Biological removal of emerging micropollutants in nitrifying activated sludge at low temperatures

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Abstract
Growing concentrations of emerging micropollutants (EMs) such as pharmaceuticals, endocrine disruptors and personal care products are found in the aquatic environment worldwide. These substances could pose the risk on humans and animals due to their chronic ecotoxicity and persistence. The main route of EMs emission into the natural waters is through effluents of wastewater treatment plants (WWTPs).
Operational conditions can significantly affect biodegradation of EMs during wastewater treatment. In particular, low temperature limits biological processes due to slower metabolic reactions and decrease of bacterial diversity in activated sludge.
In this thesis, biological removal of EMs from wastewater was studied at low temperatures (8-12°C). Different operational conditions were compared in order to increase removal efficiency and therefore enhance the quality of WWTPs effluents. Laboratory-scale Sequencing Batch Reactors (SBRs) and Membrane Bioreactors (MBRs) were used to mimic existing wastewater treatment processes. Data from full-scale WWTPs of Helsinki region were taken into account.
The study presents the removal rates for widely-used EMs ibuprofen, diclofenac, carbamazepine, estrone, 17β-estradiol and 17α-ethynylestradiol. Altogether, obtained removal efficiencies were much lower compared to published data for higher temperatures. Biodegradation studies demonstrated that EMs might accumulate in activated sludge cells at large extent. Depending on the substance, this accumulation could be followed by biodegradation or by return of the compound to the wastewaters with the cell decay. Therefore, following the concentrations of EMs in solid phase of activated sludge is necessary for the assessment of biodegradation potentials.
Sludge retention time (SRT) proved to be an effective operational tool for regulating biological processes during wastewater treatment. Prolongation of the SRT demonstrated positive effect on activated sludge performance and EMs biodegradation at low temperatures. At the same time, decrease of the temperature raised the negative pressure effect on activated sludge, limiting the removal potential of EMs as well as inhibiting nitrification. Thus, SRT optimum is closely related to the temperature fluctuations.
Contribution of different microorganisms in performance of nitrifying activated sludge and EMs removal at cold temperatures is discussed in this study. In addition to above mentioned EMs effect of antibiotics sulfadiazine and trimethoprim was studied. Described microbial communities significantly differed from typical nitrifying activated sludge at class level. EM removal potential and the need for further research of bacterial class Deltaproteobacteria and domain archaea is proposed. Overall, prolongation of SRT improved stress resistance of microbial community resulting in stable performance of activated sludge.
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Espoo, June 2018

Antonina Kruglova
List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. In addition, some unpublished data are included.


The author’s contribution

I. The first author planned the experiments together with the P. Rantanen, carried out part of experimental work and sampling, interpreted all of the results and had the main responsibility for writing and submitting the article. P. Ahlgren and N. Korhonen participated in bioreactors operation and part of the experimental work. A. Mikola and R. Vahala participated in planning of the scope of the article and commented the manuscript.

II. The first author participated in the design and construction of MBRs and planning the experiments at 12°C together with M. Riska and P. Rantanen. The first author planned and performed all of the experiments at 8°C, collected and pre-treated the samples. M. Kråkström analysed emerging micropollutants concentrations in part of the activated sludge samples. The first author calculated the micropollutants removal rates, interpreted the results, wrote the manuscript and received comments from the co-authors.

III. The first author designed and performed all of the experiments, collected and pre-treated the samples. A. Gonzalez-Martinez performed the DNA extraction and statistical analyses of sequencing data. M. Kråkström analysed emerging micropollutants. The first author interpreted the results, wrote and submitted the manuscript. Anna Mikola and R. Vahala supervised the study and commented the manuscript.

IV. The second author planned the scope of the mini-review together with P. Välitalo. The second author reviewed the data on antibiotics of high concern and on bacteria involved in wastewater treatment process, wrote the relevant chapters of the review and participated in submission process. P. Välitalo had the main responsibility for writing and submitting the article and received comments from the co-authors.

V. The first author designed and performed all the experiments, collected and pre-treated the samples, analysed the bacterial communities and wrote the manuscript. A. Gonzalez-Martinez was responsible for extraction of DNA, supervised sequencing data analyses and commented the manuscript. A. Mikola and R. Vahala provided comments on the manuscript.
Abbreviations and key terminology

AOA  Ammonium-oxidizing archaea
AOB  Ammonium-oxidizing bacteria
AMO  Ammonia monooxygenase
AS   Activated sludge
BOD  Biological oxygen demand
CAS  Conventional activated sludge
CEC  Contaminant of emerging concern
CMZ  Carbamazepine
COD  Chemical oxygen demand
DCF  Diclofenac
DO   Dissolved oxygen
E1   Estrone
E2   17β-estradiol
EE2  17α-Ethynylestradiol
EM   Emerging micropollutants
EPS  Extracellular polymeric substances
EQS  Environmental quality standard
F:M  Food per microorganism ratio
HRT  Hydraulic retention time
IBU  Ibuprofen
$K_{\text{biol}}$ Biodegradation rate constant
MBR  Membrane bioreactor
MF   Microfiltration
MLSS Mixed liquor suspended solids
NH$_4$-N Ammonium as nitrogen
NO₂⁻N  Nitrite as nitrogen
NO₃⁻N  Nitrate as nitrogen
NOB  Nitrite-oxidizing bacteria
NSAID  Non-steroidal anti-inflammatory drug
OLR  Organic loading rate
OTU  Operational taxonomic unit
PCP  Personal care product
PhAC  Pharmaceutically active compound
PO₄³⁻P  Phosphate as phosphorous
SBR  Sequencing batch reactor
SDZ  Sulfadiazine
SRT  Sludge retention time
SS  Suspended Solids
TN  Total nitrogen
TP  Total phosphorous
TMP  Trimethoprim
WFD  Water Framework Directive
WWTP  Wastewater treatment plant

**Aerobic** = Conditions or environment characterised by the presence of free oxygen, which required by aerobic organisms for growth as terminal electron acceptor

**Anoxic** = Conditions or environment characterised by the deficiency of molecular oxygen in which bacteria are promoted to use chemically bound oxygen, including nitrites and nitrates for instance, nitrates (NO₃⁻)

**Anaerobic** = Conditions or environment with absence of free oxygen in which the growth of anaerobic organisms is promoted since they are able to use as terminal electron acceptor other molecules then oxygen, such as organic matter
**Autotrophic** = Metabolism type, in which simple inorganic substances, such as carbon dioxide, used for synthesis of organic molecules with the energy of chemical reactions or sunlight

**Emerging micropollutants** = Pollutants in low/trace concentrations, which environmental importance have been recently discovered and therefore, no requirements of monitoring and discharges are yet regulated

**Heterotrophic** = Metabolism, which depends on complex organic substances as a source of carbon and energy

**Nitrification** = Process in which microorganisms oxidize ammonia (NH₃) or ammonium (NH₄⁺) in two sequential steps: first (nitritation) to nitrite (performed by ammonium oxidizing microorganisms) and then (nitratation) to nitrate (by nitrite oxidizing microorganisms)

**Nitritation** = Biological transformation of ammonia or ammonium to nitrites (NO₂⁻); the first stage of nitrification

**Nitratation** = Transformation of nitrites to nitrates (NO₃⁻); the second stage of nitrification

**Priority micropollutants** = Pollutants in low/trace concentrations with appointed risk on the environment listed in Water Framework Directive with defined environmental quality standards
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1. Introduction

1.1 Background and motivation for the research

In recent years, environmental awareness of the growing medicalization and chemicalization of global health, agriculture and industry have brought resource recovery and environmental impact assessment into major focus in society. As a result, environmental regulations on wastewater effluents are getting stricter and thus nutrient removal requirements for wastewater treatment plants (WWTPs) are constantly increasing (Fernández-Arévalo et al., 2017). In particular, removal requirements for emerging contaminants are already affecting municipal wastewater treatment requirements. However, more advanced treatment usually requires more energy and other resources such as chemicals, operational costs, etc. Additional treatment steps may also increase the environmental footprint, including space for new treatment facilities, biosolids such as treated sewage sludge with accumulated micropollutants, and so on (Clarke and Smith, 2011; Foley et al., 2010). Therefore, a high-priority task for modern society is to optimize the technologies used in the existing WWTPs to reach their maximum removal efficiency including emerging pollutants (EMs) and to avoid or minimize the need for further treatment steps.

1.2 Objectives of the study

In this project, two wastewater treatment technologies were used to simulate the conditions of different existing WWTPs and evaluate their potential for the biological removal of EMs from wastewater in low temperature conditions (8-12 °C). The main aim of the research was to develop a more efficient wastewater treatment by optimizing the operational parameters for WWTPs in Finland, other Nordic countries and some central Europe WWTPs that have climatic conditions similar to those in Nordic region.

The research questions were:

1. How can the biodegradation of potentially harmful EMs from wastewater be enhanced to avoid their release to the environment?

2. Which process conditions are optimal for elimination of emerging micropollutants from wastewater in cold regions?

3. Which groups of microorganisms are capable of improving the process of biodegradation?
The main objective was to determine the biodegradation potential of selected micropollutants in low-temperature-adapted activated sludge (AS) of the conventional wastewater treatment systems and advanced membrane bioreactors (MBRs). The results were aimed at assessing the preferable strategy for EMs removal as well as estimating the limitations of the studied technologies.

