Understanding the impact of a pressure sewer on municipal wastewater: a pre-treatment for AGS plants

Delft University of Technology
Faculty of Civil Engineering and Geosciences
MSc Thesis in Sanitary Engineering,
Water Management

Thesis Committee:
Merle de Kreuk
Jeroen Langeveld
David Weissbrodt
Sara Toja Ortega

13th May 2020,
Chiara Merola 4614747

TU Delft

“Dai diamanti non nasce niente,

dal letame nascono i fior.”

“From diamonds, nothing is born,

from manure, flowers will grow.”

Fabrizio de Andrè – Via del Campo

CONTENTS

ABSTRACT

The performance of AGS reactors treating municipal wastewater can be optimised by converting influent particulate matter into readily available substrate. This can be done via anaerobic hydrolysis and fermentation of the influent. Anaerobic processes taking place in pressure sewers are not fully understood but show the potential to act as a pre-treatment for the wastewater reaching AGS reactors. Moreover, the contribution of the influent to the hydrolytic activity of the reactor is unknown.

This research evaluated the impact of a pressure sewer on wastewater characteristics, as a possible pre-treatment of sewage before reaching the treatment plant. The variations of sewage in terms of physicochemical composition and microbial activity were monitored in a full-scale pressure sewer, focusing on the hydrolysis and fermentation of organic matter for further treatment in AGS reactors. Moreover, the contribution of the influent to the enzymatic activity of a full-scale AGS reactor was assessed.

Inaccuracies deriving from sampling on a full-scale pressure sewer might have affected the results. However, statistical analyses helped to derive trends from the collected data. The pressure sewer primarily affected the degree of fermentation of the wastewater and the concentration of suspended solids. It is hypothesised that such variations could benefit the performance of AGS reactors. Although the biodegradability and enzymatic activity of the wastewater did not improve significantly, anaerobic conveyance seemed more appropriate than aerobic transport for AGS reactors. However, the influent did not seem to have a large contribution to the total reactor activity, due to the high concentration of granular biomass.

ACKNOWLEDGEMENTS

I am very grateful towards the whole committee, Merle de Kreuk, Sara Toja Ortega, David Weissbrodt and Jeroen Langeveld, for allowing me to carry out this project. It turned out to be an incredible journey, a rollercoaster of challenging and highly motivating moments, in which I have learnt so much, as a person before than as a student.

Thank you, Dante Lopes, for not running away when you understood what exactly your internship would have been like. And thank you for ordering pizza for us when I was too concentrated on the analyses. And especially, for repeating for every obstacle that came across our way: “We’re in this together!”. You made everything much better.

I would like to thank the water authority Hoogheemraadschaap De Stichtse Rijnlanden (HDSR) for authorizing my project in the end; Maxime van Wiggen and Tonny Oosterhoff for helping me with everything that came before and afterwards and the operators, Jan van Bentum, Albert Berkhof and Jan Cloo, for all the jokes and mood lifting moments, but especially for speaking Dutch with me.

Thanks to IMD for making the sampler modification much easier and, in particular, thanks to Dennis Zwart for helping to develop the software. And to Watdan, Hadassa and all the other colleagues who provided constant support with the installation.

To my friends here in the Netherlands: I’ll never forget the moment in which I came back from the wastewater treatment plant and all of you guys were there, waiting for me with a surprise birthday party. You are simply fantastic!

Thanks mum and dad for all your creative ways of supporting me, and Ena and Carlo for being such inspiring sister and brother.

Robby, thank you for listening for hours and hours to all my blabbering without understanding a thing of what I was saying. You have no idea how useful that has been!

Lampo, Mamalu, Lupi and Martina: we made it until the end!!

LIST OF ABBREVIATIONS

α-GLU	α-glucosidase
β-GLU	β-glucosidase
AcH	Acetate
AGS	Aerobic granular sludge
AS	Activated sludge
ATP	Adenosine triphosphate
BOD_5	Biological oxygen demand, after 5 days of incubation
BNR	Biological nutrients removal
SCOD	Soluble fraction of chemical oxygen demand
TCOD	Total chemical oxygen demand
DO	Dissolved oxygen
DWF	Dry weather flow
EBPR	Enhanced biological phosphorus removal
EPS	Extracellular polymeric substances
FBT	Fermentation batch test
GAO	Glycogen accumulating organisms
HA	Humic acids
HRT	Hydraulic retention time
OHO	Ordinary heterotrophic organisms
OUR	Oxygen uptake rate
PrH	Propionate
PAO	Phosphorus accumulating organisms
PSD	Particles size distribution
SND	Simultaneous nitrification and denitrification
SNR	Simultaneous nutrients removal
SRB	Sulfate reducing bacteria
SRT	Sludge retention time
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids

VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WW	Wastewater
WWF	Wet weather flow
WWTP	Wastewater treatment plant

1 INTRODUCTION

1.1 AGS TECHNOLOGY

Biological wastewater treatment is considered the most environmentally-friendly and cost-effective method of removing nutrients from wastewaters of different types, as opposed to physicochemical systems (Lemaire et al., 2008). Activated sludge (AS) has so far been the most widely established technology in this field. There is a growing awareness, though, regarding the fact that these systems cannot cope with the current demands of rapid human population growth and urbanization, due to their high capital and operational costs and their large area requirements (van Loosdrecht & Brdjanovic, 2014). Aerobic granular sludge (AGS) was developed to tackle the need for a lower spatial footprint (de Kreuk et al., 2005). So far, AGS technologies have proved to compete with and even outperform conventional AS systems, holding great promise to replace activated sludge as a standard in the treatment of wastewater (M. Pronk, Abbas, et al., 2015).

Aerobic granules are formed by the aggregation of microorganisms into a spherical structure, maintaining the same microbial functional groups as activated sludge, but differing from flocculent biomass in terms of their larger size and density (de Kreuk et al., 2007; Winkler et al., 2013). The limitations to substrate diffusion through the granule's depth result in a layered structure in which different redox and substrate availability conditions are created. Different functional groups of microorganisms can therefore establish within a single granule, allowing for simultaneous nutrient removal (SNR) within one tank (de Kreuk et al., 2005). The dense spherical structure of AGS also determines a high settling velocity, making separate clarifiers redundant as sludge separation can quickly occur in the reactor itself. Overall, this results in advantages, not only in terms of lower spatial footprint, but also for energy consumption compared to the conventional activated sludge system (de Bruin et al., 2004).

Aerobic granules are formed and maintained in Sequencing Batch Reactors (SBR), with an increasing number of applications (Bengtsson et al., 2018). Nereda® is the commercial full-scale technology originated from the collaboration between TU Delft and Royal Haskoning DHV, based on a constant working volume sequential fed-batch process (M. Pronk, de Kreuk, et al., 2015). A typical Nereda® cycle begins with simultaneous anaerobic feeding and effluent discharge, followed by an aerated reaction phase, a settling phase and an excess sludge disposal phase.

1.2 AGS TREATING COMPLEX WASTEWATER

Initial studies showed that granulation can swiftly be achieved in lab-scale reactors fed with synthetic feeds, obtaining smooth, dense and well-settling granules (Beun et al., 1999; De Kreuk et al., 2005; Morgenroth, Sherden, van Loosdrecht et al., 1997). However, the treatment of complex wastewater like sewage leads to a significantly longer granulation period and still presents problems related to the structural and functional stability of the granules obtained (Wagner et al., 2015).

From theories on biofilm structures, it is known that a high substrate gradient between the superficial and the deeper layers of the biofilm causes preferential growth of the microorganisms at the top, resulting in filamentous and finger-like protuberances (Picioreanu et al., 1998; van Loosdrecht et al., 1995). If this phenomenon occurs with microorganisms characterized by a fast growth rate, for instance in the case of heterotrophic growth under aerobic conditions, the finger-like protuberances can reach macroscopic proportions (van Loosdrecht et al., 1995; 1997). These hinder the settling ability of granular biomass by creating less dense granules. Granular stability is also affected by the presence of substrate gradients, caused by the localised hydrolysis of particulate matter. In-depth growing bacteria are important for biofilm stability, leading to the formation of a dense and compact structure. A uniform substrate distribution within the granule, with an increased substrate penetration depth, reduces conditions of starvation and decay of the innermost colonies and leads to uniform microbial growth (Picioreanu et al., 1998). Outgrowths make so that the substrate is consumed at their tips, reducing the concentration reaching the core of the granules and

eventually causing their decay, leading to granular instability due to core breakages and the successive granule disintegration (M. Pronk, Abbas, et al., 2015).

In real wastewater, the organic load is largely present as particulate matter. As suspended solids are too complex to be directly taken up by microorganisms, this introduces a necessary and rate-limiting hydrolysis step in the formation and maintenance of granules, other than in the nutrients removal process. Filamentous outgrowths were observed in lab-scale reactors fed with high suspended solids concentrations (de Kreuk et al., 2010; Schwarzenbeck et al., 2004; Wagner et al., 2015).

As AGS technology reaches maturation, full-scale installations are increasing in number, particularly in the municipal wastewater sector (Giesen et al., 2013; Giesen et al., 2014; Li et al., 2014; M. Pronk, de Kreuk, et al., 2015; Pronk et al., 2017). Particulate matter is a major contributor to the organic material and nutrients found in real municipal wastewater. Its abundance in domestic wastewaters in the Netherlands accounts for 50% of the total COD, with values reaching up to 90% in colder or mountainous areas such as Switzerland, where in-sewer degradation is slower and steeper slopes determine a lower residence time in the sewer system. (Roeleveld & van Loosdrecht, 2001; van Nieuwenhuijzen et al., 2004). However, experiences with full-scale applications have shown that smooth, although smaller, fast-settling granules can be achieved even in the presence of considerable concentrations of suspended solids (M. Pronk, de Kreuk, et al., 2015). Figure 1 reports microscopy images of the sludge found within a full-scale AGS reactor operated in Garmerwolde, the Netherlands. In facilities treating real municipal wastewater, a mixture of flocs and granules composes the sludge, with prevalence of granular biomass (M. Layer et al., 2019). The granules obtained present only sporadic filamentous outgrowths.

It is important to notice that swift formation of new granules is observed in AGS reactors which have already completed the granulation process (Personal communication; Mario Pronk, October 2018). The issues related to the presence of particulate matter seem therefore to be more relevant during start-up periods. Conversion of suspended solids to more readily available substrate could help to mitigate these problems. Thus, introducing a pre-hydrolysis step of the influent municipal wastewater might be an option to improve AGS start-ups during future installations.

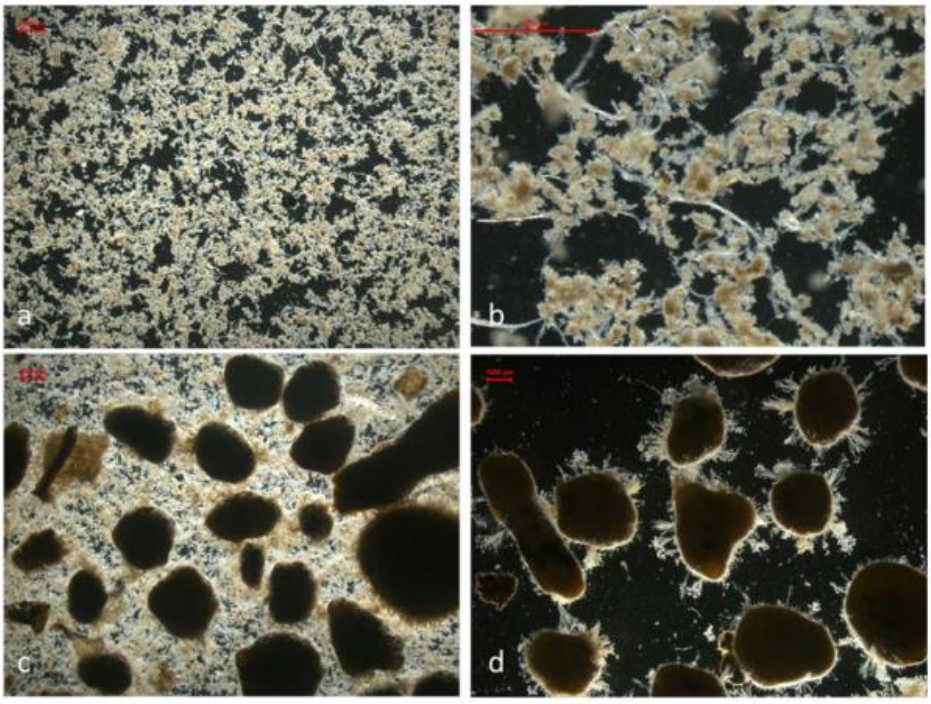


Figure 1: Sludge from the full-scale Nereda® reactor in Garmerwolde, the Netherlands. a) and b) biomass on top of the granular sludge bed; c) mixed liquours; d) granules sieved and washed with tap water. (M. Pronk, de Kreuk, et al., 2015)

1.3 AGS REACTORS OPERATIONAL CONDITIONS

A plug-flow strategy, feeding from the bottom of the reactor, increases nutrient concentrations in the settled granular bed, and is usually adopted to enhance substrate penetration in the granules and therefore microbial growth at the core (de Kreuk et al., 2010; Picioreanu et al., 1998; Pronk, Abbas, et al., 2015). Due to the stratification of the biomass during settling, this strategy also helps to provide fewer nutrients to the lighter flocculent biomass deposited on top of the sludge bed. The presence of particulate matter, however, makes so that not all substrate can directly be taken up by the settled granules. Part of the organic matter can hence rise and be trapped by the flocs, substaining their growth.

Polymer accumulating organisms, such as PAO or GAO (phosphorus and glycogen accumulating organisms), can outcompete ordinary heterotrophic bacteria (OHO) due to their potential to accumulate substrates in their cells in the absence of an electron acceptor (Oehmen et al., 2010; Wagner et al., 2015). Complete uptake of organic matter before the beginning of the aerobic phase will force OHO's to grow under substrate limitations, hindering their growth rate. The slow-growth kinetics thus achieved during the aerobic phase were proven to ensure higher biofilm density and granular stability in AGS systems (de Kreuk & van Loosdrecht, 2004; M. Pronk, Abbas, et al., 2015). The application of a feast/famine regime, with alternating feeding and reaction phases, was therefore implemented in AGS reactors cycles to promote the enrichment of storage polymer forming microorganisms, such as PAO or GAO. Both groups can help to reduce the growth kinetics during the aerobic phase, however, the first ones present the advantage of allowing for enhanced biological phosphorus removal (EBPR). Their selection has, therefore, become a commonly applied strategy to ensure reactor stability and complete biological nutrients removal (BNR) in AGS installations.

1.4 PRE-ACIDIFICATION OF THE WASTEWATER AS A PAO SELECTION STRATEGY

The term PAO refers to microorganisms sharing the same phenotypic trait, which can be further divided into specific microbial groups (Oehmen et al., 2010). The abundance of *Candidatus Accumulibacter phosphatis* has been commonly reported in EBPR processes. Tetrasphaera- related PAO's are abundant in domestic wastewaters and could therefore also be enriched in the EBPR. (Oehmen et al., 2010). The conceptual model of the AGS bacterial ecosystem from SBRs operated for full BNR under alternating anaerobic-aerobic conditions with volatile fatty acids (VFA)-based synthetic wastewater confirms the importance of these microbial groups also in AGS systems (Weissbrodt et al., 2014). Furthermore, Next Generation Sequencing (NGS) analysis also showed the abundance of these PAO's groups in a full-scale Nereda installation treating municipal wastewater (Ali et al., 2019).

The metabolism of these PAO's relies on the uptake of volatile fatty acids (VFA), such as acetate and propionate, or of amino acids (Oehmen et al., 2010; Weissbrodt et al., 2014). Hydrolysis and fermentation processes become hence crucial for the conversion of particulate and soluble organic matter into VFA and for the hydrolysis of proteins into their amino acids. Substantial anaerobic contact time should allow hydrolysis and fermentation of slowly biodegradable particulate substrates and their uptake by PAO's or GAO's (Winkler et al., 2018). Introducing a pre-acidification step before entering the reactor has therefore been considered as a viable selection strategy, allowing the extension of the anaerobic phase without affecting the whole reactor cycle and hence maintaining a high rate of treatment.

1.5 THE SEWER AS A PRE-TREATMENT STEP

The composition of the wastewater reaching the WWTP is affected by several factors. Processes of sedimentation and resuspension are among the physical processes that can affect the concentration of suspended solids (Langeveld et al., 2017). Biochemical processes taking place in the sewer network, on the other hand, dominate the transformations of organic – and to a certain extent inorganic - matter during sewage transport.

Municipal wastewater incorporates a rich microbial community (Ali et al., 2019), composed both of microorganisms originally present at WW discharge or for which growth conditions are favourable inside the sewer. Alongside the physical processes related to sewage flow, these microorganisms will therefore drive chemical and biochemical transformations of the organic matter and of those inorganic compounds that can serve as electron acceptors. (Nielsen et al., 1992; Raunkjaer et al., 1995; Tanaka & Hvitved-Jacobsen, 1998).

Sewers can, therefore, be considered an integrative part of the treatment processes, acting as pre-treatment bioreactors before the wastewater reaches the WWTP (Huisman et al., 2002; Hvitved-Jacobsen et al., 2002). Understanding the interactions between the sewer, the WWTP and the receiving water bodies can help to improve the robustness and resilience of all the units of the urban water system and has shown its potential to lead to enhanced performance and costs optimisation of the whole water treatment system. Therefore, an integrated approach to urban water systems optimization has become common practice in the last two decades (Ahnert et al., 2005; Benedetti et al., 2013; Langeveld et al., 2003).

1.6 BIOCHEMICAL PROCESSES IN THE SEWER

Sewer networks can foresee the separate collection of urban stormwater runoff or its combined transportation with municipal wastewater. In combined sewers, wastewater flow can increase by a factor of 10-100 during precipitation events. It follows that wet weather conditions increase the significance of hydraulic and solids transport processes, reducing the importance of biochemical transformations. Under Dry Weather Flow (DWF), on the other hand, the physical aspects of the sewer performance are reduced, and microbial and chemical conversions exert pronounced effects on the wastewater quality and the whole sewer as a system. (Hvitved-Jacobsen et al., 2013).

During the wastewater conveyance in a sewer system, significant changes in both quantity and quality of the organic matter and the electron acceptors occur. Heterotrophic bacterial processes dominate the transformations of wastewater components in the sewer (Hvitved-Jacobsen et al., 2013).

The wastewater composition can significantly be affected by the hydraulic residence time (HRT) inside the network, hydraulic shear forces, enzymatic hydrolysis of macromolecules and by microbial growth and respiration in both bulk water and in biofilms. (Flamink et al., 2005; Huisman et al., 2002; Nielsen et al., 1992) Though sewer processes share similarities with the corresponding processes involved in biological treatment at WWTP, it is important to notice that they also develop very differently due to the distinct environmental conditions found in sewers or various types of bioreactors (Almeida et al., 2002; Hvitved-Jacobsen et al., 2013).

A conceptual representation of the sewer environment divides it into four phases, as shown in Figure 2: the water phase, the biofilms covering the submerged walls, the sediments and the sewer atmosphere, i.e. the air filling the remaining volume of the collection system (Hvitved-Jacobsen et al., 2002, 2013; Nielsen et al., 1992; Rudelle et al., 2011). Chemical and biological sewer processes proceed in one or more of these phases and mass transfer across the interfaces takes place, too (Bachmann et al., 2007; Hvitved-Jacobsen et al., 2013). For instance, substrates contained in the bulk water can penetrate the biofilm at the walls and hydrogen sulfide or carbon dioxide there produced can be released back to the water flow. The pathways of organic matter transformation depend on many factors. Different interactions can take place according to the flow and the structural configuration of the sewer, which can be pressurised or by gravity (Rudelle et al., 2012). The sewer configuration will influence the presence and importance of the abovementioned sub-environmental phases. Moreover, the availability of different electron acceptors, such as O_2, NO_x or SO_4^{2-}, will vary greatly, determining aerobic, anoxic, or anaerobic conditions inside the sewer.

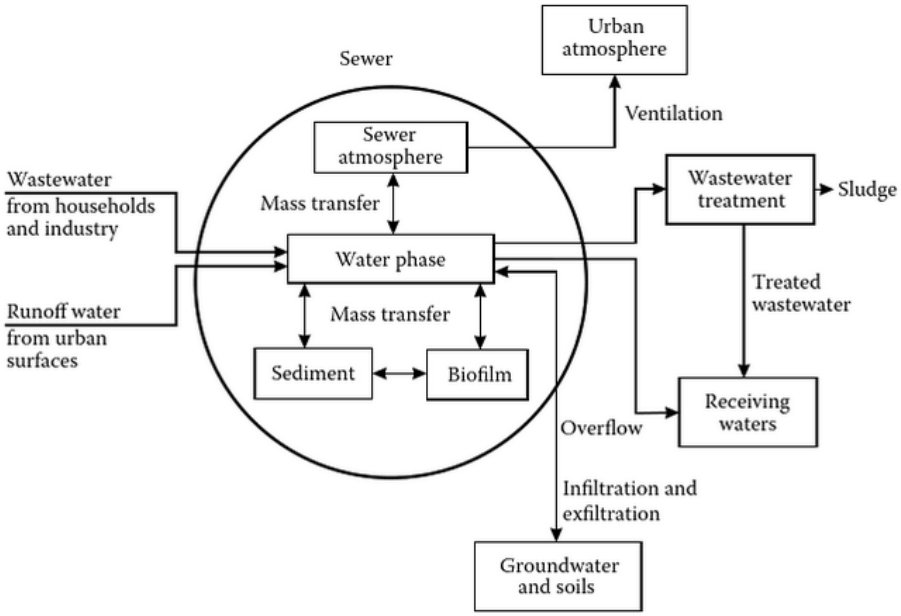


Figure 2: Sewer subsystems (phases) for the potential occurrence of sewer processes and wastewater flows between the sewer and the surrounding (urban) environment (Hvitved-Jacobsen et al., 2013).

1.6.1 Anaerobic transformations of wastewater inside sewer networks

The major pathways for anaerobic organic matter transformations in collection systems are anaerobic hydrolysis, fermentation, sulfate reduction, and methanogenesis (Rudelle et al., 2011). Hvitved-Jacobsen et al. (2013) exemplified the conversions taking place under anaerobic conditions, showing the main processes in Figure 3.

Any substrate larger than 1 kDa cannot penetrate the cell-membrane of microorganisms and is hence not readily available for microbial uptake (Sophonsiri et al., 2004). Particulate matter found in WW is usually in the form of organic polymers, which must be broken down into their mono- and oligomers before further degradation can take place (Morgenroth et al., 2002). Enzymes released into the surrounding medium, also called extracellular enzymes or exoenzymes, catalyse the breakdown process of complex matter. This process is referred to as hydrolysis. It results in the partial conversion of complex matter into smaller, more soluble molecules which can penetrate the microbial membrane, increasing the availability of substrate for the biomass. Hydrolysis can proceed under aerobic, anoxic and anaerobic conditions without altering the oxidation state of the compounds, as it does not require the presence of an electron acceptor (Hvitved-Jacobsen et al., 2013).

Fermentation is a microbial energy production process which does not require the presence of an external electron acceptor; rather, an organic compound is reduced and oxidised at the same time. Fermentation gives back low-molecular compounds, such as VFA and alcohols (Lie et al., 1997). In the absence of oxygen in sewer systems, these products can become substrate for sulfate reducing bacteria (SRB) or methanogens (Rudelle et al., 2011). SRB have been reported to grow on many different types of organic compounds. Typically, though, polymeric organic compounds – such as starch, cellulose, and proteins – are not direct substrates for SRB, who depend on other microorganisms for their hydrolysis and fermentation. Methanogens use an even more limited number of substrates for their growth, with the best-known ones being hydrogen, acetate and CO_2 (Muyzer et al., 2008).

In the presence of sulfate in excess concentrations, SRB compete with methanogens for their common substrates, hydrogen and acetate. Thanks to their higher affinity and lower threshold values for hydrogen, SRB easily outcompete hydrogenotrophic methanogens. Acetoclastic methanogens are also outcompeted by SRB, but it is important to notice that this competition is not as fast and that the two microbial groups might

coexist for prolonged periods of time within a system (Omil et al., 1998). Sulfate reducers types that can directly grow on propionate and butyrate exist, too. The dependence on syntrophic relations with fermenting communities for further reduction of the VFA is eliminated and these types of SRB hence gain a competitive advantage over other SRB and methanogens (Muyzer et al., 2008). Though on a first estimate methanogens are assumed to be confined to areas where sulfate is depleted, methane and sulfide formation have been observed to take place simultaneously (Guisasola et al., 2008; Hvitved-Jacobsen et al., 2013).

Fermentation has been observed to take place in the suspended water phase, the biofilm on the sewer walls and the sediments (Bachmann et al., 2007; Hvitved-Jacobsen et al., 1995; Rudelle et al., 2011; Tanaka & Hvitved-Jacobsen, 1998). On the other hand, due to their slow growth kinetics, SRB and methanogens would be washed away in the bulk liquid and are expected to be mainly present in the stationary parts of the sewer, i.e. the biofilm and the sediments. While methanogens have been reported to grow in the deep layers of the sediments in gravity sewers (Hvitved-Jacobsen et al., 2013), their presence in the biofilm was only observed under high temperatures of the wastewater (28°C) in a pressurised sewer with no sediments (Guisasola et al., 2008).

