THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Ideal carbon utilisation in wastewater treatment for enhanced nutrient removal

Primary filtration with filter sludge fermentation as primary treatment for a novel biofilm process

ELIN OSSIANSSON

Department of Architecture and Civil Engineering

CHALMERS UNIVERSITY OF TECHNOLOGY

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ELIN OSSIANSSON
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Department of Architecture and Civil Engineering
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 (0)31-772 1000

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ABSTRACT

Wastewater treatment is necessary to protect our receiving waters from eutrophication and oxygen deficiency, but requires space, energy and chemicals. A novel compact primary treatment for wastewater was tested, to enable resource efficient carbon utilisation for nitrogen and phosphorus removal and for methane production. Primary filtration and fermentation of filter primary sludge (FPS) at ambient temperature were studied at pilot scale to assess the efficiency of particle removal, and seasonal variation in volatile fatty acid (VFA) production by fermentation. A novel continuous biofilm process with bio-based biofilm support material was studied in combination with the primary treatment to understand how carbon management can impact enhanced biological removal of nitrogen and phosphorus.

Through the primary treatment, the VFA concentration in the wastewater could nearly be doubled. The seasonal variations in VFA production and distribution, and in the microbial community of FPS fermentation were considerable. Calculations and simulations indicated lower energy demand and volume requirement with the primary treatment, in addition to lower effluent nitrogen compared to conventional primary settling. The continuous biofilm process with two alternating moving bed biofilm reactors (MBBRs) resulted in mean nitrogen and phosphorus removals during the different operational periods of 70-85% and 57-82%, respectively, assuming complete particle removal. Aerobic and anoxic phosphate uptake was observed, signifying the presence of denitrifying polyphosphate accumulating organisms (PAOs). The FPS fermentation enables storage of the produced VFA-rich carbon source, and control of the dosage to biological nutrient removal. It was shown that VFA dosage was needed to enable high PAO activity with filtered influent, and that controlled dosage at low redox improved the phosphorus removal. The PAO activity decreased when the primary treatment was omitted. Microbial analysis showed high abundances of the PAOs *Ca.* Phosphoribacter, *Ca.* Accumulibacter and *Tetrasphaera* in the biofilm and in the effluent.

The importance of carbon management for enhanced biological nutrient removal was shown in this thesis work. The primary filtration and the addition of VFA from FPS fermentation could increase the enhanced biological removal of nitrogen and phosphorus, and reduce the needs for electricity, volume and chemicals in the wastewater treatment.

Keywords: wastewater treatment; primary filtration; primary sludge fermentation; carbon source; microbial community; moving bed biofilm reactor (MBBR); enhanced biological phosphorus removal (EBPR)

LIST OF PUBLICATIONS

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

Paper I: Ossiansson, E., Bengtsson, S., Persson, F., Cimbritz, M., Gustavsson, D.J.I. (2023) Primary filtration of municipal wastewater with sludge fermentation – Impacts on biological nutrient removal. Science of the Total Environment. 902, pp 166483. 10.1016/j.scitotenv.2023.166483

Paper II: Ossiansson, E., Persson, F., Bengtsson, S., Cimbritz M., Gustavsson, D.J.I., 2023. Seasonal variations in acidogenic fermentation of filter primary sludge. Water Research. 242, pp.120181. 10.1016/j.watres.2023.120181

Paper III: Ossiansson, E., Piculell, M., Persson, F., Bengtsson, S., Gustavsson, D.J.I., Christensson, M., Rosen, C. 2025. A continuous biofilm process for biological nitrogen and phosphorus removal with bio-based support material and carbon management. (Submitted)

Paper IV: Ossiansson, E., Piculell, M, Persson, F, Bengtsson, S., Gustavsson, D.J.I., Cimbritz, M., Dankmeyer, A., Christensson, M., Rosen, C. 2025. With or without primary treatment? Effects on enhanced biological phosphorous removal in a continuous biofilm process. (Manuscript)

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Paper II: Conceptualisation, Methodology, Resources, Visualization, Formal analysis, Investigation, Funding acquisition, Writing of original draft, Writing of review & editing, Project administration.

Paper III: Conceptualisation, Methodology, Resources, Visualization, Formal analysis, Investigation, Writing of original draft, Writing of review & editing.

Paper IV: Conceptualisation, Methodology, Resources, Visualization, Formal analysis, Investigation, Writing of original draft, Writing of review & editing.

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LIST OF ACRONYMS AND ABBREVIATIONS

AGS: aerobic granular sludge
AMPTS: automatic methane potential test system
AOB: ammonia-oxidising bacteria
BMP: biomethane potential
BNR: biological nutrient removal
BSM1: benchmark simulation model no. 1
COD: chemical oxygen demand
d: days
DO: dissolved oxygen
EBPR: enhanced biological phosphorus removal
FPS: filter primary sludge
GAO: glycogen accumulating organism
HAc: acetic acid
HAc-eq: acetic acid equivalents
HBu: buturic acid
HPr: propionic acid
HVal: valeric acid
HRT: hydraulic retention time
ICU: ideal carbon utilisation
Iso-HBu: iso-butyric acid
Iso-HVal: iso-valeric acid
MBBR: moving bed biofilm reactor
no.: number
NOB: nitrite-oxidising bacteria
pe: personal equivalents
PHA: polyhydroxyalkanoate
PAO: polyphosphate accumulating organism
RBF: rotating belt filter
RBFF: rotating belt filter with fermentation of filter primary sludge
RT: retention time
SBBR: sequencing batch biofilm reactor
SBR: sequencing batch reactor
SCOD: soluble COD measured after 1.6µm filtration
SPS: settler primary sludge
SRT: solids retention time
TS: total solids
TSS: total suspended solids
VFA: volatile fatty acid
VS: volatile solids
VSS: volatile suspended solids
WAS: waste activated sludge
WWTP: wastewater treatment plant

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TABLE OF CONTENTS

1. Introduction

1.1. Wastewater treatment

Wastewater treatment plants (WWTPs) were first built to protect people from diseases, and the receiving waters from oxygen deficiency and eutrophication. As the cities are expanding, populations increasing, and water scarcity is of growing concern, more objectives and responsibilities are arising for the WWTPs. Footprint and electricity consumption, chemical requirement and the potential for nutrient and energy recovery from wastewater have been on the minds of process engineers, researchers and plant executives for decades. Stricter effluent demands from the European Union will be implemented with consideration to the environmental status of our recipients and raise the bar for the WWTPs (EU Directive 3019, 2024). In addition, more emphasis is put on reduction of greenhouse gas emissions and resource efficiency and recovery in wastewater treatment.

How can we manage these new effluent standards with low environmental impact? In cities where the WWTP infrastructure was built in the 1960s and 1970s, the areas designated for treatment processes might be closer to, or surrounded by, housing and new infrastructure. Simultaneously, the load might have multiplied, and we need more compact technologies to manage our mission. These frames have induced an ongoing technology development within the field of municipal wastewater treatment.

1.2. Organic carbon – problems and prospects

Organic carbon is present in the influent wastewater in both particulate and soluble forms, which can be measured as chemical oxygen demand (COD). The energy content of the organic carbon has been estimated to 15% of the total energy in the wastewater (150 kWh/(person, y)), where heat is the major possibility for energy recovery (Larsen, 2015). Nonetheless, a part of the influent organic material can be recovered as methane through biogas production in anaerobic digestion of the separated sludge, and render a high product value as replacement for fossil natural gas.

Sludge can be separated from the primary treatment, and from the secondary treatment, which is the biological wastewater treatment (Fig. 1). From the typical influent COD concentration of ~500 mg/L (Henze and la Cour Jansen, 2019a), particulate organic carbon can be separated as primary sludge. The COD which enters the biological wastewater treatment is partially oxidised in the process, and partially assimilated in the biological sludge.

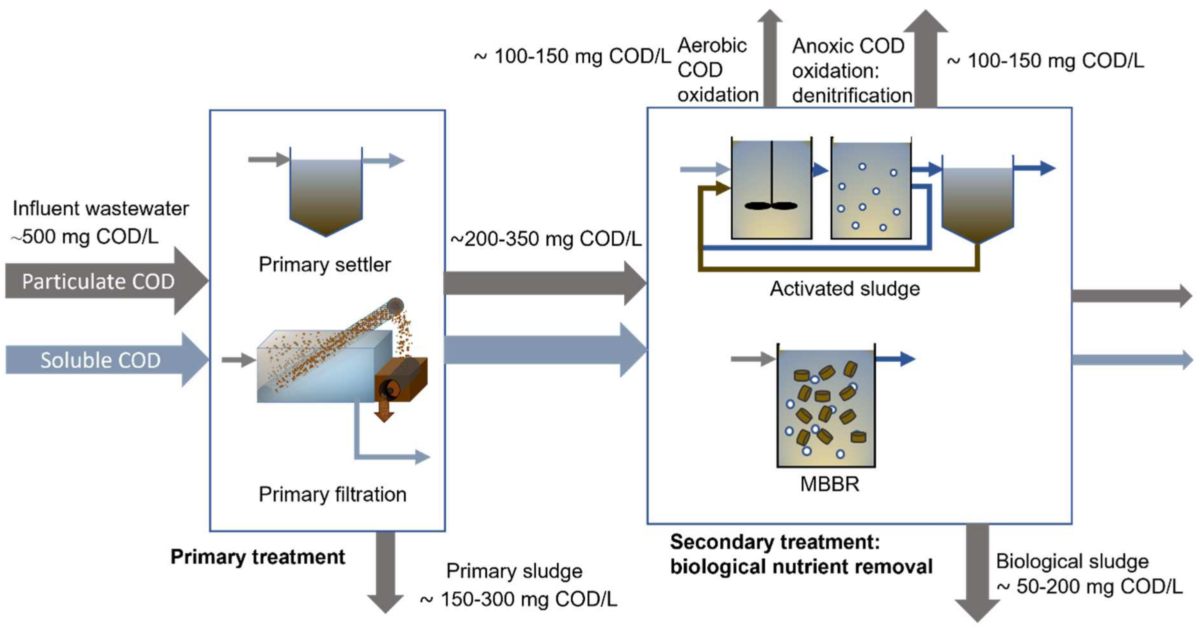

Figure 1. Overview of primary treatment and secondary treatment (biological wastewater treatment) with possible technology alternatives and typical values for COD in the wastewater and sludge derived from the wastewater (mg COD/L wastewater). The biological nutrient removal is exemplified by activated sludge with pre-denitrification, and by moving bed biofilm reactor (MBBR).

In the biological wastewater treatment process, organic carbon is needed as carbon source for biological nutrient removal (BNR). Nitrogen removal through nitrification and denitrification requires readily degradable COD for denitrification (anoxic COD oxidation, Fig.1). The theoretical demand for denitrification is 2.86 g COD/g N, while the COD demand in practice varies depending on the availability of the carbon source (Henze et al., 2008). In pre-denitrification, the nitrate is recirculated and the organic carbon in the influent wastewater can be used for nitrogen removal. The downside of the configuration is the slow hydrolysis of the particulate carbon source, and hence large volumes are required. Post-denitrification does not require recirculation or large volumes, but instead addition of carbon source, since the readily available organic carbon has been removed in the foregoing process steps. Based on a survey among Swedish WWTPs by the Swedish Water and Wastewater Association (Svenskt Vatten, 2021), the estimated total annual consumption of external carbon source in 2020 was 2 900 tons of ethanol and 6 800 tons of methanol. Considering the economic cost, the high impact on greenhouse gas emissions (Gustavsson and Tumlin, 2013) and the risk of handling flammable chemicals, the use of external carbon sources should be avoided or minimised.

Phosphorus can be removed from the wastewater chemically by addition of metal coagulants. Implementation of enhanced biological phosphorus removal (EBPR) reduces the need for coagulant addition, but entails additional carbon source need corresponding to 20 g COD/ g P (Henze and la Cour Jansen, 2019b). Polyphosphate-accumulating organisms (PAOs) perform EBPR by storing excessive amounts of polyphosphate within their cells, which they use for uptake of readily available COD. Thereby, COD is needed for their growth and for their excessive phosphate uptake from the wastewater.

The need for electricity to aerate the biological treatment increases when organic carbon is used by heterotrophic bacteria for aerobic COD oxidation (Fig. 1), and it has been shown that extensive removal of organic carbon in the primary treatment can drastically decrease the energy demand (Arnell et al., 2017; Pasini et al., 2021; Rusten et al., 2016). In order to meet strict effluent requirements for nitrogen and to apply EBPR, WWTPs need to use the influent carbon as a resource. Although the use of organic carbon for methane production and for nutrient removal oppose each other, WWTPs need to prioritise both.

2. Background

The background to the thesis work is presented by introducing the steps in wastewater treatment, and the treatment processes which are important for the understanding of following sections. The overarching focus is the use of organic carbon in these processes, and its importance for nutrient removal, energy production and energy demand at the WWTPs.

2.1. The primary step

The purpose of the primary treatment is to decrease the load of total suspended solids (TSS) containing COD, and in some cases PO_4^{3-}, to the biological wastewater treatment. The organic carbon in the influent wastewater can be in dissolved and in particulate form. In the primary treatment, a high fraction of TSS, and thereby also chemically bound energy can be removed and separated as primary sludge. Removing the particulate COD will increase the potential for energy recovery through anaerobic digestion, and decrease the need for aeration and volume in BNR. On the other hand, COD is needed as carbon source for denitrification, and for EBPR. Consequently, the wastewater composition after primary treatment sets the scene for the subsequent BNR and deserves attention.

2.2. Sedimentation

Sedimentation is widely used as primary treatment in conventional wastewater treatment. The technology is relatively simple, with rectangular or circular settling tanks equipped with mechanical sludge collectors in the bottom (Tchobanoglous et al., 2014). The mean TSS removal is typically 50-55% (Amerlinck, 2015; Patziger and Kiss, 2015). While primary settling is robust, the drawback is the areal requirement, which is high compared to other compact technologies such as primary filtration (Franchi and Santoro, 2015).

2.3. Primary filtration

Primary filtration was first tested in the 1970s (Särner, 1976), and has been developed continuously (Väänänen, 2017). The advantages compared to settlers are smaller footprint, less odour and better opportunities to control and enhance the particle separation. The drawbacks can be higher maintenance cost due to more machinery, higher electricity input and higher re-investment cost. Filters can be designed as rotating belts, drums or disks (Caliskaner et al., 2021). The primary filtration can be carried out with precedent polymer addition, which has shown to enhance particle separation (Ebeling et al., 2006; Rusten et al., 2017). The polymer addition adds to the TSS load, and therefore decreases the hydraulic capacity of the filter. Applied hydraulic loads for rotating belt filters (RBFs) without polymer addition have been lower with polymer addition (Rusten et al., 2017) compared to without (Franchi and Santoro, 2015).

The RBF filtration is affected by increased flow, as the filter cloth moves faster to be able to treat the wastewater coming into the filter. Consequently, higher flowrates have detrimental effect on the TSS removal efficiency (Rusten and Ødegaard, 2006). A higher TSS concentration in the inlet will build up a filter mat on the cloth, which makes the passing of particles more difficult, and enhances the filtration and the particle removal (Franchi and Santoro, 2015; Rusten et al., 2017). The TSS removal efficiency is therefore expected to be linked to the influent TSS.

2.4. Energy balance and methane potential

A more extensive primary treatment gives more primary sludge, and hence a higher methane production at the WWTP, as well as lower aeration demand in the biological wastewater treatment (Areskoug et al., 2025; Arnell et al., 2017; Behera et al., 2018). The primary treatment is therefore important for the BNR and for the energy balance at the WWTP. The potential to produce methane through anaerobic digestion, the so called biomethane potential (BMP) from primary sludges differs between plants, but is generally higher (300-500 NmL/g VS) compared to waste activated sludge (WAS; 170-280 NmL/g VS) from biological wastewater treatment (Calabrò et al., 2024). The primary sludge has also different properties depending on the separation process (Alizadeh et al., 2023). Filter primary sludge (FPS) has a higher content of cellulose compared to settler primary sludge (SPS; Ahmed et al., 2019), since the long fibres that are easily captured in filtration do not settle well. Primary sludges from different WWTPs with primary settlers or primary filtration have been compared, which resulted in higher BMP for FPS compared to SPS (Paulsrud et al., 2014).

The BMP does also differ between sludges from the BNR depending on influent characteristics and load and process configuration (Calabrò et al., 2024; Carlsson et al., 2016; Mottet et al., 2010). The choice of process is therefore important for the resource efficiency and energy recovery, both for primary treatment and BNR.

2.5. Sludge – a source of carbon

The energy which is withdrawn as primary sludge can be directed to anaerobic digestion for biogas production, but it can also be used for production of volatile fatty acids (VFAs) by fermentation. In practice, it means that the anaerobic production chain to methane it ended after hydrolysis and fermentation. The retention time is kept low (<10 d) to prevent methanogens from growing, and pH is naturally kept low due to the VFA production. Under these conditions, particles are degraded, hydrolysed and fermented by bacteria that utilise carbon without an external electron acceptor. Carbon is partially lost as CO_2, but the energy is largely conserved in the VFAs. Bacteria can take up and utilise VFA with ease; acetate can enter the TCA cycle, and the longer chained acids which contain more energy can be split and metabolised. PAOs can use VFAs as a precursor for energy storage in the form of polyhydroxyalkanoates (PHAs) and glycogen (Wentzel et al., 2008). Readily degradable carbon is therefore a prerequisite for the growth of PAOs and for EBPR.

2.6. Sludge fermentation

When EBPR started to be in use at larger scale during the 1990s, the need for increasing the content of readily available carbon in the wastewater arose. As a consequence, hydrolysis and fermentation of primary as well as waste activated sludge was studied by academia, and also implemented at full scale.

Fermentation at ambient temperature saves energy compared to heating the sludge, and facilitates the operation. Side-stream reactors can be used, or an in-line process in the bottom of a settling tank by increasing the sludge level and thereby also the solids retention time (SRT; Banister and Pretorius, 1998; Hey et al., 2012; Pitman et al., 1992; Tykesson et al., 2005). However, the SRT in the settler is difficult to calculate and control at a full-scale plant, and the VFA concentration in the primary sludge depends not only on the production, but also on the transfer to the wastewater phase. The concentration is the water phase is low and the production of specific VFAs is therefore difficult to measure. FPS from filtration without polymer addition has also been used as a substrate for fermentation at constant temperatures > 20°C (Bahreini et al., 2020a; Brison et al., 2022; Da Ros et al., 2020).

SPS in an in-line fermentation can be pumped to the settler surface at the inlet where the VFAs are washed out to the wastewater. Particles in fermented sludge from side-stream processes can also be separated by settling, although the settling properties are deteriorated after fermentation (Lötter and Pitman, 1992; Moser-Engeler et al., 1998). VFAs can also be separated by mechanical dewatering of the fermented sludge, which would inevitably lead to a loss of VFAs in the solid fraction. This loss of carbon source results in a major cut in the benefits from the fermentate addition (Bahreini et al., 2021; Canziani et al., 1996; Christensen et al., 2022).

