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Cultivating aerobic granular sludge in a lab-scale sequencing batch reactor

Removal of pharmaceutically active compounds, total phosphorus, total nitrogen and organic matter from a complex synthetic wastewater

Master's thesis in Infrastructure and Environmental Engineering

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MASTER'S THESIS ACEX30

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ABSTRACT

Pharmaceutically active compounds (PhACs) in wastewater effluents pose a threat to both the environment and humanity and, due to disadvantages associated with commonly implemented advanced treatment methods for PhAC removal, it is of interest to investigate advanced biological treatment alternatives for the removal of PhACs from wastewater. Since aerobic granular sludge (AGS) possesses several advantages in comparison to the conventional activated sludge process, it is of great interest to investigate as a new alternative for the biological treatment of PhACs. This master thesis project was a part of the start-up of a PhD project investigating the potential of AGS cultivated in a lab-scale sequencing batch reactor (SBR) to remove PhACs from wastewater. The overall aim of this master thesis project was to successfully cultivate aerobic granules in the lab-scale SBR, to theoretically evaluate its removal potential of nine selected PhACs and to reach a sufficient reduction of total nitrogen (TN), total phosphorus (TP) and organic matter, expressed as TOC. The performances of the lab-scale SBR were monitored for 29 days by collecting samples from the reactor during different cycle phases and effluent. The dissolved concentration of the investigated contaminants was analysed and the biomass characteristics were evaluated based on the assessment of total suspended solids (TSS), volatile suspended solids (VSS), sludge volume index (SVI), solid retention time (SRT), and sludge morphology by light microscopy. Results showed that the formation of aerobic granules was initiated, but not fully achieved during the observation time. The reactor accomplished nitrogen, phosphorus and, organic removal. High TOC (>90 %), TP (>85 %), and TN (>80 %) removals were achieved by this system. The removal potential of selected PhACs was theoretically evaluated based on relevant findings in a literature review. Overall, it could be concluded that AGS cultivated in a SBR shows potential for the removal of PhACs from wastewater due to the several unique characteristics, such as the easy adjustment of the operational parameters. However, future research is needed to fully determine the potential of using AGS cultivated in an SBR for PhAC removal.

Key words: Aerobic granular sludge, sequencing batch reactor, pharmaceuticals, pharmaceutically active compounds, wastewater, advanced biological treatment

Kultivering av aerobt granulärt slam i en labbskalig satsvis reaktor
Rening av läkemedelsrester, totalfosfor, totalkväve och organiskt material från ett komplext syntetiskt avloppsvatten

Examensarbete inom masterprogrammet Infrastruktur och Miljöteknik

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SAMMANFATTNING

Läkemedelsrester i avloppsvatten är ett hot för både miljön och mänskligheten och på grund av nackdelar med de avancerade reningstekniker som vanligtvis implementeras för läkemedelsrening är det av intresse att undersöka biologiska reningsalternativ. Det är av intresse att undersöka aerobt granulärt slam (AGS) som ett nytt alternativ för biologisk rening av läkemedelsrester då det besitter många fördelar jämfört med konventionellt aktivt slam. Det här examensarbetet var en del av uppstarten av ett doktorandprojekt som undersöker potentialen för rening av läkemedelsrester med hjälp av AGS som kultiverats i en labbskalig satsvis reaktor (SBR). Det övergripande syftet med examensarbetet var att med framgång kultivera aeroba granuler i den labbskaliga SBRen, att teoretiskt analysera reningspotentialen för nio utvalda läkemedel och nå tillräcklig rening av totalkväve (TK), totalfosfor (TF) och organiskt material. Reaktorns prestation observerades under 29 dagar genom att ta prover från reaktorn under de olika cykelfaserna och i utflödet. Koncentrationerna av de olika föroreningarna analyserades och biomassans egenskaper utvärderades genom att beräkna total suspended solids (TSS), volatile suspended solids (VSS), sludge volume index (SVI), solids retention time (SRT) och att undersöka slammets morfologi i mikroskop. Resultaten visade att granulering var påbörjad men inte fullt uppnådd under observationstiden. Reaktorn genomförde rening av kväve, fosfor och organiskt material. Hög rening av organiskt material (>90 %), TF (>85 %) och TK (>80 %) åstadkoms genom detta system. Reningen av de utvalda läkemedelsresterna analyserades teoretiskt baserat på relevant information från litteraturstudien. AGS kultiverat i en SBR kan anses ha potential för rening av läkemedelsrester på grund av granulernas unika egenskaper och att driften av SBR medför enkla justeringar av viktiga parametrar som påverkar läkemedelsrening. Mer forskning krävs för att fullt fastställa potentialen i att använda AGS kultiverat i en SBR för att rening av läkemedelsrester.

Nyckelord: Aerobt granulärt slam, satsvis reaktor, läkemedel, läkemedelsrester, avloppsvatten, avancerad rening

Contents

Preface

In this master thesis project, a literature review and experiments on the removal of pharmaceutically active compounds, total nitrogen, total phosphorus and organic matter by aerobic granular sludge cultivated in a sequencing batch reactor have been done. The project was carried out from January 2020 to June 2020 and is a part of the start-up of a PhD project carried out by Cecilia Burzio at the Department of Architecture and Civil Engineering, Division of Water Environment Technology, at Chalmers University of Technology in Sweden.

All experiments were carried out in the laboratory of the Division of Water Environment Technology at Chalmers University of Technology. We would like to give a special thanks to Cecilia Burzio for trusting us with the lab-scale SBR and letting us be a part of her PhD project as well as for supervising, supporting us in the laboratory, sharing her knowledge, and helping us planning the experiments. We would also like to thank Britt-Marie Wilén for supervising and Amir Saeid Mohammadi for laboratory support.

Göteborg June 2020

Johan Calgaro and Linn Trieb

Abbreviations

AGS - Aerobic granular sludge

AOB - Ammonium oxidizing bacteria

CEC - Contaminant of emerging concern

COD - Chemical oxygen demand

DO - Dissolved oxygen

EBPR - Enhanced biological phosphorus removal

EPS - Extracellular polymeric substances

F/M-ratio - Food-to-microorganism ratio

GAC - Granular activated carbon

GAO - Glycogen-accumulating organisms

HCl - Hydrochloric acid

HRT - Hydraulic retention time

IC - Ion chromatography

MLSS - Mixed liquor suspended solids

NaOH - Sodium hydroxide

N - Nitrogen

N_2 - Nitrogen gas

NH_4^+ - Ammonium

NH_4^+-N - Ammonium nitrogen

NOB - Nitrite oxidizing bacteria

NO_3^- - Nitrate

NO_3^--N - Nitrate nitrogen

NO_2^- - Nitrite

NO_2^--N - Nitrite nitrogen

OLR - Organic loading rate

OMP - Organic micropollutant

O_2 - Oxygen gas

O_3 - Ozone

P - Phosphorus

PAC - Powdered activated carbon

PAO - Polyphosphate-accumulating organisms

PHA - Polyhydroxyalkanoates

PhACs - Pharmaceutically active compounds

Poly-p - Polyphosphate

PO_4^{3-} - Phosphate

PO_4^{3-}-P - Phosphorus from phosphate

SBR - Sequencing batch reactor

SRT - Solids retention time

SVI - Sludge volume index

TN - Total nitrogen

TOC - Total organic carbon

TP - Total phosphorus

TSS - Total suspended solids

VFA - Volatile fatty acid

VSS - Volatile suspended solids

WWTP - Wastewater treatment plant

1. Introduction

The presence of pharmaceutically active compounds (PhACs) in wastewater effluents poses a threat to both the environment and humanity due to their adverse impact on water living organisms and risk to spread antibiotic-resistant bacteria, which calls for conventional wastewater treatment to be supplemented with advanced treatment of PhACs. This master thesis project is a part of the reactor start-up investigated in a PhD project carried out by Cecilia Burzio at the department of Architecture and Civil Engineering at Chalmers University of Technology in Sweden, that investigates the potential of aerobic granular sludge (AGS) cultivated in a sequencing batch reactor (SBR) to remove PhACs from wastewater.

1.1 Background

The overall purpose of wastewater treatment is to remove contaminants from the municipal wastewater to prevent littering of the recipient, eutrophication, and spreading of pathogens and environmentally hazardous compounds (Swedish Environmental Protection Agency [SEPA], 2008). PhACs have been detected in wastewater treatment plant (WWTP) effluents throughout the last years (SEPA, 2017). PhACs find their way into the wastewater through urine and feces and, due to conventional wastewater treatment being designed to only remove easily degradable organic matter, nitrogen (N) and phosphorus (P), they practically pass the WWTP unaffected and are released into the recipient where they pose a threat to both the environment and humanity (Cruz del Álamo et al., 2017).

Due to PhACs being persistent, and often high lipid-soluble, they can bioaccumulate in living organisms and biomagnification of some PhACs has also been found (Kaur Brar et al., 2018). Since pharmaceuticals are built to affect biochemical and physiological processes in humans or animals, they can affect other living organisms too (SEPA, 2017). For instance, contraceptives can disrupt fish's ability to reproduce (Swedish Agency for Marine and Water Management [SwAM], 2018) and, due to the exposure of antidepressants, behavioral changes among fish has been found (Baresel, Magnér, Magnusson & Olshammar, 2017). There is also a risk that antibiotics in the environment can cause spreading of antibiotic-resistant bacteria, which can be seen as a global threat to humanity (SwAM, 2018).

Thus, in the short term, the presence of PhACs in WWTP effluents can have a negative impact on water living organisms and may also pose a threat to human health. Currently, the long term effects of these compounds are unknown (Carswell et al., 2010), but the so-called cocktail effect is often discussed (Cimbritz et al., 2016). Therefore, conventional wastewater treatment has to be supplemented with advanced treatment of PhACs to reduce the loading of these compounds in the effluents (Beijer et al., 2017).

Several methods, that are either physical, biological, oxidative, or adsorptive, are available for the removal of microcontaminants in wastewater (SEPA, 2017). Literature and studies of full-scale facilities show that activated carbon and ozonation have been prioritized when implementing

advanced treatment of PhACs (Cimbritz et al., 2016). However, due to some disadvantages associated with these treatment methods, such as the formation of toxic by-products and high energy use during ozonation (SEPA, 2017) and limited possibilities of using sludge contaminated with activated carbon as a fertilizer on farmland as well as the high resource consumption and operating costs related to the use of activated carbon (Mulder, Antakyali & Ante, 2015), it is of interest to further investigate advanced biological treatment alternatives for the removal of PhACs from wastewater.

In the early 1990s, a new type of sludge was observed in aerobic biological reactors (de Bruin, de Kreuk, van der Roest, Uijterlinde & van Loosdrecht, 2004). This sludge, which showed bigger formations of flocs with a more compact structure than conventional activated sludge, is nowadays designated as aerobic granular sludge (AGS). This type of flocculated microorganisms enables faster settling times compared to regular sludge and the compact structure of AGS enables the possibility of different environmental conditions to occur within the granule. Since the properties of AGS enable the design of compact and energy-efficient treatment methods in comparison to the conventional activated sludge process that requires large surface areas, the AGS technology is of great interest to investigate as a new alternative for the biological treatment of wastewater (de Bruin et al., 2004). Due to the fact that AGS has many similarities with biofilms, one suspects that the removal of PhACs could be possible in a similar manner as what has been seen previously in biofilm processes (Falås, Baillon-Dhumez, Andersen, Ledin & la Cour Jansen, 2012; Luo et al., 2014). Thus, it is of interest to further investigate the potential of AGS to remove PhACs from wastewater.

1.2 Aim

The overall aim of this master thesis project is to increase the understanding of how aerobic granules are cultivated in an SBR and to evaluate the potential of AGS cultivated in a lab-scale SBR, constructed at the WET laboratory at Chalmers University of Technology in Sweden, to remove nine selected PhACs. The primary aim is to set-up a lab-scale SBR that successfully cultivate aerobic granules and to theoretically evaluate its removal potential of PhACs. Furthermore, the aim is also to reach a sufficient reduction of total nitrogen (TN), total phosphorus (TP), and organic matter, expressed as total organic carbon (TOC).

1.2.1 Objective

In order to achieve the aim, the objectives are to (i) gather knowledge regarding the cultivation of AGS as well as its PhAC removal potential through a literature review of relevant scientific papers, (ii) to set up and calibrate the lab-scale SBR to be optimal for aerobic granule cultivation and TN, TP, TOC and PhAC removal, (iii) to evaluate the reactor performances and biomass characteristics and (iv) to investigate the main biological processes occurring in the reactor.

The master thesis aims to answer the following research questions:

1. Are aerobic granules successfully cultivated in the lab-scale SBR?
2. Are the removal efficiency criteria fulfilled for TN, TP, and TOC?
3. Which processes were involved in removal of TN and TP?
4. What is the theoretical removal potential of selected PhACs by the lab-scale SBR?

1.3 Scope and limitations

Due to this master thesis being a part of the reactor start-up investigated in a PhD project, the decisions regarding the setup and operation conditions of the lab-scale SBR, as well as the choice of PhACs were outside the scope of this work.

The analytical investigation of the selected PhACs was initially within the scope of this master thesis project, but due to laboratory constraint due to the COVID-19 pandemic could not be carried out.

Since aerobic granules did not have sufficient time to form and the removal processes did not have enough time to stabilize, time restraint can be considered as a main factor that limited the experimental work.

2. Literature review

This chapter aims to increase the understanding of why the conventional wastewater treatment needs to be supplemented with advanced biological treatment for the removal of PhACs and highlight the AGS technology as a potential advanced biological treatment alternative.

2.1 Conventional wastewater treatment

The overall purpose of wastewater treatment is to remove contaminants from the municipal wastewater to prevent littering of the recipient, eutrophication, and spreading of pathogens and environmentally hazardous compounds (SEPA, 2008). Conventional wastewater treatment is designed to remove easily degradable organic matter, often expressed as chemical oxygen demand (COD), N and P by a combination of mechanical, biological, and chemical treatment. In Sweden, according to the regulation of treatment and control of effluent wastewater in urban areas stated by SEPA, a minimum of 70 and 75 % reduction of TN and COD or TOC, respectively, is required in urban areas with more than 2,000 pe if the effluent is discharged in freshwater or estuary and 10,000 pe if it is discharged in seawater or coastal areas (SEPA, NFS 2016:6). For TP, SEPA does not regulate any specific minimum reduction. However, the European Union demands that a minimum removal efficiency of 80 % must be ensured for TP (Schaum, 2018). During the mechanical treatment, heavy objects are settled out and buoyant materials are removed (Pell & Wörman, 2011). The purpose of chemical treatment is to precipitate P by adding metal salts to the wastewater. The chemical treatment may be performed either before, simultaneously with or after the biological treatment. The biological treatment can be performed according to two different principles where the bacterial growth is either supported by suspended solids naturally occurring in the wastewater or by solid surfaces in the tank supporting the development of a biofilm. The treatment of wastewater is performed at different levels: preliminary, primary, secondary, tertiary and advanced treatment (Riffat, 2012). A typical process chart for conventional wastewater treatment is presented in Figure 1.

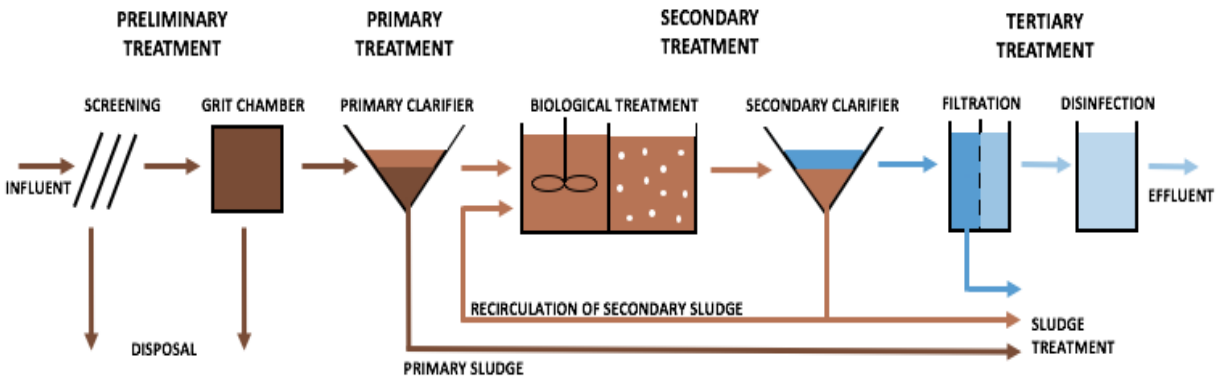

Figure 1. Typical process chart for conventional wastewater treatment. Figure by author.

2.1.1 Preliminary treatment

The purpose of preliminary treatment is to separate easily removed inorganic content such as oil, stones, gravel and trash from the wastewater and it usually consists of screens, grit chambers, flow measurement devices and storage facilities used to even out the influent water (Hopcroft, 2014; Riffat, 2012).

2.1.2 Primary treatment

During the primary treatment, a proportion of the suspended solids from wastewater, mainly organics, is physically removed in a primary clarifier in which relatively heavy objects settle out and buoyant material, such as oil, grease and plastics, float to the top (Hopcroft, 2014; Riffat, 2012). The primary treatment can be improved by the addition of chemical coagulants in the clarifier to support coagulation and flocculation of solids which increases the removal of suspended solids (Riffat, 2012).

2.1.3 Secondary treatment

The secondary treatment generally consists of a biological treatment method, where organic matter is degraded in a biological reactor, followed by a secondary clarifier in which the biomass in the form of suspended solids is removed from the effluent and partially recycled back to the reactor (Hopcroft, 2014; Riffat, 2012). It is possible to combine the secondary treatment for removal of organic matter with N and P removal, but it may require additional reactors for the removal of N through the nitrification-denitrification process (Riffat, 2012).

The biological treatment can be divided into two major categories: attached growth and suspended growth processes (Riffat, 2012). In an attached growth process, the organic matter is degraded as the wastewater gets in contact with a biofilm formed by microorganisms attached to an inert medium with high porosity and surface area. Examples of commonly used types of attached growth processes are trickling filters, biotowers, moving bed biofilm reactors (MBBRs), and rotating biological contactors (RBCs). In a suspended growth process, a suspension of microorganisms is kept in the biological reactor by using an appropriate mixing technique. The microorganisms degrade the organic matter and turn it into new biological cells, energy, and waste matter. The most commonly used type of suspended growth process is the activated sludge process.

2.1.3.1 The activated sludge process

The activated sludge process is the most widely used biological treatment method (Garnaey & Sin, 2011). During the activated sludge process, a suspension of microorganisms is responsible for the removal of contaminants present in the wastewater. The activated sludge process is conventionally designed as a continuous-flow system in which the primary effluent is led through, either via a single aerobic tank or via a series of tanks that are either aerobic, anoxic or anaerobic, depending on which contaminant to be removed (Pell & Wörman, 2011).

As illustrated in Figure 2, the basic activated sludge process consists of an aerated tank, where the suspension of microorganisms is kept, a clarifier, and a system recycling the settled solids in the clarifier back to the aerated tank (Riffat, 2012). Several variations of the activated sludge process are available and it is common to use it for removal of organic matter together with N and/or P removal.

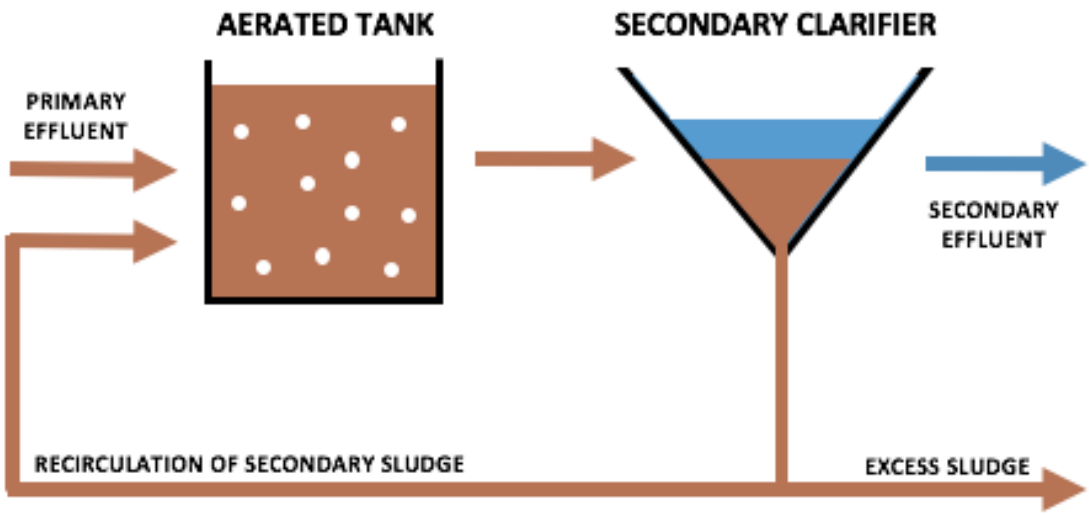

Figure 2. The basic activated sludge process. Figure by author.

2.1.3.2 Sequencing batch reactor

The activated sludge process can also be designed as a sequencing batch reactor (SBR) process (Pell & Wörman, 2011). The difference between SBRs and the conventional continuous flow system is that the treatment processes in SBRs occur sequentially in a single tank instead of in separate tanks (Riffat, 2012). The operation of SBRs consists of a sequence of fill-and-decant cycles that, as illustrated in Figure 3, typically include the following phases: fill, react, settle, decant, and sometimes, idle.

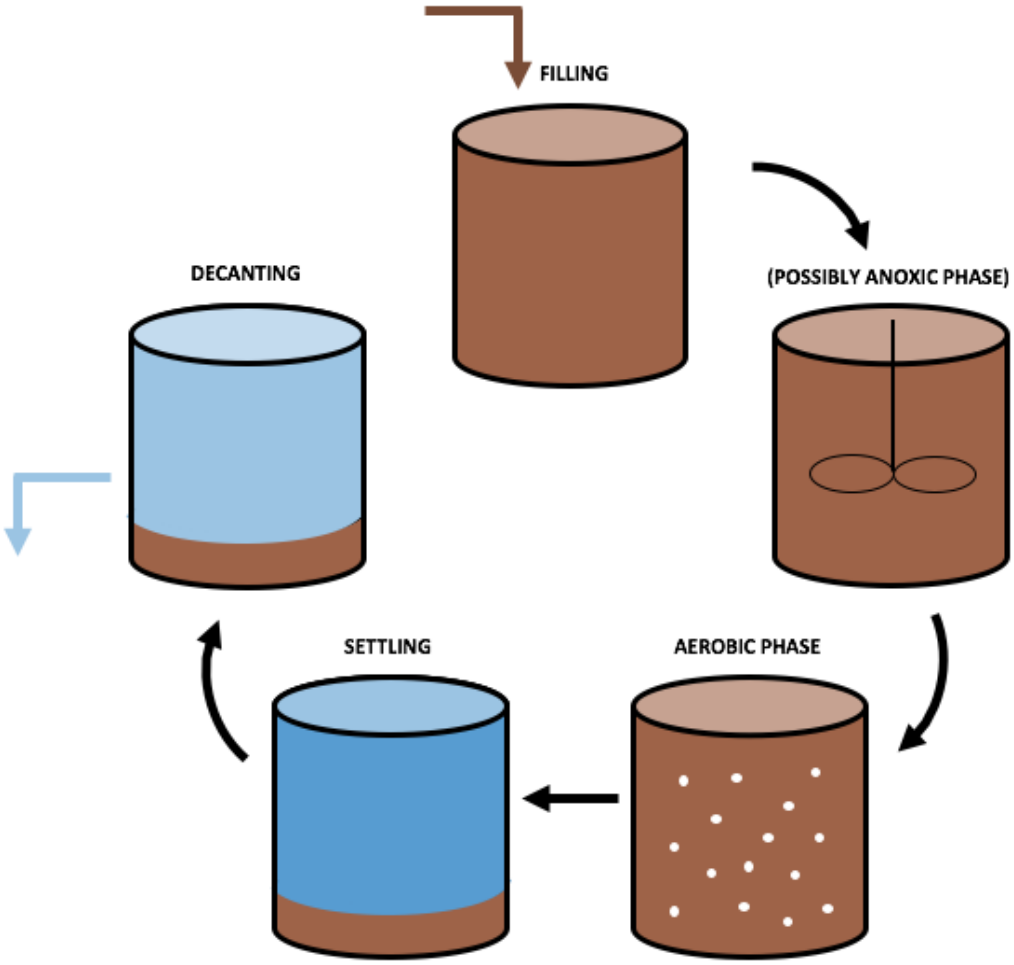

Figure 3. Phases typically included in an SBR cycle. Figure by author.