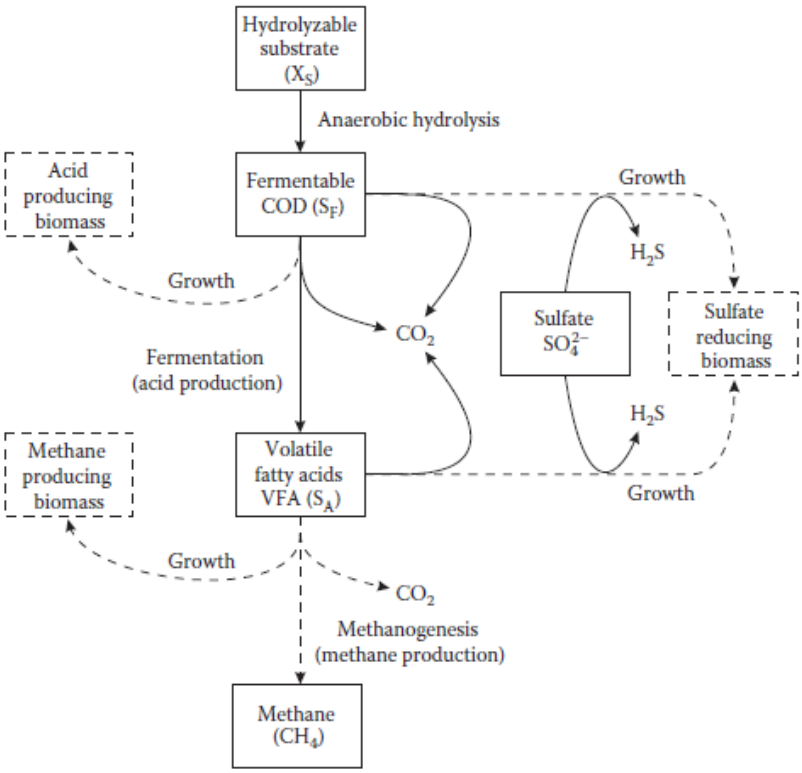


Figure 3: Simplified concept for anaerobic transformations of biodegradable organic matter in wastewater and biofilm of a sewer system. The most important parts are shown with fully drawn lines, whereas the dotted lines are typically less important for the formulation of sewer processes. (Hvitved-Jacobsen et al., 2013)

1.6.2 Influence of structural configuration on wastewater variations

Gravity sewers are partially filled and reaeration of the conveyed WW can happen via gas exchange through the surface. The presence of area characterised by highly turbulent flow is also common, increasing the oxygen content of the water. (Almeida et al., 2002; Raunkjær et al., 1997). The bulk liquid phase is consequently kept under aerobic conditions during the whole or great part of the transport. Anoxic and anaerobic conditions can take place, too, especially in the presence of deep sediments layers or in the deeper zones of a thick walls' biofilm. However, aerobic processes are the dominant ones in terms of organic matter transformations in gravity sewers (Hvitved-Jacobsen et al., 2013).

Readily biodegradable organic substrates are consumed or degraded if the wastewater is conveyed aerobically (Tanaka & Hvitved-Jacobsen, 1998). At the same time, as aerobic heterotrophic biomass is characterised by a fast growth rate (Henze et al., 1995), slowly biodegradable particulate organic matter is produced in the form of new biomass suspended in the wastewater (Hvitved-Jacobsen et al., 2002). Particle size distribution has hence been observed to shift towards larger sizes of suspended solids. Recalcitrant compounds and larger solids have been seen to reach the wastewater treatment plant and this was hypothesised to carry a detrimental effect on biological treatment. (Rudelle et al., 2011).

On the other hand, pressurised sewers are generally characterised by the absence of a gaseous phase in the sewer environment (Figure 2), as the pipe is completely filled with water. Moreover, many pressurised sewers are operated under self-cleaning strategies that prevent settling and the formation of a sediments phase.

Except for a limited initial length, strict anaerobic conditions are usually reported for pressurised sewers, making anaerobic processes the dominant transformations of wastewater compounds (Freudenthal et al., 2005; Matos & de Sousa, 1996; Nielsen et al., 1992). Anaerobic microbial processes proceed at a slow rate. At lab-scale, hydrolysis and fermentation rates have hence been observed to be fast enough to determine an accumulation of readily biodegradable substrate, such as VFA and alcohols, enhancing the wastewater quality for biological treatment (Bachmann et al., 2007; Hvitved-Jacobsen et al., 2002; Tanaka & Hvitved-Jacobsen, 1998).

Aerobic organic matter transformations were investigated in detail, building comprehensive knowledge on aerobic conversions in gravity sewers (Flamink et al., 2005; Huisman et al., 2004; Raunkjaer et al., 1995). Research on pressurised sewers, instead, has mainly been focusing on sulfide production (Hvitved-Jacobsen et al., 1995; Oosterhuis & van Loosdrecht, 2009; Sutherland-Stacey et al., 2008), leaving limited attention for anaerobic hydrolysis and fermentation.

1.7 MICROBIAL ACTIVITY TRANSFER TO AGS REACTORS

As already mentioned, municipal wastewater is characterised by a rich microbial community (Ali et al., 2019). Microorganisms enter the sewer network at discharge, directly from the wastewater sources, or by interactions with the sewer environment. Biofilm detachment or solids resuspension processes are very frequent and microorganisms grown in the sewer biofilm or in the sediments are constantly released to the suspended water phase (Ashley et al., 2004; Picioreanu et al., 1998; Rudelle et al., 2012). The receiving WWTP are therefore constantly inoculated with microbial communities deriving from the sewer (Huisman et al., 2002). However, characterisation of the microbial community found in AGS systems, both at lab- and at full-scale, showed that the microbial community composition was different from that of the influent. The effect of local environmental conditions on microbial community composition was less pronounced on microbial aggregates with shorter SRT. (Ali et al., 2019; Weissbrodt et al., 2014). This could indicate a correlation between the time spent inside the reactor and the reduction in relative abundance of the species originating in the sewer.

While sewage microorganisms might not have favourable growth conditions inside the reactor, their continuous presence is assured by a high dispersal rate provided by regular feeding of the influent (Ali et al., 2019). The influence of their activity on hydrolysis and fermentation processes inside the reactor before being inactivated or washed away, however, remains unknown.

1.8 MOTIVATION OF THE THESIS AND RESEARCH QUESTIONS

AGS technology relies on anaerobic storage of organic matter. Focusing research on anaerobic processes, such as hydrolysis and fermentation, is therefore important to improve control over the reactor stability. It would be simplistic to look at anaerobic transformations going on within AGS reactors without considering the influence that raw wastewater can have on them. Influent wastewater, in fact, is subject to biochemical conversions already within the sewer system before reaching the treatment plant. Municipal wastewater conveyed under anaerobic conditions – such as the ones created in pressurised sewers - has shown improved biodegradability. Moreover, the contribution of the influent to the hydrolytic activity of the reactor is unknown.

Anaerobic hydrolysis and fermentation can occur in two phases:

- During the anaerobic phase inside the reactor.

  Studies on the enzymatic and fermentative activity of aerobic granular sludge are necessary to evaluate its ability to hydrolyse and ferment SS. Though previous studies suggested that microbial populations inside AGS bioreactors develop away from the microbiome of their influent, the activity carried by raw wastewater might still have an impact on the observed degradation kinetics. Investigating the enzymatic and self-fermentation activity of the wastewater to be treated would thus allow to gain a deeper insight in the contribution of the influent to total reactor activity. It is therefore important to assess the contribution of raw wastewater to the degradation of particulate matter inside Nereda® reactors.

- As a pre-treatment of wastewater before entering the reactor.

  Sewer interactions can affect the amount and characteristics of suspended solids reaching the treatment plant. Though it is widely accepted that conversions of wastewater are already initiated during conveyance, previous studies on anaerobic transformations have mainly concentrated on sulfide production. Knowledge on the hydrolysis and fermentation processes inside the sewers is not yet comprehensive and is mainly based on lab studies.

An integrative approach to understanding the contribution of the sewer and of raw wastewater to AGS reactors will therefore be presented in this thesis. The theoretical background presented in this chapter led to the formulation of the following research questions:

1. What is the impact of anaerobic transformations, during wastewater transport in a pressurised sewer, on the following wastewater characteristics?
   a. Physicochemical composition, with emphasis on organic matter and solid content
   b. Fermentability
   c. Hydrolytic activity
2. Which fraction of raw wastewater displays the highest hydrolytic activity just before entering the water treatment plant?
3. Is the order of magnitude of the hydrolytic activity of the raw wastewater entering the plant comparable to that of AGS?

2 METHOD

2.1 RESEARCH STRUCTURE

Anaerobic transformations of organic matter, focusing on hydrolysis and fermentation processes, were investigated by sampling the wastewater at the inlet and outlet of a pressure sewer, indicated in Figure 4 by points ① and ②. The same locations allowed to assess possible variations in the microbial activity of the wastewater.

Primary treatment of the wastewater after it reaches the WWTP will impact its composition and possibly its activity, too. To provide a better comparison for the activities of the influent and the AGS sludge, wastewater samples were taken directly from the feeding pumps to the reactor. The sludge was collected inside the reactor, during the aeration phase to ensure complete mixing of the different microbial aggregates. The influent and sludge sampling location are shown by points ③ and ④, in Figure 4.

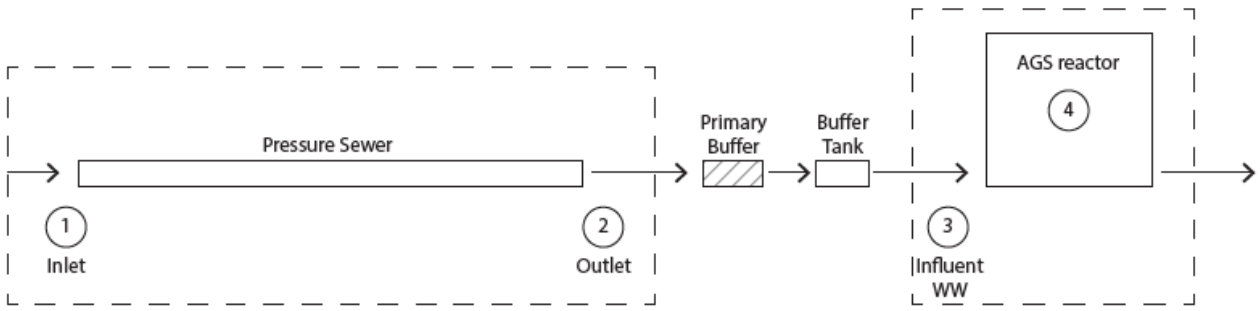


Figure 4: Schematic overview of the sampling locations in reference to the urban wastewater system. Note that the selected pressure sewer and AGS reactor were not part of the same system.

2.2 SELECTION OF THE SAMPLING LOCATIONS AND SAMPLING METHOD

Two locations in the Netherlands were selected for sampling: the pressure sewer connecting the village of Doorn to the nearest WWTP in Driebergen and the AGS treatment plant in Garmerwolde.

2.2.1 Pressure sewer

Sewer characteristics

This location was selected to assess the anaerobic transformations of raw wastewater after the conveyance through a pressure main. The physical and chemical composition of the WW and the microbial activity in terms of hydrolysis and fermentation were monitored.

The pressure main in Doorn was identified as suitable for this study because it serves a sufficiently large number of households, lacks side connections and has minimal groundwater infiltration. Industrial discharge is limited, and the flow is mainly due to domestic wastewater. A system of gravity mains conveys the wastewater from the households up until a pump sump immediately upstream of the pumping station (PS). The residence time within the gravity sewers is short, however biodegradable substrate uptake and growth of aerobic biomass can be expected. The maximum capacity of the pumps is 750 m^3/h, with usual intermittent pumping flow during DWF conditions of 200 m^3/h. A self-cleaning operation strategy involving high pumping speed ensures solids suspension, preventing the accumulation of sediments in the pipe.

Sampling

Raw wastewater was sampled at the upstream and downstream locations of the pressurised sewer stretch between the 3rd September and the 24th October 2019. Figure 5 shows a schematic of the pressure sewer and the sampling locations.

Samples were collected under comparable conditions of DWF, determined according to the definition provided by Schilperoort et al., (2012). Two samples were collected under WWF conditions to test the activity of the sewer walls biofilm under the hypothesis of sloughing phenomena.

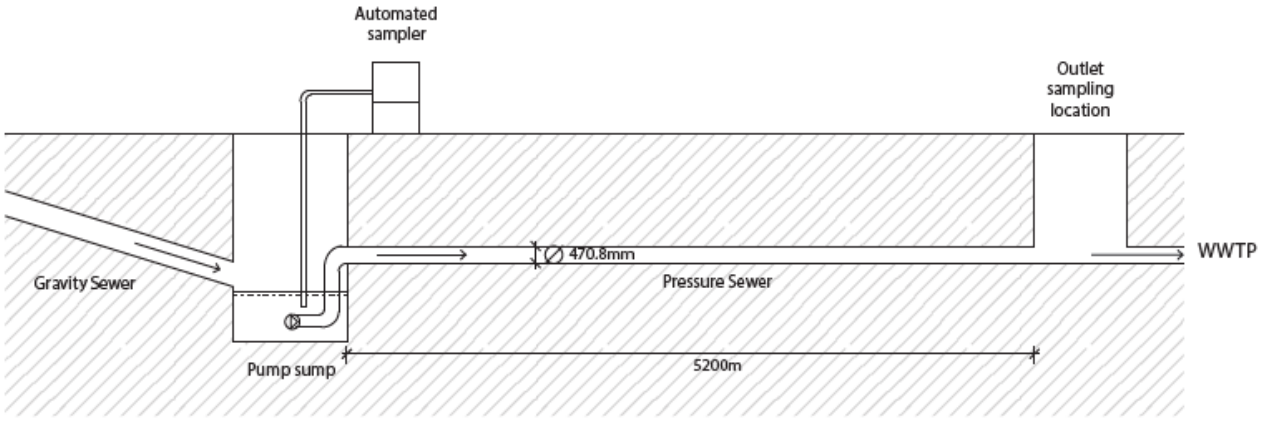


Figure 5: Schematic representation of the sampled pressure sewer (not to scale). The pressure sewer was made of a PVC pipe, with length and internal diameter reported in the figure. Sampling always occurred at the same times (10:00 and 14:00 during weekdays), with an average flow during sampling of 80 m³/h reaching the WWTP.

The initial plan involved the installation of automated samplers at the sewer inlet and outlet locations. However, it was not possible to install the sampler at the WWTP. The sampling port was located vertically below the pipe (Figure 6), causing high settling of solids which immediately clogged the sampler line. For future scopes, it would be ideal to find a location with a sampling outlet located horizontally at the side of the pipe. However, online sampling directly from the pipe is not recommended in any case for raw wastewater.

Grab samples were taken instead at the sewer outlet. Samples were always collected on weekdays at the same time (10:00 and 14:00) to limit the variations of flow and concentrations due to the daily and weekly WW variability. Due to hygiene and safety conditions, it was not possible to manually sample the sewer. Wastewater was collected from the sampling latch for the influent to the WWTP. Three pumping stations contributed to the influent of the WWTP in Driebergen. All the pumps were switched off with the help of the operators, allowing the flow to arrive only from Doorn for fifteen minutes before sampling.

Figure 7 shows the automated vacuum sampler, a “Liquistation” from Endress + Hauser, that was installed by IMD at the inlet of the sewer to collect hourly samples, provided flow was available. An OPLC controller from IMD was used to set the starting and the stopping times of sampling and to record the sampling data. The decision to collect grab samples was taken to match the type of samples collected at the outlet of the sewer. The sampler was therefore modified with the help of the installing company to be able to collect grab samples, similarly to the outlet ones. DO and T data were collected during the sampling period, from the beginning of September until the 2nd October 2019, when the probe broke. Continuously recorded flow-data was measured online at the inlet location, in connection with the pumps activity. This data was available throughout the whole campaign and was retrieved daily, right after sampling at the outlet location. This allowed to take the hydraulic retention time (HRT) in consideration when matching the inlet and outlet samples.

Enzymatic assays, BOD_5, ATP and PSD analysis were carried out within 5 hours after collection, storing samples at 4°C in the meantime. The remaining measurements were done after a maximum storage time of 2 days at 4°C, or longer sample preservation at -20°C. Frozen samples were thawed at room temperature.

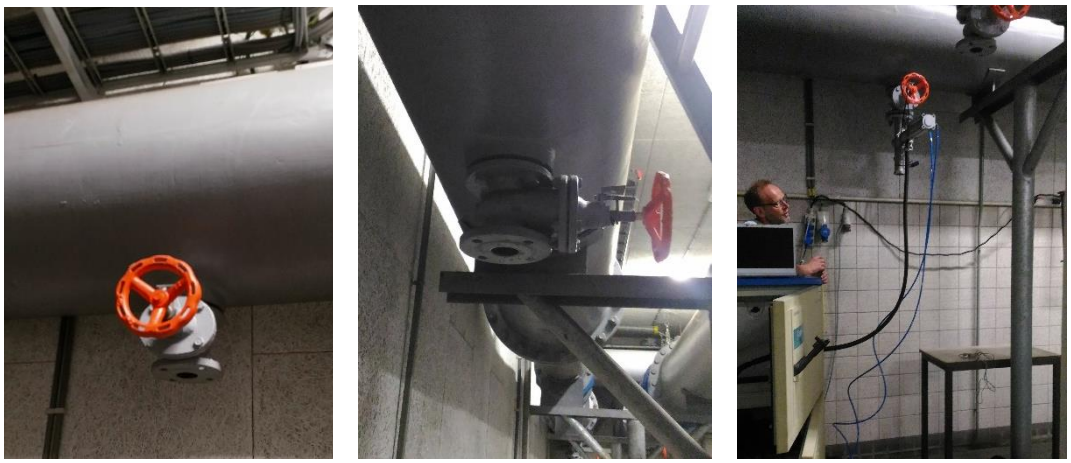


Figure 6: Sampling valve underneath the pressure main at the wastewater treatment plant. The installed on-line sampler (right) could not operate due to the elevated settling of suspended solids and the impossibility of installing a flushing mechanism due to the safety regulations for the basement of the WWTP.

Figure 7: Automated vacuum sampler installed at the upstream location of the pressure sewer.

2.2.2 AGS treatment plant

AGS biomass and influent enzymatic activities were measured at the WWTP of Garmerwolde, NL, between the 30th April and 9th May 2019. Half of the influent to the treatment plant is treated by two Nereda® reactors. The plant characteristics were described by Pronk, de Kreuk, et al. (2015). Wastewater is transported to the WWTP of Garmerwolde by means of six pressurised sewer lines (Veel, 2018). The domestic wastewater reaching the plant has therefore undergone long anaerobic sewer conveyance.

Grab samples of the influent were collected after screening and grit removal. As mentioned in the introduction, Nereda® reactors are operated in sequential batch mode. The biomass was collected during

the aerobic phase, ensuring complete mixing of the sludge. The analyses were performed within 8 hours from sampling, storing them at 4°C before the beginning of the tests.

Figure 8: Nereda reactor in Garmerwolde during the aerobic reaction phase when sampling occurred.

2.3 ENZYMATIC ASSAYS

2.3.1 Method selection

Enzyme activities can be determined by measuring specific enzymatic conversion of synthetic substrates to coloured products that are quantified photometrically (Bisswanger, 2014). Though newer methods exist, based on automatic enzyme activity readings by means of a 96-well plate, a literature review showed that this innovative method is not yet suitable for this research. Instant reading of the enzymatic activity requires the absence of particles in the analysed samples, introducing additional error during the enzymes extraction step which might be of different magnitudes for different sized particles. This would make it difficult to compare the activity of different fractions of the WW. Moreover, no method has been developed yet to extract enzymes from granular sludge. For consistency of the results, all sludge fractions and the WW were analysed using the same method.

2.3.2 Samples preparation

AGS was fractionated into the following fractions: large granules (particles of diameter >1000 µm), small granules (200-1000 µm) and flocs (45-200 µm). The activity of mixed liquors was also assessed for all the substrates, after a dilution to around 4 g/L. The bulk liquid was obtained by letting 2 L of influent settle for 2 hours in a beaker with capacity 2 L and then collecting the supernatant with a syringe, making sure that the tube would not go below half of the beaker's height. No variations were made to influent wastewater for α- and β-glucosidase assays, while it was concentrated for protease and lipase assays, as the particles were hypothesised to carry more activity. The concentration of the influent was achieved by settling for 2 hours and discarding the supernatant, keeping only a final volume of 250 mL. All the fractions thus obtained were buffered with 20 mM Tris-HCl and the initial pH was adjusted to 7.5.

2.3.3 Assay procedure

Hydrolytic activity of the wastewater and of the AGS fractions was measured by means of enzymatic assays. The enzymes targeted were α-glucosidase, β-glucosidase, lipase and protease. This was done by means of chromogenic artificial substrates: *p*NP-α-glucopyranoside, *p*NP-β- glucopyranoside, *p*NP-palmitate and azocasein. Air-tight vials of 40 mL were used, adding controlled amounts of biomass (i.e. the analysed sample) and substrate and flushing for 1 minute with N_2 gas to ensure anaerobic conditions. Substrate was added in excess concentrations to ensure the detection of the maximum hydrolytic activity. Samples were collected

at the beginning and at regular intervals throughout the assay. The reaction was immediately stopped by addition of TCA, furthermore, enabling prolonged storage of the samples. At the end of the assay, all collected samples were stored at -20°C. When later processing the samples, they were thawed at room temperature, then centrifuged and filtered with syringe filters at 0.45 µm. 2 M NaOH addition allowed to read the absorbance of the collected samples in a Thermo Scientific spectrophotometer Genesys 10S UV-Vis.

For p-nitrophenol conjugates (*p*NP-α-glucopyranoside, *p*NP-β- glucopyranoside and *p*NP-palmitate) hydrolysis of 1 mol of the synthetic substrate is accompanied by the release of 1 mol pNP. Activity can therefore be assessed by linear regression of the measured absorbance vs time. For all pNP conjugate substrates, calibration curves were constructed using known amounts of p-nitrophenol (Sigma-Aldrich) and diluting the solutions with equal volumes of TCA.

Azocasein, on the other hand, does not hydrolyse according to a 1:1 proportion or substrate and released dye. A double conversion was therefore required, obtained by the preparation of two calibration curves from two equal samples in which the effect of protease was assessed differently. In one sample, the concentration of the dye release by the activity of protease was measured via absorbance with the spectrophotometer. The other parallel sample was treated with Folin & Ciocalteu's reagent to determine the equivalent released concentration of Tyrosine. Absorbance due to the colour of the dye could therefore be converted to the concentration of Tyrosine. It must be noted that this method could lead to a large underestimation of the actual concentrations of hydrolysed proteins, as only one amino acid is measured.

2.4 FERMENTATION BATCH TESTS

Fermentation Batch Tests (FBT) were carried out to assess the self-solubilisation and self-fermentation rates of the wastewater, based on its total and soluble COD content. Wastewater samples collected at the upstream and downstream locations of the pressure sewer of Doorn, matched according to the WW retention time in the pipe, were analysed. The samples were left at room temperature until the desired temperature had been restored after storage at 4°C. Triplicate tests were performed for each of the collected samples. The bottles were capped by air-tight stoppers equipped with a sampling port. The initial volume was set at 250±1 mL and a constant sample volume was collected throughout the experiments. Initial and final pH values were recorded. The tests were performed at room temperature (20°C) and anaerobic conditions were obtained by flushing with N_2 gas for 2 minutes. The bottles were continuously stirred by means of magnetic plates and samples were taken at the beginning of the test and every 40 minutes for 240 minutes. Final volumes and CO_2 concentrations were measured.

The production of VFA and the variations in SCOD were monitored. Moreover, NH_4^+ was measured as an indicator for protein hydrolysis.

2.5 OXYGEN UPTAKE RATE

The samples were allowed to reach room temperature after being stored at 4°C for 1-2 days. Oxygen saturation (8.5 mgO_2/L) was reached by high speed stirring by means of magnetic stirring plates. Substrate saturation conditions were obtained by adding a concentrated solution of $NaAc{\cdot}3H_2O$ and demi-water, for a final concentration of the WW of at least 2 gCOD/L including the COD already present in the WW. This was done to reach 0-order kinetics and test for the maximum OUR of the WW. The $NaAc{\cdot}3H_2O$ powder used was bought from Sigma-Aldrich (Darmstadt, Germany). Two OUR tests were run in parallel under the same environmental conditions in two 140 mL respirometers. Each respirometer was monitored by an FDO-925 Optical IDS dissolved oxygen sensor and both sensors were connected to the same WTW multimeter, recording DO values per second. A computer logged DO concentrations per time. OUR were calculated from these measurements by linear regressions on DO concentration vs time measurements, as described by Vollertsen & Hvitved-Jacobsen (1999).

2.6 E. COLI QUANTIFICATION

Wastewater and sludge samples were pre-treated with sonication in order to separate the microorganisms and prevent underestimation of Colony Forming Units (CFU). Pulses of 30 seconds and 10 seconds of pause were applied at 40% of the maximum intensity for a total of 5 minutes of sonication time (pauses were not included in the time count). This was however seen to cause a significant reduction of the detected bacteria. Preliminary experiments were repeated pre-treating the samples with a Potter-Elvehjem-type tissue grinder. 200 µL of the pre-treated samples were inoculated on Chemocult Agar plates, and incubated for 24h at 37°C.

Decimal dilutions were performed in series for the samples before sonication or biomass grinding. These were namely 10^{-2} for the influent and 10^{-1} for small granules. No dilution was performed on flocs and big granules.

2.7 PHYSICAL-CHEMICAL ANALYSIS OF THE WASTEWATER

Total and volatile solids (TS and VS) concentrations and Total and Volatile Suspended Solids (TSS and VSS) were measured according to the procedure described in the Standard Methods (APHA, 2005).

