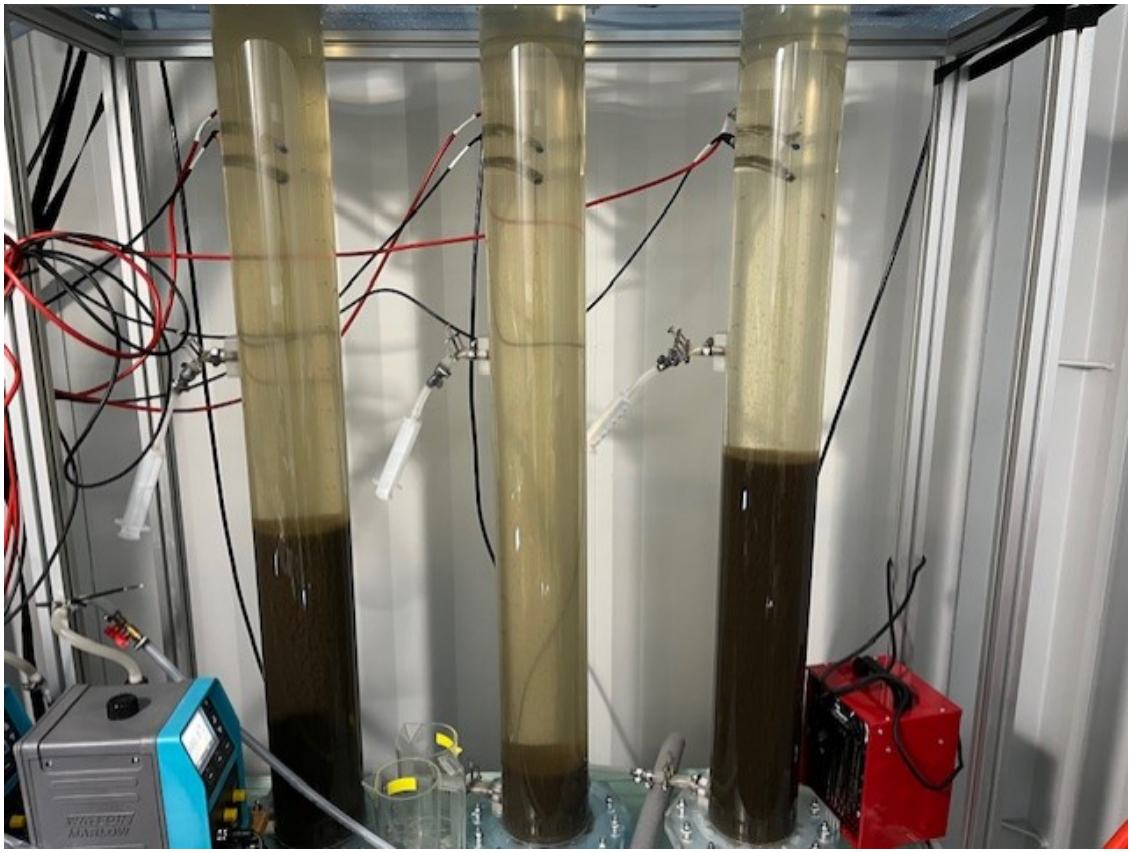
CHALMERS
UNIVERSITY OF TECHNOLOGY



Aerobic Granular Sludge Start-up under Varying Wastewater Compositions

A Pilot Study at Ryaverket WWTP

Master's thesis in Infrastructure and Environmental Engineering

SANDRA NASTOVSKA

DEPARTMENT OF ARCHITECTURE AND CIVIL ENGINEERING
Division of Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Master's Thesis ACEX30
Gothenburg, Sweden 2026

CHALMERS
UNIVERSITY OF TECHNOLOGY

Department of Architecture and Civil Engineering
Division of Water Environment Technology
Chalmers University of Technology
Master's Thesis ACEX30
Gothenburg, Sweden 2026

Master's thesis 2026

AGS Start-up under Varying Wastewater Compositions

A Pilot Study at Ryaverket WWTP

SANDRA NASTOVSKA

CHALMERS
UNIVERSITY OF TECHNOLOGY

Department of Architecture and Civil Engineering
Division of Water environment technology
ACEX30
Chalmers University of Technology
Gothenburg, Sweden 2026

AGS Start-up under Varying Wastewater Compositions
A Pilot Study at Ryaverket WWTP
SANDRA NASTOVSKA

Supervisor: Britt-Marie Wilén, Division of Water Environment Technology
Veronica Gast, Division of Water Environment Technology
Susanne Tumlin, Gryaab
Examiner: Frank Persson, Division of Water Environment Technology

Master's Thesis 2026
Department of Architecture and Civil Engineering
Division of Water Environment Technology
ACEX30
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone +46 31 772 1000

Gothenburg, Sweden, 2026

AGS Start-up under Varying Wastewater Compositions
SANDRA NASTOVSKA
Department of Architecture and Civil Engineering
Chalmers University of Technology

Abstract

Aerobic granular sludge (AGS) is a promising technology for biological wastewater treatment that offers advantages over activated sludge treatment in terms of compactness, energy efficiency and treatment performance. In this study, the start-up of three pilot scale AGS reactors at Ryaverket wastewater treatment plant was investigated, where the effect of seeding culture and wastewater composition on granulation and treatment performance was evaluated. The reactors were operated with activated sludge or mature granules, and with raw or pre-sedimented wastewater.

The results showed that nitrification and carbon degradation were effectively established in all reactors, while denitrification and biological phosphorus removal were deficient, probably due to suboptimal operating conditions. The reactor with mature granules generally showed the best treatment performance, but overall, no reactor achieved sufficient treatment as the outgoing wastewater exceeded the limit values for total nitrogen and total phosphorus. The use of pre-sedimented wastewater was considered to be disadvantageous for biomass build-up due to the low substrate available. In addition, the results showed that starting with mature granules achieve better purification, which can also be established more quickly.

Keywords: Aerobic Granular Sludge (AGS), Start-up Strategy, Granule Formation, Pilot Study, Biological Wastewater Treatment, Activated Sludge Seeding, Inoculation of Granules.

Acknowledgements

I would like to express my sincere gratitude to my supervisors, Veronica and Britt-Marie at Chalmers University of Technology, for their guidance, support, and constructive feedback throughout this project, and to my examiner Frank for his valuable input and review. Their expertise, encouragement, and knowledge have been greatly appreciated and have contributed to the completion of this work. I would also like to express my sincere gratitude to everyone at Gryaab who shared their experience, and valuable insights throughout this project. I am equally grateful to everyone at the WET Lab at Chalmers University of Technology for their support in the laboratory, their assistance during the experimental work, and for providing access to the facilities and resources that made this work possible.

List of Acronyms

Below is the list of acronyms that have been used throughout this thesis listed in alphabetical order:

AGS	Aerobic granular sludge
AOB	Ammonia oxidizing bacteria
AS	Activated sludge
ATP	Adenosine triphosphate
COD	Chemical oxygen demand
dGAO	Denitrifying glycogen-accumulating organism
dPAO	Denitrifying phosphorus-accumulating organism
DOC	Dissolved organic carbon
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
GAO	Glycogen-accumulating organism
IC	Ion chromatography
MLSS	Mixed liquor suspended solids
NOB	Nitrite oxidizing bacteria
PAO	Phosphorus-accumulating organism
PHA	Polyhydroxyalkanoates
SBR	Sequencing batch reactor
SND	Simultaneous nitrification and denitrification
SRT	Solids retention time
SVI	Sludge volume index
TOC	Total organic carbon
TP	Total phosphorus
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

Contents

List of Figures

List of Tables

1
Introduction

1.1 Background

Access to clean water is essential for human health and functioning ecosystems. With a growing global population and increasing urbanization, the load on wastewater treatment plants (WWTP) is continuously increasing (Bengtsson et al., 2018). This drives the need for treatment methods that are both effective and efficient. Discharges of insufficiently treated wastewater carry many risks (Seviour & Blackall, 1999), and stricter treatment requirements from authorities are driving the need for technological development in the industry (Sepúlveda-Mardones et al., 2019). For many years, activated sludge (AS) has been the global standard for biological wastewater treatment, but the technology faces challenges related to large space requirements and high energy consumption (Nancharaiah & Sarvajith, 2019). As a solution to these limitations, aerobic granular sludge (AGS) has emerged as a promising technology that requires 50-75 % less space and 50-60 % less energy. This is because the purification of nitrogen, phosphorus and carbon takes place in one tank and at the same time (de Kreuk et al., 2005). Despite the technology's great potential, AGS is still in a relatively early stage of full-scale implementation and is considered relatively untested, especially in Nordic conditions. Therefore, more research is needed to increase the understanding of important aspects, such as start-up strategies (Bengtsson et al., 2018).

1.2 Aim and Objectives

The aim of this master's thesis is to investigate the start-up and development of AGS in different wastewater compositions, in a pilot scale. The study is conducted at Ryaverkets wastewater treatment plant and involves three parallel AGS reactors operated with different types of wastewater. Reactor 1 was fed raw wastewater and seeded with activated sludge from Ryaverket WWTP, reactor 2 was fed pre-settled wastewater and seeded activated sludge and reactor 3 was fed raw wastewater and seeded granules. The project was carried out in connection with Gryaab's plans to implement AGS at Ryaverket WWTP. Gryaab wanted to investigate whether granules could be grown from the plant's own activated sludge, and to identify the most advantageous start-up strategy for the system. In addition, evaluating the influence of different wastewater compositions was of interest to inform decisions about the placement and configuration of the AGS within the treatment plant.

The focus is on evaluating how variations in water composition affect granule formation, sludge characteristics, and process performance during the start-up phase. Furthermore, how the three reactors differ in removal of carbon, phosphorus and nitrogen will be studied. The sub objectives of the project include:

- Analysing and comparing the removal performance of each reactor regarding phosphorus, nitrogen and carbon.
- Comparing the start-up of pre-settled wastewater and raw wastewater.
- Comparing the start-up of inoculation with mature granules and activated sludge seed.

1.3 Limitations

The study was conducted at a pilot scale at Ryaverket's wastewater treatment plant, which means that the results are not necessarily directly transferable to full scale AGS plants. The study was also limited to the analysis of selected process parameters linked to AGS start-up. Another limitation was the time frame, which may have affected the ability to evaluate stable granulation.

2
Theory

2.1 Biological Wastewater Treatment

Wastewater treatment can be done by various methods such as chemical treatment, physical treatment, and biological treatment. In biological wastewater treatment, microorganisms that naturally occur in the wastewater are used to remove pollutants including phosphorus, nitrogen, and carbon. In this thesis, the focus is on a biological treatment process that removes all three of those elements from the wastewater in a single reactor. This technology is aerobic granular sludge.

2.1.1 AGS

AGS consists of microbial granules that enable removal of carbon, nitrogen, and phosphorus and are operated in a sequential batch reactor (SBR) (de Blois et al., 2022). AGS consists of compact granules with good sinking properties where the particles sink with a velocity of around 12-145 m/hr (Bengtsson et al., 2018). The technology can be applied to high sludge concentrations and can withstand high organic loading rates, as well as operate at short sedimentation times (Bengtsson et al., 2018). However, AGS can require much more time as approximately four times more time for operation and maintenance was required for the AGS compared to activated sludge in the AGS plant in Österröd (de Blois et al., 2022). The most common way to start AGS are through inoculation with activated sludge or inoculation with granules (Bengtsson et al., 2018). When inoculated with activated sludge, it takes longer for microorganisms to create dense granules (Nancharaiah & Sarvajith, 2019).

The foundation for AGS was established in 1997 when it was discovered that granules could be used in a sequenced batch reactor under aerated conditions (Morgenroth et al., 1997). The technology was then further developed in the Netherlands, where the Nereda process was established (Sepúlveda-Mardones et al., 2019). The technology has expanded and is present in around 90 full-scale plants, with the first in Sweden being established in 2018 in Österröd (de Blois et al., 2022). There are a number of studies on the operation of AGS at full-scale plants, but there is still some insufficient knowledge about how AGS works with regard to chemical oxygen demand (COD), nitrogen and phosphorus removal in municipal wastewater (Bengtsson et al., 2018). Furthermore, most studies have been conducted on synthetic wastewater at lab scale under controlled conditions (Nancharaiah & Sarvajith, 2019).

2.1.1.1 The SBR/AGS Cycle

AGS is operated in a SBR often in a cycle consisting of three different phases (Nancharaiah & Sarvajith, 2019). The process can be applied to granules with the goal of maintaining or increasing them, or to activated sludge with the aim of creating stable granules (Areskoug et al., 2024). In the first phase filling occurs, which is done through a sludge bed (Royal HaskoningDHV, n.d.). Simultaneously decantation takes place, where the water that is filled in the bottom pushes out water and particles at the top of the reactor and thus creates a plug flow. This phase is called the fill and draw phase. The following phase is the aerobic phase and is when the reactor is aerated. In the aerobic phase, there is a lot of oxygen available, which allows different processes that are presented in figure 2.1 together with the granule. Lastly, the particles are allowed to settle and then the process is repeated (Areskoug et al., 2024). The SBR cycle is on the market as Nereda (Royal HaskoningDHV, n.d.).

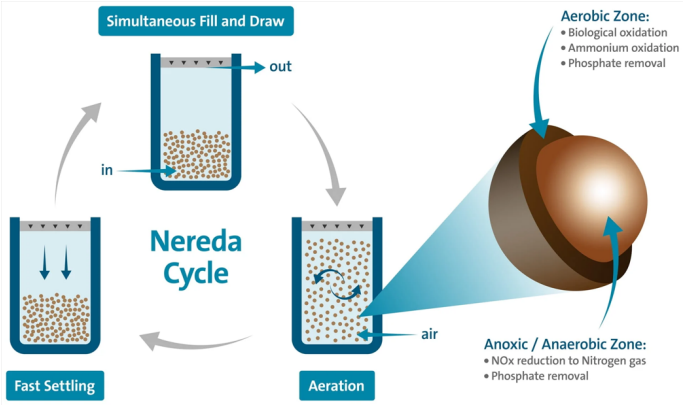


Figure 2.1: The SBR cycle and the processes in the granule. Retrieved from *Nereda technology how it works* (Royal HaskoningDHV, n.d.).

Table 2.1 presents different purification rates and output levels for several Nereda plants, both full-scale and pilot plants. The purification rate refers to the percentage reduction in the concentration out compared to in.

Table 2.1: Purification rate and output levels for several Nereda plants.