Depending on the treatment objective, the fill phase may be static, mixed, or aerated and is accomplished by adding the influent wastewater to the mixed liquor suspended solids (MLSS) remaining from the previous cycle (Vigneswaran, Sundaravadivel & Chaudhary, 2009). The reaction phase is generally accomplished through vigorous aeration. However, depending on required concentrations of dissolved oxygen (DO), the reaction phase can be either aerated or mixed to achieve high respectively low DO concentrations. When the desired level of effluent quality is achieved, the solids are separated from the treated wastewater during the settling phase. The use of the SBR as a clarifier without any inflows or outflows is a major advantage in comparison with the conventional activated sludge process. The treated wastewater is discharged from the reactor during the decant phase. An idle phase may take place before the reactor is filled again to waste sludge and to provide aeration or mixing, depending on the treatment objectives.

2.1.4 Tertiary treatment

Tertiary treatment of the secondary effluent may include removal of residual suspended solids, disinfection to reduce pathogens, and additional nutrient removal (Riffat, 2012). Tertiary treatment is not required for all treatment plants (Hopcroft, 2014).

2.1.5 Advanced treatment

Advanced treatment methods are used as an additional treatment of the effluent from the secondary treatment due to the toxicity of a certain compound or if the water is to be reused (Riffat, 2012). Examples of advanced treatment methods are activated carbon adsorption and ion exchange.

2.2 Pharmaceutically active compounds in wastewater

PhACs are used as therapeutics in human and animal medicine and have been detected in WWTP effluents throughout the last years (SEPA, 2017). PhACs find their way into the wastewater, either as a whole substance or metabolite, through urine and feces. Due to conventional wastewater treatment being designed to only remove easily degradable organic matter, N and P, the majority of PhACs practically pass unaffected and are thereafter released into the recipient (Cruz del Álamo et al., 2017).

Due to PhACs being persistent, and often high lipid-soluble, they can bioaccumulate in living organisms and biomagnification of some PhACs has also been found (Kaur Brar et al., 2018). Since pharmaceuticals are built to affect biochemical and physiological processes in humans or animals, they can affect other living organisms too (SEPA, 2017). For instance, contraceptives can disrupt fish's ability to reproduce (SwAM, 2018) and, due to the exposure of antidepressants, behavioral changes among fishes has been found (Baresel, et al., 2017). There is also a risk that antibiotics in the environment can cause spreading of antibiotic-resistant bacteria, which can be seen as a global threat to humanity (SwAM, 2018).

Thus, in the short term, the presence of PhACs in WWTP effluents can have a negative impact on water living organisms and may also pose a threat to human health. Currently, the long term effects of these compounds are unknown (Carswell et al., 2010), but the so-called cocktail effect, that is that compounds in a mixture can cause amplified, attenuated or other unknown effects than those that every single compound may cause by their own, is discussed (Cimbritz et al., 2016). Therefore, conventional wastewater treatment has to be supplemented with advanced treatment of PhACs to reduce the loading of these compounds in the effluents (Beijer et al., 2017).

2.2.1 Use, occurrence, removal, and toxicity of selected pharmaceutically active compounds

PhACs are organic compounds which present a broad range of characteristics. A detailed description of the medical use, occurrence in wastewater, removal potential, and toxicity of nine selected PhACs is presented in this section.

2.2.1.1 Carbamazepine

Carbamazepine is used to control epileptic seizures and to treat trigeminal neuralgia and episodes of mania or mixed episodes during bipolar 1 disorder (U.S. National Library of Medicine [U.S. NLM], 2020a). It may also be used to treat other conditions like depression, posttraumatic stress disorder (PTSD) and restless leg syndrome.

Studies have shown that carbamazepine is one of the most frequently detected PhAC in WWTP effluents as well as in rivers (Ternes, 1998; Heberer, 2002; Metcalfe et al., 2003). Carbamazepine has been detected in wastewater and sludge, both unchanged and as metabolites, and previous studies on the removal of carbamazepine from wastewater have shown that the elimination of this compound is known to be hard and removal rates are usually low (Onesios, Yu & Bouwer, 2009). A study carried out by Kent and Tay (2019) tested the removal of carbamazepine and other PhACs by AGS. The results from the study showed promising PhAC removal of all involved compounds except for carbamazepine which was removed from the wastewater at a rate <10 %. Carbamazepine also showed to have a negative impact on the aerobic granule formation which is important for the achievement of optimal treatment with AGS.

2.2.1.2 Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that reduces pain, fever, and inflammation (U.S. NLM, 2020d). It is used to treat arthritis, menstrual pain, and migraine headaches. Since diclofenac is classified as a contaminant of emerging concern (CEC), it was a part of the previous Watch List of the EU Water Framework Directive (Lonappan, Brar, Das, Verma & Surampalli, 2016; Sousa, Ribeiro, Barbosa, Pereira & Silva, 2018; Li, Zhang, Liu & Ding, 2019). Nearly 75 % of the consumed diclofenac enters the environment (Schmidt, Hoffmann, Garbe & Schneider, 2018) and, due to its high hydrophilicity and stability, it is likely to persist in the aquatic environment (Madikizela, Tavengwa & Chimuka, 2017; Tiedeken, Tahar, McHugh & Rowan, 2017).

Studies of the removal rates of WWTPs have found that some effluents have lower concentrations of diclofenac than influents (Anumol, Vijayanandan, Park, Philip & Snyder, 2016; Papageorgiou, Kosma & Lambropoulou, 2016; Pereira, Silva, Lino, Meisel & Pena, 2016; Kapelewska et al., 2018) whilst, in some effluents, the concentrations are significantly increased compared to the influents (Fernández, Fernández, Laca, Laca & Díaz, 2014; Lindholm-Lehto, Ahkola, Knuutinen & Herve, 2016; Kołecka, Gajewska, Stepnowski & Caban, 2019; Paíga et al., 2019). The latter may be explained by metabolites of diclofenac converting back to the parent compound (Lorenzo-Toja et al., 2015; Chiffre, Degiorgi, Bulete, Spinner, & Badot, 2016).

Due to the conjugation process, an accumulation of metabolites of diclofenac in WWTPs has been found (Sathishkumar et al., 2020). Diclofenac metabolites present in the environment may have higher toxicity than the parent compound (Scheurell, Franke, Shah & Huhnerfuss, 2009; Schulze et al., 2010). However, the metabolites tend to show a higher removal efficiency at the WWTP compared to their original state (Kołecka et al., 2019).

2.2.1.3 Ciprofloxacin

Ciprofloxacin is an antibiotic of the fluoroquinolones class used to treat certain bacterial infections such as pneumonia, gonorrhea, typhoid fever, and infectious diarrhea (U.S. NLM, 2020b). Ciprofloxacin is one of the most widely used antibiotics worldwide (Githinji, Musey & Ankumah, 2010). It is one of the leading quinolone antibiotics of choice in hospitals and it is also a common compound used as veterinary medicine. Fluoroquinolones have been detected in domestic

wastewater at concentrations ranging from 600 to 1000 ng/L (Batt, Kim & Aga, 2007; Seifrtová, Pena, Lino & Solich, 2008; Gros, Petrović & Barceló, 2009; Kümmerer, 2009a) and ciprofloxacin alone have been reported in hospital effluents at concentrations up to 124 μg L^{-1} (Hartmann et al., 1999).

The exposure of ciprofloxacin, as well as other antibiotics, to the aquatic environment, promotes antibiotic-resistant bacteria and genes which in turn exacerbates the situation of antibiotic resistance (Kümmerer, 2009b; WHO, 2014). Antibiotic resistance is a global public health threat that is currently increasing. Therefore, ciprofloxacin, as it is one of the most commonly used antibiotics, is of great importance for the study of PhAC removal from wastewater and hospital effluents are to be considered as a critical source for the release into the environment (Santos et al., 2013).

2.2.1.4 Sulfamethoxazole

Sulfamethoxazole is an antibiotic of the sulfonamide class used to treat certain bacterial infections (U.S. NLM, 2020g).

Sulfamethoxazole is usually found in WWTP effluents due to its enhanced persistence and concentrations as high as 5 μg L^{-1} have been reported during the last decade (Loos et al., 2013; Verlicchi, Al Aukidy & Zambello, 2012a). It is stated that sulfamethoxazole is strongly resistant to biodegradation in WWTPs (Pérez, Eichhorn & Aga, 2005) and a study carried out by Göbel, Thomsen, McArdell, Joss, and Giger (2005) showed sulfamethoxazole to have low sorption to activated sludge in the secondary treatment step resulting in high effluent concentrations from the studied WWTP. Furthermore, two similar studies found the removal efficiency of sulfamethoxazole by WWTPs to be 26 and 42 % respectively (Xu et al., 2007; Gulkowska et al., 2008).

As already mentioned, exposure of antibiotics into the environment exacerbates the situation of antibiotic resistance (Kümmerer, 2009b; World Health Organization [WHO], 2014).

2.2.1.5 Citalopram

Citalopram is an antidepressant of the selective serotonin reuptake inhibitors (SSRI) class used to treat depressions (U.S. NLM, 2020c). It may also be used to treat other conditions like eating disorders, premenstrual dysphoric disorder, alcoholism, and panic disorder. Citalopram is one of the world's most consumed drugs for the treatment of depression (Beretsou et al., 2016) and several studies carried out in different countries have reported the presence of citalopram in wastewater influents and effluents, sewage sludge and surface waters (Silva, Lino, Meisel & Pena, 2012, Silva, Pereira, Meisel, Lino & Pena, 2015).

Due to bioaccumulation, low levels of antidepressants in aqueous ecosystems currently raise a concern about their potential long-term risks to aquatic organisms (de Solla et al., 2016; Minguez et al., 2015). Citalopram has been shown to be less toxic compared to other SSRIs, but it is also stated to be tested in relation to its toxicity less than to other compounds (Christensen, Faaborg-

Andersen, Ingerslev & Baun, 2007). Christensen et al. (2007) suggest that the transformation products formed from citalopram may be more toxic than the parent compound. However, a study carried out by Osawa, Carvalho, Monteiro, Oliveira and Florêncio (2019) indicated most transformation products of citalopram being equal or less toxic than citalopram. Further, it is concluded that more studies on the transformation products of citalopram are needed in order to state its toxicity.

Biodegradation is considered to be the most important removal mechanism for citalopram since studies on volatilization and sorption have found these removal mechanisms to be insignificant (Alvarino et al., 2015; Hörsing et al., 2011; Hörsing, Kosjek, Andersen, Heath & Ledin, 2012). There have been studies made showing a removal rate ranging from 40-70 % elimination (Suarez, Lema & Omil, 2010; Suarez, Reif, Lema, J & Omil, 2012; Beretsou et al., 2016). All three studies concluded biodegradation to be the reason for the majority of the removal of citalopram.

2.2.1.6 Sertraline

Sertraline is, as well as citalopram, an antidepressant of the SSRI class used to treat depressions, obsessive-compulsive disorder, panic attacks, PTSD, and social anxiety disorder (U.S. NLM, 2020f). It may also be used to treat headaches and sexual problems.

As already mentioned, low levels of antidepressants in aqueous ecosystems raise a concern about their potential long-term risks to aquatic organisms due to bioaccumulation. A study carried out in Canada by Metcalfe et al. (2010) measured the concentration of sertraline, as well as other PhACs, downstream from two WWTPs in the province of Ontario. The concentrations of sertraline and its metabolite, desmethylsertraline, in the samples were low in relation to other compounds and no results showed concentrations >0.1 μg L^{-1}. However, a study carried out by de Solla et al. (2016) measured the concentration of PhACs in wild and caged freshwater mussels one and three years later at the same site. Sertraline was among a few other compounds detected at the highest concentrations in the mussels. This was stated to likely be caused by bioaccumulation in the living organisms. Furthermore, other studies have also shown sertraline to accumulate both in aquatic organisms and sediment and increasing to levels that may affect feeding behavior, locomotor activity, predator-prey interactions, and reproduction of the exposed organisms (Hedgespeth, Nilsson & Berglund, 2014; Kwon & Armbrust, 2008; Minguez et al., 2015).

In 2019, a study by Paíga et al. (2019) investigated the removal efficiency of 83 different PhACs from a WWTP consisting of primary, secondary (activated sludge) and tertiary (UV exposure) treatments. Results found that sertraline, together with two other compounds, was determined to enable the highest risk levels of all investigated PhACs in the effluent flow from the WWTP. The risk quotient assessed for the PhACs in the study was based not only on the influent and effluent concentrations but also on the ecotoxicity of the compounds.

2.2.1.7 Venlafaxine

Venlafaxine is an antidepressant of the selective serotonin and norepinephrine reuptake inhibitors (SNRI) class used to treat depressions, generalized anxiety disorder, social anxiety disorder and panic disorder (U.S. NLM, 2020i). It may also be used to treat hot flashes.

As already mentioned, due to bioaccumulation, low levels of antidepressants in aqueous ecosystems raise a concern about their potential long-term risks to aquatic organisms. Venlafaxine has been observed at concentrations up to 2.9 $\mu g\ L^{-1}$ in the effluent of 5 municipal WWTPs around Canada (Lajeunesse, Smyth, Barclay, Sauvé & Gagnon, 2012) and studies have shown that venlafaxine is neuroendocrine disruptive to rainbow trout at concentrations near 1 $\mu g\ L^{-1}$ (Melnyk-Lamont, Best, Gesto & Vijayan, 2014).

There is evidence that the removal of venlafaxine from wastewater is inefficient (Parrott & Metcalfe, 2017). The concentration of venlafaxine in influent and effluent wastewater was investigated in two different studies from Canada and the U.S. Venlafaxine was observed at concentrations of 1.1 $\mu g\ L^{-1}$ and 0.8 $\mu g\ L^{-1}$ in the influent and effluent, respectively, at a municipal WWTP in the province of Ontario in Canada (Metcalfe et al., 2010). At a municipal WWTP in Colorado, venlafaxine was detected at concentrations of 0.93 $\mu g\ L^{-1}$ in the influent and 0.87 $\mu g\ L^{-1}$ in the effluent (Schultz & Furlong, 2008).

2.2.1.8 Metoprolol

Metoprolol is a beta-blocker used to treat high blood pressure, prevent chest pain, improve survival after a heart attack, and in combination with other medications, heart failure (U.S. NLM, 2020e). It may also be used to treat migraine headaches and irregular heartbeat and movement disorders caused by medication of mental illness.

Several studies indicate that beta-blockers are incompletely degraded in WWTPs (Ternes, 1998; Stolker et al., 2004; Wiegel et al., 2004; Thomas & Hilton, 2004; Ashton, Hilton & Thomas, 2004; Bendz, Paxeus, Ginn & Loge, 2005; Fono & Sedlak, 2005; Castiglioni et al., 2006; Roberts & Thomas, 2006) and a high degree of persistence for metoprolol have been reported in the aquatic environment (Bendz et al., 2005).

Gabet-Giraud, Miège, Jacquet, and Coquery (2013) studied the spreading of beta-blockers in recipients from three different WWTPs. The study showed metoprolol to be one of the most persistent beta-blockers due to it being shown to not have been degraded in samples taken 2 km from the studied WWTP outfalls. The majority of the other beta-blockers analyzed in the study were mostly degraded in samples taken at this distance from the WWTP.

It is stated that the ecotoxicological effects of beta-blockers have not yet been fully established (Santos, 2010). However, Santos (2010) states that the drug may cause deleterious effects on organisms such as fish, invertebrates, and green algae. Furthermore, older studies on beta-blockers have confirmed that these drugs are to be considered as Endocrine Disruptive Compounds (EDCs)

as they have been proved to affect testosterone levels in male organisms (Rosen, Kostis & Jekelis, 1988; el-Sayed et al., 1998).

2.2.1.9 Atenolol

Atenolol is, as well as metoprolol, included in the class of medication called beta-blockers and is most commonly used to treat chest pain, stabilize high blood pressure and improve survival after heart attacks (U.S. NLM, 2020h). It may also be used to prevent migraine headaches and heart failure.

In terms of occurrence, concentrations of beta-blockers in the ng L^{-1} to μg L^{-1} range have been reported in influents and effluents of WWTPs located in the U.S., Canada, Germany, and Finland (Huggett, Khan, Foran & Schlenk, 2003; Lee, Sarafin & Peart, 2007; Ternes, 1998; Vieno, Tuhkanen & Kronberg, 2006). These reports also highlight the lack of elimination by conventional biological treatment in WWTPs for this type of compound.

2.2.2 Chemical and physical characteristics of pharmaceutically active compounds

The main chemical and physical characteristics of PhACs, such as protonation constant (pK_a), octanol-water partition coefficient (Log K_{ow}), solubility (S_w), sludge–water distribution coefficient (Log K_d), reaction rate constant (k_{biol}) and molecular charge at pH 7, may be used to predict their expected behavior during wastewater treatment (Verlicchi et al., 2012a). These characteristics, which affect the tendency of a compound to remain in the dissolved phase, to adhere to flocs and/or particles or to biodegrade, may vary for PhACs within the same class of medications. Thus, PhACs belonging to the same class of medications may not necessarily be expected to show similar removal efficiencies. The main chemical and physical characteristics of the selected PhACs are listed in Table 1.

Table 1. Chemical and physical characteristics of the selected PhACs. A dash indicate that no information was found.

PhAC	pK_a [-]	Log K_{ow} [-]	S_w 25°C [mg L^{-1}]	Log K_d [-]	k_{biol} [L gSS^{-1} d^{-1}]	Charge at pH 7 [-]
Carbamazepine	13.90 [b]	2.45 [k]	17.66 [k]	0.1 [h]	≤0.10 [f] <0.03-<0.06 [i] <0.005-<0.008 [j]	Neutral [k]
Diclofenac	4.150 [a]	4.51/0.70 [k]	4.52 [k]	1.2 [h]	<0.04-1.2 [i] <0.002-<0.10 [j]	Negative [k]
Ciprofloxacin	6.380 [d]	0.40 [f]	1.148 10^4 [k]	4.3 [g]	-	Positive/neutral [k]
Sulfamethoxazole	5.700 [c]	0.89 [e]	3 942 [k]	2.1-2.7 [g] 2.3-2.6 [h]	0.30 [i]	Neutral/negative [k]
Citalopram	9.780 [n]	2.51 [m]	5.88 [n]	-	-	Positive [m]
Sertraline	9.850 [n]	4.81 [m]	3.80 [n]	-	-	Positive [m]
Venlafaxine	14.42 [n]	2.74 [n]	230 [n]	-	-	-
Metoprolol	9.600 [l]	1.88 [k]	4 777 [k]	-	0.35-0.40 [f]	Positive [k]
Atenolol	9.600 [l]	0.16 [k]	685.2 [k]	-0.68 [e]	1.10-1.90 [f]	Positive [k]

References: [a]Avdeef et al. 2000; [b]Jones et al. 2002; [c]Huber et al. 2003; [d]Nowara et al. 1997; [e]Vieno et al., 2007; [f]Wick et al., 2009; [g]Le-Minh et al., 2010; [h]Suarez et al., 2008; [i]Suarez et al., 2010; [j]Abegglen et al., 2009; [k]Verlicchi et al., 2012b; [l]Petrovic & Barcelò, 2007; [m]Magnér et al., 2016; [n]DrugBank, 2020.

As a simple criterion, k_{biol} <0.1 L gSS^{-1} d^{-1} and k_{biol} >10 L gSS^{-1} d^{-1} indicates poor and very good degradability, respectively, while k_{biol} between 0.1 and 10 L gSS^{-1} d^{-1} indicates quite good degradability (Verlicchi et al., 2012b). Log K_{ow} <2.5 and Log K_{ow} >4 indicates high hydrophilic compound and high lipophilic compound, respectively, while Log K_{ow} between 2.5 and 4 indicates moderate hydrophilic compound. Log K_d <2.7 indicates low adsorption potential while Log K_d >2.7 indicates high adsorption potential.

2.2.3 Removal mechanisms and operational parameters affecting the removal of PhACs in biological processes

The main removal mechanisms of PhACs occurring in a conventional biological reactor are biodegradation, mainly due to co-metabolic processes, and sorption on the sludge (Verlicchi et al., 2012a). Biodegradation depends on time, pH, temperature, and concentration (Riffat, 2012). Hence, the biological degradation of these compound correlates to the characteristics of the biomass, the compounds, the plant configuration, and operational parameters. Sorption on the sludge may occur due to absorption or adsorption and depends on several factors, such as pH, redox potential, stereochemical structure, and chemical characteristics of both the sludge and sorbed compound (Kümmerer, 2009c). Absorption is caused by hydrophobic interactions between the aliphatic and aromatic groups of a compound and the lipophilic cell membrane of the microorganisms or the lipid parts of the suspended solids, while adsorption is due to electrostatic

interactions between positively charged groups of chemicals and the negatively charged surfaces of the microorganisms.

Since the removal efficiency during biological treatment is affected by several operational parameters, it is hard to link the chemical and physical characteristics of PhACs to their correlated removal efficiency (Tadkaew, Hai, McDonald, Khan & Nghiem, 2011). This section describes how the operational parameters of a WWTP can affect the removal of PhACs in general.

2.2.3.1 Biomass concentration and solids retention time

A longer solids retention time (SRT) in activated sludge systems such as membrane bioreactor (MBR) and AGS-treatment technologies have been shown to promote the adaptation of a broader range of different kinds of microorganisms (Kreuzinger, Clara, Strenn & Kroiss, 2004; Weiss & Reemtsma, 2008). A longer SRT has also been shown to promote the presence of slow growing bacteria. The presence of a more diverse set of microorganisms and slow-growing species in the system is suggested to enable a greater removal capacity for PhACs. A study carried out by Kimura, Hara, and Watanabe (2007) found that diclofenac was removed from wastewater more efficiently in an MBR system with longer SRT compared to a conventional activated sludge system with a short SRT. The reason for the greater removal of diclofenac was due to the composition in the MBR sludge which improved the sorption capacities of the sludge. A study by Clara, Strenn, Ausserleiter, and Kreuzinger (2004) also compared PhAC removal capacity between an MBR and a conventional activated sludge system. In this study, the same SRT was set for both systems, and no differences in PhAC removal were shown. This result was concluded as evidence that the reactor type is of less importance compared to SRT for PhAC removal.