Soluble concentrations were obtained by filtering the samples by means of a cross-flow filter at 0.45 µm. Samples for WW characterisation were also pre-treated by membrane filtration at 1kDa to assess the concentration of organic matter readily available for microbial uptake.

Hach-Lange photochemical test-kits were used to test total and soluble COD, BOD_5, NH_4-N and TN. Products number were LCK514, LCK555, LCK303 and LCK338, respectively. ATP was measured by luminometry, with the LuminUltra QG21W-50C test kits and an LB9509 luminometer from Aqua-Tools. High-performance liquid chromatography (HPLC) was used to assess the VFA content of the wastewater. Proteins were measured according to the modified Lowri method proposed by Frolund et al. (1995), while carbohydrates concentration was determined as glucose equivalent using the enthrone-sulfuric acid method by Dubois et al. (1956). The developed colour absorbance was measured against a Tris blank with the Thermo Scientific spectrophotometer Genesys 10S UV-Vis, at 750 nm for proteins and 490 nm for carbohydrates. Calibration curves were constructed with known concentrations of the mentioned compounds, which were related to the measured absorbance by linear interpolation.

The particle size distribution (PSD) of wastewater samples pre-sieved at 1000 µm was assessed using the Particle Size Analyser Blue Wave S3500 from Microtrac (Montgomeryville, USA). The measurement was carried with a 25% flow rate.

2.8 GRANULAR SIZE DISTRIBUTION

The granular size distribution (GSD) of AGS from the Nereda reactor in Garmerwolde was assessed, fractionating the sludge according to the size ranges shown in Table 1. A stack of sieves was used, separating the biomass by applying moderate pressure with tap water. TS and VS were determined for the resulting sludge fractions and the respective weight percentage with respect to the total was calculated.

Table 1: Size ranges for the fractionation of the AGS in its microbial aggregates

Sludge fraction	Particles diameter size range (µm)
Large granules	>1000
Small granules	200-1000
Flocs	45-200

2.9 WASTEWATER FRACTIONATION

The enzymatic activity of different WW fractions was measured on samples matched via the HRT from the inlet and the outlet of the pressure sewer of Doorn. The TS, VS and cellular ATP concentrations were measured, too, to provide further information on inorganic/organic matter content and viable organisms distribution in each fraction. The wastewater fractionation was repeated in triplicates.

According to the definition by van Nieuwenhuijzen et al. (2004), solids are considered settleable when their diameter is >45 µm. A known volume of sewage was therefore sieved at 45 µm, resuspending the retained solids in an equal volume of water, and keeping the permeate as the fraction <45um. The bulk liquid fraction of the wastewater was the permeate of a cross-flow filter at 0.45 µm.

3 RESULTS AND DISCUSSION

3.1 DOORN-DRIEBERGEN SEWER SYSTEM AND SAMPLING OVERVIEW

3.1.1 The pressure sewer was predominantly anaerobic, with turbulent flow-regime

The sampling period on the sewer main connecting the village of Doorn, NL, to the nearest WWTP in Driebergen, NL, was between the 3rd September and the 24th October 2019.

The average DO of the WW entering the sewer was 0.8 mgDO/L under DWF conditions. Due to a fault in the DO probe, it was not possible to monitor the oxygen concentration after the 2nd October. The maximum capacity of the pumps was 750 m^3/h, beyond which the wastewater was either buffered, in the pump sump (see Figure 5) and in the storage capacity of the upstream gravity sewer or redirected to overflow. The highest DO value recorded during the monitoring period was of 3.4 mgDO/L, corresponding to a storm which lasted for 8 hours at 600 m^3/h. The flow and DO behaviour during the storm are reported in Figure 9 (Top: 9th of August).

Wastewater was pumped intermittently. The average flow during pumping was around 200 m^3/h for DWF. Even in the absence of rain, the sewer was always operated under turbulent conditions to cause resuspension of solids and provide a self-cleaning effect of the duct.

From the OUR measured at the inlet of the pressure main (see Appendix I), it was possible to calculate the redox conditions inside the pressure main according to the model provided by Matos & de Sousa (1996). The initial DO was estimated to be consumed within the first 19 minutes inside the pipe under DWF and 25 minutes under WWF. Besides, anoxic conditions require the absence of DO and the presence of nitrates. Such conditions are typically only found when artificially implemented to control sulfide formation (Hvitved-Jacobsen et al., 2013; Oosterhuis & van Loosdrecht, 2009). It can, therefore, be assumed that after the first half an hour during DWF the sewer stretch of Doorn was strictly anaerobic.

3.1.2 Overview of the collected samples

All the collected WW was conveyed overnight in the pressurised main, with an HRT of 12-14h. The WW temperature did not show high oscillations during the whole sampling campaign, with an average T of 20.1±0.6°C.

Sloughing phenomena were hypothesised to increase during rain events, hence two samples were purposely collected under WWF with the aim to assess biofilm activity (Hvitved-Jacobsen et al., 2013; Nielsen et al., 1992; van Loosdrecht et al., 1997). Figure 9 shows an example of flow and DO conditions of the day with the most intense storm event registered during the sampling period, for the WW taken under DWF and for the two WWF samplings.

In both WWF cases, the rain events took place after a long period of DWF (see 13th and 16th September in Figure 9). Coincidentally, the WW sampled at the outlet of the sewer on the 13th September afternoon could be matched back to the WW entering the sewer just before the storm started. The inlet sample automatically collected by the sampler on 16th September afternoon, instead, was taken at the peak intensity of the storm event, allowing to see the effect of transport in the sewer of diluted WW (Section 3.2.4).

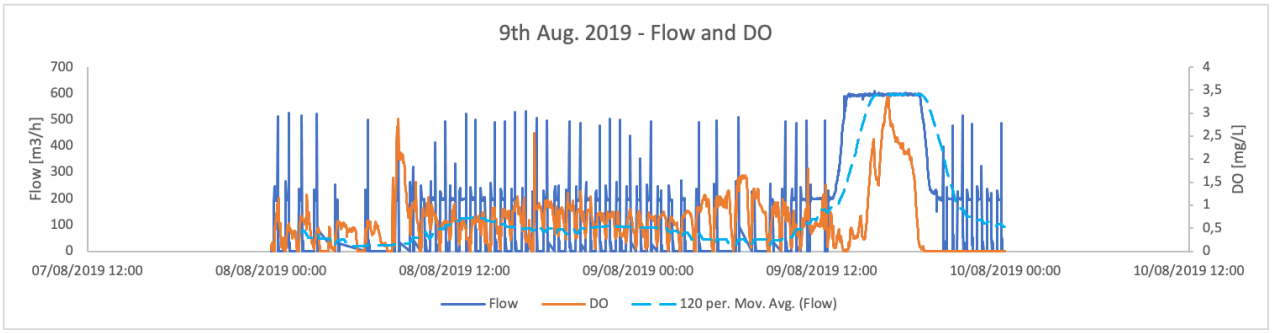
Unlike the initial hypothesis, no significant amounts of biofilm were found in the collected samples, which could, however, still provide interesting data.

The microbial activity of the sample collected at the beginning of the storm event was confirmed to be the same as that of DWF samples. It has been reported that a biofilm developed under anaerobic conditions is

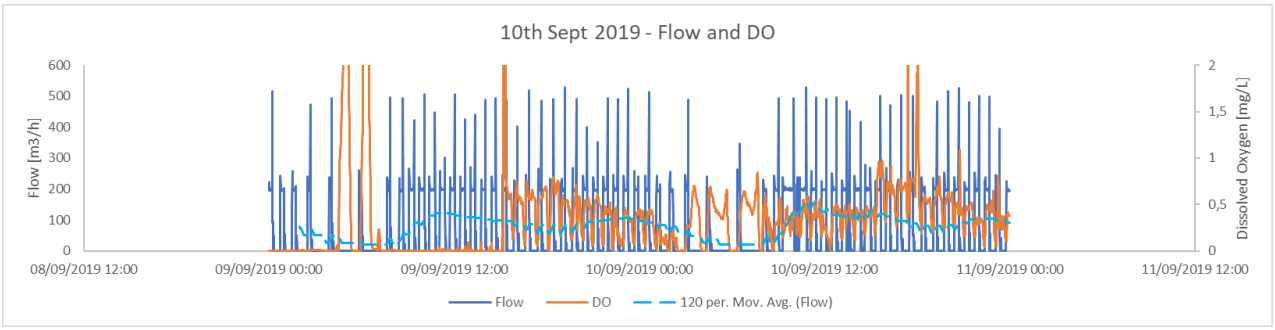
typically rather smooth and less than 500 µm in thickness (Hvitved-Jacobsen et al., 2013). The sloughing phenomena due to turbulence increase during storm events might not have led to significant increases in the biofilm concentration in the WW as the biofilm never managed to grow protuberances that could detach.

In total 16 samples were collected under DWF and 2 samples under WWF. The WWF samples were used for enzymatic assays, while the DWF samples were analysed in more detail, performing different kinds of analyses. An overview of the gathered samples, the corresponding flow, T and DO data and the type of analysis carried out on them can be found in Appendix I.

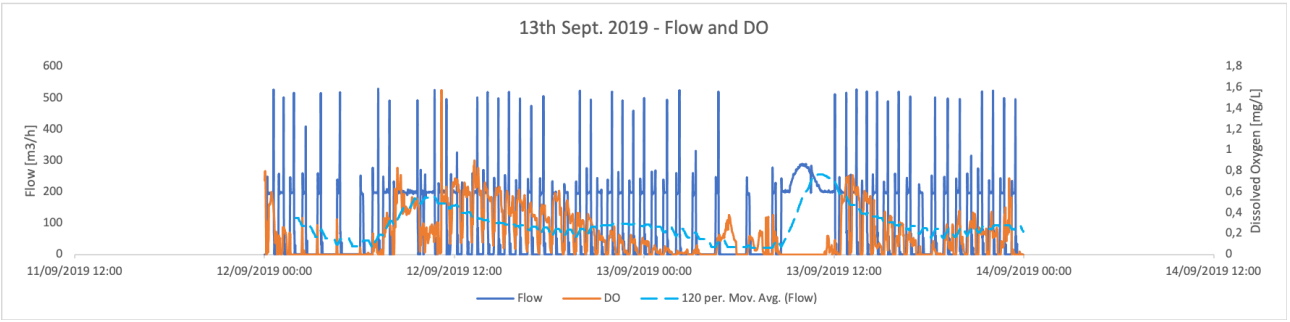
a)



b)



c)



d)

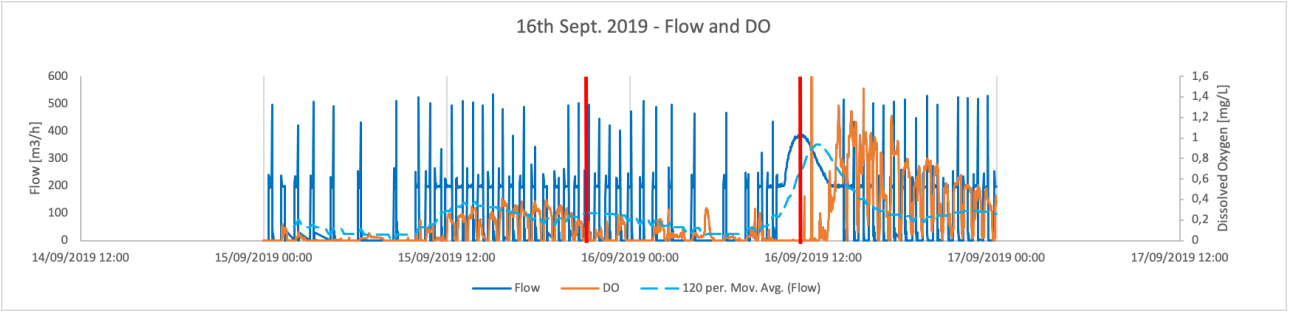


Figure 9: Flow and dissolved oxygen data from online measurements at the inlet of the sewer on a) the day with the most intense storm event during the sampling period b) a DWF day, c) the WWF day on 13th September; and d) the second WWF day, the 16th September. Two samples were collected each day and the red vertical lines indicate the time at which the inlet samples were collected. The sampling time of the outlet samples can be calculated from the HRT. The distance between one dark blue peak and the other indicates the frequency with which pumps needed to activate to cope with the wastewater flow. The flow variations can be more easily understood from the average flow, reported by the dashed line in light blue (moving average over 2 hours). The zones with continuous flow indicate storm events, in which the pumps were operated continuously. On the 13th and 16th of September, the red lines on the right overlap with the continuous flow zones, indicating that samples were collected during a precipitation event. In both cases the DO probe clogged for the initial phase of the storm, returning nul values.

3.2 CHANGES IN THE WASTEWATER MICROBIAL ACTIVITY

3.2.1 Drop in ATP concentrations after sewer transport

The adenosine triphosphate (ATP) content of viable organisms was measured at the inlet and the outlet of the pressure sewer. These values were indicative of the energetic level of the microorganisms right after the aerobic sewer and their state after the extended anaerobic conveyance in the pressure sewer. The results reported in Figure 10 showed that the WW at the inlet of the pressure sewer contained almost 2.5 times more activity than average domestic sewage (Whalen et al., 2006). However, a reduction in cellular ATP content from 125±26 ng/mL to 51±17 ng/mL was observed on the samples collected at the outlet. Shifting of aerobic biomass to a dormant state inside the force main could explain the findings. Moreover, according to Archibald et al., (2001), the ATP level is relatively constant in bacterial cells growing steadily, but it increases with increasing growth rate. As anaerobic microorganisms have a slower growth rate, their ATP production might be reduced. Lower ATP concentrations in the WW might not indicate a decrease in the biomass concentration, rather a shift towards (facultative) anaerobic microorganisms.

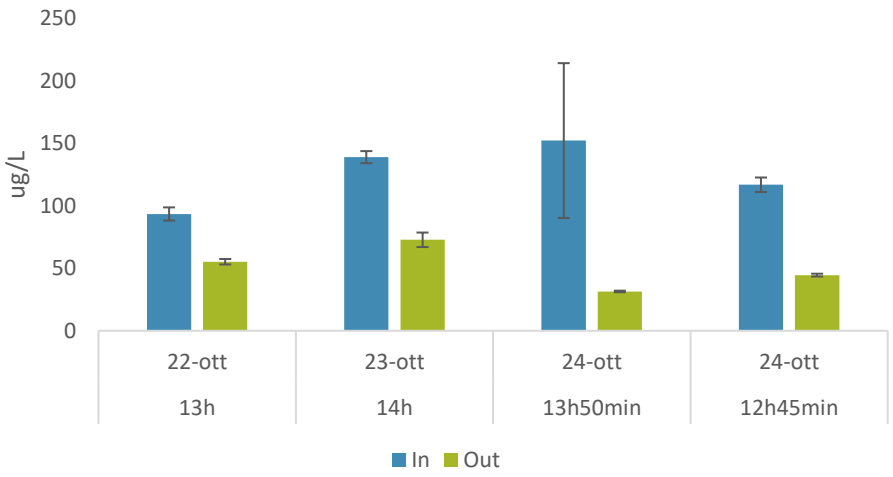


Figure 10: Cellular ATP concentrations (living microorganisms) in the wastewater at the inlet and the outlet of the sewer

3.2.2 Fermentability and solubilisation rate of the WW could not be identified

The self-fermentation rate of the wastewater was studied via fermentation batch tests (FBT), carried out in duplicates over a set of six WW samples. The samples included three inlet and three outlet samples, creating three matching pairs obtained considering the HRT in the pressure sewer. There were two original purposes of the FBT: isolate the fermentation activity of the WW bulk from that of the sewer biofilm and evaluate the hydrolytic and fermentation performance of the WW within 1 hour of anaerobic feeding phase in AGS reactors.

Despite having extended the monitoring beyond the target time (60 min) to allow more fermentation products, the concentrations measured were still too low. Results of the FBT showed a very poor fitting of the data and neither the fermentation nor the solubilisation rate of the WW could be identified. Although it seemed like the WW activity fell below the detection limit or it was not present at all, these results might have been deceiving.

A report of all the acetate, SCOD and NH_4^+-N concentrations measured at regular intervals during the test can be found in Appendix III.

A full WW characterisation was carried out on the same samples used for the FBT. It was therefore possible to compare the initial FBT measurements (t_0) of AcH, SCOD and NH_4^+-N to the composition of the WW right

after sampling in situ (Table 2). Although both data sets should have been the same at the beginning of the batch test, it was noticed that the initial SCOD was lower in all the FBT bottles, while the initial acetate concentration was higher for inlet samples and lower for outlet ones. NH_4^+-N had remained stable on all samples, apart from the last where it increased after storage. Furthermore, the AcH/SCOD ratio increased in the inlet samples and remained constant in the outlet ones.

The reason for the observed differences could be due to a lag before the beginning of the fermentation batch tests. After storage in the fridge, samples were acclimatised before starting the test to achieve an initial temperature of 20°C. This lasted around 2 hours. After that, preparation of the bottles, initial pH measurements and nitrogen flushing took another 1h15min circa.

During the lag time before the fermentation batch test, there could have been unmonitored fermenting activity in the wastewater. This would explain the higher VFA and lower SCOD on the inlet samples compared to their equivalent samples used for wastewater characterization. On the other hand, the samples from the outlet showed equal or lower VFA than on their equivalent WW characterization samples, suggesting that VFA were consumed before the beginning of the test and without further solubilisation. The differences between the behaviour of the inlet and outlet samples could be explained due to their content of easily fermentable COD – the outlet samples seem to have less fermentable COD and therefore in the lag phase, as well as during the test, no perceivable VFA production is observed.

This observation suggests that the fermentation activity of the inlet WW might be considerable. The increase in AcH observed in the WW in less than 3 hours before the beginning of the FBT was between 10 and 22 mgCOD/L for the sewer inlet samples. This was pretty similar to the net increase in acetate observed after the passage through the sewer, with an average HRT of 13h, which ranged from 16 to 62 mgCOD/L.

The VFA production observed in the sewer stretch might in large part be due to the fermentation activity of the WW itself, rather than deriving from processes inside the pressure main, such as biofilm activity. However, the acetate production seen in these samples was quite large compared to other similar studies. Rudelle et al., for instance, found a production of AcH of 5 mgCOD/L in the first 3 hours at 20°C (Rudelle et al., 2011).

As it was not possible to monitor the environmental conditions before the beginning of the FBT under which the changes occurred, these data should be handled with care and it is suggested to make new experiments to draw robust conclusions in this regard.

a)

		AcH				SCOD				NH4-N			
		WW Ch.		FBT		WW Ch.		FBT		WW Ch.		FBT	
		Avg	*St. Dev.*	*Avg*	*St. Dev.*	*Avg*	*St. Dev.*	*Avg*	*St. Dev.*	*Avg*	*St. Dev.*	*Avg*	*St. Dev.*
In	13h50min	22	-	44	-	325	2	263	-	55,5	0,8	56,6	-
Out	13h50min	70	-	59	-	314	5	268	-	62,5	0,9	61,6	-
In	12h45min	5	-	15	-	149	3	142	-	31,3	0,4	32,2	-
Out	12h45min	72	-	67	-	320	3	267	-	52,5	1,2	62,0	-

b)

AcH/SCOD		WW Ch.		FBT	
		Avg	St. Dev.	Avg	St. Dev.
13h50min	In	6,8%	n.a.	16,3%	1,6%
	Out	22,1%	n.a.	21,0%	0,6%
12h45min	In	3,5%	n.a.	10,0%	1,0%
	Out	22,5%	n.a.	24,0%	2,0%

Table 2: a) Acetate, SCOD and ammonium concentrations as measured during the WW characterisation and as initial values for the fermentation batch test. The quantities for which no replicates were measured are reported without standard deviation. b) Comparison of the fractions of acetate over the soluble COD as measured for the WW characterisation and for the FBT.

WW Ch. = Wastewater characterisation; FBT = Fermentation batch test; AcH = Acetate; SCOD = Soluble COD; In and Out correspond to the inlet and outlet of the sewer.

3.2.3 Variations in enzymatic activity were enzyme-dependent

A screening test was done to choose which enzymes to work with for the analysis of the pressure sewer connecting Doorn to Driebergen. The activities of four types of enzymes measured in the wastewater reaching the WWTP in Driebergen were compared to results previously obtained in Garmerwolde. Data can be found in Appendix IV. Alpha- and Beta- glucosidase activities were selected in this study to assess eventual variations in the enzymatic activity of domestic wastewater due to the conveyance in an anaerobic sewer. Glucosidases, in fact, could be quantified with a lower margin of error than the other considered enzymes and were hence considered more suitable to evaluate changes in the wastewater activity.

Figure 11 provides an overview of the α- and β- glucosidase activities measured at the inlet and outlet of the pressure sewer during this study. The samples were collected 4 times under DWF and 2 times under WWF conditions (see Section 3.1.2). The deducted hydrolysis rates presented a high variability, even within similar types of samples and over the one-week sampling period. Although enzymatic activity variations have never been assessed in sewer systems before, the results of this research are in line with the observations from previous studies on activated sludge and influent to WWTP (Frolund et al., 1995; Gómez-Silván et al., 2013; Kreutz et al., 2016).

Hydrolytic activity was measured per unit of WW volume. Beta-glucosidase volumetric activities at the pipe inlet during DWF conditions were in average double as high as values observed for Alpha-glucosidase. It was not possible to identify a recurrent behaviour for the changes in volumetric activity of α-glucosidase between the pump sump and the influent sampling point at the WWTP. Contrarily, it seemed that β- glucosidase enzymes decreased after transport.

Under WWF conditions, no statistically significant difference was observed between in and out samples, neither for α- nor for β-glucosidases. This was true for the samples of both the 13th (sampled at the beginning of the precipitation event) and the 16th September (sampled at the flow peak during the rain event). This could be an indication of the fact that such a short HRT might not be enough to induce any difference in the wastewater. High hydrolytic activity was expected under WWF conditions due to sloughing phenomena of the walls' biofilm. Slime detachment might have been found earlier in the flush or, as mentioned in Section 3.1.1, it might not have been considerable due to the dense biofilm growth under high hydrodynamic shear stress inside the force main. These results should, anyhow, be interpreted cautiously since only two WWF samples, of different nature, were studied.

The biomass-specific α-glucosidase and β-glucosidase activities, based on the concentration of cellular ATP, are reported in Figure 12. The specific activity for α-glucosidase was seen to follow an increasing trend in all DWF samples. The opposite trend was observed for β-glucosidase specific activities, which in DWF were observed to decrease after the anaerobic conveyance.

As the volumetric activity did not vary, but the biomass-specific activity increased, α-glucosidase enzymes might mainly be released by facultative anaerobic biomass. On the other hand, β-glucosidase enzymes might mainly be secreted by aerobic biomass and undergo an inhibition or degradation process under anaerobic conditions.

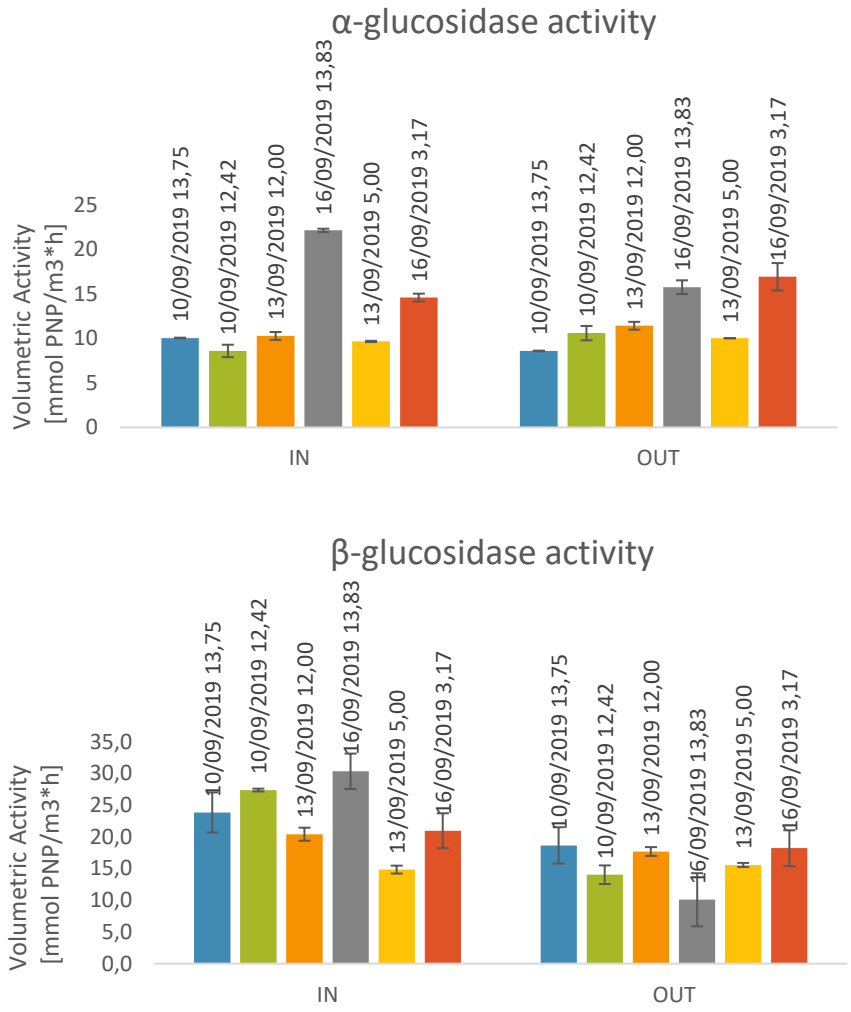


Figure 11: Volumetric activities measured on the samples collected at the inlet and outlet of the sewer for a) α-glucosidase and b) β-glucosidase. The first four bars in each cluster represent DWF samples, while the last two (yellow and red bars) correspond to WWF samples (see also HRT values reported next to each bar).