Facility	**COD** %	**TN** %	**TP** %	**SS** %	**NH_4-N** mg/L	**NO_3-N** mg/L	**NO_2-N** mg/L	**References**
Epe, NL (Full)	96.9	>94.7	97.2	>98.5	0.1	<4	-	[1, 2]
Garmerwolde, NL (Full)	89.2	86	90.3	96.4	1.1	<5	-	[1, 5, 6]
Lubawa, PL (Full)	97	87.2	95.4	-	0.4	1.6	0.5	[1, 7]
Gansbaai, RSA (Full)	94	90	>80	>98	<1	5–10	-	[1, 2]
Vroomshoop, NL (Full)	92.4	76	89.9	96.8	1.4–3	<2	-	[1, 3]
Österröd, SE (Full)	∼81	∼77	∼85	∼90	0.6	5.9	0.1	[5]
Ryaverket, SE (Pilot)	91.6	83.7	92.3	98.1	0.33	2	0.08	[4]

Notes: For Österröd, data refers to flow-weighted average values [5]. The values from Ryaverket pilot are for period IN 3a, which is with incoming raw wastewater [4].

Reference list:

[1] Kazimierowicz & Dębowski (2022).
[2] Lashkarizadeh (2014).
[3] Pronk et al. (2017).
[4] Areskoug et al. (2024).
[5] de Blois et al. (2022).
[6] Pronk et al. (2015).
[7] Świątczak & Cydzik-Kwiatkowska. (2018).

2.1.2 Activated Sludge

Activated sludge (AS) is the most widely used biological wastewater treatment technology and consists of sludge flocs, which are loose and irregular, consisting of EPS, filamentous bacteria and fast growing heterotrophs (Nancharaiah & Reddy, 2018). AS consists of two main parts, which are aeration basins and sedimentation basins (Nancharaiah & Reddy, 2018). In addition to these, AS systems also incorporate an unaerated (anoxic) zone to enable denitrification (Nancharaiah & Reddy, 2018). In the aeration basin, the wastewater is blended into a mixed liquor, which is a mixture of wastewater and biomass (Sustarsic, 2009). After aeration, the water is transferred to a sedimentation basin, where particles and sludge are allowed to fall to the bottom, which, after sedimentation, are scraped while the water near the surface is allowed to flow out (Sustarsic, 2009). Furthermore, some of the scraped sludge is reused and pumped back to the aeration basin to maintain the amount of microorganisms (Sustarsic, 2009). The remaining fraction is removed from the system to control the sludge age, which is the average time that the microorganisms remain in the system (Sustarsic, 2009). The particles on the surface are called scum and are also removed for further treatment (Sustarsic, 2009).

The aeration enables nitrification but also decomposition of organic material which, among other things, is converted to the energy form adenosine triphosphate (ATP) (Nancharaiah & Sarvajith, 2019). ATP is used by microorganisms to form flocs, which then develop into activated sludge and is important for biological phosphorus purification (Seviour & Blackall, 1999). The activated sludge process removes organic matter, nitrogen and phosphorus, but different tanks with different environments are required as flocs are usually completely aerobic and lack anaerobic and

anoxic parts (Nancharaiah & Reddy, 2018). Their sedimentation rate varies but is usually below 5 m/h (Kazimierowicz & Dębowski, 2022). This results in requiring several sedimentation tanks to separate the sludge (Kassim et al., 2022). This is because at high flows the flocs do not have time to settle sufficiently before the water leaves the basin, which can result in flocs following the outgoing water (Kassim et al., 2022).

Moreover, the sludge flocs are porous and often less than 0.2 mm in size (Nancharaiah & Reddy, 2018). In addition to these properties, flocs are sensitive to the composition of the wastewater (Seviour & Blackall, 1999). However, flocs are good at hydrolysing particles due to their high specific surface area (de Blois et al., 2022). Furthermore, activated sludge produces more excess sludge than AGS (Sarma et al., 2017), and the technology is less energy efficient, using between 20-50% more energy (Nancharaiah & Sarvajith, 2019). Since multiple tanks are needed AS also requires more space than AGS, around 25-75 % more (Nancharaiah & Sarvajith, 2019).

2.2 Granule Formation

Microbial granules have different internal zones due to transport diffusion leading to concentration gradients (Nancharaiah & Sarvajith, 2019). The outer part of the granule is aerobic, meaning there is oxygen, and is where nitrification occurs (Nancharaiah & Sarvajith, 2019). Further in, there is only access to bound oxygen, i.e. an anoxic environment (Nancharaiah & Sarvajith, 2019). At the core of the granule, the environment can be anaerobic, which favours slow growing organisms such as PAO and GAO (Nancharaiah & Sarvajith, 2019). The different environments in the granule enable the removal of phosphorus, nitrogen and organic carbon to take place in the same tank and at the same time. Furthermore, the different environments of the granule can enable simultaneous nitrification and denitrification (SND) (Nancharaiah & Sarvajith, 2019). However, this requires that the access to carbon sources is sufficient and that the granules are large enough so that different zones are formed.

Granules are created by cells coming into contact and attaching to each other through bonds (Lashkarizadeh, 2015). The bacteria then form extracellular polymeric substances (EPS), which act as a glue and further strengthen the composition (Lashkarizadeh, 2015). The granules grow larger and more compact as more bacteria attach and are shaped by hydraulic forces (Nancharaiah & Reddy, 2018). They consist of different layers with different microorganisms for instance, bacteria, fungi, protozoa, and diatoms, and consist of lysed and dead cells in the core (Khan et al., 2013). The aerobic granule sinks faster than activated sludge (de Kreuk et al., 2005). The creation of granules enables resistance against chemical toxicity and inhibition (Khan et al., 2013).

On the other hand, there are many challenges with granule formation, where several parameters can affect growth and stability. A major problem is that the granules can disintegrate due to loss of stability (Li et al., 2014), which can cause loss of biomass (Sanchez-Sanchez et al., 2023). An important factor for the stability of the granules

is shear force, where high shear force is beneficial as it improves stability and growth by promoting the formation of EPS (Khan et al., 2013). Another parameter that is also important is how much food the microorganisms have access to, where a higher food to microorganism ratio results in larger and more stable granules and faster granulation (Khan et al., 2013).

There are several different studies on AGS and the time required to achieve stable granulation. At the full scale AGS plant in Garmerwolde in the Netherlands, it took around 5 months to form stable granules that could be maintained through the Nereda process (Pronk et al., 2015). In Österröd, which was Sweden's first full scale AGS plant, it took approximately 3 months to reach full treatment capacity when inoculated with mature granules, while the reactor inoculated with activated sludge was restarted with mature granules after 9 months because it took too long to achieve stable granulation (de Blois et al., 2022). In the pilot plant at Ryaverket, the reactor was inoculated with mature granules and stable granulation was achieved after approximately a week (Areskoug et al., 2024).

2.2.1 EPS Matrix

The EPS matrix consists of biopolymers secreted by microorganisms (Nancharaiah & Sarvajith, 2019), and is like a biological glue that binds microbial cells together in dense, spherical aggregates, i.e. granules (Khan et al., 2013). The main components are proteins and polysaccharides, but lipids and nucleic acids also occur in the matrix (Kazimierowicz & Dębowski, 2022). For granules, there are mainly two important EPS types, which are alginate like exopolymers (ALE) and granulans (Nancharaiah & Sarvajith, 2019). The EPS matrix is dense, which results in limited diffusion of oxygen into the granule, creating the different layers of the granule (Nancharaiah & Reddy, 2018). Furthermore, the matrix also provides protection against environmental disturbances and toxic compounds (Nancharaiah & Reddy, 2018).

2.2.2 Trigger Forces

To ensure that stable granules are created, the AGS reactors can be subjected to trigger forces. Trigger forces are factors that induce and initiate granulation by changing the phenotype, i.e., physiological traits and behaviours of microorganisms (Nancharaiah & Sarvajith, 2019).

2.2.2.1 Feast and Famine

The bacteria in the water can be subjected to a process, called feast and famine. During the feast period, the bacteria receive a lot of nutrients from organic matter, which the bacteria store internally (Nancharaiah & Sarvajith, 2019). At this stage, electron donors are abundant while electron acceptors such as oxygen and nitrate are absent (Nancharaiah & Sarvajith, 2019). This period occurs in the feeding phase of the SBR cycle and is often called anaerobic filling, since the wastewater is fed into the reactor from the bottom through a sludge bed, as in a plug flow (de Blois

et al., 2022). This causes a concentration gradient, which favours granulation, as nutrients diffuse more easily into the granule (de Blois et al., 2022). Furthermore, organic material is absorbed by microorganisms because oxygen is not available. The organisms store organic matter internally as polymers, often in the form of polyhydroxyalkanoates (PHA) (de Blois et al., 2022). The anaerobic filling also favours slow-growing organisms such as GAO and PAO (de Blois et al., 2022).

In the famine period, the bacteria are starved, which makes them use the stored energy to convert carbon, which occurs during the aeration phase (de Blois et al., 2022). In this stage, no electron donor is supplied while electron acceptors are readily available. Aeration is a central part of the AGS cycle and generates hydrodynamic shear forces through rising air bubbles, which stimulates EPS production (Nancharaiah & Reddy, 2018). These shear forces also contribute to shaping the granules into dense and round structures. By adding oxygen, the dissolved oxygen (DO) increases, which is necessary for the biological processes that purify wastewater (Seviour & Blackall, 1999). The amount of oxygen added can vary, but higher levels result in higher shear forces (Show et al., 2012) and usually concentrations around 1.5-3 mg/L are implemented (de Blois et al., 2022). In addition, there are different strategies for aeration, where a common one is intermittent aeration, that is, short aeration pulses. These pulses create agitation and facilitate the contact of granules and sludge with nutrients. Furthermore, intermittent aeration has been shown to improve nitrogen removal by up to 10 % compared to continuous aeration (de Blois et al., 2022). In addition, intermittent aeration favours ammonium oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB), enabling partial nitrification and denitrification (PND) (Nancharaiah & Sarvajith, 2019). Furthermore, slow-growing bacteria such as GAO and PAO are favoured during the famine phase, which are important for phosphorus purification, denitrification, and ensuring stable granules (Nancharaiah & Sarvajith, 2019). The reactors are exposed to the feast and famine process during each cycle.

2.2.2.2 pH

pH is also a trigger force and affects the granule formation where low pH favours fungi granules (Khan et al., 2013) resulting in fluffier granules with weaker structure (Lashkarizadeh, 2015). A pH range between 7 and 8 is considered optimal for bacteria dominant granules that are compact and stable (Khan et al., 2013). Higher pH around 8.5-9 can cause disintegration and inhibit the granulation process (Hailei et al., 2006). Nitrogen removal processes are pH dependent. Nitrification, which involves the oxidation of ammonium to nitrate, consumes alkalinity and thereby lowers pH (Seviour et al., 1999; Cheremisinoff, 1997). Denitrification, on the other hand, restores alkalinity and raises pH (Cheremisinoff, 1997).

2.2.3 Selection Forces

Another way to ensure stable granules is through selection forces, which means that a selection is made to maintain stable and good granules (Nancharaiah & Sarvajith, 2019). This can be done in many ways, but some examples are by varying the

sedimentation time, taking out sludge, controlling the temperature, and controlling the DO concentration.

2.2.3.1 Sedimentation Time

One way to select granules is through their sinking properties. Compact and larger granules sink quickly and by applying a short sedimentation time, granules with poorer sinking properties are removed (Nancharaiah & Sarvajith, 2019). A sedimentation time of between 2 and 10 minutes is often applied to ensure a proper selection (Nancharaiah & Sarvajith, 2019). Moreover, it also favours slow growing organisms such as PAO and GAO (Nancharaiah & Sarvajith, 2019).

2.2.3.2 Sludge Removal

In the AGS cycle, a withdrawal is made in the decantation phase where wastewater is removed together with particles that have not settled, but other sludge withdrawals can also be made, which further select fast-sinking granules by removing lighter flocculent sludge (de Blois et al., 2022). By removing particles with poorer sinking properties, the growth of the granules is accelerated by making more substrate available to them (Khan et al., 2013). In addition, the sludge age can be controlled through withdrawal (de Blois et al., 2022). Sludge removal can be made in different phases of the SBR cycle, where withdrawals in the aeration phase result in a reduction of the sludge content and removal of larger granules that can cause instability (de Blois et al., 2022). Newly formed granules, also called protogranules, are very small and have poor sinking properties (van Djik et al., 2022). Although these granules may exhibit the correct morphology, they risk being selected out during the sedimentation phase, which can inhibit further granulation development, as the starting points disappear (van Djik et al., 2022). In addition, sludge removal can lead to the removal of slow-growing organisms that are necessary for development (Ekholm et al., 2022).

2.2.3.3 Temperature

The optimal temperature for granulation is around 20-30 °C (Bengtsson et al., 2018). At temperatures below 10 °C, the activity of microorganisms is significantly lower, thus the granulation process is more difficult and slower (de Blois et al., 2022). At these low temperatures, it is difficult to form granules from inoculation of flocculated sludge, in addition filamentous growths increase (de Blois et al., 2022). Furthermore, the sinking properties of the granules deteriorate as the viscosity of the water increases, thus making the selection more difficult to carry out (de Blois et al., 2022).

2.2.3.4 DO

By controlling the dissolved oxygen concentration, filamentous growths can be prevented (Khan et al., 2013), and selection can be made for slow-growing bacteria (Bengtsson et al., 2018). A lower DO creates a larger anoxic zone in the granule, limiting the growth of fast growing aerobic heterotrophs at the granule surface (Bengtsson et al., 2018), which favors slow growing organisms, such as dPAO (Pronk

et al., 2015). Previous studies show variations in the DO level required for optimal granulation, but 2-3 mg/L is often considered optimal (Lashkarizadeh, 2015). In the pilot study at Ryaverket, the DO concentration needed to be increased from 2 to 3 mg/L to maintain stable nitrification during cold periods (Areskoug et al., 2024). It is important to note that the effect of DO is often linked to other factors such as aeration, which makes it difficult to isolate the role of DO (van Dijk et al., 2022).

2.2.3.5 Selective Feeding

Selective feeding is also part of the SBR cycle and is done by feeding in a plug flow where the granules at the bottom are exposed to the most substrate as the filling takes place there (van Djik et al., 2022). The smaller granules thus have less contact time and therefore take up less substrate, resulting in further development of the granules with good sinking properties (van Djik et al., 2022).

2.3 Nitrogen Removal

Nitrogen in wastewater can be transformed and removed by a variety of biological and chemical processes. This chapter describes the most important processes for nitrogen removal, including nitrification, denitrification, simultaneous nitrification and denitrification (SND), partial nitrification and denitrification (PND) and alternating nitrification and denitrification (AND). The anaerobic ammonium oxidation process (Anammox) is also explained.