A higher biomass concentration in a biological treatment system decreases the food-to-microorganism ratio (F/M-ratio) (Verlicchi et al., 2012a). A consequence of a lower F/M-ratio is that the shortage in biodegradable substances in relation to the number of microorganisms present may induce the microorganisms to metabolize poorly degradable compounds (Weiss & Reemtsma, 2008). In turn, this will increase the removal of persistent PhACs. Furthermore, Göbel, McArdell, Joss, Siegrist, and Giger (2007) highlight that a reduced F/M-ratio in combination with a high SRT may result in better elimination of some PhACs due to co-metabolism processes.

2.2.3.2 pH

The pH value can have a significant impact on the behavior of PhACs due to the presence of different functional groups within the same molecule (Verlicchi et al., 2012a). Depending on the pH value, the molecule can be either neutral, anionic, cationic, or zwitterionic, which changes its physical, chemical, and biological characteristics (Kümmerer, 2009c, Cirja, Ivashechkin, Schäffer & Corvini, 2008). Tadkaew, Sivakumar, Khan, McDonald, and Nghiem (2010) studied the impact on pH ranging from 5 to 9 on the removal of selected PhACs by a submerged MBR. The study showed that the removal efficiency of the ionizable PhACs diclofenac, sulfamethoxazole, ibuprofen, and ketoprofen was highly pH-dependent while it was relatively independent for the non-ionizable compound carbamazepine. The ionizable PhACs showed a high removal efficiency at pH 5 which could be linked to their speciation behavior. At this pH, these compounds mainly

exist in their hydrophobic form. This means that they could easily adsorb to the activated sludge which results in a higher removal efficiency compared to the removal efficiency achieved at higher pH.

2.2.3.3 Temperature

Biological reactions are strongly affected by temperature and seasonal variation on the removal efficiency of some PhACs has been observed (Vieno, Tuhkanen & Kronberg, 2005). Hai et al. (2011) investigated the removal of some PhACs by a lab-scale MBR fed by synthetic wastewater during temperature variations. The study showed that the removal of most hydrophobic PhACs was stable during operations under the temperature ranging from 10-35 ^{o}C while the removal of less hydrophobic PhACs showed a higher variation. Castiglioni et al. (2006) analyzed removal data from six different WWTPs in Italy and found that the removal efficiency of amoxicillin, atenolol, bezafibrate, enalapril, furosemide, ibuprofen, ranitidine, and SMX are significantly higher during summer than during winter. Ciprofloxacin, hydrochlorothiazide, and ofloxacin showed similar removal efficiencies during the two seasons. However, it is unknown if the temperature dependency observed for biological degradation of the common contaminants, such as organic matter, N and P, also applies to the removal of antibiotics or PhACs in general (Göbel et al., 2007, Tauxe-Wuersch, De Alencastro, Grandjean & Tarradellas, 2005).

2.2.3.4 Treatment configuration

Nitrifying bacteria have been found to be able to co-metabolize a variety of persistent compounds (Batt, Kim & Aga, 2006; Perez et al., 2005). High removal efficiencies of PhACs have been suggested to correlate with high levels of N removal (Batt et al., 2006; Clara, Kreuzinger, Strenn, Gans & Kroiss, 2005). To optimize the removal of N and COD, wastewater treatment processes used for complete biological nutrient removal are characterized by different zones with aerobic, anoxic, and anaerobic conditions, which may influence the removal of PhACs as well (Zwiener & Frimmel, 2003). Suárez et al. (2010) divided PhACs into three different groups based on their potential to be removed during biological treatment: (1) highly biodegradable compounds under aerobic and anoxic conditions (ibuprofen, fluoxetine, and natural estrogens), (2) highly biodegradable compounds under aerobic conditions, but persistent in anoxic conditions (diclofenac, naproxen, ethinylestradiol, roxithromycin, and erythromycin) and (3) resistant compounds to biological transformations (sulfamethoxazole, trimethoprim, carbamazepine, and diazepam).

2.3 Current advanced treatment of pharmaceutically active compounds

Several methods, that are either physical, biological, oxidative, or adsorptive, are available for the removal of microcontaminants in wastewater (SEPA, 2017). However, literature and studies of full-scale facilities show that activated carbon and ozonation have been prioritized when implementing advanced treatment of PhACs (Cimbritz et al., 2016). This section further explains the processes of activated carbon and ozonation together with the advantages and disadvantages associated with each technique.

2.3.1 Activated carbon

Activated carbon may be used either through dosing with powdered activated carbon (PAC) or by filtering through granulated activated carbon (GAC) (Cimbritz et al., 2016). PAC is a well-tested advanced treatment method in which powdered activated carbon is added to the wastewater (Abegglen & Siegrist, 2012). Usually, PAC is added to a contact tank followed by a clarifier or added directly into the activated sludge treatment followed by a polishing filter (Boehler et al., 2012; Metzger, 2010; Löwenberg et al., 2016). PAC can also be added directly into a sand filter. When PAC is added, microcontaminants present in wastewater adsorb onto the activated carbon due to hydrophobic and electrostatic interactions. Further, flocs are created whereupon they settle and/or are filtered from the wastewater. Depending on the setup of the PAC treatment, a flocculant addition tends to be needed in order to enable good settling and efficient filtration.

A promising setup for PAC treatment is to initially dose 15 gPAC m^{-3} into a biological reactor and recycle PAC waste from the post-treatment back into the reactor (Boehler et al., 2012). This setup was tested in a Swiss study and the use of recycling showed improved removal efficiency of 30 to 50 % compared to single-stage application. Furthermore, the study states that an optimally configured PAC treatment should be seen as a feasible technology for the removal of microcontaminants with up to 80 % elimination. However, in Sweden, the economical value and desire to recycle the sludge for agricultural purposes is restricting the possibilities of dosing PAC into the biological treatment step (Kårelid, Larsson & Björlenius, 2017).

The process of using GAC as a treatment method consists of implementing filters in which GAC is added (U.S. Environmental Protection Agency [U.S. EPA], 2000). As well as for PAC, adsorption is the physical process that enables the removal of contaminants by GAC. One advantage with GAC filters is the possibility to regenerate the activated carbon. This is in many cases done by transporting the used carbon to a facility where regeneration is accomplished by thermal means and organic matter present within the GAC is oxidized and removed.

GAC has shown to be promising in terms of microcontaminant removal (Alt et al., 2016) and is, therefore, also a strong possible alternative for the adoption of WWTPs to manage expected future removal demands for PhACs. With an empty bed contact time (EBCT) longer than 25 minutes, GAC filters can ensure microcontaminant removal >80 % for up to 25,000 bed volumes on biologically treated wastewater (Benstoem et al., 2017; Bitterwolf, Boehler, Siegrist & Joss, 2017; Wunderlin, Joss & Fleiner, 2017). However, during certain conditions, GAC generates hydrogen sulfide from bacterial growth which creates problems related to corrosion and odors (U.S. EPA, 2000). Furthermore, the used carbon may also present a land disposal problem.

Even though PAC is shown to be a feasible and efficient alternative for PhACs removal, the capital and operating costs of such a treatment technology are in the higher spectrum even for smaller WWTPs (<100,000 pe) (Mulder, Antakyali & Ante, 2015). Extra costs for coal, chemicals, and sludge treatment are also to be expected. Therefore, ozonation, even though it entails approximately two times higher energy use, has a lower variable cost. This is also true for when ozonation is compared to GAC.

2.3.2 Ozonation

Ozonation is accomplished by dosing ozone (O_3) to the biologically treated wastewater (Abegglen & Siegrist, 2012). Approximately 3-10 $gO_3\ m^{-3}$ of wastewater is commonly dosed. Since the already biologically treated wastewater includes dissolved organic carbon (DOC), a decrease and inactivation of PhACs in/from the water body is enabled by partial oxidation. During ozonation, both molecular O_3 and hydroxyl radicals are present (Barry, Hristovski & Westerhoff, 2014). Chemicals of emerging concern, such as PhACs, tend to react slowly with O_3 due to the hydroxyl radicals controlling the oxidation. Therefore, a higher concentration of hydroxyl radicals is desirable to promote removal efficiency. However, the oxidation process that removes and inactivates contaminants during ozonation also produces potentially toxic by-products due to reactions with background DOC (Abegglen et al., 2009; Benner & Ternes, 2009; Krauss, Longree, Van Houtte, Cauwenberghs & Hollender, 2010; Lee & von Gunten, 2016). To eliminate the negative effects from these by-products, post-treatment is necessary and, usually, this is implemented by having a sand filter directly after the ozonation tank in the process configuration (Huber et al., 2005; Hollender et al., 2009; Stalter, Magdeburg & Oehlmann, 2010; Stalter, Magdeburg, Quednow, Botzat & Oehlmann, 2013; Zimmermann et al., 2011).

2.4 Aerobic granular sludge

As stated in Section 1.1, there are some disadvantages associated with the advanced treatment methods commonly implemented for the removal of PhACs, such as the formation of toxic by-products and high energy use during ozonation (SEPA, 2017) and limited possibilities of using sludge contaminated with PAC as a fertilizer on farmland as well as the high resource consumption and operating costs (Mulder et al., 2015) related to the use of activated carbon. Thus, it is of interest to further investigate advanced biological treatment alternatives for the removal of PhACs from wastewater.

Aerobic granules are defined as "[...] aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs" (de Kreuk, Kishida & van Loosdrecht, 2007). Aerobic granules consist of components of microbial origin as well as active microorganisms and the aggregates are formed without adding a carrier material to the wastewater. The settleability and minimum size of aerobic granules should be such that the difference between the sludge volume index (SVI) after 10 respectively 30 minutes of settling is very small.

2.4.1 Aerobic granule formation and physio-chemical characteristics

Generally, the formation of aerobic granules is initiated due to hydrodynamic shear forces inducing the production of extracellular polymeric substances (EPS) and cell surface hydrophobicity which forces the bacteria cells to attach to each other (Nancharaiah & Reddy, 2017).

2.4.1.1 Mechanisms of aerobic granule formation

As illustrated in Figure 4, Liu and Thay (2002) propose that the mechanisms of aerobic granule formation can be described to occur in four different steps were (1) the bacteria cells attach to each other, (2) aggregates are formed due to attractive forces between the bacteria cells, (3) the aggregates are matured due to microbial forces and (4) the aggregate is formed to a three-dimensional structure.

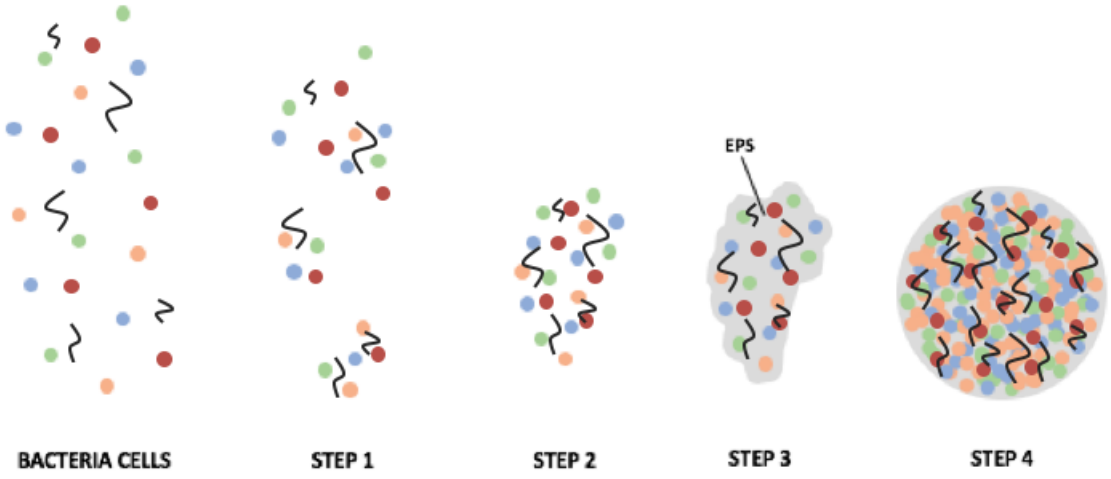

Figure 4. The aerobic granule formation. Figure by author.

During the first step, the bacteria cells attach to each other due to hydrodynamic forces, gravity, thermodynamic forces, diffusion, and the natural mobility of the bacteria cell (Liu & Thay, 2002). The attractive forces forming aggregates during the second step can be divided into physical, chemical, and biochemical forces. The physical forces include surface free energy, hydrophobicity, surface tension, cross-linking bridges, opposite charge attraction, and van der Waal forces, while the chemical forces comprise ionic pairing, hydrogen bonding, triple ion formation and bridge formation between particulates. The biochemical forces involved are cellular surface dehydration and cellular membrane fusion. During the third step, the aggregates are matured due to metabolic changes, production of a matrix of EPS, and cellular cluster growth. The three-dimensional structure of the aggregates formed during the fourth step is shaped by hydrodynamic forces.

2.4.1.2 Structure, morphology and characteristics of aerobic granular sludge

The average diameter of a typical aerobic granule is usually between 0.2 and 3 mm (Winkler, Kleerebezem, Strous, Chandran, & van Loosdrecht, 2013a) although aerobic granules of size up to 25 mm have been observed (Dangcong, Bernet, Delgenes & Moletta, 1999; Tay, Liu, & Liu, 2001a; Tay et al., 2001b; Zhu & Wilderer, 2003; Torregrossa, Di Bella, Viviani & Gnoffo, 2007). An advantageous characteristic of AGS is its higher density and specific gravity compared to flocculated sludge, which enables faster solid-liquid separation (Nancharaiah & Reddy, 2017). The aerobic granules also have a high porosity that allows for mass transfer between the microorganism EPS complex if the AGS is cultivated in an SBR operated with alternating aerobic and anaerobic phases (Winkler, Bassin, Kleerebezem, van der Lans & van Loosdrecht, 2012). The mass transfer further enables the stratification of aerobic, anoxic, and anaerobic layers in the aerobic granule due to a DO gradient across the depth of the granule, see Figure 5.

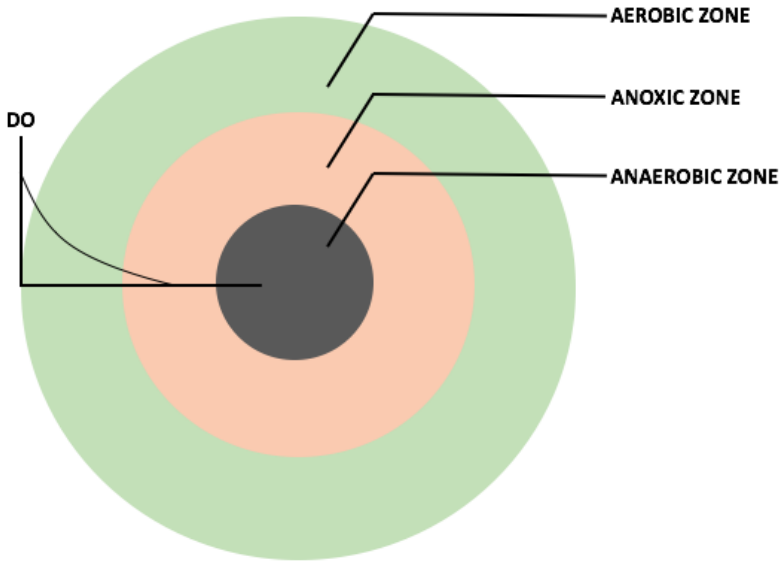

Figure 5. Aerobic, anoxic, and anaerobic zones of an aerobic granule. Figure by author.

One of the most defining features of AGS is the aggregated mass of microorganisms formed in the matrix of EPS. This composition makes the AGS similar to biofilms, with the difference that aerobic granules do not need any carrier material to grow and form into its characteristic spherical or ellipsoidal shape (Mannina, Ekama, Ødegaard & Olsson, 2018). EPSs are natural polymers that are mainly composed of polysaccharides and proteins (Miao, Yang, Tao & Peng, 2019). Usually, the stability of granules is determined by the protein to polysaccharide ratio. Polysaccharides have an easier molecular structure compared to proteins and are more susceptible to biological and chemical degradation. Therefore, a low protein to polysaccharide ratio indicates aerobic granules with a matrix of fragile structural elements. Furthermore, the polysaccharides are mainly distributed in the outer edge of the aerobic granule and the proteins in the center (McSwain, Irvine, Hausner & Wilderer, 2005).

2.4.2 Microbial community and conversion processes

The microbial selection in AGS is triggered by multiple operational parameters such as organic loading rate (OLR), F/M-ratio, COD/N-ratio, SRT, settling time, redox conditions, and substrate type (Winkler et al., 2013b). Furthermore, the microbial community in AGS is dependent on the treatment configuration in terms of what the AGS system is intended to remove as well as the organic composition of the wastewater that is being treated (Wilén, Liébana, Persson, Modin & Hermansson., 2018). AGS systems are generally applied for COD removal, COD and N removal or simultaneous COD, N, and P removal. The specific system, and especially how it exposes the microorganisms to contaminants, together with the incoming wastewater and its concentration of contaminants will entail different compositions and properties of AGS (Wilén, Gapes & Keller, 2004).

If aerobic granules are cultivated in an SBR operated with a primary anaerobic phase, the granule composition, in terms of microorganisms, firstly consists of an anaerobic zone in which easily

degradable organic substances are stored in the form of PHAs (Oehmen et al., 2007). The PHAs later serves as a carbon source and electron donor during denitrification in the anoxic zone and are crucial for the enabling of simultaneous nitrification and denitrification (Third, Burnett & Cord-Ruwisch, 2003). When the SBR is operated with an aerobic phase, the aerobic granule will form an inner anoxic zone and an outer aerobic zone where the inner zone mainly will consist of denitrifying microorganisms, polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) and the outer zone will include nitrifying microorganisms due to the presence of DO (Nancharaiah & Reddy, 2017). However, it is possible for organic substances to diffuse into the inner zones of the granule and since PAOs and GAOs can grow in anoxic conditions and are not dependent on the presence of oxygen, it is possible for PAOs and GAOs to exist both in the inner and outer part of the aerobic granule (de Kreuk, Picioreanu, Hosseini, Xavier & van Loosdrecht, 2007; de Kreuk, Heijnen & van Loosdrecht, 2005; Lemaire, Webb & Yuan, 2008; Lemaire, Yuan, Blackall & Crocetti, 2008). The location of nitrifying microorganisms, denitrifying microorganisms, PAOs, and GAOs inside an aerobic granule can be seen in Figure 6.

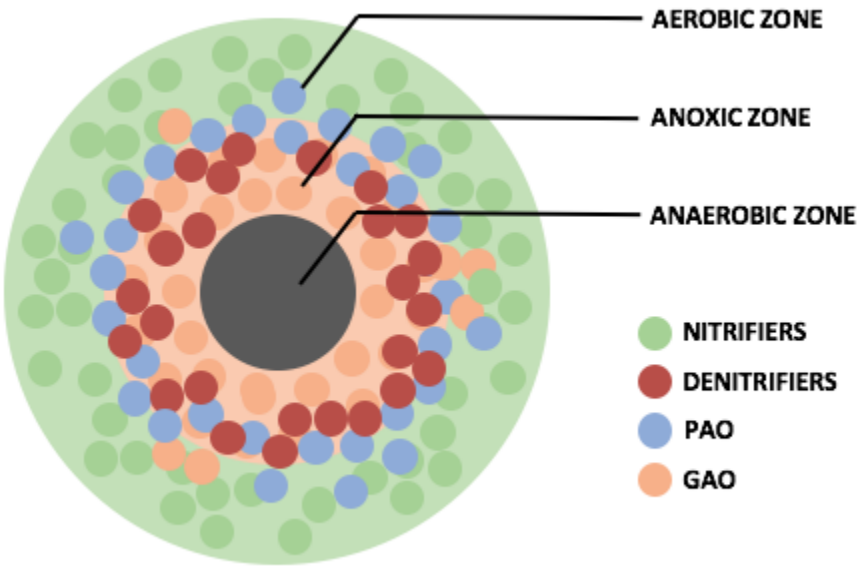

Figure 6. Location of different microorganisms inside an aerobic granule. Figure by author, based on Nancharaiah and Reddy (2017).

This stratification of aerobic, anoxic, and anaerobic layers in the aerobic granule is what allows for simultaneous COD, N, and P removal (Winkler et al., 2013b; Nancharaiah & Reddy, 2017), by simultaneous nitrification-denitrification, anammox process and biological phosphorus removal, see Figure 7.

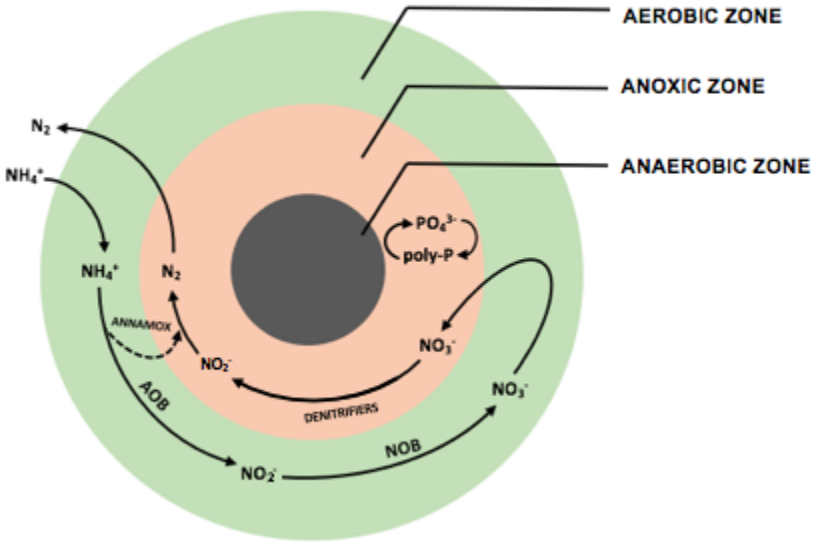

Figure 7. Simultaneous nitrification-denitrification, anammox process, and biological phosphorus removal inside an aerobic granule. Figure by author, based on Nancharaiah and Reddy (2017).

2.4.2.1 The nitrification-denitrification process

Simplified, nitrification is the process of how ammonium (NH_4^+) is converted into nitrate (NO_3^-) and denitrification is the process of how NO_3^- is converted into nitrogen gas (N_2) (Hermansson, Sörensson, Lindgren, Mattsson & Wik, 2006). In order for denitrification to occur, nitrification is first required unless N is already present in the water in the form of nitrate (NO_3^-) (Gray, 2005). Nitrification is performed in aerobic conditions and includes two chemical reactions. First, NH_4^+ is oxidized by ammonium oxidizing bacteria (AOB) into nitrite (NO_2^-) and thereafter NO_2^- is oxidized by nitrite oxidizing bacteria (NOB) and turned into NO_3^-. The chemical equation for the reactions can be seen in equation (1) and equation (2).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

Denitrification is performed by denitrifying bacteria in anoxic conditions with the presence of organic matter (Hermansson et al., 2006). When the organic matter is consumed, NO_3^- is used as electron acceptor and N_2 is formed. The chemical equation for the denitrification process can be seen in equation (3).

$$NO_3^- + 1.25CH_2O \rightarrow OH^- + 0.75H_2 + 1.25CO_2 + 0.5N_2 \quad (3)$$

Since organic matter is consumed in the process, a high rate of TOC is also removed from the wastewater by denitrification.