The second objective was to spot the critical process parameters in the enhancement of the micropollutant removal and from the results draw conclusions on further application of the two studied treatment technologies.

The third objective was to evaluate the contribution of certain microorganisms in biological removal. In this context, the microorganisms with higher sensitivity are of particular importance. Therefore, a special focus was put on nitrifying bacteria.

1.3 Outline of the thesis

The following chapters comprise the scope of this thesis.

Chapter 2 presents the overview of the emerging micropollutants issue in municipal wastewaters. In Chapter 3, principles of emerging micropollutants biological removal in wastewater treatment plants are presented. Chapter 4 briefly describes the research schedule, experimental design and analytical methods used in this thesis. The detailed descriptions of the materials and analyses are presented in the original papers: I, II, III and, V.

The results obtained in this research and described in papers I-V are discussed in Chapter 5.

Chapter 6 presents the main conclusions of the thesis and suggestions for further research and future applications.
2. Emerging micropollutants

2.1 Micropollutants in the aquatic environment

With the development of more advanced detection methods, more and more pollutants in trace amounts were reported in wastewaters, raising new challenges in their release in the water environment and reuse. Harmful substances at concentrations ranging from ng L$^{-1}$ to a few μg L$^{-1}$ are commonly termed micropollutants. Before the early 1990s, growing concentrations of organic micropollutants (commonly used dyes, solvents, pesticides, etc.) and heavy metals were of great concern and were appointed as priority micropollutants in European regulations (Vergili, 2013).

During the last decades, increasing attention was given to so-called emerging micropollutants (EM) such as pharmaceutically active compounds (PhACs) and personal care products (PCP), antibiotics, steroids and hormones, pesticides, perfluorinated substances, polychlorinated alkanes and other industrial and household chemicals, whose diversity and consumption are growing every year (Clarke and Smith, 2011; Gorito et al., 2017; Luo et al., 2014). Nowadays, EMs are the big concern of drinking water industries due to their occurrence in sources of drinking water supplies (Vergili, 2013). WWTPs are reported to be the primary sources of many EMs in the aquatic environment (Luo et al., 2014).

2.2 Emerging micropollutants in municipal wastewater treatment

EMs enter municipal WWTPs by different paths, including domestic and hospital effluents, landfill leachates, agricultural runoffs, etc. (Luo et al., 2014). In principal, municipal WWTPs consist of primary treatment including screening and sedimentation for the removal of suspended in water solids, followed by secondary treatment, where conventional organic pollutants and nutrients are removed via biological processes. Since conventional WWTPs are not designed to remove EMs, persistent and mass-consumed substances pass through the treatment stages and end up in effluents from where they spread to the environment, as shown in Figure 1 (Barbosa et al., 2016).
Several possible negative effects of EMs dissemination to the environment have raised researchers’ attention and society’s concerns. The toxicological effects of WWTP effluents containing EMs have been observed in fish, crustaceans, mussels, algae and bacteria (Maya et al., 2017; Petrie et al., 2015). Additionally, due to their persistence, EMs can accumulate in environmental waters affected by WWTPs discharges (rivers, groundwaters, etc.), causing chronical toxic effects as well as bioaccumulate in aquatic organisms. Furthermore, a mixture effect of EMs may exhibit a greater toxic effect compared to individual compounds. The use of receiving waters as raw sources for drinking water production, irrigation and other agricultural uses bring additional risks of food safety and public health should be considered (Qi et al., 2015). Finally, the antibiotic resistance crisis has a direct link to the release of antibiotics to the environment and WWTPs are the important contributors to antibiotics dissemination (Berendonk et al., 2015; Michael et al., 2013).

2.3 Emerging micropollutants of high concern

Among the EMs occurring in municipal wastewaters, two parameters commonly define substances of highest concern. Firstly, it is the most commonly consumed medicine, such as over-the-counter and mostly prescribed drugs as well as widely used pesticides.
Secondly, there are the substances that persist in all typical wastewater treatment steps. These substances are most frequently found in samples of environmental waters.

In particular, over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and estrogens are among the most typically reported EMs in municipal influents, effluents and effluent-receiving water bodies (Fent et al., 2006; Hamid and Eskicioglu, 2012). For instance, carbamazepine (CMZ) and ibuprofen (IBU) are of high interest, since hundreds of tons of these drugs are produced annually and they are detected in environmental waters all over the world (Vergili, 2013). Therefore, a particular focus of this research was given to the removal of these substances during wastewater treatment.

No considerable removal was reported for PhACs and hormones during primary treatment (Luo et al., 2014). During secondary treatment the main mechanisms of EMs elimination is attributed to biodegradation and sorption (Verlicchi et al., 2012). In general, anti-inflammatories and the antibiotics remain in the water, whereas estrogens are adsorbed to the sludge (Carballa et al., 2004). Additionally, for some of them such as diclofenac (DCF) and CMZ, so-called “negative” removal was frequently reported (discussed in Chapter 1.1.2.2), meaning that higher concentrations are detected in effluents compared to influent concentrations (Kasprzyk-Hordern et al., 2009; Sipma et al., 2010). Finally, the toxic effect of these EMs can affect the bacteria in AS, inhibiting biological processes and diminishing WWTP performance (Katipoglu-Yazan et al., 2013; Tobajas et al., 2016).

Summarized literature data on the presence and removal of the above-mentioned EMs is presented in Table 1. It can be noticed, that IBU, estrone (E1) and 17β-estradiol (E2) have rather high removal efficiencies (70-100%), whereas the removal of DCF, CMZ and 17α-ethinylestradiol (EE2) can drastically vary from no removal (or even accumulation in treated wastewater) up to 60-80%.

| Table 1 | The reported concentrations and removal rates of the contaminants included to the list of EMs of high concern |
| --- | --- | --- | --- | --- |
| Category of EMs | Compound name | Influent (μg L⁻¹) | Effluent (μg L⁻¹) | Removal (%) |
| Analgesic and anti-inflammatory | Ibuprofen (IBU) | < 0.004–603¹ | ND–55¹ | 72-100¹ |
| | Diclofenac (DCF) | < 0.001–94.2¹ | < 0.001–0.69¹ | < 0–81.4¹ |
| Anticonvulsant | Carbamazepine (CMZ) | < 0.04–3.78¹ | < 0.005–4.60¹ | < 0–62.3¹ |
| Steroid hormones | Estrone (E1) | 0.01–0.17¹ | < 0.001–0.08¹ | 74.8–90.6¹ |
| | 17β-Estradiol (E2) | 0.002–0.05¹ | < 0.001–0.007¹ | 92.6–100¹ |
| | 17α-Ethinylestradiol (EE2) | 0.001–0.003¹ | < 0.001–0.002¹ | 43.8–100¹ |
| Antibiotics | Sulfadiazine (SDZ) | 0.01² | 0.01-0.56³,⁴ | 23.7-100³,⁵ |
| | Trimethoprim (TMP) | 0.06–6.80¹ | < 0.01–3.05¹ | < 0–81.6¹ |

¹(Luo et al., 2014); ²(Oliveira et al., 2015); ³(Zhou et al., 2013); ⁴(Gao et al., 2012); ⁵(Verlicchi et al., 2012)
The large variation in the removal rate suggests that there is the possibility to improve the removal of EMs for sustainable wastewater effluent quality. Additionally, the uncertainty of the removal rates could be linked to the fact that most of the studies only analyse the water phase removal whilst there are evidences that a significant fraction of EMs can be adsorbed/absorbed by a solid phase (microorganisms) (Suárez et al., 2008). Finally, climate, population and other specific conditions of each wastewater treatment process may affect the removal of EMs, and therefore region-specific data on the fate of these substances in WWTPs should be considered (Lin et al., 2018).

2.4 Existing policies

The European Water Framework Directive (WFD) is the basis of the European Union legislation on water. Water contaminants are divided into priority substances with a proven risk to the environment and existing regulations and the contaminants of emerging concern (CEC) recommended for closer attention.

The recent WFD (DIRECTIVE 2013/39/EU, 2013), includes the list of priority substances with the defined environmental quality standards (EQS), i.e. concentration limits for surface waters, as well as emphasised the need to monitor the CECs. In 2015 the directive was updated with a list of 17 CECs, which were combined into the Watch List (DECISION 2015/495/EU, 2015) for collecting European Union-wide monitoring data (Gorito et al., 2017). Among them, one NSAID - DCF and estrogens E1, E2 and EE2. In addition to that, there are several examples of existing legislation at the local level, where some EMs have EQS.

For instance, in Switzerland the upgrade of more than 100 WWTPs for the removal of EMs has been regulated (Logar et al., 2014). In the German Water Association (DWA), the topic of micropollutants is also dealt with at various levels. As a result, there are WWTPs that are already in operation, with micropollutants removal in Germany, Baden-Württemberg, and several others are under construction or at different planning stages (DWA-BW, 2017). Furthermore, the Danish municipalities composed a guideline for the regulation of hospital wastewater discharges to public sewers. In the guideline, the list of limit values for 40 PhACs have been set including IBU, CMZ and DCF (DHI, 2017).

However, in other European countries upcoming regulations are still under discussion.
3. Removal of emerging micropollutants in biological wastewater treatment

3.1 Biological wastewater treatment

Most of the municipal WWTPs across the developed world consist of primary treatment followed by an AS process, which employs a diverse microbial community, maintained in suspension (by mixing or aeration) to ensure their continuous contact with wastewater (Seviour and Nielsen, 2010).