2.3.1 Nitrification and Denitrification

Nitrification is a biological purification process consisting of two steps and is carried out by autotrophic bacteria. In the first step, ammonium is converted to nitrite, which is done by AOB (Nancharaiah & Reddy, 2018). Nitrification occurs in the aeration phase of the AGS cycle (de Blois et al., 2022). In an AGS system, there is a lack of oxygen in the reactor after the aeration, resulting in nitrite oxidizing bacteria (NOB) oxidizing nitrite to nitrate, also using oxygen as the electron acceptor (Nancharaiah & Reddy, 2018). The DO can thus be controlled to be low enough to support simultaneous nitrification and denitrification. Both processes requires oxygen and inorganic carbon, where carbon deficiency can result in incomplete nitrification (Nancharaiah & Reddy, 2018). Some nitrifying bacteria, known as complete ammonia oxidizers (comammox), are capable of carrying out both steps, oxidizing ammonium all the way to nitrate (de Blois et al., 2022). The nitrogen removal process is shown in equations 2.1 and 2.2.

$$\text{Step 1 (AOB):} \quad 2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \tag{2.1}$$

$$\text{Step 2 (NOB):} \quad 2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{2.2}$$

The lack of dissolved oxygen, but the availability of bound oxygen, creates an anoxic environment, located further towards the centre of the granule (Nancharaiah &

Sarvajith, 2019). This environment enables denitrification, where organic material serves as the electron donor (Henze et al., 2001). The denitrification process occurs in four steps. In the first step, nitrate is reduced to nitrite by denitrifying bacteria, which use nitrate as their electron acceptor (Henze et al., 2001). Thereafter, nitrate is converted to nitrogen gas (Henze et al., 2001). The process proceeds through a stepwise reduction chain in which nitrite is converted to nitric oxide, further reduced to nitrous oxide, and finally to nitrogen gas (Henze et al., 2001). Denitrification is performed by a variety of bacteria, including denitrifying phosphorus-accumulating organisms (dPAO) and denitrifying glycogen-accumulating organisms (dGAO) (Bengtsson et al., 2018). Denitrification can occur both in the anoxic zones of the granules and in flocculated activated sludge, although the process is generally less stable and efficient in flocculated structures (Dasgupta et al., 2020). Equations 2.3-2.6 show the different steps in denitrification.

$$\text{Step 1:} \quad NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \tag{2.3}$$

$$\text{Step 2:} \quad NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O \tag{2.4}$$

$$\text{Step 3:} \quad 2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O \tag{2.5}$$

$$\text{Step 4:} \quad N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O \tag{2.6}$$

2.3.2 Simultaneous Nitrification and Denitrification

SND is a process where nitrification and denitrification occur simultaneously, which can occur in AGS due to the different layers of the granule (de Blois et al., 2022). In the outer aerobic layer of the granule there are AOB and NOB, which convert ammonium to nitrate (de Blois et al., 2022). Further towards the core, where it is anoxic, reduction of nitrate to nitrogen gas occurs with the help of denitrifying bacteria (de Blois et al., 2022). In order for the different layers with different environments and SND to occur, the granules need to be mature and larger than 1 mm (Nancharaiah & Reddy, 2018). In addition, the DO content cannot be too high as the anoxic zone can be destroyed (Nancharaiah & Reddy, 2018).

2.3.3 Alternating Nitrification and Denitrification

Alternating nitrification and denitrification is a method of removing nitrogen and is applied through intermittent aeration where nitrification occurs during aeration and denitrification occurs after aeration in the anoxic environment (Bengtsson et al., 2018). A limitation of the method is that there may be a lack of internally stored carbon at the end of the cycle, which is necessary for denitrification (Bengtsson et al., 2018). Additionally, AND can reduce nitrous oxide emissions compared to SND and the method can reduce the nitrogen concentration by 10 % more than SND (Bengtsson et al., 2018).

2.3.4 Partial Nitrification and Denitrification

In PND not all steps of the nitrification process occur, instead ammonium is oxidized to nitrite which is then directly reduced to nitrogen gas by heterotrophic bacteria

(Nancharaiah & Reddy, 2018). This process occurs when there is a shortage of NOB, which can happen if the DO concentration is too low (Nancharaiah & Reddy, 2018). PND requires approximately 25 % less oxygen and 40 % less carbon compared to the classic nitrification and denitrification (Nancharaiah & Reddy, 2018).

2.3.5 Anammox

Ammonium can also be directly oxidized to nitrogen gas through a process called anaerobic ammonium oxidation (Anammox), which is an autotrophic process, meaning that no organic carbon is required (Nancharaiah & Sarvajith, 2019). Anammox is possible due to the different layers of the granules and occurs in the core of the granule where the zone is anoxic. The process begins with some ammonium being oxidized to nitrite in the outer oxygen rich layer of the granule (Nancharaiah & Sarvajith, 2019). The ammonium and nitrite then diffuse towards the anoxic core of the granule, where the anammox reaction occurs. Ammonium acts as an electron donor, while nitrite acts as an electron acceptor, which enables direct conversion to nitrogen gas (Nancharaiah & Sarvajith, 2019).

2.4 Phosphorus Removal

Phosphorus removal from wastewater can be achieved by chemical or biological processes, or a combination of these. In AGS it is often achieved through biological phosphorus purification (bio-P), also called EBPR (Enhanced biological phosphorus removal). Biological phosphorus purification is carried out as follows. First, an uptake of easily degradable organic material, such as VFA, is carried out by PAO bacteria (Nancharaiah & Sarvajith, 2019). The uptake occurs during the anaerobic phase (feast phase) and the organic material is stored as polyhydroxyalkanoates (PHA/PHB), which requires energy (Nancharaiah & Sarvajith, 2019). The energy for the storage is created by the breakdown of polyphosphate which has been stored internally in the bacteria, where the breakdown results in a release of orthophosphate (Bengtsson et al., 2018). In the aerobic phase (famine phase), PAO can utilize its stored organic material, i.e. PHA or PHB, to take up orthophosphate and store it as polyphosphate, where more orthophosphate is taken up than the amount that was previously released (de Blois et al., 2022). The different layers of the granule, specifically the anoxic one, also enable phosphorus uptake by dPAO that can use nitrite or nitrate as an electron acceptor for phosphorus uptake (Nancharaiah & Sarvajith, 2019). This process is more energy efficient as no oxygen is used and carbon utilization is more efficient (Nancharaiah & Sarvajith, 2019).

The process can be complicated when using pre-sedimented wastewater as the availability of readily degradable organic material may be too low (de Blois et al., 2022). A too low temperature (< 10 °C) can also cause a lack of available organic material. In addition, PAO competes with GAO bacteria for the organic material, where GAO does not contribute to phosphorus removal (de Blois et al., 2022). Furthermore, there is competition with denitrifying bacteria as well, especially if nitrate remains from the previous cycle (Seviour & Blackall, 1999).

2.5 Organic Carbon Removal

Organic carbon removal occurs through a combination of biological processes, such as hydrolysis, fermentation and aerobic decomposition, anaerobic decomposition and physical processes such as adsorption, sedimentation and decantation. In addition, the previously described processes for nitrogen and phosphorus removal also contribute to the removal through uptake.

2.5.1 Aerobic and Anaerobic Decomposition

Aerobic decomposition involves the oxidation of organic matter by heterotrophic bacteria and oxygen in the aerobic layer of the granule, a process called respiration (Ramesh, 2020). This process creates energy, which is used for microbial growth and as energy reserves (Seviour & Blackall, 1999). Another important part of aerobic decomposition is ammonification, where heterotrophic microorganisms break down organic material that contains nitrogen, and convert it to ammonium (Cheremisinoff, 1997).

Anaerobic degradation occurs through different steps. First, hydrolysis occurs where particles are broken down by enzymes from bacteria, resulting in dissolved molecules that can be absorbed (de Blois et al., 2022). Granules have a lower ability to hydrolyse than activated sludge flocs due to their compact structure, which results in a smaller specific surface area (de Blois et al., 2022). After the hydrolysis, fermentation occurs, which is in the anaerobic or anoxic zone in the granule (Nancharaiah & Sarvajith, 2019). The final step is acidogenesis where fatty acids and alcohols form acetate, among other things (Kazimierowicz & Dębowski, 2022).

2.5.2 Adsorption

The organic material is also removed through a physical process where dissolved organic material sticks to the surface of the granule, also called adsorption (Bengtsson et al., 2018). This occurs because the EPS matrix captures particles and binds them (Khan et al., 2013), which often occurs in the outer layers of the granule (Bengtsson et al., 2018). For activated sludge flocs, adsorption can occur further in towards the center because the flocs are less dense (Bengtsson et al., 2018).

2.5.3 Sedimentation and Decantation

Another physical purification process is sedimentation and decantation, which is part of the AGS cycle. By applying a short sedimentation phase, only the heavy granules have time to settle (de Blois et al., 2022). Organic material that has not been absorbed by the granules settles very slowly and is thus removed in the decantation phase. The wastewater can also undergo pre-sedimentation, in which suspended solids and a portion of the organic carbon are removed by settling (de Blois et al., 2022).

2.6 Wastewater Composition

An important component of the perfomance and the process design of AGS is the composition of wastewater. The organic matter content and available carbon are particularly important, where high concentrations of available carbon favour granulation (de Blois et al., 2022). Raw wastewater and pre-sedimented wastewater differ in composition. During pre-sedimentation, a large proportion of the organic material is separated, resulting in lower substrate levels in the pre-sedimented water (de Blois et al., 2022). This has been observed in practice at both Österröd (de Blois et al., 2022) and Ryaverket's AGS pilot (Areskoug et al., 2024). In addition the suspended particles amount, particle size, and the presence of substances such as fat can also differ (de Blois et al., 2022; Areskoug et al., 2024). Furthermore, carbon, often in the form of volatile fatty acids (VFAs), is used for nitrogen and phosphorus removal. However, the concentration of VFAs is often insufficient in WWTPs as well as in Swedish wastewater (de Blois et al., 2022). At the Ryaverket pilot AGS plant, VFAs levels in the incoming raw wastewater were so low that they could not be detected, that is, below 10 mg/L (Areskoug et al., 2024).

Synthetic wastewater often consists of water and carbon sources such as acetate or glucose and ethanol (de Blois et al., 2022). The synthetic water enables direct uptake of carbon, while in the raw wastewater, particles must first be hydrolysed (Ekholm et al., 2022). Furthermore, the raw wastewater results in smaller and more irregular granules compared to synthetic wastewater (Bengtsson et al., 2018). The studies on AGS with synthetic water have highlighted that SND is a major advantage of the technology, however, SND is not as comprehensive in real wastewater (de Blois et al., 2022). In real wastewater, changes occur constantly, such as temperature or flow, thus the results of the studies on synthetic water can be difficult to transfer to real wastewater and to full scale plants. Furthermore, the bacteria need a carbon source to be able to create granules, which is often controlled and monitored closely in synthetic water, while it can be lacking in wastewater (de Blois et al., 2022).

3
Methods

3.1 Pilot design

The AGS cycle implemented was 6 hours, and the times for sedimentation, feeding and aeration were changed several times during the course of the project, but was started with 30 min of sedimentation, 35 min of feeding and the remaining time of aeration. The project was carried out in three reactors with a volume of 22.5 L each. The reactors were connected to two buffer tanks of approximately 200 L, where one contained pre-sedimented wastewater and the other raw wastewater. The buffer tanks were supplied via hose systems connected to pumps placed in Ryaverket's raw and pre-sedimented wastewater, respectively. The pumps had a maximum capacity of 13.7 m^3/min, which was used. Figure 3.1 shows pictures of the pilot.

Figure 3.1: Pictures of how the pilot is constructed showing the hose system, arduino box, reactors and buffer tanks.

Each reactor was equipped with three outlets, of which the lowest one was not used. The upper outlet, at 160 cm, was used for decantation. Figure 3.2 shows a process diagram of the pilot plant where the sampling points and outlets, amongst other things, are marked. The effluent samples were taken from the top outlet from each reactor. At the middle of the reactors, at a volume of 17 L, there were probes for continuous measurement of pH, temperature and DO. Data collection was carried out using an Arduino based system that was connected to the probes, the aeration

system and and the pumps feeding the reactors, allowing simultaneous monitoring and control of the process. The middle outlet was used for taking biomass samples during the aeration phase.

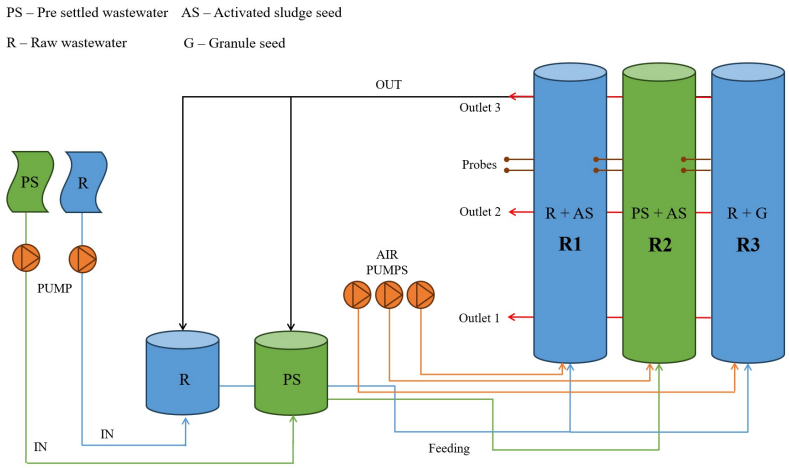


Figure 3.2: Process diagram of the pilot plant.

3.1.1 Changes and Disruptions

Table 3.1 summarizes changes and disruptions during the course of the project. The sampling of incoming wastewater was adjusted on several occasions during the project. During the first three weeks, sampling was carried out through a valve arrangement that had two connections, one connected to the bottom of the reactor and one to the incoming wastewater. This approach proved to be unsatisfactory, as biomass risked being carried into the samples and made it difficult to obtain representative samples. The sampling methodology was changed on 27 February to sample directly from the buffer tanks, which improved the representativeness of the incoming water. On 11 March, the sampling valves on the inlet lines were replaced with T-valves to simplify and standardize sampling.

During a period, biomass was removed from the reactors during aeration and was done to maintain the sludge age in the reactors. This continued for about 4 weeks, but was interrupted when a trend was observed where the biomass concentration gradually decreased over time. The sludge withdrawal varied in volume between different occasions, but was always carried out during aeration and so that the volume in the reactors decreased to 20.5 L. It was also noted that significant amounts of biomass had accumulated on the walls of the reactor with raw wastewater and activated sludge, which meant that the measured biomass may underestimated the actual amount. After around 6 weeks, the concentration of DO was observed to be too high and was lowered from 6 mg/L to around 3 mg/L using the Arduino based control system. The aeration strategy was then modified successively and the last change was a control system that regulated the DO of 3 mg/L through intermittent aeration. This was done by adding oxygen when the DO dropped below 2 mg/L and re-agglutinating when DO levels exceeded 3 mg/L. There was also an initial aeration of 3 minutes that ensured sufficient mixing.