2.7. Activated sludge

In the biological wastewater treatment, microorganisms assimilate and convert nitrogen and COD to biomass and gases, which are removed from the wastewater. Phosphorus can be removed by assimilation or by excess phosphate uptake by PAOs if EBPR is applied, and is removed with the WAS. Activated sludge is the most widely used BNR process and was first installed in 1914 (Daigger, 2014). Since then, it has been built in different configurations and been developed continuously. The process can be designed to use the influent carbon for denitrification and hence avoid addition of external carbon source. New installations of activated sludge are still being built, but the drawback of the technology is the larger footprint compared to processes with biofilm technology (Bengtsson et al., 2019).

2.8. Enhanced biological phosphorus removal

Phosphate is removed from the wastewater by assimilation and by EBPR in the biological treatment. EBPR eliminates or reduces the need for coagulant dosage in the chemical phosphorus removal, and can thereby contribute to economic and environmental savings for the WWTPs.

PAOs achieve EBPR by taking up excessive amounts of phosphate from the wastewater in aerated conditions to build up an internal storage of polyphosphate. The polyphosphate is used in anaerobic conditions as energy to take up COD (Wentzel et al., 2008). The COD is also stored within their cells as biopolymers. Compared to heterotrophic bacteria, which are unable to take up substrate anaerobically, PAOs have an important advantage if easily available COD is present under anaerobic conditions. Wastewater treatment processes for EBPR are therefore designed to provide alternating aerobic and anaerobic conditions, and to ensure that PAOs have access to readily degradable COD under anaerobic conditions (Henze and la Cour Jansen, 2019b). In EBPR configurations, an anaerobic zone can be added as a first step to allow PAOs access to the influent VFAs and the readily degradable COD (Wentzel et al., 2008), following the principle of an anaerobic selector which was first implemented in South Africa (Barnard, 1976).

Different groups of PAOs have been identified, with different capabilities for carbon source uptake and storage of biopolymers, among them are *Ca.* Accumulibacter, *Ca.* Dechloromonas, *Ca.* Phosphoribacter and *Tetrasphaera* (Ruiz-Haddad et al., 2024).

2.9. Processes for enhanced biological nitrogen and phosphorus removal

EBPR in combination with enhanced biological nitrogen removal is widely applied in activated sludge (Wentzel et al., 2008). The COD is then used for denitrification and for EBPR in a configuration allowing for anaerobic uptake of readily degradable COD by PAOs. The inclusion of nitrogen removal is challenging, since competing organisms can have access to nitrite and nitrate (NO_{2+3}^-) as electron acceptors, and use the NO_{2+3}^- and COD for denitrification. As a consequence, EBPR failure has been observed due to the lack of anaerobic conditions (Arnz et al., 2001; Guerrero et al., 2012) Although nitrogen removal is also a target, the substrate uptake by PAOs must be ensured in the process. There are several process alternatives for nitrogen removal and EBPR in continuous activated sludge (Wentzel et al., 2008).

The varying aerobic, anoxic and anaerobic conditions in the enhanced BNR of nitrogen and phosphorus is not only problematic. It opens up the possibility for anoxic dephosphatation by denitrifying PAOs, simultaneously removing nitrogen and phosphorus. Denitrifying PAOs use the carbon source more efficiently compared to heterotrophic denitrifies and non-denitrifying PAOs, and considerable savings in carbon source demand and in aeration can be achieved (Kuba et al., 1996).

2.10. Alternating processes for biological nutrient removal

In an alternating process for biological wastewater treatment, two coupled reactors with continuous inflow are operated in series with alternating flow direction, or with flow over only one of the reactors to optimise the effluent values. An activated sludge process with alternating reactors which are aerated intermittently to allow to aerobic and anoxic conditions for nitrogen removal was developed in the 1970s (Bundgaard et al., 1983). Over the years, the alternating system has shown the advantages of flexibility and stability, with high nitrogen removal as a result (Petersen et al., 1993). Alternating reactor systems are flexible and allow for phase control in a manner that is similar to sequencing batch reactors (SBRs), but since the flow is continuous, there is no need for buffer volumes.

EBPR in alternating processes for activated sludge was first seen already in the 1980s through phosphate release in the non-aerated phases (Bundgaard et al., 1983; Jansen and Behrens, 1980). An anaerobic reactor was added prior to the two main reactors to favour COD uptake by PAOs (Arvin and Kristensen, 1985). The system for controlling the reactor phases (Nielsen et al., 1994; Thornberg et al., 1993) was expanded to introduce an anaerobic phase after the NO_{2+3}^- has been depleted, and thereby promote EBPR in an alternating system without a separate volume for phosphate release (Ingildsen et al., 2006; Rosen et al., 2006).

2.11. Enhanced biological phosphorus removal in biofilm processes

Biofilm processes for BNR give microorganisms a vast area to grow on, and the possibility for several conditions to coexist in the same reactor, in different depths of the biofilm. The biofilm is protected from washout, and the amount of biomass in the reactor can be high compared to conventional activated sludge. Implementation of EBPR in a biofilm process is complex, since three different redox conditions need to be included for combined phosphorus and nitrogen removal: anaerobic, anoxic and aerobic.

The most widespread EBPR biofilm process at full scale is aerobic granular sludge (AGS), where the biofilm forms granules (Pronk et al., 2015). Other process solutions for biofilm EBPR with biofilm on biofilm support material, in moving bed biofilm reactors (MBBRs), have been proposed (Goncalves and Rogalla, 1992; Humbert et al., 2018; Saltnes et al., 2017). Carriers for biofilm growth can be practical since granulation is not needed. Furthermore, the sludge production as well as the methane potential from the produced sludge can be high with MBBR (Carlsson et al., 2016).

There are relatively few experimental studies with real wastewater on MBBR processes including high nitrogen removal and EBPR. Sequencing batch biofilm reactors (SBBRs) have been tested, resulting in nitrogen and phosphorus removals of 54 and 75% (Pastorelli et al., 1999) and 20 and 81% (Fanta et al., 2021), respectively, with addition of VFA to the process. Removal efficiencies for nitrogen and phosphorus of 70 and 68% (Joeng et al., 2003), and 70 and 86% (Humbert et al., 2018) has been achieved in SBBRs without VFA addition. Continuous processes are attractive, since no volumes are needed for flow equalisation. A continuous MBBR process for EBPR has been tested at larger scale (Saltnes et al., 2017), but to the best of the author’s knowledge, no continuous MBBR process for enhanced removal of both nitrogen and phosphorus from municipal wastewater has been presented.

2.12. A novel biofilm process for nitrogen and phosphorus removal

Bio-based carriers of different origins have been tested as an alternative to fossil-based plastic biofilm carriers (Jagaba et al., 2021). There are, however, several requirements that need to be met for a biofilm support material: stability, high area to volume ratio, suitable density, and absence of harmful substances. Fossil-based carriers are more widely used for MBBRs at full scale compared to bio-based carriers, but the interest in more sustainable support materials for biofilm has brought an ongoing development in this field. A novel alternating MBBR process with bio-based support material has been developed (Cella^{TM}, Veolia Water Technologies). The first full-scale plant with Cella^{TM} for COD and nitrogen removal (without EBPR) was started up in 2024 at Svinninge WWTP (Denmark).

Including EBPR in the novel continuous biofilm process was appealing to study, based on the experience of EBPR in alternating activated sludge reactors (Rosen et al., 2006). There are differences between activated sludge and MBBR processes. In activated sludge, particulate carbon can be captured in flocs and fermented in anaerobic conditions to be available for PAOs. MBBRs are invented to provide a compact treatment with lower hydraulic retention time (HRT), and particulate carbon is washed out from the process faster compared to in activated sludge. The alternating process with EBPR has an anoxic denitrification phase prior to the anaerobic phase. This changes the prerequisites for EBPR in an alternating system, where much of the influent carbon is taken up during the anoxic phase, rather than in the anaerobic phase.

Extensive particle removal in the primary treatment combined with biofilm and short HRT provide a compact and energy efficient process (Ødegaard, 2000), but makes it more difficult to include EBPR in the alternating process due to the shortage of carbon source. The primary filtration with FPS fermentation was therefore considered to be a suitable supplement, as it allowed for dosage of VFA as carbon source for PAOs.

The effluent from an MBBR contains the treated wastewater, but also biological sludge detached from the biofilm, as well as particles. A post treatment is needed to separate the particles, and to remove more phosphate from the wastewater if needed. For this purpose, different technologies have been used, such as sedimentation, flotation and filtration (Ivanovic and Leiknes, 2012). Each of the technologies have different area requirements, electricity usage and need for chemical addition. In the MBBR process, the particle size distribution is shifted towards larger particles which facilitates the post-treatment (Ødegaard et al., 2012). The type of biofilm carrier in the MBBR can influence the effluent particle size characteristics (Arabgol et al., 2022), and the effluent COD size distribution in the wastewater is therefore interesting to study for a novel biofilm process.

2.13. Research gaps

Chemically enhanced primary filtration in RBF has been tested and applied at full scale, but the separated FPS has acquired less interest from a research point of view. The only known pilot-scale study with fermentation of FPS (Da Ros et al., 2020) was conducted without polymer addition prior to filtration. It has been shown that the particle removal in chemically enhanced RBF filtration is higher, as well as the potential for energy recovery from the FPS (Rusten et al., 2017). The possible gain of using FPS from chemically enhanced filtration for production of carbon source has not yet been studied.

Although fermentation at ambient temperature has been applied at many WWTPs (Ekholm et al., 2022; Pitman et al., 1992; Tykesson et al., 2005), the seasonal variation in yields and production of specific VFAs have not been investigated thoroughly before. Numerous lab-scale studies in batch reactors are also valuable, but the results may not be valid for a continuous process at ambient, and thereby transient temperature. The seasonal variation in VFA distribution and production most likely impact the BNR, and is of general interest for WWTPs which have implemented or are looking into implementing primary sludge hydrolysis-fermentation. Despite this, the seasonal variations in primary sludge fermentation have not been studied before in detail.

The impact of temperature on microbial community in fermentation of primary sludge has been studied occasionally (Huang et al., 2021), but the effects over time from temperature changes and different retention times were unknown. The primary treatment technology can also affect the microbial community (Brison et al., 2022). Even though the microbial composition is likely to be affected by the seasonal variations in primary sludge fermentation at ambient temperature, no research on this subject had yet been found by the author.

Side-stream fermentation of primary sludge with a high total solids (TS) is appealing to decrease the volume requirement. However, the issue of separating the produced carbon source and direct it to BNR has not been thoroughly addressed. In case the WWTPs would lose a large fraction of the produced VFAs in separation of the solids, it would affect the required volumes for fermentation or the enhancement of nutrient removal through carbon source addition. It is therefore desirable to develop a separation of the carbon source without significant losses or practical obstacles. Mixing the fermented sludge with wastewater and separate the particles by filtration is a viable option, that had not yet been tested.

Owing to the advantages of both EBPR and of MBBR, productive attempts have been made to combine the two (Humbert et al., 2018; Saltnes et al., 2017). However, the up-scaling and spread of these processes have not yet accelerated. Further research is needed in this field to drive the progress and provide WWTPs with resource efficient and compact alternatives. The Cella^{TM} process was new and not presented in any previous publication by the time of this study. In addition, no attempt had been done to include EBPR in the process.

Continuous MBBR processes are compact and do not require equalising volumes, but there is a gap regarding processes for high nitrogen removal and EBPR in continuous MBBR. Apart from a lab-scale study with synthetic wastewater (Iannacone et al., 2021), and a continuous MBBR with focus on EBPR (Saltnes et al., 2017), there are no previous research published on this subject.

The carbon source derived from side-stream fermentation can be stored and dosed to the BNR, which opens up new possibilities to control the dosing and direct the VFAs to PAOs. In many full-scale processes, the anaerobic zone is placed before the anoxic and aerobic conditions and the PAOs have first-hand access to the carbon source. In alternating processes or SBRs, the gain of controlling the carbon source addition may be even more noticeable. Research on this subject is scarce, only one example of carbon source control could be found by the author (Choi et al., 2012), and none for biofilm EBPR.

The impact from primary treatment on BNR is less studied for biofilm processes, and the few studies that can be found show that in-line fermentation has a strong positive impact on EBPR (Ekholm et al., 2022) and that primary treatment can cause lower removal of both nitrogen and phosphorus compared to untreated influent (Kosar et al., 2022). For MBBR, only one publication could be found by the author on the primary treatment's impact on nitrogen removal (Rusten et al., 2016). The impact on EBPR was not yet studied, although it could be influential.

3. Aims and objectives

The overarching aim of the thesis was to increase the understanding of how carbon management impacts the enhanced biological nitrogen and phosphorus removal and the energy balance of the wastewater treatment process.

By combining the advantages of carbon management (primary filtration, fermentation of FPS and control of carbon source dosage), the aim was to increase knowledge of, and to develop the BNR in the novel continuous biofilm process to achieve high nitrogen removal, stable EBPR, low footprint and low chemical consumption.

The objectives formulated as research questions were as follows:

- How effective is the chemically enhanced particle removal in primary filtration by RBF? What particles are removed, and how can the separation be predicted by modelling? What are the characteristics of the separated FPS?

- How is fermentation of FPS affected by seasonal variations concerning VFA yield and distribution as well as microbial community assembly? How much VFA can be produced over the year, and how high is the nutrient release?

- Can the fermented sludge be added to the wastewater and the suspended solids separated by filtration?

- How are the requirements for volume and electricity in activated sludge affected by primary treatment with RBF and RBF with FPS fermentation, compared to conventional primary settling?

- Can the novel biofilm process result in high nitrogen removal and stable EBPR with wastewater primary filtration and addition of VFA to mimic fermented FPS?

- How can the dosage of VFA to the biofilm process be optimised to benefit EBPR while maintaining nitrogen removal?

- What microorganisms related to nitrogen and phosphorus removal are present in the biofilm?

- How is the nutrient removal affected by receiving untreated influent wastewater compared to application of primary filtration and VFA addition?

4. Research at pilot scale - plant design and operation

Primary filtration, fermentation of FPS and enhanced biological removal of nitrogen and phosphorus were studied at pilot scale. The pilot plants for primary treatment and BNR (Fig. 2) are described in detail in **Paper I-IV**. They are presented here to give a more practical background to their design and operation, and hopefully some useful ideas for future pilot-scale tests. Since the author was in charge of the building and operation of the pilot plant for primary treatment, this part is more detailed. In addition, a batch test for fermentation which was not included in any of the papers, is described here.

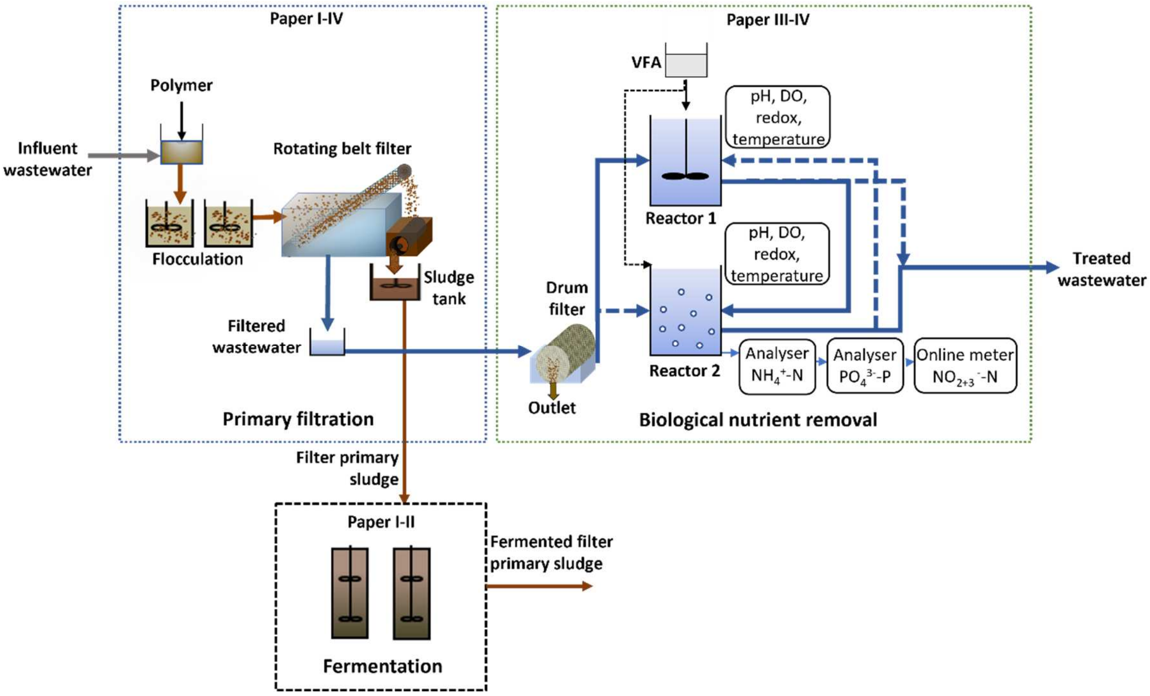

Figure 2. Overview of the pilot plant for primary filtration, fermentation and biological nutrient removal.

4.1. Overview

A pilot plant was built at Källby WWTP (Lund, southern Sweden) to study wastewater primary treatment and fermentation of FPS in the project Ideal Carbon Utilisation (ICU) which was the first part of the PhD period. The ICU pilot plant (Fig. 2) included chemically enhanced primary filtration with an RBF and FPS filtration, as well as the possibility to recycle fermented sludge to the influent wastewater as a means of transferring the VFAs in the wastewater and separate the fermented particles (5.2.1.). It was operated for two years in order to evaluate the filtration and the hydrolysis-fermentation. The aim was to assess this new process regarding carbon source production, particle separation and impact on BNR.

The biofilm BNR was realised during the second part of the PhD project through cooperation with Veolia Water Technologies in the project FramBliK. The BNR pilot for the novel Cella^{TM} process was added after the primary filtration (Fig. 2). To facilitate the pilot plant operation, VFA was dosed as a mixture of chemicals, and the fermentation of FPS was not operated during this study.

Pilot-scale experiments were chosen to enable long-term study of the processes with real wastewater under conditions close to a full-scale WWTP. RBF filtration has been conducted at bench-scale (Rusten and Lundar, 2014), but a pilot-scale RBF with a mean load of 850 personal equivalents (pe) was chosen to provide a more representative scale. The FPS is thick and requires pipes with a larger diameter to be pumped into the fermentation reactors. At lab-scale, the feeding of the reactors would most likely have been manual, resulting in dynamics more similar to a semi-batch process rather than a continuous process. With the large scale of the RBF, the FPS was pumped to fermentation with short intervals. Moreover, the larger scale of the fermenters resulted in more realistic reactors temperatures, which was important in the study of ambient temperature fermentation. The scale of the biofilm reactors was smaller, about 5 pe. For the research on the BNR, the scale was still large enough to use the same kinds of pumps and the same online meters as in a full-scale installation.