The main biological removal processes in AS include carbon removal and nitrification of ammonium (mostly under aerobic conditions), denitrification of nitrates to molecular nitrogen (under anoxic conditions) and removal of phosphates in anaerobic AS stages (Saunders et al., 2016; Verlicchi et al., 2012). The presence of functional bacterial groups determines the efficiency of substrate-specific removal (Barra Caracciolo et al., 2015). Consequently, microbiological techniques help to enhance the efficiency of biological removal processes by identifying the key bacteria of studied AS. Some species may improve certain removal processes, whereas other species inhibit them for example, due to bulking and foaming (Saunders et al., 2016). However, conventional microbiological methods such as staining and microscopy are unable to detect so-called “unculturable” bacteria, i.e. the bacteria which lose their ability to grow or are changing their metabolic mechanisms when extracted from the original environmental sample (Stewart, 2012). Due to these limitations during past decades, these methods are being replaced with molecular biological methods, shading light on the total microbial community of studied environments such as AS.

Nowadays, microbial population analyses development and applications allows for an understanding and close following of the removal cycles of the main wastewater pollutants. In particular, key groups of microorganisms responsible for nitrification (the indispensable process of nitrogen removal cycle in WWTPs) are identified. The main oxidizers of ammonium to nitrite are ammonium-oxidizing bacteria (AOB). Additionally, ammonium-oxidizing archaea (AOA) and several heterotrophic bacteria contribute to this step of nitrification (Zhang et al., 2011). Nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB). The efficiency of this wastewater treatment process significantly depends on the microorganism population and follows the specific requirements of each group’s optimum operation conditions, including temperature and substrate concentrations. Novel sequencing techniques such as Illumina sequencing provide the data on microorganisms and their abundance in the natural samples, clarifying the main metabolic pathways and bioaugmentation potential of AS for the removal of particular substances (Cydzik-Kwiatkowska and Zielińska, 2016).
3.2 Removal of emerging micropollutants in activated sludge

One of the main parameters determining the removal efficiency of EMs is the way they interact with the solid particles including purposely added adsorbents and AS microorganisms (Carballa et al., 2004). This parameter, together with toxicity and complexity of the structure, will affect bioavailability and therefore the efficiency of biodegradation of the micropollutant (Barret et al., 2010). For instance, high persistence of DCF and CMZ to biodegradation can be caused by the presence of chlorine and multi-aromatic rings in the molecules (Kimura et al., 2005).

The first step of the biodegradation process is the uptake of EMs followed by enzymatic reactions (Luo et al., 2014). Due to their trace concentration levels, EMs cannot be used as sole carbon or energy source but rather in metabolic reactions on a mixed substrate (Figure 2,a) or in co-metabolic processes when EMs are modified by the microorganism but not used as a carbon source (Figure 2, b) (Hamid and Eskicioglu, 2012).

Biodegradation rates are commonly described by biodegradation rate constants ($K_{biol}$) and depend on the type and amount of microorganisms (Margot et al., 2015), toxicity (concentration) of the EM and operation conditions (discussed in Chapter 1.1.3). However, there are several reported studies showing no correlation between complexity of molecular structure and biodegradability of EMs (Sipma et al., 2010). Therefore, the total effect of the EMs structure on its removal is unclear.

3.3 ‘Negative’ removal

An increase in the EM load in effluents compared to influent was witnessed in several studies for DCF, CMZ, estrogens and antibiotics including TMP and sulphonamides (Göbel et al., 2007; Polesel et al., 2016, 2017). The explanations for the negative values of removal are often linked to frequently occurring conjugation of EMs. Deconjugation of the transformation products back to parent compounds via abiotic reactions or mediated by AOB and heterotrophic bacteria in WWTPs lead to higher EM concentrations in effluents (Stadler et al., 2012).

Therefore, calculated removal efficiencies themselves can be misleading and controversial. In this context, there is a need for further study of the conditions favouring the desired direction of the removal process following the intermediate transformation products.
3.4 Proposed mechanisms of biodegradation

At this moment, knowledge of EM biodegradation mechanisms is limited. Likewise, it is unclear which microorganisms are responsible for the removal of discussed EMs. However, multiple data are available proving the significant effect of the bacterial community composition on the removal efficiency of PhACs and steroid hormones (Suarez et al., 2010).

Additionally, certain mechanisms were proposed and several bacterial species have been suggested as active contributors to EMs removal. In particular, several heterotrophic bacterial species demonstrated the ability to remove estrogens and PhACs (Iasur-Kruh et al., 2011; Larcher and Yargeau, 2013), and the significance of autotrophic AOBs presence was highlighted repeatedly (Fischer and Majewsky, 2014; Park et al., 2017; Silva et al., 2012). Although some of the AOBs were able to remove EMs, experiments with IBU and EE2 removal showed the inability of pure AOB cultures to degrade EMs proving the importance of other groups of bacteria in AS (Gaulke et al., 2008). Supporting these data, the co-metabolic activity of AOBs was reported for acidic PhACs, among which were IBU and DCF, sulfonamide antibiotics and estrogens (Kassotaki et al., 2016; Quintana et al.,...
Furthermore, cooperation of both mechanisms enhanced the removal of these EMs compared to separate studies on heterotrophic mineralisation and AOB co-metabolic degradation (Hamid and Eskicioglu, 2012; Tran et al., 2014). Therefore, the commonly proposed EMs biodegradation mechanism includes the first co-metabolic step mediated by AOBs, followed by metabolic reactions carried out by heterotrophic bacteria (Park et al., 2017). However, further studies are required to support this proposition and to find the key bacterial species, involved in co-metabolic and metabolic reactions for the discussed EMs.

3.5 Comparison of activated sludge technologies

AS technologies have been developing over the past century from only organic material removal (COD) with a short sludge retention time (SRT) to complex systems with anoxic and aerobic steps and efficient nitrogen and phosphorus removal. The conventional activated sludge process (CAS), including AS and settling steps (Figure 3, a), is the most commonly used secondary treatment process, with a reported higher potential for EMs removal compared to trickling filters, bio-contactors and stabilization ponds (Hamid and Eskicioglu, 2012).

Over the past 50 years, MBR technology, in which the AS process coupled with membrane filtration for liquid-solid separation (Figure 3,b), has become an alternative for the CAS process. MBR technology is effectively applied for handling highly concentrated wastewaters, complete removal of pathogens and viruses, decreasing odour and reduction in WWTP area (Sipma et al., 2010). Due to these advantages, accelerating attention is paid to the application of the MBRs for municipal and industrial WWTPs (Alvarino et al., 2018).

**Figure 3** Principle configuration of conventional CAS process (a) and MBR (b)
Many studies tried to compare the removal efficiency of different AS technologies for the removal of EMs. As a consequence of large deviations among the removal efficiency data, evaluations of the technologies for EMs removal also greatly vary and sometimes contradict each other. In general, most of the data for the discussed groups of EMs show a higher removal potential of MBRs compared to CAS and sequencing batch reactor (SBR) systems (Figure 4). However, as seen from Figure 4, the differences in the removal efficiencies in most cases are insignificant and could not improve the removal process to a valuable extent.

The commonly used membrane materials in MBR are hydrophilic or neutrally charged and not expected to affect the EMs removal (Alvarino et al., 2018). Therefore, the EMs removal mechanisms are neither based on nor related to membrane filtration. The benefits of MBR technology for EMs removal could be linked to the biomass concentration and thus the food-per-microorganism (F:M) ratio (Chapter 1.1.3.1), to the more advanced bacterial community due to a longer SRT (Chapter 1.1.3.2), or to the improved separation of EMs incorporated with colloids and particulates of AS (Taheran et al., 2016).

Figure 4: Comparison of MBRs (SRT from 30d to infinite), CAS and SBR (SRT from 1d to 16 d) for the removal of different EMs. SMX: Sulfamethoxazole; ETM: Erithromycin; GFZ: Gemfibrozil; BZF: Bezafibrate; ATL: Atenolol; MTP: Metoprolol. Numbers in brackets represent the number of references considered for each bar. Modified from Cecconet et al. (2017)
3.6 Operational parameters related to biodegradation

There are multiple studies proving that operational parameters such as SRT, hydraulic residence time (HRT), redox conditions, temperature, pH and others may affect the biodegradation rates of EMs (Luo et al., 2014; Suárez et al., 2008). The first two parameters are also closely connected to the F:M ratio and the removal efficiency of EMs. However, due to the inconsistency of the experimental parameters there is no clear knowledge about these links yet (Besha et al., 2017).

3.6.1 Organic loading

Organic loading rate (OLR) (kgBOD·d⁻¹·m⁻³) is directly linked to the food-per-microorganism (F:M) ratio (kgBOD·gMLSS⁻¹·d⁻¹) in AS and could correlate with biodegradation rates of EMs. Co-metabolic EMs removal requires presence of sufficient amount of growth substrates and co-factors (Margot et al., 2015). At the same time, relatively low organic loading (i.e., the low amount of degradable compounds per unit volume) can force microorganisms to metabolize more complex molecules through co-metabolic reactions as well as favour the growth of oligotrophic species with specific mechanisms of using substrates presented in very low concentrations (Sipma et al., 2010). However, severe lack of a substrate would limit the diversity of metabolic and co-metabolic reactions and therefore reduce the removal potential.