Table 3.1: Timeline of disruptions and operational changes.

Date	Type of event	Description
26-01-2026	Start	R1 and R2 were started. DO set to 10 L/min.
27-01-2026	Adjustment	DO reduced to 6 L/min.
04-02-2026	Start	R3 was started with granules.
11-02-2026	Disturbance	Air entered into reactors and mixed the biomass, may affected the effluent samples.
18-02-2026	Disturbance	pH calibration error in R1 and R2, may have affected previously recorded data.
27-02-2026	Adjustment	Adjustment of conversion rate (40%), feeding time (60 min), sedimentation time (5 min) and aeration flows (R1: 200, R2: 192, R3:189 mL/min). Sampling moved to buffer tank. Cycle was stopped. Mixed liquid was removed until below the probes. Liquid loss occurred in R2 due to handling difficulties.
03-03-2026	Adjustment / Sludge extraction	Intermittent aeration was implemented (1.5 min on/off). Sludge extraction from R3 ($\sim$2.5 L). 3.5 L of sludge was removed from all reactors.
06-03-2026	Adjustment / Sludge extraction	New aeration cycle (45 s on, 2 min off). Approximately 6 L sludge removed from each reactor.
09-03-2026	Adjustment	DO-regulation (3 mg/L). Automatic switching between aeration and mixing.
11-03-2026	Adjustment / Sludge extraction	2 L removed per reactor. A lot of biomass observed on the walls of R1. Change valves to T-valves for influent sampling.
13-03-2026	Sludge extraction	Sludge removal.
16-03-2026	Sludge extraction / Sampling	2 L sludge removed from all reactors. Sampling for influent was performed in influent lines and buffer tank.
18-03-2026	Sludge extraction	Withdraw 2 L from each reactor.
23-03-2026	Sludge extraction	Removed 5 L from R1, 3.5 L from R2 and 3.75 L from R3.
25-03-2026	Disturbance / Sludge extraction	Around 2 L of sludge removed from reactors. Pump for pre-sedimented wastewater broke. Replaced pump which caused a short circuit $\rightarrow$ no feeding to R2 overnight.
26-03-2026	Adjustment	Pump replaced with a new one.
27-03-2026	Disturbance / Sludge extraction	2 L sludge removed from reactors. Pumps were found to be off for 36 hours and were restarted.

3.2 Analyses

To evaluate the treatment performance and biomass development in the AGS reactors, several analyses were carried out. These aimed to characterize both the biomass and the composition of the wastewater over time. The biomass was evaluated by measurements of suspended matter and sedimentation capacity, as well as by microscopic analysis to follow the development of the granules. The activity analysis batch test was carried out to assess the capacity of the biomass regarding nitrification, denitrification and biological phosphorus removal. The composition and degree of wastewater treatment were analysed by ion chromatography and measurement of total organic carbon. The respective analysis methods are described in the following chapters.

3.2.1 MLSS and VSS

The biomass concentration was retrieved by performing a mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) analysis, which was done by taking samples for influent, effluent and in the reactor during aeration. The analysis was done for duplicates of each sample and a duplicate of ultra pure water. Then a certain volume of the samples was filtered through 0.2 µm filters with the help of vacuum suction which helps to capture suspended particles. All filters were dried in an oven at 105 °C for 2 hours and then the filters were weighed again to obtain the total amount of suspended solids (MLSS). To determine the organic content, i.e. VSS, the filters were annealed in an oven at 550 °C, where the organic material was burned (Leitrim County Council, 2009). After cooling, the filters were weighed again, and the weight loss corresponds to the organic part of the sludge. Where MLSS was derived through equation 3.1 and VSS through equation 3.2.

$$MLSS = \frac{m_{mlss} - m_{filter}}{V_{sample}} \tag{3.1}$$

where:

- m_{mlss} = mass of the filter after oven drying (g)
- m_{filter} = weight of the filter (g)
- V_{sample} = volume of added sample (L)

$$VSS = \frac{m_{mlss} - (m_{vss} + m_{correction})}{V_{sample}} \tag{3.2}$$

where:

- m_{mlss} = mass of the filter after oven drying (g)
- m_{vss} = mass of the filter after combustion in a furnace (g)
- $m_{correction}$ = correction factor for the amount of filter burned (g)
- V_{sample} = volume of added sample (L)

3.2.2 SVI

The biomass in each reactor was analysed by taking the sludge volume index (SVI) for 30, 10 and 5 minutes. Each reactor was marked for each L so that the sludge volume could be easily retrieved. The sludge volume together with the reactor volume and the concentration of the biomass were then used to determine the sludge volume index. This was done using equation 3.3.

$$SVI = \frac{\frac{V_{sludge}}{V_{reactor}} \times 1000}{VSS} \tag{3.3}$$

where:

- V_{sludge} = Sludge volume after sedimentation (L)
- $V_{reactor}$ = Reactor volume (L)
- 1000 = Conversion factor from L/L to mL/L
- VSS = Volatile suspended solids (g/L)

3.2.3 Microscopy

For microscopic analysis of the biomass, samples were taken from the aeration phase in each reactor. A small amount of sludge was then obtained using a pipette and placed on a slide. The sample was then studied under the light microscope (Nikon) at magnifications of 2x and 10x to observe the sludge structure, granule formation, and amount of granules or flocs, as well as filamentous growths. Pictures were taken for documentation, mainly where the presence of granules or signs of granule formation could be identified.

3.2.4 IC

Ion chromatography (IC) (Dionex ICS-900) was used to analyse the ions in the samples. The ion chromatography analysis focused on the anions nitrate, nitrite and phosphate and the cation ammonium. The samples were first filtered through a 0.2 µm PES membrane syringe filter and then diluted. The conductivity of all samples was measured and the highest conductivity was used to dilute all samples so that they fell below the conductivity of 100 µS/cm. The samples were then inserted into the ion chromatography machine in numerical order, with the first and last vial containing ultra-pure water. Each ion was identified based on its retention time, and the concentration is determined by comparing the size of the peaks.

3.2.5 TOC, DOC and TN

Total organic carbon (TOC), dissolved organic carbon (DOC) and total nitrogen (TN) were analysed to quantify the amount of organic carbon with an TOC/total N analyzer (Shimadzu, ASI-V). For DOC, the samples were filtered through a 0.2 µm PES membrane syringe filter. The samples were diluted 10 times with deionized water and then placed in the TOC analyser, where the organic carbon is oxidized to

carbon dioxide (Shetty & Goyal, 2022). The carbon dioxide concentration is then analysed, thus giving the TOC and DOC content in the sample.

3.2.6 COD

The Chemical Oxygen Demand (COD) was only analysed once during the course of the project. The COD analysis was performed for incoming wastewater samples, filtered incoming wastewater, and filtered effluent. For the COD analysis an oxidant was added to the sample, and then heated under controlled conditions to ensure complete oxidation of the organic material. After the reaction, the amount of oxidant consumed was quantified, which was proportional to the COD content of the sample. Data was also collected for incoming wastewater from Ryaverket's wastewater treatment plant for the pre-settled and the raw wastewater.

3.2.7 Batch Test

The batch test was performed to evaluate the potential activity of the biomass in the reactors and to assess its capacity for nitrification, denitrification, and bio-P. The analysis serves as a complement, as it provides additional support for interpreting the results in terms of overall process performance. The analysis was done to see the biomass potential with regard to nitrification, denitrification, and bio-P. The exact amounts of solutions and compounds added in the experiment are presented in Table A.1 in the Appendix.

The batch test was performed on the biomass. Biomass together with wastewater was taken out of each reactor and allowed to settle. The wastewater was then separated from the biomass and the effluent wastewater was taken from Ryaverket's wastewater treatment plant outlet. The biomass was tested in batch tests in both the effluent water from Ryaverket and the reactor's wastewater. As the amount of biomass in the reactors varied, different amounts of mixed wastewater from the aeration phase were taken from each reactor, so that the amount of biomass was approximately the same. The test was performed in a batch, where sludge samples from the treatment process were mixed with water and specific substrates depending on the process to be studied (van Loosdrecht et al., 2016). The setup consisted of flasks that were in a water bath at 20 °C. Half of the flasks were used for the bio-P test, while the remaining half were used to assess the potential for nitrification and denitrification. Prior to the experiment, samples were collected to quantify the biomass in terms of MLSS and VSS.

For the bio-P test a trace solution was added to the flasks. Aeration was then initiated for 1 hour. Thereafter, sodium acetate was added as a carbon source, after which the aeration was switched with nitrogen sparging that was applied for 2 hours. Samples were collected at 1, 15, 30, 45, 60, and 120 minutes during the aeration phase of the experiment. In the batch tests, both the bio-P and nitrogen removal experiments were conducted using the same flasks and the experiment was started at 14:40, with aeration applied until 16:40, after which nitrogen sparging was

initiated. For the nitrification and denitrification potential ammonium was added to the flasks as a nitrogen source, after which aeration was applied for 2 hours, where samples were collected every 30 minutes. Thereafter aeration was switched to nitrogen sparging, and sodium acetate was added as a carbon source. Nitrogen sparging was maintained for 2 hours, during which samples were also collected every 30 minutes.

3.2.8 Temperature, pH and DO

In each reactor, probes were installed approximately in the middle of the reactor. These logged data for temperature, pH and DO and the probes were calibrated every month. The probes were connected to an Arduino system which logged the data in a google sheet. DO was controlled through the Arduino system. The system was programmed so that a constant DO level of 3 mg/L was maintained in each reactor. The system added oxygen through aeration if necessary to maintain the level, otherwise no aeration occurred except for the initial aeration which aimed to mix all the liquid in the reactor and to prevent gas formation and biomass sticking to the walls of the reactor.

4
Results

4.1 Operating Conditions

The results for the operating conditions are presented in the appendix. To provide a more detailed picture of the systems, zoomed-in time intervals are also presented where the variations over a few days and within individual cycles can be studied in more detail. It should be noted that a calibration error likely occurred up until day 40.

The project was started at the end of January during cold temperatures and completed in April, which causes variations in temperature due to seasonal changes. The temperature profile presented in figure A.1 generally shows a similar course in all reactors with a variation of temperature from approximately 6 to 21 °C. In addition to the seasonal variation, the temperature also showed a cyclical variation within each operating cycle and during the day, seen in figure A.2. Furthermore, in figure A.3, it is shown how the DO concentration has varied. The figure shows that the DO concentration was very high and scattered at the beginning of the project and also shows when the DO was changed. In addition, figure A.4 shows how the DO concentration varies over a number of days. Lastly, figure A.5 presents how the pH concentration has varied. At the beginning of the measurement period, R1 has very low and spread pH values. From around day 40, the pH stabilizes and becomes more similar between the reactors. Figure A.6 presents the pH variations in the cycles.

4.2 Biomass Characterization

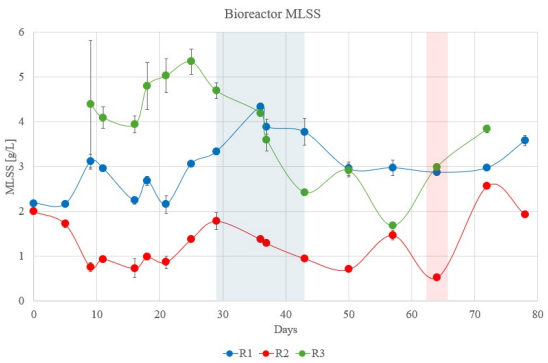
This chapter presents the results of the biomass development in the three AGS reactors during the course of the project. MLSS and VSS are reported to describe the changes in biomass concentration over time, followed by SVI as an indicator of sedimentation capacity and granule formation. Finally, microscopic images are presented to illustrate the development and formation of the granules.

In the results presented in the following chapter, two periods have been marked, where the blue area represents the operational change period. During this period, several modifications were made to the operation, including adjustments to process settings, aeration strategy, and sludge management. The red area represents a temporary shutdown caused by a pump failure, resulting in an interruption of the influent feed for approximately 36 hours. A detailed description of these changes is

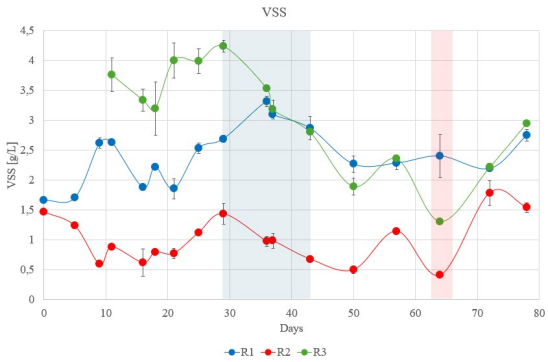
provided in Table 3.1.

4.2.1 MLSS and VSS

Throughout the project, notable differences in biomass concentration were observed between the three reactors. R3 maintained the highest MLSS and VSS concentrations, while R2 exhibited the lowest values for both parameters, as shown in figure 4.1. R1 displayed a slow but steady increase in both MLSS and VSS over the course of the project. The system adjustments, resulted in a decrease in MLSS in all reactors, where especially R3 showed a decrease in both MLSS and VSS. The pump failure caused a decrease in MLSS in R3 and an increase in R2. The corresponding pattern was also observed in the VSS data for the same time period. The results presented are from samples taken from fully mixed reactors during the aeration phase, which is referred to as bioreactor in the figure. The error bars represent the standard deviation calculated from duplicate samples.



(a) MLSS from the bioreactor for reactor 1, 2 and 3 with error bars.

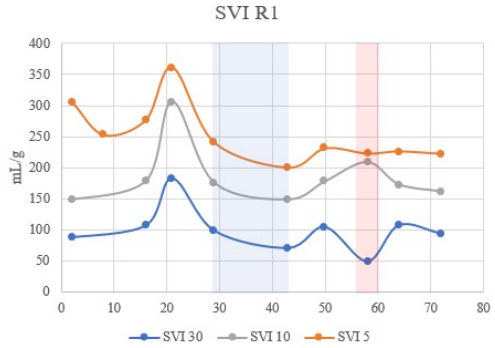


(b) VSS for reactor 1, 2 and 3 with error bars.

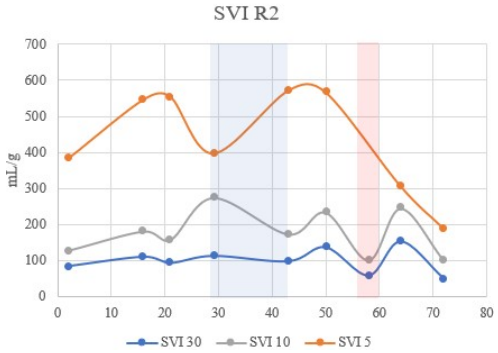
Figure 4.1: MLSS and VSS concentrations in R1, R2 and R3 throughout the project with error bars. The blue area indicates the period of system adjustments and the red area a period of pump failure.