4.2. The ICU pilot plant for primary treatment

4.2.1. Building the pilot plant

Building the pilot plant, from idea to operation, required about a year of working with design, procurement, construction and automation. The drawings were made to scale in PowerPoint and used as basis for the constructors. Pipes of plastics, and hoses were used for the wastewater, while the pipes for sludge were built in steel to withstand pressure and wear.

The SF1000 RBF filter (Salnes Filter), which had been used before for pilot-scale tests was proposed by Salsnes filter. The corresponding size of the fermentation reactor tanks to enable treatment of all the produced sludge fitted well with available reactor tanks of 3 m^3 each from Sjölunda WWTP. The two reactor tanks were placed just outside of the building where the flocculation, filtration and pumps were situated Fig. 3a). The inlet to the reactors were at 1.3 m, and the outlet valve was at the bottom of the reactors to prevent accumulation of solids in the reactors. The top of the reactors could be accessed from a platform.

A room next to the sand separation at the WWTP was available for the pilot plant. It was considered suitable since it had a chute in the floor, and a slope to allow filtered wastewater to flow down to the grit chamber next to the building. On the other hand, it was small, and the limited space was a challenge both under construction and under operation (Fig. 3b). The pump for FPS out from the sludge tank was placed on the floor next to the tank, but the two pumps for fermented sludge out from the reactors were fastened on the wall to save space. There was no room for the polymer makeup plant, which was put in the sand separation room next door. This proved to be a good solution since leakage of polymer could have caused a slippery and hazardous floor in the pilot plant room.

Figure 3. a) Mounting of the reactors for hydrolysis and fermentation. b) The pilot plant room during electrical installation.

4.2.2. Operation

The ICU pilot plant (Figs 2-4, **Paper I-IV**) was built in 2019 and operated in two periods during 2020-2022 and 2023-2025 (Table 1-2). Wastewater was pumped after screening, and no recirculation stream from the sludge treatment at the plant was included. This raw wastewater was pumped to the building where the flocculation and RBF were placed. Since the aim was to evaluate a realistic case, the inflow was proportional to the main plant, but with minimum and maximum limits of 6-8 and 16-20 m^3/h, respectively.

4.2.3. Polymer addition

Cationic polymer (Kemira Kemi AB) was added both based on flow (g/m^3) and on mass load calculated from the flow and the online TSS meter (**Paper I-II**). The TS of the polymer solution which was 0.1-0.2% was measured regularly to tune in the right dose. Prior to the first operational period, several different polymers were tried out in batch testing in order to find a chemical which would render efficient flocculation and high-strength flocs. An anionic polymer which gave efficient flocculation in the lab proved to be unsuitable in pilot-scale as the flocs were too weak and fell apart before the filtration. The 40% polymer solution was mixed with water in a mixer unit (Polymore). The polymer concentration was analysed weekly during the first operational period, and monthly during the second operational period. In the SCADA system, the polymer concentration was used as an input to the dosing control. Two tanks in series were used for flocculation, with a volume of 0.8 m^3 each and mixing of 70 and 50 rpm respectively. This resulted in an HRT in flocculation of 9±3 min. Addition of coagulant (FeCl) was tested during the start-up of the RBF, before the fermentation was in operation. Since it resulted in FPS with low TS, and since the aim of the project was to facilitate EBPR, coagulant dosage was omitted during the study.

4.2.4. Filtration

For this study, an RBF was chosen for the primary filtration since it produces an FPS with a high TS (Paulsrud et al., 2014). The RBF (Salsnes Filter) was operated with a 350 µm pore size filter at fixed water level (200-230 mm) which controlled belt speed. The pore size waschosen based on recommendation by the technology supplier as the most widely used at WWTPs. Because of the polymer addition, particles were aggregated, and the sludge cake on the filter further enhanced removal of particles smaller than the pore size of the cloth. The filter was equipped with air compressor as the filter sludge was removed from the filter by air (air knife). A washing sequence was carried out 2-3 times per day during the first hours in the morning when the loading was low to remove particles and polymer from the filter cloth. During continuous operation high pressure waster was used, but occasionally hot water was applied to remove fat. A sludge screw transported the FPS to a sludge tank with working volume of 0.15–0.38 m^3 and HRT of 0.3 ± 0.1 d. The back of the filter, where the sludge screw and air knife were placed was cleaned weekly.

Figure 4. a) Flocculation tanks and rotating belt filter at the ICU pilot plant for wastewater primary treatment. b) Fermentation reactors.

4.2.5. Hydrolysis-fermentation

Since the study was performed at ambient temperature, two reactors for hydrolysis-fermentation were used to assess the impact of HRT in parallel operation at different HRTs. Thus, the reactors were operated with similar substrate and at similar temperatures. The reactors (R1, R2) were placed outdoors but insulated to avoid cooling during low temperatures. They were fed intermittently every 2 h after sludge withdrawal and operated at a working volume of 1.5-2.5 m^3 each. In practice, the volume exchange each feeding was small (3-5%) compared to the retention time of 3-5 d, and the operation was more comparable to a continuous reactor rather than an SBR. During sludge withdrawal from the bottom of the reactors, the flow was measured and the volume summed up until it reached the set value. The feeding of FPS was pumped from the sludge tank until the set reactor level was attained. Flow measurement of the influent FPS was avoided since it would have led to an increased risk of clogging. Although the distance to the reactors was just a few meters and 90 degree turns in the piping were avoided, clogging still happened. Shorter stops in the operation occurred when rags passed the screens, ended up in the FPS and clogged the pipes. The fermented FPS had a notably lower viscosity than the influent FPS and clogging in the pipes for effluent FPS was rare, despite that the Promag W 400 flow meters (Endress & Hauser) had smaller dimensions compared to the pipes. The steel pipes (40 mm in diameter) were equipped with heat tracing and insulation to prevent freezing during the Nordic winter. Nevertheless, a cold spell with temperature as low as -13℃ in February 2021 caused freezing of the pipes and a stop in the reactor operation during two weeks.

4.2.6. Produced gas during fermentation

The reactors were designed to be operated either with an open or with a closed headspace. When the headspace was closed, the effluent gas passed a valve which kept the headspace pressure at 5-10 mbar. The gas flow was measured with Gallus G1 (Itron) and passed a vessel for condensate collection before it was let out 2 m above the platform. The pilot plant was also prepared for online measurement of methane and carbon dioxide in the effluent gas. However, the gas production was too low compared to the requirement of the online meter, and it could not be operated continuously.

Due to the potential of both methane and hydrogen production in the fermentation with closed headspace, the effluent gas was considered as explosive, and security measures were taken. For example, equipment in the security zones were of ATEX classification, and inlet as well as effluent pumps were interlocked to online measurements of temperature in the gas condensate vessels. Grab samples of the headspace gas was taken on three occasions to measure the methane content with gas chromatograph Agi490 (Micro GC).

4.2.7. Recirculation of fermented sludge

From November 2020 to June 2021, the fermented sludge was recycled to the wastewater inlet tank (5.2.1.). As a result, the FPS contained both fermented FPS and fresh FPS. Addition of fermented sludge was tested to evaluate mixing with wastewater and separation in RBF as a method of separating fermented particles from the produced carbon source without substantial loss of carbon source. By recycling the fermented sludge, the SRT became longer and the HRT and the effect on fermentation yield could be assessed (**Paper I**).

4.2.8. Automation

The automation for the plant was quite complex, with some solutions which worked well and could be applied elsewhere. For example, the set flow to the pilot plant was calculated from the signal for the main WWTP flow but normalised to the average flow. The setpoint in the SCADA system was therefore the long-term average flow to the pilot plant.

The pump for dosing of polymer solution to the wastewater required inputs for polymer concentration and maximum pump capacity. The setpoints were dose per m^3 wastewater and per g TSS load, from which the required pump flow was calculated in the control system. Consequently, the calculated dose from the control system was close to the real flow.

Although online flow measurement was not feasible due to the risk of clogging, this setup could give reliable online data for the dosing. The problem with flow measurement was also encountered for the viscous FPS. To control the influent pumping to the fermentation reactors, the more reliable pressure meters at the bottom of the reactors were used to pump in sludge up to the set level.

4.2.9. Operational challenges

Overall, the pilot plant could be operated as intended, although operational problems caused temporary interruptions and trouble. Some of the encountered problems that might be useful for planning of future pilot plants are listed here.

Owing to the two parallel pumps for the influent wastewater, the inflow to the pilot plant could be upheld apart from short periods. In the beginning of the trial, clogging in the sludge effluent pipe caused overflow of FPS from the sludge tank during one weekend. The wastewater inflow was thereafter stopped automatically when the sludge tank reached a set level, to avoid the nuisance of sludge overflow.

The influent, which was expected to be free of rags and stones after screening, also contained unpleasant surprises because of operational problems at the main WWTP screening. From the tank where the influent from the WWTP flowed in, a hose was connected down to a sampling bucket for influent wastewater. The sampler was placed on the floor, and could only be sampled from below. The flow to the sampling bucket was sometimes stopped by rags or stones which entered the valve from the tank. Despite the installation of a coarse grid, the hose needed regular backwashing. Sand and gravel also entered the sampling bucket, which in practice served as a grit chamber.

Even though the inlet pumps were placed upstream of the reject water inlet from the sludge handling at the WWTP, it happened at times that the influent wastewater was contaminated with sludge, due to high flow of reject water full of untreated sludge or reject water. The flocculation tanks were then full of settled sludge and needed to be emptied and cleaned.

Due to the shape of the reactors, the mixers had long shafts. This led to problems during periods with poor performance of the WWTPs screens, when rags entered the pilot plant and ended up in the reactors’ mixer blades. Because of the long shafts, the mixers started to wobble, and the attachment to the reactor top was put under stress. The reactors needed to be emptied, and the rags were removed by long hooks from the top of the reactors. This could only be done in between operational periods as it would otherwise have interrupted the continuous process.

4.2.10. Experimental plan for pilot plant operation

The two reactors R1 and R2 for hydrolysis-fermentation were first operated at the same HRTs of 5 days during a verification period (Table 1) to verify that the process performance was similar under similar conditions. As the aim was to follow the seasonal variations of the fermentation at ambient temperature, one of the reactors was operated at the same retention time of 5 days during one year (**Paper II**). R2 had shorter HRTs of 3 days and 2 days during the assessment of different HRTs in Q4-5. When the fermented sludge was recycled to the inlet, different HRTs and SRTs could be evaluated in Q6-8 (**Paper I**).

Table 1. Experimental periods for the study on fermentation of FPS.

		2020		2021					2022		
		Q1	Q2	Q3	Q4	Q5	Q6		Q7		Q8
R1	HRT; SRT (d)	5;5					3	3; 5	5; 7	6; 8	6; 9
R2	HRT; SRT (d)	5;5			3;3	2;2	3	3; 5	5; 7	6; 8	6; 9
Period		Seasonal variation (R1)						Recirculation of sludge, prolonged SRT (R1, R2)			
		Verification			Different HRTs (R1, R2)						

4.2.11. Batch tests for fermentation and methane production

Batch tests for fermentation of FPS, SPS as well as fermented sludge from R1 (HRT: 5d) and R2 (HRT: 3d) lasted for 7 days. The aim was to quantify and compare the methane production from different sludges during fermentation, and to follow the solubilisation over time. For this purpose, the sludges were divided into two parallel trials: one in gastight reactors for analysis of methane production in an Automatic Methane Potential Test System (AMPTS; BPC Instruments), and one in mixed and covered, but not completely gastight reactors where samples were taken out frequently to follow the fermentation.

pH in the non-gastight reactors was controlled at 5.3 by manual addition of HCl or NaOH once or twice per day. Initial reactor volumes were 300 mL in the AMPTS (in duplicates) and 900 mL in the pH- controlled reactors. During sampling, 40 mL was taken out for pH measurement. Thereafter, the sludge was centrifuged at 4500 rpm for 5 min, and filtered through Munktell filters 110116 (Ahlström Munksjö) and 0.45 µm syringe filters RC 25 Minisart (Sartorious) for analysis NH_4^+-N, PO_4^{3-}-P with ion chromatograph ECO IC and 863 Compact Autosampler (Metrohm) and COD analysis with cuvettes LCK114 (Hach). TS and volatile solids (VS) were analysed by heating at 105℃ during 24 h and thereafter at 550℃ during 2 h.

4.2.12. Calculations

The temperature dependencies of hydrolysis and fermentation were calculated as in activated sludge models (Rieger et al., 2013) and could be adjusted to concentrations in Eq. (1), since the HRT was not varied in a short-term perspective (**Paper I-IV**).

$$\mathrm{VFA} = \mathrm{VFA}_{20} \cdot \theta^{(\mathrm{T}-20°\mathrm{C})} \quad (1)$$

4.3. The pilot plant for enhanced biological nutrient removal

The pilot plant for the novel continuous biofilm process (Cella$^{\mathrm{TM}}$, Veolia Water Technologies) was placed in a container next to the grit chamber at Källby WWTP, close to the ICU pilot plant for primary treatment. Wastewater from the ICU pilot was pumped to the pilot plant for enhanced biological removal of nitrogen and phosphorus with biofilm on bio-based support material.

During the second operational period for the pilot plant, August 2023- 2025, the primary filtration was operated in the ICU pilot, without the fermentation reactors (Fig 2, **Paper III-IV**). Since the fermentation yields had been assessed during the first operational period, it was feasible to mimic the fermentate addition by adding a mixture of acetate, propionate and butyrate (**Paper III-IV**).

4.3.1. Drum filter

The wastewater from the primary filtration was pumped at a constant flow to a 1000 µm drum filter HDF801 (Hydrotech). It was included as safety measure to protect the subsequent biofilm reactors from particles which would have accumulated in the reactors. The drum filter was operated in automatic mode, with backwashing at high water level inside the drum. Drinking water was applied for backwashing, but the flow was negligible compared to the wastewater flow. The filter cloth was inspected weekly and cleaned manually if needed. No impact of the drum filter on the wastewater was observed when the RBF was in operation day 23-460 (Table 2. When the RBF was put out of operation day 460 to evaluate the process with untreated wastewater, it was seen that the drum filter removed more COD than was expected, and the drum filter was therefore omitted as well.

4.3.2. The novel continuous biofilm process

In the Cella™ process for BNR (Fig. 2, Fig. 5, **Paper III-IV**), the wastewater flowed continuously through the process with two alternating reactors (R1, R2). Either the water flowed through R1, into R2 and then out, or the reversed way: into R2, through R1 and then to the outlet. The flow could also go through one of the reactors only, to optimise the effluent quality (Fig. 5b). The operational cycle (Fig. 5b) was either controlled with set times for the different phases, or operated with automatic control of the phase shifts. The cycles of different phases allowed for aerobic conditions (nitrification and phosphate uptake by PAOs), anoxic conditions (denitrification and phosphate uptake by denitrifying PAOs) and anaerobic conditions (substrate uptake by PAOs).

The two reactors with a volume of 0.6 m^3 each (height 0.8 m) were equipped with the novel bio-based support material for biofilm biomass (AnoxK™C). New support material was added in the beginning of the study. Different pumps were used to feed R1 and R2, and tree-way valves were set to control the flows out from each reactor to either R2/R1 or to the outlet. Hubgrade (Krüger A/S, Veolia Water Technologies) was applied during the periods of automatic phase control. One of the reactors was equipped with online measurements (Hach) of NH_4^+-N, NO_{2+3}^--N and PO_4^{3-}-P (Fig. 2). Both reactors were equipped with online meters for dissolved oxygen (DO) and redox potential (LDO2 and pHD respectively, Hach). Due to the scale, control of aeration is challenging, and different control modes for the airflow were applied during the study.

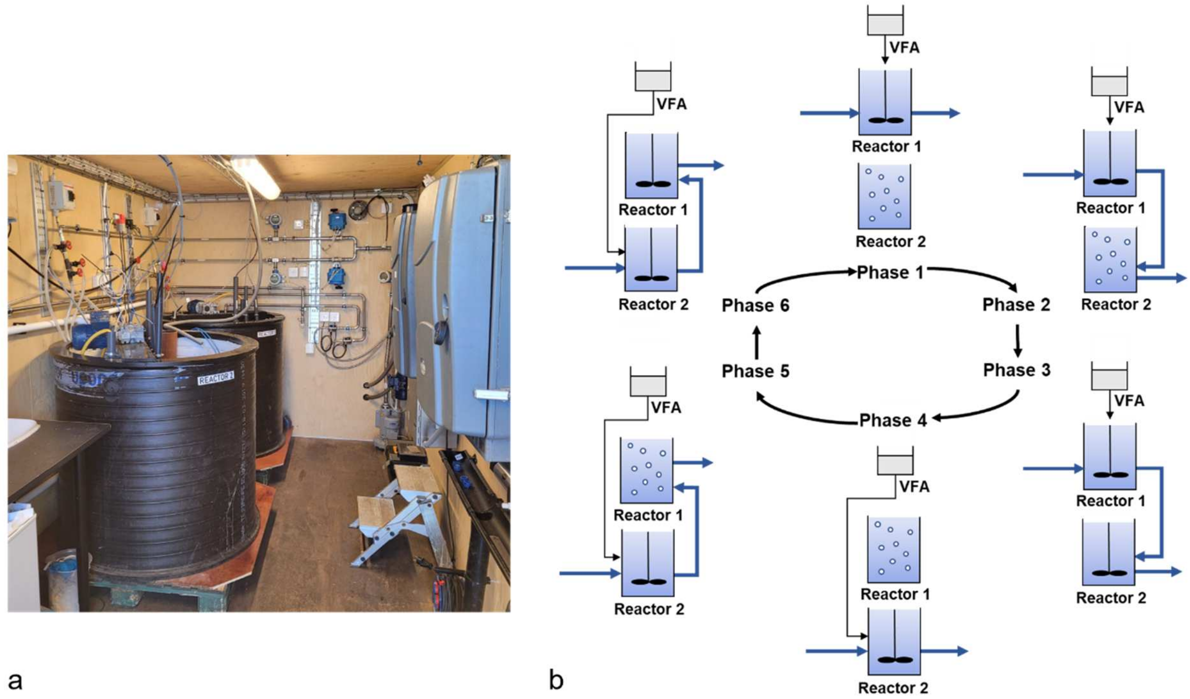

Figure 5. a) The two alternating pilot reactors the CellaTM process. b) The different phases of one operational cycle in the alternating biofilm process, showing the flows, aeration and dosage of volatile fatty acid (VFA).