Moreover, OLR is one of the important parameters since it affects biomass growth and therefore the production of extracellular polymeric substances (EPS) by bacteria. EPS may have impact on EMs removal mechanisms by influencing EMs sorption, bonding biomass and supporting microbial interactions (Cydzik-Kwiatkowska and Zielińska, 2016; Alvarino et al., 2018). In MBR systems increase of EPS in AS is one of the predominant causes of membrane fouling together with the other AS characteristics (Meng et al., 2009).

3.6.2 Sludge retention time

SRT can affect the diversity, size and content of a microbial community. Slow-growing bacteria are able to use more diverse enzymatic reactions and metabolic pathways which lead to biodegradation of more persistent substances (Margot et al., 2015).

Most of the studies proved that SRT has a great impact on the removal of the above-listed groups of EMs (Besha et al., 2017; Taheran et al., 2016). At the same time, overall the reported data are contradictory since there are studies showing no effect of SRT on biodegradation rates (Servos et al., 2005; Weiss and Reemtsma, 2008). These results could be explained by the specific molecular structure of some EMs, such as the presence of multi-aromatic rings (Falås et al., 2016). The other reason could be non-systematic data in presenting different experimental conditions for different substances. In some studies, compared SRT values could not reach critical values for the removal since the other factors
affecting the growth rates (e.g. temperature, concentration and toxic effect of EM) were not taken into account.

It is important to mention that optimizing the SRT is a trade-off between removal efficiency and energy consumption. Within a certain range, the SRT also has an effect on sludge production due to low biological activity (Besha et al., 2017).

Another important limiting factor in MBR can be the fouling of the membrane. Prolongation of the SRT increases the biomass concentration and sludge viscosity, which increases the rate of membrane fouling (Besha et al., 2017; Meng et al., 2009). At the same time, studies of MBRs with short SRTs (3-5 days) demonstrated higher amounts of EPS compared to the optimum SRT of 20-50 days in most of the studies (Meng et al., 2009). However, the optimum SRT may vary under different operational conditions (HRT, F:M, biomass growth rate, etc.)

3.6.3 Aerobic conditions and nitrification

The presence, deficiency or absence of free oxygen indicate aerobic, anoxic and anaerobic conditions and determine the main metabolic pathways contributing to biological removal. Aerobic conditions have been proved to enhance degradation rates, especially for EMs with lower biodegradability such as DCF and EE2 (Besha et al., 2017; Suarez et al., 2010). In many published studies EMs removal was also linked to nitrification process efficiency (Dawas-Massalha et al., 2014; Fernandez-Fontaina et al., 2012; Kassotaki et al., 2016; Park et al., 2017; Tran et al., 2014).

Overall, studies show that nitrifying conditions have positive effect on EMs removal in different AS systems (CAS, SBR, MBR, fixed bed reactor and so on) (Besha et al., 2017; Luo et al., 2014). Considering the excellence of aerobic conditions, EMs removal could be related to the ammonium oxidation step of nitrification. The proposed explanations include co-metabolic reactions with AOBs and presence EMs utilisers among nitrifying bacteria (Chapter 1.1.2.3). The ammonium monoxygenase (AMO) enzyme, secreted by AOBs to catalyse ammonia, was proposed as also contributing to degradation of EMs (Fernandez-Fontaina et al., 2012; Vieno and Sillanpää, 2014).

In addition to the above-mentioned mechanisms, abiotic nitration of EMs in the presence of nitrites was proposed for DCF and EE2 (Vieno and Sillanpää, 2014). This mechanism could explain the ‘negative’ removal of DCF (Chapter 1.1.2.2) by the return of nitration products (nitro-derivatives of DCF) to the parent compound after a decrease in nitrite concentration in AS (Barbieri et al., 2012).

3.6.4 Temperature

Seasonal temperature variations noticeably affect EMs removal efficiency due to the dependence of metabolic reactions kinetics on temperature (Luo et al., 2014; Sari et al., 2014; Vieno et al., 2005; Zhou et al., 2013). Considering that a large number of WWTPs are located in areas, where the temperature is in the range of 8-12°C for at least half of the
year and the environment of cold climate areas is potentially more vulnerable, it is necessary to study the long-term effect of this factor on WWTPs performance. One of the proposed strategies to compensate for the low temperature effect by optimizing other parameters was the increase in mixed liquor suspended solids (MLSS) concentration with raising the SRT (Luo et al., 2014). This could be achieved by implementing MBR.

Low-temperature operation was proven to cause severe membrane fouling in both lab-scale and full-scale studies (Sun et al., 2014). This could be attributed to increasing sludge viscosity, changes in EPS production, reducing sludge stabilization and particle size in AS, as well as to a change of bacterial community (e.g. an increase in filamentous bacteria) under pressure of low temperature (Zhang et al., 2014). However, the performance of WWTPs with MBR technology in cold climate zones where year-round water temperatures are slightly higher than 10 °C is still poorly studied.

The temperature of wastewater was proven to influence the microbial community structure and gene functional patterns of AS as well as to be one of the most important factors affecting the abundance of nitrifying bacteria (Cydzik-Kwiatkowska and Zielińska, 2016; Fredriksson et al., 2017). A significant decrease in nitrifiers in AS was shown at 13 °C (Zhang et al., 2014). Considering the importance of co-metabolic mechanisms of AMO bacteria in EMs removal (Chapter 1.1.2.1), this could significantly inhibit the removal of the discussed EMs. Furthermore, not only bacterial composition correlates with wastewater temperature but also microbial (Cydzik-Kwiatkowska and Zielińska, 2016).

Nonetheless, the correlation between the EMs removal efficiencies and the temperature is still unclear and therefore the removal rates of EMs at certain temperatures cannot be predicted yet (Besha et al., 2017).
4. Materials and methods

The research was started in August of 2012 with an extensive study of the available literature regarding the chemical, physical and ecotoxicological properties of EMs. Target compounds from several groups of the EMs of high concern were selected according to their presence in the environmental waters, proven toxicological impact and potential to biodegradation optimization. Experimental schedule and studied parameters are summarized in Table 2.

<table>
<thead>
<tr>
<th>Year</th>
<th>Q</th>
<th>Studied emerging micropollutants</th>
<th>Pilot studies</th>
<th>Full-scale WWTP data</th>
<th>Article</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>t'</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SRT SBR MBR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>3</td>
<td></td>
<td>Literature review</td>
<td></td>
<td>I, II</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>Experimental design</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>1</td>
<td>Ibuprofen</td>
<td>12°C 12 d 30 d</td>
<td>Operation data collection</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Diclofenac</td>
<td>30 d</td>
<td>Effluent sampling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Carbamazepine</td>
<td>60 d</td>
<td>Removal experiments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>1</td>
<td>Ibuprofen</td>
<td>8°C 14 d 90 d</td>
<td>Literature review</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Diclofenac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Estrone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Carbolin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>1</td>
<td>Sulfadiazine</td>
<td>90 d</td>
<td></td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Trimethoprim</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>2016</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
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</tr>
</tbody>
</table>

During the year 2013 the removal of IBU, DCF and CMZ at 12°C was studied in full-scale AS (I) as well as SBR and MBR pilot-scale AS (I, II). Experiments under 8°C were started in May of 2014. Three estrogenic compounds were added to the study of micropollutants removal (II). CMZ was excluded from further studies since no removal was reached under all of studied laboratory conditions. Microbial analyses were started in
2015 (III). Literature survey on antibiotics (IV) and the effect of SDZ and TMP exposure on AS (V) were performed during 2016 – 2017.

4.1 Design and operation of laboratory-scale bioreactors

For the comparison of AS removal performance under different operational conditions four equal pilot-scale SBR reactors and two equal MBR reactors were studied in temperature-controlled chambers and fed with synthetic influent. Studies were started at 12°C in presence of IBU, DCF and CMZ. Two MBRs and two SBRs were operated with same OLR whereas two other SBRs were operated with higher OLR. After the six months of operation, two SBRs with higher OLR showing lower EMs removal potential were excluded from the studies (I). The experiments were continued at the same conditions with lower concentrations of the three EMs (discussed in Chapter 3.2). Then the temperature conditions were changed to 8°C and after two months of adaptation three estrogens were added to the wastewater. After two more months experiments with removal of EMs were continued during the next six months, four of those two pilot reactors were operated without the addition of EMs and two were operated with the addition of EMs (II, III). For the next stage of the research, AS was operated several months without the addition of EMs. In the final experimental stage, four pilot reactors were operated under pressure of the two antibiotics TMP and SDZ (V).

Detailed description of the synthetic influent composition is presented in I. EMs concentrations and all the operational conditions for each experiment are listed in I, II, III, V.

4.1.1 Sequencing batch reactors

SBRs were installed in parallel as shown in Figure 5. Influent peristaltic pump and effluent pumps were calibrated according to the desired influent flow and HRT.

In I and II four SBRs were operated in order to compare different OLRs, whereas in III and V only two of SBRs (lower OLRs, showed better performance) were used in the study. The detailed description of SBR operation is presented in I, III. SRT values chosen for SBRs operation represented the typical SRT range for Finnish nitrifying WWTPs (I, Table 4).
Aeration and peristaltic pumps work schedule was controlled by timer according to the operational cycle.

The cycle length was chosen according to the biological step time in reference Finnish WWTPs (I).

The SBR operational cycle is illustrated in Figure 6.