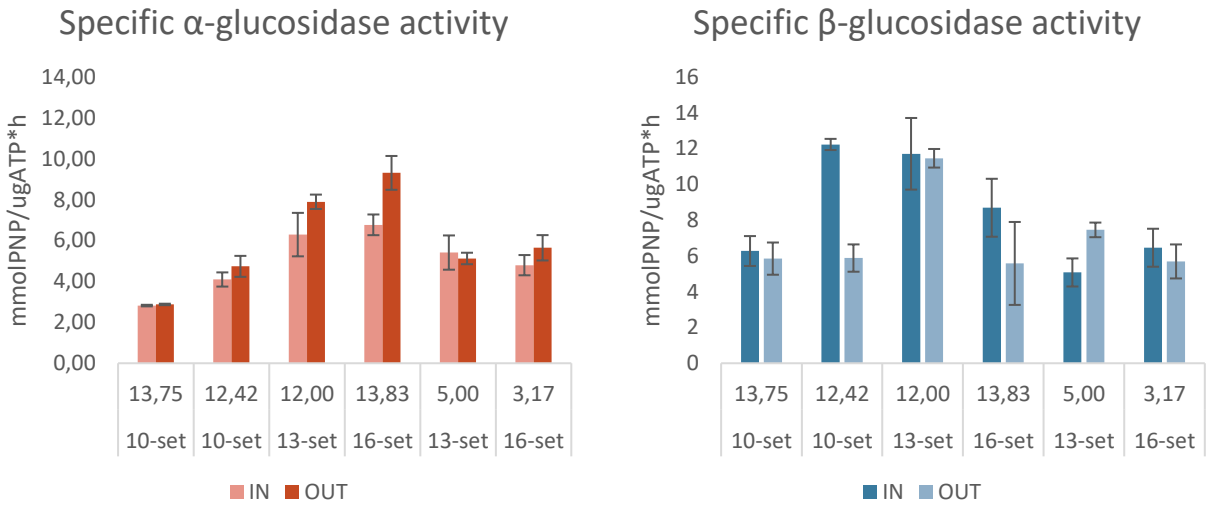


Figure 12: Biomass-specific activities measured on each sample, with values reported for the sewer inlet and outlet. The last two sampling dates correspond to WWF samples (13th Sept HRT 5h and 16th Sep HRT 3h10minutes).

Left: for α-glucosidase; Right: for β-glucosidase

3.3 VARIATIONS IN THE WASTEWATER PHYSICAL-CHEMICAL COMPOSITION DUE TO ANAEROBIC CONVEYANCE

This section reports the variations observed between the inlet and the outlet of the sewer in terms of solid content, organic matter (COD, BOD, VFA) and nitrogen (TN and NH_4). ANOVA tests showed high variability among replicate samples. This was expected due to the weekly and daily variability of domestic wastewater (Gruber et al., 2005; Henze et al., 1995; Schilperoort et al., 2012). The effect of the pressurised sewer conveyance was hence determined per each sampling, performing t-tests to compare inlet and outlet values for each of the measured parameters.

3.3.1 Exclusion of the samples of the 24th October, HRT 12h45min

The inlet and the outlet samples collected during the second sampling set on the 24th of October, with an HRT of 12h45min, showed a different behaviour compared to the other samples. From the results reported in this chapter section it is possible to make the following observations:

- All organic matter values were lower than average on the last inlet sample, apart from BOD. This could be explained by the sampling time at the inlet of the sewer. While the other samples had been collected by the automated sampler between 19.45 and 21.30, the second inlet sample of the 24th October was taken at 00:56. The results of a 24h monitoring campaign conducted on 3 DWF days (Appendix V), showed that TCOD concentrations decreased every day around 1am, when the evening peak finished.
- Contrary to the remaining samples, TS, TCOD, SCOD, BOD, COD of particles <1kDa and even particulate proteins increased after the sewer (Figure 20).
- Soluble carbohydrates decreased, but without any corresponding change to their particulate form.
- The PSD curve shifted towards smaller sizes, following a different trend from the rest.

Increases in the total COD and total solids might be indicative of sampling errors or variations in the wastewater composition. As the actual reasons for these variations remain elusive, the samples collected during the second set on the 24th of October were excluded from further discussions in this chapter. However, they were reported together with the remaining data for transparency of the results.

3.3.2 Solid content was reduced after conveyance in the sewer

It must be noted that in this study only sanitary solids and kitchen organics were sampled, defined according to the classification proposed by Ashley et al., (2004). Larger solids, such as towels, rags etc., were excluded by the sampling method.

Figure 13 shows the total and volatile solid content of the wastewater. Volatile solids were found to decrease from 455±145 mg/L to 318±140 mg/L after the sewer. While inorganics did not seem to be affected by the residence within the pressure main (the ash content of the samples remained the same), the VS/TS ratio changed from 0.60±0.3 to 0.47±0.09, indicating a reduction in organic matter content in the wastewater.

Similarly, TSS and VSS were lower in the samples collected at the outlet of the sewer, with initial TSS of 398±64 mg/L and final of 243±40 mg/L. These TSS and VSS values were on the high end compared to the usual DWF sewage composition (Henze et al., 1995; Roeleveld & van Loosdrecht, 2001).

Suspended solids were almost completely organic, with a stable VSS/TSS ratio of 0.90±0.4 and 0.92±0.5 before and after.

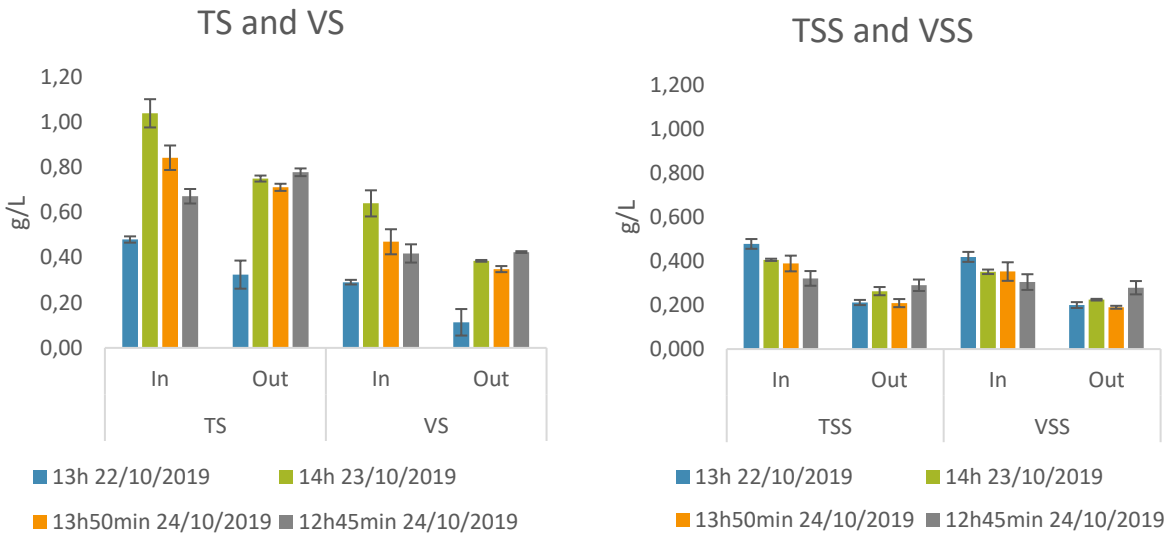


Figure 13: Left: Total and volatile solids; Right: Total and volatile suspended solids.

These values were measured on the samples used for the full WW characterisation, like described in Section 3.1.2. Standard deviations refer to triplicate analysis on each sample.

3.3.3 Particles below 1000 µm did not show any significant size reduction

As seen in Figure 14, all the PSD measurements for particles below 1000 µm showed two main peaks in particles sizes: one around 200 µm and one varying between 40 and 60 µm, depending on the day. Similar results were reported by Verbanck et al., (1990), who found peaks in the particles 15-45 µm and 125-500 µm.

Excluding the last sample (24th October, HRT 12h45min) like discussed at the beginning of this chapter, only the first sample (22nd Oct) showed an overall shift in the PSD curve towards smaller particles sizes. This could equally be due to inaccurate sampling or to the internal processes of the sewer. However, other processes such as feed pumping have been reported to reduce particles size, too (García-Mesa et al., 2010). Previous studies regarding the changes in PSD after sewer conveyance were not found.

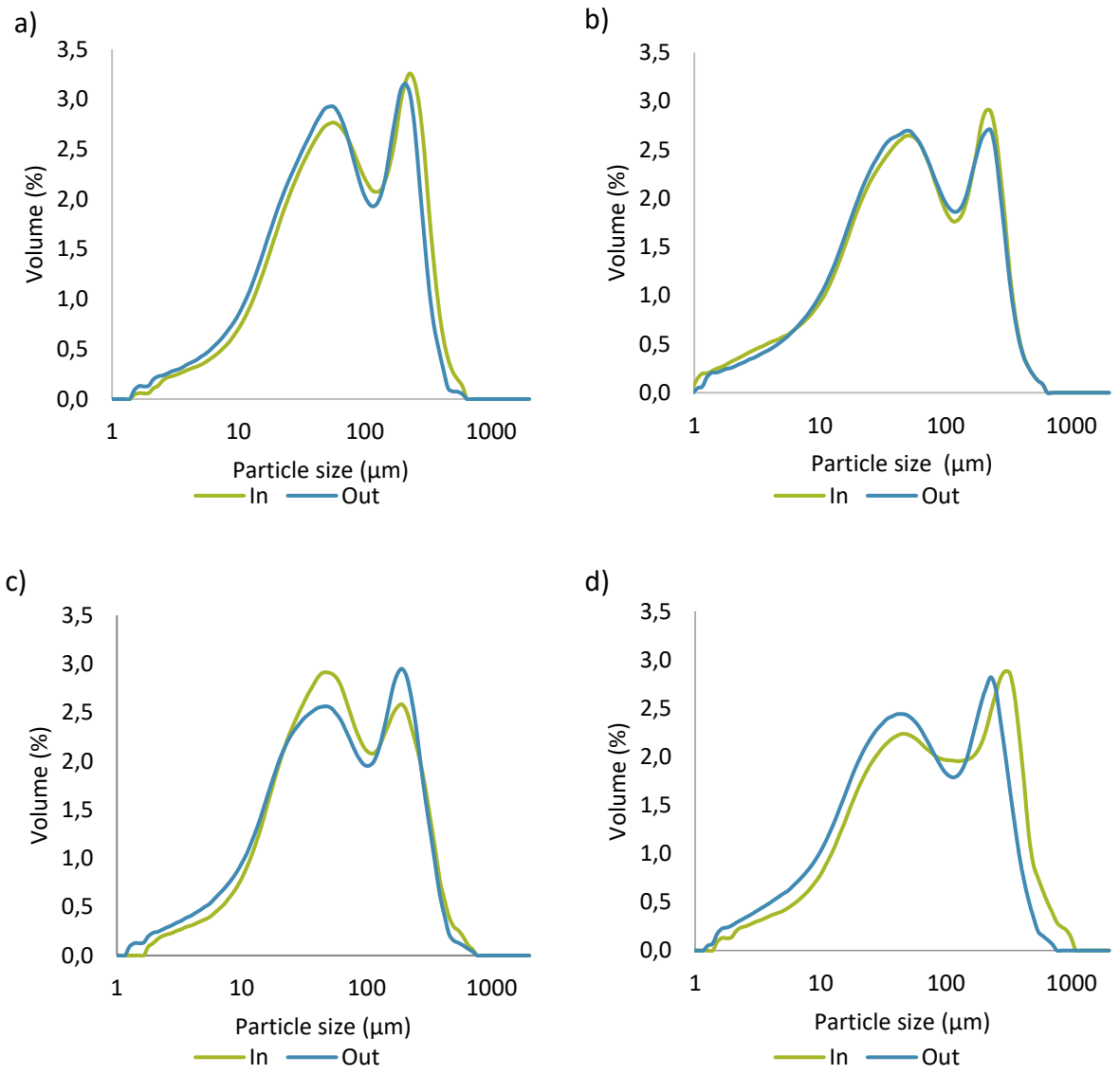


Figure 14: Particle size distribution (PSD) curves, in volume %, for the WW characterisation samples:
a) *22nd October - HRT=13h20min; b) 23rd October; HRT= 14h; c) 24th October - HRT 13h50min; d) 24th October – HRT= 12h45min*

3.3.4 The COD and BOD concentrations decreased

Figure 15 provides an overview of the different fractions of COD analysed in the wastewater, on DWF samples as described in Appendix I. The collected domestic wastewater had not undergone any primary treatment and it was of medium-high strength (Henze et al., 1995). When excluding the last sample, as described in section 3.3.1, all the concentrations of the different fractions of COD were lower on the samples taken at the sewer outlet. The TCOD decreased from 885±180 to mgCOD/L to 604±63 mgCOD/L, while the SCOD showed a much smaller decrease, passing from 336±92 mgCOD/L to 270±62 mgCOD/L.

The biodegradability of the wastewater was rather high when entering the sewer, with BOD of 252±42 mg/L, however, it had decreased to 192±33 mg/L at the outlet of the pressure sewer. The COD of particles smaller than 1kDa, hence readily available for microbial uptake, consisted of up to one-fourth of the total organic matter. This fraction remained stable after the anaerobic transport, however, their actual concentration decreased.

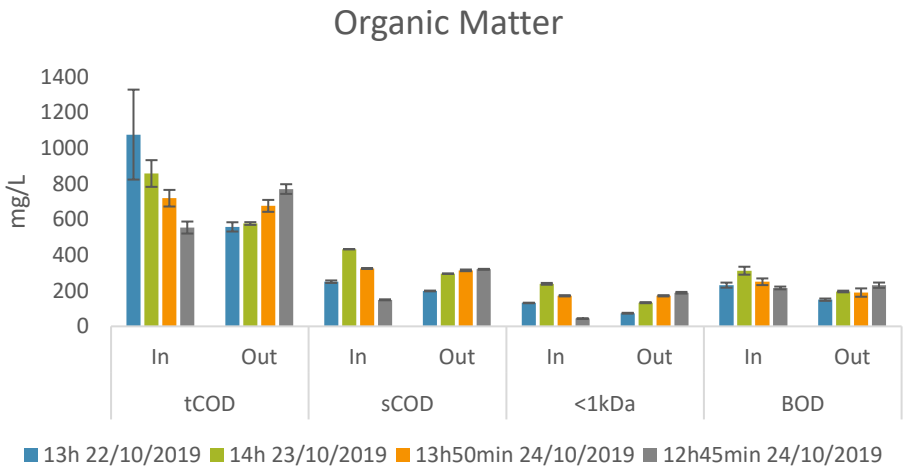


Figure 15: COD measurements from left to right, on the: total and soluble fractions of the WW samples and for the particles of size <1kDa. Outermost right: biological oxygen demand of the WW samples. All measurements are compared on inlet and outlet samples. Standard deviations refer to triplicate measurements on the same sample.

3.3.5 Increase in acetate and propionate

Figure 16 shows the VFA concentrations measured in the wastewater flowing in and out of the sewer main over the different sampling dates. Acetate and propionate were the major fermentation products observed, similarly to what was found in raw domestic sewage batch test studies (Bachmann et al., 2007; Freudenthal et al., 2005; Rudelle et al., 2011). However, in these batch tests increases in VFA were lower than those found in this study, ranging from 15-28 mg/L after 13h at 20°C (same conditions as this research). Similar results, instead, were observed in a full-scale pressure sewer on a study regarding sulfide production (Hvitved-Jacobsen et al., 1995).

The sample of the 23rd showed an inverse trend compared to the others, with initial AcH and PrH concentrations higher at the inlet than the outlet. The reason for this remains elusive. As explained in further detail in Section 3.3.1, the sample of the 24th October with HRT 12h45min was excluded from the analysis. On the remaining days, the average increase in acetate was 44±25 mgCOD/L. The maximum increase of AcH was of 67 mgCOD/L, i.e. almost 14 times the initial concentration. On the other two days, the increase corresponded to 348±34% of the initial AcH concentration. Similar results were found only by Narkis et al., (1980) who, however, carried their tests at 25°C.

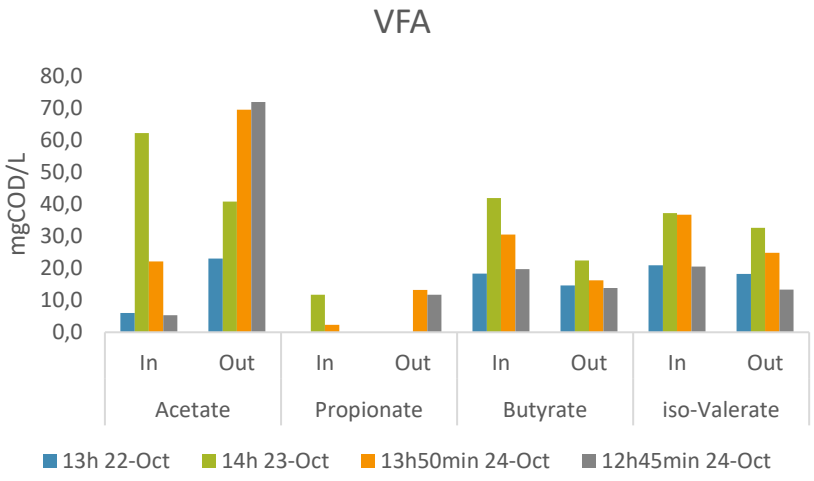


Figure 16: Volatile Fatty Acids measured at the inlet and the outlet of the sewer, reported as COD equivalents

3.3.6 Particulate carbohydrates concentrations were lower, while no significant changes were observed for proteins

Total and soluble proteins and carbohydrates were measured in the samples collected for wastewater characterization. Figure 17 illustrates the results.

Proteins were mainly found in the form of suspended solids, with a minimum observed particulate fraction of 70%. No significant changes were seen after the sewer, neither in total nor soluble concentrations. Carbohydrates were roughly equally distributed between particulate and soluble compounds. Unlike proteins, total carbohydrates did show a statistically significant change, decreasing from 106±24 mgCOD/L to 71±14 mgCOD/L after transportation. As no significant differences in soluble carbohydrates were detected, the variation in total concentration was due to a reduction in the particulate matter after the sewer.

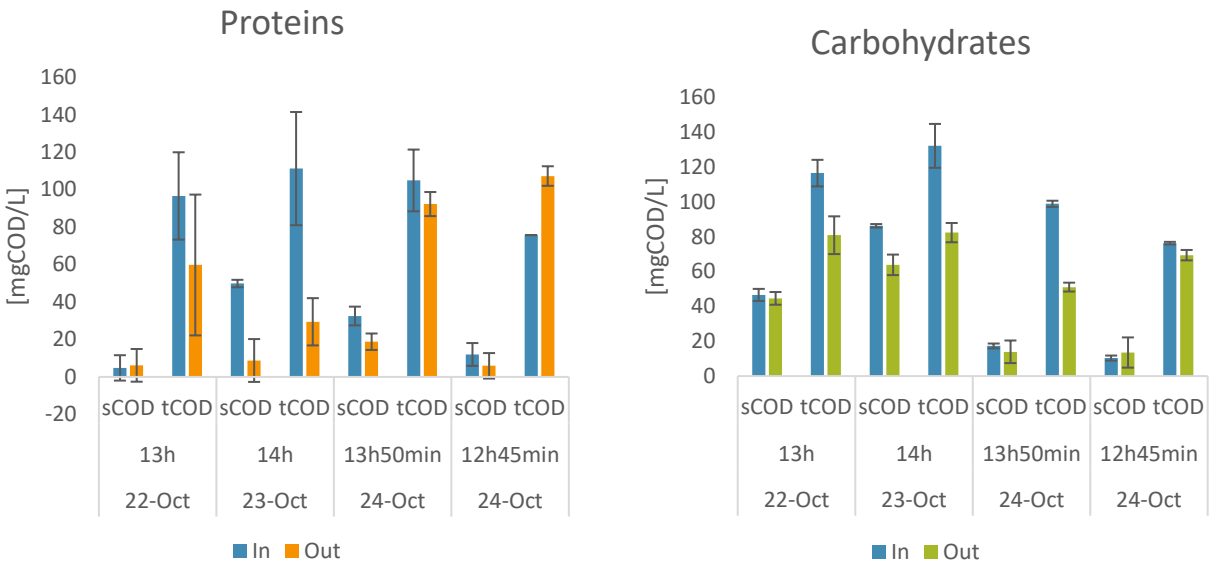


Figure 17: Total and soluble proteins and carbohydrates concentrations measured at the inlet and the outlet of the sewer. Values were reported as COD equivalents, with conversion factors by Sophonsiri et al., 2004.

3.3.7 Increase in ammonium

As illustrated in Figure 18, $NH4^{+}$-N entered the sewer in concentrations of 51.2±11.2 mg/L, increasing to an average of 55.2±10.0 mg/L when reaching the WWTP. The total nitrogen (TN) remained rather constant, with values of 75.2±9.1 mg/L and 73.7±3.0 mg/L before and after the sewer (data not shown).

Ammonification can occur due to several processes in anaerobic systems. Potential processes in the considered sewer stretch could be urea degradation, protein hydrolysis and amino-acid fermentation. However, protein concentrations showed that their hydrolysis was not very significant.

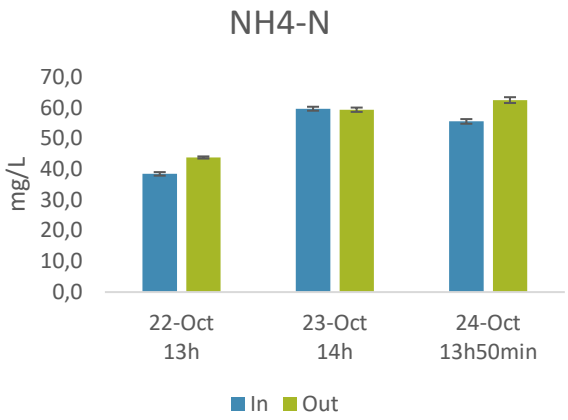


Figure 18: Variations in the NH4+-N concentrations of the wastewater, at the inlet and outlet of the pressure sewer

3.4 OVERALL VARIATIONS OF THE WASTEWATER AFTER THE SEWER CONVEYANCE

3.4.1 Higher measurements uncertainty in full-scale systems

The variations observed in this study might have been influenced by sources of error deriving from the experimental method. Additional sources of error in this full-scale research include:

- **Different types of sampling methods used for inlet and outlet samples**

  A vacuum automated sampler was used to collect the wastewater at the inlet of the sewer each hour. Manual grab samples, instead, were collected at the sewer outlet at the receiving WWTP. This difference in sampling methods might have affected the quality of the sampled WW. Vacuum samplers have been reported to limit the ability to collect a representative sample of solids in the WW (Ashley et al., 2004). Moreover, vacuum conditions might induce volatilisation of the VFA's, reducing their concentration in the WW.

- **Non-accurate match of the wastewater entering and exiting the sewer**

  Domestic wastewater presents significant variability, not only at the seasonal or weekly level, but also throughout the day. Due to the automated sampling installed at the inlet of the sewer, wastewater entering and exiting times could only be matched at an average accuracy of ±20 minutes. Exceptional discharges or the intrinsic WW variability could therefore have affected the differences in the samples. Moreover, grab samples of 1L were taken both manually and by the automated sampler. Due to the heterogeneity of the WW flow, the collected samples might not have been representative of the whole volume flowing at that moment.

  It is not possible to correct for the spatial heterogeneity of the WW volume at a given time, inducing errors in a grab sample. However, from the 24h monitoring campaign it is possible to give a rough estimate of the maximum error due to inaccurate sampling timing as the maximum observed difference in TCOD from one hour to the other was around 240 mgCOD/L.

- **Sampling limitations to the size of the collected suspended solids**
  Only smaller solids, historically defined as sanitary solids as described by (Ashley et al., 2004), could be collected with the manual and automated spot sampling methods. Though it was not possible to monitor it, the influence of larger solids on the WW quality cannot be excluded.

- **Low number of replicate samples**

  Despite the effort to take as many samples as possible within the available time, the number of replicates for each type of analysis is still very low, making it difficult to overcome the WW variability.

The above-mentioned factors might have influenced the observations described in this chapter. The accuracy of the observations will, therefore, be lower than for lab-scale studies. The data required cautious handling, however, it was still possible to qualitatively discuss overall behaviours.