4.2.2 SVI

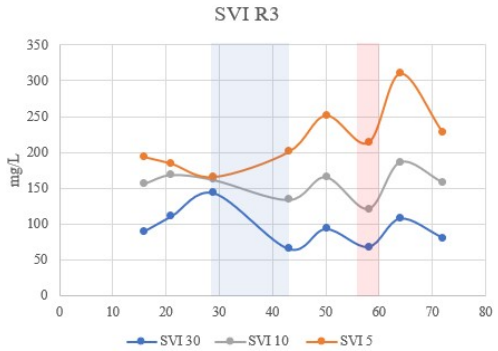
The sludge volume index shows how well the sludge settles and the results show that R2 exhibited the highest SVI values throughout the study period, and a large difference between SVI 5, SVI 10, and SVI 30, seen in figure 4.2. In contrast, R3 showed the lowest and most stable SVI values, with a small difference between SVI 5 and SVI 30. During the operational change period, SVI generally decreased for all reactors. A decrease in SVI was observed in all reactors during the pump failure which may have been caused by no new particles and wastewater being added to the reactors. Overall there was a decreasing trend in SVI for all reactors. It should be noted that biomass was repeatedly observed adhering to the reactor walls, which may have affected the measured values.



(a) The SVI for 5, 10 and 30 min for Reactor 1.



(b) The SVI for 5, 10 and 30 min for Reactor 2.



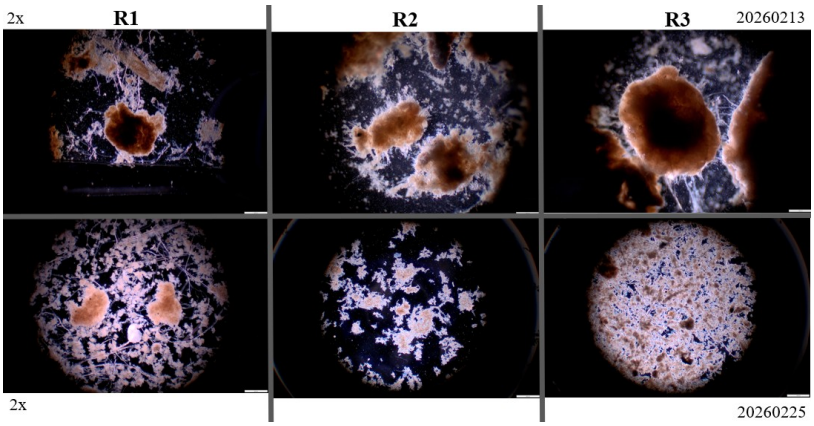
(c) The SVI for 5, 10 and 30 min for Reactor 3.

Figure 4.2: The SVI for 5, 10 and 30 minutes for reactor 1, 2 and 3. The blue area indicates the period of system adjustments and the red area indicates the period of pump failure.

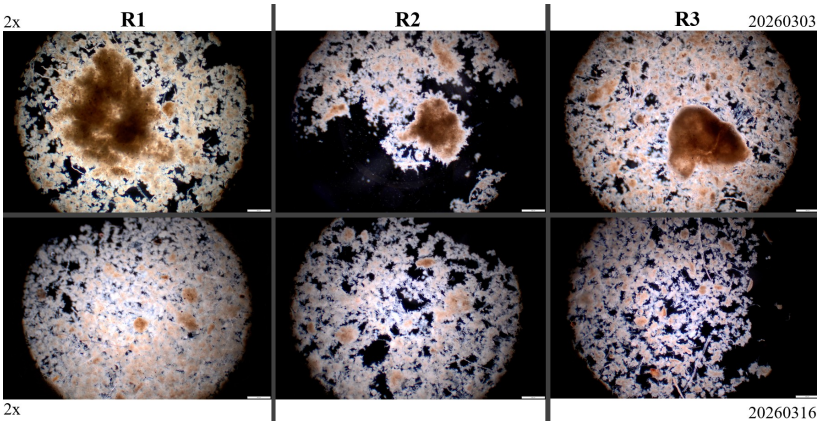
4.2.3 Microscopy

Microscopic analysis was performed to follow granule formation and biomass composition in the reactors over time. Granule formation progressed and after 18 days, well defined structures of varying sizes were observed in all reactors, which is presented in figure 4.3. After 30 days of operation, the granule size had decreased compared to the previous sampling, however, flocs and signs of biomass compaction were detected in R1 and R2, while some granules remained in R3. Larger structures were again observed in all reactors after 37 days of operation, but had decreased in

both size and occurrence by day 50. In R2 a more loosely structured biomass with flocs and filamentous bacteria were detected, while R1 and R3 exhibited rounder and compacter aggregates. Day 0 in the figure corresponds to the start of operation for R1 and R2, while R3 was started on day 9. Furthermore, the samples on day 18 were taken from the bottom of the reactors, while all other samples were taken from the middle of the reactors. All microscopy images were captured at 2x magnification, with a scale bar of 500 µm. The granule in R3 at day 18 corresponds to a size of 2000 µm in length and 1000 µm in width.



(a) Biomass after 18 days and 30 days of operation at 2x magnification.



(b) Biomass after 37 days and 50 days of operation at 2x magnification.

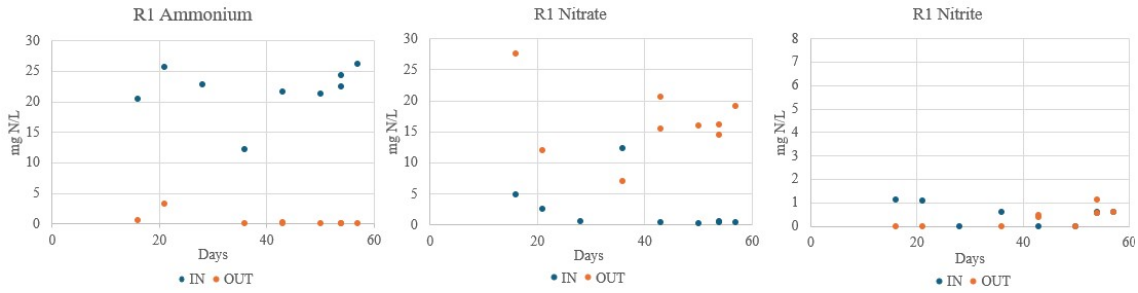
Figure 4.3: Microscopic images of the biomass in the AGS reactors (R1, R2 and R3) for specific dates at 2x magnification.

4.3 Wastewater Treatment and Performance

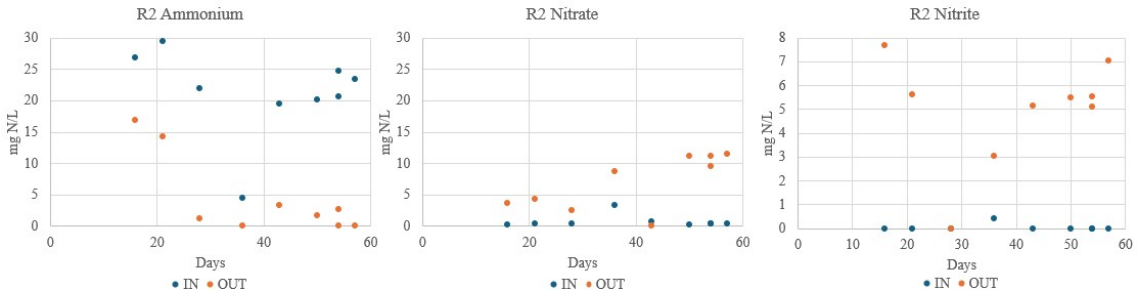
This chapter presents the results from the wastewater characterization and treatment performance evaluation, which has been divided into what is removed, that is, nitrogen, phosphorus and carbon.

4.3.1 Nitrogen

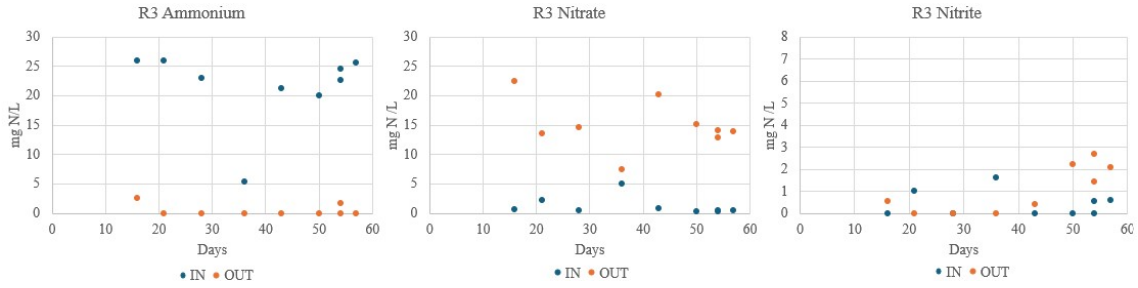
The results for ammonium, nitrate and nitrite are shown for the three reactors in figure 4.4. R1 and R3 showed an ammonium removal of around 98 %, while the corresponding in R2 was approximately 79 %. The average concentration of the effluent was 0.45 mg NH4-N/L for R1, 4.44 mg NH4-N/L for R2 and 0.143 mg NH4-N/L for R3. Regarding nitrate, no removal was observed, instead an increase was demonstrated in the outgoing wastewater for all reactors. The outgoing nitrate levels were highest in R1 and lowest in R2. For nitrite, a removal can be seen in R1 and R3 up to 36 days of operation, after which the concentration increases and is higher in the outgoing samples than in incoming. The concentration of nitrite remains quite stable around 1 mg NO2-N/L in R1, and around 1-2 in R3. In R2 the concentrations vary more and the outgoing concentrations are around 5-8 mg NO2-N/L.



(a) The concentrations for ammonium, nitrate and nitrite for reactor 1.



(b) The concentrations for ammonium, nitrate and nitrite for reactor 2.



(c) The concentrations for ammonium, nitrate and nitrite for reactor 3.

Figure 4.4: Nitrite, nitrate and ammonium concentrations for influent and effluent in the AGS reactors (R1, R2 and R3) during the course of the project.

The total nitrogen concentrations in the effluent and influent for all three reactors are presented in figure A.7. The effluent TN concentrations varied during the project but overall a decrease was observed in all reactors until day 30. On day 30, the TN concentrations for R1 and R3 were below the limit value of 8 mg/L, while R2 exceeded the limit value for the entire study period. After day 30, the purification efficiency decreased for all reactors.

In the batch test, the nitrification and denitrification capacity was analysed. The experiment was started at 14:40, with aeration applied until 16:40, after which nitrogen sparging was initiated. The result presented in 4.5 shows that ammonium decreases over time, which demonstrates that nitrification is present in all reactors however, the removal is not complete. In addition, nitrate was almost fully removed, suggesting that denitrification functioned effectively in all reactors. Nitrite concentrations also decreased, although complete removal was not achieved in any of the reactors. Overall, the lowest concentrations of ammonium and nitrite were observed in R1, while R2 exhibited the highest concentrations, but all reactors showed potential for nitrification and denitrification.

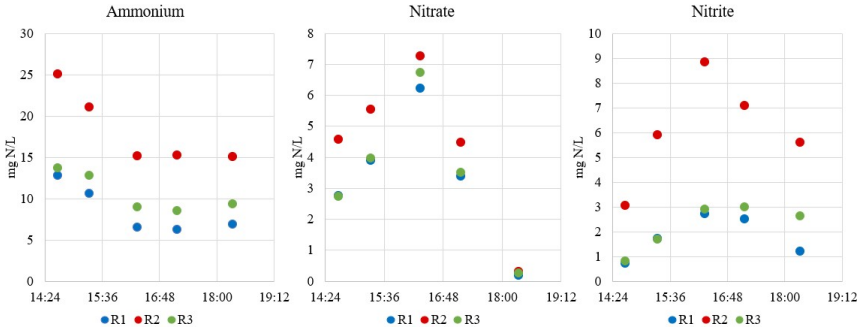


Figure 4.5: Ammonium, nitrate and nitrite concentrations during the batch test for all reactors.

4.3.2 Phosphorus

Phosphate removal was observed to varying degrees in the reactors. In R1 the total removal efficiency over the entire study period was around 27 % where the start of the removal process could be seen after day 36 in figure 4.6. In R2, the removal varied with both reductions and increases, and the total removal efficiency was around 5 %. R3 had a stable removal throughout the period, with a removal efficiency of 42 %. The average concentrations of the outgoing wastewater was approximately 0.96 mg PO4-P/L for R1, 1.16 for R2 and 0.76 for R3. R1 and R2 exceeded the total phosphorus limit value of 0.3 mg/L throughout the entire study period. A similar trend was observed for R3, with the exception of one measurements where the concentration fell below the limit.

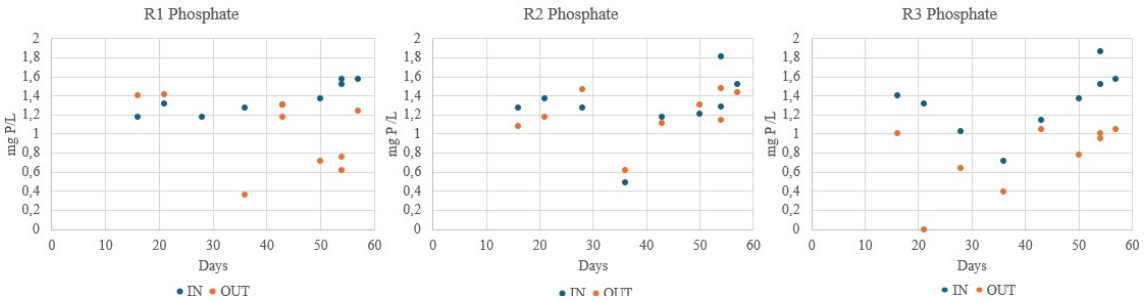


Figure 4.6: The phosphate concentrations for reactor 1, 2 and 3 for influent and effluent samples for the course of the project.

In the batch tests, both the bio-P and nitrogen removal experiments were conducted using the same flasks and acetate levels were also analysed, shown in figure A.8. The experiment was started at 14:40, with aeration applied until 16:40, after which nitrogen sparging was initiated. The results showed that the biomass actively took up acetate. For the phosphate concentrations, shown in figure 4.7, all three reactors show signs of bio-P potential, but none of them show full removal, where R1 and R3 have the most potential and R2 slightly lower.