2.4.2.2 The anammox process

N can also be removed by anaerobic ammonium oxidation (anammox). The anammox process was discovered in 1995 and have, since then, been extensively researched and is now suggested as a sustainable and innovative alternative to the traditional technology implemented for N removal in wastewater (Mulder, van de Graaf, Robertson & Kuenen, 1995; Jin, Yang, Yu & Zheng, 2012). The advantages of the anammox process are stated to be a higher N removal rate together with smaller space requirements and low operational costs (Jetten et al., 2005; Joss et al., 2009; van der Star et al., 2007). During the anammox process, NH_4^+ is oxidized to NO_2^-, which is further reduced to N_2 (Pal, 2017), which can be seen in equation (4).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (4)$$

2.4.2.3 Biological phosphorus removal

Biological P removal is considered to be a cost-effective and environmentally sustainable alternative to chemical treatment (Bunce, Ndam, Ofiteru, Moore & Graham, 2018). The biological removal of P can be carried out in the activated sludge process by a method called enhanced biological phosphorus removal (EBPR). The main concept includes the recirculation of sludge through anaerobic and aerobic conditions (Barnard, 1975). In the anaerobic phase, organic matter is fermented into volatile fatty acids (VFAs) (Mino, van Loosdrecht & Heijnen, 1998; van Loosdrecht, Nielsen, Lopez-Vazquez & Brdjanovic, 2016). The VFAs are then stored intracellularly as polyhydroxyalkanoates (PHAs) by PAOs. The energy necessary for this biotransformation is provided by the breakdown of the two intracellularly stored polymers polyphosphate (poly-P) and glycogen (gly), which enables phosphate (PO_4^{3-}) to be released. The stored PHAs are later used as an energy source by PAOs in the aerobic phase for PO_4^{3-} uptake and poly-P storage. Finally, PO_4^{3-} is removed from the wastewater by removing the poly-P rich activated sludge. Figure 8 shows a conceptual scheme over the PAO's metabolism during EBPR carried out in the activated sludge process.

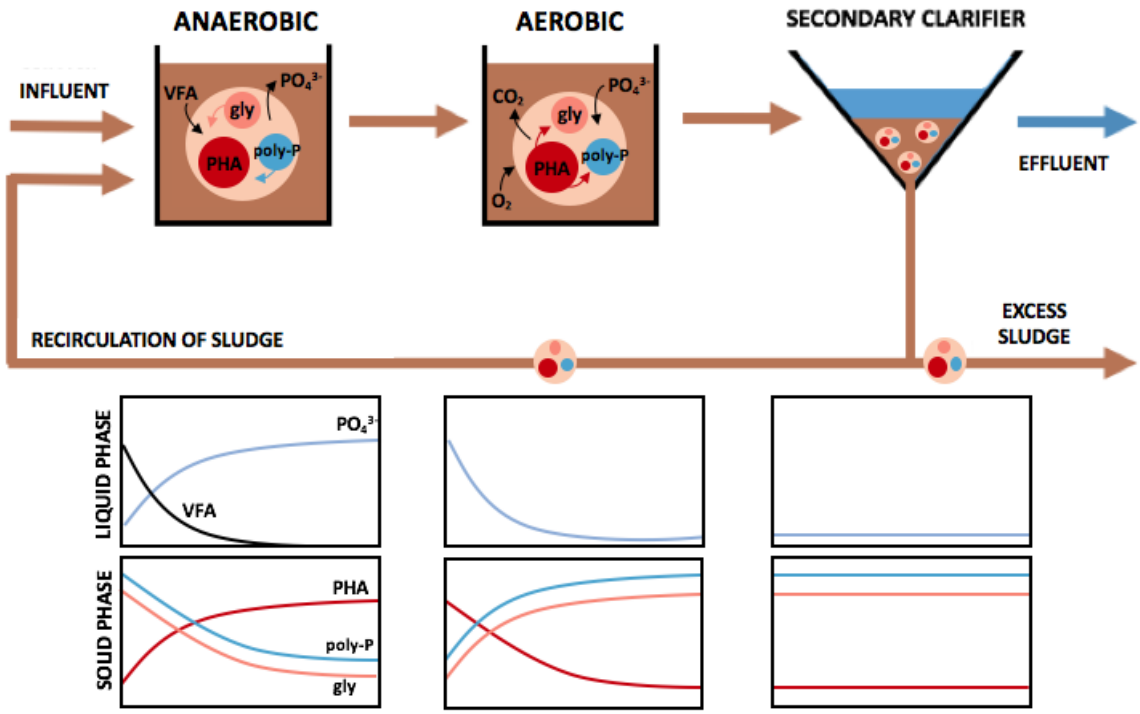

Figure 8. Conceptual scheme over the PAO's metabolism during EBPR carried out in the activated sludge process. Figure by author, based on Lopez-Vazquez (2009 and Meijer (2004).

2.4.3 Operational parameters affecting granulation

Aerobic granules are spontaneously formed in a WWTP when certain combinations of conditions are fulfilled (Bengtsson et al., 2017). Several operational parameters can affect the granulation process, such as hydraulic retention time (HRT), DO concentration, OLR, temperature, hydrodynamic shear forces, and growth rate.

According to Pan, Tay, He, and Tay (2004), stable aerobic granules are formed when HRT is between 2 and 12 hours and the formation of AGS is possible with DO concentration ranging from 0.7-1 up to 2-7 mg L^{-1} (Winkler et al., 2018). However, DO concentrations lower than 2-5 mg L^{-1} may lead to the formation of instable aerobic granules (Hailei, Guangli, Guosheng & Feng, 2006; McSwain & Irvine, 2008; Mosquera-Corral, de Kreuk, Heijnen & van Loosdrecht, 2005).

For stable aerobic granules to form, a moderately high OLR is suitable. Tay, Pan, He, and Tay (2004) investigated the effect of OLR on aerobic granulation by operating four sequential aerobic sludge blanket reactors (SASBRs) with OLR of 1, 2, 4 and 8 kg COD $m^{-3}d^{-1}$. The study showed that the best aerobic granules were formed at OLR of 4 kg COD $m^{-3}d^{-1}$. At OLR of 1 and 2 kg COD $m^{-3}d^{-1}$, no aerobic granules were detected while instable aerobic granules were formed at OLR of 8 kg COD $m^{-3}d^{-1}$. This was also observed by Li, Yang, Li, and Gu (2008) during the investigation of granulation in glucose-fed AGS reactors showing that OLR of 4.5 kg COD $m^{-3}d^{-1}$ resulted in faster granulation and aerobic granules with more and larger pores compared to OLR of 1.5 and 3 kg COD $m^{-3}d^{-1}$.

According to de Kreuk et al. (2005), the performance of an AGS reactor can be affected by changes in temperature. It is possible to maintain stable aerobic granules during relatively low temperatures (8 ^{o}C) if the granulation process is started up at higher degrees before it is successively lowered. If the process is started up at low temperatures, the aerobic granules become irregular shaped and instable.

Franca, Pinheiro, van Loosdrecht and Lourenco (2017) highlight the selection of microbial communities with a low maximal growth rate as a key factor for the formation of stable aerobic granules. For instance, if slow-growing nitrifying bacteria are selected, the stability of aerobic granules can be significantly improved (Liu, Yang & Tay, 2004). Aerobic granules also become more stable and compact if they are exposed to high hydrodynamic shear forces that arise from liquid/gas flow or attrition between particles (Liu & Tay, 2002).

2.4.4 Aerobic granular sludge for the removal of pharmaceutically active compounds

The biological removal of PhACs from wastewater has mainly been evaluated by treatment methods such as the conventional activated sludge process (Osachoff et al., 2014) and MBRs (Cheng, Lee, Kuo & Wu, 2015). Fewer studies have evaluated the performance and potential of the AGS technology related to the treatment of wastewater containing such microcontaminants (Kong, Zhi-Bin, Shu & Miao, 2015, Moreira et al., 2015, Shi, Xing, Wang & Wang, 2013). However, previous studies on AGS performance have shown that AGS is promising for the treatment of toxic compounds (Duque et al., 2011). The dense physical structure of aerobic granules has been shown to protect the interior of the granule from shock loadings of toxic compounds due to the mass transfer limitation that the structure enables (Adav, Lee, Show & Tay, 2008; Beun et al., 1999). This capability makes AGS an ideal biological treatment option for compounds that are poorly biodegradable, (Morgenroth, Sherden, van Loosdrecht, Heijnen & Wilderer, 1997; Tay, Moy, Jiang & Tay, 2005) and recently, the AGS technology has shown to be able to remove PhACs from wastewater by biotic and abiotic processes (Amorim et al., 2016).

The possibility to biotransform CECs with the AGS technology is still an unexplored area in which more research is needed (Mery-Arya, Lear, Perez-Garcia & Astudillo-Garcia, 2019). However, it is of interest to investigate the ability of the AGS technology for PhAC removal. Microbial communities with greater taxonomic richness have shown to improve the transformation of microcontaminants in wastewater at higher rates (Johnson et al., 2015) and the degradation of aromatic compounds in AGS is declared to likely be stimulated in the nitration process by heterotrophs (Ramos, Suárez-Ojeda & Carrera, 2016). These sorts of findings highlight the importance of understanding the microbial community in AGS, and, especially if, or how, it can be manipulated in order to enhance the degradation of CECs, such as PhACs (Mery-Araya et al., 2019). The microbial composition and diversity is mainly influenced by the organic composition of the wastewater (Wilén, et al., 2018) and, therefore, the PhAC removal capability of AGS systems with different carbon sources have been investigated in recent studies (Mery-Araya et al., 2019; He, Zhang, Wei, Zhao & Pan, 2019). The study carried out by Mery-Araya et al. (2019) found that AGS cultivated with glycerol showed to give a significant increase in the

biotransformation of CECs compared to the other studied AGSs cultivated with acetate, 2-propanol and 1:1:1 mixture of glycerol, acetate and 2-propanol. The presence of the bacteria *Enterobacteriaceae* was suggested to be the key dominant family increasing biodegradability in AGS grown in glycerol.

In another study carried out by He et al. (2019), manganese-oxidizing aerobic granular sludge (Mn-AGS) was cultivated and tested on a set of organic micropollutants (OMPs) including three types of commonly prescribed pharmaceuticals (17α- ethinylestradiol (EE2), tetracycline (TC) & chloramphenicol (CAP)). The Mn-AGS was successfully cultivated by continuously adding Mn(II) to an SBR operating for 55 days and degradation rates of OMPs were compared to conventionally cultivated AGS from the same reactor. Results showed that degradation rates of EE2, TC and CAP were 1.3-3.9 times higher in an SBR with Mn-AGS compared to AGS and the removal of COD was not significantly impacted. However, N and P removal were not subject of analysis in the study, thus the impact of Mn(II) addition in relation to the removal of nutrients was not covered. Furthermore, Mn-AGS was found to inhibit the microbial activity of some OMPs degradation, which leads to much lower degradation rates compared to conventional AGS. Therefore, the authors conclude that some OMPs may not be removed by Mn-AGS.

Kent and Tay (2019) proposed that the removal mechanism of AGS for CECs commonly detected in wastewater, specifically 17α- ethinylestradiol (EE2), 4- nonylphenol (NP), and CBZ in this study, is changing over time. A mixture of EE2, NP and CBZ was added to synthetic wastewater in an SBR with AGS. The removal rate and mechanisms of the AGS were assessed. The initial removal mechanism was shown to be adsorption. However, due to limited sludge growth, the adsorption decreased as available sites were occupied. Finally, the maximum adsorption capacity of the aerobic granules was reached whereupon degradation took over as the leading removal mechanism. Similar trends for the removal mechanism in AGS have been seen in other studies with the compounds fluoroquinolones (FQs) (Amorim et al., 2014) and cationic dye (Sun et al., 2008). In relation to this, a study by Zhao et al. (2015) found that the adsorption capacity of AGS increased when aerobic granules were formed into larger aggregates with more surface area. The surface of granular sludge includes a large number of functional groups, such as amino-, carboxyl- and phosphate groups, which are providing adsorption sites for PhACs (Gao, Zhang, Su, Chen & Peng, 2010) and by increasing the surface area of the aerobic granules, the adsorption sites increases and, thus, removal by adsorption can be enhanced. The removal rates for each substance that was tested in the study by Zhao et al. (2015) showed to improve as the aerobic granule surface area increased. The investigated substances in the study were a mixture of PhACs and other persistent substances from personal care products (PCPs). Furthermore, the study highlights the importance of finding ways of stimulating both the adsorption as well as the biodegradation capability of AGS since each individual PhAC and PCP showed to have a unique removal behavior where some were more responsive to adsorption and some to biodegradation and vice versa.

3. Materials and method

The experimental work was performed using a lab-scale SBR, constructed at Chalmers WET laboratory, cultivating AGS. The first challenge was to start up the reactor and cultivate AGS. During the first period, the focus was mainly to control biomass washout and SRT, by regulating the time of the settling phase, and to control the system in terms of DO level and pH value. The second challenge was to reach stable removal performance in terms of TOC, TN and TP. This chapter describes the configuration of the lab-scale SBR, how it was calibrated before start-up, operational settings and composition of the complex synthetic wastewater as well as the sampling routine and analytical methods used.

3.1 Reactor description and reactor start-up

The lab-scale SBR was operated in 6 hours cycles at a controlled room temperature of 21 ^{o}C, average pH of approximately 7 ± 0.3 and an average DO level of approximately 0 and 20 ± 10 % during the anaerobic fill phase and aerobic reaction phase, respectively.

The reactor was inoculated with activated sludge from a full-scale municipal WWTP in Kungsbacka, Sweden. The measured concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) in the activated sludge were 4.68 gTSS L^{-1} and 3.86 gVSS L^{-1}, and SVI of the activated sludge after 5, 10 and 30 minutes of settling was calculated to be 210.0, 206.0 and 180.5 mL g^{-1}. Complex synthetic wastewater providing total COD, TN and TP concentrations of 600 mg COD L^{-1}, 42 mg TN L^{-1} and 6 mg TP L^{-1}, respectively, micronutrients and selected PhACs were pumped in at the bottom of the reactor.

The level of DO and the pH value were monitored throughout the cycle and adjusted during the reaction phase. In order to adjust the reactor pH, two bottles containing sodium hydroxide (X M NaOH) and hydrochloric acid (X M HCl) were connected to two pumps dosing acid and base when the measured value was outside the limit. The goal was to get an average pH value of 7 ± 0.3 during the whole cycle. The level of DO was monitored through the whole cycle and controlled during the reaction phase to get an average DO level as close as possible to 20 % during aeration. If the level of DO was reported too high in the reactor, N_2 was injected and if it was reported too low, air was added. When the DO level was in the desired range, the gas was circulated to provide mixing by the means of a pump. The air N_2 and recirculated gas from the reactor were mixed in a gas mixing vessel and consequently pumped to the bottom of the reactor. Before any gas reached the reactor it was led through a rotameter in order for the volumetric flow rate to be measured. The flow was kept constant by the means of a rotameter delivering a gas flow of 3 L s^{-1}. A data acquisition hardware was programmed to report measurements from the reactor to the computer every 60 seconds.

The reactor scheme can be seen in Figure 9.

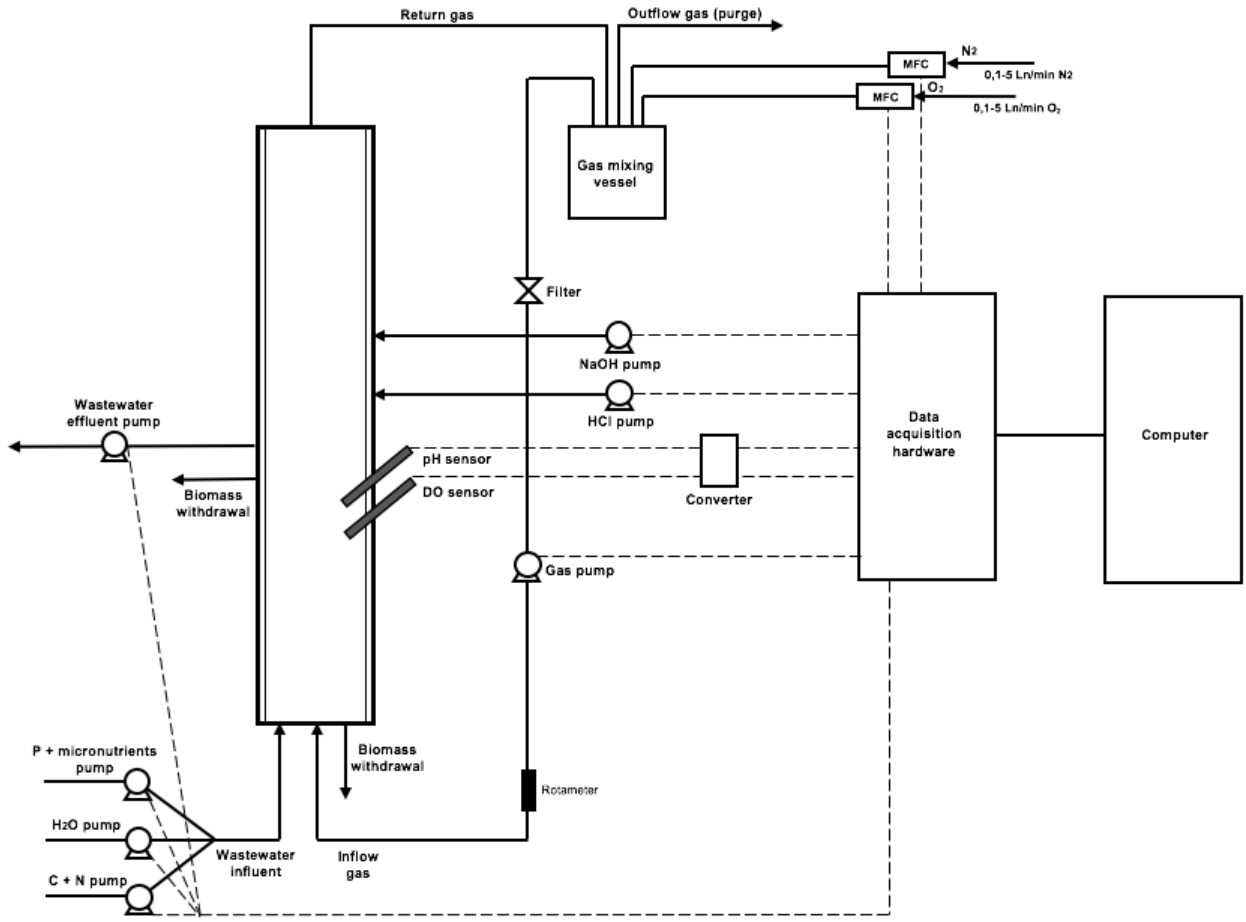

Figure 9. Lab-scale SBR scheme (Burzio, 2020).

3.1.1 Cycle setup

The operation of the reactor consisted of a sequence of fill-and-decant cycles including the following phases: anaerobic fill, aerobic react, settle, decant and idle. The total cycle time was 6 hours and the time of each phase can be seen in Table 2.

Table 2. Time of each phase.

Phase	Time [min]
Anaerobic fill	90
Aerobic reaction	212 - 242
Settle	45 - 15
Decant	10
Idle	3

During the anaerobic fill phase, the reactor was fed with the complex synthetic wastewater to a volume of 3.1 L. The filling was anaerobic and static to provide the conditions needed for the uptake of VFAs and breakdown of intracellularly stored poly-P and glycerol releasing PO_4^{3-} during the biological removal of P. The aerobic reaction phase was accomplished through aeration with air and nitrogen, mainly to achieve the aerobic conditions necessary for nitrification before aerobic granule formation, simultaneous nitrification-denitrification once aerobic granules are formed, and uptake of PO_4^{3-} released during the anaerobic fill phase, but also to mix and provide the shear forces needed to form and maintain stable aerobic granules. During the settling phase, the biomass was allowed to settle to the bottom of the reactor. Based on the start-up settling strategy by Lochmatter and Holliger (2014), the time of the settling phase was set to 45 minutes at the beginning of the experimental run and was gradually decreased to finally reach 15 minutes, in order to avoid washout of slow growing bacteria. The initial strategy was to decrease the settling time if SRT ≥ 20 days and increase the aerobic reaction phase accordingly to maintain the 6 hour cycle time. However, during the experimental run, the SRT had to be maintained ≥ 20 days to be able to wash out enough biomass in order to stimulate the selection of biomass with good settling characteristics. To reach an exchange ratio of 50 %, half of the reactor volume was emptied during the decant phase. The days of adjustment of the settling and aerobic reaction phases are presented in Table 3.

Table 3. Date of every change in phase time for the settling and aerobic reaction phase.

Day	Settling time [min]	React time [min]
1	45	212
8	35	222
19	25	232
25	15	242

3.1.2 Required pump flows and pumps calibration

The three different pumps dosing the solution containing the carbon and nitrogen sources, the micronutrient and phosphorous solution and water, respectively, had to be calibrated in order to set the pump flows required to reach the target wastewater influent concentrations and volume. To calculate the flow each pump had to generate, equation (5) was used.

$$Q_{sub.sol} = \frac{V_{inf}}{CF \times t_{fill}} \text{ (mL min}^{-1}\text{)} \quad (5)$$

Where V_{inf} is the influent volume (mL), CF is the concentration factor (-) and t_{fill} is the anaerobic fill phase time (min).

The water pump flow was then calculated using equation (6).

$$Q_w = \frac{V_{inf} - Q_{C+N.sol} - Q_{p+mn.sol}}{t_{fill}} \text{ (mL min}^{-1}\text{)} \quad (6)$$

Where V_{inf} is the influent volume (mL), $Q_{C+N.sol}$ is the C + N solution flow (mL min^{-1}), $Q_{P+mn.sol}$ is the P + micronutrient solution flow (mL min^{-1}) and t_{fill} is the anaerobic fill phase time (min).

The pump calibration procedure can be seen in Figure 10. To validate that the pump settings provided accurate influent concentration, influent samples were analysed by IC.

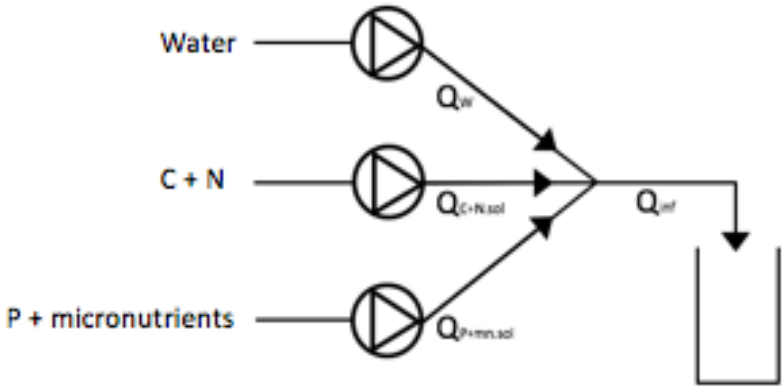

Figure 10. Schematic of the pump validation procedure.

3.2 Complex synthetic wastewater composition

The reactor was fed with complex synthetic wastewater based on a recipe by Layer et al. (2019). Layer et al. (2019) compared the start-up of AGS systems fed by different synthetic and municipal wastewaters characterized by increasing complexity in terms of non-diffusible organic substrate and concluded that, for laboratory studies, the complex synthetic wastewater used in their study can be a suitable substitute for real municipal wastewater. The recipe for the complex synthetic wastewater used in this thesis provides total COD, TN, and TP concentrations of 600 mg COD L^{-1}, 42 mg TN L$^{-1,}$ and 6 mg TP L^{-1}, respectively. The specific substrate recipe for the different solutions can be seen in Table 4.

