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Assessment of biogas potential for aerobic granular sludge and activated sludge at Gryaab

Master thesis in Infrastructure and Environmental Engineering

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Abstract

The population of the Gothenburg region is expected to increase in the coming years, which will lead to increasing flows into the Rya Wastewater Treatment Plant (WWTP). Therefore, the treatment capacity will be increased by building a parallel WWTP on new land. One of the wastewater treatment technologies considered to be implemented is aerobic granular sludge (AGS). AGS is a promising biotechnology that has demonstrated several advantages when compared to the conventional activated sludge (CAS) process such as lower energy usage and area requirements. A broader understanding of the biochemical methane potential (BMP) of the two different types of sludge produced by the AGS process, waste aerobic granular sludge (WAGS) and mixed aerobic granular sludge (Mixed AGS), is necessary as there are few previous studies available. The BMPs of these two AGS-sludge fractions were compared to primary sludge (PS) and waste activated sludge (WAS). Additionally, the effect of primary clarification on the BMP of the AGS-sludges was elucidated by sampling the AGS-sludge when the pilot plant was fed with incoming or pre-clarified wastewater. The results indicated that PS had the highest BMP (365 ± 7 ml CH_4/gVS) of all the sludge fractions. From all the AGS-sludge fractions the highest BMP (223 ± 19 ml CH_4/gVS) was obtained from the WAGS-sludge when the pilot plant was fed with incoming wastewater without primary clarification. This value was 1.64 times lower than the BMP of the PS. The BMP of the WAGS-sludge was affected by primary clarification as a higher BMP result (223 ± 19 ml CH_4/gVS) was observed when the pilot was fed with incoming wastewater when compared to its BMP when the pilot was fed with pre-clarified water (185 ± 10 ml CH_4/gVS). The BMPs of the mixed AGS-sludge were not affected by primary clarification (213 ± 19 ml CH_4/gVS with pre-settled wastewater and 208 ± 15 ml CH_4/gVS with incoming wastewater). Mixed AGS-sludge was the substrate that took the most time in being digested, taking 21 days to reach a daily production of <1% of the accumulated volume of CH_4. The results showed that AGS-sludges are biodegradable, however their biodegradability was lower in comparison to WAS and PS.

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Keywords: aerobic granular sludge, biogas, biochemical methane potential, biodegradability, activated sludge, Gothenburg

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Johanna Arita
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Abbreviations

AS: Activated Sludge

AGS: Aerobic Granular Sludge

AMPTS: Automatic Methane Potential Test System

BMP: Biochemical Methane Potential

EPS: Extracellular polymeric substances

MBR: Membrane bioreactor

OLR: Organic Loading Rate

PE: population equivalent

PS: Primary sludge

SBR: Sequencing Batch Reactor

TS: Total Solids

VS: Volatile Solids

WAS: Waste Activated Sludge

WAGS: Waste Aerobic Granular Sludge

WWTP: Wastewater treatment plan

1. Introduction

The growth of human population is directly proportional to the expansion of the agricultural, industrial, and domestic sectors. The anthropogenic activities linked to these sectors produce one of the surest resources available due to its unavoidable production: wastewater (Obotey Ezugbe & Rathilal, 2020). Health, environmental, and climate-related hazards can occur if wastewater is not treated in an appropriate manner (IWA, n.d.) Therefore, it is necessary to invest in effective wastewater treatment processes. However, wastewater treatment is costly since it requires inputs of resources such as energy. Moreover, WWTPs produce large amounts of sludge that must be dispatched (Gnaneswar, 2015). The management of sludge can account for more than 50% of a WWTP's operational costs (Val Del Río et al., 2014) and municipal wastewater treatment is responsible for around 5% of the electricity demand in some countries (Tavares Ferreira et al., 2021). In Sweden, c. 200 000 tons of sewage sludge (dry matter) is generated annually (SOU, 2020), and 30% of it is used for agriculture (Eurostat, 2022).