3.4.2 No net solubilisation of the wastewater

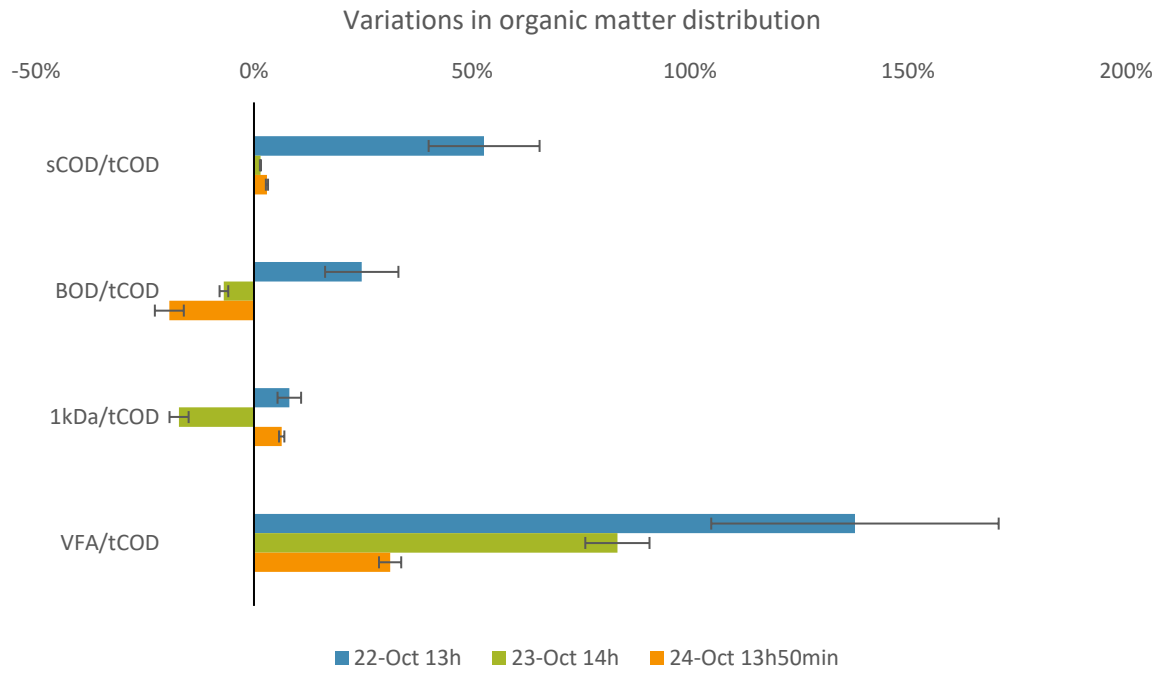


Figure 19: Ratio of the variation between inlet and outlet for different organic fractions to the TCOD measured in the inlet sample. The standard deviation derives from the error propagation of the standard deviations of TCOD and of the considered organic fractions, each found from triplicate measurements on the respective sample.

The solubilisation of a wastewater stream is considered to affect the following characteristics:

- Lower concentration of total suspended solids
- Smaller particles size
- Increased concentration of soluble COD
- Higher SCOD/TCOD ratio

As shown in Figure 15, after transportation the SCOD, BOD_5 and COD <1kDa decreased on all the considered samples - excluding the last one like explained in Section 3.3.1. The PSD curves did not show a significant shift towards smaller sizes and no significant change was observed in the SCOD/TCOD ratio after the sewer either, apart from on the first day (see Figure 19). On that day, the drastic reduction in TCOD could have determined the increase in the ratio. Though TSS and VSS decreased after the sewer, no net solubilisation of the WW was, therefore, observed for particles smaller than 1000 µm. It is not possible to completely exclude hydrolysis processes; however, it is possible that the hydrolysis rate might have been lower than the microbial uptake rate.

Though excluded from the PSD measurements, the WW matrix also includes particles larger than 1000 µm. As larger solids present a smaller surface to volume ratio, their structure will limit the accessibility to hydrolytic enzymes and their hydrolysis kinetics will be even slower than that of fine solids (Angelidaki & Sanders, 2004). No net solubilisation is hence assumed to have happened for the whole WW volume.

When excluding settling for particles <1000 µm, as will be discussed in further detail later in this section, their removal can be justified by either entrapment on the sewer walls' biofilm or microbial degradation. In the second case, fine solids would be hydrolysed by the exo-enzymes present in the WW and then be taken up for microbial growth or catabolic functions.

Methanogenesis has been reported to occur in the deeper layer of biofilms or of sewer sediments (Hvitved-Jacobsen et al., 2013; Nielsen et al., 1992; Tanaka & Hvitved-Jacobsen, 1998). However, as described in

Section 3.1.1, pressure sewers tend to form smooth and dense biofilm structures, resulting in only thin layers of slime around the sewer walls. Under these conditions and the temperatures observed in this study, methane production might not have been very significant in the considered sewer stretch. Previous studies reported methanogenesis to be negligible under pressure sewer conditions in Denmark (Hvitved-Jacobsen et al., 2013; Tanaka & Hvitved-Jacobsen, 2002). It must be noted, though, that in one study conducted in Australia, with average sewage temperature above 26°C, methane was observed to contribute considerably more than sulfide reduction to the loss of SCOD in pressure sewers (Guisasola et al., 2008).

In general, sulfate reducing bacteria (SRB) have been identified as the main consumers of the readily available soluble substrate in pressure mains with anaerobic conditions (Hvitved-Jacobsen et al., 2013; Nielsen et al., 1992; Rudelle et al., 2011). Sulfate reduction was seen to lead to an underestimation of the production of readily available soluble substrate by up to 28%, justifying good part of the observed reduction in SCOD, BOD_5 and COD of particles readily available for microbial uptake (Hvitved-Jacobsen et al., 2013).

Humic substances

It is important to note that humic acids were 21±4% of TCOD and 51±8% of SCOD entering the sewer (Figure 20). Humic substances are difficult or impossible to degrade anaerobically (Gonzalez et al., 2018; Rosenblum et al., 2018; Zahmatkesh et al., 2016). In the considered system, the maximum attainable increase in readily biodegradable substrate of the WW without oxidative processes could not have been expected to exceed 83% of TCOD, out of which maximum 43% of the SCOD could be fermented.

Humic substances affect the anaerobic degradability of organic matter as they cause enzymes immobilization and consequently a slower hydrolysis rate (Azman et al., 2015; Frolund et al., 1995; Gonzalez et al., 2018). Moreover, apart from enzymes, other dissolved protein-like components could also be trapped by dissolved humic-like components, forming supramolecular assemblies and reducing the microbial degradability of proteins in terms of accessibility to their structure (Gonzalez et al., 2018; Wang et al., 2015).

The large presence of humic acids in the considered WW will therefore partly explain the low solubilisation extent and the lower reduction in soluble proteins concentrations after the sewer, compared to soluble carbohydrates.

Carbohydrates hydrolysis

Hydrolysis of carbohydrates was studied in more detail. Enzymatic activity targeting α- and β-glycosidic bonds was measured in the WW. Calculations of the expected carbohydrate concentration that could be hydrolysed in the sewer were carried out based on the measured enzymatic activities and taking starch and cellulose as representative compounds for carbohydrates with α- and β-glycosidic bonds, respectively, and adding their contributions. The alpha- and beta-glucosidase activities measured in the wastewater would be enough to hydrolyse up to 150 mg/L of carbohydrates. This should, however, be interpreted as an overestimation: the activities measured in the assay are maximum hydrolysis rates since the assays were performed at excess substrate concentrations and easily accessible substrates. Moreover, despite the presence of enzymatic activity, the rate of hydrolysis will also be limited by the availability of free accessible surface area and by the overall structure of the solid substrate (van Lier et al., 2008).

The particulate carbohydrates were, indeed, always lower in the outlet sample. Apart from the last day, when only a 15% decrease occurred, on the other days particulate carbohydrate concentrations were reduced by 67% in average, and up to 92%, of the incoming concentration. This was, however, not met by an increase in soluble carbohydrates. Soluble carbohydrates are thermodynamically favourable substrates for microorganisms and their microbial uptake in the sewer might have had similar kinetics to their hydrolysis (Muyzer et al., 2008).

Particles below 1000 µm did not seem to be affected by significant settling

Solubilisation of the wastewater is influenced by the increase in SCOD and reduction in particulate organics. The possible settling issues mentioned as related to the sampling method, could therefore have had a big impact on the interpretation of the results mentioned in this section.

However, the particles size distribution (PSD) results showed no significant sedimentation - maximum 0,2% in volume for particles of size around 200 µm, while smaller particles did not seem to be affected. It is important to notice, though, that SS larger than 1000 µm were not included in the PSD measurement and their sedimentation cannot be excluded.

Due to the recalcitrant characteristics of humic acids (HA), microbial degradation is not a possible degradation pathway and their eventual disappearance from the WW could only be attributed to physical removal processes, such as entrapment on the walls' biofilm or sedimentation. These characteristics make them a good indicator for particles in the same size range as HA's, whose size is known to be between 300-600 µm (Klučáková, 2018). No significant variation was seen for HA's either.

Overall, it is likely that the SS of size <1000 µm contained in the WW samples did not undergo significant settling. It seems probable them that the variations in the soluble fraction could be attributed to in-sewer processes or to inaccurate matching of the WW.

3.4.3 Acidification of the wastewater

As discussed in Section 3.3.5, on every day apart from the second, AcH and PrH concentrations increased considerably compared to their initial values and their ratio over the total and soluble COD became higher (Figure 19). These results were in agreement with the observations by Narkis et al. (1980) and Rudelle et al. (2011), who found that WW collected from pressure mains with longer retention times had higher VFA concentrations. Despite an overall decrease in soluble COD, there was an increase in the proportion of fermentation products such as AcH and PrH (Figure 20), showing that SCOD was fermented in the pressure sewer.

The production of AcH and PrH over the SCOD available in the samples ranged between 0.07 and 0.18 mgVFA/mgCOD. As mentioned in the section regarding the variations in VFA concentrations, laboratory and full-scale studies presented different results. The lab batch tests reported in literature showed an accumulation of SCOD (Bachmann et al., 2007; Freudenthal et al., 2005; Rudelle et al., 2011). On the opposite, both published results on a full-scale pressure sewer (Hvitved-Jacobsen et al., 1995) and the results of this study showed that SCOD concentrations decreased at the outlet of full-scale pressure sewers. Moreover, the VFA production was lower compared to what was observed in the full-scale pressure sewers. This might be justified by the presence of biofilm on the sewer' walls, however, further research will be required to elucidate the relative contributions of bulk wastewater and biofilm to the biochemical processes occurring in pressure sewers.

As discussed in (Section 3.4.1), the VFA production might have been overestimated due to the volatilisation of the compounds under vacuum conditions in the automated sampler. However, even considering the volatilization of VFA during vacuum sampling, the increase in VFA during the conveyance in the sewer would be significant.

Tanaka and Hvitved-Jacobsen (1998) reported that a high VFA concentration in the WW corresponded to a high sulfide production and viceversa. Acetate uptake by SRB cannot be excluded in the considered sewer system. However, acetic acid was the dominant VFA in all samples, as illustrated in Figure 16. Its generation might have been a by-product of the metabolic activity of acidogenic bacteria but also of SRB. Species such

as Desulfovibrio and Desulfotomaculum have, in fact, been reported to incompletely oxidise a number of carbon compounds into acetate for their growth (Charaklis & Marshall, 1990; Muyzer et al., 2008).

Significant acidification was observed in the considered system, despite the very low hydrolysis rates previously discussed. It is possible, therefore, that hydrolysis might have been the rate-limiting step for the fermentation of organic matter. Similar conclusions were found by Rudelle et al. (2011) and Narkis et al. (1980), from batch test studies performed on raw sewage collected from pressure sewers with different characteristics.

3.4.4 Biodegradability reduction

Biodegradability was defined in this study according to the ready availability of substrate to microorganisms and the capacity of the WW to make new substrate available.

Opposite to other studies, the readily biodegradable substrate of the wastewater seemed to be slightly reduced after the passage in the pressure sewer (Hvitved-Jacobsen et al., 2002; Tanaka & Hvitved-Jacobsen, 1998). Despite an increase in VFA's, the BOD_5 concentrations decreased and in general the BOD/TCOD ratio became lower, too (see Figure 19). Only on the first day, the BOD/TCOD and the SCOD/TCOD ratio followed an inverse trend and increased. This increase seemed to be due to a very high reduction in TCOD, which might have included the removal of non-readily biodegradable solids (possibly larger than 1000 µm), such as seeds or leaves.

A lower VS/TS ratio was observed in the WW after sewer transport. The organic matter readily available for microbial uptake decreased, shown by the COD <1kDa values in Figure 15. As mentioned in the solubilisation section above, microbial uptake of organic matter might have reduced the biodegradability of the WW, as the rate of SS hydrolysis seemed to be slower. The maximum combined BOD_5 uptake by heterotrophs, during the initial aerobic conditions, and by denitrifiers, in case of nitrogen oxides in the incoming wastewater, was estimated to be less than 2 mg/L, based on the measured initial DO of 0.8 mgDO/L and an estimated initial concentration of nitrate of 1.5 mg/L. On the other hand, SRB and possibly methanogens might have converted into CO_2 and CH_4 significant amounts of readily available organic matter, justifying significant part of the observed decrease in BOD_5 concentrations (Guisasola et al., 2008; Muyzer et al., 2008).

The sedimentation of particles >1000 µm could have affected the overall biodegradability of the WW, too, as settleable solids were found to be mainly composed of organic matter (VS/TS ratio of 0.96).

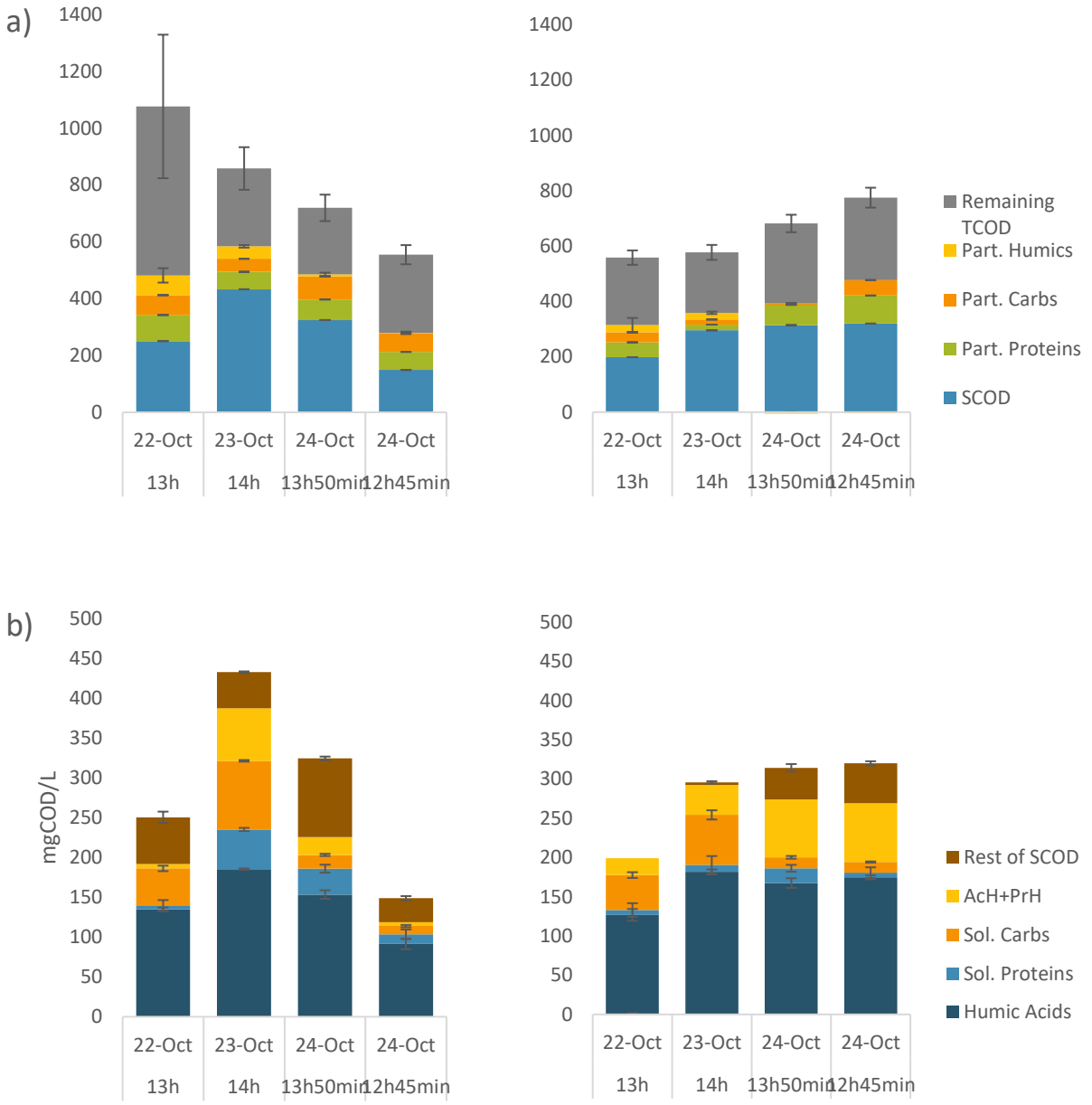


Figure 20: Left: Sewer inlet, Right: Sewer outlet. All values were reported as COD equivalents.
a) Particulate proteins, carbohydrates and humic acids as fractions of total COD.
b) Humic acids, soluble proteins and carbohydrates, acetate and propionate as fractions of soluble COD.

Table 3: Total and soluble COD values of each sample. Values in mgCOD/L

		22-Oct 13h		23-Oct 14h		24-Oct 13h50min		24-Oct 12h45min	
		Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
TCOD	In	1076	252	858	75	719	47	555	34
	Out	558	26	577	8	676	34	771	27
SCOD	In	251	7	433	1	325	2	149	3
	Out	199	2	296	2	314	5	320	3

3.5 EXPECTED IMPACTS OF THE WASTEWATER VARIATIONS ON AGS REACTORS

Transport in a pressure sewer led to a significant increase in acetate and propionate concentrations in the wastewater. Increased VFA concentrations in the feed to AGS reactor have been associated to multiple benefits. Acetate and propionate are substrates for storage-polymers forming organisms, PAO and GAO, who possess a competitive advantage over ordinary heterotrophic organisms (OHO), thanks to their ability to take up organic substrate under anaerobic conditions (Mino et al., 1998; Stokholm-Bjerregaard et al., 2017). Their presence in AGS microbial communities has been associated with improved granular stability (de Kreuk & van Loosdrecht, 2004). Higher VFA concentrations in the feed can favour the selection of PAO's in AGS reactors. In turn, higher PAO's abundances in the biomass will result in higher storage of polyphosphates under aerobic conditions, enhancing the efficiency of phosphorus removal. Moreover, VFA's can be more easily converted into polyhydroxyalkanoates (PHA). Growth on these storage polymers has been hypothesised to help the formation of larger granules (Layer et al., 2020; Pronk, Abbas, et al., 2015). This would favour the formation of larger anoxic zones needed for the denitrification of nitrogen compounds, increasing the simultaneous nitrification and denitrification (SND) rates and hence improving the nitrogen removal efficiencies (Layer et al., 2020). The exact estimation of the effects of higher VFA concentrations on full-scale AGS reactors goes beyond the scope of this study. However, it is likely that the fermentation of wastewater in the pressure sewer might help to improve the performance of AGS reactors in multiple ways.

As described in the introduction, particulate substrate can lead to the formation of filamentous outgrowths on the surface of granules. This has been associated with hindered settleability and reduced growth at the core, resulting in granular breakage (Pronk, Abbas, et al., 2015). Incomplete hydrolysis during the anaerobic phase will result in the inability for microorganisms to take up the organic substrate. The residual particulate substrate will continue to be hydrolysed during the aerobic phase. The simultaneous presence of organic substrate and oxygen will favour the growth of OHO, resulting in excessive formation of flocculant biomass and causing instabilities in the aerobic granular sludge (de Kreuk & van Loosdrecht, 2004). The decrease in suspended solids concentration is expected to reduce the occurrence of these drawbacks and therefore to be beneficial for AGS reactor performance.

As discussed in further detail in the previous sections, municipal wastewater also showed a reduction in soluble and biodegradable substrate at the outlet of the pressure sewer. However, it is interesting to notice that pressure sewers injected with oxygen or nitrates with the aim of H_2S production control also showed a sudden consumption of COD or BOD (Oosterhuis & van Loosdrecht, 2009; Sharma et al., 2008; Tanaka & Takenaka, 1995). Sharma et al., (2008) suggested that reducing the aerobic length by moving the oxygen injection point resulted in higher VFA concentrations at the end of the pipe. Anaerobic transport of municipal wastewater might therefore not only be favourable due to its impact on the degree of fermentation and reduction in suspended solids, but also because of the lower decrease in biodegradable substrate for microbial uptake in further biological wastewater treatment facilities.

The enzymatic activities of lipase and protease were detected to be low in the raw wastewater conveyed in the pressure sewer. Hydrolysis of these fractions of the organic matter will therefore be limited before reaching the AGS reactor. This was partially confirmed by the unvaried concentrations of proteins at the inlet and the outlet of the considered pressure sewer. Hydrolysis is recognized as being the rate-limiting step for microbial substrate uptake (Morgenroth et al., 2002; Vollertsen et al., 1999). Therefore, despite the increase in VFA at the sewer outlet, it does not seem possible to decrease the length of the anaerobic phase in AGS reactors.

Despite the possible contribution of the microorganisms in the sewer walls' biofilm to the VFA production, the wastewater also seemed to have considerable fermentative activity. Pre-treatment of the WW with hydrolysing biomass could allow a significant increase in the incoming concentrations of VFA's to AGS reactors.

Activated sludge enzymatic activity has been widely reported in literature (Goel et al., 1998; Kreutz et al., 2016; Morgenroth et al., 2002), making sludge addition to a pre-acidification tank a viable option to overcome hydrolysis limitations and reach full fermentation potential of domestic sewage. Proteins solubilisation seemed to be very limited in the considered sewer stretch. Henze & Mladenovski, (1991) found that the hydrolysis by AS of nitrogenous compounds in raw WW was reduced, but not inhibited, under anaerobic conditions. Providing contact with activated sludge would likely lead to the solubilisation of part of the proteins not hydrolysed inside the sewer, improving the wastewater characteristics before being fed to the AGS reactor.

3.6 ACTIVITY OF DIFFERENT SIZE FRACTIONS OF THE WASTEWATER

In order to answer the third research question, alpha-glucosidase activity was measured on different size fractions of the WW: settleable solids (>45 µm), particles <45 µm and the soluble fraction(<0.45 µm), also referred to as bulk liquid for the sake of this study. The biomass-specific α-glucosidase activity obtained, based on VS content, is shown in Figure 21,a below.

a) b)

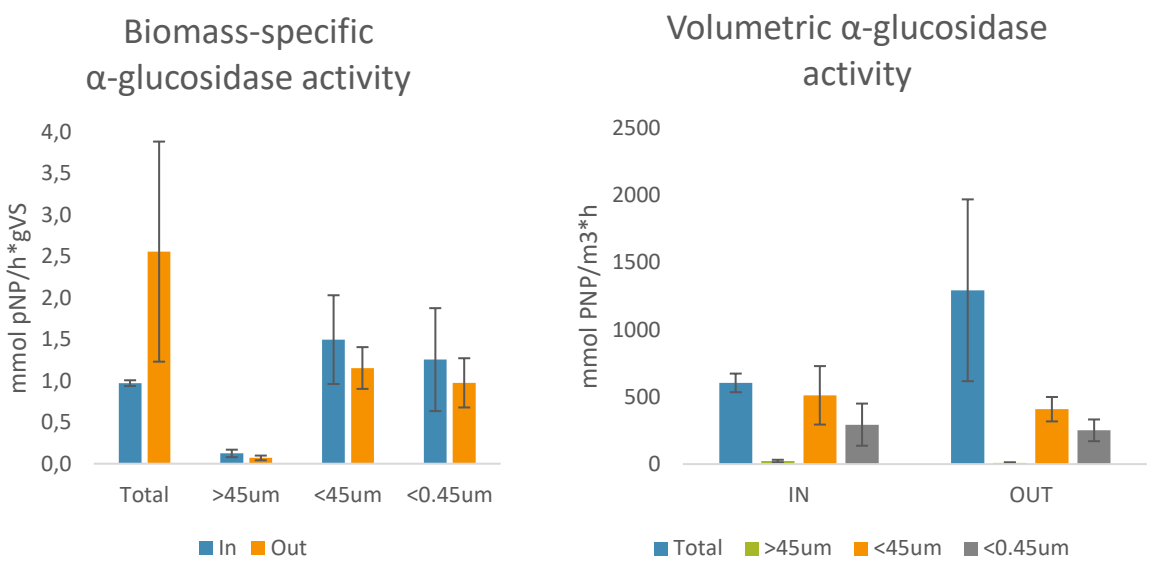


Figure 21: a) Biomass-specific α-glucosidase activity, based on VS content, of the fractionated raw wastewater. Note that the fraction of solids <45um includes the dissolved ones <0.45um, also referred to as bulk liquid of the wastewater.

b) Volumetric alpha-glucosidase activity, or alpha-glucosidase activity per unit of volume.

Alpha-glucosidase biomass-specific activities of bulk liquid and of solids <45 µm were the highest. No significant difference was observed between them before entering the sewer. After transport, however, a reduction in the biomass-specific activity of the bulk was observed, but not of the solids <45µm. As this fraction includes the bulk, the activity might have been compensated by an increased activity of the fine solids, i.e. particles with size contained between 0.45µm and 45µm. Settleable solids had the lowest biomass-specific activity out of the considered WW fractions.

The volumetric α-glucosidase activity was calculated from the biomass-specific enzymatic activity and the concentration of solids (VS) in the fractions. Figure 21,b illustrates the volumetric contributions of each fraction to the α-GLU activity of the total wastewater, at the inlet and the outlet sampling locations. The particles <45 µm were detected to have the highest influence on the volumetric α-GLU activity of the total

WW sample. Moreover, it is important to notice that the fraction of particles below 45µm included those <0.45 µm, i.e. the bulk liquid, and that the volumetric α-GLU activity of the bulk liquid was around 60% of that of the fraction <45 µm. The highest contribution to the volumetric α-GLU activity of the total wastewater sample was, therefore, given by the soluble fraction. The remaining contribution appeared to be mainly given by the enzymatic activity of fine solids, with settleable solids showing negligible α-GLU activity.