4.3.3. VFA dosage

VFA was dosed to the reactor which received inflow (Fig. 5b), when the primary filtration was in operation. The composition of the VFA mixture was set from the yearly averages for acetate and propionate. Butyrate was added, representing the summed COD fractions of butyrate, iso-valerate and valerate as yearly averages. The VFA addition (mg COD/L wastewater) had also been established (**Paper I**), and the temperature correlation curve was used to calculate the addition.

For the VFA addition to the biofilm EBPR process at pilot-scale (**Paper III**), the results of the VFA increase were recalculated to VFA-COD for the wastewater temperature dependence VFA_{20}=43 mg VFA-COD/L with Θ =1.10±0.01. In addition, the VFA increase was calculated from the flows of FPS and wastewater, and the seasonal variation in flow to the WWTP was therefore considered in the estimation which made the temperature dependence with respect to the wastewater even higher. The VFA dose was adjusted weekly to the average wastewater temperature.

The VFA was dosed either with a continuous flow (constant), or limited to redox below a set-point (redox) during the different periods of the study (Table 2). The redox-based control for VFA dosage was applied to direct the carbon source to the anaerobic periods, allowing for a higher VFA dose during these phases.

4.3.4. Sampling of effluent

The sampling of effluent from the biofilm reactors was optimised over time to obtain representative composite samples for both the particulate and the soluble fractions (**Paper III**).

Since the effluent also contained detached biomass including PAOs, phosphate could be release during the sampling time. At a full-scale WWTP, the equalising volume prior to post-treatment would not be large enough to enable phosphate release, and this effect was therefore undesirable at the pilot plant.

In the start-up period, samples were withdrawn from the upper phase of the effluent trap, thereby probably unaffected by phosphate release. The particulate fractions, on the other hand, were not representative with this method, and a sampling bucket was installed on day 143 to allow for improved measurement of the particulate fractions. Despite the cold storage of the collected samples in the fridge, it was noticed that the phosphate concentration was overestimated with this method due to phosphate release in the bottom of the sampling bucket, which was shaken prior to sample collection and filtration. The sampling was evaluated day 299-359 with 20 samples taken both from the upper phase with a syringe before shaking the sample, and filtration of the shaken sample as was done before. This evaluation showed that the sample collection did not affect NH_4^+-N (P=0.97). The effluent phosphate was 0.2±0.2 mg PO_4^{3-}-P/L higher in the shaken sample, with a relatively lower impact on values > 2 mg PO_4^{3-}-P/L (Fig. 6). Results from sampling of the effluent supernatant for filtration and analyses of soluble compounds was therefore applied from day 299 to avoid a slight overestimation of PO_4^{3-}-P.

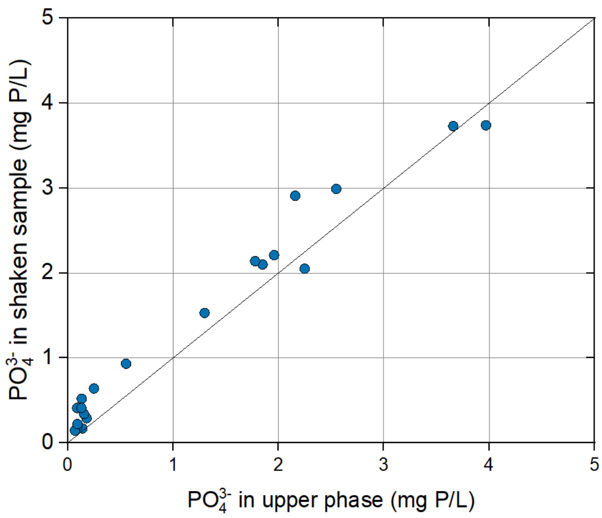

Figure 6. Phosphate concentration in the shaken sample versus PO_4^{3-}-P concentration in the upper phase of the sampling bucket.

4.3.5. Experimental plan

The influent wastewater to the biofilm BNR was filtered, and VFA addition was applied during the first 1.5 years, Q1-Q6 (Table 2). Control of VFA addition based on redox was started Q4 (**Paper III**). The importance of the primary filtration and VFA addition was tested by omitting it Q7-8 (**Paper IV**).

In **Paper III**, the time was divided into operational periods A-F, with different modes of phase control and different strategies for dosage of VFA (Table 4). Period F/PII was used for evaluation of strategies for VFA dosing in **Paper III**, and the same period was used for comparison of process performance and BNR effluent particle separation in **Paper IV**.

Table 2. Experimental periods for the study on BNR with and without primary filtration and VFA addition.

	2023		2024				2025	
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Period	A	A-C	C-D	D	E	F/PI	PII	
Paper			**III**			**III/IV**	**IV**	
Primary filtration								
VFA addition								
Redox-control of VFA								
No primary treatment								

4.4. Limitations

The applied methods were chosen to provide results enabling comparisons based on time, temperature, between different operational modes and different modes of control. Limitations of different kinds were associated with the methods, of which some are discussed below.

Calculations and simulations were applied to assess the impact of primary treatment on biological wastewater treatment with activated sludge (**Paper I**). The simulations of the activated sludge process were performed with benchmark simulation model no. 1 (BSM1; Jeppsson et al., 2006) as the basis. The activated sludge model no 1 (ASM1; Henze M. et al., 1987) applied in BSM1, does not include EBPR or phosphorus, and the effluent nitrogen of 16 mg/L is high compared to the legal requirement for WWTPs within the European Union (EU Directive 3019, 2024). Even with the resizing of the volumes according to the widely used DWA design standard (DWA, 2016) with a set effluent of 7 mg N/L at 10°C, the effluent nitrogen was higher: around 13 mg N/L. BSM1 was chosen since it is well defined and widely applied for simulations in the literature, but it should be seen more as a means for relative comparisons rather than representative of a real process. Similarly, the energy analysis was also made in relative terms, since the electricity needed in sludge handling and anaerobic digestion was omitted for all cases.

The pilot plant offered the opportunity to test processes under conditions similar to those of a full-scale plant, but it also had some inherent limitations. Since the temperature and the wastewater composition and flow varied over time, the comparisons between periods and different operational modes were less straightforward compared to a lab-scale study. This problem was addressed by having two parallel fermentation reactors (**Paper II**), allowing to test different HRTs in parallel at the same temperature and influent composition. Variations in environmental conditions occurred to the same extent in both reactors and the differences in performance at different HRTs could be assessed by comparison of the two reactors. Although operational disturbances (described in 4.2.9) may have affected the results, they are often encountered in pilot-scale studies.

The biological nutrient removal (**Paper III-IV**) was tested in two reactors, but only one process line. Therefore, the redox-based control of VFA dosage was tested intermittently in period F, shifting between the control modes with an interval of a few days, to avoid the effects of changing temperature and influent characteristics. With this method, the wastewater samples and online measurements were affected not only by the current method for dosing, but also on the storage products in PAOs which had been accumulated during the last day. Since the time for which the values could have been affected by the previous dosing conditions was difficult to set, the evaluation periods were the same as the periods of the control strategy.

Due to limitations in the project, measurement of VFA in the influent wastewater was not conducted during the study of BNR, although it could have been valuable. It would have been complicated to operate the fermentation and to remove the fermented particle at the pilot plant, as there was only one filter and refermentation of sludge should be avoided. The dosed VFA mixture could most likely give a similar effect on EBPR as the fermentate, but contained no other soluble COD (SCOD), no nutrients or small particles which could have affected the outcome. The storage of polyphosphate, PHA and glycogen by PAOs and glycogen-accumulating organisms (GAOs) in the biofilms would have been interesting to study and compare for different VFA dosing strategies, but was out of scope of this project.

5. Results and discussion

The results from both pilot plant studies (**Paper I-IV**) are presented and discussed in this section, with the aim of giving a comprehensive overview and linking the different periods and papers.

5.1. Primary filtration

The chemically enhanced primary filtration was studied in detail during the first operational period when the FPS was fermented (**Paper I-II**). The removal efficiencies of TSS and COD were assessed from 24-h composite samples, and the removal of particles in different size fractions was studied. The results from this long-term experiment were also used to model the particle removal and provide tools for future studies and to facilitate full-scale operation.

5.1.1. Particle separation

During the first year of operation, the influent TSS was varying both in the composite 24 h samples and also during the days (Fig. 7a, **Paper I**). The flow-proportional operation, with a daily pattern which was matching the main WWTP, gave higher TSS in the filtered wastewater during late mornings until midnight and lower during the early hours (Fig. 7a). Since 24 h composite samples were representative for the whole day, they were suitable for evaluation.

The concentrations of filtered COD and ammonium nitrogen were similar in the influent and in the filtered wastewater, and this was further strengthened by P- values > 0.2 in pairwise student t-tests (**Paper I**). This supports the validity of the methods for sampling as well as chemical analysis. It does also suggest that there was no significant degradation of COD due to oxygen intrusion during the flocculation or filtration.

The mean removal efficiency of TSS was 64 ± 10% (**Paper I**), and thus higher compared to the removal efficiency in the primary settlers at the main WWTP (Fig. 7b), which was similar to the 50-55% reported by others for primary sedimentation (Amerlinck, 2015; Patziger and Kiss, 2015). The RBF performance was in line with previous studies of chemically enhanced primary filtration (Franchi and Santoro, 2015; Rusten et al., 2017), which was higher than the 20-50% removal obtained without polymer addition (Franchi and Santoro, 2015). Corresponding removal efficiencies of total phosphorus and total nitrogen were 8.5 ± 7.8% and 18 ± 8%, respectively.

The separated FPS (**Paper II**) had a TS of 4.5 ± 0.6%, which is thicker compared to SPS (Paulsrud et al., 2014), and a COD to VS ratio of 1.47± 0.35 g COD/g VS, which is in the range of what has been reported for SPS (Ucisik and Henze, 2008).

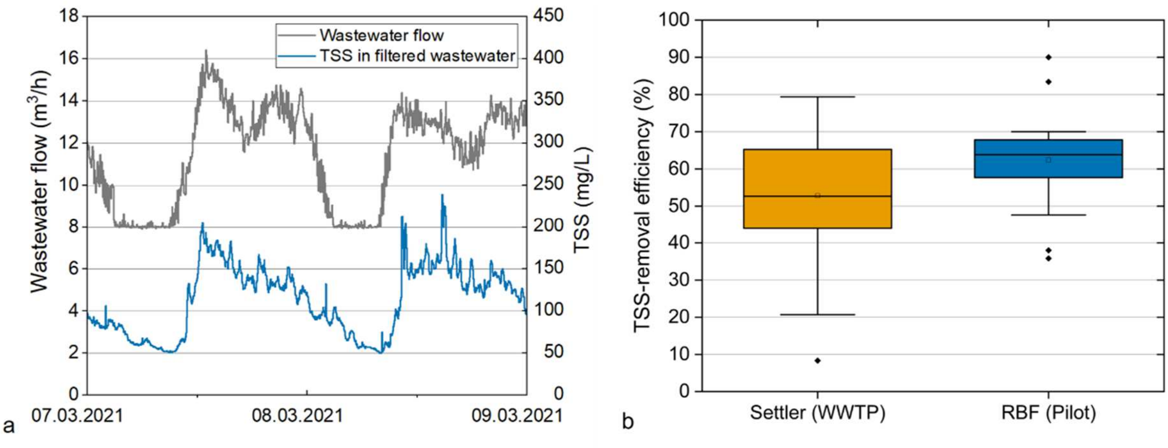

Figure 7. a) Online data of influent wastewater flow and filtered wastewater TSS during operation without recirculation of fermented sludge 07.03.2021-09.03.2021. b) Mean TSS removal efficiencies in the primary settler at Källby WWTP and in the RBF filter at the pilot plant.

5.1.2. COD size fractionation and wastewater characteristics

The flocculation with polymer and the build-up of a sludge mat on the filter allowed for removal of much smaller particles than the pore size of 350 µm. Fractionation of COD in different particle sizes in the influent wastewater and after the RBF showed that particles larger than 10 µm were removed efficiently in the primary filtration (Figs 8-9, **Paper I**). For the 11 characterised samples, the overall COD removal efficiency was ~50%, while the removal of particles >10 µm was ~85-95% (Fig. 8). Based on 101 24-h samples over a year, the COD removal efficiency in the primary filtration was 44±9% (**Paper I**).

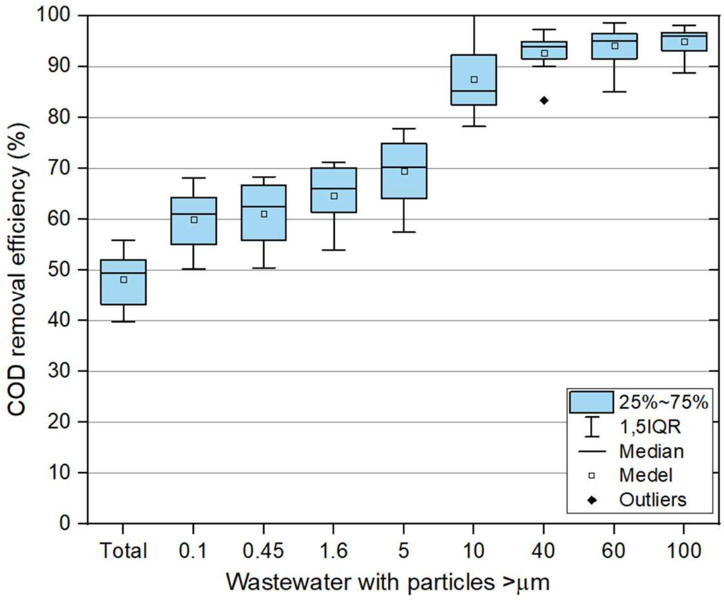

Figure 8. COD removal efficiency of particles in different size fractions after RBF filtration, derived from COD characterisation of influent and filtered wastewater during one year.

Characterisation of COD was also performed to study the shift in particle size from influent wastewater to filtered wastewater (BNR influent) and to BNR effluent (**Paper IV**). The influent wastewater composition was similar to the fractionation in other studies (Razafimanantsoa et al., 2014; van Nieuwenhuijzen et al., 2004), meaning that the results can be applicable for other WWTPs. The overview of measurements from both **Paper I** and **Paper IV** (Fig. 9) showed that the COD size distributions for influent wastewater were comparable in **Paper I** and in **Paper IV** during period I (PI) with primary filtration. The samples in period II (PII) without primary filtration in **Paper IV** displayed higher and more variable COD content. However, the more frequently analysed samples for chemical parameters (Table 3) showed that the total COD concentrations were not dissimilar during **Paper I** (PII) in **Paper IV,** while the SCOD differed slightly. The variable COD distribution in PII without filtration could therefore be an effect of the fewer samples. It can be observed that the filtered wastewater composition shifted to soluble COD and smaller particles <10 µm (Fig. 9). The composition of the effluent from BNR is discussed in 5.3.8. and in **Paper IV**.

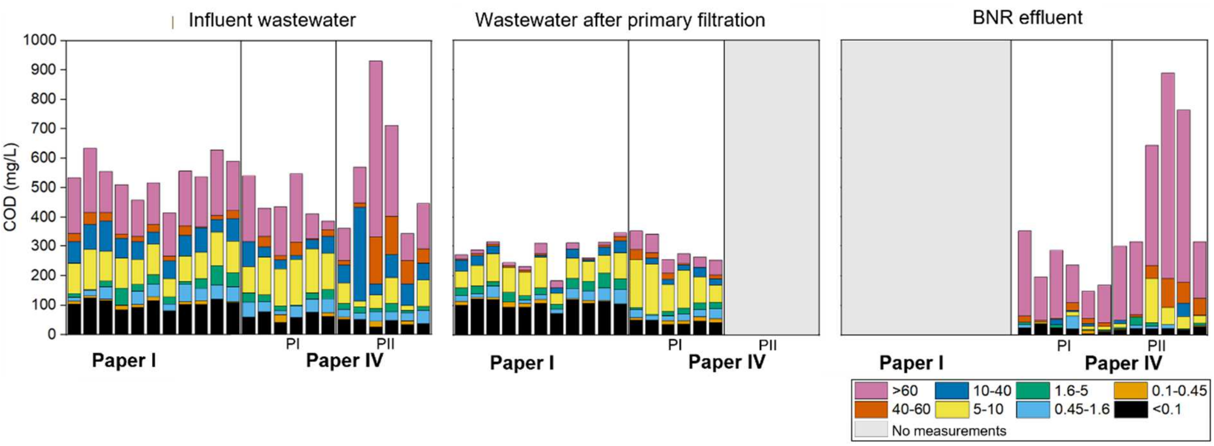

Figure 9. COD characterisation of influent wastewater, wastewater after primary filtration with rotating belt filter, and BNR effluent after biological nutrient removal, derived from measurements shown in **Paper I** and **Paper IV** (period I, PI, with primary filtration and period II, PII, without primary filtration).

Table 3. Chemical parameters for influent wastewater in **Paper I** and **Paper IV** (Period II, PII, without primary filtration) shown as mean± standard deviation (No.).

Parameter	Unit	Paper I		Paper IV (PII)	
Influent Ntot	mg/L	55.7±9.6	(103)	54.2±8.0	(14)
Influent NH_4^+	mg N/L	39.0±7.1	(102)	34.0±4.1	(14)
Influent Ptot	mg/L	6.7±1.3	(102)	7.7±2.4	(14)
Influent PO_4^{3-}	mg P/L	-	-	3.7±0.7	(14)
Influent TSS	mg/L	242±60	(102)	381±112	(14)
Influent COD	mg/L	528±110	(102)	511±102	(14)
Influent soluble COD <1.6 µm	mg/L	146±29	(30)	116±47	(14)
Influent COD <20 µm	mg/L	225±42	(99)	-	-

5.1.3. A model for the separation

An exponential model for TSS removal efficiency in RBF filtration without chemical addition has been proposed (Behera et al., 2018), and rendered a good fit versus the influent TSS concentration (Da Ros et al., 2020). This exponential model proved to be less useful in our study (R^2=0.46; **Paper I**). Instead, a simpler model of the TSS removal efficiency in mg/L showed a strong linear correlation (R^2=0.91) to the influent TSS (**Paper I**). In general, a simpler model is preferable to a more complex model and is more likely to be applied in practice. Compared to only using the mean TSS removal efficiency for calculation of the effluent TSS, the linear model has the advantage of crossing the x-axis at a level which better predicts the effluent values for low influent TSS (Fig. 10). The results are highly variable for the filtered wastewater, and the poor fit of the models should be considered. Nonetheless, the model can be useful for calculation of mass balances and amount of produced FPS, and for estimations of the TSS and COD in the filtered wastewater.