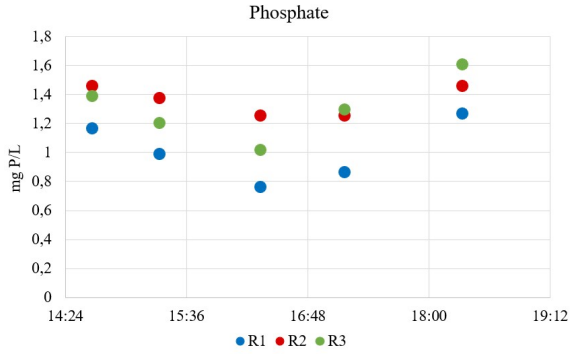


Figure 4.7: The phosphate concentrations in the reactors (R1, R2 and R3) during the batch test.

4.3.3 Carbon

The total organic carbon concentrations are shown in figure 4.8. For all reactors, the effluent TOC concentrations range between 20–40 mg/L. The influent TOC concentrations for R1 and R3 are expected to be very similar, as they are fed simultaneously from the same buffer tank. However, differences are observed, which is unexpected given that they consist of the same raw wastewater. Overall, all three reactors appear to remove the majority of TOC, indicating that the degradation of organic matter functions well across all systems. The DOC results are presented in figure A.9. Similar to the TOC results, the effluent DOC concentrations were comparable between all three reactors. Furthermore, the effluent DOC levels were generally similar to the corresponding effluent TOC concentrations.

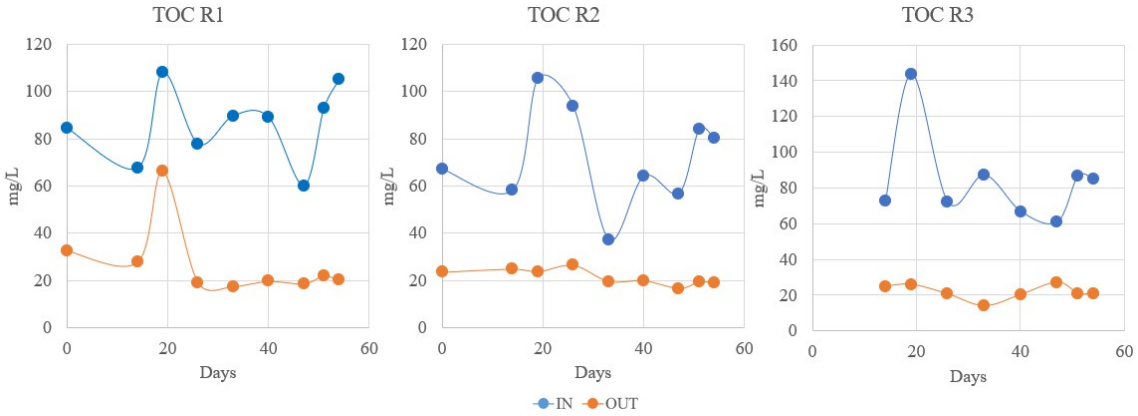


Figure 4.8: TOC concentrations for influent and effluent samples for R1, R2 and R3 for the course of the project.

The chemical oxygen demand analysis carried out after 72 days of operation show that a significant proportion of the organic material is removed in all reactors. The results are shown for samples of unfiltered incoming wastewater, filtered incoming wastewater, and filtered outgoing wastewater which are presented in figure 4.9. The effluent COD levels are similar for all three reactors, while the influent COD is lower for R2 which is expected since it consists of pre-sedimented wastewater. In addition, the influent COD for R1 and R3 shows some unexpected differences, despite both reactors being fed the same raw wastewater. Furthermore, since the analysis is based on filtered outgoing samples, some of the organic material may have already been removed before measurement, which means that the results mainly reflect the dissolved and fine particulate fraction of COD.

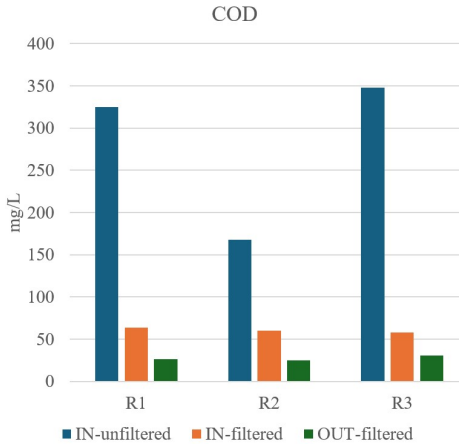


Figure 4.9: The COD for reactor 1, 2 and 3 for filtered and unfiltered samples of influent and filtered effluent.

The incoming COD levels are shown in figure 4.10 and are taken from Ryavaerket wastewater treatment plant's data for unfiltered incoming raw wastewater and incoming pre-settled wastewater. It can be seen that the COD level is higher for the raw wastewater and that the levels have varied, in a cyclical pattern, during the course of the project.

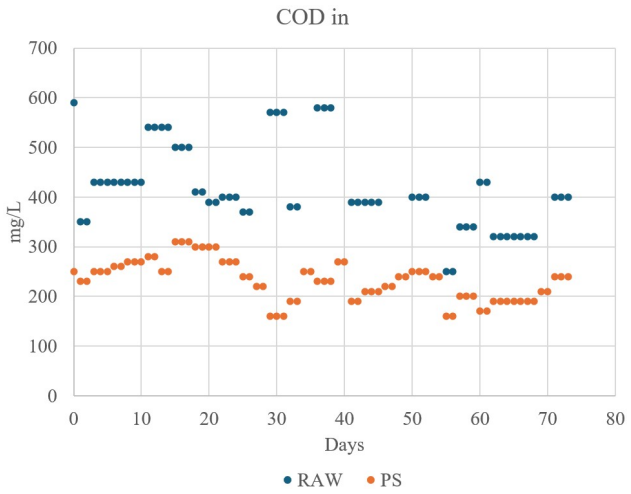


Figure 4.10: The COD for incoming raw wastewater and pre-settled wastewater, retrieved from Ryaverket's data.

5
Discussion

5.1 Nitrogen Removal

The results, figure 4.4, showed a high removal of ammonium in all reactors. The slightly lower removal of ammonium in R2, may be related to differences in wastewater composition and biomass activity. Since pre-sedimentation removes a portion of the organic matter, it may have influenced the biological process performance. Furthermore, despite effective ammonium removal, no removal of nitrate was observed in the reactors. The high nitrate accumulation together with the low ammonium content, indicates that denitrification has been limited or absent as there is no consumption of nitrate. The limited removal of nitrate implies that sufficient anoxic conditions were not established or that there was not enough carbon for denitrification. Furthermore, R2 differs from the other reactors by exhibiting lower nitrate levels but, at the same time, very high nitrite concentrations in the effluent which can be seen in figure 4.4. This indicates an imbalance in the nitrification process, leading to the accumulation of nitrite. All reactors had similar influent ammonium concentrations, meaning differences in ammonium availability cannot explain the differences. In addition, PND can be suspected due to the nitrite accumulation and lower nitrate formation, but it cannot be determined whether a complete nitrogen reduction to nitrogen gas has occurred, as this was not analysed, thus the hypothesis of PND cannot be confirmed. Furthermore, SND was probably limited or absent in R1 and R3, as nitrate accumulation was observed.

The substrate availability also has an impact on nitrogen removal. R1 and R3, which consisted of raw wastewater, generally showed a higher COD load compared to R2, which was fed with pre-sedimented wastewater, which can be seen in figure 4.9 and 4.10. The readily degradable fraction of organic matter is particularly important for denitrification, as it acts as an electron donor for the reduction of nitrate to nitrogen gas (Campos et al., 2019). The lower COD concentration in pre-sedimented wastewater may thus have limited the availability of readily available substrate in R2, which may contributed to the more limited nitrogen removal, figure 4.4. The effluent TOC and COD levels, figure 4.8 and 4.9, are similar in all reactors, which indicates that the decomposition of organic material works well and is relatively independent of the wastewater composition and sludge character. Furthermore, some of the carbon may have been utilized in competing biological processes, such as the growth of heterotrophic bacteria or GAO activity, further limiting the fraction available for denitrification (Ekholm et al., 2024).

5.1.1 Operating Conditions

Previous studies have shown that the optimal oxygen concentration for nitrification and denitrification is around 2–3 mg/L (Lashkarizadeh, 2015). During the course of the study, the oxygen content was adjusted from an initial 6 mg/L to 3 mg/L. In addition, the aeration method was changed from constant aeration to intermittent aeration controlled around a DO of 3 mg/L. Since the system supplied air when the DO concentration fell below 2 mg/L, oxygen free or oxygen deficient periods may have been too short for effective denitrification. This is also supported by the results from the batch test, which showed that the biomass in all reactors (R1, R2 and R3) had the capacity for denitrification. Therefore, the results indicate that the limited denitrification was mainly due to the control of the process, with the high oxygen content likely being the main cause.

The temperature varied between approximately 6 and 16 °C during the study, figure A.1. Since the start-up was initiated during winter and completed during spring, the nitrification and denitrification processes may initially have been limited by temperature. Nitrification in particular is sensitive to low temperatures, which may have contributed to instability (Seviour et al., 1999). In AGS systems, temperature also affects the granulation process (de Blois et al., 2022) and thus indirectly nitrogen removal, since the establishment of stable granules is important for creating the layers in the granule that enable SND. Furthermore, at temperatures below approximately 10 °C, microbial activity is generally reduced, which can hinder the formation of granules. In addition, selection may be impaired as sedimentation properties may be affected by low temperatures due to increased water viscosity (de Blois et al., 2022).

Throughout the study period, the pH was relatively stable between 6.2 and 7.2 in all reactors. However, a pH of around 7-8 is considered optimal, as it favours the formation of more compact and stable granules (Khan et al., 2013), while a lower pH can promote a more filamentous and looser structure in the biomass (Lashkarizadeh, 2015). Overall, the results indicate that the pH conditions were sufficient but possibly slightly low for fully efficient and stable removal in the AGS reactors.

5.2 Phosphorus Removal

Phosphate removal was generally low in all reactors, figure 4.6, with all effluent levels exceeding the requirements for TP, which is 0.3 mg/L. The batch test showed that the biomass in all reactors had some potential for biological phosphorus removal however, there was not any complete phosphate removal. The results, figure 4.7, showed that R1 and R3 had the best removal, where the better removal in R1 may be due to the raw wastewater containing higher levels of readily available organic material, which favours PAO activity and bio-P (Bengtsson et al., 2018). In R2, which consisted of pre-sedimented wastewater, the phosphate removal was lower. This may have limited the ability of PAO to store carbon and thus impaired subsequent phosphate uptake (Ekholm et al., 2022). R3, which was inoculated with

granules and fed with raw wastewater, also showed signs of bio-P activity. Despite the presence of granules, no effective phosphate removal was achieved, which may indicate that the granules were not yet fully stabilized.

In addition, a biomass loss was observed after the operational change period, mainly in R3, where granule loss occurred. This may have negatively affected the biological activity of the reactor and thereby contributed to the limited removal of phosphate. The limited removal in all reactors may further be due to insufficient anaerobic conditions and relatively high oxygen levels in the systems. The presence of oxygen or nitrate during the filling phase may have inhibited PAO activity and thereby limited the establishment of effective bio-P (Seviour et al., 1999). This suggests that the process conditions during the start-up phase were not yet optimal for stable bio-P.

Carbon serves as an important source for PAO, where it is stored as PHA and enables phosphate uptake (Loosdrecht & Nielsen, 2015). The results from the batch test for acetate concentrations, figure A.8, showed clear consumption in all reactors, but despite this, the phosphorus removal was not complete. This suggests that acetate was not efficiently converted but was probably also consumed by competing microorganisms such as GAO. Furthermore, the COD content was lower for R2, which consisted of pre-sedimented wastewater, thus contributing to a slightly lower bio-P potential in reactor 2.

5.2.1 Operating Conditions

Operating conditions are also important for the establishment and stability of bio-P. Here too, the low temperature can affect the phosphorus removal due to impaired granulation and reduced microbial activity (de Blois et al., 2022). Reduced microbial activity at low temperatures can also lead to slower selection of PAO and provide competitive advantages to less desirable microorganisms, which in turn can contribute to poorer phosphorus uptake (Ekholm et al., 2024). Also the fluctuating temperature during the cycles might have had some effects. Furthermore, DO is a crucial parameter for enabling the alternating anaerobic and aerobic conditions required for bio-P. If DO levels are too high during the anaerobic phase, this can interfere with phosphate release, while insufficient oxygen supply can limit phosphate uptake (Seviour et al., 1999). Operating conditions such as pH, DO and temperature are likely to have influenced the bio-P process. However, the results of the batch tests indicated that the main limitation was likely that the biomass was still in an early development phase and had not yet established a sufficiently stable and active PAO population.

5.3 Organic Matter Removal

Despite differences in influent TOC and COD, the results showed similar effluent concentrations, figure 4.8, 4.9 and 4.10, indicating that organic matter degradation works well and is relatively independent of wastewater composition and sludge character. Since the effluent TOC levels were similar in all reactors, the results indicate

that the process for the separation of organic matter was relatively robust and not affected as much by the operating conditions and operational changes as nitrogen removal or phosphorus removal.

5.4 Comparison

In the following chapter, a comparison is made of, the start-up with granules compared to activated sludge. Furthermore, the start-up with raw wastewater and pre-sedimented wastewater is compared. Finally, the reactors are compared with other pilot and full-scale AGS plants in the world. However, it should be emphasized that in practice it is difficult to completely isolate the effect of the seeding culture and wastewater composition from other system parameters, such as operating conditions, which should be taken into account when interpreting the results.

5.4.1 Seeding Culture

The following section aims to compare start-up with activated sludge and start-up with mature granules through an analysis of R1 and R3. These two reactors were chosen for the comparison as they are operated with identical wastewater composition, which minimizes the influence of the substrate as a confounding factor and enables a more accurate evaluation of the importance of the seeding culture. The comparison covers both sludge characteristics and treatment performance.

When comparing the reactors with respect to sludge characteristics, R3 showed better settling properties, figure 4.2, and higher biomass concentrations, figure 4.1, compared to R1 and R2. Furthermore, R3 showed the lowest and most stable SVI values and showed the best sludge compressibility. This is consistent with the expectation that mature granules gives an advantage in the form of an already established granular structure with good settling properties (van Dijk et al., 2022). The operational changes implemented around days 28–42 affected the SVI values in both reactors, indicating that external disturbances have an impact on the sludge characteristics regardless of the seeding culture. Furthermore, in the microscopic analysis compact granules were observed in R3 directly and continuously throughout the project until day 48, at which point several smaller aggregates were observed instead. This shift was after the operational change period suggesting that a certain biomass loss had occurred and some disintegration of the granules as a result of the implemented system changes.

In R1, a gradual development from loose floc material to more defined aggregates appears over time, which suggests an ongoing biomass build-up process, figure 4.3. In addition, the steady increase in MLSS and VSS, figure 4.1, in R1 also indicates a gradual build-up of biomass. The results show that start-up with mature granules in R3 gave a clear advantage compared to start-up with activated sludge in R1 and R2, especially regarding sedimentation properties and biomass concentration from

an early stage.