Table 4. Specific substrate concentrations for the complex synthetic media.

Component	Concentration [g L^{-1}]
Sodium acetate ($C_2H_3NaO_2$)	0.19
Sodium propionate ($C_3H_5NaO_2$)	0.12
Glucose ($C_6H_{12}O_6$)	0.14
Peptone	0.12
Ammonium chloride ($(NH_4)Cl$)	0.09
Calcium chloride hydrate ($CaCl_2.H_2O$)	0.02
Magnesium sulfate ($MgSO_4$)	0.02
Potassium chloride (KCl)	0.03
Potassium dihydrogen phosphate (KH_2PO_4)	0.01
Dipotassium hydrogen phosphate (K_2HPO_4)	0.02
Sodium hydrogen carbonate ($NaHCO_3$)	0.20

To avoid bacterial growth in the substrate storage bottles, the P species were stored separately from the N and carbon species. The specific substrate recipes for the C + N solution and P solution were prepared in 20-fold and 100-fold concentration, respectively, in 1L storage bottles with Milli Q water. Selected PhACs were added to the C + N solution, also in 20-fold concentration, providing final concentrations of 10 μg L^{-1} in the influent wastewater. After the growth of microorganisms was observed in the C + N bottle at the beginning of the experiment, the C + N solution was filtered and the bottle and connecting pipe were autoclaved to further provide a sterile environment. In the P solution, 200 mL of the Milli Q water was substituted by 100 mL, i.e. 1 mL L^{-1} in 100-fold concentration, of the micronutrient solution seen in Table 5. The recipe for the micronutrient solution is based on a recipe by Tay, Liu, and Liu (2001a).

Table 5. Recipe for the micronutrient solution.

Component	Concentration [g L^{-1}]
Boric acid (H_3BO_3)	0.05
Zinc chloride ($ZnCl_2$)	0.05
Copper chloride ($CuCl_2$)	0.03
Manganese sulfate monohydrate ($MnSO_4.H_2O$)	0.05
Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	0.05
Aluminum chloride ($AlCl_3$)	0.05
Cobaltous chloride hexahydrate ($CoCl_2.6H_2O$)	0.05
Nickel chloride ($NiCl_2$)	0.05

3.3 Sampling

To evaluate the performance of the lab-scale SBR, both liquid and biomass samples were collected along the different phases during the operation of the lab-scale SBR. Liquid samples were collected from the influent and effluent in order to determine the removal efficiency and during the end of the anaerobic fill phase in order to evaluate the biological P removal. However, since no mixing was provided during the anaerobic fill phase, the samples intended to represent the anaerobic conditions in the results were collected 1-2 minutes after the start of the aerobic reaction phase to provide mixing before sampling. This was done based on the assumption that any aerobic biodegradation process possibly taking place during this time period would be negligible. Biomass samples were collected during the aerobic reaction phase and from the effluent, in order to evaluate the sludge characteristics, growth, and evolution over time. From the reactor, liquid samples were collected from the middle port while an equal share of biomass were collected from the middle and the bottom port and then mixed.

The variety of samples collected during the experimental run can be seen in Table 6. For all parameters included in the sampling routine, duplicate samples were collected twice a week, except for sludge morphology which was analyzed once a week with 30 replicates. The influent samples were also collected on a regular basis to assess the incoming wastewater conditions.

Table 6. Sampling routine.

Parameter	Sampling phase	Frequency	Number of replicates
TSS & VSS (reactor)	End of aerobic reaction phase	Twice a week	2
TSS & VSS (effluent)	Decant phase	Twice a week	2
$SVI_{10,30}$	Settling phase	Twice a week	-
Sludge morphology	Aerobic reaction phase	Once a week	30
TOC & TN (effluent)	Decant phase	Twice a week	2
TOC & TN (influent)	Influent	Every second week/twice a week	2
TOC & TN (anaerobic conditions)	1-2 minutes after start of aerobic reaction phase	Twice a week	2
NH_4^+-N, NO_2^--N, NO_3^--N & PO_4^{3-}-P (effluent)	Decant phase	Twice a week	2
NH_4^+-N, NO_2^--N, NO_3^--N & PO_4^{3-}-P (influent)	Influent	Every second week/twice a week	2
NH_4^+-N, NO_2^--N, NO_3^--N & PO_4^{3-}-P (anaerobic conditions)	1-2 minutes after start of aerobic reaction phase	Twice a week	2

3.4 Sample analysis

3.4.1 Liquid sample analysis

The liquid samples were analyzed in order to determine the concentration of soluble contaminants in the influent and effluent as well as at the end of the anaerobic feeding. This section describes the analytical methods used to determine the concentrations of NH_4^+-N, NO_3^--N, and NO_2^--N as well as TN, TP, and TOC.

According to the method specified by the International Organization for Standardization (ISO, 1992), ion chromatography (IC) was used to determine the concentrations of NH_4^+, NO_3^-, NO_2^- and PO_4^{3-} in order to calculate the concentrations of NH_4^+-N, NO_3^--N, NO_2^--N, TN, and TP. The samples were filtered through a syringe filter with 0.2 µm pore size and diluted until the conductivity was <200 µS cm^{-1} before it was placed in the ion chromatograph. Calibration of the instrument response and calculations of concentrations can be seen in Appendix, Section 1.1.

The concentration of TN was also determined together with the concentration of TOC measured in the TOC analyzer.

The theoretical concentration at the end of the anaerobic fill phase, which is a mix of the influent and the recycled water, provided that no biological processes occurred, was calculated using equation (7).

$$C_{end.an.fill.ph.} = 0.5\left(c_{inf} + C_{ave.eff}\right) \text{ (mg L}^{-1}\text{)} \quad (7)$$

Where $c_{end.an.fill.ph.}$ is the theoretical concentration at the end of the anaerobic fill phase, provided that no biological processes occurred (mg L^{-1}), 0.5 is the exchange ratio of 50 %, c_{inf} is the influent concentration (mg L^{-1}) and $c_{ave.eff}$ is the average effluent concentration (mg L^{-1}).

3.4.2 Removal efficiency

In order to evaluate if the removal criteria of TN, TP, and TOC was fulfilled, the removal efficiency for every contaminant was calculated using equation (8).

$$Removal\ efficiency = (1 - \frac{c_{eff}}{c_{inf}}) \times 100 \text{ (\%)} \quad (8)$$

Where c_{eff} is the effluent concentration (mg L^{-1}) and c_{inf} is the average influent concentration (mg L^{-1}).

Further, the average removal efficiency criteria for TOC, TN and TP was compared to the regulated limits stated to be 75, 70 and 80 %, respectively, in Section 2.1.

3.4.3 Biomass sample analysis

Biomass samples were analyzed in order to evaluate sludge characteristics, growth, and morphology during the operation of the lab-scale SBR. TSS and VSS were calculated according to standard method 2540 D and 2540 E, respectively (American Public Health Association [APHA], 1998). A more detailed explanation of the method can be seen in Appendix, Section 1.2.

According to standard method 2710 D (APHA, 1998), SVI is measured by filling a 1 L graduated cylinder with the sludge sample and reading the volume occupied by sludge from the graduated cylinder after 30 minutes of settling. SVI is then calculated using equation (9).

$$SVI_{30} = \frac{SV_{30}}{TSS} \text{ (mL g}^{-1}\text{)} \quad (9)$$

Where SVI_{30} is the SVI after 30 minutes (mL g^{-1}), SV_{30} is the volume occupied by sludge after 30 minutes (mL L^{-1}) and TSS is the concentration of TSS in the reactor (gTSS L^{-1}).

During this work, the reactor itself was used as a graduated cylinder and the volume occupied by sludge after 10 and 30 minutes was measured during the settling phase and later calculated using equation (10). When the time of the settling phase was decreased to <30 minutes, SVI_{30} was substituted with SVI_{25}.

$$SVI_x = \frac{\frac{SV_x}{V_R}}{TSS} \text{ (mL g}^{-1}\text{)} \quad (10)$$

Where SVI_x is the SVI after x minutes (mL g^{-1}), SV_x is the volume occupied by sludge after x minutes (mL L^{-1}), V_R is the reactor volume (L) and TSS is the concentration of TSS in the reactor (gTSS L^{-1}).

To evaluate the sludge age, SRT was calculated using equation (11) (Layer et al., 2019).

$$SRT = \frac{V_R \times TSS_R}{Q_{ex} \times TSS_{ex} + Q_{eff} \times TSS_{eff}} \text{ (days)} \quad (11)$$

Where V_R is the reactor volume (L), TSS_R is the TSS concentration in the reactor (gTSS L^{-1}), TSS_{ex} is the TSS concentration of the excess sludge (gTSS L^{-1}), TSS_{eff} is the concentration of TSS in the effluent (gTSS L^{-1}), Q_{ex} is the excess sludge flow (g day^{-1}) and Q_{eff} is the effluent flow (g day^{-1}).

Depending on the frequency of SRT calculations, the summarized volume of excess sludge was calculated using equation (12).

$$Q_{ex} = \frac{V_{ex}}{x} \text{ (L day}^{-1}\text{)} \quad (12)$$

Where V_{ex} is the summarized volume of all samples taken out from the reactor during the time interval x (L) and x is the time interval between the last and the current calculation of SRT (days).

To assess the sludge morphology and microbial community, the biomass was observed with light microscopy at 2x and 20x magnification, respectively. Two drops of biomass were pipetted onto three slides each and a cover glass was placed on top of each slide before it was placed under the light microscope.

4. Results

4.1 pH and dissolved oxygen

The results for the DO level and pH value during a randomly selected cycle is presented in Figure 11. The pH value decreased during the first 60 minutes of the anaerobic fill phase, to finally stabilize during the last 30 minutes. During the aerobic phase, the pH level increased and was kept stable by the system controlling pH until the start of the settling phase. During the settling, decant and idle phases, the pH gradually decreased. The average pH value during the anaerobic fill phase, the aerobic reaction phase, and the settling, decant, and idle phases combined were 6.72 ± 0.03, 7.20 ± 0.07, and 7.06 ± 0.07, respectively, during this specific cycle. The average pH value during the whole cycle was 7.08 ± 0.19.

For DO, the average level was 0 ± 0.04 % during the settling, decant and idle phases. For the aerobic phase, the average DO level was 20.38 ± 4.50 %. The average DO level during the anaerobic fill phase was 0.28 ± 0.09 %.

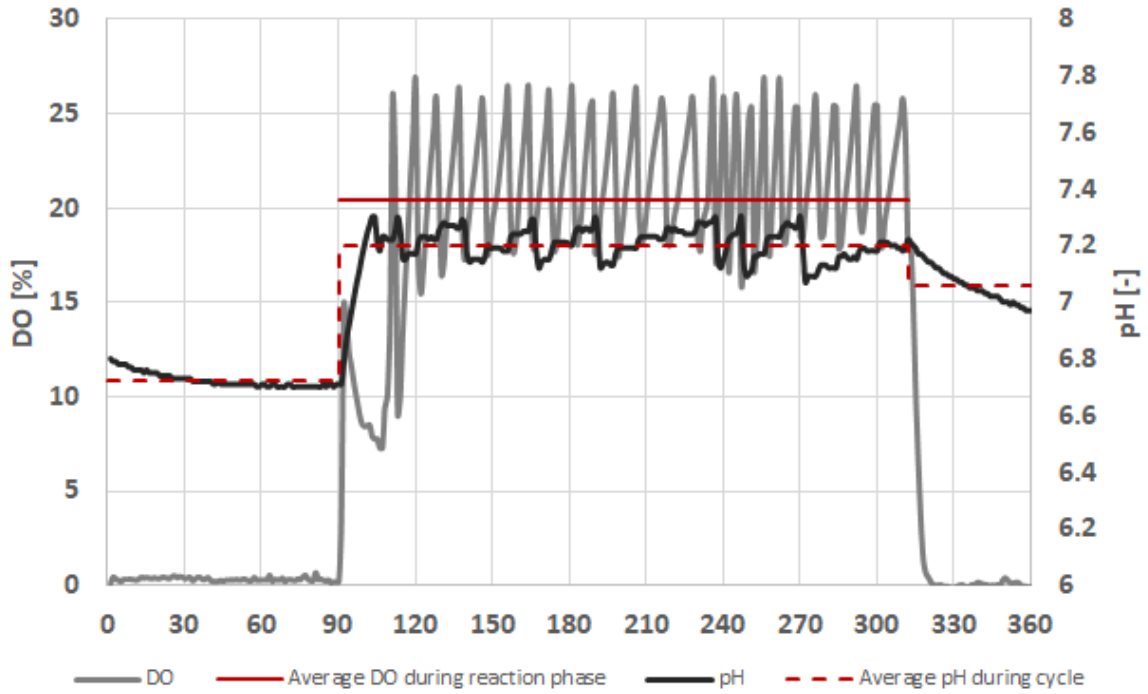

Figure 11. DO level and pH value in the different phases during a randomly selected cycle.

4.2 Liquid samples

4.2.1 Influent

The influent concentration of TN over time during the experimental run is presented in Figure 12. The average influent concentration of TN measured with the TOC analyzer and the ion chromatograph was 6 and 53 % lower than the influent target of 42 mgNH_4^+-N L^{-1}, respectively. The average influent concentration of TN was 39.50 ± 9.68 and 19.70 ± 2.86 mgTN L^{-1} for the TOC-analyzer and IC, respectively. The results show that the influent concentration of TN was fluctuating throughout the experimental run and that the influent concentrations of TN measured with IC shows a similar, but almost twice as low, pattern as the TOC analyzer measured concentrations of TN. Since the concentrations of TN measured with the TOC analyzer were higher and more close to the target concentration than the concentrations measured with IC, it was assumed that the TOC measured value is more accurate.

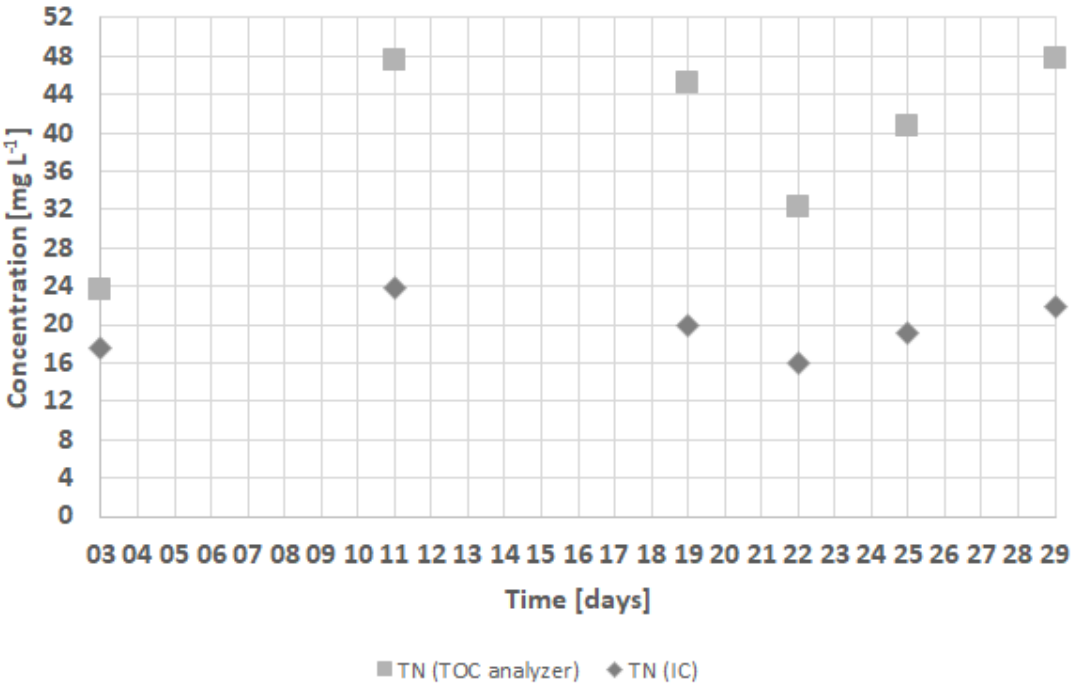

Figure 12. Influent concentration of TN over time.

The influent concentration of TOC throughout the experimental run is presented in Figure 13. The results show that the influent concentration of TOC was fluctuating throughout the experimental run with an average influent concentration of 250.25 ± 101.14 mgTOC L^{-1}.

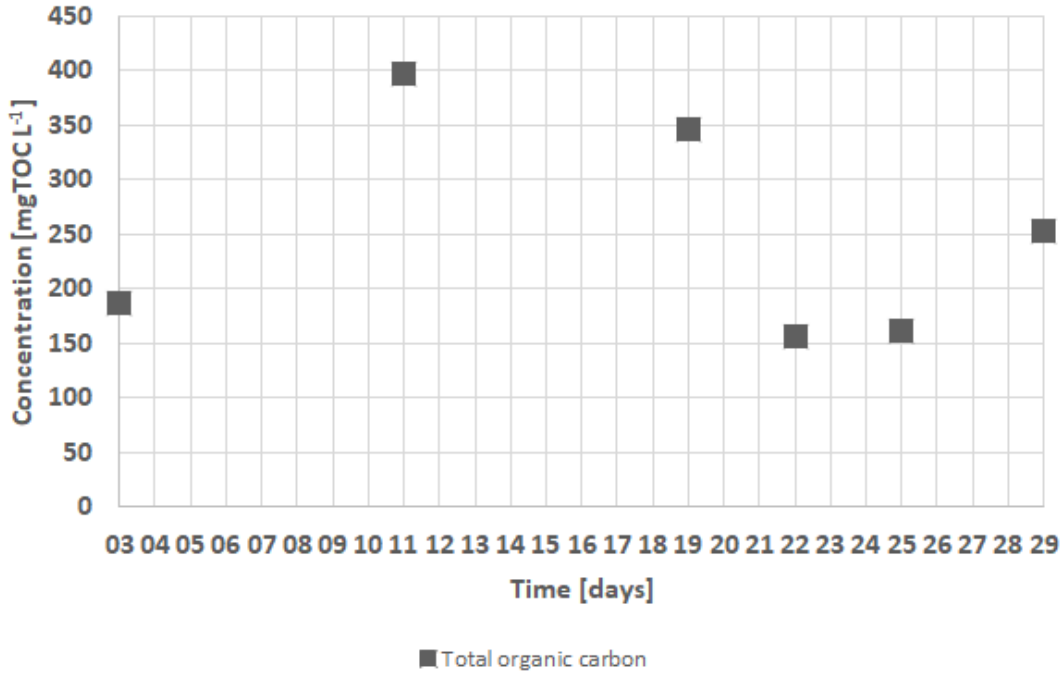

Figure 13. Influent concentration of TOC over time.

The influent concentration of TP over time during the experimental run is presented in Figure 14. The average influent concentration of TP was 41.7 % higher than the target of 6 $mgPO_4^{3-}$-P L^{-1} with an average influent concentration of 8.50 ± 0.98 $mgPO_4^{3-}$-P L^{-1}. The results show that TP remained quite stable in the influent. However, on day 29, no TP was detected in the influent.

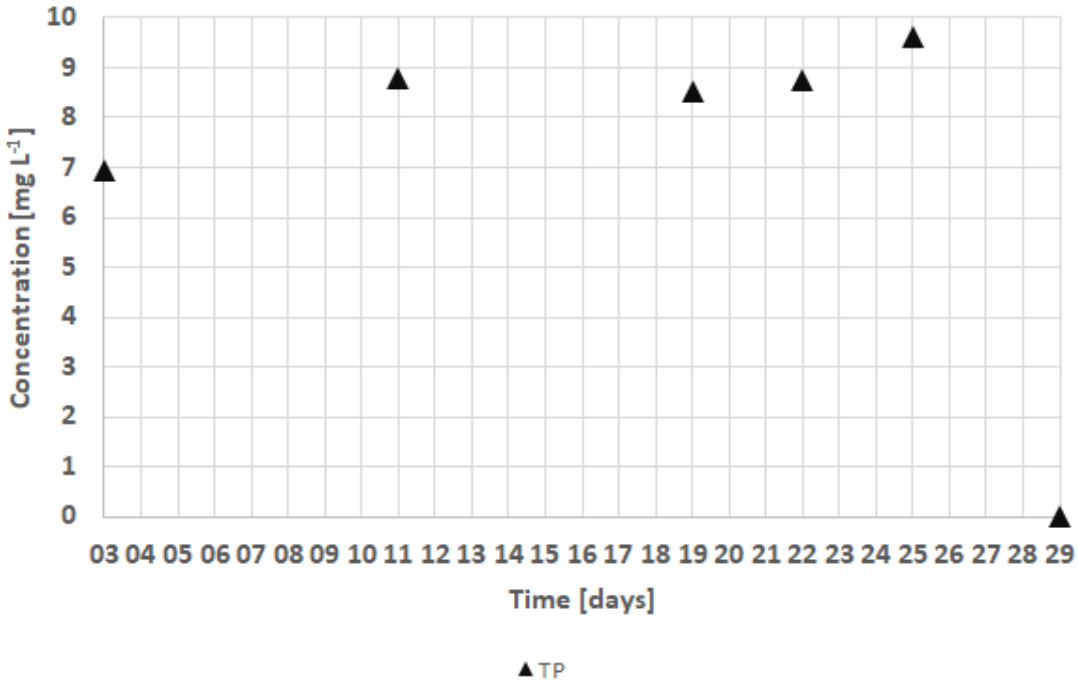

Figure 14. Influent concentration of TP over time.

4.2.2 Anaerobic fill phase

The concentrations of NO_3^--N, NH_4^+-N and TN together with the theoretical concentration at the end of the anaerobic fill phase over time are presented in Figure 15. NO_2^--N was never detected at the end of the anaerobic fill phase. NO_3^--N was only detected on day 15 when the concentration was 1.41 mgNO_3^--N L^{-1}. Further, the results show that the concentration of TN was fluctuating throughout the experimental run. At the end of the anaerobic fill phase, the measured concentration of TN was lower than the theoretical concentration for most sampling occasions.

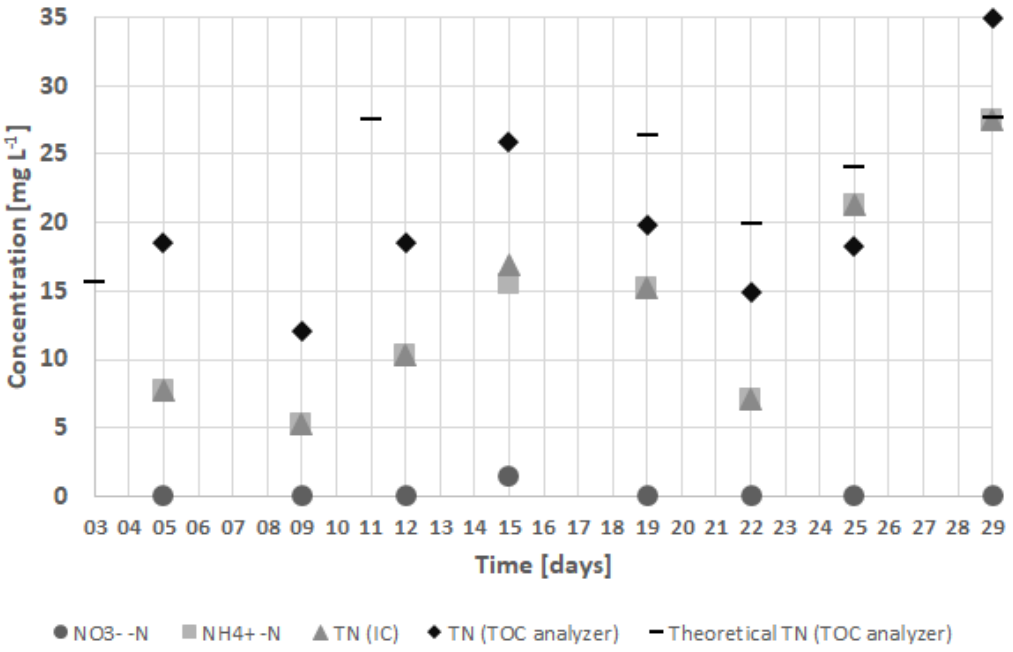

Figure 15. Concentrations of NO_3^--N, NH_4^+-N, and TN over time during the end of the anaerobic fill phase in relation to the theoretical concentration.