4.1.2 Membrane bioreactors

Two identical pilot-scale MBRs were designed and constructed from acrylic glass with 15 L working volume in accordance to the shape and size of small-scale submerged flat-sheet microfiltration (MF) membranes provided by Kubota Membrane Europe as illustrated in Figure 7.
Figure 7. Schematic illustration of the MF membrane and MBR filled with tap water with the membrane installed (on the left). MBR after start-up with AS (on the right).

Special characteristics of the membrane affecting operational conditions are presented in Table 3.

Influent pumps are operated continuously. Effluent pumps are controlled by low (start) and high (stop) level switches, providing enough relaxation for the membranes. Membrane characteristics and design principles have been described in II and IV.

Table 3 Microfiltration membrane parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main material</td>
<td>Chlorinated Polyethylene</td>
</tr>
<tr>
<td>Membrane sheet surface area</td>
<td>0.11 m²</td>
</tr>
<tr>
<td>Dry weight</td>
<td>0.4 kg</td>
</tr>
<tr>
<td>Pore size</td>
<td>0.4 μm</td>
</tr>
<tr>
<td>Aeration intensity demand</td>
<td>0.2 – 0.8 m³·m⁻²·h⁻¹</td>
</tr>
<tr>
<td>Max flux with clean water</td>
<td>1.0 m³·m⁻²·d⁻¹</td>
</tr>
<tr>
<td>Design flux</td>
<td>0.5 m³·m⁻²·d⁻¹ / 20.8 LMH</td>
</tr>
<tr>
<td>Operational flux</td>
<td>0.14 m³·m⁻²·d⁻¹ / 5.7 LMH</td>
</tr>
<tr>
<td>Design flow</td>
<td>55 l·d⁻¹</td>
</tr>
<tr>
<td>Operational flow</td>
<td>15 l·d⁻¹</td>
</tr>
</tbody>
</table>
4.2 Full-scale activated sludge studies

Three types of full-scale AS were used in this research. Seeding sludge for preliminary experiments with IBU removal in SBRs was taken from Suomenoja WWTP treating mainly municipal wastewater in the city of Espoo. Removal efficiency of full-scale process was studied in Viikinmäki WWTP treating mainly municipal wastewater of Helsinki. Seeding sludge for laboratory simultaneous experiments with MBR and SBR pilot-reactors was taken from semi-scale Evac MBR treating wastewater from an office building. Important parameters of WWTPs operation are presented in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suomenoja (L)</th>
<th>Viikinmäki (L)</th>
<th>Evac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q [m³ d⁻¹]</td>
<td>100 000</td>
<td>280 000</td>
<td>2.25</td>
</tr>
<tr>
<td>SRT [d]</td>
<td>14</td>
<td>16</td>
<td>≥100</td>
</tr>
<tr>
<td>Temperature [°C]</td>
<td>12±1</td>
<td>12±1</td>
<td>28±2</td>
</tr>
<tr>
<td>pH</td>
<td>6.0-8.0</td>
<td>6.0-8.0</td>
<td>6.0-8.0</td>
</tr>
</tbody>
</table>

Influent water:
- TN [mg l⁻¹]: 60, 46, 177
- TP [mg l⁻¹]: 7.8, 6.3, 30
- CODCr [mg l⁻¹]: 486, 528, 2006

Effluent water:
- TN [mg l⁻¹]: <20, <20, 101
- TP [mg l⁻¹]: <0.4, <0.3, 12
- CODCr [mg l⁻¹]: <75, <75, 112

4.3 Micropollutants removal analysis

The removal experiments with target EMs are extensively described in I, II, IV. In principle, for evaluation of the removal efficiency, the known volume of AS from full-scale WWTP or in pilot-scale reactor was spiked with determined amount of target EMs. AS adapted and not adapted to EMs was studied under different laboratory conditions: at 12 and 8°C, at two OLRs and operated with different SRTs between 12 and 90 days.

Mixed liquor samples were withdrawn from the batch volume several times during following hours (up to 24h). Samples were immediately preserved by acidifying and freezing to -20°C until further analysis.
**LC-MS/MS analysis**

EMs concentrations were analysed using ultra pressure liquid chromatography-tandem mass spectrometry UPLC-MS/MS (triple-quadrupole) multiple reaction monitoring (MRM) technology after solvent extraction with methanol. Analyses were performed in two laboratories. The samples from the removal experiments at 12°C were analysed in Ramboll Analytics, Finland as described in I. Samples in all the experiments at 8°C were analysed in Laboratory of Organic Chemistry, Åbo Academy, Turku. The detailed protocol of the separated analyses of water and solid phase of AS is presented in II.

**Biodegradation rate calculations**

Biodegradation potentials of EMs were evaluated and compared using pseudo first-order biodegradation rate constant $K_{biol}$ (l gMLSS⁻¹d⁻¹) which was calculated as described in I, II, IV. The total removal (%) was calculated as presented in I considering absence of sorption process. Differences between the calculated values were identified by analyses of variances (ANOVA) with significance level of 5% (p <0.05).

**4.4 Bacterial community analysis**

Key bacterial and archaeal groups for EMs removal under low temperature conditions were studied by comparing AS from pilot reactors with different removal efficiency. Microbial community of seed-sludge-providing WWTP was sampled (in duplicates) and analysed as a reference. The data were presented as an average ± standard deviation.

**Samples preparation and DNA extraction**

Duplicate AS samples for microbial analyses were centrifuged and frozen according to IV. Frozen samples were sent to the Department of Civil Engineering, University of Granada, Granada, Spain for the DNA extraction as described in IV and V. After the extraction, DNA was frozen and sent to the Research & Testing Laboratory of Lubbock, TX, USA for the Illumina sequencing (MiSeq/HiSeq). Primers and PCR protocol for domain Bacteria described in IV and V. Primers and PCR protocol for the domain Archaea presented in V.

**Bioinformatics**

Raw data from sequencing were quality checked and operational taxonomic units (OTUs) were affiliated for study of bacterial community affected by IBU, DCF, E1 and EE2 as well as similarities and dissimilarities between the bacterial communities were calculated in the Department of Civil Engineering, University of Granada as extensively described in IV. For study of antibiotics effect on microbial community, raw reads were quality filtered and processed into OTUs in Research & Testing Laboratory as described in V.
5. Results and discussion

5.1 Performance of pilot reactors

Summarized data on the performance of MBRs and SBRs during long-term operation are presented in Figure 8. Stable COD removal (85±10%) was observed under all experimental conditions in both wastewater treatment technologies. It could be concluded that the studied factors (including the effect of environmentally relevant concentrations of EMs) have no negative effect on the performance of heterotrophic bacteria of AS. In contrast, nitrogen removal efficiency was noticeably disturbed by temperature changes. Additionally, negative effects on nitrogen removal efficiency and collection of nitrites in the SBR effluents were observed after the additional spiking of micropollutants on the days of the experiments, despite the fact that AS was adapted to environmentally relevant EM concentrations.

The performance of ammonium oxidation was followed through ammonium removal efficiency, and nitrite oxidation efficiency was evaluated (starting from the 16th week) by monitoring effluent concentrations of nitrites. Since the initial AS was taken from the full-scale MBR (Evac) plant operated at 28 ± 2°C, expected inhibition of nitrification followed the inoculation of the AS to the pilot reactors operated in 12°C conditions. Partial removal of ammonium appeared in MBRs after four weeks of adaptation and 99 ± 1% removal in the next two days, whereas SBRs did not show any removal of ammonium.

To speed up the acclimation process, 1 L of MBR sludge was inoculated at each SBR, after which partial oxidation of ammonia was detected in the SBRs. Total removal of ammonia was reached in the SBR in the next four weeks (I).

It should be noted, that the effect of IBU, DCF and CMZ in wastewater could also inhibit the nitrification process during the adaptation period as well as slow down the acclimation of AS in SBRs. The same observation applies to the short decrease in the ammonium removal (from 99% to ~80%) when EMs including estrogens were added to wastewater after four months of operation without EMs exposure to the sludge.
Figure 8 Average COD removal (upper graph) and ammonium removal (lower graph) for MBRs and SBRs during the experimental period (excluding technical breaks between the experimental stages in which analyses were not performed). Rectangular grey areas represent an addition of EMs in wastewater. Dotted arrows show the events of additional EMs spiking to AS during the experiment.
An operational temperature drop from 12 °C to 8 °C did not affect the removal of ammonium as well as additional concentrations of IBU, DCF, CMZ and estrogenic compounds during the experiments. Once the AS is adapted to these EMs, ammonium oxidizers are not affected by their presence in the environment. In contrast, the addition of antibiotics to wastewater (without preliminary adaptation) noticeably affected ammonium removal in both MBR and SBRs (V), which is consistent with the conclusion from the literature review that AOB is the group of AS bacteria most sensitive to antibiotics (IV). The negative effect was expectedly delayed due to the slow growth of nitrifying bacteria. The other reason for the delay could be due to the use of the growth factor reserve in AS (as discussed in IV). Furthermore, the reason for the inhibition delay in MBRs could be the high concentration of bacteria, and therefore the presence of more bacteria with the ability to store folic acid, important cofactor for DNA synthesis whose metabolism is inhibited by TMP and SDZ.