Producing biogas from sludge through anaerobic digestion (AD) is one of the most appropriate solutions to reduce the amount of sludge produced and is a significant step towards the development of sustainable energy (Tyagi & Lo, 2013). Furthermore, greenhouse gas emissions from WWTPs decrease with the correct implementation of anaerobic digestion in their sludge management process since organic wastes generate large amounts of methane as they decompose and escape into the atmosphere (Environmental and Energy Study Institute, 2017). Biogas is distinct from other renewable energy sources since during its production, organic waste material is reduced and the remaining digested sludge can be used as fertilizer if the quality is good enough (Taleghani & Shabani Kia, 2005). If there are no upgrading processes available, biogas can be burned on-site to heat buildings or the digester itself. It can be used for combined heat and power (CHP) operations, or transformed into energy by using a combustion engine, fuel cell, or gas turbine (USDA, 2014). When CO_2 and other trace gases are removed from biogas, biomethane is obtained and can be injected into an existing natural gas grid or used as vehicle fuel. In Sweden, 138 WWTPs produce biogas and contribute to 35% of the total biogas production (The Swedish Gas Association, 2020). This biogas is currently used for vehicle gas, heat, or electric power (Energimyndigheten, 2011).

A novel and promising biotechnology for the treatment of industrial and municipal wastewater named aerobic granular sludge (AGS) has gained increasing interest in the last two decades. This is due to its capability of performing simultaneous nitrification, denitrification and phosphorus removal which leads to 20-50% energy usage reduction when compared to that of activated sludge (Pronk et al., 2017). Two different types of sludge are produced during the AGS process: Mixed aerobic granular sludge (Mixed AGS) and Waste Aerobic Granular Sludge (WAGS). However, few studies have been conducted before to evaluate their biogas potential (Hamza et al., 2022). Therefore, in this master thesis the biogas potential of both WAGS-sludge and mixed AGS-sludge from a pilot plant located at the Rya WWTP was assessed and compared to primary sludge (PS) and the waste activated sludge (WAS) produced from activated sludge process at the Rya WWTP.

1.1. Aim

The aim of the master thesis was to assess the biogas potential of WAGS-sludge, mixed AGS-sludge, PS, and WAS at the Rya WWTP. PS and WAS were sampled from the full-scale plant and the AGS-sludges were sampled from a pilot plant located at the Rya WWTP. Different configurations of the AGS pilot setup were explored to fulfill this aim. WAGS-sludge, the flocculent sludge that is removed every cycle, and Mixed AGS-sludge, sludge under fully mixed conditions during the aeration phase, were tested when the pilot was fed with either incoming (crude) or pre-settled wastewater. PS and WAS were tested as well for comparison with these AGS-sludges. The biomethane production was measured by anaerobically digesting two setups of samples with AMPTS II equipment, see Chapter 3. The results provided by the AMPTS II were used to compare the biomethane potential of both technologies.

The following research questions were addressed for this project:

1. What is the methane production potential from the waste sludges of the aerobic granular sludge pilot plant and how does it relate to that of the primary sludge and waste activated sludge at the Rya WWTP?
2. How is the methane production of the different sludge fractions of aerobic granular sludge affected by the characteristics of the influent wastewater?

1.2. Limitations

The limitations for this master thesis project are listed below:

- This master thesis project was performed only with sludge samples from the Rya WWTP. Therefore, the results are only representative of the Rya WWTP.

- The AGS-sludge samples were taken from a pilot plant and not from a full-scale plant. However, the biological characteristics were assumed to be the same as from a full-scale plant.

- Inoculum samples used to prepare mixtures were taken from a digester at the Rya WWTP already adapted to PS and WAS.

2. Background

2.1. Gryaab AB

Gryaab AB is a municipally owned company. The municipalities of Ale, Göteborg, Härryda, Kungälv, Lerum, Mölndal, Partille, and Bollebygd cooperate to treat wastewater from industries and households before being discharged into the sea to comply with stringent discharge consents. The company has around 120 employees distributed in five different departments (Gryaab AB, 2021).

The incoming wastewater is treated with respect to degradable organic material, phosphorus, and nitrogen. Once the water is treated it is led via a tunnel to the Göta River estuary (Gryaab AB, 2022b). Gryaab has discharge limit values per year, tertial, and as 3-year average masses presented in Table 1 (Dag Lorick, personal communication, June 2, 2022).

Table 1. Gryaab's discharge limit values.

