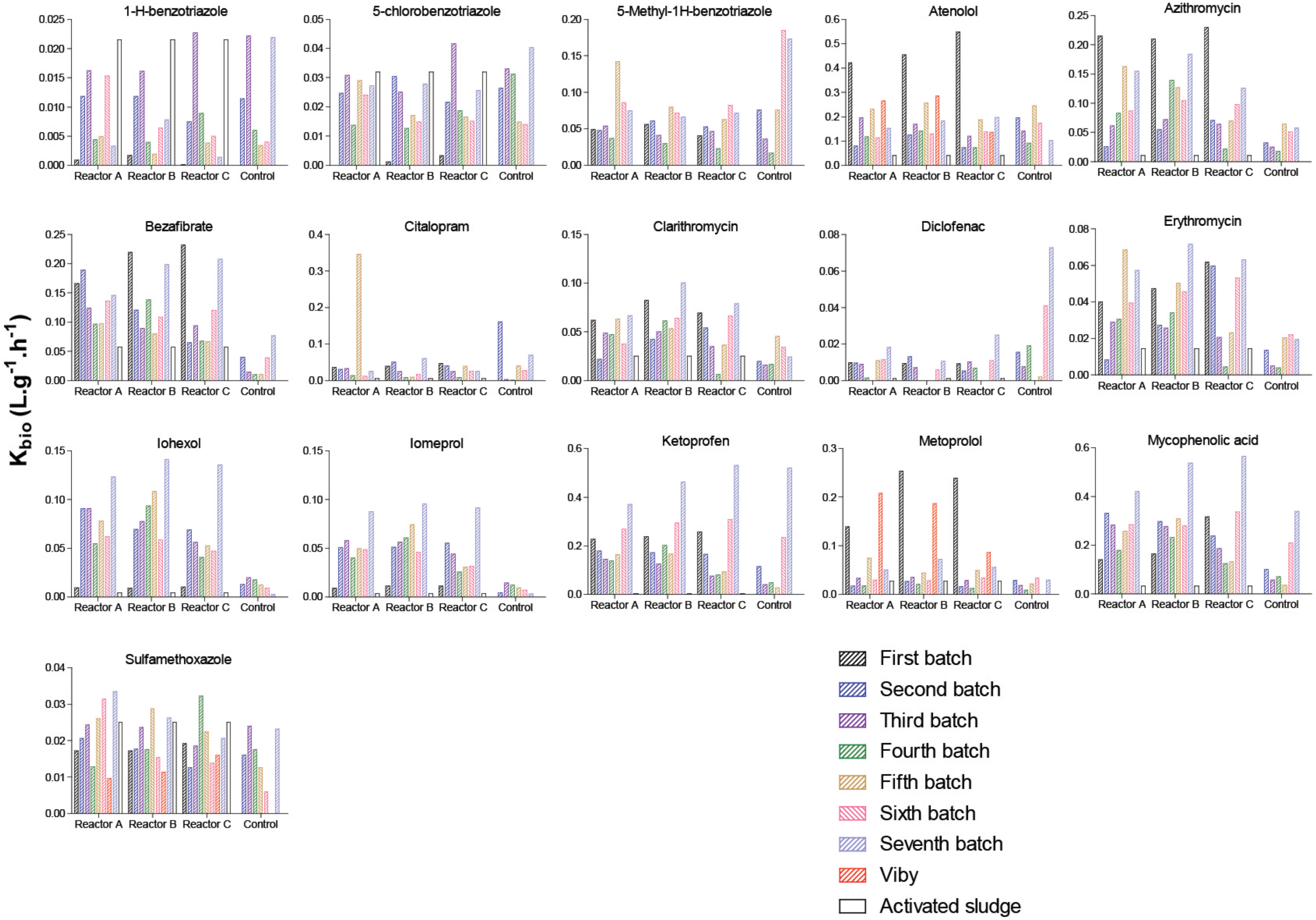

Figure: Removal rates of different pharmaceutical compounds normalised to the amount of biofilm on the carriers (Kbio). Each batch number refers to the seven operational phases (TABLE 13). Activated sludge for Herning WWTP and a previous pilot experiment (Viby) are included for comparison.

MerEFF – Environmental treatment of wastewater effluents - MiljøEffektiv Rensning af afløb fra renseanlæg EFFluenter

The eXeno™ technology concept was implemented at pilot scale as a polishing step at Herning Water. Reduction of pharmaceuticals of 93% was documented by this strict biological solution. In addition, ammonia was reduced between 78-93% as well as inert COD (6-36%). Advantages are low operational costs, low carbon footprint and environmental and sustainable technology.

eXeno™ teknologikonceptet blev implementeret i pilotskala som efterpolering trin ved Herning Rens. 93% reduktion af lægemidler blev dokumenteret ved denne biologiske proces. Tillige blev ammonium-indhold reduceret mellem 78-93% og tilsvarende inerte COD (6-36%). Fordelene er lave driftsomkostninger, lavt carbon footprint og er tillige en miljøvenlig og bæredygtig teknologi

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