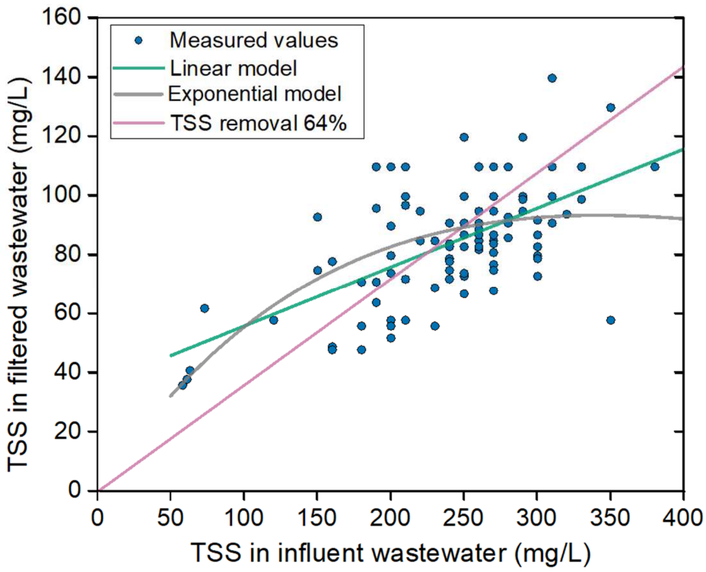

Figure 10. Measured values of TSS in filtered wastewater versus TSS in the influent wastewater. Model predictions with linear model, exponential model and percentual removal are shown.

5.2. Fermentation of filter primary sludge

The fermentation of FPS at ambient temperature was studied with focus on the yields of VFA and SCOD, VFA distribution, nutrient solubilisation and microbial community assembly.

5.2.1. Impact of retention time

The fermentation of FPS was stable, and VFA was produced at the tested HRTs of 2-5 days (**Paper I-II**). As the two fermentation reactors were operated at different HRTs , and also with different SRT due to recirculation of fermented sludge back to the influent (Fig. 11a), the impact of both HRT and SRT could be evaluated (**Paper I**). An increase of the HRT from 2 to 3 days did not result in a notable difference in VFA yield (mg HAc-eq/ g VS), whereas an HRT of 5 days increased the yields of VFA and SCOD with ~40 and 50%, respectively compared to 3 days HRT (**Paper II**). If volumetric yield is the goal, a short HRT would still render more carbon source (**Paper II**). The increase in flow with 2-3 days HRT gave a higher volumetric productivity compared to 5 days HRT.

When recirculation of the fermented sludge was applied, the mean SRT of the solids was increased. The already degraded substrate was not as productive as the new sludge, leading to an overall decrease of 65-70% in VFA yield (Fig 12). Since the yield from refermented sludge proved to be low, it is not recommended to separate the solids and hydraulic retention time. There are no directly comparable studies with FPS fermentation in continuous reactor operation at different retention times. It has been seen in batch tests that the SCOD yield can increase with ~50% with four days HRT compared to two days (Bahreini et al., 2020b), but the impact from SRT had not been shown previously.

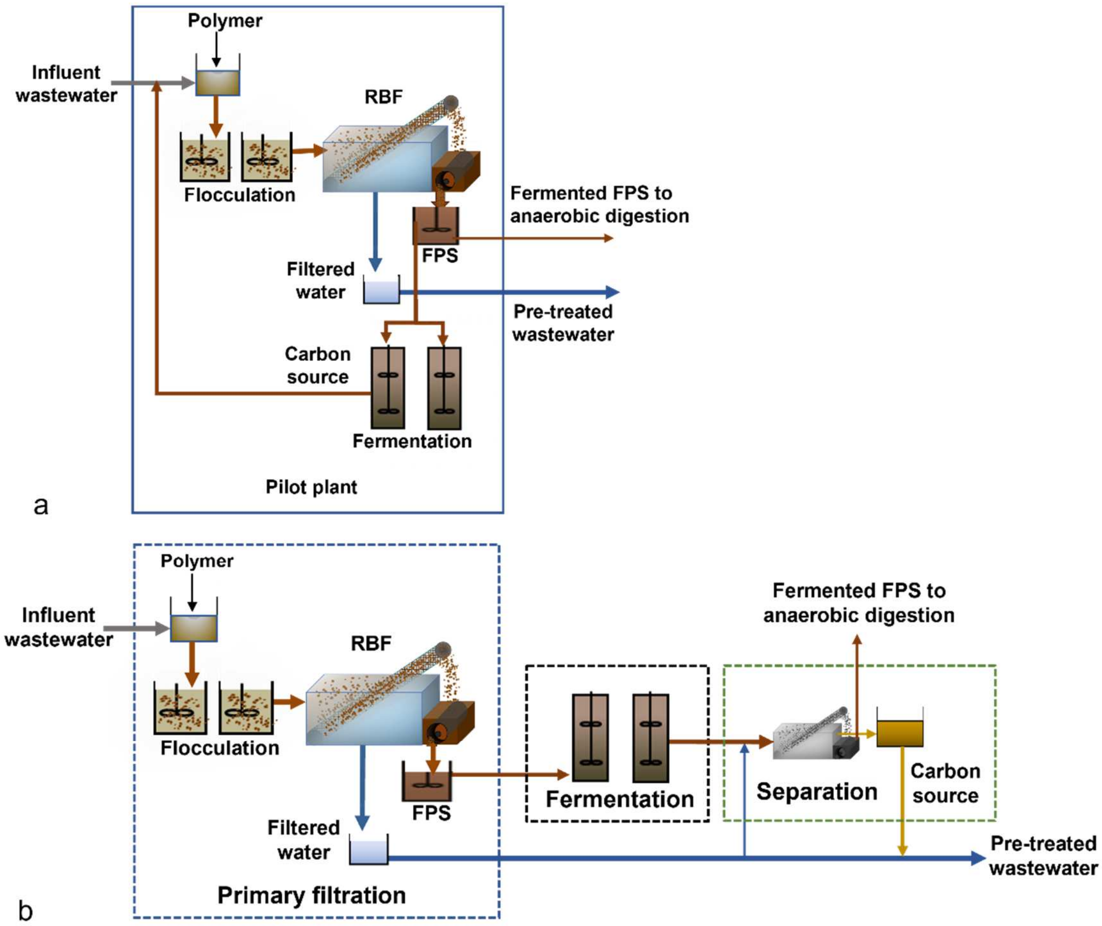

Figure 11. Overview of primary filtration with fermentation of filter primary sludge (FPS) and separation of fermented FPS for increase of carbon source to the wastewater a) at the pilot plant, b) as proposed for full-scale installations.

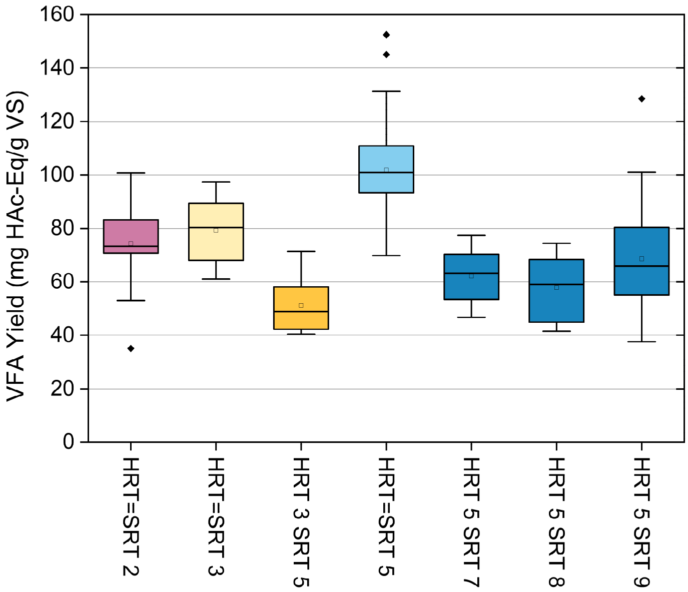

Figure 12. Volatile fatty acid (VFA) yields in filter primary sludge fermentation normalised to 20℃ for different hydraulic and solids retention times (HRT and SRT).

5.2.2. Seasonal variations in yield

Knowledge about the seasonal variations in primary sludge fermentation is useful when assessing the impact of planned installations, and when operating and evaluating existing processes. The yields of SCOD and of VFAs were clearly seasonally dependent (Fig. 13a, **Paper II**), as the ambient temperature in the fermentation reactors varied between 16-29°C. The VFA yield ranged from 110-270 mg VFA-COD/g VS_{in}, with average over the year of 242±40 mg COD/g VS_{in} at 5 days HRT. The temperature dependency (Eq. 1) could be estimated to Y_{20}=172±4 mg VFA-COD/g VS_{in} and ϴ=1.033±0.005 for the reactor temperature (Fig. 13a, **Paper II**).

The hydrolysis seemed less influenced by temperature, and SCOD displayed a weaker correlation of Y_{20}=231±7 mg COD/g VS_{in} and ϴ=1.020±0.006 (R^2=0.20; Fig. 13a). The corresponding ϴ for hydrolysis in wastewater calculated from constants for 10 and 20°C in ASM2d is 1.04 (Henze et al., 1999), and the calculated ϴ from FPS fermentation at different temperatures (Bahreini et al., 2020b) gives a similar value, which strengthens the validity of the results. Notably, the temperature correlation for primary sludge fermentation at ambient and transient temperature had not been shown before. To the best of the author's knowledge, no other study with long-term operation of side-stream primary sludge fermentation at ambient temperature has been published to this date.

The yields of soluble COD and VFA in the study were comparable to previous studies of FPS fermentation, although they were performed at higher and constant temperatures of 37°C (Crutchik et al., 2018; Da Ros et al., 2020). In conclusion, the results show that FPS fermentation at ambient temperature can be a viable alternative for carbon source production.

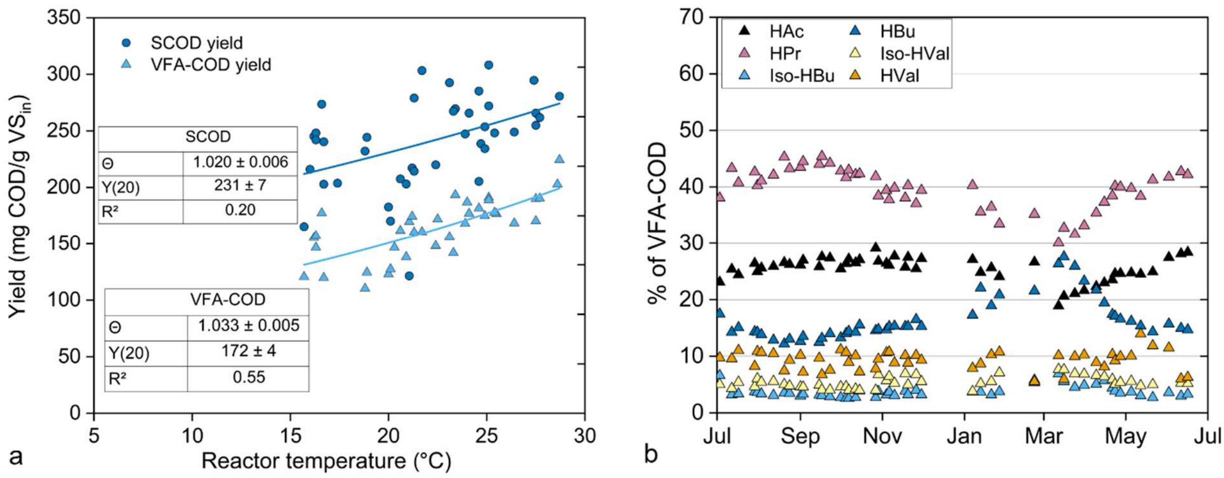

Figure 13. a) Yields during one year of operation at 5 days HRT of soluble COD (SCOD) and VFA-COD versus reactor temperature, b) Seasonal variation in VFA-COD distribution at 5 d HRT .

5.2.3. VFA composition

The mean VFA distribution for one year of fermentation at 5 days HRT was (as % of VFA-COD): acetic acid (HAc) 26±1%, propionic acid (HPr) 41±3%, butyric acid (HBu) 15±2%, iso-butyric acid (Iso-HBu) 3.4±0.8%, valeric acid (HVal) 9.3±1.4% and iso-valeric acid (Iso-HVal) 5.0±0.8%. The VFA distribution varied over the year (Fig. 13b, **Paper II**), with lower fractions of HAc and HPr at lower temperatures (Fig. 13b). The VFA distribution in previous studies has shown different impact of increasing temperature, with either increasing or decreasing fractions of longer-chained VFAs (Cokgor et al., 2009; Ferreiro and Soto, 2003; Huang et al., 2021). Interestingly, the productions of acetate and propionate displayed strong temperature correlations of Θ=1.042±0.007 and Θ=1.054±0.007 respectively, while no correlations could be found for the longer chained acids (**Paper II**). The underlying mechanism for the changes in the VFA distribution at different temperatures could thereby be shown in the study.

It has been observed that the type of carbon source may influence EBPR and denitrification (Elefsiniotis and Wareham, 2007; Moser-Engeler et al., 1998; Vargas et al., 2011), which empathises the relevance of increased knowledge of the temperature dependencies in VFA distribution. In this study, the temperature dependencies in primary sludge fermentation at ambient temperature were shown for different VFAs.

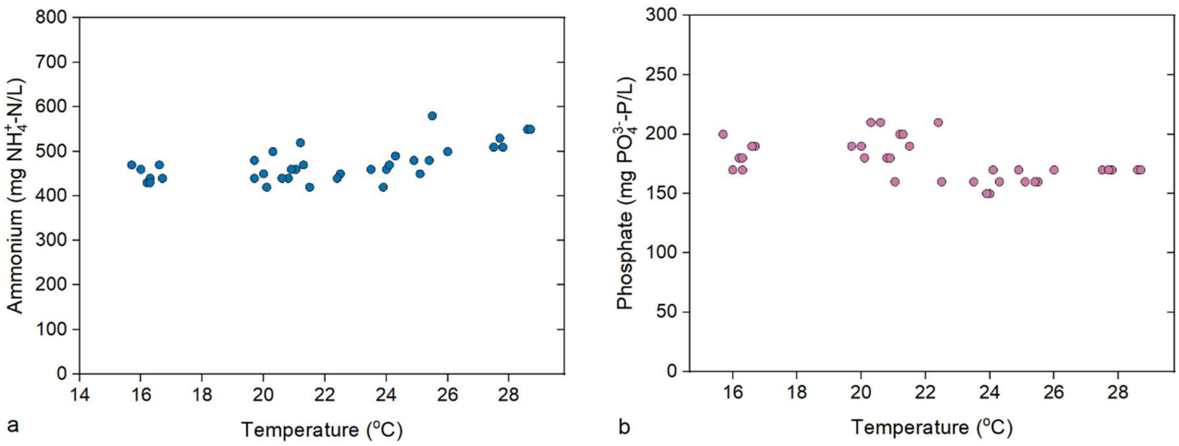

Figure 14. Concentrations versus the reactor temperature for a) NH_4^+-N, b) PO_4^{3-}-P.

5.2.4. Nutrient solubilisation

The solubilisations both nitrogen and phosphorus were rather stable during the year, with no clear temperature dependencies (Fig. 14, **Paper II**). This resulted in a pattern where the ratios of SCOD to both ammonium and phosphate were higher at higher temperatures, ranging from 20-30 g SCOD/g NH_4^+-N and 40-80 g SCOD/g PO_4^{3-}-P (**Paper II**). Even though the ratios were still higher than what is required for denitrification and EBPR, the carbon source availability is lower in winter when it is needed the most. This seasonal variation had not been highlighted previously, although it would impact nutrient removal in WWTPs with primary sludge fermentation.

5.2.5. Microbial community

During the first part of the pilot trial, the two reactors for hydrolysis-fermentation were operated at the same HRT of 5 days, which resulted in similar microbial communities between them (**Paper II**). When the HRT in R2 was shortened to 3 days and 2 days, the dissimilarity between the microbial communities in the reactors rose. Still, the difference between influent sludge and both reactors was distinct, and the selection towards bacteria within *Bacteroidota* was clear (Fig. 15, **Paper II**). There was a seasonal pattern for the bacterial community in the influent sludge while the pattern in R1 with 5 days HRT seemed to be impacted by both season and a continuous change.

Among the few other long-term studies found on seasonal impact on primary sludge microbial community composition, one is for primary sludge as influent to anaerobic digestion (Wang et al., 2018), where *Proteobacteria*, *Firmicutes* and *Bacteroidota* were found in high abundance and with a seasonal pattern, as in this study. The same genera were found in fermentation at mesophilic temperature and 3 days HRT (Maspolim et al., 2015). Furthermore, it has been observed that both FPS and SPS fermentation can result in similar microbial composition (Brison et al., 2022). An out selection of *Proteobacteria* in fermentation was shown in this study (Fig. 15, **Paper II**), analogous to what was seen by others (Brison et al., 2022; Maspolim et al., 2015). This study could thereby both confirm previous findings and contribute with new knowledge regarding the impact from temperature and HRT on the bacterial selection in fermentation of primary sludge.

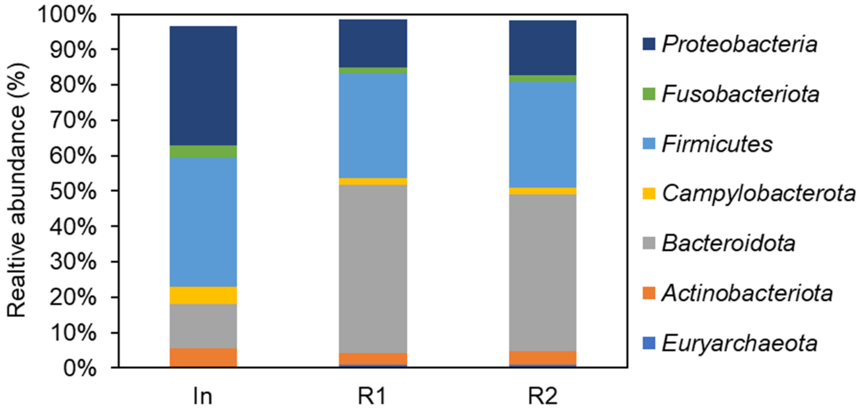

Figure 15. Average composition over time at the phylum level of phyla exceeding 1% average relative abundance in influent and in fermentation reactors R1 and R2 (Figure S7, **Paper II**).

5.2.6. Batch trial: solubilisation and methane production

A batch trial of fermentation was conducted with SPF, fermented FPS and SPS as substrates. The results show the yields of the substrates, and the methane productions which were measured in parallel batch-tests.

A further extension of the fermentation time could render more SCOD from the sludge, both from R1 with 5 days HRT and from R2 with 3 days HRT (Fig. 16a). After additionally 3 days, the SCOD seemed to level out, or to be consumed, meaning that an increase of the HRT in the fermentation up to 8 days could give an increased yield of carbon source. A comparison between SPS and FPS indicated that the soluble COD yield was comparable during the first two days, but higher for FPS after 3 and 4 days. After 7 days, the SCOD yield was decreased, and substantially lower for SPS compared to FPS. The corresponding hydrolysis constants were 0.04 /d for SPS and 0.07 /d for FPS. Other studies have reported both higher VFA yield for FPS compared to SPS (Brison et al., 2022) and lower yield (Bahreini et al., 2020a). Increased retention time for R1 and R2 beyond 4 days did not result in a major increase of phosphate and ammonium (data not shown).