A high activity of the soluble fraction suggests that hydrolysis of α-glycosidic bonds in the wastewater might occur mainly due to exoenzymes released in the environment by hydrolysing microorganisms. Similar results have been reported previously in literature. For instance, Larsen & Harremoës, (1994) found that when using starch (a macromolecule characterised by α-glycosidic bonds) as a substrate, bulk liquid hydrolysis was the mechanism for transforming non-diffusible organic matter into biofilm diffusible substrate. Moreover, Guellil et al. (2001) localised the activity of α-glucosidase in the colloidal fraction of domestic wastewater. Preliminary results (data not shown) indicated the possibility of bulk liquid contributing to the enzymatic activity of wastewater also in terms of protease and lipase. However, further research would be required to confirm these results.

In terms of AGS reactor performance, the localisation of enzymes in the bulk liquid of the wastewater implies that enzymes might be washed away after a short period of time (one or few cycles). Their contribution to the total enzymatic activity of the reactor will hence be limited in time.

These results might be enzyme specific, as shown by Guellil et al. (2001) who localised the activity of protease and of α-glucosidase in the AS matrix and in the colloidal fraction of domestic wastewater, respectively.

Overall, no significant changes in the relative activity carried by the different particles size fractions were observed before and after passing through the sewer. It was interesting to notice, however, that most of the active organisms were detected in the fraction of solids <45 µm at the inlet of the sewer and in the settleable solids fraction (>45 µm) at the outlet (see Figure 22). The settleable solids might have included detached pieces of biofilm after the sewer. Another possible explanation could be that the microbial population might have entered the sewer suspended in the WW, while it seemed to have colonized the settleable solids after leaving it. In both cases, this change was not followed by a shift in the biomass-specific enzymatic activity of α-glucosidase. Even if a higher active biomass to VS ratio was measured in the >45 µm fraction after the sewer, this biomass did not correspond to alpha-glucoside hydrolysers.

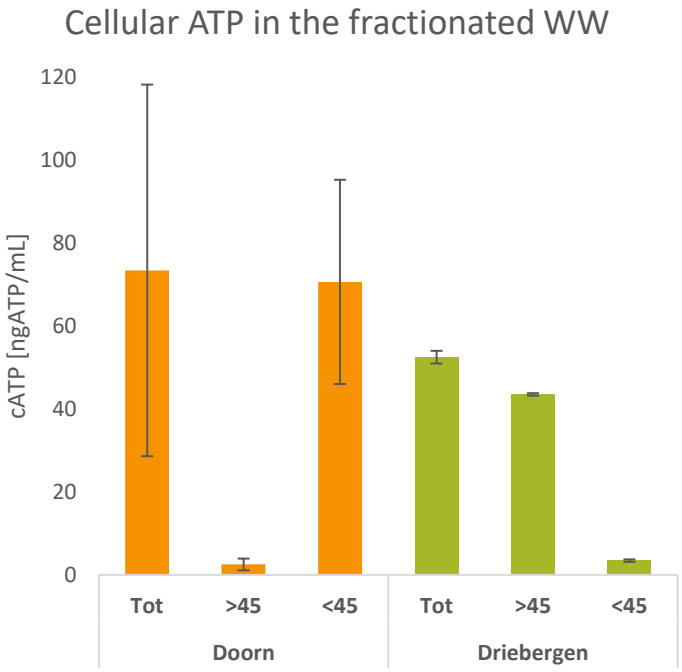


Figure 22: Concentrations of adenosine triphosphate in the fractionated wastewater, at the inlet (Doorn) and the outlet (Driebergen) of the sewer. Sizes are reported in micrometers and refer to particles' diameter.

3.7 CONTRIBUTION OF THE INFLUENT TO THE ENZYMATIC ACTIVITY IN THE AGS REACTOR

AGS collected from the Nereda reactor in Garmerwolde was divided into several sludge size fractions, as described in the method section. Enzymatic assays were then carried out on each of the fractions and the influent to the Nereda.

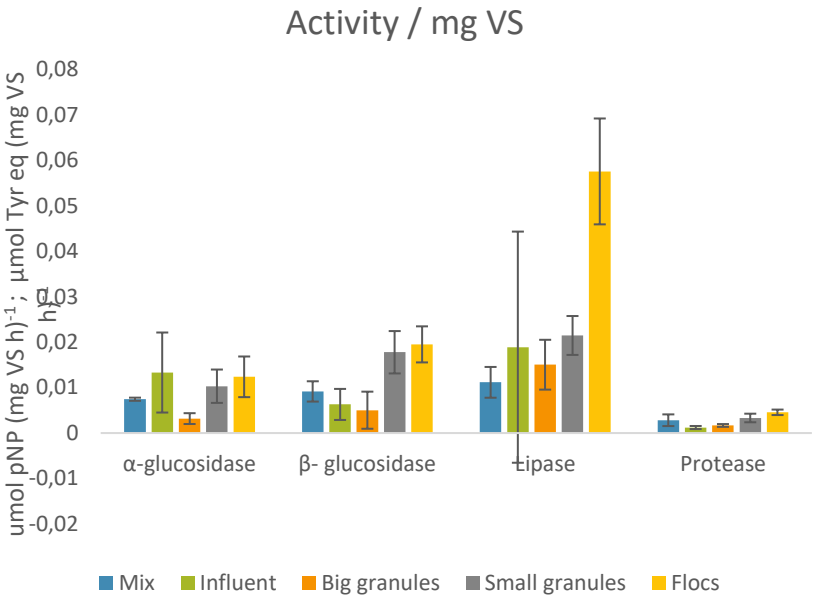


Figure 23: Biomass-specific enzymatic activity of the influent and the AGS fractions

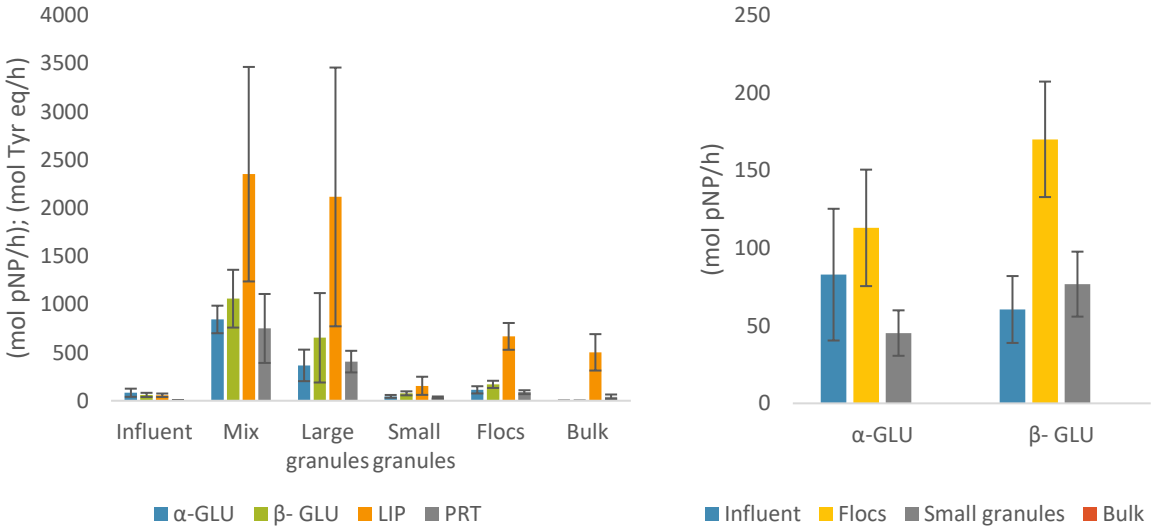


Figure 24: (Left) Contributions to total reactor enzymatic activity by the different AGS fractions and the influent. The figure reports the estimated values for all the considered enzymes, as shown in the legend.
(Right) Zoom on the contributions of influent, small granules, flocs and bulk liquid to total reactor α- and β-glucosidase activities. Bulk liquid was seen to have no glucosidases activity.

The total activity in the reactor was calculated from the specific activities of the different AGS fractions and the biomass content of each fraction and the mixed liquors, in terms of volatile solids (VS). The influent activity was calculated from the volumetric activity and the volume fed per cycle. The bulk activity was calculated volumetrically, too, as it does not contain biomass. The bulk volume was calculated from the SVI of AGS, assuming that all the volume not occupied by the sludge would be bulk.

The biomass-specific activities (activity/mgVS) for the measured enzymes are reported in Figure 23. The influent activity/mgVS of α- and β-glucosidase was comparable to those of the different AGS fractions and of the mixed liquors. In terms of lipase, the biomass-specific activity of the influent showed a very high standard deviation. This was possibly due to the assay method and not due to an actual variability of the wastewater. No conclusions could be drawn in this case. On the other hand, the biomass-specific protease activity of the influent was not of the same order of magnitude as the other AGS fractions, suggesting that protease activity might have mainly been developed within the reactor itself.

The results of the granular size distribution (GSD) and the volume of influent fed per cycle allowed to calculate the contributions to the total reactor enzymatic activity for each AGS fraction and the influent (see Figure 24, left). Despite its low detection in the influent, for all AGS fractions lipase gave the highest contribution to total enzymatic activity in the reactor out of all measured enzymes. Though the microbial population excreting lipase exo-enzymes seemed to have mainly been growing on large granules, the biomass-specific activities of flocs and reactor bulk liquid were also significant.

Due to the high concentration of granular biomass, large granules showed the highest contribution to the total enzymatic activity of the reactor. In general, the contribution to the total enzymatic activity of the reactor by the aerobic granular sludge was much higher than that of the influent, for all the enzymes. Similar results were observed between raw wastewater and the AS matrix. However, while Nybroe et al. (1992) found influent esterase and dehydrogenase activities to be roughly half those of AS, the enzymatic activities of the influent in Garmerwolde ranged in the order of magnitude one or two times smaller than the mixed AGS. The contribution of the influent to total reactor enzymatic activity, therefore, appeared to be negligible for all the considered enzymes, when compared to the mixed liquors.

In terms of α- and β-glucosidase, influent, small granules and flocs had similar contributions to total reactor enzymatic activity (Figure 24, right). This could be due to the interactions between influent particles and the two sludge fractions. E. Coli were used as an indicator of the microorganisms in the influent, plating the influent and each sludge fraction to see where the E. Coli from the influent ended up. However, the pre-treatment with sonication was seen to kill part of the bacteria. New preliminary results from samples pre-treated with biomass grinding seemed to suggest that most of the influent microbiome ended up in the small granules fraction of AGS. Moreover, microscope pictures of small granules showed that influent debris were trapped in this fraction (Figure 25), more than in other ones. Flocs, to a lower extent than small granules, trapped influent debris, too, such as fibers. In a previous study on the same reactor as the one considered in this study, Ali et al. (2019) saw that immigration of microorganisms originating from the sewer was limited. However, despite all sludge fractions being subjected to the same bacterial dispersal rate from the source habitat (influent), immigration played a larger role on microbial aggregates with shorter retention times in the AGS reactor. The activities of small granules and, to a lower extent, flocs might have been overestimated by the enzymatic assay method as substrate might have been additionally hydrolysed by influent particles. Further analysis should, however, be done to prove any existing relation and the data should be interpreted with caution.

It was interesting to notice that neither α- nor β-glucosidase activities were detected in the bulk liquid of AGS, contrary to what was seen in the influent. From the study in Doorn, it was concluded that α-GLU activity was mainly found in the soluble fraction of the wastewater. If these results held also in Garmerwolde, the enzymes in the influent must have either been integrated into one of the sludge fractions or have decayed in the reactor, reducing the free exoenzymes concentrations in the bulk and hence explaining the lack of activity. Extracellular polymeric substances (EPS) have, in fact, often been reported to immobilise and accumulate enzymes (Frolund et al., 1995; Goel et al., 1999; Morgenroth et al., 2002). It could also be that the low α-GLU activity seen in the settleable solids fraction of the WW in Doorn and Driebergen is site-specific. If glucosidase activity were carried by the particles in Garmerwolde, this would explain why no activity was detected in the bulk liquid.

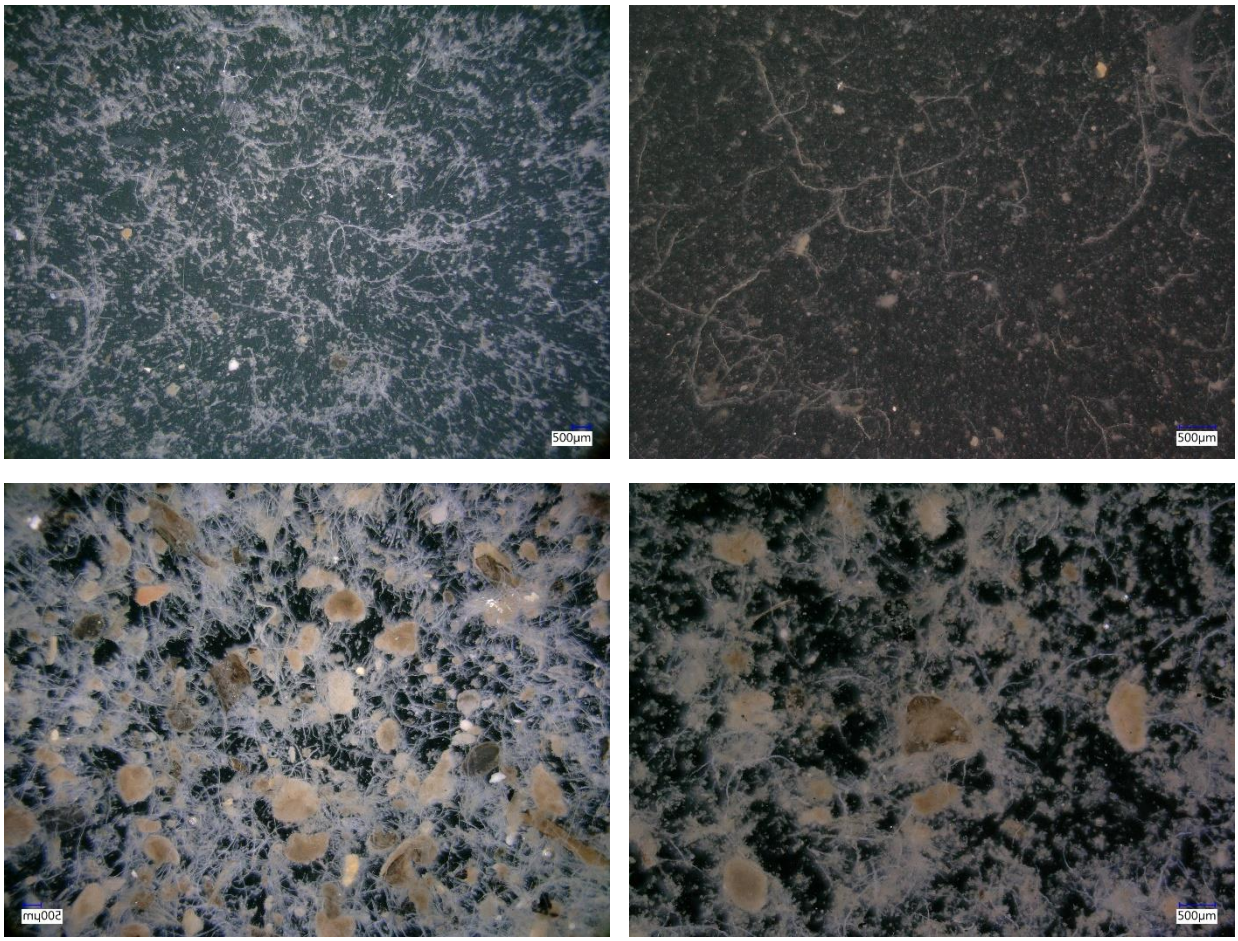


Figure 25: Microscope pictures at 20x (left) and 40x (right) resolution of the influent to the AGS reactor (Top) and of the small granules fraction of the sludge (Bottom). Influent and sludge samples were taken on the same day. The pictures at resolution 20x show samples taken on the 9th of May 2019 and the ones at 40x show samples collected on the 2nd May 2019.
Pictures by Sara Toja Ortega, with permission.

4 CONCLUSIONS

This research was carried out to understand the effect of transport in a full-scale pressure sewer on the characteristics of municipal wastewater, as a pre-treatment for AGS reactors. So far, anaerobic hydrolysis and fermentation processes inside sewer systems have received limited attention. Moreover, the impact of the influent on the enzymatic activity of AGS reactors remains unclear.

The hydrolysis and fermentation of wastewater in a pressure sewer were evaluated. Moreover, the development of hydrolytic and fermentation activity during transport was assessed. In particular, hydrolysis was investigated in terms of enzymatic activity for the full wastewater volume and fractionated samples. Finally, a comparison of the hydrolytic activity of the influent to that of AGS sludge fractions was made to provide an assessment of the contribution of the influent to the total hydrolytic activity of the reactor.

The considered pressure sewer proved to be a highly dynamic system, with large variations in terms of wastewater composition and microbial activity. Statistical data analysis was used to distinguish significant changes from intrinsic wastewater variability. Though biases of the sampling method cannot be excluded, the conclusions from the first part of this study can be considered significant at a qualitative level. The sampling method had a smaller bias on the results of the second part of the study, regarding the enzymatic activity of the influent and the AGS reactor. Moreover, the extensive time and resources requirements of the research limited the number of replicate results. Collecting additional samples might be beneficial for developing further knowledge on sewer processes and their effect on AGS reactors.

The measured hydrolysis rates were lower than the VFA production observed in the sewer. Significant fermentation of the wastewater was observed, leading to an accumulation of volatile fatty acids in the wastewater reaching the WWTP. High VFA concentrations have been hypothesised to bring several benefits to aerobic granules, such as higher P-removal, larger and possibly more stable granules and higher SND rates. Moreover, there was a reduction of around one third in the concentration of suspended solids. Challenges associated with the treatment of WW containing particulate matter include granular instabilities or poor sludge settleability. Though the particles did not show a significant reduction in size, a reduced SS concentration might help to mitigate these drawbacks. Transport under anaerobic conditions in pressure sewers is, therefore, expected to enhance AGS reactors performance. It should be noted that the biodegradable and the soluble fractions of the COD in municipal wastewater decreased after transport in the pressure sewer. However, the reduction in BOD and SCOD was less prominent than values generally observed in gravity sewers.

Due to deterioration of the samples before the beginning of the fermentation batch test, it was not possible to determine the self-fermentation rate of the wastewater. The causes for this remain unclear. However, the observations regarding the failed test, in addition to the full-scale observations of increased VFA after the sewer, suggested that the fermentation activity of the wastewater might be considerably high, even compared to that of the sewer biofilm.

In terms of hydrolytic activity development under anaerobic conditions, the sewer did not seem to bring appreciable benefits. Preliminary results suggested that neither alpha nor beta-glucosidase would show significant differences after a short residence time in the sewer (< 5 hours). When sampling WW that had an HRT comparable to the maximum in the selected sewer (around 14 hours), the observed variations differed according to the considered enzyme and showed either a reduction or no significant change in volumetric hydrolytic activity.

The biomass-specific enzymatic activities of the influent wastewater were comparable to those of the mixed liquors and the distinct AGS fractions in terms of α- and β-glucosidase, but not for lipase and protease. Furthermore, the contribution of the influent to the total enzymatic activity of the reactor seemed to be

negligible for all the considered enzymes, mainly due to the large concentration of sludge biomass inside the reactor.

Overall, the results of this study suggest that transport of municipal wastewater in a pressure sewer can have a positive impact on its degree of fermentation and concentration of suspended solids. Despite a reduction in biodegradable and soluble substrate, the decrease was lower than what observed in pressure sewers in which aerobic conditions were controlled by oxygen injection. Therefore, anaerobic conveyance seemed more appropriate than aerobic transport for AGS reactors. Nonetheless, no significant benefit of anaerobic transport was observed on the enzymatic activity of glucosidases of the WW. Based on these observations, it is not suggested to reduce the length of the anaerobic feeding phase or to skip a pre-acidification tank step.

5 RECOMMENDATIONS

5.1 PRACTICAL CONSIDERATIONS

The criteria for selecting the sewer stretch should include the possibility to carry out the same type of sampling on either end of the sewer. Continuous measurements by means of online sensors, placed directly in the sewer, are to be favoured for their ability to follow the wastewater variability and reduce the limitations of matching inlet and outlet samples. However, collecting such measurements is currently limited to certain parameters due to a lack of appropriate sensors. For many types of analysis, sensors robust enough to withstand the harsh conditions of the sewer environment have yet to be developed. For those cases, collecting flow-proportional samples by means of automated samplers might help to reduce the inaccuracies deriving from matching inlet and outlet samples. In-line samplers are, however, not recommended for raw wastewater. The attempt to install an in-line automated sampler showed that clogging will happen within the first sampling.

Finally, if the project has been stopped for some time before being resumed, it is important to check whether the people involved in the preliminary arrangements are still working in the same position. The turnover might be higher than expected.

5.2 SUGGESTIONS FOR FURTHER RESEARCH

- Lab and full-scale studies on anaerobic processes in sewer systems present differences in terms of the VFA production and the behaviour (consumption or accumulation) of soluble substrate. The main difference seems to be related to the presence of a biofilm in contact with the bulk wastewater. Research on sewer biofilms has mainly been conducted in reference to gravity sewers. However, studying the microbial community composition and activity of the biofilm in pressure sewers might uncover useful knowledge to better understand and predict anaerobic processes affecting wastewater transport.

- The production of H_2S was not investigated in this thesis. Hydrogen sulfide, however, is commonly produced in pressure sewers and has been reported to cause several issues in activated sludge plants. Among these, H_2S was hypothesised to cause chemical phosphorus precipitation. The effect of H_2S on AGS reactors should be further investigated.

- Although removal efficiencies in AGS reactors allow this technology to easily meet the effluent standards, effluent suspended solids concentrations are generally more elevated than what observed in AS systems. Fats, oil, and grease (FOG) were found to be among the factors affecting this. In this study, lipids concentrations in the wastewater were not measured and the enzymatic activity of lipase was not investigated in depth. Gaining further knowledge regarding the behaviour of lipids and lipids-targeting enzymes before the wastewater treatment plant might help the optimisation of AGS reactor operation.

BIBLIOGRAPHY

Ahnert, M., Kuehn, V., & Krebs, P. (2005). Identification of overall degradation in sewer systems from long-term measurements and consequences for WWTP simulations. *Water Science and Technology*, *52*(3), 153–161. https://doi.org/10.2166/wst.2005.0072

Ali, M., Wang, Z., Salam, K. W., Hari, A. R., Pronk, M., Van Loosdrecht, M. C. M., & Saikaly, P. E. (2019). Importance of species sorting and immigration on the bacterial assembly of different-sized aggregates in a full-scale Aerobic granular sludge plant. *Environmental Science and Technology*, *53*(14), 8291–8301. https://doi.org/10.1021/acs.est.8b07303

Almeida, M. do C., & Butler, D. (2002). In-Sewer Wastewater Characterization and Model Parameter Determination Using Respirometry. *Water Environment Research*, *70*(3), 295–305. https://doi.org/10.2175/106143002X140035

Angelidaki, I., & Sanders, W. (2004). Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Biotechnology*. https://doi.org/10.1007/s11157-004-2502-3

Archibald, F., Méthot, M., Young, F., & Paice, M. G. (2001). A simple system to rapidly monitor activated sludge health and performance. *Water Research*, *35*(10), 2543–2553. https://doi.org/10.1016/S0043-1354(00)00542-X

Ashley, R. M., & Bertrand-Krajewski, J. L. Hvitved-Jacobsen, T. Verbanck, M. (2004). *Solids in sewers - Characteristics, Effects and Control of Sewer Solids and Associated Pollutants*. (R. M. Ashley, J. L. Bertrand-Krajewski, T. Hvitved-Jacobsen, & M. Verbanck, Eds.). IWA Publishing.

Azman, S., Khadem, A., Zeeman, G., van Lier, J., & Plugge, C. (2015). Mitigation of Humic Acid Inhibition in Anaerobic Digestion of Cellulose by Addition of Various Salts. *Bioengineering*, *2*(2), 54–65. https://doi.org/10.3390/bioengineering2020054

Bachmann, R. T., Saul, A. J., & Edyvean, R. G. J. (2007). Investigating and modelling the development of septic sewage in filled sewers under static conditions : A lab-scale feasibility study. *Science of the Total Environment*, *388*, 194–205. https://doi.org/10.1016/j.scitotenv.2007.08.004

Benedetti, L., Langeveld, J., Comeau, A., Corominas, L., Daigger, G., Martin, C., … Vanrolleghem, P. A. (2013). Modelling and monitoring of integrated urban wastewater systems: Review on status and perspectives. *Water Science and Technology*. https://doi.org/10.2166/wst.2013.397

Bengtsson, S., de Blois, M., Wilén, B.-M., & Gustavsson, D. (2018). Treatment of municipal wastewater with aerobic granular sludge. *Critical Reviews in Environmental Science and Technology*, *48*(2), 1–48. https://doi.org/10.1080/10643389.2018.1439653

Beun, J. J., Hendriks, A., Loosdrecht, M. C. ., Morgenroth, E., Wilderer, P. A., & Heijnen, J. J. (1999). Aerobic granulation in a sequencing batch reactor. *Water Research*, *33*(10), 2283–2290.