Results	Phosphorus	Nitrogen	COD
Concentrations (mg/l, to be achieved as yearly basis as well as during tertial 2)			
Total 2021	0.18	6.6	6.4
Permit	0.3	8	10
Gryaab's goal	< 0.25	< 7	< 9
Amounts (tons, to be achieved as a 3-year average)			
Total 2021	23.7	854	824
Permit	40	1,000	1,300
Gryaab's goal	< 35	< 980	< 1,200

The Rya WWTP plant currently treats wastewater from 812,960 people (Gryaab AB, 2022a), and this number is expected to increase in the coming years. To increase the plant's capacity, it is planned to build a parallel treatment plant next to the existing facility, the project is named Nya Rya (Gryaab AB, n.d.-b)

2.2. Wastewater Treatment Process at the Rya WWTP

The removal of organic matter is one of the most important tasks WWTPs must perform. Its decomposition in receiving water bodies consumes dissolved oxygen and releases nutrients that feed algae growth (Lowe, 2004). Eutrophication is the over-enrichment of surface waters with mineral nutrients. This results in an excessive production of autotrophs such as algae and cyanobacteria (Correll, 1999). The main organic components in wastewater are 50% proteins, 40% carbohydrates, 10% fats and oils, and trace amounts of priority pollutants (Shon et al., 2006). Regarding nitrogen, most of it is found in wastewaters in the form of ammonia (Winkler & Straka, 2019). It must be tackled by WWTPs to prevent environmental problems such as eutrophication, toxic algae blooms, and higher emissions of greenhouse gases (Baron et al., 2013). Phosphorus can also cause eutrophication. Its removal can be done through either chemical or biological methods, or a combination of both (Yeoman et al., 1988).

At Gryaab the water is purified mechanically, physically, chemically, and biologically with the aim of removing organic matter, nitrogen, and phosphorus. The treatment process begins with the transportation of wastewater from the eight different municipalities through a 13 kilometers long tunnel system which is diverted into the WWTP. The tunnel system has a slope of 0.1%, meaning the water flows naturally down into the treatment plant. The water entering the plant is pumped with four pumps with a maximum capacity of 4.7 m^3/s – 6.0 m^3/s each (Gryaab AB, 2020). The flow into the plant varies

from 2-16 m^3/s depending on the amount of stormwater into the treatment due to the predominant combined sewers in the region (D. Lorick, personal communication, April 17, 2023).

2.2.1. Preliminary Treatment

After entering the WWTP, the wastewater first passes through a coarse bar screen with a two centimeters grid mesh. Here, paper towels and other big items such as plastics, sticks, or rags are removed. Afterwards, the wastewater passes through an aerated sand trap with the objective of separating medium sized solid particles such as sand, stone, and grit. This is followed by a fine grate bar with a gap of 2 mm. The collected solids from the preliminary treatment process are washed, pressed, and stored before being incinerated (Gryaab AB, 2020).

2.2.2. Primary Treatment

During primary treatment the wastewater flows into twelve rectangular-shaped sedimentation tanks with a total volume of 22,670 m^3 and an area of 5,800 m^2 (Gryaab AB, 2020). Organic matter such as dissolved toilet paper, feces, and food scraps sink to the bottom and primary sludge is formed. The sludge at the bottom of the tanks is pumped to the biogas reactors and the water is pumped to the secondary treatment (Gryaab AB, n.d.-c).

2.2.3. Secondary Treatment

More than 90% of municipal wastewater treatment plants around the world use activated sludge as their core treatment process (Liu, 2003) due to its proven effectiveness. At the Rya WWTP the secondary treatment consists of a high-loaded BOD removal activated sludge basin, nitrifying trickling filters, nitrifying moving bed biofilm reactor, denitrifying moving bed biofilm reactor, an anammox reject water treatment line, and secondary sedimentation basins.

After passing through the primary sedimentation, iron sulphate is added to the water for chemical precipitation of phosphorus after which the water flows into the three activated sludge basins which are separated into anoxic and aerobic zones. The first area is unaerated and due to the lack of oxygen the bacteria use nitrate for respiration, transforming into nitrogen gas that is released to the atmosphere. This process is called denitrification. After denitrification the water enters the oxic basins where the bacteria break down organic matter with oxygen and multiply via cell division, forming new activated sludge (Gryaab AB, 2020). The necessary oxygen is transferred in this basin in order to oxidize organic material in the reactor and prevent the settling of sludge flocs. The role of microorganisms is vital as they consume organic matter and produce carbon dioxide (van Handeel & van der Lubbe, 2012).

After on average one hour and a half of retention time, polymer is added to the water to improve flocculation and the water flows into the secondary sedimentation basins, which have a total volume of 72,200 m^3. Here, the wastewater is retained on average for three hours with the aim of removing the biomass either as return sludge which is sent back to the anoxic basing or as waste activated sludge sent to the pre-sedimentation tanks or to the sludge handling process if high flows are present (Gryaab AB, 2020)

After the secondary sedimentation basins, one part of the water flows into the nitrifying trickling filters and the other part flows into the nitrifying moving bed bioreactors to receive post-nitrification treatment (Gryaab AB, 2020).