An overall pattern that emerges when comparing the reactors (R1 and R3) is that the choice of seeding method primarily affects processes that require well developed granules with different layers, while simpler processes can be established relatively independently of the start-up strategy. This is demonstrated by the TOC removal in the reactors, which are very similar, indicating that organic matter degradation is established quickly regardless of whether the reactor is inoculated with granules or activated sludge. Similarly, R1 achieves an ammonium removal equivalent to R3, suggesting that nitrification can also be established effectively without mature granules. In terms of MLSS separation, R3 performs slightly better than R1, which is probably due to the compact structure and high density of the granules, which allows for significantly faster and more efficient sedimentation. However, it should be emphasized that the operating time is likely insufficient to fully evaluate the long-term differences between the start-up with granules and activated sludge. With continued operation and maturation of the granules in R1, the differences in denitrification and total nitrogen removal would probably become more pronounced, which would provide a more accurate picture of the performance of the respective start-up strategies.

5.4.2 Water Composition

The following section compares the effect of wastewater composition by comparing R1 and R2, where R1 consists of raw wastewater and R2 pre-sedimented wastewater. The comparison between R1 and R2 indicates that wastewater composition has an impact on sludge characteristics, where the smaller amount of organic material in the pre-sedimented wastewater (R2) disadvantages the build-up of biomass and granules compared to the raw wastewater (R1). This is also reflected in the VSS concentrations, figure 4.1b, where R2 has lower values than R1 throughout the whole study period. The amount of organic material in R2 limits the substrate availability for the microorganisms and thus also their growth. Furthermore, regarding the sedimentation properties, R2 had higher and more unstable values for SVI than R1 during large parts of the operating period.

When comparing the treatment performance between R1 and R2, it can be seen that the treatment performance is lower in R2. Overall, the results indicate that wastewater composition has an impact on the treatment performance, where the reduced organic content in pre-sedimented wastewater limits the biomass build-up and thus the biological treatment capacity, but that the degradation of organic matter works well regardless of the wastewater composition. The combined effect of low biomass and poorer sedimentation properties in R2 illustrates how the wastewater, as a result of pre-sedimentation, can have a negative impact on the sludge characteristics and removal performance. The wastewater composition is therefore an important parameter for the development of the biomass and therefore also for the start-up of AGS.

5.4.3 Other AGS Plants

The comparison with full scale and pilot AGS plants is limited to reactor 3, as this is the only reactor with granules. Although stable granulation has not yet been achieved, it is the reactor that most closely resembles the conditions in the referenced plants. The comparison provides an indication of the treatment potential that can be expected with continued operation of the reactor. Where other reactors exhibit comparable results for specific parameters, these will also be noted.

Table 2.1 shows the effluent concentrations for various parameters for some AGS plants in the world, both pilot and full-scale, which both exhibit high treatment performance. The full scale plants have effluent ammonium concentrations of 0.1-3 NH4-N mg/L, effluent nitrate concentrations of 1.6-10 NO3-N mg/L, and effluent nitrite concentrations remain below 0.5 NO2-N mg/L. The pilot at Ryaverket (Areskoug et al., 2024) had effluent ammonium concentrations of 0.33 NH4-N mg/L, an effluent nitrate concentration of 2 NO3-N mg/L, and an effluent nitrite concentration of 0.08 NO2-N mg/L, which is similar to the performance of the full-scale plants. Thus demonstrating that AGS can achieve high nitrogen removal even at pilot scale. R3 shows an average effluent ammonium content of 0.143 NH4-N mg/L and is comparable to the other AGS plants. R1 also has comparable ammonium effluent concentration that was 0.45 NH4-N mg/L. This indicates that nitrification works well in both R1 and R3. For other nitrogen parameters, however, they do not achieve the effluent levels reported for the other AGS plants. However, comparisons between full scale plants and pilots should be made with caution. Full-scale systems generally have better conditions for stable hydraulics, temperature control, and operational optimization, which contributes to more consistent and efficient treatment. While smaller scale systems are more sensitive to disturbances and variations.

5.5 Sources of Error and Uncertainties

Several uncertainties and potential sources of error should be considered when interpreting the presented results. The operating period is a limitation, as biological systems may require longer time to be established and stabilize. Therefore, the observed trends and differences between the reactors should be considered as indicators of the development direction rather than as representative of the full purification potential. Furthermore, several disturbances occurred and several operational changes were made, thus there are also uncertainties in the interpretation of the results. These events affected all reactors to varying degrees and make it difficult to distinguish the effects of seeding method and wastewater composition from the effects of operational disturbances.

There are also uncertainties surrounding the sampling, as the method was changed on several occasions during the course of the study, which is presented in table 3.1. However, disturbances and changes have been taken into account when interpreting the results. Furthermore, several samples were taken per week to obtain more ro-

bust results. In addition, operational disturbances and method changes that were assessed to have the greatest impact have been marked directly in the figures to facilitate the interpretation of the results. Duplicate samples were taken for VSS and MLSS, which allowed calculation of standard deviation and inclusion of error bars in the respective figures. For the other parameters, no duplicate samples were taken, which means that no estimate of the measurement uncertainty can be presented for these. Grab samples were used which might be a limiting factor, especially with large variations in temperature during the cycles. In addition, since the sampling was conducted in the morning, temperatures were generally lower, which could result in poorer treatment performance.

It should be mentioned that only phosphate was analysed in this study, which means that other phosphorus fractions in the system, were not included in the analysis. Since total phosphorus (TP) includes all phosphorus forms, the actual phosphorus removal in the reactors may therefore deviate from the measured phosphate levels. However, since phosphate is part of TP and the measured phosphate levels in the effluent exceeded the discharge requirements for TP, the results indicate that the overall phosphorus removal in the systems was not sufficient, even if other phosphorus fractions were not analysed.

Furthermore, TOC includes all organic carbon, including both biodegradable material (Shetty and Goyal, 2022) and fractions such as lignin that are not directly biologically available (Kazimierowicz and Dębowski, 2022). Thus, TOC is limited to assessing the biological availability of organic material. COD reflects the complete oxidation of organic matter, including fractions that are not biodegradable, and is also not good at assessing the available organic matter in this context. BOD would provide a better indication of the biodegradable organic matter, but is not practical to analyse. Furthermore, only one COD analysis was performed during the project period, which limits the possibility of drawing robust conclusions. However, COD data are available for incoming raw and pre-sedimented wastewater, which gives a better indication of differences in available organic matter between the inflows, where the pre-sedimented water exhibits lower COD and thus a reduced amount of available organic material.

5.6 Further Studies

To more thoroughly evaluate the potential of AGS and the factors affecting start-up and treatment performance, several complementary studies could be conducted. For example, an extended operating period would have provided a better evaluation of the full treatment capacity, and of the stability and disintegration of granules during long-term operation. In addition, it may be useful to continue investigating how good performance can be achieved even at low temperatures and high flows, which is particularly relevant for the application of AGS in Nordic countries. Furthermore, climate change is expected to lead to an increased frequency of intense precipitation and thus greater flow variations (Trenberth, 2005), thus studies on the robustness of AGS systems under extreme hydraulic conditions are an additional area that may

be interesting to study further.

A more comprehensive analytical characterization would have been of interest, as it could have provided a deeper understanding of the underlying biological processes. How pharmaceutical residues, endocrine disruptors, other micro pollutants, but also difficultly biodegradable compounds such as PFAS, are handled in AGS systems could be further investigated. Another interesting aspect to study is the influence of individual parameters on granulation and purification performance. In this project, several operating parameters were changed simultaneously, making it difficult to establish which parameter caused what. Systematic studies where, for example, sedimentation time or aeration strategy are varied one at a time would have given a clearer picture of the influence of each parameter, and thus enabled a better optimization of the operating conditions in AGS systems.

6
Conclusion

All reactors showed good ammonium removal and efficient organic matter degradation, indicating that these processes are established relatively independently of starting material and wastewater composition. However, denitrification was deficient in all three reactors, which is likely a result of operating conditions, since there was potential for denitrification. Furthermore, the phosphorus removal was not enough in any of the reactors, which is probably explained by a combination of insufficient PAO population and unfavourable operating conditions. Overall, the treatment performance was not sufficient to meet the applicable emission limit values, but the reactors showed a positive development and improved performance compared to the initial phase, indicating that continued operation could result in further improved treatment.

Regarding the importance of wastewater composition, the results show that the use of pre-sedimented wastewater resulted in a disadvantage when starting up AGS systems, as the low substrate availability makes biomass build-up more difficult. Raw wastewater thus show more potential for an efficient start-up. Regarding the seeding culture, the reactor with mature granules generally performed best. It should be noted that the results have several uncertainties due to the limited operating period, operational disturbances and method changes during the course of the project. Further studies with, among other things, longer operating time and more analysis may therefore be useful in order to draw more conclusions about the potential of the AGS technology.

Bibliography

Areskoug, T., Nunes, J., Asp, T., & Tumlin, S. (2024). *Ags-pilot ryaverket 2022–2023: Slutrapport – utan sekretess* (Slutrapport). Gryaab.

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), 119–166. https://doi.org/10.1080/10643389.2018.1439653

Cheremisinoff, N. P. (1997). *Biotechnology for waste and wastewater treatment*. Noyes Publications.

Dasgupta, S., Podder, A., & Goel, R. (2020). Response of an aerobic granular and conventional flocculated reactors against changing feed composition from simple composition to more complex. *Chemosphere*, *253*, 126694. https://doi.org/10.1016/j.chemosphere.2020.126694

de Blois, M., Bengtsson, S., Gunnarsson, K., Engström, S., Ekholm, J., Persson, F., Wilén, B.-M., van Erp, T., & Gustavsson, D. (2022). *Tillämpning av aerobt granulärt slam i sverige: En fullskalestudie* (SVU-rapport No. 2022-13). Svenskt Vatten AB. Stockholm.

de Bruin, L., De Kreuk, M., Van Der Roest, H., Uijterlinde, C., & Van Loosdrecht, M. (2004). Aerobic granular sludge technology: An alternative to activated sludge? *Water Science and Technology*, *49*(11-12), 1–7. https://doi.org/10.2166/wst.2004.0790

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

Ekholm, J., Persson, F., de Blois, M., Modin, O., Pronk, M., van Loosdrecht, M. C. M., Suarez, C., Gustavsson, D. J. I., & Wilén, B.-M. (2022). Full-scale aerobic granular sludge for municipal wastewater treatment – granule formation, microbial succession, and process performance. *Environmental Science: Water Research & Technology*, *8*(12), 3138–3154. https://doi.org/10.1039/d2ew00653g

Ekholm, J., Persson, F., De Blois, M., Modin, O., Gustavsson, D. J. I., Pronk, M., Van Loosdrecht, M. C. M., & Wilén, B.-M. (2024). Microbiome structure and function in parallel full-scale aerobic granular sludge and activated sludge processes. *Applied Microbiology and Biotechnology*, *108*(1), 334. https://doi.org/10.1007/s00253-024-13165-8

Hailei, W., Guangli, Y., Guosheng, L., & Feng, P. (2006). A new way to cultivate aerobic granules in the process of papermaking wastewater treatment. *Bio-*

chemical Engineering Journal, *28*(1), 99–103. https://doi.org/10.1016/j.bej.2005.10.002

HaskoningDHV, R. (n.d.). Nereda technology how it works. Retrieved May 21, 2026, from https://nereda.haskoning.com/en/technology/nereda-technology-how-it-works

Henze, M., Harremøes, P., la Cour Jansen, J., & Arvin, E. (2001). *Wastewater treatment: Biological and chemical processes* (3rd ed.). Springer.

Kassim, N. M., Anuar, A. N., Halim, H., Sharani, N. A., Md Aris, A., & Chin, K. B. (2022). To optimize sequencing batch reactor (sbr) and monitor aerobic granular sludge (ags) development for domestic wastewater treatment. *IOP Conference Series: Earth and Environmental Science*, *1091*(1), 012059. https://doi.org/10.1088/1755-1315/1091/1/012059

Kazimierowicz, J., & Dębowski, M. (2022). Aerobic granular sludge as a substrate in anaerobic digestion—current status and perspectives. *Sustainability*, *14*(17), 10904. https://doi.org/10.3390/su141710904

Khan, M. Z., Mondal, P. K., & Sabir, S. (2013). Aerobic granulation for wastewater bioremediation: A review. *The Canadian Journal of Chemical Engineering*, *91*(6), 1045–1058. https://doi.org/10.1002/cjce.21729

Lashkarizadeh, M. (2015). *Operating ph and feed composition as factors affecting stability of aerobic granular sludge* [Master's thesis]. University of Manitoba.

Leitrim County Council. (2009). *Wastewater sampling method* (Attachment E.2.2). Leitrim County Council. County Leitrim, Ireland.

Li, J., Ding, L.-B., Cai, A., Huang, G.-X., & Horn, H. (2014). Aerobic sludge granulation in a full-scale sequencing batch reactor. *BioMed Research International*, *2014*, 1–12. https://doi.org/10.1155/2014/268789

Lobos, J., Wisniewski, C., Heran, M., & Grasmick, A. (2008). Sequencing versus continuous membrane bioreactors: Effect of substrate to biomass ratio (f/m) on process performance. *Journal of Membrane Science*, *317*(1–2), 71–77. https://doi.org/10.1016/j.memsci.2007.09.014

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. https://doi.org/10.1016/S0043-1354(97)00216-9

Nancharaiah, Y. V., & Sarvajith, M. (2019). Aerobic granular sludge process: A fast growing biological treatment for sustainable wastewater treatment. *Current Opinion in Environmental Science & Health*, *12*, 57–65. https://doi.org/10.1016/j.coesh.2019.09.011

Nancharaiah, Y., & Kiran Kumar Reddy, G. (2018). Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. *Bioresource Technology*, *247*, 1128–1143. https://doi.org/10.1016/j.biortech.2017.09.131

Ni, B.-J., Xie, W.-M., Liu, S.-G., Yu, H.-Q., Wang, Y.-Z., Wang, G., & Dai, X.-L. (2009). Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low-strength municipal wastewater. *Water Research*, *43*(3), 751–761. https://doi.org/10.1016/j.watres.2008.11.009

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

Pronk, M., 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), 987–996. https://doi.org/10.2166/wpt.2017.101

Ramesh, V. (2020). *The function of key microbial guilds in full- scale wastewater treatment reactors with aerobic granular sludge* [Master's thesis, Chalmers university of technology].