The concentration of TOC together with the theoretical concentration at the end of the anaerobic fill phase over time is presented in Figure 16. At the end of the anaerobic fill phase, the measured concentration of TOC was lower than the theoretical concentration for most sampling occasions.

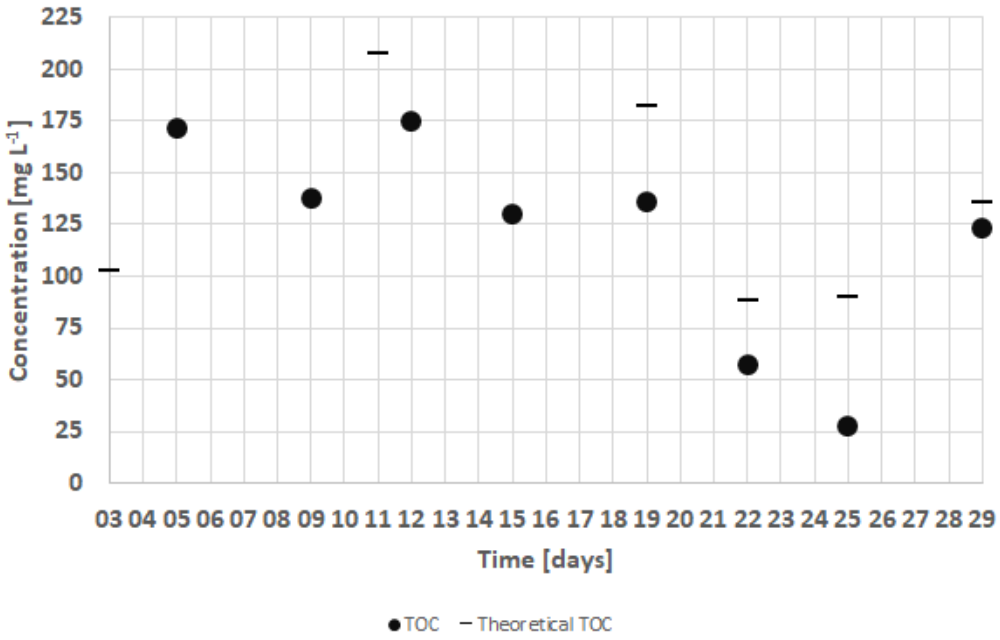

Figure 16. Concentrations of TOC over time during the end of the anaerobic fill phase in relation to the theoretical concentration.

The concentration of TP together with the theoretical concentration at the end of the anaerobic fill phase over time is presented in Figure 17. At the end of the anaerobic fill phase, the measured concentration of TP was equal to or lower than the theoretical concentration.

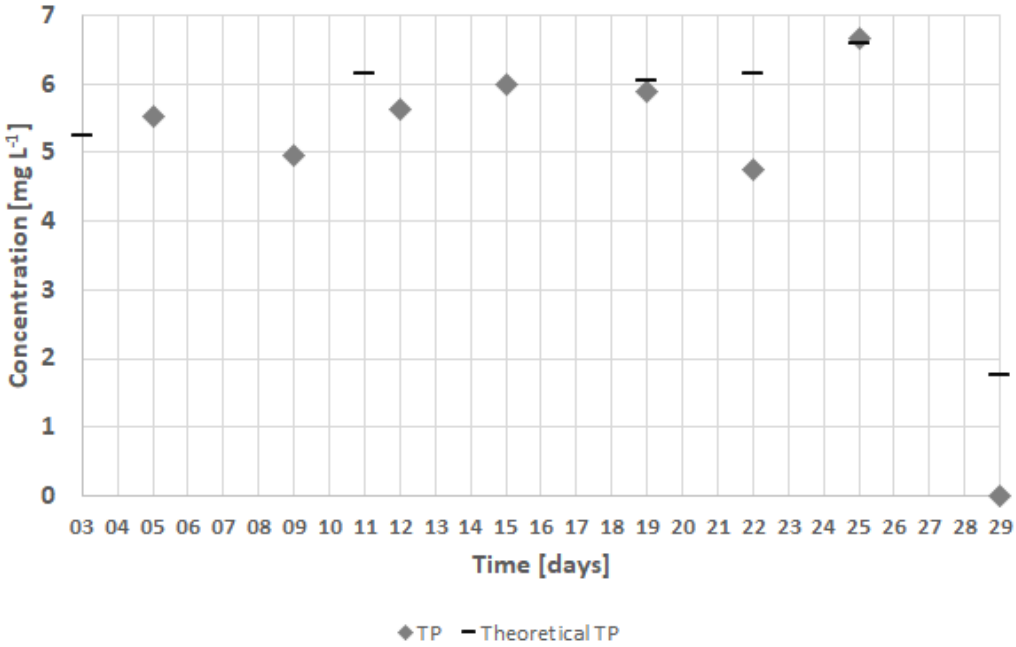

Figure 17. Concentration of TP over time during the end of the anaerobic fill phase in relation to the theoretical concentration.

4.2.3 Effluent

Effluent concentrations of NO_3^--N, NH_4^+-N and TN over time together with the average influent TN during the experimental run is presented in Figure 18. NO_2^--N was never detected in the effluent. In the effluent, 81 % of TN was reduced on average, with significantly lower reduction of 19.20 and 39.70 % on day 25 and 29, respectively.

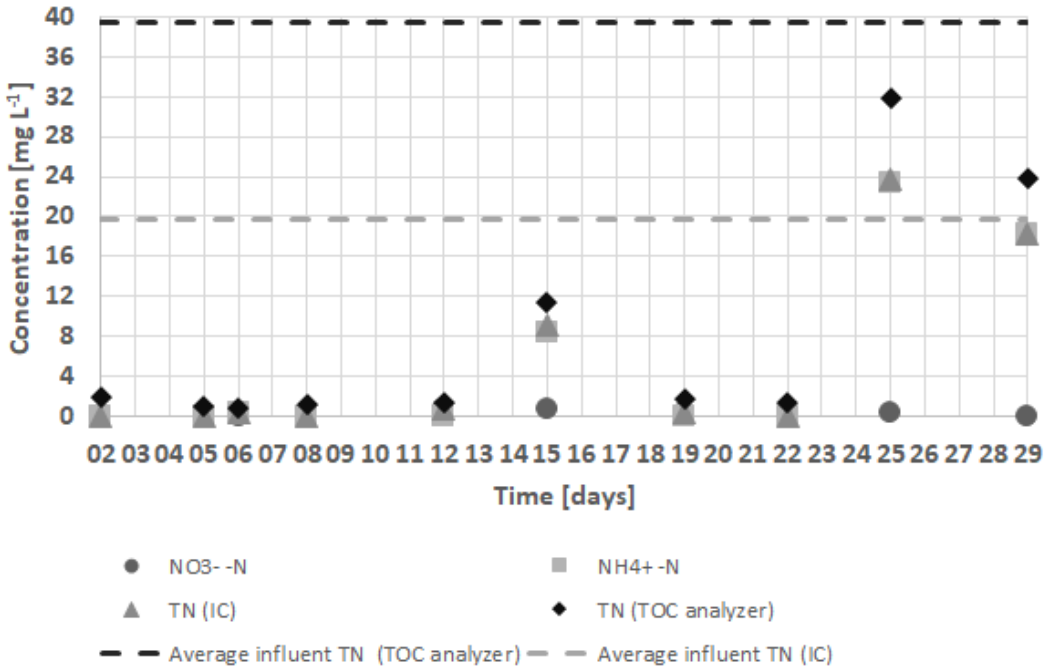

Figure 18. Effluent concentrations of NO_3^--N, NH_4^+-N, and TN over time, in relation to the average influent concentration.

Effluent concentrations of TOC over time together with the average influent TOC during the experimental run is presented in Figure 19. In the effluent, 92 % of TOC was reduced on average.

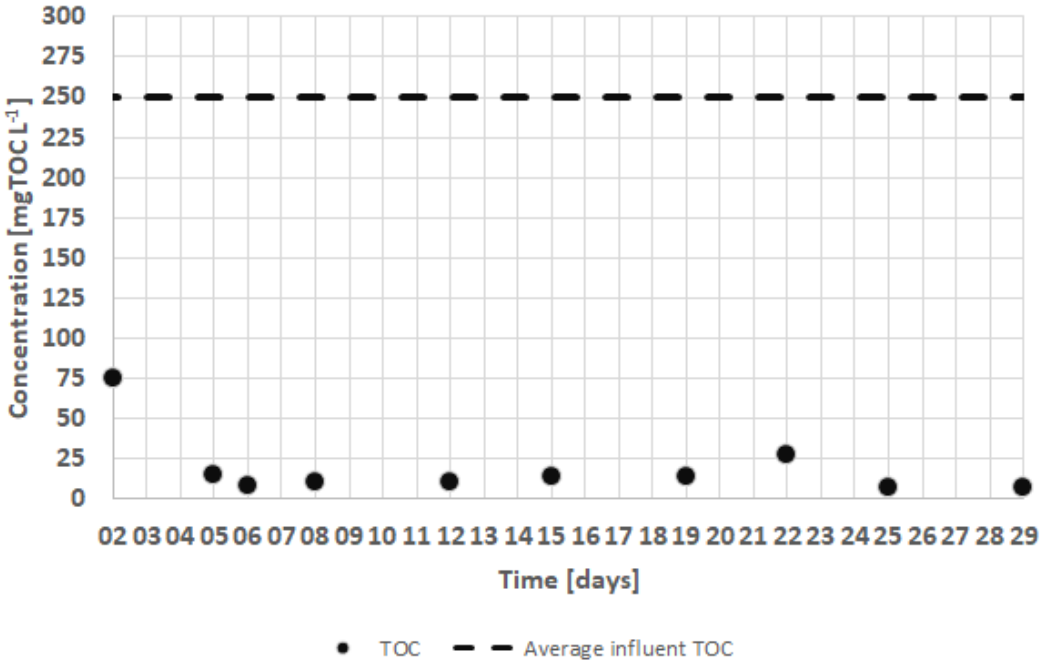

Figure 19. Effluent concentration of TOC over time, in relation to the average influent concentration.

Effluent concentrations of TP over time together with the average influent TP during the experimental run is presented in Figure 20. In the effluent, 86.00 % of TP was reduced on average, with significantly lower reduction of 52.00 % on day 8.

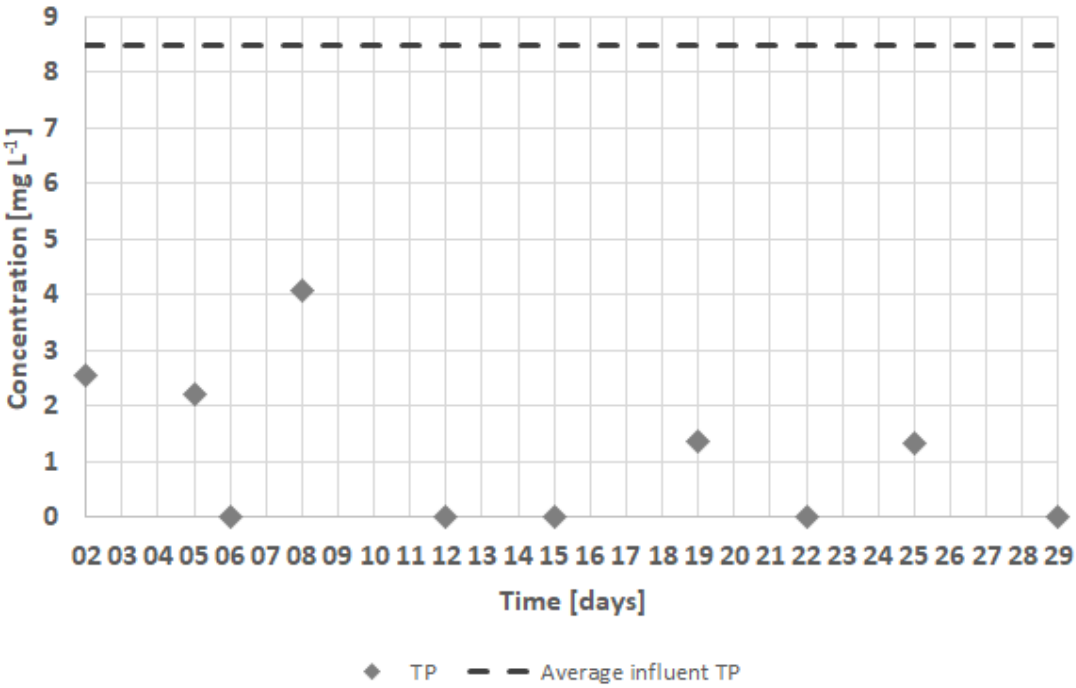

Figure 20. Effluent concentration of TP over time, in relation to the average influent concentration.

4.2.4 Removal efficiency

The removal efficiency for TOC, TP, and TN throughout the experimental run are presented in Table 7. The average removal efficiency over the experimental run was 92 % for TOC, 86 % for TP and 81 % for TN measured with the TOC analyzer. Hence, the removal efficiency criteria were fulfilled for all contaminants.

Table 7. Removal efficiency for TOC, TP, and TN over time, calculated with the average measured influent concentrations and day-specific effluent concentration as well as the determined removal efficiency criteria.

Day	TOC [%]	TP [%]	TN (TOC analyzer) [%]
2	67	70	95
5	94	74	97
6	97	100	98
8	96	52	97
12	96	100	97
15	94	100	71
19	94	84	96
22	89	100	96
25	97	84	19
29	97	100	40
Average	**92**	**86**	**81**
Criteria	**75**	**80**	**70**

4.3 Biomass samples

4.3.1 Total suspended solids and volatile suspended solids

The concentrations of TSS and VSS in the reactor and effluent are presented in Figure 21. The average concentrations were 3.85 gTSS L^{-1} and 3.59 gVSS L^{-1} in the reactor and 0.18 gTSS L^{-} and 0.19 gVSS L^{-1} in the effluent, respectively. The concentration of TSS in the reactor initially decreased to finally stabilize around 3.50 gTSS L^{-1}. The fraction of VSS in the reactor was increased over time during the experimental run.

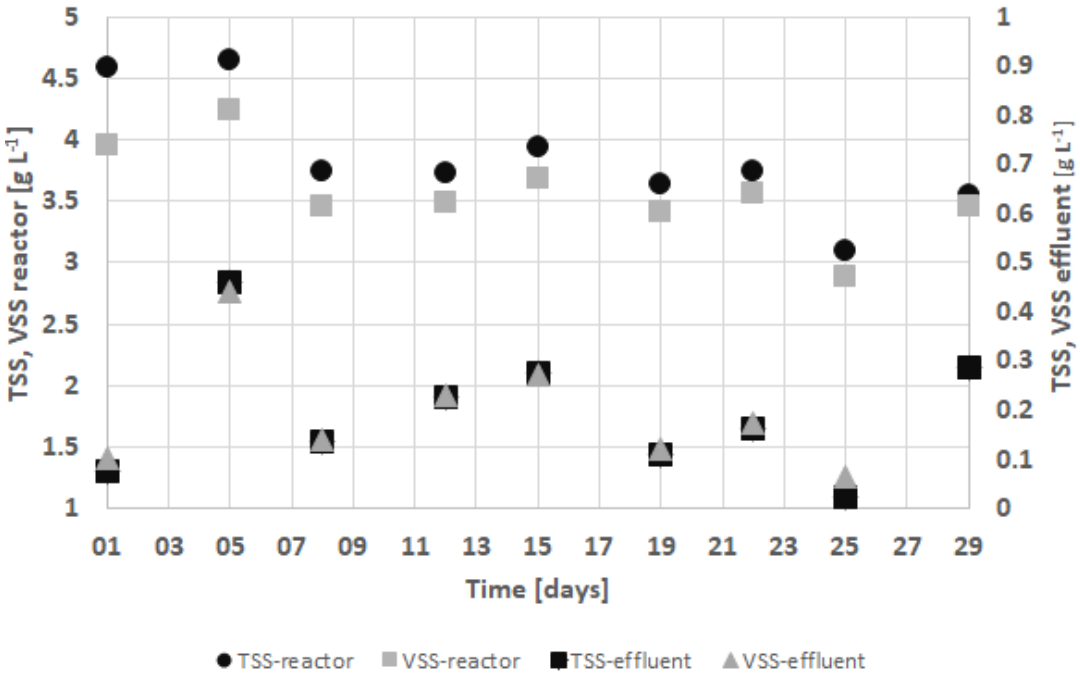

Figure 21. Concentrations of TSS and VSS in the reactor and effluent over time.

4.3.2 Solids retention time

SRT throughout the experimental run, in relation to the time of the settling phase, is presented in Figure 22. For every time the settling phase time was decreased, a decrease of SRT followed with an increase of SRT within the settling phase can be observed. When the settling phase was 45, 35, and 25 minutes, the average SRT was 11.0, 8.8 and 19.6 days, respectively. The average SRT during the experimental run was 11.6 days.

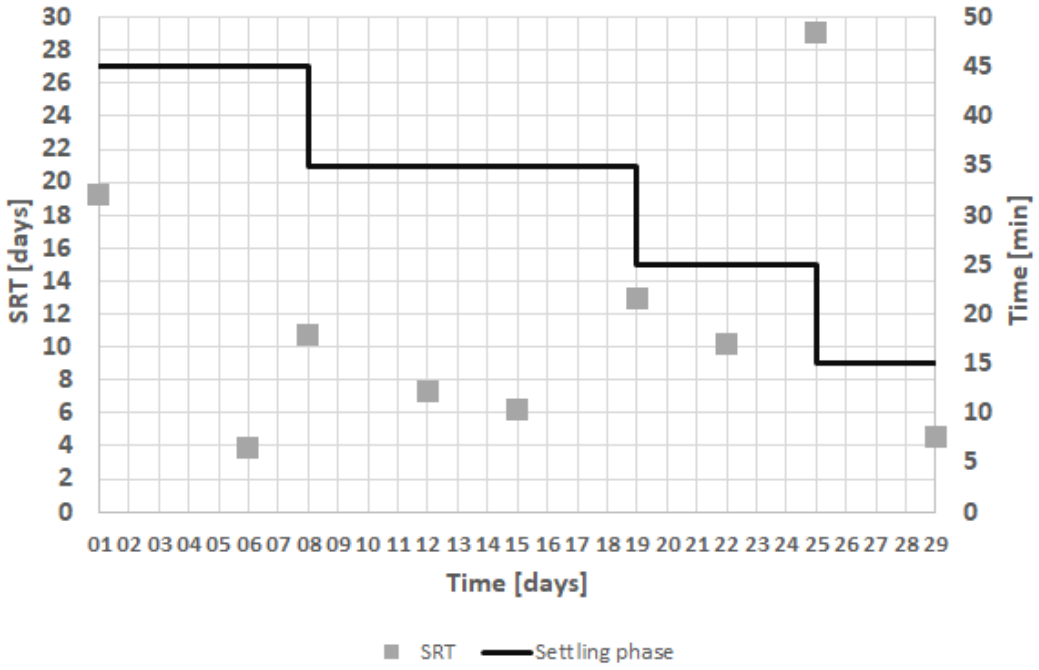

Figure 22. SRT over time, in relation to the time of the settling phase.

4.3.3 Sludge volume index

SVI throughout the experimental run is presented in Figure 23. SVI_{25} and SVI_{30} were <165 mL g^{-1} on all days with the average value of SVI_{25} and SVI_{30} combined being 137.6 ± 21.8 mL g^{-1}. The difference between SVI_{10} and SVI_{30} gradually increased over time.

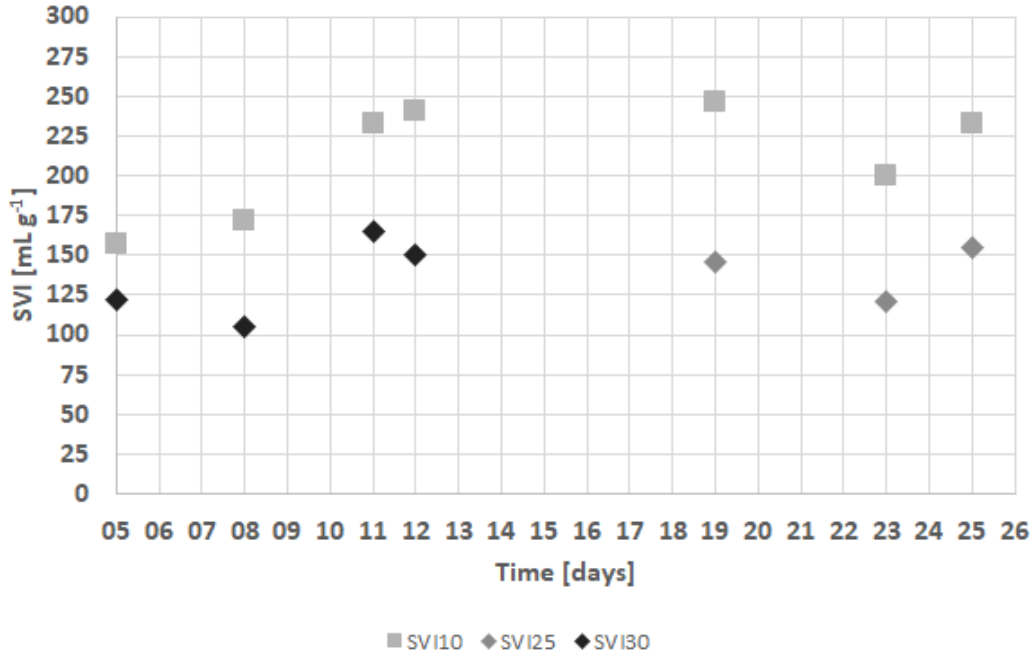

Figure 23. SVI_{10}, SVI_{25} and SVI_{30} over time.

4.3.4 Sludge morphology

Figure 24 shows the evolution of the sludge morphology from day 5 to day 30. Each picture for a specific day was chosen from a selection of 30 pictures from the same sampling occasion and is assumed to be the most representative image of the state at that time. On day 5, the structure was loose and irregular and further, on day 12, 19 and 25, the sludge gradually became more structured as more compact flocs were formed. More representative pictures of the morphology can be seen in Appendix, Section 2.1.