Bisswanger, H. (2014). Enzyme assays. *Perspectives in Science*, *1*(1–6), 41–55. https://doi.org/10.1016/j.pisc.2014.02.005

Charaklis, W. G., & Marshall, K. C. (1990). *Biofilms*. (W. G. Charaklis & K. C. Marshall, Eds.), *Wiley Series in Ecological and Applied Microbiology (Book 5)* (1 edition). USA: Wiley-Interscience;

de Bruin, L. M. M., de Kreuk, M. K., van der Roest, H. F. R., Uijterlinde, C., & van Loosdrecht, M. C. M. (2004). Aerobic granular sludge technology : An alternative to activated sludge? *Water Science & Technology*, *49*(December 2014), 7–13.

de Kreuk, M. K., Heijnen, J. J., & van Loosdrecht, M. C. M. (2005). Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. *Biotechnology and Bioengineering*, *90*(6), 761–769.

https://doi.org/10.1002/bit.20470

de Kreuk, M. K., Kishida, N., Tsuneda, S., & van Loosdrecht, M. C. M. (2010). Behavior of polymeric substrates in an aerobic granular sludge system. *Water Research*, *44*(20), 5929–5938. https://doi.org/10.1016/j.watres.2010.07.033

de Kreuk, M. K., Kishida, N., & van Loosdrecht, M. C. M. (2007). Aerobic granular sludge – state of the art. *Water Science & Technology*, *55*(8–9), 75–81. https://doi.org/10.2166/wst.2007.244

De Kreuk, M. K., Pronk, M., & Van Loosdrecht, M. C. M. (2005). Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures. *Water Research*, *39*(18), 4476–4484. https://doi.org/10.1016/j.watres.2005.08.031

de Kreuk, M. K., & van Loosdrecht, M. C. M. (2004). Selection of slow growing organisms as a means for improving aerobic granular sludge stability. *Water Science and Technology*, *49*(11), 9–17.

Flamink, C., Langeveld, J., & Clemens, F. (2005). Aerobic transformations in sewer systems: Are they relevant? *Water Science and Technology*, *52*(3), 163–170. https://doi.org/10.2166/wst.2005.0073

Freudenthal, K., Koglatis, J., Otterpohl, R., & Behrendt, J. (2005). Prediction of sulfide formation in sewer pressure mains based on the IWA Anaerobic Digestion Model No. 1 (ADM1). *Water Science and Technology*, *52*(10–11), 13–22. https://doi.org/10.2166/wst.2005.0674

Frolund, B., Griebe, T., & Nielsen, P. H. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology*, *43*, 755–761.

García-Mesa, J. J., Poyatos, J. M., Delgado-Ramos, F., Muñio, M. M., Osorio, F., & Hontoria, E. (2010). Water quality characterization in real biofilm wastewater treatment systems by particle size distribution. *Bioresource Technology*, *101*(21), 8038–8045. https://doi.org/10.1016/j.biortech.2010.05.071

Giesen, A., de Bruin, L. M. M., Niermans, R. P., & van der Roest, H. F. (2013). Advancements in the application of aerobic granular biomass technology for sustainable treatment of wastewater. *Water Practice & Technology*, *8*(1), 47–54. https://doi.org/10.2166/wpt.2013.007

Giesen, A., Loosdrecht, M. C. M. Van, Bruin, L. M. M. De, Pronk, M., & Roest, H. Van Der. (2014). Full-scale Experiences With Aerobic Granular Biomass Technology For Treatment Of Urban And Industrial Wastewater. In *Singapore International Water Week*. Singapore.

Goel, R., Mino, T., Satoh, H., & Matsuo, T. (1998). Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Research*, *32*(7), 2081–2088. https://doi.org/10.1016/S0043-1354(97)00425-9

Goel, R., Mino, T., Satoh, H., & Matsuo, T. (1999). Modeling hydrolysis processes considering intracellular storage. *Water Science and Technology*, *39*(1), 97–105. https://doi.org/10.2166/wst.1999.0022

Gómez-Silván, C., Arévalo, J., Pérez, J., González-López, J., & Rodelas, B. (2013). Linking hydrolytic activities to variables influencing a submerged membrane bioreactor (MBR) treating urban wastewater under real operating conditions. *Water Research*, (47), 66–78. https://doi.org/10.1016/j.watres.2012.09.032

Gonzalez, A., Hendriks, A. T. W. M., van Lier, J. B., & de Kreuk, M. (2018). Pre-treatments to enhance the biodegradability of waste activated sludge: Elucidating the rate limiting step. *Biotechnology Advances*. Elsevier Inc. https://doi.org/10.1016/j.biotechadv.2018.06.001

Gruber, G., Winkler, S., & Pressl, A. (2005). Continuous monitoring in sewer networks an approach for quantification of pollution loads from CSOs into surface water bodies. *Water Science and Technology*, *52*(12), 215–223. https://doi.org/10.2166/wst.2005.0466

Guellil, A., Boualam, M., Quiquampoix, H., Ginestet, P., Audic, J. M., & Block, J. C. (2001). Hydrolysis of wastewater colloidal organic matter by extracellular enzymes extracted from activated sludge flocs. In

Water Science and Technology (Vol. 43, pp. 33–40). IWA Publishing. https://doi.org/10.2166/wst.2001.0334

Guisasola, A., de Haas, D., Keller, J., & Yuan, Z. (2008). Methane formation in sewer systems. *Water Research*, *42*(6–7), 1421–1430. https://doi.org/10.1016/j.watres.2007.10.014

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M. C., & Marais, G. v. R. (1995). Wastewater and biomass characterization for the Activated Sludge Model No. 2 biological phosphorus removal. *Water Science and Technology*, *31*(2), 13–23.

Henze, M., & Mladenovski, C. (1991). Hydrolysis of particulate substrate by activated sludge under aerobic, anoxic and anaerobic conditions. *Water Research*, *25*(1), 61–64. https://doi.org/10.1016/0043-1354(91)90099-C

Huisman, J. L., & Gujer, W. (2002). Modelling wastewater transformation in sewers based on ASM3. In *Water Science and Technology* (Vol. 45, pp. 51–60). IWA Publishing. https://doi.org/10.2166/wst.2002.0093

Huisman, Jacco L., Gasser, T., Gienal, C., Kühni, M., Krebs, P., & Gujer, W. (2004). Quantification of oxygen fluxes in a long gravity sewer. *Water Research*, *38*(5), 1237–1247. https://doi.org/10.1016/j.watres.2003.11.012

Hvitved-Jacobsen, T., Raunkjær, K., & Nielsen, P. H. (1995). Volatile fatty acids and sulfide in pressure mains. *Water Science and Technology*, *31*(7), 169–179. https://doi.org/10.2166/wst.1995.0226

Hvitved-Jacobsen, T., Vollertsen, J., & Matos, J. S. (2002). The sewer as a bioreactor – a dry weather approach. *Water Science & Technology*, *45*(3), 11–24.

Hvitved-Jacobsen, T., Vollertsen, J., & Nielsen, A. (2013). *Sewer processes: microbial and chemical process engineering of sewer networks, Second Edition*. CRC Press LLC. Retrieved from https://content.taylorfrancis.com/books/download?dac=C2011-0-05358-X&isbn=9781439881781&format=googlePreviewPdf

Klučáková, M. (2018). Size and Charge Evaluation of Standard Humic and Fulvic Acids as Crucial Factors to Determine Their Environmental Behavior and Impact. *Frontiers in Chemistry*, *6*(JUL), 235. https://doi.org/10.3389/fchem.2018.00235

Kreutz, J. A., Böckenhüser, I., Wacht, M., & Fischer, K. (2016). A 1-year study of the activities of seven hydrolases in a communal wastewater treatment plant: trends and correlations. *Applied Microbiology and Biotechnology*, *100*(15), 6903–6915. https://doi.org/10.1007/s00253-016-7540-6

Langeveld, J., Daal, P. Van, Schilperoort, R., Nopens, I., Flameling, T., & Weijers, S. (2017). Empirical Sewer Water Quality Model for Generating Influent Data for WWTP Modelling. *Water*, *9*(3). https://doi.org/10.3390/w9070491

Langeveld, J. G., Clemens, F. H. L. R., & Van Der Graaf, J. H. J. M. (2003). Interactions within the wastewater system: Requirements for sewer processes modelling. In *Water Science and Technology* (Vol. 47, pp. 101–108). IWA Publishing. https://doi.org/10.2166/wst.2003.0231

Larsen, T. A., & Harremoës, P. (1994). Degradation mechanisms of colloidal organic matter in biofilm reactors. *Water Research*, *28*(6), 1443–1452. https://doi.org/10.1016/0043-1354(94)90312-3

Layer, M., Adler, A., Reynaert, E., Hernandez, A., Pagni, M., Morgenroth, E., … Derlon, N. (2019). Organic substrate diffusibility governs microbial community composition, nutrient removal performance and kinetics of granulation of aerobic granular sludge. *Water Research X*, *4*(100033). https://doi.org/10.1016/j.wroa.2019.100033

Layer, Manuel, Villodres, M. G., Hernandez, A., Reynaert, E., Morgenroth, E., & Derlon, N. (2020). Limited

simultaneous nitrification-denitrification (SND) in aerobic granular sludge systems treating municipal wastewater: Mechanisms and practical implications. *Water Research X*, *7*(100048). https://doi.org/10.1016/j.wroa.2020.100048

Lemaire, R., Webb, R. I., & Yuan, Z. (2008). Micro-scale observations of the structure of aerobic microbial granules used for the treatment of nutrient-rich industrial wastewater. *ISME Journal*, *2*(5), 528–541. https://doi.org/10.1038/ismej.2008.12

Li, J., Ding, L., Cai, A., Huang, G., & Horn, H. (2014). Aerobic Sludge Granulation in a Full-Scale Sequencing Batch Reactor. *BioMed Research International*, (Article ID 268789). https://doi.org/http://dx.doi.org/10.1155/2014/268789

Lie, E., & Welander, T. (1997). A method for determination of the readily fermentable organic fraction in municipal wastewater. *Water Research*, *31*(6), 1269–1274. https://doi.org/10.1016/S0043-1354(96)00092-9

Matos, J. S., & de Sousa, E. R. (1996). Prediction of dissolved oxygen concentration along sanitary sewers. *Water Science and Technology*, *34*(5–6), 525–532. https://doi.org/10.2166/wst.1996.0592

Mino, T., Van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*. Elsevier Sci Ltd. https://doi.org/10.1016/S0043-1354(98)00129-8

Morgenroth, E., Kommedal, R., & Harremoës, P. (2002). Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment - A review. In *Water Science and Technology* (Vol. 45, pp. 25–40). IWA Publishing. https://doi.org/10.2166/wst.2002.0091

Morgenroth, E., Sherden, T., Van Loosdrecht, M. C. M., Heijnen, J. J., & Wilderer, P. A. (1997). Aerobic granular sludge in a sequencing batch reactor. *Water Research*, *31*(12), 3191–3194.

Muyzer, G., & Stams, A. J. M. (2008, June 7). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology*. Nature Publishing Group. https://doi.org/10.1038/nrmicro1892

Narkis, N., Henefeld-Fourrier, S., & Rebhun, M. (1980). Volatile organic acids in raw wastewater and in physico-chemical treatment. *Water Research*, *14*(9), 1215–1223. https://doi.org/10.1016/0043-1354(80)90179-7

Nielsen, P. H., Raunkjaer, K., Norsker, N. H., Jensen, N. A., & Hvitved-Jacobsen, T. (1992). Transformation of wastewater in sewer systems - A review. In *Water Science and Technology* (Vol. 25, pp. 17–31). IWA Publishing. https://doi.org/10.2166/wst.1992.0111

Nybroe, O., Jørgensen, P. E., & Henze, M. (1992). Enzyme activities in waste water and activated sludge. *Water Research*, *26*(5), 579–584. https://doi.org/10.1016/0043-1354(92)90230-2

Oehmen, A., Carvalho, G., Lopez-Vazquez, C. M., van Loosdrecht, M. C. M., & Reis, M. A. M. (2010). Incorporating microbial ecology into the metabolic modelling of polyphosphate accumulating organisms and glycogen accumulating organisms. *Water Research*, *44*(17), 4992–5004. https://doi.org/10.1016/j.watres.2010.06.071

Omil, F., Lens, P., Visser, A., Pol, L. W. H., & Lettinga, G. (1998). Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotechnology and Bioengineering*, *57*(6), 676–685. https://doi.org/10.1002/(SICI)1097-0290(19980320)57:6<676::AID-BIT5>3.0.CO;2-I

Oosterhuis, M., & van Loosdrecht, M. C. M. (2009). Nitrification of urine for H2S control in pressure sewers. *Water Practice and Technology*, *4*(3), 1–7. https://doi.org/10.2166/wpt.2009.059

Picioreanu, C., van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Mathematical Modeling of Biofilm Structure

with a Hybrid Differential-Discrete Cellular Automaton Approach. *Biotechnology and Bioengineering*, *58*(1), 101–116.

Pronk, M., Abbas, B., Al-zuhairy, S. H. K., Kraan, R., Kleerebezem, R., & van Loosdrecht, M. C. M. (2015). Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. *Applied Microbiology and Biotechnology*, *99*(12), 5257–5268. https://doi.org/10.1007/s00253-014-6358-3

Pronk, M., de Kreuk, M. K., de Bruin, B., Kamminga, P., Kleerebezem, R., & van Loosdrecht, M. C. M. (2015). Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Research*, *84*(August), 207–217. https://doi.org/10.1016/j.watres.2015.07.011

Pronk, Mario, Giesen, A., Thompson, A., Robertson, S., & Van Loosdrecht, M. (2017). Aerobic granular biomass technology: Advancements in design, applications and further developments. *Water Practice and Technology*, *12*(4). https://doi.org/10.2166/wpt.2017.101

Raunkjaer, K., Hvitved-Jacobsen, T., & Nielsen, P. H. (1995). Transformation of organic matter in a gravity sewer. *Water Environment Research*, *67*(2), 181–188. https://doi.org/10.2175/106143095x131330

Raunkjær, K., Nielsen, P. H., & Hvitved-Jacobsen, T. (1997). Acetate removal in sewer biofilms under aerobic conditions. *Water Research*, *31*(11), 2727–2736. https://doi.org/10.1016/S0043-1354(97)00128-0

Roeleveld, P. J., & van Loosdrecht, M. C. M. (2001). Experience with guidelines for wastewater characterisation in The Netherlands. *Water Science and Technology*, *45*(6), 77–87.

Rosenblum, J., Lee, J., Martin, J., & Bisesi, M. S. (2018). Correlation Between Levels of Humic Acid and Fecal Indicator Bacteria: A Potential Predictor of Biosolids Stabilization. *Environmental Engineering Science*, *35*(7), 663–672. https://doi.org/10.1089/ees.2017.0281

Rudelle, E., Vollertsen, J., Hvitved-Jacobsen, T., & Nielsen, A. H. (2011). Anaerobic Transformations of Organic Matter in Collection Systems. *Water Environment Research*, *83*(6), 532–540. https://doi.org/10.2175/106143010X12681059116699

Rudelle, E., Vollertsen, J., Hvitved-Jacobsen, T., & Nielsen, A. H. (2012). Modeling anaerobic organic matter transformations in the wastewater phase of sewer networks. *Water Science and Technology*, *66*(8), 1728–1734. https://doi.org/10.2166/wst.2012.378

Schilperoort, R. P. S., Dirksen, J., Langeveld, J. G., & Clemens, F. H. L. R. (2012). Assessing characteristic time and space scales of in-sewer processes by analysis of one year of continuous in-sewer monitoring data. *Water Science and Technology*, *66*(8), 1614–1620. https://doi.org/10.2166/wst.2012.115

Schwarzenbeck, N., Erley, R., & Wilderer, P. A. (2004). Aerobic granular sludge in an SBR-system treating wastewater rich in particulate matter. *Water Science & Technology*, *49*(11–12), 41–46.

Sharma, K. R., Yuan, Z., de Haas, D., Hamilton, G., Corrie, S., & Keller, J. (2008). Dynamics and dynamic modelling of H2S production in sewer systems. *Water Research*, *42*, 2527–2538. https://doi.org/10.1016/j.watres.2008.02.013

Sophonsiri, C., & Morgenroth, E. (2004). Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters. *Chemosphere*, *55*, 691–703. https://doi.org/10.1016/j.chemosphere.2003.11.032

Stokholm-Bjerregaard, M., McIlroy, S. J., Nierychlo, M., Karst, S. M., Albertsen, M., & Nielsen, P. H. (2017). A critical assessment of the microorganisms proposed to be important to enhanced biological phosphorus removal in full-scale wastewater treatment systems. *Frontiers in Microbiology*, *8*(APR), 718. https://doi.org/10.3389/fmicb.2017.00718

Sutherland-Stacey, L., Corrie, S., Neethling, A., Johnson, I., Gutierrez, O., Dexter, R., … Hamilton, G. (2008).

Continuous measurement of dissolved sulfide in sewer systems. *Water Science and Technology*, *57*(3), 375–381. https://doi.org/10.2166/wst.2008.132

Tanaka, N., & Hvitved-Jacobsen, T. (2002). Anaerobic transformations of wastewater organic matter and sulfide production - Investigations in a pilot plant pressure sewer. In *Water Science and Technology* (Vol. 45, pp. 71–79). IWA Publishing. https://doi.org/10.2166/wst.2002.0057

Tanaka, N., & Takenaka, K. (1995). Control of hydrogen sulfide and degradation of organic matter by air injection into a wastewater force main. *Water Science and Technology*, *31*(7), 273–282. https://doi.org/10.2166/wst.1995.0243

Tanaka, Naoya, & Hvitved-Jacobsen, T. (1998). Transformations of wastewater organic matter in sewers under changing aerobic/anaerobic conditions. *Water Science & Technology*, *37*(1), 105–113.

van Lier, Jules, B., Mahmoud, N., & Zeeman, G. (2008). Anaerobic wastewater treatment. In *Biological Wastewater Treatment: Principles Modelling and Design*. London: IWA Publishing.

van Loosdrecht, M. C. M., & Brdjanovic, D. (2014). Anticipating the next century of wastewater treatment. *Science*, *344*(6191), 1452–1453. https://doi.org/10.1126/science.1255183

van Loosdrecht, M. C. M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L., & Heijnen, J. J. (1995). Biofilm structures. *Water Science and Technology*, *32*(8), 35–43.

van Loosdrecht, M. C. M., Picioreanu, C., & Heijnen, J. J. (1997). A more unifying hypothesis for biofilm structures. *Microbiology Ecology*, *24*, 181–183.

van Nieuwenhuijzen, A. F., van der Graaf, J. H. J. M., Kampschreur, M. J., & Mels, A. R. (2004). Particle related fractionation and characterisation of municipal wastewater. *Water Science and Technology*, *50*(12), 125–132.

Veel, L. P. J. (2018). *Impact of the sewer on the start-up of a Nereda plant*. Delft.

Verbanck, M., Vanderborght, J. P., & Wollast, P. (1990). Size distribution of suspended particles in combined sewers during dry and wet weather. In Y. Iwasa & T. Sueishi (Eds.), *Drainage Systems and Runoff Reduction* (pp. 89 1-896). Osaka.

Vollertsen, J., Almeida, M. do C., & Hvitved-Jacobsen, T. (1999). Effects of temperature and dissolved oxygen on hydrolysis of sewer solids. *Water Re*, *33*(14), 3119–3126.

Vollertsen, J., & Hvitved-Jacobsen, T. (1999). Stoichiometric and kinetic model parameters for microbial transformations of suspended solids in combined sewer systems. *Water Research*, *33*(14), 3127–3141.

Wagner, J., Weissbrodt, D. G., Manguin, V., Ribeiro da Costa, R. H., Morgenroth, E., & Derlon, N. (2015). Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors. *Water Research*, *85*, 158–166. https://doi.org/10.1016/j.watres.2015.08.030

Wang, Z., Cao, J., & Meng, F. (2015). Interactions between protein-like and humic-like components in dissolved organic matter revealed by fluorescence quenching. *Water Research*, *68*, 404–413. https://doi.org/10.1016/j.watres.2014.10.024

Weissbrodt, D. G., Shani, N., & Holliger, C. (2014). Linking bacterial population dynamics and nutrient removal in the granular sludge biofilm ecosystem engineered for wastewater treatment, *88*, 579–595. https://doi.org/10.1111/1574-6941.12326

Whalen, P. A., Whalen, P. J., & Tracey, D. R. (2006). Cellular ATP – A Superior Measure of Active Biomass for Biological Wastewater Treatment Processes. In *Proceedings of the Water Environment Foundation* (pp. 3025–3037). https://doi.org/10.2175/193864706783751177

Winkler, M. H., Kleerebezem, R., Bruin, L. M. M. De, Verheijen, P. J. T., Abbas, B., Habermacher, J., & van

Loosdrecht, M. C. M. (2013). Microbial diversity differences within aerobic granular sludge and activated sludge flocs. *Applied Microbiology and Biotechnology*, 7447–7458. https://doi.org/10.1007/s00253-012-4472-7

Winkler, M. K. H., Meunier, C., Henriet, O., Mahillon, J., Suárez-Ojeda, M. E., Del Moro, G., … Weissbrodt, D. G. (2018, March 15). An integrative review of granular sludge for the biological removal of nutrients and recalcitrant organic matter from wastewater. *Chemical Engineering Journal*. Elsevier B.V. https://doi.org/10.1016/j.cej.2017.12.026

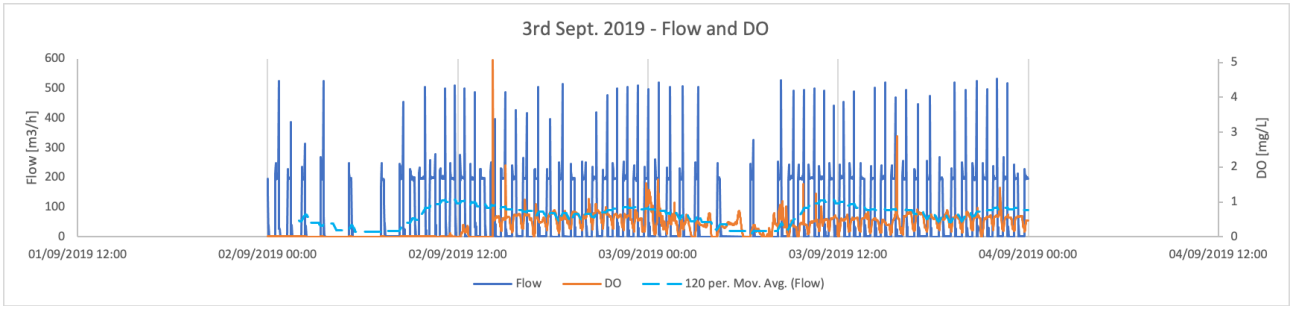
Zahmatkesh, M., Spanjers, H., Toran, M. J., Blánquez, P., & van Lier, J. B. (2016). Bioremoval of humic acid from water by white rot fungi: exploring the removal mechanisms. *AMB Express*, *6*(1), 118. https://doi.org/10.1186/s13568-016-0293-x

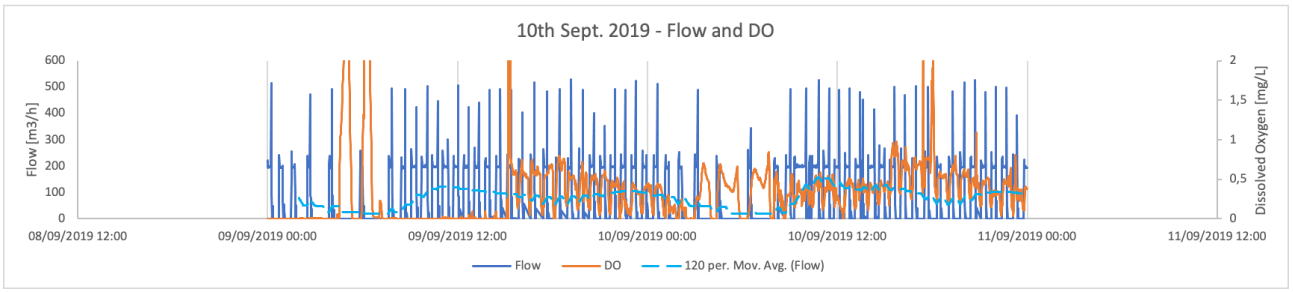
APPENDIX I - SAMPLING OVERVIEW

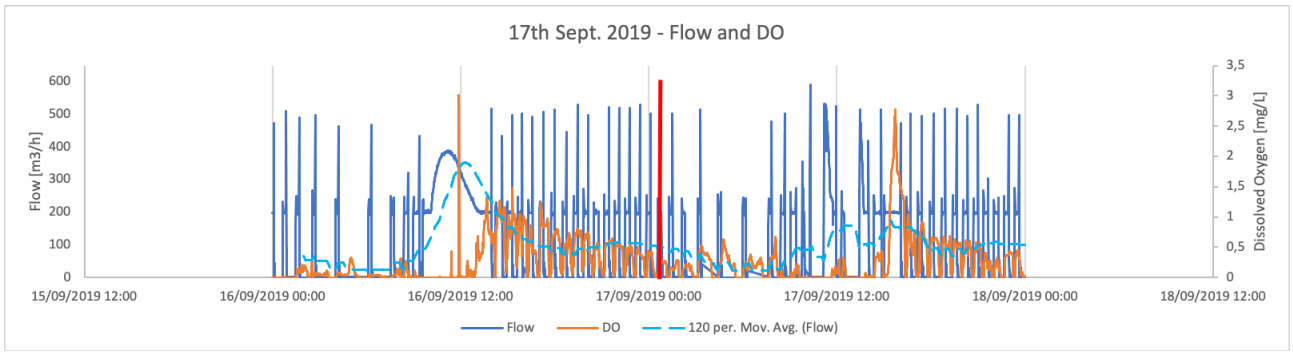
Table 4: Overview of the collected samples