The trickling filters are 7.2 meters deep, have a volume of 16,500 m^3 and a capacity of 7 m^3/s. They possess a high water-plastic contact surface where bacteria can thrive. The ammonia in the water is converted to nitrate in the process called nitrification. The water treated at the trickling filters is later sent back to the activated sludge basins for pre-denitrification (Gryaab AB, 2020).

The nitrifying moving bed biofilm reactors are basins filled with plastic carriers where bacteria can grow. The basins have a total volume of 10,800 m^3 and a capacity of 5.0 m^3/s. The reject water from the dewatering process performed after anaerobic digestion can be sent back to this area, however, it is normally sent to the anammox process where the anammox bacteria convert ammonia directly into nitrogen gas (Gryaab AB, 2020).

Following the two nitrifying processes mentioned above the water is sent to the post-denitrification moving bed biofilm reactors where plastic carriers treat the nitrate in water produced from the nitrification process by converting it into nitrogen gas with the addition of an external carbon source such as methanol. The total volume of the post-denitrification basins is 11,000 m^3 with a capacity of 4.0 m^3/s (Gryaab AB, 2020).

2.2.4. Tertiary Treatment

After the secondary treatment, the water is sent to the disc filter plant which consists of 32 disc filters on rotating filter cloths with a mesh width of 15 μm. This is the final step of the treatment process, where suspended solids are removed and effluent quality is improved before the water is finally released to the Göta River (Gryaab AB, 2020).

The management and handling of the sludge produced from the plant is explained in Section 2.7.