Sanchez-Sanchez, C., Moreno-Rodríguez, E., Ortiz-Cruz, J. A., & Moeller-Chávez, G. E. (2023). Development of aerobic granular sludge for real industrial/-municipal wastewater treatment. *Water Science & Technology*, *87*(9), 2328–2344. https://doi.org/10.2166/wst.2023.121

Sarma, S. J., Tay, J. H., & Chu, A. (2017). Finding knowledge gaps in aerobic granulation technology. *Trends in Biotechnology*, *35*(1), 66–78. https://doi.org/10.1016/j.tibtech.2016.07.003

Sepúlveda-Mardones, M., Campos, J. L., Magrí, A., & Vidal, G. (2019). Moving forward in the use of aerobic granular sludge for municipal wastewater treatment: An overview. *Reviews in Environmental Science and Bio/Technology*, *18*(4), 741–769. https://doi.org/10.1007/s11157-019-09518-9

Seviour, R. J., & Blackall, L. L. (Eds.). (1999). *The microbiology of activated sludge*. Springer Netherlands. https://doi.org/10.1007/978-94-011-3951-9

Shetty, A., & Goyal, A. (2022). Total organic carbon analysis in water – a review of current methods. *Materials Today: Proceedings*, *65*, 3881–3886. https://doi.org/10.1016/j.matpr.2022.07.173

Show, K.-Y., Lee, D.-J., & Tay, J.-H. (2012). Aerobic granulation: Advances and challenges. *Applied Biochemistry and Biotechnology*, *167*(6), 1622–1640. https://doi.org/10.1007/s12010-012-9609-8

Sustarsic, M. (2009). Activated sludge. *Chemical Engineering Progress*, *105*(1), 26–35.

Świątczak, P., & Cydzik-Kwiatkowska, A. (2018). Performance and microbial characteristics of biomass in a full-scale aerobic granular sludge wastewater treatment plant. *Environmental Science and Pollution Research*, *25*(2), 1655–1669. https://doi.org/10.1007/s11356-017-0615-9

Trenberth, K. E. (2005). The impact of climate change and variability on heavy precipitation, floods, and droughts. *Encyclopedia of hydrological sciences*, *17*, 1–11.

Van Dijk, E. J., Haaksman, V. A., Van Loosdrecht, M. C., & Pronk, M. (2022). On the mechanisms for aerobic granulation - model based evaluation. *Water Research*, *216*, 118365. https://doi.org/10.1016/j.watres.2022.118365

van Loosdrecht, M. C. M., Nielsen, P. H., Lopez-Vazquez, C. M., & Brdjanovic, D. (Eds.). (2016). *Experimental methods in wastewater treatment*. IWA Publishing.

Varão Moura, A., Da Silva, J. D. S., & Gubert, P. (2022). Ion chromatography: Principles and instrumentation. *Orbital: The Electronic Journal of Chemistry*, 110–115. https://doi.org/10.17807/orbital.v14i2.15871

A

Appendix

Table A.1: Amounts of reagents added in the bio-P and nitrification/denitrification Batch tests.

Reagent	**Compound**	**Bio-P test (g)**	**Nitrogen test (g)**
Trace solution	KCl	0.36	0.36
Trace solution	$FeSO_4$	0.20	0.20
Trace solution	$MgSO_4$	0.90	0.90
Trace solution	$CaCl_2$	0.14	0.14
Ammonia feed	NH_4Cl	-	0.38
Sodium acetate	$NaCH_3COO$	1.67	1.67
Phosphate feed	KH_2PO_4	0.07	-
Phosphate feed	K_2HPO_4	0.08	-

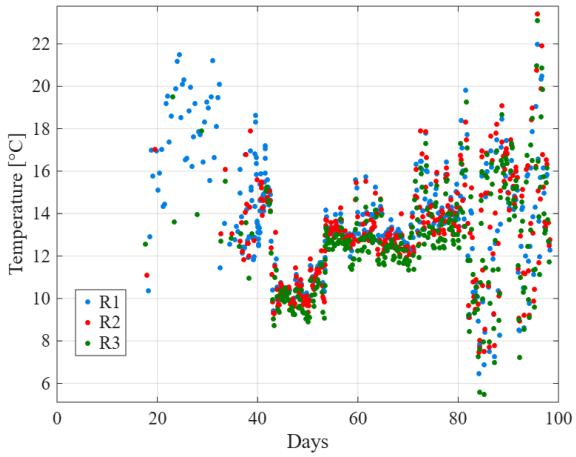


Figure A.1: Temperature for reactor 1, 2 and 3 for the entire study period.

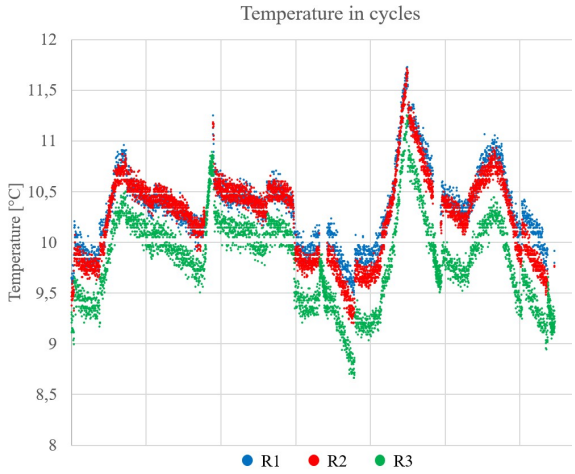


Figure A.2: Temperature variation for reactor 1, 2 and 3 during the day and in the cycles.

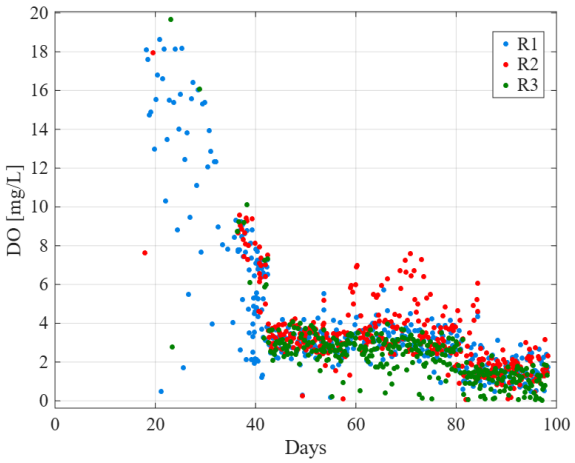


Figure A.3: Dissolved oxygen (DO) concentration for reactor 1, 2 and 3.

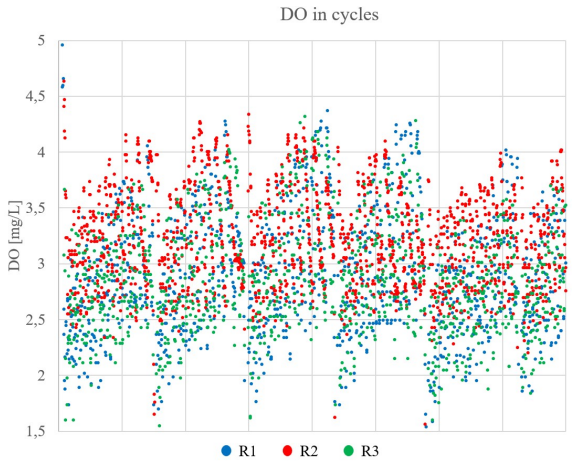


Figure A.4: Dissolved oxygen (DO) variation for reactor 1, 2 and 3 in the cycles.

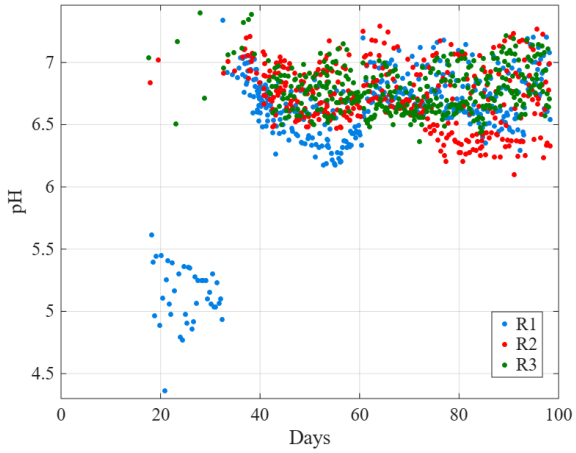


Figure A.5: The pH for reactor 1, 2 and 3 during the course of the project.

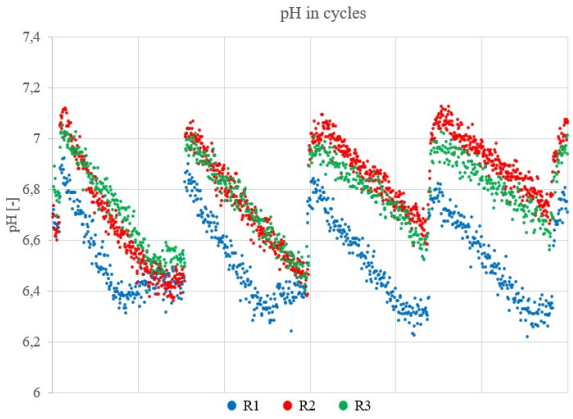


Figure A.6: The pH variations for reactor 1, 2 and 3 in the cycles.

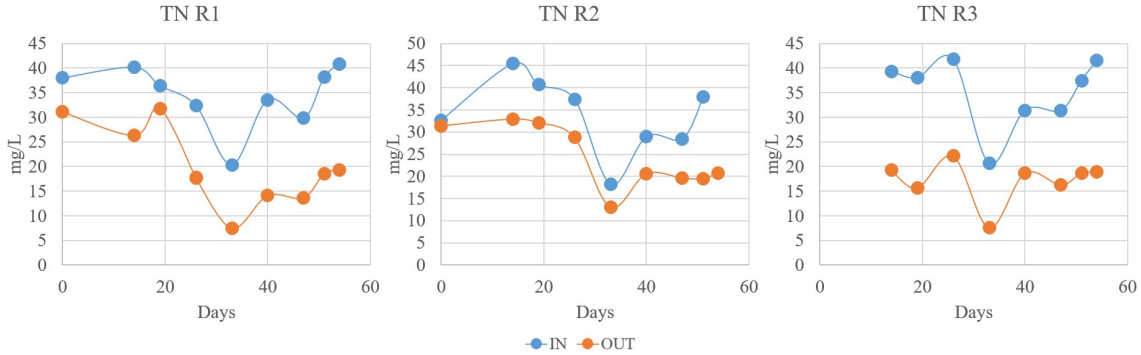


Figure A.7: TN concentrations for reactor 1, 2 and 3 for effluent and influent.

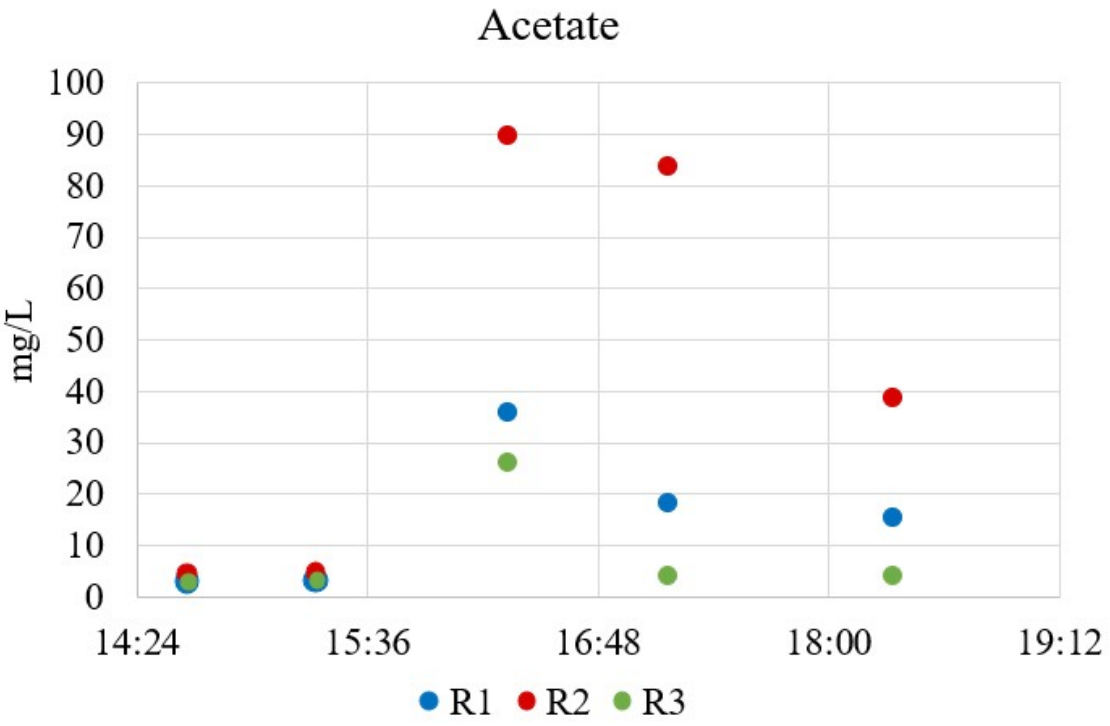


Figure A.8: The acetate concentrations in the reactors (R1, R2 and R3) during the batch test.

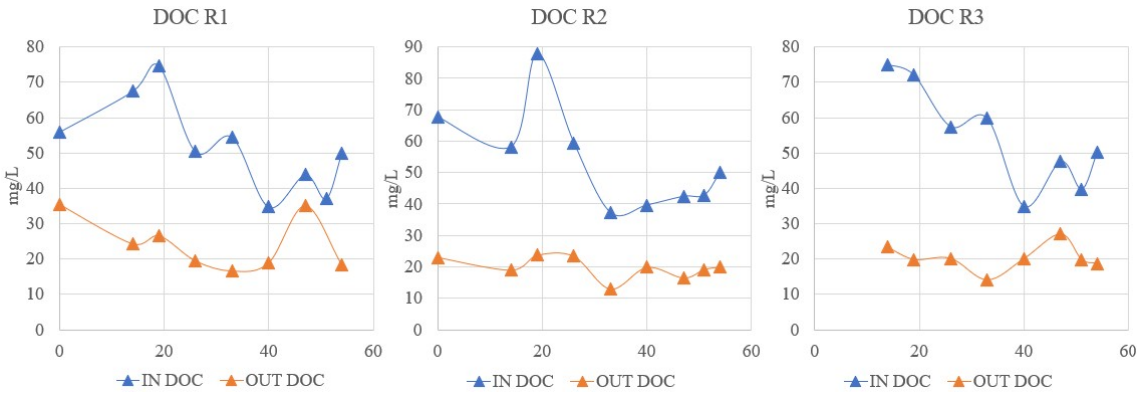


Figure A.9: The DOC concentrations for influent and effluent samples for R1, R2 and R3.

DEPARTMENT OF ARCHITECTURE AND CIVIL ENGINEERING
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden
www.chalmers.se

CHALMERS
UNIVERSITY OF TECHNOLOGY