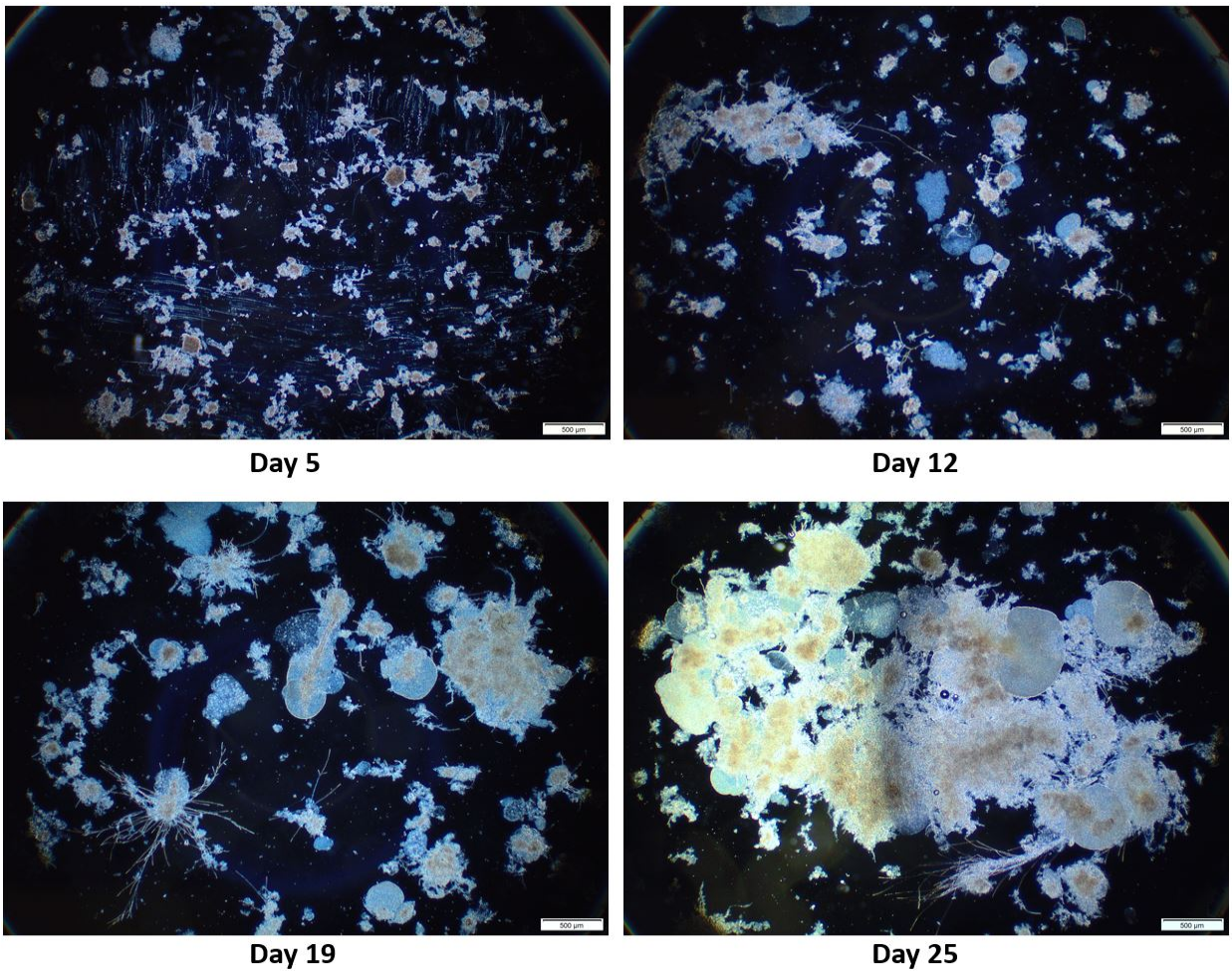

Figure 24. Evolution of the sludge morphology (2× magnification) from day 5 to day 25. The scale bar represents the length of 500 µm.

5. Discussion

5.1 pH and dissolved oxygen

As mentioned in Section 3.1, the target for the DO level during the aerobic reaction phase was 20 % and as close as possible to 0 % during the other phases. It is evident from Figure 11 that the aimed DO level was fulfilled since the average DO level during the aerobic reaction phase was 20.38 ± 4.50 %, 0.28 ± 0.09 % during the anaerobic fill phase, and 0 ± 0.04 % during the other phases. The slightly increased DO level during the anaerobic fill phase might be explained by air bubbles, observed during several occasions in the influent pipe, entering the reactor. However, after 11 days of operation, the DO sensor was detached from the reactor in order to be cleaned and re-calibrated. During the calibration check, it showed that the DO sensor underestimated the DO level by almost 50 %, which means that the DO level during aeration might have been higher than desired. According to the DO sensor suppliers, the reason for this is that the sensor has a drift up to 50 % when exposed to a DO level < 20 %. Hence, the result might not reflect the real DO level at all times during the experimental run.

As mentioned in Section 3.1, the aim was to reach a pH value of 7 ± 0.3 during the whole cycle. Figure 11 shows that the average pH value was within the range during the anaerobic fill phase, aerobic reaction phase and settling, decant and idle phases combined. During the aerobic reaction phase, when the pH was controlled with the addition of acid or base, the average pH value was 7.2. Further, during the other phases, when pH was not controlled, the pH value decreased over time but never exceeded the lower limit of the target value. As opposed to the DO sensor, the pH sensor remained stable throughout the experimental run, hence, it can be assumed that the result reflects the real pH value at all times.

5.2 Liquid samples analysis

5.2.1 Influent

Since the average influent concentration of TN measured with the TOC analyzer is much closer to the target influent concentration than the concentration of TN measured with IC, the results indicate that the TOC analyzer generated a more accurate result for TN than the ion chromatograph. The reason why the ion chromatograph detected a significantly lower value of TN might be due to the levels of NO_2^- and NO_3^- sometimes being lower than the detection limit of the ion chromatograph. This could be due to the samples being overly diluted before testing. Figure 12 clearly shows an uneven distribution of influent TN during the experimental run which is most likely due to the unstable performance of the influent water pump. Since the influent water pump did not generate a constant flow rate, it was tested and re-calibrated throughout the experimental run. The influent water pump was fluctuating since the total pump head required for filling the reactor gradually increased as the water level in the water tank decreased. The water tank was elevated and placed above the pump to decrease the total pump head required. This measure managed to marginally stabilize the distribution of water into the reactor. The filled reactor volume

was however still fluctuating over time and, for future work, it is recommended to install a float valve in the water tank to keep the water level constant during filling. As is the case with TN, Figure 13 clearly shows that the influent TOC was unevenly distributed over the experimental run due to the unstable performance of the influent water pump. Since microbial growth was observed in the C + N bottle and in the pipes delivering the feed, the lower TOC and TN concentration could also be due to biodegradation.

The low standard deviation, in comparison to TOC and TN, for the average influent concentration of TP indicate that the higher concentration factor used for the preparation of the P + micronutrient solution, lower flow and smaller pipe diameter used for distribution generated a higher resistance to changes of the water pump flow rate compared to the C + N setup. With this in mind, the rpm value for the pump distributing the P + micronutrient solution could have been lowered and adjusted for an influent concentration closer to the target value.

Since the duplicate influent samples from day 29 were placed last in the ion chromatograph, they were the last analyzed samples during a big run of many samples. Hence, the reason for PO_4^{3-}-P not being detected at day 29 might be due to the anion eluent being totally consumed during the separation of ions in the earlier samples, meaning that there was not enough anion eluent left for the last samples. However, since PO_4^{3-}-P also was not detected at the end of the anaerobic fill phase at day 29, which can be seen in Figure 17, the more likely explanation would rather be that the P + micronutrient pump was mistakenly off during this day and, therefore, no PO_4^{3-}-P was present in the reactor nor the influent.

5.2.2 Anaerobic fill phase analysis

Figure 15 shows that the concentration of TN, measured with the TOC analyzer, at the end of the anaerobic fill phase was below the theoretical concentration for most sampling occasions. This means that NH_4^+, as the only contributor to TN in the influent, was consumed. Since almost no oxygen was present, the nitrification processes could not solely have been the reason for why NH_4^+ was consumed. Instead, the most reasonable explanation is that NH_4^+ was consumed during the anammox process. As mentioned in Section 2.4.2.2, during the anammox process, NH_4^+ is oxidized and then reduced to N_2 during strictly anaerobic conditions using NO_2^- as the electron acceptor, see equation (4). Since NO_2^--N was never detected in the effluent, no NO_2^- is likely to have remained in the reactor for the next anaerobic fill phase. Instead, the presence of some oxygen, caused by air bubbles entering the reactor during the anaerobic fill phase, may have enabled nitration, i.e. the first part of the nitrification process where NH_4^+ is oxidized to NO_2^- using oxygen as the electron acceptor, see equation (1), to occur. If so, NH_4^+ was converted to NO_2^- during the anaerobic fill phase and later converted into N_2 by the anammox process. Since the nitration process releases H^+, the gradually decreased pH value observed during the anaerobic fill phase, see Figure 11, could be explained by the nitration process. However, the main reason for why the results show a low TN concentration in this phase is assumed to be due to insufficient mixing before sampling. The samples representing the end of the anaerobic fill phase were collected from the middle of the reactor 1-2 minutes after the start of the aerobic reaction phase to provide mixing of the influent plug flow and recycled treated wastewater. If sufficient mixing was

not achieved during this short period, the concentration of TN would be lower in the middle of the reactor than at the bottom. Hence, there might not even have been a consumption of TN during the anaerobic fill phase. This should also be considered when analyzing the biological removal of TOC and TP.

Also, up to 1.7 mg NH_4^+-N $g^{-1}VSS^{-1}$ can be sorbed on to the biomass (Bassin, Pronk, Kraan, Kleerebezem & van Loosdrecht, 2011). Thus, based on the average concentration of VSS in the reactor, up to 6 mgNH_4^+ L^{-1} could have been sorbed onto the biomass, see Appendix, Section 1.3, for calculations. This should also be considered when analyzing the effluent concentration of TN.

As mentioned in Section 2.4.2.3, the concentration of TOC was expected to decrease during the anaerobic fill phase due to the uptake of VFAs by PAOs and GAOs. The concentration of PO_4^{3-} was, on the other hand, expected to increase due to the breakdown of stored poly-p, which provides the energy necessary for the PAOs to take up VFAs, enabling PO_4^{3-} to be released. The results in Figure 16 shows that TOC in general was decreased in relation to the theoretical concentration at the end of the anaerobic fill phase. Therefore, since a high removal efficiency was achieved for TP, the uptake of VFAs by PAOs likely occurred. However, the results in Figure 17 shows that the concentration of PO_4^{3-}-P at the end of the anaerobic fill phase was not increased as expected in relation to the theoretical concentration. The main reason for why the results are not showing an increased concentration of PO_4^{3-}-P is assumed to be due the insufficient mixing before sampling, i.e. the real concentration of PO_4^{3-}-P at the end of the anaerobic fill phase might have been higher and a release of PO_4^{3-} can be assumed to have occurred. Furthermore, another reason why the concentration of PO_4^{3-}-P was not increased as expected at the end of the anaerobic fill phase could be the formation of struvite crystals ($MgNH_4PO_4.6H_2O$). Struvite crystals can be formed if magnesium (Mg^{2+}) and NH_4^+ is present to react with the released PO_4^{3-}, which leads to PO_4^{3-} being precipitated as a part of struvite instead of staying released in the mixed liquor (Batista & Jeong, 2006). Since $MgSO_4$ was included in the complex synthetic wastewater, all the components for the formation of struvite crystals are present in the reactor. Thus, this is also a possible explanation for why the concentration of PO_4^{3-}-P was not increased as well as for why the concentration of NH_4^+-N was decreased at the end of the anaerobic fill phase. The influent concentration of $MgSO_4$ was calculated to, theoretically, be able to precipitate 4.18 mgPO_4^{3-}-P L^{-1} and 1.89 mgNH_4^+-N L^{-1}, which makes the concentration of $MgSO_4$ in the influent high enough for 50 % of the influent PO_4^{3-} and 4.8 % of the influent NH_4^+ to precipitate, as assumed, during the anaerobic fill phase, see calculations in Appendix, Section 1.4. However, if struvite crystals were formed, they would have been visible under the light microscopy. So, since no crystal formation was shown during the microscope investigation, the main reason for why the results are showing lower concentrations of PO_4^{3-}-P and NH_4^+-N at the end of this phase is concluded to be due to insufficient mixing before sampling.

5.2.3 Effluent

The results in Figure 18 shows that both nitrification and denitrification was achieved at almost all sampling occasions since the concentration of TN in the effluent was significantly decreased in comparison with the concentration of TN at the end of the anaerobic fill phase and little or no NO_2^--N and NO_3^--N was present. Since denitrification should not occur before aerobic granules with inner anoxic zones were formed, a higher effluent concentration of NO_3^--N was expected due to nitrification being the only process working during aeration before AGS is formed. However, since it is evident that the concentration of TN was reduced in the effluent, the flocculated sludge in the reactor may have formed already an inner anoxic part which enabled simultaneous nitrification-denitrification. If this was not the case, it is assumed that some denitrification took place during the settling phase, especially at the beginning of the experimental run when the settling phase was 45 minutes, since this is the only phase with possible anoxic conditions after the anaerobic fill phase. The effluent concentration of NH_4^+-N was significantly higher on day 25 and 29, which indicate that the nitrification process was not sufficient these days. The reason for this may either be due to nitrifiers being inhibited or that the time of the settling phase was decreased to 15 minutes which might have caused flush out slow growing bacteria, such as nitrifiers.

It should also be mentioned that, on day 25, the effluent concentration of TN is higher than the concentration of TN at the end of the anaerobic fill phase, which is not reasonable. This can be explained by the fact that the effluent samples occasionally were collected during the cycle previous to the cycle where the anaerobic fill phase samples were collected.

Furthermore, Figure 19 shows that a high removal of TOC was achieved during the experimental run, which further indicate that denitrification was enabled during the aerobic reaction phase.

Figure 20 shows that an overall high removal of TP was achieved during the experimental run, which indicate that the uptake of PO_4^{3-} by PAOs was enabled during the aerobic reaction phase. The effluent concentration of TP was higher in the beginning of the experimental run, which might be explained by lack of PAOs in the inoculated activated sludge.

5.2.4 Removal efficiency

It is clear from Table 7 that the average removal efficiencies are well above the removal efficiency criteria. For TN, the removal efficiency measured with the TOC analyzer is assumed to give the most accurate result since the ion chromatograph systematically measured lower concentrations of TN and, in many cases, did not detect some of the investigated ions in samples with low concentrations, such as the effluent samples. It can be concluded that the lab-scale SBR managed to achieve sufficient treatment of TOC, TP and TN even though selected PhACs were present in the complex synthetic wastewater from the start of the experimental run. Thus, it can also be concluded that the presence of the selected PhACs in the complex synthetic wastewater does not seem to inhibit the biological treatment processes at concentrations ≤ 10 µg L^{-1}.

5.3 Biomass samples analysis

5.3.1 Total suspended solids, volatile suspended solids and solids retention time

The concentration of TSS in the reactor was expected to, after an initial decrease, increase over time, which, as can be seen in Figure 21, was not the case since the concentration of TSS in the reactor initially decreased to finally stabilize around 3.5 gTSS L^{-1}. However, a small increase over time can be observed for all samples collected at days with the same time of settling, seen in Figure 22, even though the effluent concentration of TSS increased gradually during the same periods. Hence, growth of biomass in the reactor is validated, which indicates that an increase in the concentration of TSS in the reactor over time would occur when the time of the settling phase was set to a constant time. The fraction of VSS was increased during the experimental run, which was expected since no solids were present in the complex synthetic wastewater and some salt may have been precipitated. It is important to notice that, occasionally, the effluent concentration of VSS was higher than the effluent concentration of TSS, which is not possible since VSS is a fraction of TSS. This observation indicates inaccurate measurements, which was probably due to the fact that some of the filter weight was lost during drying and ignition. In order to prevent this, the filters should have been cleaned with reagent-grade water and ignited at 550 ^{o}C before the measurements.

The time of the settling phase was decreased whenever the effluent concentration of TSS was <0.15 gTSS L^{-1}. Furthermore, Figure 22 shows that SRT was below 20 days for almost the whole experimental run, which means that the selection of biomass with good settling characteristics was, to some extent, prioritized over the selection of slow growing bacteria.

5.3.2 Sludge volume index

It is observed that SVI_{25} and SVI_{30} were <165 mL g^{-1} for all measurements, which is within the range of 100-200 mL g^{-1}, which is classified as “light" sludge (Wanner, 1998). Hence, SVI_{25} and SVI_{30} throughout the experimental run, see Figure 23, was decreased in comparison to SVI_{30} of the activated sludge before inoculation which was 180.5 mL g^{-1}. The difference between SVI_{10} and SVI_{30} was, however, gradually increased over time in comparison to the activated sludge before inoculation for which the values of SVI_{10} and SVI_{30} was 206 and 180.5 mL g^{-1}, respectively. Hence, the characteristics of the biomass clearly indicate that aerobic granules are not fully formed since, as mentioned in Section 2.4, the settleability and minimum size of aerobic granules should be such that the difference between the SVI_{30} and SVI_{10} is very small. Also, since the biomass began to look more light and fluffy over time and the microscope examination frequently showed the presence of filamentous bacteria, one reason for the unimproved settling characteristics may be due to filamentous sludge bulking, which is often a reason for a high SVI (Hahn, Hoffmann & Odegaard, 1998).

However, it is worth mentioning that all the assessments of SVI, except for day 11, were carried out by dividing the reactor volume occupied by sludge after 5, 10, 25, and 30 minutes, respectively, by the aimed total reactor volume. Since the influent flow was fluctuating over time during the experimental run, the actual reactor volume was occasionally different than the aimed reactor

volume, meaning that the measured SVI may not reflect the real SVI at all times. The actual reactor volume was often higher than the aimed volume, which makes it likely that a slightly better overall settling behavior of the sludge is probable. Also, since the settling phase time was decreased to 25 minutes from day 19, the SVI_{30} could not be assessed. Therefore, it was substituted with SVI_{25} for the remaining days and, since the values for SVI_{25} were close to the values for SVI_{30}, it can be assumed that SVI_{30}, if measured, would have been slightly decreased from day 19 and forward.

5.4 Sludge morphology analysis

Figure 24 clearly shows how the activated sludge was gradually formed into more compact flocs over time, thus, it becomes evident that the formation of aerobic granules was initiated but, due to the lack of the characteristic spherical shapes, not fully achieved. Hence, the aim of successfully cultivating aerobic granules in the lab-scale SBR was not fulfilled. The main reason for this is probably due to time restraints since, due to the microscopy exam showed that the formation of AGS was initiated, it is likely to assume that aerobic granules would eventually be fully formed if the reactor was operated for a longer time period.

However, some operational parameters may also have been affecting the granulation process. As concluded in Section 5.3.1, the selection of biomass with good settling characteristics was, to some extent, prioritized over the selection of slow growing bacteria during the operation of the reactor. This might also have had a negative impact on the granulation process due to, as mentioned in Section 2.4.3, the selection of slow growing bacteria being a key factor for the formation of AGS since it significantly improves the stability of the aerobic granules. The presence of carbamazepine might also have been affecting the aerobic granule formation, since, as mentioned in Section 2.2.1.1, carbamazepine has been shown to have a negative impact on the granulation process. Also, the filamentous bacteria are assumed to have made it harder for the biomass to form into granules due to filamentous sludge bulking.

5.5 Potential for the removal of selected pharmaceutically active compounds

In order to evaluate the potential for removal of selected PhACs in the lab-scale SBR, it is necessary to analyze both the physicochemical characteristics of the incoming selected PhACs presented in Table 1 as well as the operational parameters of the reactor. One of the main influencing operational parameters is SRT since, as mentioned in Section 2.2.3.1, a longer SRT promotes the selection of a broader range of microorganisms and slow growing bacteria, which enables a higher removal of PhACs. Thus, during the experimental run, SRT was low in the context of stimulating the removal of PhACs. However, since SRT can be controlled by adjusting the exchange ratio, biomass withdrawal and the time of the settling phase, the enabling of a sufficient SRT for the removal of PhACs is possible by changing these operational parameters. Also, SRT would most certainly become longer as aerobic granules are formed and better settling characteristics are obtained. Since a longer SRT keep the biomass in the system for a longer period, the growth of biomass can be assumed to increase accordingly, which increases the amount of microorganisms in the reactor. Therefore, if assuming that the incoming organic matter is constant,

the F/M-ratio would decrease as SRT increase which, as also mentioned in Section 2.2.3.1, have been stated to be preferable for biodegradation of poorly degradable substances due to co-metabolism processes. It shall also be mentioned that an optimal removal of PhACs through adsorption requires fully formed aerobic granules in the lab-scale SBR since, as stated in Section 2.4.4, the adsorption capacity for AGS increases as aerobic granules are formed. This is due to the many functional groups, such as amino-, carboxyl- and phosphate groups, included on the surface of granular sludge, which are providing adsorption sites for PhACs.

As mentioned in Section 2.2.3.2, the pH value has a significant impact on the physical, chemical, and biological characteristics of ionizable PhACs since the charge of these compounds is different at different pH values. Since activated sludge is known to have a negatively charged surface at pH values between 6.7-8.5 (Figoli, Hoinkis & Bundschuh, 2016), the adsorption of positively charged compounds are favorable at this pH. Therefore, the adsorption potential of the selected PhACs needs to be analyzed with respect to the average pH value in the SBR during the experimental run. As can be seen in Table 1, citalopram, sertraline, metoprolol, atenolol and ciprofloxacin are positively charged at pH 7, hence, there is a potential for these PhACs to be removed by adsorption in the SBR since the average pH value was 7 ± 0.3 during the experimental run. For ciprofloxacin, log K_d is >2.7, see Table 1, which, as stated in Section 2.2.2, further indicate high adsorption potential. For diclofenac and sulfamethoxazole, which are negatively charged at pH 7, a lower pH would be favorable for removal by adsorption, which, as mentioned in Section 2.2.3.2, also has been observed in previous studies. Since carbamazepine is non-ionizable, the removal of this compound is, more or less, independent of pH since the constant neutral charge makes adsorption unlikely. Instead, biodegradation should be the main removal mechanism for carbamazepine. However, as mentioned in Section 2.2.3.4 carbamazepine is considered to be resistant to biological degradation, which is also supported by the low k_{biol}, seen in Table 1, which, as mentioned in Section 2.2.2, indicates poor degradability. For sulfamethoxazole, metoprolol, and atenolol, the values for k_{biol} are within the range of 0.1-10 L gSS^{-1} d^{-1}, see Table 1, which indicate quite good degradability. However, as mentioned in Section 2.2.3.4, sulfamethoxazole is considered to be resistant to biological degradation and is therefore assumed as unlikely to be degraded biologically in the SBR.

In summary, the removal potential of carbamazepine by the lab-scale SBR is assumed to be low due to its resistance to biological degradation and constant neutral charge. Furthermore, the removal potential for diclofenac is also assumed to be low due to its negative charge at pH 7, low log K_d, and k_{biol}. For sulfamethoxazole, the removal potential is also assumed to be low due to its low adsorption potential together with indications on poor biodegradability. Furthermore, due to positive charge at pH 7 and k_{biol} indicating quite good degradability, the removal of atenolol and metoprolol by both adsorption and biodegradation is assumed to be possible. For sertraline and citalopram, due to positive charge at pH 7, the removal by adsorption is assumed to be possible, but unlikely, due to log K_d being <2.7 and, as mentioned in Section 2.2.1.5, the removal of citalopram by sorption having been systematically declared as insignificant in previous research papers. For ciprofloxacin, the removal by adsorption is assumed to be likely due to positive charge at pH 7 and log K_d indicating a high adsorption potential.