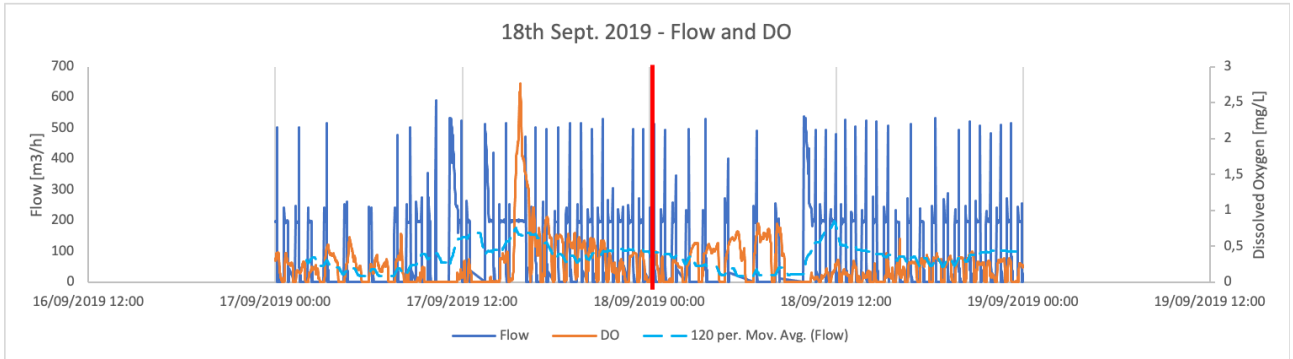
DATE	HRT	DWF/WWF	TYPE OF ANALYSIS	REMARKS
09/08/2019	13h	DWF	α&β Enzymatic Assays	
03/09/2019	13h40min	DWF	α&β, Lip and Pr Enzymatic Assays	
10/09/2019	13h45min	DWF	α&β Enzymatic Assays	
10/09/2019	13h10min	DWF	α&β Enzymatic Assays	
13/09/2019	11h40min	DWF	α&β Enzymatic Assays	
13/09/2019	5h	WWF	α&β Enzymatic Assays	
16/09/2019	13h50min	DWF	α&β Enzymatic Assays	
16/09/2019	2h55min	WWF	α&β Enzymatic Assays	
17/09/2019	13h50min	DWF	OUR	
17/09/2019	12h55min	DWF	OUR	
18/09/2019	12h55min	DWF	OUR	Morning sampling WW discarded due to very dark colour
22/10/2019	13h20min	DWF	Fractionated WW α Enzymatic Assay + WW characterization	Maintenance works between 11 and 13 to remove air from sewer
23/10/2019	14h	DWF	FBT + WW characterization	
24/10/2019	13h50min	DWF	FBT + WW characterization	
24/10/2019	12h45min	DWF	FBT + WW characterization	
21/11/2019	10h25min	DWF	WW fractionation	No flow data available between 12:00 (20.11) and 6:55 (21.11)
21/11/2019	10h25min[1]	DWF	WW fractionation	
22/11/2019	13h15min	DWF	WW fractionation	
22/11/2019	\	DWF	WW fractionation	Doorn samples

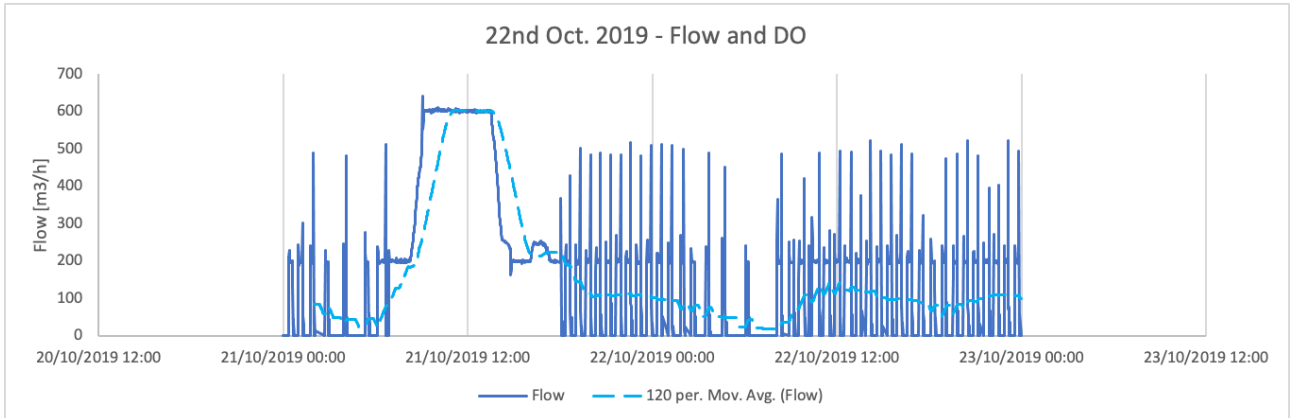
[1] Flow data was not recorded between 12:00 of the 20th November and 6:55 of the 21st November 2019. The HRT of the samples was therefore calculated from the total volume of water reaching the WWTP from Doorn between the 20th and the 21st Nov, measured online from 9am to 9am. This is likely to be an underestimation, as the flow during the night is usually lower than day flows, leading to longer retention times.

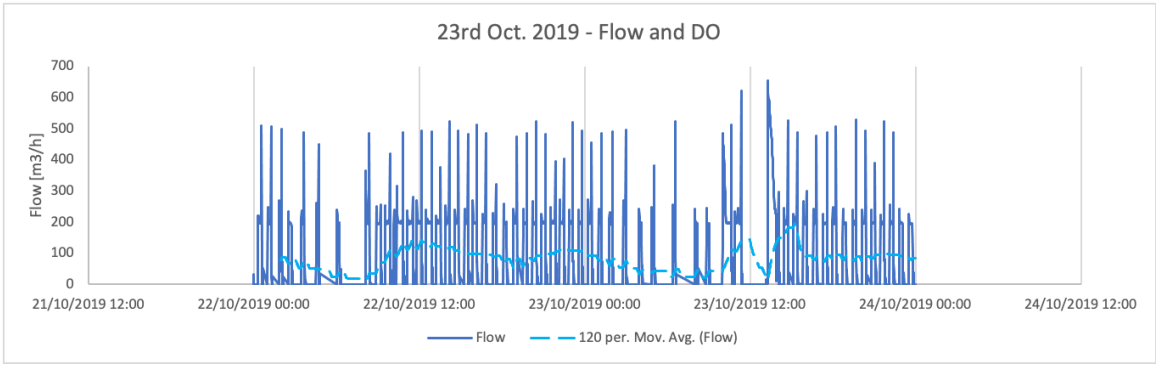

3rd Sept. 2019 - Flow and DO
Flow [m3/h]
DO [mg/L]
01/09/2019 12:00
02/09/2019 00:00
02/09/2019 12:00
03/09/2019 00:00
03/09/2019 12:00
04/09/2019 00:00
04/09/2019 12:00
Flow
DO
120 per. Mov. Avg. (Flow)

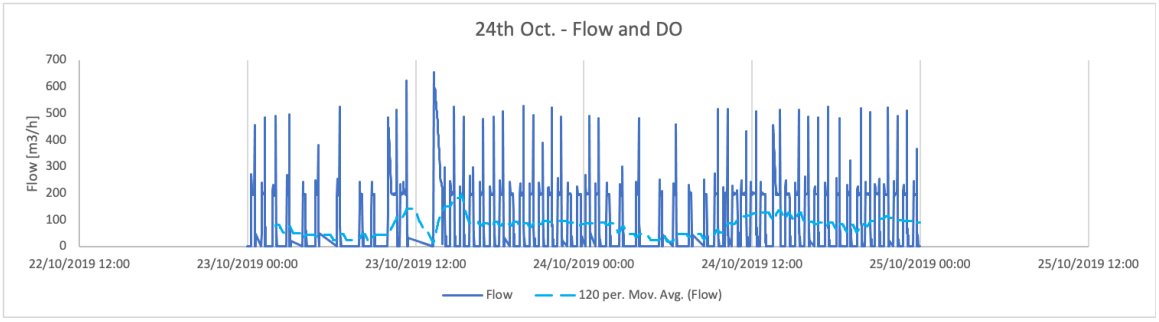

10th Sept. 2019 - Flow and DO
Flow [m3/h]
Dissolved Oxygen [mg/L]
08/09/2019 12:00
09/09/2019 00:00
09/09/2019 12:00
10/09/2019 00:00
10/09/2019 12:00
11/09/2019 00:00
11/09/2019 12:00
Flow
DO
120 per. Mov. Avg. (Flow)


17th Sept. 2019 - Flow and DO
Flow [m3/h]
Dissolved Oxygen [mg/L]
15/09/2019 12:00
16/09/2019 00:00
16/09/2019 12:00
17/09/2019 00:00
17/09/2019 12:00
18/09/2019 00:00
18/09/2019 12:00
Flow
DO
120 per. Mov. Avg. (Flow)


18th Sept. 2019 - Flow and DO
Flow [m3/h]
Dissolved Oxygen [mg/L]
16/09/2019 12:00
17/09/2019 00:00
17/09/2019 12:00
18/09/2019 00:00
18/09/2019 12:00
19/09/2019 00:00
19/09/2019 12:00
Flow
DO
120 per. Mov. Avg. (Flow)


22nd Oct. 2019 - Flow and DO
Flow [m3/h]
20/10/2019 12:00
21/10/2019 00:00
21/10/2019 12:00
22/10/2019 00:00
22/10/2019 12:00
23/10/2019 00:00
23/10/2019 12:00
Flow
120 per. Mov. Avg. (Flow)





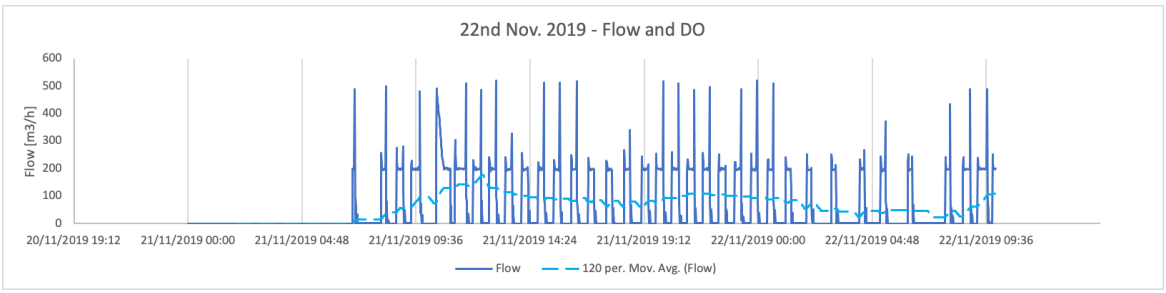


Figure 26: Flow and dissolved oxygen data on the different days of sampling

APPENDIX II – OXYGEN UPTAKE RATE

The maximum oxygen uptake rate (OUR) of the WW was measured to assess the impact of the pressure sewer on aerobic microorganisms and, as described in Section 3.1.1, to gain a deeper understanding of the electron acceptor conditions inside the main. Due to the low volumes collected by the automated sampler, these tests could not be conducted on the same samples used for the WW characterisation and six separate samples (three matching WW samples sets) were used for these measurements.

Table 5 reports the measured OUR values. All rates followed 1st order kinetic behaviour, with minimum R^2 value of 0,9947. The average OUR at 20°C for the WW at the sewer inlet was 6.30±0.15 $mgO_2/L \cdot h$, in line with previous studies of domestic wastewaters, where OUR values had been measured in the range of 2-25 $mgO_2/L \cdot h$ (Hvitved-Jacobsen et al., 2013; Matos & de Sousa, 1996).

After the anaerobic sewer, the OUR values were variable, ranging from 4.59±0.30 to 9.29±0.73 $mgO_2/L \cdot h$. Positive OUR values after the sewer imply that at least part of the aerobic heterotrophic biomass entering the sewer was maintained alive under the anaerobic conditions during transport. This phenomenon can easily be understood, as municipal WW contains many facultative aerobic microorganisms that can survive anaerobic periods (Ali et al., 2019; Tanaka & Hvitved-Jacobsen, 1998).

Table 5: Oxygen uptake rate measurements for the WW entering and exiting the force main

	17/09/2019 13 h 50 min		17/09/2019 13 h		18/09/2019 13 h	
[mg/L·h]	Average	St.Dev.	Average	St.Dev.	Average	St.Dev.
In	-6,31	0,14	-6,15	0,40	-6,45	0,35
Out	-6,29	0,34	-9,29	0,73	-4,59	0,30

APPENDIX III - FERMENTATION BATCH TEST RESULTS

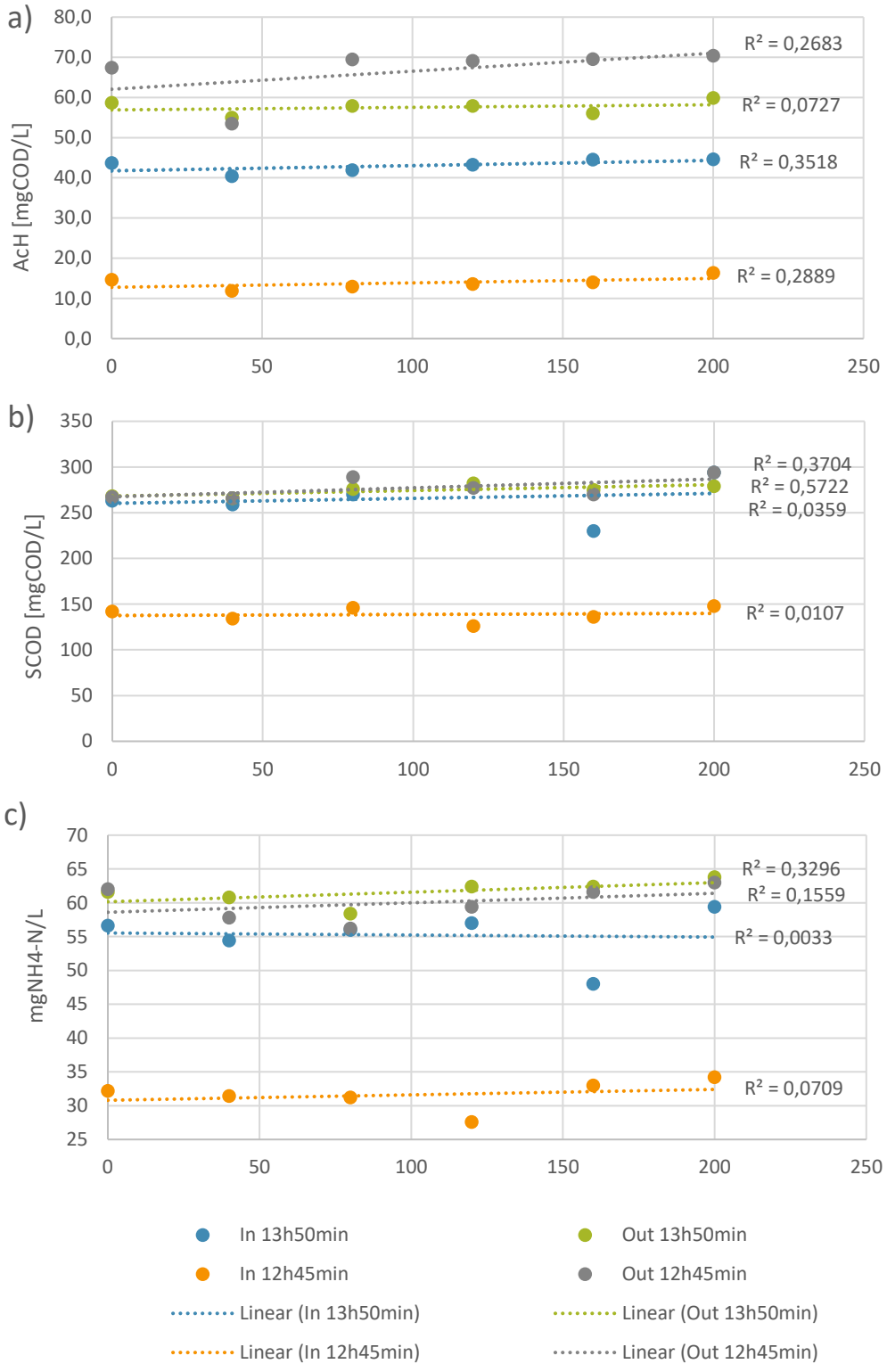


Figure 27: Concentrations changes during the fermentation batch test, over the total experiment duration of 200 minutes. The linear fitting of the data was very poor, with R^2 values reported aside to the right.
a) Acetate; b) Soluble COD; c) Ammonium.

APPENDIX IV - ENZYMATIC FINGERPRINT OF THE WASTEWATER

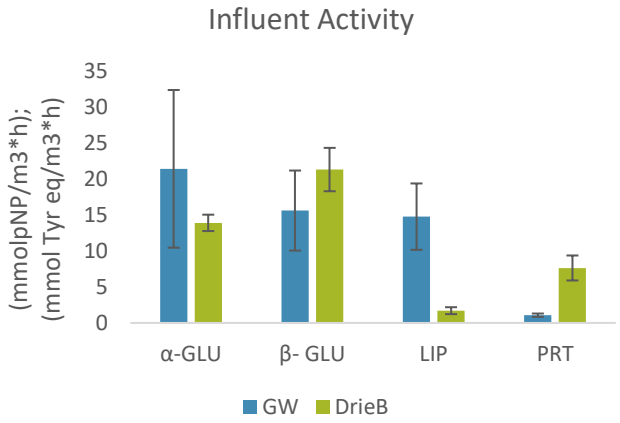


Figure 28: Volumetric enzymatic activity of the wastewater, for all the measured enzymes in Driebergen and Garmerwolde

Figure 28 reports the enzymatic activities of the influent to the Nereda reactor in Garmerwolde, compared to the activities measured in the wastewater reaching the WWTP in Driebergen. Glucosidases had the highest activities in both wastewaters.

Lipase behaved differently in the two locations, but in both cases it showed values much lower or equal to those of the alpha- and beta-glucosidase, varying from 15±5 mmol*p*NP/m^3·h in GW to only 2±0 mmol*p*NP/m^3·h, in Driebergen. Similarly, protease showed very low activity both in Garmerwolde (1±0 mmol*p*NP/m^3·h) and in Driebergen (8±2 mmol*p*NP/m^3·h). It is generally reported in literature that protease and esterases activity in raw wastewater and activated sludge is higher than glucosidases (Frolund et al., 1995; Nybroe et al., 1992). The WWTP in these published studies, however, received their influent from mixed gravity and force sewer systems. As both systems analysed in this study received WW from pressurised mains, lipids and proteins degradation might have been limited by anaerobic conditions during transport.

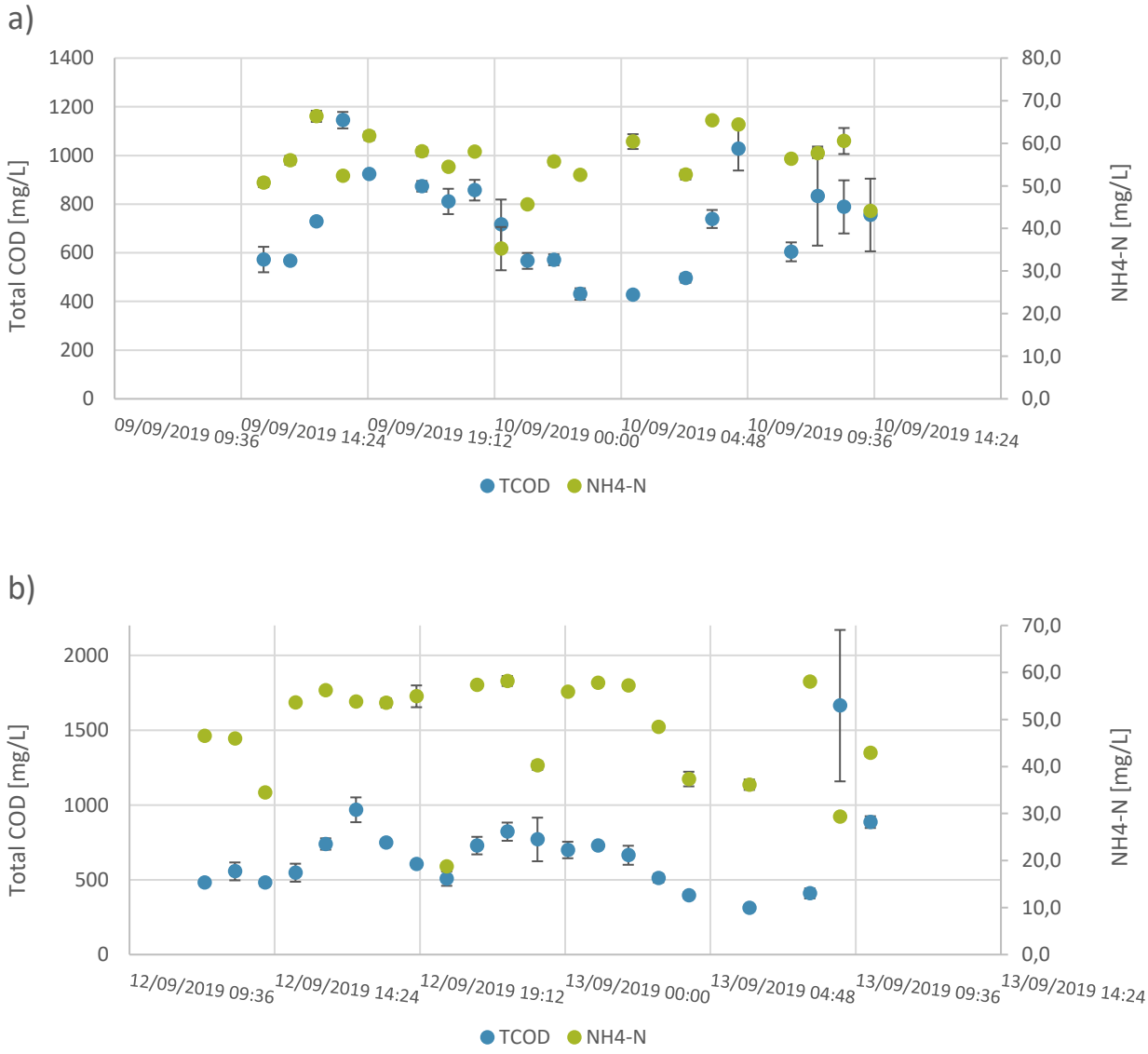
APPENDIX V - DOMESTIC WASTEWATER 24H MONITORING IN DOORN, NL

Domestic wastewater was collected at the end of the gravity sewer connecting the town of Doorn, the Netherlands, to the pumping station leading to the nearby WWTP. A vacuum sampler collected grab samples of 1L once per hour for 24h, provided flow was available. Total COD and NH_4-N were monitored over the 24h samples of four days. The following Table 1 reports the weather conditions during the samplings:

Table 6: Sampling dates and flows

Sampling Date	Flow
10th September 2019	Dry Weather Flow, no significant precipitations
13th September 2019	DWF, with small storm during the afternoon
15th September 2019	DWF followed by an overnight storm
16th September 2019	Overnight storm followed by DWF

Figure 28 shows the trends in TCOD and NH_4-N over the different sampling dates.



c)

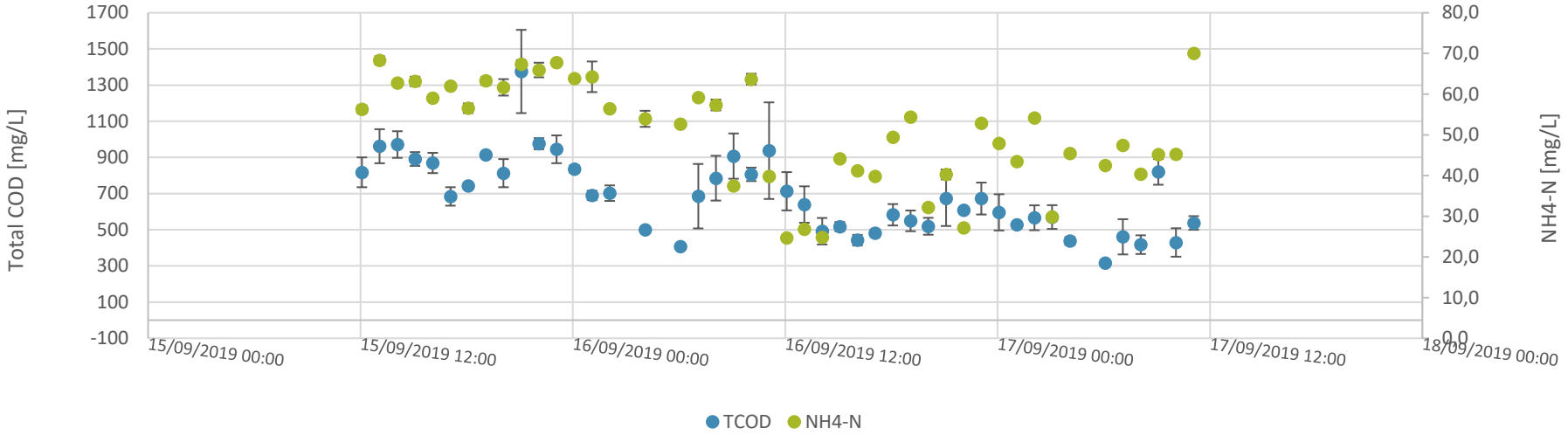


Figure 29: Total COD and NH_4^+-N behaviour of domestic wastewater samples collected in Doorn:

a) over 24h, starting at 15.15 of the 9th September until 14.15 ofthe 10th September

b) 24h sampling between the 12th September 2019 at 12.05 and the 13th September 2019 at 11.05

c) 48h sampling, from 12.00am of the 15th September and 11am of the 17th September 2019