In addition, the negative effect of antibiotics on AS of MBRs was noticeably shorter compared to SBRs. The ammonium removal decreased after three weeks of MBR operation with antibiotics and the 99% removal efficiency was recovered in the next four weeks, whereas in SBRs the ammonium removal dropped dramatically down to zero removal with only partial recovery to 15±10% removal during the rest of the experiment (Figure 8, V). Since ammonium removal was also limited in SBRs during the start-up period before the MBR sludge inoculation event, it is evident that regrowth of an efficient nitrifying community would require a longer SRT.

Nitrite oxidation efficiency appeared to be highly disturbed by the decrease in temperature from 12 °C to 8 °C. Faster recovery of nitratation efficiency was observed in the AS of MBRs. As Figure 8 (lower graph) illustrates, high amounts of nitrates were rapidly detected in all the effluents after the change in temperature conditions. Lately, Chen at al. (2018) showed a similar dynamic of ammonium-oxidizing / nitrite-oxidizing activities at low temperature. In long-term 10°C operation AOB recovered better than NOB, whilst nitratation was limited.

High nitratation efficiency recovered in MBRs after about 4 weeks of adaptation, and stable total removal (<0.3 mg L⁻¹ in effluent) was reached in the next 10 weeks. In contrast, no nitratation recovery was observed for 10 weeks in SBRs. For this reason, 1 L of MBR sludge was inoculated to SBR sludge to boost community adaptation. Partial recovery of nitratation was immediately detected and full removal of nitrite was reached in the next 10 weeks of operation.

Suspended solids (SS) removal in SBRs was at the stable level of 97±1%, except for the first one to two weeks after the temperature changes when it decreased no more than 2±1%. SS removal in MBR effluents was at the stable level of 99.99±0.01% showing the membrane integrity despite the changes in the conditions and long-term operation.
5.2 Biological removal of emerging micropollutants

Full-scale studies included comparison of IBU, DCF and CMZ concentrations in influent, AS from the anoxic zone, AS from the aerobic zone (the end-point of the biological stage) and effluent concentrations. The process scheme and sampling points are illustrated in Fig. 1 in I. Samples were taken from the three parallel lines of the AS process. The results showed ~95% removal of IBU and no removal of DCF and CMZ. At the same time, \( K_{\text{biol}} \), obtained from 24 h laboratory experiments with Viikinmäki AS (Table 3 in I), were lower than reported in most of the studies (Smook et al., 2008; Suarez et al., 2010).

It is important to mention that the detected concentrations of EMs were similar in AS samples and in the final effluent samples (Fig. 2 in I), proving that EMs concentrations extracted from the solid phase of AS where insignificant. Therefore, no sorption or adsorption to the sludge was involved in the removal of IBU, DCF and CMZ, which is consistent with the literature data (Sipma et al., 2010). A comparison of AS samples showed that most of the IBU (80-85%) was removed in the first anoxic zone biological treatment, whereas in the aerobic zone the remaining 10-15% of the IBU was removed.

\( K_{\text{biol}} \) values similar to full-scale or even lower were obtained for the pilot AS. \( K_{\text{biol}} \) values obtained in laboratory conditions for estrogens were also many times lower than reported in the literature for higher temperatures (Estrada-Arriaga and Mijaylova, 2011). This difference can be related to low-temperature conditions, which slow down metabolic reactions of microorganisms (I). A comparison of \( K_{\text{biol}} \)-s under different laboratory conditions is presented in Figure 9.

Preliminary adaptation to EMs had a positive effect on the removal of IBU in SBRs (Fig. 3 of I) and the removal of E1 in both SBRs and MBRs (III). Adapted AS in both cases demonstrated 30 - 50% higher removal rates compared to non-adapted AS. No effect of adaptation to IBU on AS with 90d SRT was observed (III). Adaptation of AS to DCF, CMZ and EE2 had no effect on the removal efficiency of these compounds. Lower OLR improved IBU removal in SBRs; however that did not affect removal rates in MBRs. These data could be explained by a higher F:M ratio in SBRs compared to MBRs. For instance, in the presence of an easily available substrate (F:M ≥ 0.1 kgBOD· gMLSS \(^{-1} \cdot \text{d}^{-1}\)), mixed substrate utilization, involving the removal of EMs could be suppressed.
Despite the different operational parameters, concentrations of CMZ were at the stable range of calculated spiked amounts in all the experiments. Between experiments, concentrations decreased to the calculated background concentrations from wastewater, as presented in Figure 10, showing no removal or accumulation in AS cells or flocs (I).
Figure 10: Average concentrations of CMZ in AS before and during the experiments; and in effluents before the experiments. Experimental period 1 included three experiments with higher concentrations of CMZ (10 μg L⁻¹ in wastewater and 20 μg L⁻¹ spiked in the experiment). Experimental period 2 included two experiments with lower concentrations of CMZ (1 μg L⁻¹ in wastewater and 5 μg L⁻¹ spiked in the experiment).

Effluent concentrations of CMZ were very similar to the concentrations in AS as well as calculated concentrations. Previously, Li et al. reported that partial removal of CMZ in MBRs (up to 10%) dropped to zero removal with the decrease in temperature under 15 °C. However, the exact SRT was not reported (Li et al., 2011). The results of this study show that prolongation of SRT up to 90 days cannot compensate for the negative effect of low-temperature conditions on CMZ removal.

A positive effect on the removal efficiencies of IBU, DCF and E1 was obtained with the increase in the SRT to 90 days compared to shorter SRTs (II). No significant differences were noticed between the SRTs of 12, 14, 20 and 60 days for all EMs except DCF, which could be removed only when the SRT was at least 30 days. No effect of SRT on EE2 removal was detected (III). At the same time, despite the relatively short SRT (16 days), the full-scale AS showed the highest values of removal efficiencies and removal rate constants. It could be related to greater diversity of substrates in real wastewater composition and therefore broader metabolic reactions.

A temperature drop from 12° to 8° led to a noticeable decrease in IBU and DCF removal efficiencies supporting the theory of the crucial effect of low temperatures on EMs removal rates. In this sense, the positive effect of prolonged SRT compensated for the negative effect of low temperature, which is consistent with Luo et al. (2014). At the same time, no difference in the efficiency of the removal from the water phase in SBRs and
MBRs during 24-h experiments could be noticed, demonstrating >80% removal in all the cases, as shown in Figure 11 (on the left). At the same time, already 0.5 h after spiking the EMs to AS, the removal from water reached 60% to 80% for the studied EMs. Therefore, these removal efficiencies could be mainly attributed to either sorption or abiotic reactions and not biodegradation. The total removal from both water and solid phases of AS (Figure 11, on the right) revealed that most of the spiked DCF and EE2 was left in the solid phase of AS, showing low total removal (≤30%); whereas a percentage of removed IBU and E1 increases during 24 h showing that efficient biodegradation took place. Additionally, in all the studied cases, the total removal efficiencies after 24 h were 10-30% higher in the MBR samples compared to the SBR samples.

![Figure 11](image)

**Figure 11** Removal (%) of the EMs in environmentally relevant concentrations at 8°C after 24 h. Comparison of the results calculated from water phase of AS (on the left) and total removal of EMs from AS (on the right). Error bars represent standard deviation between data from all the successful experiments.

Altogether, pilot studies supported the results from full-scale data showing the importance of AS solid phase measures for interpreting EMs removal efficiencies. Furthermore, these results showed that the capacity of microorganisms to degrade EMs is different in AS with an SRT of 14 days compared to AS with an SRT of 90 days, which can be explained by the differences in microbial communities. Different species of slow-growing oligotrophic AS and a wider range of enzymes allow new co-metabolic relationships to develop leading to deeper EMs biodegradation. At the same time, these results show the additional positive impact of effective SS separation on the effluent quality in MBR processes due to mechanical separation of cells, which are able to take in and collect a big proportion of EMs.
5.3 Formation of transformation products

In addition to the remarkably lower removal rates obtained at lower temperatures compared to the literature data (I), the possible limiting effect of the studied operational parameters on the EMs mineralisation was studied (II). Two main IBU transformation products (hydroxy-IBU and carboxy-IBU) and the nitro product of DCF (nitro-DCF) were measured during the removal experiments.

At 12 °C, no transformation products of IBU were detected in AS of MBRs, and less than 5% of spiked IBU was left in the form of hydroxy-IBU and carboxy-IBU in AS of SBRs after 24 h. At 8 °C, the concentration of present transformation products slightly increased. In MBRs, less than 2% of the spiked IBU presented in AS were in the form of hydroxy-IBU (no carboxy-IBU). In SBRs during 24 h, about 10% of IBU was collected in AS as carboxy-IBU and about 2% as hydroxy-IBU (Fig. 1 in II).

Nitro-DCF was not detected in MBRs in either of the studied temperature conditions. In SBRs the amount of nitro-diclofenac increased in lower temperature. However it stayed in a low range (≤4% of spiked DCF at 12 °C and ~20% at 8°C) in both cases (Fig. 3 in II).

To support the hypothesis of abiotic nitration of DCF and accumulation of the parent compound at high concentrations of nitrites, nitrite concentration (NO₂⁻) was followed during the experiments in the SBRs with dissolved oxygen (DO) concentration ranging from 2 mg L⁻¹ to 8 mg L⁻¹. Decrease of DO led to limited nitratation and nitrite accumulation in the AS.