In fermentation, a fraction of the COD is transformed to hydrogen gas (Batstone et al., 2002), which can be further processed by methanogens and lost as a methane emission unless it is recovered or destroyed. The methane productions from SPS and FPS at day 3 accounted for 21.5 and 16.8 mg COD/g VS_{in} (Fig. 16b), or 7% and 11% of the produced SCOD, respectively, which is in the same level as previously reported values (Eastman and Ferguson, 1981; Kaspar and Wuhrmann, 1978; Maspolim et al., 2015). In conclusion, the results indicate that fermentation of FPS may give higher SCOD potential and lower methane emission compared to SPS.

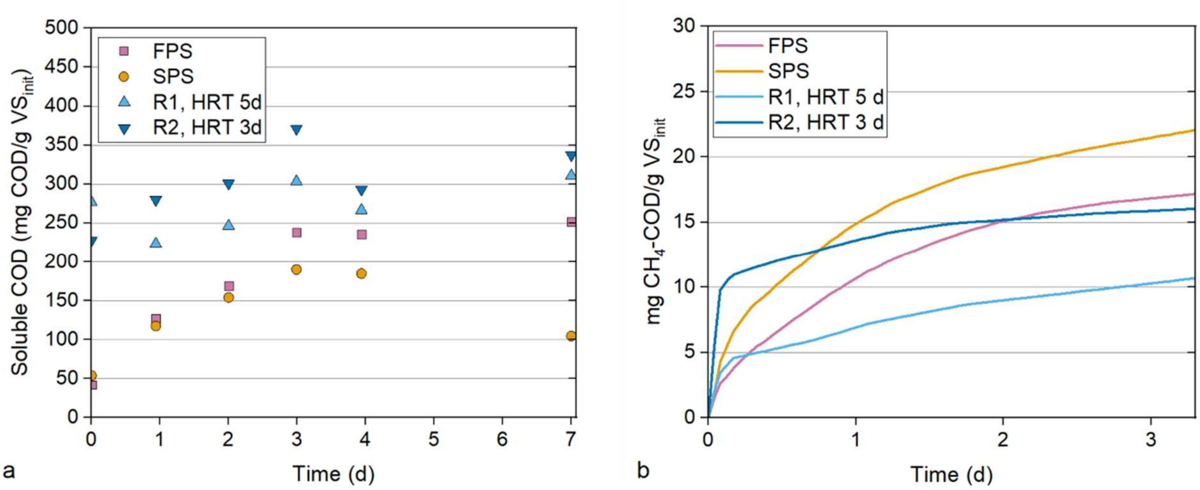

Figure 16. a) Soluble COD during batch trial with filter primary sludge (FPS), settler primary sludge (SPS) and fermented FPS at 3 and 5 d HRT. b) Produced methane during from fermentation in batch mode (mean of duplicates).

5.2.7. Gas and methane production in the pilot plant

Grab samples (no.=7) of the headspace gas from the reactors during the period with extended SRT of 9 days and HRT of 6 days in the reactors resulted in 13-22% CH_4, and the remaining part CO_2. According to the online analyser, the CH_4 fraction was 17-30%. Similar gas composition of 20% CH_4 has been reported for 3 days HRT (Eastman and Ferguson, 1981). During the same period, the gas production was 0.03-0.05 $Nm^3/(m^3, d)$. In October-November 2021, R2 was operated as a closed reactor with an HRT of 3 days, which resulted in a higher volumetric gas production of 0.6-0.8 $Nm^3/(m^3, d)$. Although the methane production was low, it is recommended to cover the reactors for hydrolysis-fermentation to avoid odour and to reduce climate impact. It has been highlighted that methane emissions from anaerobic conditions in EBPR can have an environmental impact (Högstrand et al., 2024).

5.2.8. Separation of fermented particles

In studies where the VFAs as separated from the sludge by thickening, the losses of VFAs have been considerable (Bahreini et al., 2021; Canziani et al., 1996; Christensen et al., 2022). The novel method of mixing the fermented sludge with wastewater to retrieve the VFAs in the liquid phase before separating the fermented particles (Fig. 11) proved to be feasible (**Paper I**). Although the TSS removal efficiency in RBF filtration was slightly lower with this method (61±14%), the gain was to avoid loss of produced VFAs and make full use of the fermentation process.

The pilot plant setup enabled testing of the separation method, but as discussed in 5.2.1., the VFA yield from the already fermented particulate fraction was lower compared to FPS from influent wastewater. At a full-scale implementation, it is therefore recommended to apply a side-stream separation of fermented solids, and to direct this filter sludge to anaerobic digestion (Fig. 11b, **Paper I**).

The wastewater with high VFA content retrieved from the separation may be stored for a short period before it is used as carbon source for EPBR and/or denitrification. The advantage of the side-stream configuration is the possibility for carbon management in the process, to direct the carbon source to where and when the need occurs in the BNR.

5.2.9. Increase of VFA owing to fermentation and its temperature dependency

The increase in wastewater VFA concentration owing to addition of fermentate was calculated to 11-25 mg HAc-eq/ L with fermentation at ambient temperature and 5 days HRT, assuming no loss of VFA in the separation of fermented solids (**Paper I**).

The VFA concentrations in the influent wastewater were too low to be measured as separate acids, but titration could provide results which made it possible to relate the VFA addition to the background concentration. The influent wastewater displayed a strong temperature dependency for the background VFA concentration of VFA_{20}=31±1 mg HAc-eq/L and Θ=1.12±0.02 (**Paper I**). Although the VFA in the influent wastewater affects the BNR, the seasonal variations and temperature dependency has not been shown before to the best of the author's knowledge. The VFA production in the sewer grid and the composition of primary sludge is site specific for WWTPs, but the results in **Paper I** and **Paper II** are still interesting as references for researchers and process engineers.

The VFA increase owing to FPS fermentation was Y_{20}=24±1 mg HAc-eq/L and Θ=1.08±0.01 based on the wastewater temperature (**Paper I**). The advanced primary treatment can potentially nearly double the VFA compared to the influent concentrations, and is comparable to the VFA increase reported for in-line fermentation in primary settlers (Bouzas et al., 2007; Hey et al., 2012).

5.3. Enhanced biological removal of nitrogen and phosphorus

5.3.1. Overview

Nitrogen removal and EBPR were studied for the novel biofilm process with two alternating reactors (Fig. 5) during two years of pilot plant operation. The first 460 days, primary filtration and VFA addition was applied (Table 4), to represent a primary treatment with removal of particulate carbon and FPS fermentation for production of VFA as carbon source for BNR (**Paper III**). The VFA dose was adjusted to follow the temperature dependency for fermentation at ambient temperature. The VFA was either added continuously to the reactor which received influent wastewater or only at low redox for uptake during anaerobic conditions. During the last operational period, no primary treatment or VFA addition was applied in order to assess the impact on nitrogen and phosphorus removal (**Paper IV**). Days 460-504 were omitted from the study due to the deviating influent characteristics (4.3.1.). It can also be noted that the temperature, HRT and influent concentrations varied over time. Periods F/period I (PI) and period II (PII) were more similar in this respect, which was a prerequisite to study the difference between the process with and without primary filtration and VFA addition.

Table 4. Operational modes for the biofilm process during different periods, including chemical parameters for influent and effluent wastewater.

	Primary filtration and VFA addition						No primary treatment
Day	0-111	112-135	136-211	211-279	280-380	381-460	504-550
Period	A	B	C	D	E	F/PI	PII
Paper			**III**			**III, IV**	**IV**
Cycle control	Set	Automatic	Set	Automatic	Set	Set	Set
VFA dosage strategy	Constant, with temp	Constant, with temp	Constant, with temp	Constant, with temp. redox from d 240	Redox, varying dose	Redox /constant, with temp	-
Temperature (°C)	17.7±2.0	13.8±1.4	14.4±1.1	16.6±2.6	21.6±1.6	19.0±1.9	18.5±1.3
HRT (h)	5-14	13.3±1.3	11.5±2.4	12.8±2.6	14.2±1.7	15.7±2.2	15.3±3.3
Number of samples	38	8	26	24	38	31	14
Influent Ntot (mg/L)	47.6±14.9	32.4±5.3	38.6±8.0	43.8±7.2	47.1±6.5	50.5±9.1	54.2±8.0
Influent Ptot (mg/L)	7.6±4.6	4.4±1.1	4.5±1.1	5.3±0.8	5.9±0.9	5.9±1.2	7.7±2.4
Influent TSS (mg/L)	201±156	136±37	103±58	116±20	120±24	122±34	381±112
Influent COD (mg/L)	350±206	232±42	222±73	279±39	293±43	306±57	511±102
Influent soluble COD (mg/L)	91±46	56±11	72±20	99±16	103±26	117±23	116±47
VFA dosed (mg COD/L influent)	0-50	22±2	27±8	52±55	75±45	44±22	-
TIN (mg N/L)	-	6.6±3.2	11.7±5.1	11.7±4.0	7.3±2.6	7.9±2.8	8.9±3.5
PO_4^{3-} (mg P/L)	-	0.8±0.4	1.3±0.5	2.3±1.0	1.8±1.4	1.8±1.0	2.5±0.5
N removal (%)	-	81±6	70±10	72±10	85±5	84±5	84±6
P removal (%)	-	82±12	68±15	57±18	67±23	68±18	64±13

The reactors were operated with phases of anoxic, anaerobic and aerobic conditions (Fig. 5, Fig. 17). In the anoxic phase, nitrified wastewater in the reactors was mixed with the influent wastewater. The effluent from this reactor was let either to the next reactor, or to the outlet. If the NO_{2+3}^- had been depleted in the anoxic phase, an anaerobic phase could take place, in which the influent and the VFA (if added) served as carbon source for PAOs. The release of their polyphosphate reserves led to a peak in PO_4^{3-} concentration in the reactor, while the NH_4^+ had been increasing continuously in the unaerated phases. To avoid discharge of treated wastewater with high NH_4^+ and PO_4^{3-} concentrations, the reactor was first aerated without any in- or outflow, prior to the next phase when the inflow came from the other reactor, and the outflow could be let out as treated effluent. The durations and the sequence of the phases were set for most parts of the study, but an automatic phase control for the cycle was also tested (Table 4).

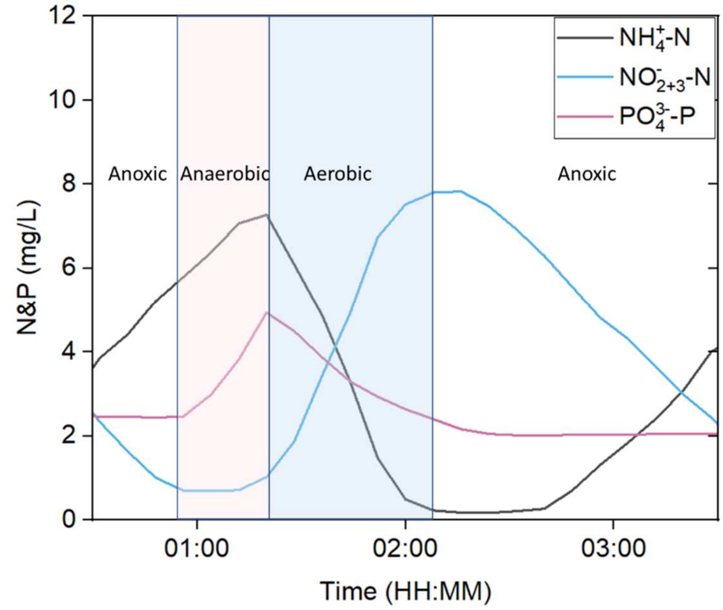

Figure 17. Online measurements of NH_4^+-N, NO_{2+3}^--N and PO_4^{3-}-P during a typical cycle of operation.

5.3.2. Start-up

The organic loading rate was high during the first weeks of operation, and the added biofilm support material to enabled quick biofilm growth (**Paper III**). When biofilm was established, the hydraulic retention time was increased gradually. The phase cycle during start-up was set with a long unaerated time, aiming to favour PAOs rather than growth of nitrifiers. This strategy resulted in visible phosphate releases during anaerobic periods after 6 weeks, and decreasing phosphate in the effluent. Nitrification and denitrification were observed after 11 weeks. Hence, the start-up of the novel biofilm process was as fast as expected from previous studies of biofilm EBPR with other types of support material (Castillo et al., 2000; Gonzalez-Martinez and Wilderer, 1991; Villard et al., 2023).

5.3.3. Nitrogen removal

With primary filtered wastewater and continuous VFA addition, the mean total nitrogen removal was 70-85% during periods B-F (Table 4, Fig. 18, **Paper III-IV**). Different phase control strategies were tested, which showed that high nitrogen removal of 81±6% can be achieved with automatic control targeting low effluent nitrogen. Since the automatic shifting of phases would have hindered the evaluation of strategies for VFA addition, set values for the phase lengths were applied during most part of the study. Fixed phases could also give high nitrogen removal efficiencies of 85±5% (period E, day 280-380) and 84±5% (period F/II, day 381-460). Automatic control which was not adjusted for nitrogen removal resulted in lower removal efficiency and higher standard deviation of 70-72±10% in periods C-D.

Overall, the results showed that the nitrogen removal was stable and high (>80%) when the phase settings were targeting for low effluent nitrogen. Since nitrate can inhibit the substrate uptake by PAOs (Wentzel et al., 2008), high nitrogen removal is difficult to combine with high phosphorus removal. In other studies of MBBR with both nitrogen removal and EBPR, the nitrogen removals were 20-70%, and hence lower compared to our study (Fanta et al., 2021; Humbert et al., 2018; Joeng et al., 2003; Pastorelli et al., 1999). With optimised automatic phase control, the nitrogen removal efficiency in the novel biofilm process could probably be improved further.

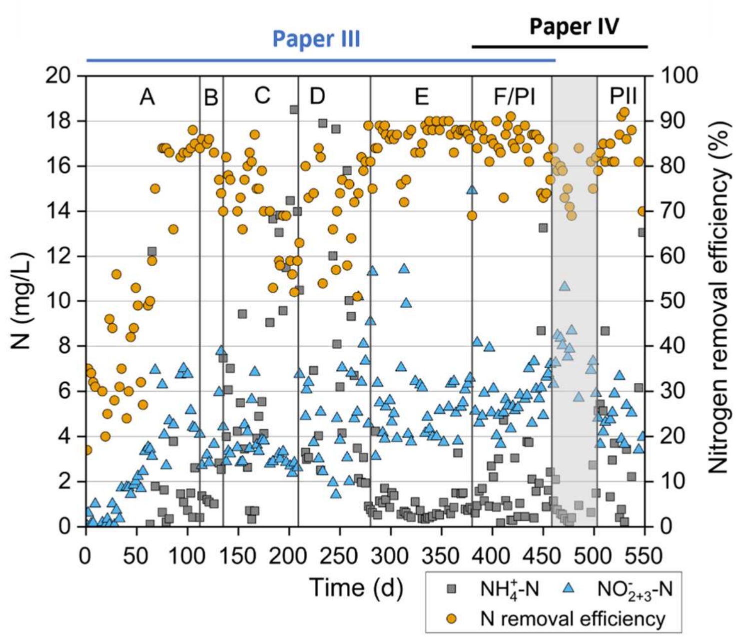

Figure 18. Effluent nitrogen and nitrogen removal efficiency for the different experimental periods during the pilot plant operation.

5.3.4. Phosphorus removal

The phosphorus removal efficiency varied between 57-82% in the different periods (Table 4). It can be noted that the effluent PO_4^{3-} varied both between sampling occasions and between periods (Fig. 19a, **Paper III-IV**). With an estimated fraction of phosphorus in heterotrophic biomass of 0.01 g P/g COD (Henze and la Cour Jansen, 2019c), the assimilated phosphorus was calculated from the effluent particulate COD, assuming that all effluent particles are biomass. Furthermore, the difference between the particulate effluent phosphorus and the assimilation was calculated as an estimation of the phosphorus uptake which could be attributed to EBPR. The assimilated phosphorus mounted up to 1-3 mg/L, whereas the phosphorus uptake owing to EBPR was 0-4 mg/L and clearly dependent on the fraction of phosphorus in the biomass (Fig. 19b).

It was concluded that EBPR gave an increased phosphorus removal, but the low COD to P ratio in the influent limited the PAOs. With primary filtration, the influent wastewater was low in particulate COD, and the soluble COD was also low: 60-120 mg COD/L compared to the ~200 mg COD/L that can be expected in municipal wastewaters (Henze and la Cour Jansen, 2019a). With a need of 20 mg COD/mg P in EBPR (Henze and la Cour Jansen, 2019b), the added VFA would be enough to remove 1-3 mg P/L. A drastic increase in effluent and online measurements of PO_4^{3-} was seen when the dosing of VFAs was stopped after day 460 (Fig. 19a, **Paper IV**).

Thus, it was concluded that VFA addition (FPS fermentation) was required to sustain stable EBPR with chemically enhanced primary filtration for a wastewater with comparable characteristics as in this study.

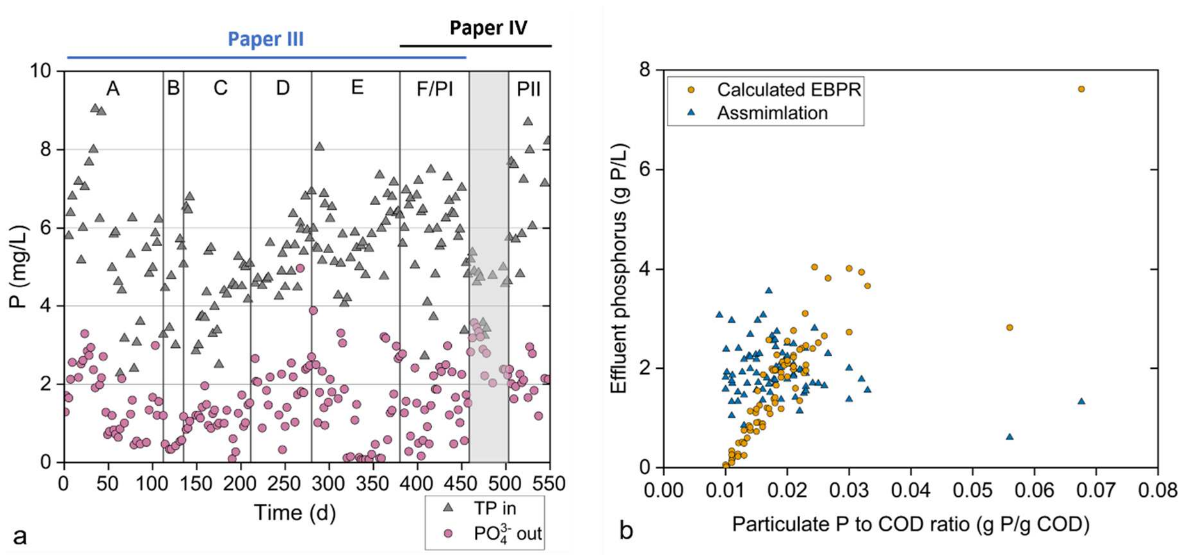

Figure 19. a) Influent total P and effluent PO_4^{3-} different experimental periods during the pilot plant operation. b) Effluent particulate phosphorus which is calculated as assimilated or particulate phosphorus which is not assimilated during periods E-F.