For venlafaxine, little information on physicochemical characteristics was found during the literature review, thus, no assumption regarding its potential removal mechanisms can be drawn. The lack of information available for venlafaxine, as well as for some of the other selected PhACs, makes the theoretical evaluation of removal potential hard. There is clearly a lack of knowledge when it comes to removal pathways of specific PhACs. Also, available information is often contradictory, which further highlights that the behavior of PhACs during wastewater treatment depends on several parameters and is difficult to predict. Therefore, analysis of the PhACs in the effluent is necessary to accurately assess the removal potential of those compounds by the lab-scale SBR.

6. Conclusions

The cultivation of aerobic granules in the lab-scale SBR was initiated and a gradual increase of more compact flocs could be seen over time during the experimental run. However, since no granules were fully formed, the aim of successfully cultivating aerobic granules was not achieved. This is suggested to be a consequence of time restraints which led to a too short experimental run. Also, the presence of PhACs in the influent feed and filamentous sludge bulking might have hampered the granulation process.

The aim of reaching a sufficient removal of TOC, TN and TP was fulfilled since the removal efficiency criteria of 75, 70 and 80 % for TOC, TN, and TP, respectively, was reached with average removal efficiencies of 92 % for TOC, 81 % for TN and 86 % for TP. During the anaerobic fill phase, the results showed concentrations of TN and TP lower or equal than the theoretical concentrations, which is concluded to be due to insufficient mixing before sampling. There is also a possibility that the concentration of TN was decreased by nitration and the anammox process during this phase. A significantly decreased concentration of TN in the effluent suggests that nitrification and denitrification occurred. In the beginning of the experimental run, nitrification occurred during the aerobic reaction phase and denitrification was probably enabled during the settling phase. Later during the experimental run, simultaneous nitrification-denitrification was probably enabled during the aerobic reaction phase due to the formation of inner anoxic layers in the flocculated sludge. There is also a possibility that some NH_4^+-N was sorbed onto the sludge. TP was removed by EBPR. For future work, to more fully understand which biological processes that occurred during the different phases, it is recommended to perform a cycle study. It is also recommended to do an analytical investigation of VFAs to be able to calculate COD.

Furthermore, the theoretically evaluated removal potential for the selected PhACs showed varying results. Overall, the removal potential of all PhACs is assumed to increase as granules start to form due to increased adsorption as well as better settling characteristics leading to a longer SRT and increase of biomass growth, which in turn is concluded to lead to a lower F/M-ratio, which is preferable for biodegradation of poorly degradable substances. It is also concluded that an analytical investigation of the removal potential, carried out by analyzing the effluent concentration of PhACs and studying of adsorption mechanisms, would have been necessary in order to accurately assess the removal potential of PhACs by the lab-scale SBR, which is recommended for future work.

Since the performance of the influent water pump fluctuated over time, a float valve is recommended to be installed in the water tank in order to keep the water level constant and achieve a more stable influent flow in the future. It is also recommended to seal the pipe network as well as the water and substrate solution bottles from air leakage and to change the type of DO sensor since the one used in this work only had a drift up to 50 % when exposed to a DO level below 20 %. In order to provide sufficient mixing of the anaerobic fill phase samples, it is also recommended to collect future samples both from the middle and the bottom of the reactor.

It can be concluded that AGS cultivated in an SBR shows potential for the removal of PhACs from wastewater due to several unique characteristics of AGS and the easy adjustment of the operational parameters affecting the removal of PhACs enabled by the SBR. However, future research is needed to fully determine the potential of using AGS cultivated in an SBR for PhAC removal. Since this was the first time this type of lab-scale SBR was operated at Chalmers WET laboratory, this master thesis project may serve as a useful source of information for future research on advanced biological wastewater treatment for PhACs in general and on AGS cultivated in a lab-scale SBR at Chalmers in particular.

7. References

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8. Appendix

1. Calculations

1.1 Ion chromatography

The diluted samples were placed in the ion chromatograph together with a blank solution and standard solutions in concentrations of 0.5, 1 and 1.5 mM. The instrument response was calibrated by establishing a calibration curve and validated if the relationship between the instrument response and the known blank and standard solutions was linear. The linearity of the calibration curve was assessed by calculating the regression line. The ions in the samples was then identified by comparing the retention times with those of the standard solutions.

The IC results for the diluted concentration of PO_4^{3-} in mmol L^{-1} was converted to the undiluted concentration of TP in mgPO_4^{3-}-P L^{-1} using equation (13).

$$[TP] = [PO_4^{3-}] \cdot M_P \cdot DF \text{ (mgPO}_4^{3-}\text{-P L}^{-1}\text{)} \quad (13)$$

Where [TP] is the concentration of TP (mgPO_4^{3-}-P L^{-1}), [PO_4^{3-}] is the diluted molar concentration of PO_4^{3-} (mmol L^{-1}), M_P is the molar mass of P (g mol^{-1}) and DF is the dilution factor (-).

The diluted concentrations of NH_4^+, NO_2^- and NO_3^- in mmol L^{-1} were converted to the undiluted concentrations of N in mgNH_4^+-N L^{-1}, mgNO_2^--N L^{-1} and mgNO_3^--N L^{-1}, respectively, using equation (14)-(16).

$$[NH_4^+ - N] = [NH_4^+] \cdot M_N \cdot DF \text{ (mgNH}_4^+\text{-N L}^{-1}\text{)} \quad (14)$$

Where [NH_4^+-N] is the concentration of N from NH_4^+ (mgNH_4^+-N L^{-1}), [NH_4^+] is the diluted molar concentration of NH_4^+ (mmol L^{-1}), M_N is the molar mass of N (g mol^{-1}) and DF is the dilution factor (-).

$$[NO_2^- - N] = [NO_2^-] \cdot M_N \cdot DF \text{ (mgNO}_2^-\text{-N L}^{-1}\text{)} \quad (15)$$

Where [NO_2^--N] is the concentration of N from NO_2^- (mgNO_2^--N L^{-1}), [NO_2^-] is the diluted molar concentration of NO_2^- (mmol L^{-1}), M_N is the molar mass of N (g mol^{-1}) and DF is the dilution factor (-).

$$[NO_3^- - N] = [NO_3^-] \cdot M_N \cdot DF \text{ (mgNO}_3^-\text{-N L}^{-1}\text{)} \quad (16)$$

Where [NO_3^--N] is the concentration of N from NO_3^- (mgNO_3^--N L^{-1}), [NO_3^-] is the diluted molar concentration of NO_3^- (mmol L^{-1}), M_N is the molar mass of N (g mol^{-1}) and DF is the dilution factor (-).

The undiluted concentration of TN was then calculated using equation (17).

$$[TN] = [NH_4^+ - N] + [NO_2^- - N] + [NO_3^- - N] \text{ (mgTN L}^{-1}\text{)} \quad (17)$$

Where [TN] is the concentration of TN (mgTN L^{-1}).

1.2 Total suspended solids and volatile suspended solids

The preparation of the glass fiber filter step was, however, skipped since it was carried out once before the experiment started and the filter weight loss after cleaning and ignition at 500 ^{o}C was judged as neglectable. Hence, a glass microfiber filter was first weighed before the sample was pipetted onto it. The filter was then placed in a filtration apparatus and vacuum was applied to remove all traces of water. Thereafter, the filters were carefully removed from the filtration apparatus and placed in an aluminum dish and dried in the oven for 1 hour at 105^{o}C. After the drying, the filter was placed in a desiccator to balance the temperature before it was weighted. Thereafter, the filter was placed in an aluminum dish and ignited in the furnace for 15 minutes at 550^{o}C. After the ignition, the filter was placed in a desiccator to balance the temperature before it was weighted again. Finally, TSS and VSS were calculated using equation (18) and (19).

$$TSS = \frac{(A-B)\times 1000}{C} \text{ (gTSS L}^{-1}\text{)} \quad (18)$$

Where A is the weight of the filter + dried residue (g), B is the weight of filter (g) and C is the sample volume (mL).

$$VSS = \frac{(A-B)\times 1000}{C} \text{ (gVSS L}^{-1}\text{)} \quad (19)$$

Where A is the weight of the filter + dried residue before ignition (g), B is the weight of residue + weight of the filter after ignition (g) and C is the sample volume (mL).

1.3 Ammonium sorption

Up to 1.7 $mgNH_4^+$-N $g^{-1}VSS^{-1}$

$VSS_{average}$ = 3.59 gVSS L^{-1}

=> Sorption = 3.59*1.7 = 6.103 $mgNH_4^+$-N L^{-1}

1.4 Struvite precipitation

$[MgSO_4]$ = 0.02 g L^{-1}

Molar mass ($MgSO_4$) = 120.366 g mol^{-1} => 0.02/120.366 = 0.135*10^{-5} mol L^{-1}

V_{fill} = 1.55 L => 0.135*10^{-5}*1.55 = 20.932*10^{-5} mol Mg^{2+}

Struvite: $Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4.6H_2O$ => 1:1:1 molar ratio

Molar mass (P) = 30.974 g mol^{-1}

=> [PO_4^{3-}-P] = (30.974*20.932*10^{-5})/1.55 = 4.18 $mgPO_4^{3-}$-P L^{-1}

Molar mass (N) = 14.0067 g mol^{-1}

=> [NH_4^+-N] = (14.0067*20.932*10^{-5})/1.55 = 1.89 $mgNH_4^+$-N L^{-1}

2 Light microscopy

2.1 Morphology

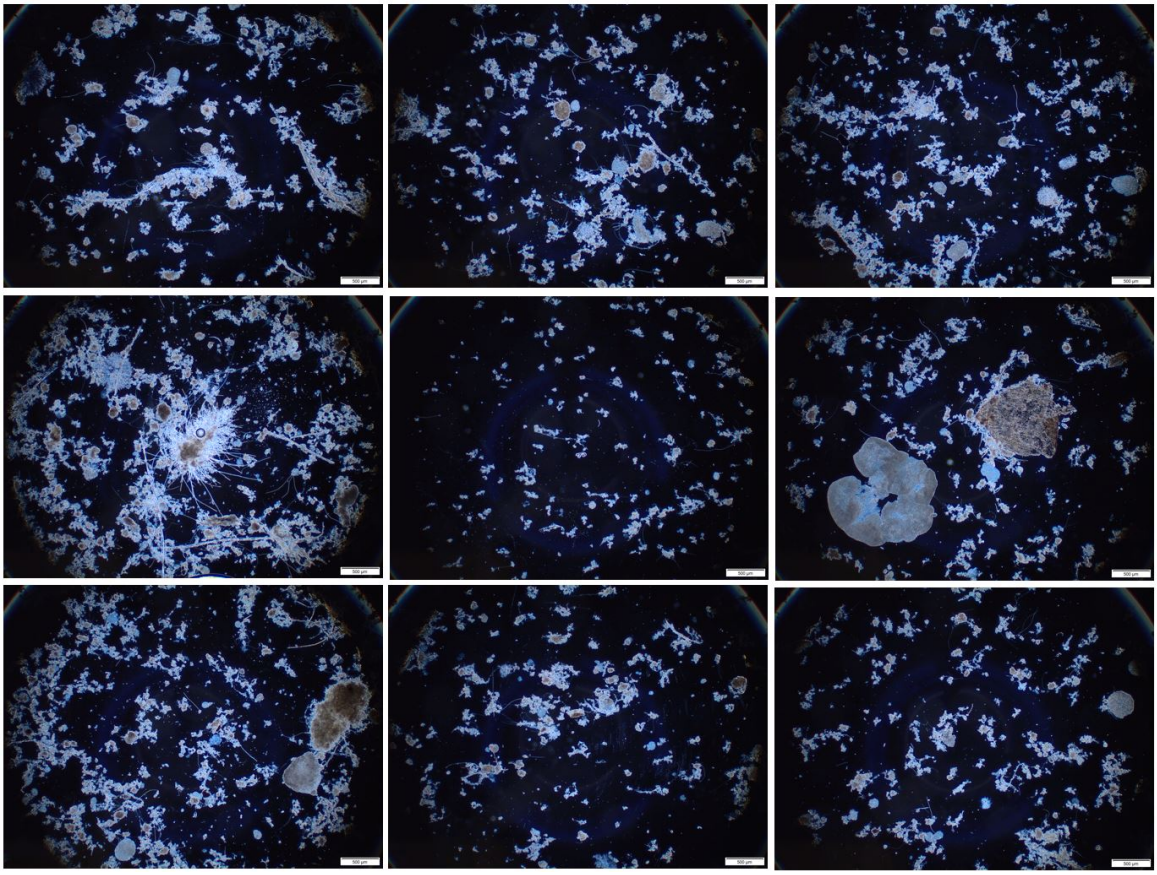

Figure A1. Sludge morphology day 5, magnification 2X.

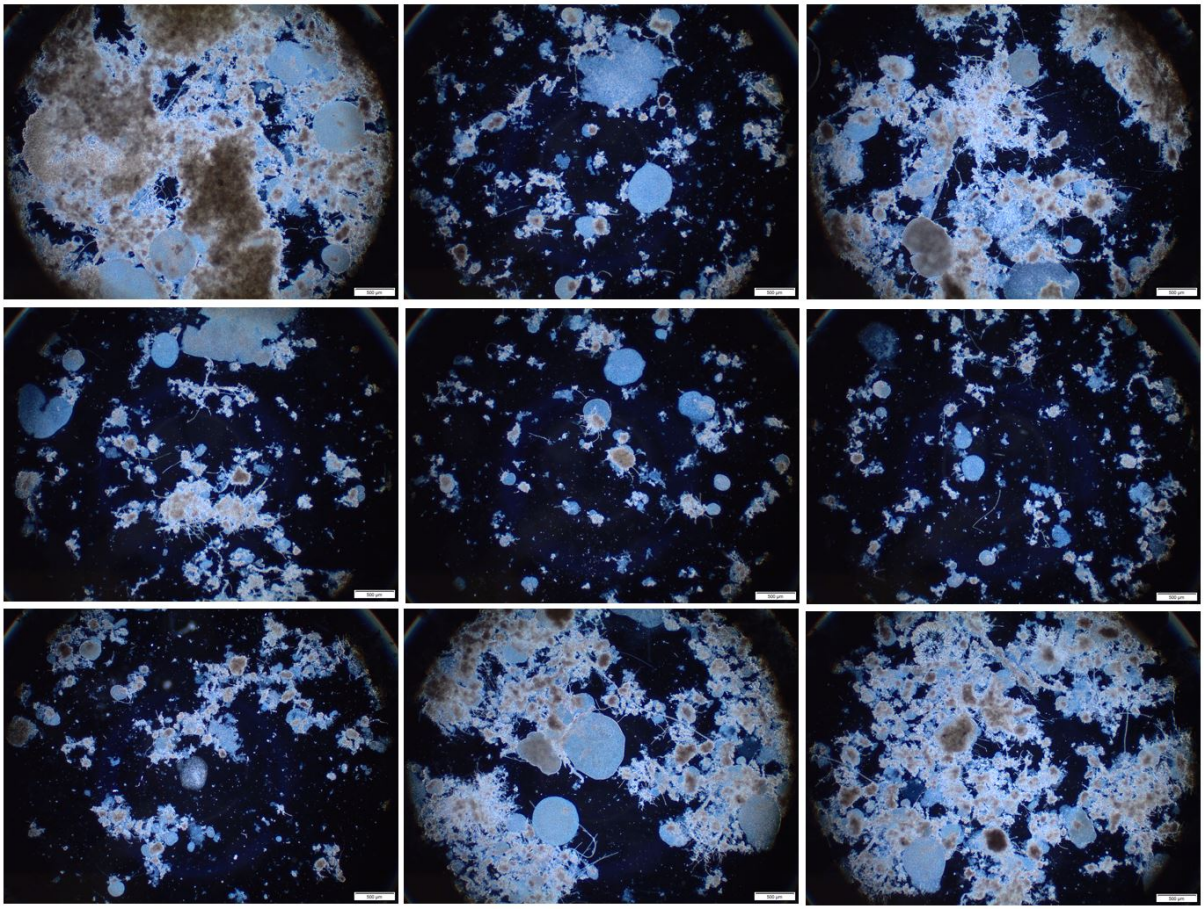

Figure A2. Sludge morphology day 12, magnification 2X.

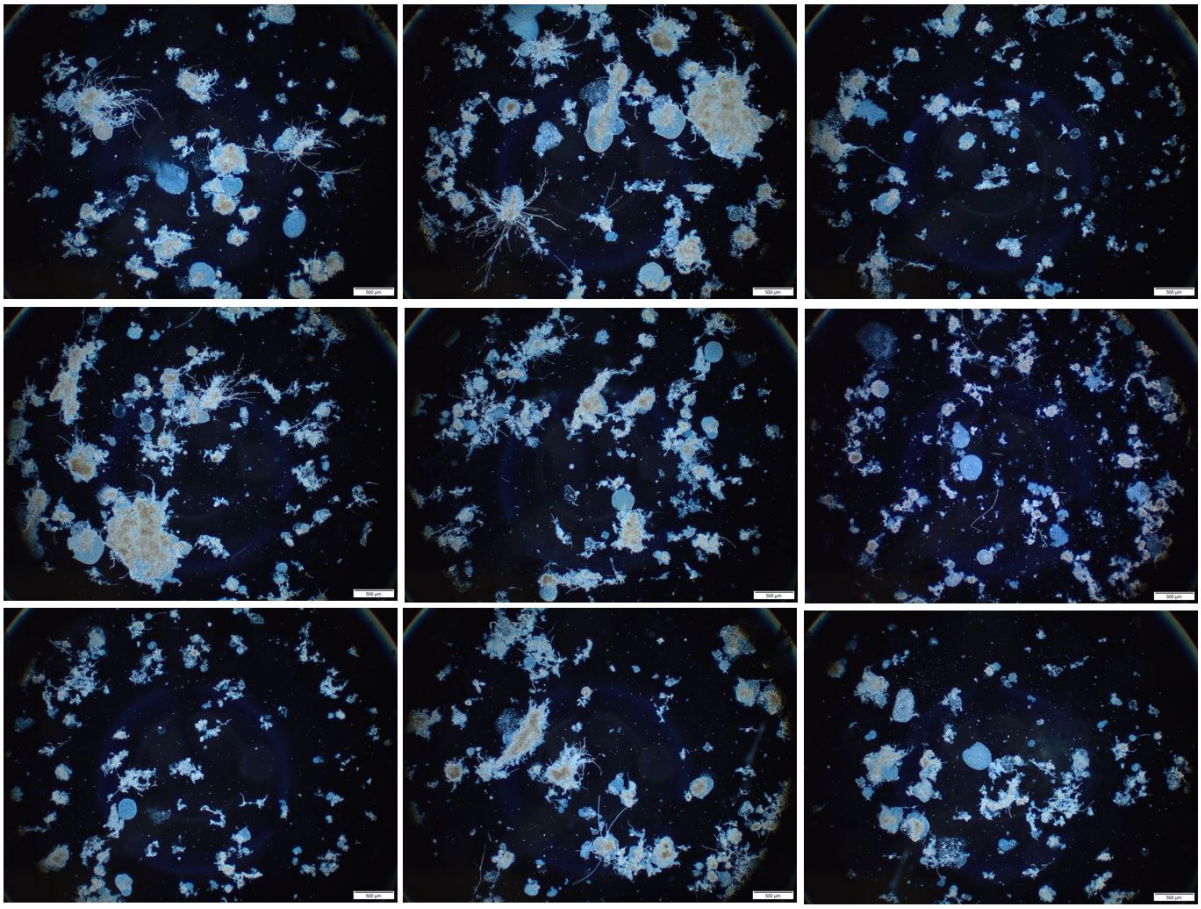

Figure A3. Sludge morphology day 19, magnification 2X.

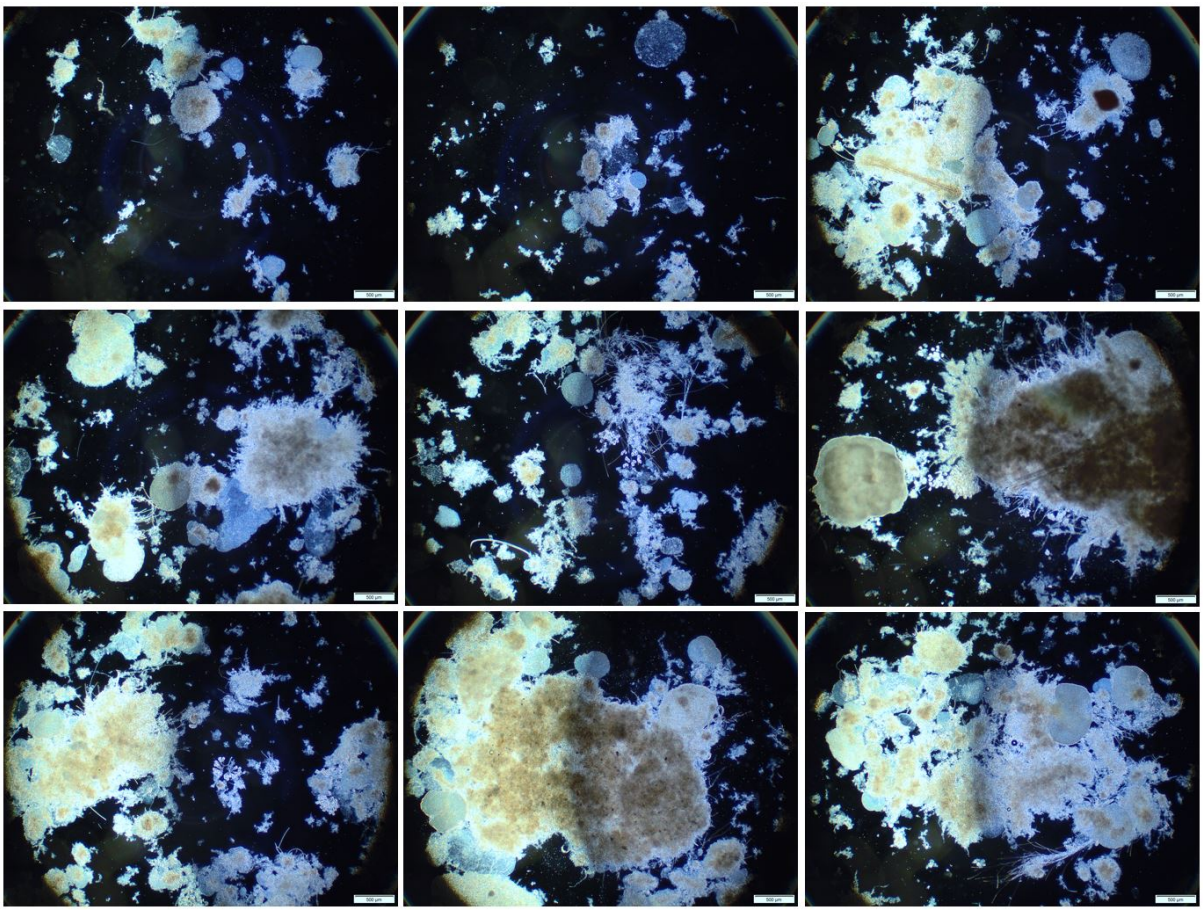

Figure A4. Sludge morphology day 25, magnification 2X.

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