As shown in Figure 12, no significant difference in nitro-DCF and DCF concentrations was detected between removal experiments at nitrite concentrations of <0.3 mg L⁻¹ and >3 mg L⁻¹. Even though the tendency toward a lower concentration of the parent compound and higher content of nitro-DCF could be noticed in the presence of higher nitrite concentration, the deviation between the samples is too high to draw any conclusions. In this experimental design, no significant effect of nitrite concentration (and therefore the reversible abiotic nitration of DCF) could be proven.
However, despite the literature data that this process takes place under anaerobic conditions (Barbieri et al., 2012), formation of nitro-DCF was observed as well in presence of DO. Therefore, the mechanism of abiotic transformation under aerobic conditions requires further exploration.

5.4 Microbial communities of activated sludge

As discussed in Chapter 1.1.2, the microbial community structure and dynamics correlate to the removal of different substances, and therefore an understanding of the EMs removal mechanisms can benefit from comprehensive knowledge on the effect of these substances on microbial populations. In this thesis, the effect of IBU, DCF, E1, E2 and EE2 on the bacterial communities of AS were studied as well as the effect of antibiotics SDZ and TMP on bacterial and archaeal population dynamics.
5.4.1 Community dynamics at the phylum level

Altogether, six bacterial phyla were identified in the AS samples. The results are summarized in Figure 13 and Figure 14.

As expected for nitrifying AS, Proteobacteria, Actinobacteria and Bacteroidetes represented the three most dominant phyla, covering from 70% to 98% of the bacterial communities of all the samples. A noticeable decrease in Actinobacteria in the presence of antibiotics was detected in both the SBR and MBR bacterial communities (V). In contrast, Bacteroidetes increased in the presence of antibiotics in all of the samples as well as in the SBR samples in the presence of other studied EMs. In addition to the dominant phyla, Firmicutes appeared in the AS in the absence of EMs, representing up to 7% of the communities (III). Bacterial communities of MBRs included two more phyla not presented in SBRs: Chloroflexi and Gemmatimonadetes (Figure 13, III). Most likely, the lack of these two bacterial groups in SBRs correlates to the SRT difference. This conclusion is in agreement with Phan et al. (2016), who in their recent study showed preferable growth of Chloroflexi at SRTs longer than 25 days. The highest abundance of Chloroflexi (17%) in MBRs was detected in the absence of EMs for six months, whereas in the presence of EMs and in samples two months after the last EMs addition, this phylum appeared in less than 2% of the community. In contrast, Gemmatimonadetes were not appearing in the absence of micropollutants while representing up to 3% of the community in the presence of EMs (Figure 13), showing the resistance and selective advantages of growing on EMs-polluted wastewaters. These tendencies were partly different from published in literature observations on the phyla level. For instance, an increase in Firmicutes and Actinobacteria as well as the decrease in Proteobacteria were reported in the presence of pharmaceuticals such as NSAIDs as well as antibiotics (Xia et al., 2015; Jiang et al., 2017). At the same time, a study by Jiang et al. (2017) supported the data on preferable growth of Bacteroidetes. Since the reported studies were conducted at 19±2 °C, low-temperature conditions could play an additional selective role, preventing Actinobacteria and Firmicutes from competitive growth despite their ability to degrade EMs.

5.4.2 Community dynamics at the class level

Proteobacteria were represented by Alpha-, Beta- and Gammaproteobacteria in SBR reactors with the additional class Deltaproteobacteria in MBRs (Figure 13, Figure 14). The amount of Alphaproteobacteria tended to decrease in the presence of EMs whilst Betaproteobacteria became the dominant class of Proteobacteria.

Additionally, Deltaproteobacteria increased noticeably in the presence of all studied EMs and totally disappeared after six months of operation without the addition of EMs (Figure 13, III, V). Gammaproteobacteria tended to favour the presence of antibiotics (increased in abundance up to 13% of the community), whereas in addition of IBU, DCF and estrogens the class was not identified in SBRs and presented as a minor class in MBRs (<1.4% of the total bacterial community). Previously published studies supported the inhibiting effect of IBU on the growth of Firmicutes and Gammaproteobacteria in natural bacterial communities. However, the sole addition of DCF and IBU (in a concentration
range similar to this study), was reported to cause the preferable growth of *Alphaproteobacteria* accompanied by the decrease in *Betaproteobacteria*, while in this study the opposite effect was observed (Barra Caracciolo et al., 2015).

### 5.4.3 Community dynamics at the genus level

Along with the above discussed results, on the genus level bacterial communities vastly differed from existing literature data (**III, V**). Despite the efficient nitrification performance, psychrophilic heterotrophic bacteria represented the majority of the bacterial community. Most of the abundant genera were previously reported as able to grow down to 4-10 °C and lower. However, several genera (such as *Tipidiphilus sp.* and *Terracoccus sp.*) were never previously reported at temperatures lower than 15 °C (**III**). Both genera were not present in AS after six months of not adding EMs to wastewater and during the addition of antibiotics (**V**). Similarly, most of the abundant genera were different in the presence of IBU, DCF, E1 and EE2 and in the absence of EMs in AS for six months. As well, different genera were abundant in the presence of antibiotics. Therefore, the dynamics of the microbial community seem to depend on the type of EMs. However, since other natural factors could affect the results, this effect should still be confirmed by repeating the test.

Only three abundant genera were represented in at least 1% of the community in all of the AS samples, despite the presence and type of EMs: *Rhodobacter sp.*, *Microbacterium*, sp. and *Dechloromonas sp.* Members of last two genera are reported to play a significant role in the nitrification process (**III**) and they are known for the removal of aromatic compounds and could be the key contributors to both ammonia and EMs removal at low temperatures.

At the same time, typical autotrophic nitrifying bacteria (AOBs and NOBs) represented altogether less than 0.5% of the communities and in some samples less than 0.1%. Nevertheless, the nitrification efficiency was good and the decrease in abundance did not affect the nitrification efficiency (**III**). Therefore, high nitrification rates could be linked to microorganisms with nitrifying activity.
Figure 13 Average relative abundance of the most abundant bacterial classes/phyla in AS of MBRs fed with wastewater with the addition of EMs and without EMs. The duration of the conditions presented in brackets. Dotted-line frame shows classes of phylum *Proteobacteria*. 
Figure 14. Average relative abundance of the most abundant bacterial classes/phyla in AS of SBRs fed with wastewater with the addition of EMS and without EMS. The duration of the conditions presented in brackets. Dotted-line frame shows classes of phylum *Proteobacteria*.
Temperature is the most probable reason for the limited growth of typical AOBs and NOBs. However, AOBs have previously been reported in high abundance at 10°C and even 5 °C (Chen et al., 2018). Therefore, the presence of EMs could have an additional inhibiting effect on the typical nitrifying community. Such inhibition effects were also previously reported. For instance, in experiments with PhACs in concentration of 5 μg L⁻¹, *Nitrospira* was reported to grow only in reactors without these EMs (Vieno and Sillanpää, 2014). In contrast, Xia et al. (2015) claimed that *Nitrospira* sp. is an important bacteria for treating sewage containing antibiotics due to its antibiotics resistance, which could not be proved in this study possibly due to low temperature pressure. Wang and Gunsch in the studies of *Nitrosomonas europaea* suggested that AOB in SBRs are more resistant to PhACs than the pure culture of *N. europaea* (Wang and Gunsch, 2011a). At the same time, a mixture of PhACs showed a higher inhibiting effect on the nitrifying community compared to the individual effect of the same PhACs at higher concentrations (Wang and Gunsch, 2011b).

Alternatively, an increase of antibiotic resistant *Archaea* diversity was observed in AS samples after the addition of antibiotics to wastewater (V). In particular, known AOA *Nitrososphaera viennensis* was found in MBR samples after 30 days of antibiotics exposure, representing about 22% of the archaeal community. Previously, a study of eight WWTPs showed that the *Nitrososphaera* cluster is the most predominant AOA species of AS (Gao et al., 2013). However, no *Archaea* was found in SBR samples, which is consistent with Park et al.’s (2017) speculation that long SRT (>15 days) may facilitate AOA growth in AS. Fredriksson et al. (2012), in their study of full-scale AS, found low concentrations of *Archaea* despite an SRT of 5-7 days. However, no AOA species were found, and inhibiting effects of temperature during the winter season as well as a decrease in SRT were stated. At the same time, Sims et al. (2012), in their study of ammonium-oxidizing communities, showed that in the winter season, AOA outcompete AOB due to their higher tolerance to low temperatures. These results could explain the inability of the AS nitrifying community from SBRs to adapt to low temperature conditions and the positive but temporary effect of MBR sludge (containing AOA) inoculation (discussed in Chapter 5.1).

It is important to note that microbial communities vary noticeably even between simultaneously operated equal bioreactors under identical conditions. Additionally, the dynamic changes continually happen under steady-state conditions inside each reactor over time. These changes give the background community dynamics, which should not be mixed with the effect of the studied parameters. Therefore, in our study only the most abundant groups of microorganisms were discussed, and only the changes, taking place in all the reactors operated at the same conditions are mentioned.
5.5 Engineering significance and practical implications

The magnitude of the risks arising from the accumulation of EMs in municipal wastewaters is hard to underestimate. Most of the discussed EMs are essential for modern society life and therefore their consumption is difficult to restrict or noticeably decrease, even if some regulation could come in the near future. Therefore, the need for the technology that is capable of improving EMs removal is beyond doubt and calls for extensive research on this subject.