5.3.5. Activity of PAOs

There was PAO activity, measured as phosphate release in batch tests, present during the whole study since the start-up (Fig. 20a, **Paper III-IV**). A peak occurred in periods E and F/PI, when the process temperature was higher, as well as addition of VFA. In these periods, the VFA was dosed predominantly at low redox conditions. As the VFA addition was removed after period F/PI, the activity decreased and remained low during PII with untreated effluent. The results cannot be normalised to biomass or biofilm area, and direct comparison with biomass from other processes is therefore not straightforward. The final concentrations of phosphate after VFA addition were, however, comparable to what has been reported for MBBR (Humbert et al., 2018; Pastorelli et al., 1999; Saltnes et al., 2017).

Batch tests for phosphate uptake in anoxic or aerobic conditions (**Paper III**) showed that the anoxic to aerobic uptake ratio was 0.39-0.87, which is high compared to what has been measured in alternating activated sludge plants (Lanham et al., 2018). Only one other similar process with continuous MBBR and EBPR could be found (Saltnes et al., 2017), but with another process configuration, showing considerable anoxic phosphate uptake. Although the novel biofilm process was operated with an aerated phase after the phosphate release, the results show that denitrifying PAOs could be active and contribute with simultaneous nitrogen and phosphorus removal. The stratification in a biofilm can enable processes to occur in different depths of the biofilm, and anoxic dephosphatation can be enhanced in biofilm processes (Costa et al., 2019; de Kreuk et al., 2005; Winkler et al., 2015).

The type of carbon source can impact the denitrifying PAO activity. Propionate, which contain more chemical energy per molecule than acetate, can lead to production of more glycogen (Carvalho et al., 2007; Vargas et al., 2011) and give an improved phosphorus removal as well as anoxic phosphate uptake by denitrifying PAOs (Girard et al., 2012; Vargas et al., 2011). A mixture of acetate, propionate and butyrate which was used in this study has been shown to increase the anoxic dephosphatation compared to acetate only in activated sludge SBR (Freitas et al., 2005). On the other hand, the literature is not coherent since propionate has also led to decreased anoxic phosphate uptake compared to acetate as carbon source (Cokro et al., 2017; Wu et al., 2010).

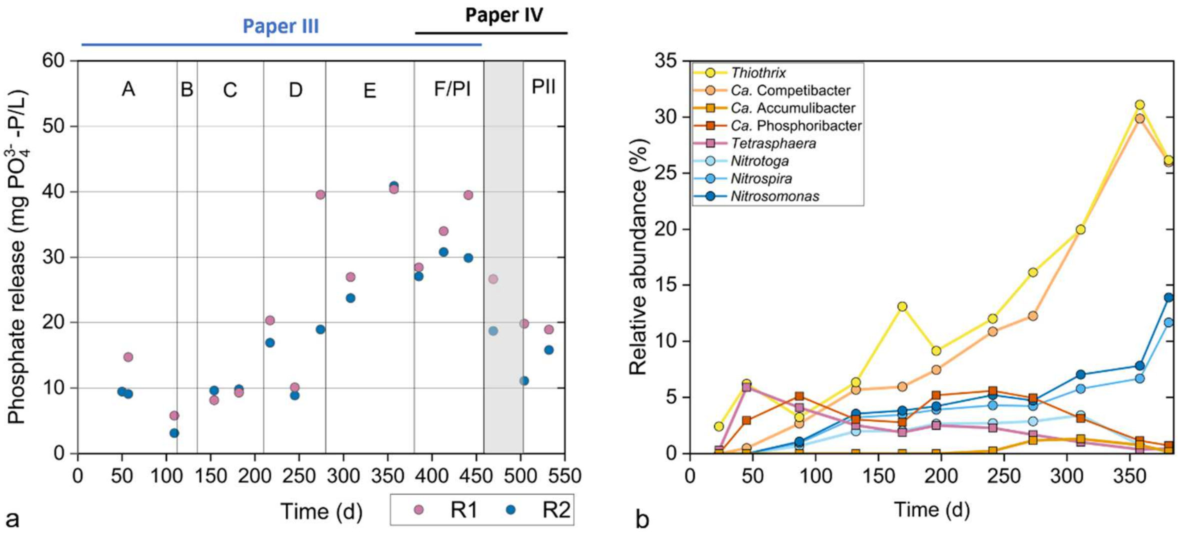

Figure 20. a) Concentration of phosphate after 1.5 h batch tests with acetate addition to support material with biofilm from reactors R1 and R2. b) Relative abundancies of mapped bacteria associated with removal of nitrogen and phosphorus, shown as mean of content in biofilm from R1 and R2.

5.3.6. Microbial community

Analysis of the microbial communities in the reactor biofilms resulted in similar composition between the two reactors (**Paper III**). PAOs within *Ca.* Phosphoribacter (previously known as part of *Tetrasphaera*), *Ca.* Accumulibacter and *Tetrasphaera* were measured in relatively high abundance both in the reactor biofilms (Fig. 19b) and in the effluent. There are few studies of the microbial communities in biofilm EBPR with carrier material, but *Ca.* Phosphoribacter has been observed in a continuous MBBR process (Villard et al., 2024). *Tetrasphaera* are common in EBPR plants worldwide (Nielsen et al., 2019; Singleton et al., 2022). The drop in abundance of known PAOs towards the end of the measurement period around day 390 was unexpected, considering the high phosphate release in batch tests during the period (Fig. 20a). This raises the question whether there were others, yet unmapped PAOs in the biofilms.

Ca. Competibacter displayed the highest abundance during the summer around day 300-350 during the period of higher VFA addition, which can be expected as it has been shown before that *Ca.* Competibacter are more competitive at high temperature and substrate access (Lopez-Vazquez et al., 2009; Nielsen et al., 2019).

Ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB) were also found in the biofilms (Fig. 20b) but not in the effluent. A shift from *Nitrotoga* to *Nitrospira* occurred in the summer, as *Nitrospira* has shown a competitive advantage at higher temperature (Wegen et al., 2019).

5.3.7. Carbon management: VFA addition

The VFA addition had a strong positive effect on the biofilm EBPR (Fig. 21, **Paper III**). It was also tested in period E to increase the VFA dose above what would be available from FPS fermentation at ambient temperature and 5 days HRT. The phosphate removal efficiency was clearly dependent on the amount of added VFA, and an increase to >100 mg VFA-COD/L could give a phosphorus removal efficiency of >90% (Fig. 21a, **Paper III**). The importance of VFA addition for EBPR was also observed in studies of SBBRs with municipal wastewater (Fanta et al., 2021; Pastorelli et al., 1999).

When EBPR is applied in alternating processes where the anaerobic phase follows the anoxic phase, the VFA in the influent wastewater is not only directed to PAOs, but partly used up for denitrification. Adding the external VFA at a constant rate to the inflow was tested during the first 240 days, resulting in stable EBPR (**Paper III**). A faster denitrification allows for more time in the anaerobic phase, but the added value of VFAs as carbon source for denitrification proved to be less important compared to using the VFA for EBPR (**Paper III**). In batch tests the addition of VFA to the anoxic phase only gave a slightly higher denitrification rate compared to wastewater without VFA, and the phosphate release with influent wastewater as the only carbon source was very small (Fig. 21b, **Paper III**). On the contrary, adding VFA during the anaerobic phase gave a very pronounced phosphate release. Addition of VFA below a setpoint for redox was therefore implemented and tested intermittently from day 240.

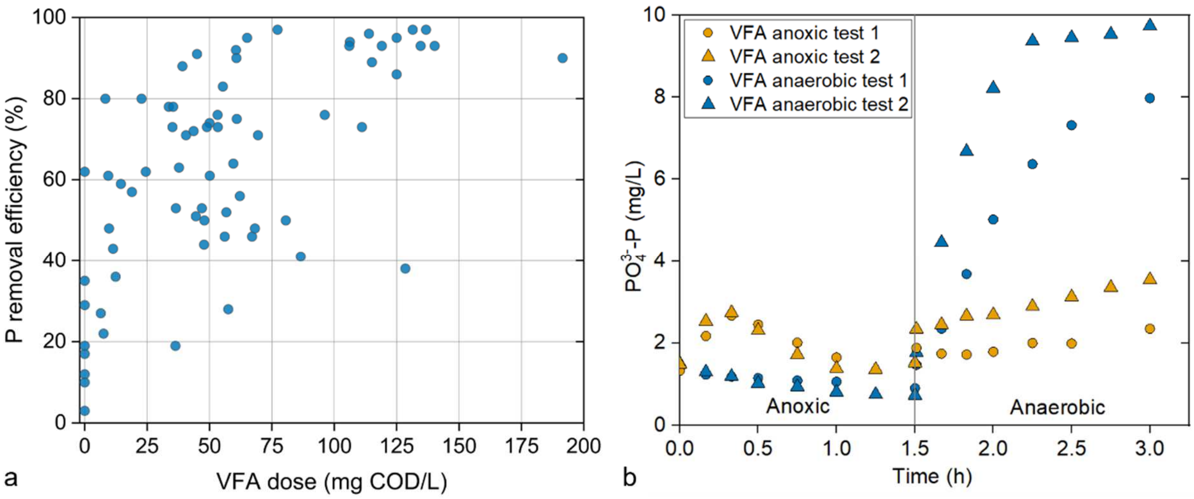

Figure 21. a) Phosphorus removal efficiency versus VFA dose during days 280-474. b) PO_4^{3-} concentration in batch tests 1 and 2 with real wastewater and VFA addition either during anoxic or anaerobic conditions.

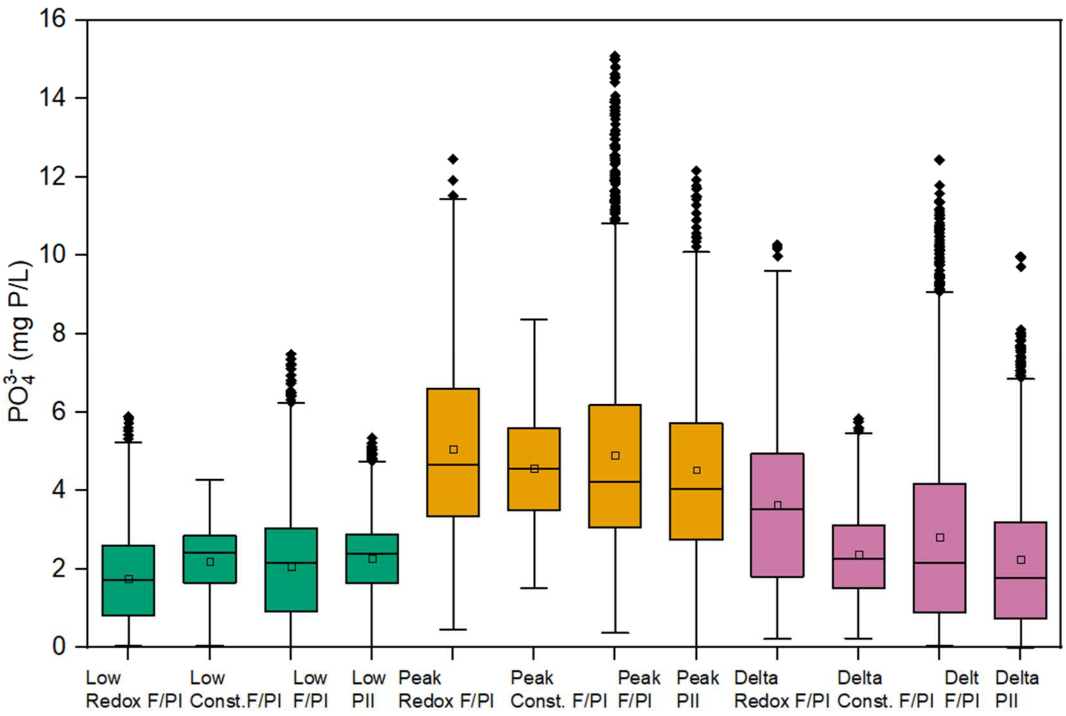

Figure 22. The lowest and peak values and their difference (delta) of online PO_4^{3-} measurements during each operational cycle in period F/I with constant or redox controlled VFA addition, for the whole period F/I and for period II without primary filtration and VFA addition.

The impact from the redox-based VFA dosage in comparison with constant dosage was studied in the pilot by intermittent periods with the different control strategies (**Paper III**). The online analysis of NO_{2+3}^- was not affected by the timing of VFA addition, but the impact on PO_4^{3-} was clear, as it could be reduced to 1.8±1.2 mg P/L during aerated periods with redox control compared to 2.4±0.9 mg P/L during constant dosage (Fig. 22). The difference between peak values and low values (delta), which is a measure of PAO activity, rose to 3.5±2.3 mg PO_4^{3-}-P/L with redox-based dosing compared to 2.3 ±1.2 mg PO_4^{3-}-P/L with constant dosing (Fig. 22). Statistical ANOVA analysis confirmed the difference between low values, peak values and their difference delta (**Paper III**). Only one comparable study has been found by the author on strategy for carbon source dosing, in SBR with activated sludge (Choi et al., 2012), showing that addition during the anaerobic phase was more effective than addition to the anoxic phase. In **Paper III**, the gain of using redox to control VFA addition in an alternating biofilm process was demonstrated clearly. The impact from carbon source dosing strategy is valuable to know for future installation of the new biofilm process if EBPR is applied, but also for alternating activated sludge plants with EBPR.

The disturbance of rainfall led to higher flow of a more diluted influent wastewater. During and after these events, higher redox and a lack of anaerobic phases resulted in high effluent PO_4^{3-}. The control at the pilot plant allowed to set a maximum daily VFA dose, a pump flow and a setpoint for redox to start the VFA dosing below. When redox control of VFA dosage was applied, the dosing stopped under and after rain events, and the setpoint for redox was increased manually to restart the dosing and regain the EBPR. It is reasonable not to waste VFA under conditions of short HRT and high redox. On the other hand, the dosing needs to be started again to shorten the time period of poor EBPR and to make use of the advantage that the carbon source addition can bring. It is therefore recommended to adapt the setpoint according to redox, flow and the available amount of carbon source.

5.3.8. The primary treatment's impact

To evaluate the impact of the primary filtration and VFA addition on nitrogen and phosphorus removal as well as particle removal from the BNR effluent, untreated influent without VFA addition was applied in the last part of the experimental period for the biofilm BNR pilot plant operation (Table 2, Table 4, **Paper IV**).

The nitrogen removal was maintained at the same level of 84 ± 5-6% in PII without primary treatment (Fig. 16), although the effluent total inorganic nitrogen was slightly increased (8.9±3.5 compared to 7.9±2.8 mg N/L) due to a higher load. The effluent ammonium became higher and more variable without primary treatment, and the effluent nitrate decreased (Fig. 18, Fig. 23). It has been observed before that denitrification in MBBR was not impaired by RBF primary treatment, while nitrification was improved (Rusten et al., 2016). A 78% higher mean aeration flow was also required in our study (**Paper IV**) to uphold same mean DO and the high level of nitrification without primary filtration. As expected, the sludge production from BNR displayed a considerable increase without primary treatment.

More of the phosphorus was assimilated in PII compared to PI (**Paper IV**), while the EBPR was less active (Fig. 19a, Fig. 20a). Although the ratio of COD to P was higher without primary treatment, the carbon was not as available to PAOs. This was seen in increased effluent PO_4^{3-} with effluent concentrations of 1.8±1.0 mg P/L with primary treatment and 2.5±0.6 mg P/L without (Fig. 23), and in lower batch tests phosphate releases during the last experimental period (Fig. 20a). The online measurements of PO_4^{3-} also differed between the periods I and II (Fig. 22), with less dynamics during the cycles in PII. The rate of PO_4^{3-} uptake, which was dependent on the PO_4^{3-} release, could reach higher with primary filtration and VFA addition (**Paper IV**). Thereby, it could be concluded that the advanced primary treatment with filtration and VFA addition was beneficial for EBPR.

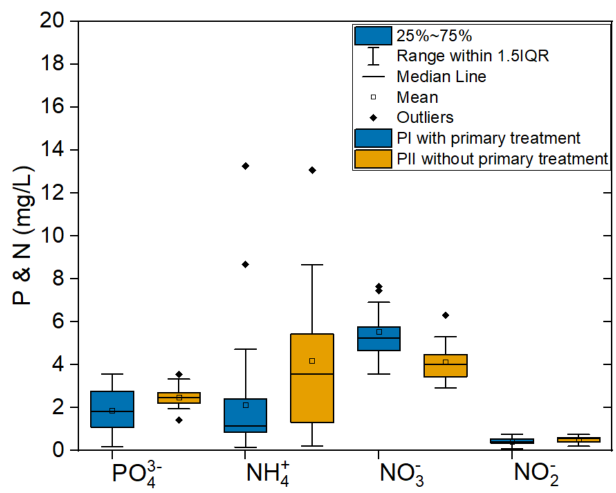

Figure 23. Overview of effluent values from BNR in PI with primary filtration and VFA addition, and period II without primary treatment (Figure S 1a, in **Paper IV**).

The particle distribution in the MBBRs shifted towards larger particles in the effluent (**Paper IV,** Fig. 9), analogous to previous findings (Åhl et al., 2006). Without primary filtration in PII, the effluent became turbid with a more wide-spread distribution in particle sizes. Hence, the absence of primary treatment would impact the need for chemicals and equipment in particle removal from the BNR effluent (**Paper IV**). Lab-scale tests with particle separation through chemically enhanced sedimentation, dissolved air flotation (DAF) and filtration resulted in high removals of turbidity and phosphorus with all methods (**Paper IV**). The results indicate that the effluent from the novel biofilm process can be treated as other MBBR effluents, for which several different particle separation methods has been applied at full scale (Ødegaard et al., 2012).

5.4. Energy recovery and demand

Energy can be recovered from the organic carbon in primary- and biological sludge by biogas production through anaerobic digestion. The potential for energy recovery as methane can be measured as BMP, which is presented in this section. Calculations and simulations were applied for estimation of the of primary treatments' impact on demand for electricity in an activated sludge process (**Paper I**). Moreover, results from the pilot plant trial with biofilm BNR, operated with or without primary treatment, were used to discuss the effects on the energy balance (**Paper IV**).