Overall, our results showed that the impact of the low-temperature conditions is of crucial importance to evaluate and optimize biodegradation processes in WWTPs. A cold environment puts hard pressure on a broad spectrum of microbial metabolic reactions, and this effect is stronger as the temperature is lower. Therefore, the further the temperature is from the optimum range, the greater the effect of only a few degrees difference on the removal kinetics (I, II).

Since the environmental and region-specific conditions (temperature, presence and type of EMs in wastewaters) are hardly possible to change, the process design focuses on the controllable parameters such as HRT, SRT, OLR, pH and so on. However, even though it is easier to control these parameters, when it comes to implementing new set points, there are certain limitations related to the correlation with other wastewater treatment processes (for instance, optimal pH) or the existing infrastructure (e.g. HRT). From this point of view, the SRT is the most important operational tool, which allows for the modification of several sludge characteristics, such as F:M factor, adaptation properties, microbial diversity (III) and EMs bioaccumulation and biodegradation, partly compensating for the negative effect of cold climate conditions (II, III). Unlike the OLR, which affected the removal only in the particular case of SBRs (I), prolongation of SRT was beneficial for most of EMs removal in all of the experiments.

Furthermore, our study also showed that the fate of EMs in the water phase and the solid phase of AS significantly differ from each other (II). This applies regardless of the low sorption potential and therefore leads to several consequences. First, the removal efficiency and calculated biodegradation rates may vary depending on the method of removal evaluation. This may cause variations in reported removal rates among the studies as well as differences in estimations of the process parameters’ effects on the removal. In particular, this could explain the misconception of the insignificant temperature impact on EMs removal (I-III). It is important to take into account the treatment process design when choosing the method for the removal efficiency evaluation. Biological treatment estimation will require a solid phase analysis, whereas physical-chemical treatment efficiency can be calculated by comparing water samples. Second, the end-of-the-pipe measures lack the understanding of the EMs fate in AS leaving the problem of sludge handling unsolved. At the same time, biological treatment step (AS) is indispensable for the removal of nitrogen and soluble organic matter in most of WWTPs. Therefore, despite the efficiency of physical-chemical methods, the control of EMs in AS is still necessary.
According to the results of our research, one potential way to enhance biodegradation rates could be based on increasing the SRT and implementing bioaugmentation of highly specific microorganisms for total biomineralisation of EMs. In this scenario, presuming successful bioaugmentation, the footprint of the wastewater treatment process would significantly decrease. Additionally, it would allow for the minimisation of risks linked to the land application of WWTP biosolids as fertilizer or soil amendment. However, the huge variety of EMs and constant appearance of new PhACs require a more complex approach. For instance, recalcitrant and non-biodegradable compounds such as CMZ (I, II) should be treated separately.

Therefore, the biodegradation potential must be considered in regulation. In this sense, one of the parameters for prioritising contaminants would be the ability of the bioprocesses to remove EMs, not only from the effluent water but also from the solid phase of AS. Accordingly, it can be recommended that the non-biodegradable substances are excluded from over-the-counter sales or totally restricted if possible (e.g. if there are alternatives of treatment). If the biodegradation limiting factor is temperature, the restrictions could be region-specific. This approach is aimed at using the bioprocesses to their maximum potential because they already exist in most of the WWTPs and they offer a possibility to limit costs and harmful side effects. Lowering the amount of the remaining substances after AS treatment could be expected to result in lower energy costs for the additional advanced physical-chemical treatment steps and a lower risk of the toxic by-products' formation.

The study of microbial communities in AS revealed several effects of the low-temperature conditions and supported the advantage of prolongation the SRT. Whilst temperature limits bacterial diversity through selective advantage of psychrophilic microorganisms, prolongation of SRT increases diversity allowing bacteria to grow slower and to adapt to environmental conditions. For instance, some of the essential bacterial classes for the nutrient and EMs removal (Deltaproteobacteria, Gammaproteobacteria) in this study required an SRT of at least 30 days for sufficient growth in AS (III, V). Applying an SRT ≥30 day in WWTPs in cold regions in addition to more sustainable nitrogen removal and up to the 70% higher removal of EMs discussed above (at SRT = 90 days) would decrease the amount of wasted AS. At the same time, application of such SRTs (30-90 days) would affect the aeration basin sizes (increased at least by 2-3 times), settler sizes or require MBR implementing. Additionally, in the application of the MBR process, there remains the question of the optimal SRT for membrane fouling control in the studied conditions.

Despite the great potential of microorganisms to mineralise a large diversity of the substances, there are several challenges in the engineering of the microbial community with a specific metabolic capacity and a stable removal performance for long-term application. To begin with, the vulnerability of highly specialized microbial bacteria to operational conditions leads to immense bacterial shifts. In particular, an atypical nitrogen-removing community at low temperatures and under the pressure of EMs was observed in our study (III, V). Process development towards highly selective microbial processes, for instance anammox bacteria and aerobic granule forming bacteria, might add even more challenges and requires a detailed study of the bacterial ecology.
Next, continuous population dynamics leads to inconstancy in the experimental data. Drawing conclusions about the links between operational parameters, bacterial population and biodegradation efficiency is very risky without multiple replications of the experiments and following the control processes without the studied factors involved. Bouchez et al. (2000) stated that ‘despite the fact that the same sludge was split between two twin reactors, each ecosystem may have evolved differently, and these differences could represent the inherent experimental variability of the environmental sample being dealt with’.

For instance, in our study (III), despite stable laboratory conditions, microbial communities in the reactors not subjected to EMs experienced shifts in dominant genera for undetermined reasons, and communities under pressure of the same EMs evolved differently. This phenomenon stands close to another related challenge – bioaugmentation. Not only is there a huge lack of knowledge about specific metabolic and co-metabolic reactions among EMs-removing microorganisms, selecting the bioaugmented microorganisms is very challenging. Moreover, in view of the present understanding on how to control the changes taking place in the microbial community, the probability of bioaugmented microorganisms to successfully integrate into the AS community without disturbing the ecosystem equilibrium is limited.

Finally, Archaea seems to have an important role in biodegradation processes under challenging conditions; however, little is known about this subject. Furthermore, there is a big knowledge gap on the antibiotic resistance agents that end up in the treated waters and wasted biosolids.
6. Conclusions and suggestions for future work

The main aims of this study were to assess the biological removal of EMs during wastewater treatment under conditions typical for Finland and to find the optimal operational parameters for the efficient removal processes by comparing two existing wastewater treatment technologies. The research objectives were to gain knowledge on how to enhance biodegradation of EMs by optimizing process parameters and which microorganisms could be the possible contributors to EMs removal in cold temperatures. The major findings of this research are summarised as follows:

1. AS with an SRT of at least 30 days was more efficient compared to AS with an SRT of 12 days (at 12 °C) and 14 days (at 8 °C) for the removal of all EMs except CMZ, whose removal was insignificant in all the experiments.

2. The removal rates of most of the EMs (except CMZ and E1) increased with the increase in the SRT to 90 days. The low-temperature conditions can be partly compensated for by applying technologies with a longer SRT operation. However, within the experimental time it was not possible to increase removal rates close to the ranges published in the literature for the temperature diapason of 16-25 °C. Higher removal rates, however, could also be explained by the fact that often only the removal from water phase of AS has been studied, whereas this study showed that up to 85% of EMs (depending on the substance) are collected in the solid part of AS. In case of DCF and EE2 removal from solid part of AS was inefficient (≤30%). Thus, these amounts of EMs can eventually return to water with cell decay or due to reversible adsorption as well as escape from the secondary clarifiers with small SS fines.

Therefore, in conditions studied in this thesis, the short SRT processes presented an increased risk of EM content in sludge. Prolongation of SRT during cold seasons, when the temperature causes a stronger pressure on highly specialised microorganisms, would lead to more sustainable effluent quality. Since, typical SRT in Finnish nitrifying WWTPs is between 14±2 days, applying 2-6 times longer SRT would require an increase of the WWTP sizes or implementation of advanced AS technologies such as MBR. The selection of the optimum SRT should depend on the EMs of concern, the temperature range and the duration of the coldest season.

An environmental risk assessment associated with EMs should include the potential of these substances to accumulate in AS cells and therefore escape the wastewater treatment process with fine SS particles in effluents. Depending on this parameter and toxicity of the substances in wastewaters, liquid-solid separation technology should be considered.
3. Co-metabolic reactions with AOBs, proposed as an important EMs biodegradation mechanism, in this study could not enhance biodegradation of EMs at low temperatures since known AOBs were only present in low-temperature-adapted AS in extremely low concentrations. Nevertheless, the EMs biodegradation could still be linked to nitrification although related to co-metabolic activity of other unidentified nitrifying bacteria as well as AOA.

4. Microbial studies showed that a longer SRT increases the persistence of the microbial community to stress factors such as temperature and the presence of toxic substances (EMs). These results mean that performance of the adapted and optimized AS in MBRs is easier to predict when applied to real conditions compared to AS with a shorter SRT.

According to our studies, the potential EMs-removing microorganisms may belong to Deltaproteobacteria as well as Archaea. Therefore, further studies of these groups of microorganisms are suggested. At the same time, vulnerability of nitrifying bacteria as well as Actinobacteria and Firmicutes to studied conditions was observed. Since these are important groups of bacteria involved in the AS process, it would be beneficial to test the possibilities of bioaugmentation for these groups of organisms.
References


Biological removal of emerging micropollutants in nitrifying activated sludge at low temperatures

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