5.4.1. Energy recovery as biomethane from the produced sludge

The BMP of the FPS as well as the fermented FPS was studied in a parallel master's thesis project (Blom, 2022). The first BMP test conducted during the study resulted in low BMP for the cellulose which was used as a reference, and it was concluded that the inoculum did not have enough activity to measure the full BMP. Nonetheless, the ratio between FPS and fermented primary sludge methane production during this test and the theoretical BMP based in sludge characterisation could be used for estimation of the BMP for fermented FPS. Fermentation had a negative effect on the BMP, as expected considering the loss of COD as H_2 during the process (Batstone et al., 2002), and the estimated BMP for fermented FPS was set to 85% of the BMP for FPS before fermentation in **Paper I**.

The measured BMP potential for FPS in the second and more reliable BMP test (Table 5) was used as basis for calculations of the plantwide COD balance in **Paper I**, where a BMP of 320 NmL CH_4/g VS was applied for FPS and 270 NmL/g VS for fermented FPS.

A third BMP test of the FPS was performed in a master's thesis project (Murugan, 2025) when the RBF was operating as primary treatment for the BNR, resulting in 340 NmL CH_4/g VS (Table 5). Inoculum was collected from Sjölunda WWTP during both studies, and the similar BMP for the FPS strengthens the validity of the results. Furthermore, both results were in line with literature values for FPS of 320-390 NmL/g VS (Paulsrud et al., 2014; Taboada-Santos et al., 2019). A comparison with the SPS was made during the first master's thesis (Blom, 2022), indicating that the BMP of SPS was considerably lower (250 NmL CH_4/g VS) compared to FPS (Table 5). The batch tests with SPS and FPS (5.2.6) point in the same direction of higher energy potential in FPS, as in a comparison between several WWTPs with settler or RBF (Paulsrud et al., 2014).

It was clear that the separated BNR sludge from the effluent of the novel biofilm process could give high BMP with primary treatment, and even higher without with primary treatment (Murugan, 2025). The BMP of 290 NmL CH_4/g VS in PI (test no. 3) is in the higher range of the 170-280 NmL CH_4/g VS for various activated sludge (Calabrò et al., 2024), but lower than the 300-380 NmL CH_4/g VS measured for high-rate activated sludge and MBBRs (Carlsson et al., 2016).

Another BMP test (no.4) was performed in PII, displaying a BMP potential for the BNR sludge of 390 NmL CH_4/g VS (Table 5). It is not likely that the BMP of the partially oxidised BNR sludge could be higher than the BMP for FPS. The reference waste activated sludge from Källby WWTP was frozen prior to the first BMP test, and thawed in the same manner before both the tests in PI and PII (Murugan, 2025). However, the BMP differed between the two periods (150 vs 180 NmL CH_4/g VS), and it was therefore not possible to make a straightforward comparison between the BMP of the BNR sludge with and without primary filtration and VFA addition.

It can be concluded that the energy recovery potential of the BNR sludge from the Cella™ process is high compared to the reference waste activated sludge. If the reference sludge is used to normalise to BMP from BNR sludge in PII, the result is similar to the BMP of the primary sludge.

Table 5. Biomethane potential at mesophilic temperature for filter primary sludge (FPS), settler primary sludge (SPS) and BNR sludge from a biofilm process with and without initial primary filtration.

Substrate	Test no.	Blom (2022) (NmL CH_4/g VS)	Murugan (2025) (NmL CH_4/g VS)
FPS (no polymer)	2	250	-
FPS (polymer)	2	320	340
SPS	2	210	-
BNR sludge with primary filtration (PI)	3	-	290
BNR sludge without primary filtration (PII)	4	-	390
Reference waste activated sludge (PI)	3	-	150
Reference waste activated sludge (PII)	4	-	180

5.4.2. Effect of primary treatment on nitrogen removal with activated sludge

The BSM1 model (Jeppsson et al., 2006) is a model for activated sludge with nitrogen removal, which is easily available and open for others (Jeppsson, 2009) to adapt and make comparisons with the results from **Paper I**. The calculated required volumes (DWA, 2016) for nitrogen removal with activated sludge were based on the standard influent to BSM1, assuming that 50% of the TSS has been removed in the settled BSM1 influent (Gernaey et al., 2015). Compared to the standard influent with settler, required volumes were 11% lower with RBF primary treatment and 18% lower with RBF and FPS fermentation (**Paper I**).

The impact from primary treatment on the required volumes depends on the influent composition and the effluent requirements, meaning that the possible gain in volume reduction must be calculated for each case. The case of a pre-denitrification as in BSM1 is a common WWTP configuration, and interesting as a base case with nitrogen removal. However, the FPS fermentation would probably be more valuable for a WWTP with EBPR, where carbon source in the form of VFA is of more importance. Models for activated sludge including EBPR are available (e.g. Henze et al., 1999), but they are more complex and not as widely applied as models for nitrogen removal only.

The energy recovery of primary sludge as methane through anaerobic digestion was calculated to similar values for settler and RBF with fermentation (Fig. 24, **Paper I**), based on BMP measurements (Blom, 2022) and the WAS production from the simulations. It shows that the loss of energy in fermentation may be balanced by the increased production of primary sludge with chemically enhanced filtration. Electricity requirement for primary treatment and biological wastewater treatment with nitrogen removal could be decreased by 11% with RBF and by 13% with RBF and fermentation, compared to sedimentation as primary treatment. The electricity requirement for aeration could be decreased by 18% for the case with RBF and fermentation (RBFF) compared to settler, and by 22% compared to the case with settler and dimensioning to reach the same simulated effluent nitrogen as with RBFF (Fig. 24, **Paper I).**

The primary sludge fermentation displayed a negative effect on the methane production, which must be taken into account. On the other hand, the environmental impact from external carbon source and the economic cost are high, and the use of a readily available carbon source decreased the need for volume and electricity in the biological wastewater treatment. Since the case with only RBF filtration rendered the most favourable energy balance (Fig. 24, **Paper I**) it is preferred if there is enough readily available carbon source in the primary treated wastewater. If the carbon source addition from fermented sludge is not needed for nutrient removal, the addition of carbon would be a load and not an asset.

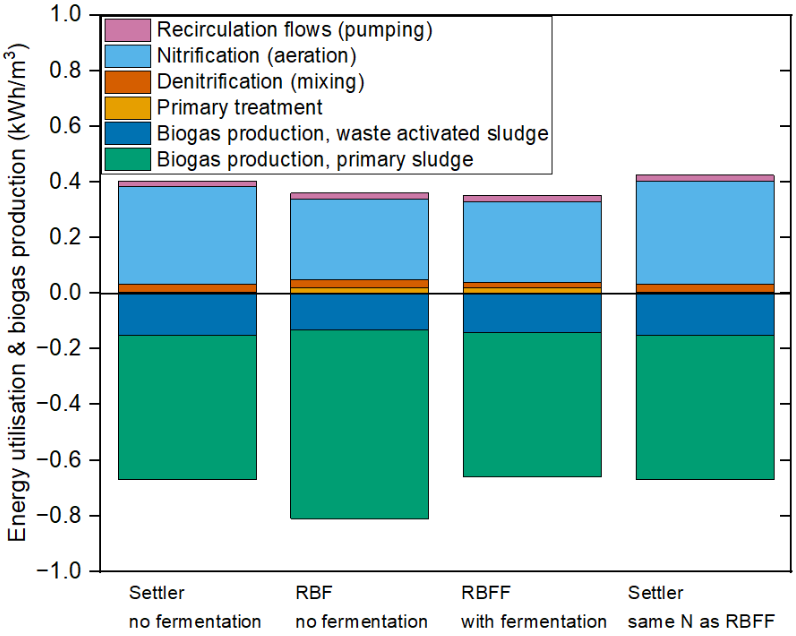

Figure 24. Energy demand in primary treatment and biological nutrient removal, and biogas production based on simulations and calculations of biogas potential for an activated sludge process with pre-denitrification with settler RBF, RBF with fermentation of FS (RBFF) and settler with dimensioning to achieve the same effluent nitrogen as with RBFF.

5.4.3. Energy demand and sludge production at pilot-scale

Primary filtration was favourable for the energy balance of the plant (**Paper I**), and fermentation rendered carbon source for BNR (**Paper I-II**). On the other hand, primary filtration and fermentation requires investments and resources for daily maintenance. For smaller WWTPs, the inputs may not be reasonable compared to the advantages. Even for a larger WWTP, the advantages depend on the local process for energy and chemicals as well as the limitation of area at the site. The case with primary filtration and fermentation could therefore be compared to a base case with raw influent wastewater, to quantify the impact on nutrient removal as well as the energy balance and post-treatment.

The aeration at the pilot plant was not representative for a full-scale plant due to the difference in air diffusers and reactor height as well as controllability of the system. Despite this, the difference in airflow between periods could be used to give more insight in the energy demand for aeration under different conditions. The increase in COD when primary filtration was omitted caused an increase in aeration demand of 78% in the biofilm EBPR pilot study (**Paper IV**). In order to provide a more representative comparison, the aeration demand would need to be studied at larger scale and for a longer time period.

The intention was to use the influent and effluent values of particulate and soluble COD for calculation of the sludge production and the COD balances for the biofilm process with primary filtration and fermentation of FPS and for the biofilm process with untreated influent. However, the high variability of the COD in combination with the sampling method and interval study resulted in an unreasonably high effluent particulate COD during PII, and a reliable COD balance could not be conducted. The effluent COD and TSS during period F/I were less variable, at 240±53 and 177±50 mg /L, respectively. Recalculated to methane yield for the biological sludge (Murugan, 2025) with 75% VS/TS, it would correspond to ~0.4 kWh/m^3 wastewater. In addition, the primary sludge after fermentation could produce methane in the same range. Compared to the theoretical study of activated sludge (**Paper I**), the contribution of the biological sludge to the total methane production was higher with the biofilm process, where fast-growing bacteria are detached from the biofilm's outer layer. Although it should be considered that the study in **Paper I** was based on the BSM1 influent, which holds a comparably high TSS concentration compared to the Källby WWTP influent, the results indicate that the possibility for energy recovery from the biological sludge could be high with the novel biofilm process.

In conclusion, both the theoretical simulations and the experimental studies of biofilm BNR could confirm that the primary filtration with carbon source addition could give lower effluent nitrogen and decreased energy requirement compared to sedimentation as primary treatment (**Paper I)** and compared to untreated influent **(Paper IV**). The gain of the primary treatment and of the carbon source addition is highly dependent of the influent wastewater characteristics, and fermentation of FPS should be applied if it is needed for biological nutrient removal.

6. Conclusions and outlook

6.1. Answers to the research questions

- How effective is the chemically enhanced particle removal in primary filtration by RBF? What particles are removed, and how can the separation be predicted by modelling? What are the characteristics of the separated FPS?

- Chemically enhanced RBF primary treatment could efficiently remove particles >10 µm and result in high TSS removal of 64 ± 10%. The separated TSS could be predicted by a linear model. The FPS had a TS of 4.5±0.6%, and a ratio of 1.47±0.35 g COD/g VS.

- How is fermentation of FPS affected by seasonal variations concerning VFA yield and distribution as well as microbial community assembly? How much VFA can be produced over the year, and how high is the nutrient release?

- The seasonal variation in VFA production and VFA increase in the wastewater were strong. The temperature dependency of VFA production at ambient temperature (16-29°C) could be determined to Y_{20}=172±4 mg VFA-COD/g VS_{in} and Θ=1.033±0.005 (Eq. 1). The temperature correlations for productions of acetate and propionate from FPS were clear, whereas no correlations could be found for the longer chained VFAs. This was reflected in a seasonal variation in the VFA distribution.

There was a selection in the microbial community in the fermentation reactors towards *Bacteroidota* and an out selection of *Proteobacteria*. A continuous change in the bacterial community could be seen, as well as a seasonal pattern.

Nutrient release in the fermentation was stable over the year, resulting in 20-30 g SCOD/g NH_4^+-N and 40-80 g SCOD/g PO_4^{3-}-P.

Can the fermented sludge be added to the wastewater and the suspended solids separated by filtration?

Through hydrolysis and fermentation of the FPS, the primary treatment could nearly double the VFA concentration in the wastewater for the studied WWTP. It was feasible to separate the fermented particles by filtration. The TSS removal was slightly lower when the fermented sludge was added to the wastewater, and the variability increased but the particle separation was still fully functioning. It is recommended to add the fermented sludge to already filtered wastewater and apply side-stream filtration of the fermented solids. The separated solids can be directed to anaerobic digestion, as it was shown that recirculation of fermented solids to the fermenter decreased the VFA yield.

- How are the requirements for volume and electricity in activated sludge affected by primary treatment with RBF and RBF with FPS fermentation, compared to conventional primary settling?

The volume decrease in activated sludge treatment with nitrogen removal was calculated to 11% with RBF and 18% with RBF and fermentation for the case with BSM1 influent. The corresponding saving in electrical energy requirements in primary and secondary treatments were 11 and 13% with RBF and RBF with fermentation, respectively.

- Can the novel biofilm process result in high nitrogen removal and stable EBPR with wastewater primary filtration and addition of VFA to mimic fermented FPS?

Yes, the novel biofilm process with alternating MBBRs could give both high nitrogen removal and stable EBPR with primary filtration and VFA addition. For a wastewater with low SCOD concentration, fermentation of primary sludge is needed to promote EBPR when primary filtration is applied prior to the alternating MBBR process. Anoxic phosphate uptake was measured, which shows that denitrifying PAOs were active in the biofilm.

- How can the dosage of VFA to the biofilm process be optimised to benefit EBPR while maintaining nitrogen removal?

Side-stream fermentation allows for storage of carbon source and control of the VFA addition. Directing the VFA to anaerobic periods strengthened the positive impact on EBPR compared to constant dosing to the alternating biofilm process.

- What microorganisms related to nitrogen and phosphorus removal are present in the biofilm?

Microbial analysis showed a continuous increase in *Nitrotoga* and *Nitrospira* in the biofilm. *Nitrosomonas* were increasing until the summer periods, when there was a decline in abundance. Regarding genera associated with EBPR, high abundances of the PAOs *Ca.* Phosphoribacter and *Tetrasphaera* were observed in the biofilm and in the effluent, and presence of *Ca.* Accumulibacter.

- How is the nutrient removal affected by receiving untreated influent wastewater compared to application of primary filtration and VFA addition?

-The novel biofilm process could be operated with high nitrogen removal both with primary filtration and VFA addition, and without any primary treatment. Primary treatment with filtration and VFA addition was advantageous for EBPR compared to an untreated influent, and resulted in a lower mean effluent PO_4^{3-}. In addition, the aeration requirement was considerably higher without primary filtration of the influent wastewater.

6.2. An outlook on the significance of the results

-The feasibility of RBF combined with FPS fermentation at ambient temperature could be shown, as well as the yields of VFAs and soluble COD at different HRT.

-The temperature dependencies for the production of VFAs from primary sludge, which had not been shown before, are useful to evaluate WWTPs that already apply primary sludge fermentation and for WWTPs where primary sludge fermentation is planned or considered.

-The seasonal differences in COD to nutrient ratio in the fermented sludge and the difference in VFA distribution is likely to affect both nitrogen removal and EBPR, yet these relations had not been demonstrated before.

-The proposed method for VFA separation from the fermented solids, where the fermentate is mixed with wastewater and filtered, was feasible and can be recommended in order to minimise losses of the produced carbon source for BNR. It is suggested to use separate filters for this purpose, since a longer SRT in fermentation led to decreased yield in fermentation of filter sludge.

-It was shown that chemically enhanced RBF combined with FPS fermentation can reduce volume for BNR with activated sludge and give similar methane production compared to a base case with primary settler. Since the need to produce energy can be a decisive point at WWTPs, this study gives useful input.

-A novel biofilm process with biobased carriers and continuous flow proved to give high nitrogen removal as well as EBPR, both with primary filtration and VFA dosage and with no primary treatment. The effluent phosphate concentration was, however, lower with the novel primary treatment. The possibility of applying both biofilm on support material, alternating flow process and EBPR is new, and the nitrogen removal was high compared to other studies of MBBR and EBPR.

-The value of side stream fermentation which allows for storage and controlled dosing of VFAs to the EBPR process could be shown. Adding the VFAs directly at low redox conditions was beneficial for the phosphate release and uptake compared to constant VFA dosing. These results are useful when planning for new installations, and side-stream fermentation can be applied for both settling and filtration as primary treatment. In a process with alternating flow and EBPR in activated sludge, which is already applied at full-scale, the possibility of controlling the carbon source availability can be advantageous to further improve the effluent values.

6.3. Suggestions for further research

The proposed solution for separation of fermented sludge, mixing with filtered wastewater and separation in filter has been tested in this study to evaluate the feasibility. This process needs to be studied further with the proposed design to evaluate the proportions of fermented sludge and wastewater as well as the filter cloth pore diameter, polymer addition and the applicable TSS load. It was seen in this study that fermentation reduced the viscosity of the FPS, meaning that pumping and heating in heat exchanger would be facilitated. However, the characteristics of the separated sludge after dilution with filtered wastewater and re-separation are yet to be evaluated.

Fermentation at ambient temperature gave lower ratio of VFAs to soluble COD at lower temperatures. Only VFAs were added to the MBBRs, and not the other soluble COD and nutrients which would be included at a full-scale WWTP, their impacts were not studied experimentally. Since the soluble COD could potentially be used both for denitrification and for EBPR, the real impact of fermentate addition could be more pronounced. This would, however, need to be evaluated in further studies.

The VFA mixture added to the BNR (**Paper III-IV**) was constant in the fractions of acetate, propionate and butyrate although it was shown that they varied in proportions with temperature. The mixture was kept constant in the pilot plant trial to facilitate evaluation of the different operational periods. Since the seasonal variation in VFA composition is likely to have an impact on the BNR, it would be interesting to study.

The environmental impact from EBPR with different solutions for internal carbon production is interesting to compare, since they may result in different amounts of methane emission and reductions of coagulant addition. The risk of methane emissions from EBPR has been highlighted (Högstrand et al., 2024), but there is a lack of experimental measurements and knowledge about how process design and control could influence the climate impact.

The alternating MBBR process with EBPR is still in an early phase of development compared to full-scale processes. Consequently, no direct comparison was made with the AGS process which is in a later stage of development. The phase control of the alternating reactors can be further applied and developed to meet the conditions in the biofilm process, where the HRT is shorter and the phosphate concentration during phosphate release may be higher compared to an activated sludge process. With application of automatic phase control and further development of control and recommended settings, the effluent values could potentially be further improved.

Denitrifying PAO activity was measured in the MBBR biofilm, but the optimisation of the process control to take full advantage of simultaneous nitrogen and phosphorus removal was out of scope in this study. The potential to decrease energy demand and use the carbon source more efficiently can hopefully drive further development with focus on denitrifying PAOs in MBBRs.

7. References

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