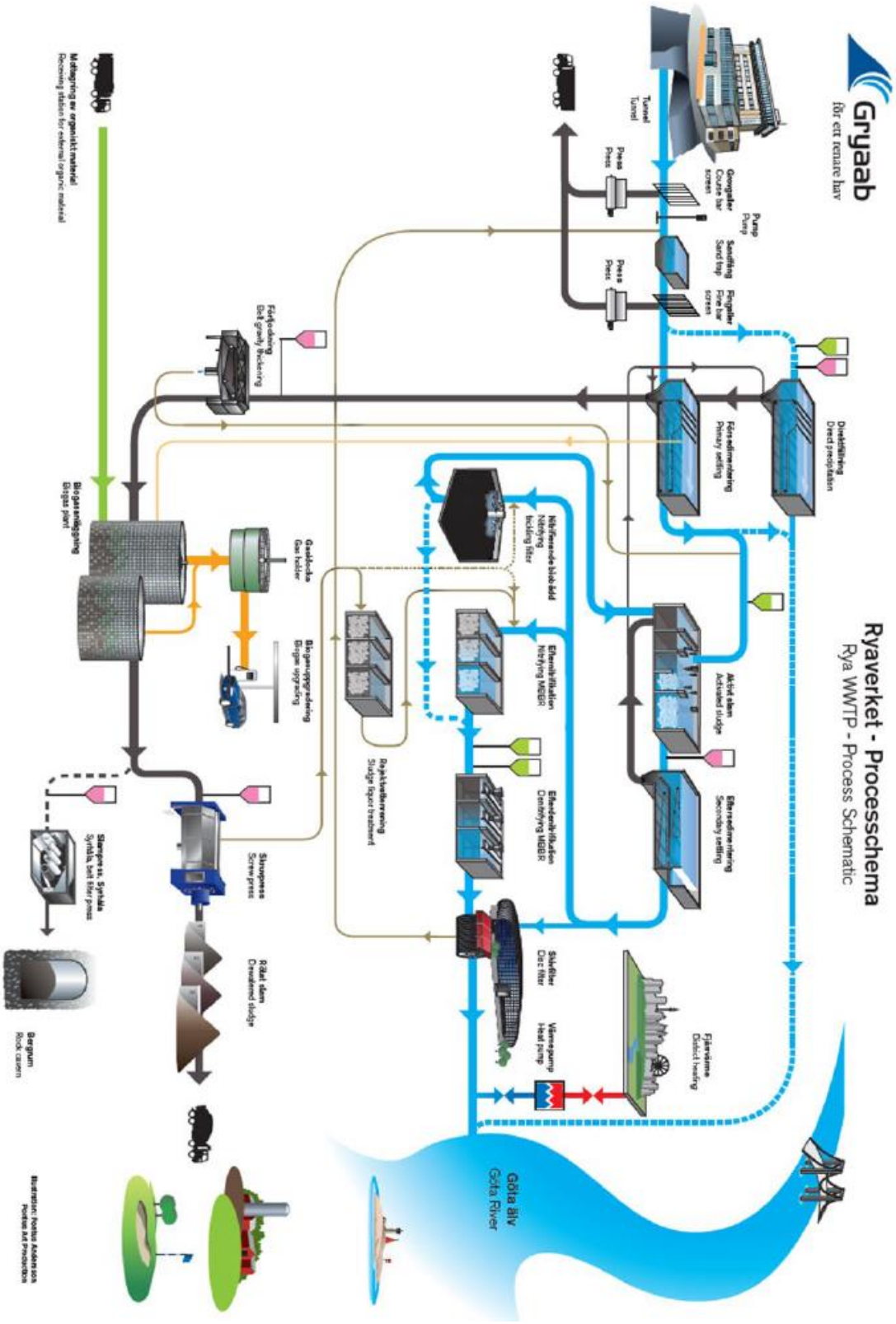

Figure 1. Process Schematic at the Rya WWTP. Taken from "Miljörapport 2022" (Gryaab AB, 2022b).

2.3. Nya Rya

The Nya Rya is an expansion project of the existing Rya WWTP. Considering there is no space left for construction at the site of the existing plant, the new parallel plant will be built on new land (Gryaab AB, n.d.-b). Currently, different wastewater treatment technologies are being compared for aspects such as environmental burdens, and costs (Gryaab AB, n.d.-a). One of the considered technologies is aerobic granular sludge. The biogas potential assessed in this master thesis project is one of the factors that stakeholders will take into consideration when designing the new plant if AGS is chosen to be the main process.

The Nya Rya project is expected to be completed in four different stages: Investigation, pre-design, actual implementation, and submission of the program to the operation organization. The new treatment plant will be fully implemented in year 2036 (Gryaab AB, n.d.-b).

2.4. Aerobic Granular Sludge

The aerobic granular sludge process is a biological wastewater treatment technology where aerobic granules are formed by the immobilization of different microbial groups. This technology was developed to address issues regarding the conventional activated sludge process, such as sludge-water separation and high land footprint. There are several advantages associated with this technology, such as the compact microbial structure of the granules and their high settling velocities, the ability to perform nitrification, denitrification, and phosphate removal at the same time and in one tank reducing energy costs and space, and lower sludge production (Nancharaiah & Sarvajith, 2019). The main disadvantages associated with this technology are related to granulation. The long start-up process of granule formation, the proneness of granule stability to deterioration due to different factors such as low hydrodynamic shear forces (Liu & Tay, 2002) and low temperatures could affect the process in a full-scale facility (Show et al., 2012).

2.4.1. Granule formation

Aerobic granules consist of aerobic, anoxic, and anaerobic microbial layers allowing the removal of carbon, nitrogen, and phosphorus within a single reactor. They have a size of > 200 μm and a settling velocity of 90 m/h (Hamza et al., 2022). For granulation to occur a combination of conditions must take place. Some of these factors are high substrate concentrations, short settling time, high shear stress, and low growth rates (Obotey Ezugbe & Rathilal, 2020).

The most common option to achieve high substrate concentrations are Sequencing Branch Reactors (SBRs). This way, microorganisms are exposed to incoming wastewater with high concentration of organic matter, nitrogen, and phosphorus under each operating cycle (Zhang et al., 2020). The most common AGS process, Nereda® operates with three cycle components: simultaneous influent feed and effluent withdrawal, aeration and reaction, and fast settling phase (Hamza et al., 2022).

To enhance granulation, reactors must be operated with a short settling time for the biomass. This way, rapidly settling granules are retained in the system and suspended microorganisms are washed out with the effluent water (Beun et al., 1999).

The application of low growth rates leads to stable granulation. This can be achieved by exposing the reactor to the “feast and famine process”, which consists of the exposure of biomass to high and low concentration of organic matter in the wastewater. The microorganisms that can store organic matter during the anaerobic phase and use it during aerobic grow phase are the fundaments of granulation. This leads to a reduction in biomass growth and forms strong and stable granules (Bengtsson et al., 2018). In a full-scale granulation reactor, it is necessary to implement a long famine phase to solidify the structure of the granules (Sun et al., 2021).

High shear forces create stable, dense and smooth granules. This can be obtained with increasing aeration velocities (Liu & Tay, 2002). This shear stress mainly exerts influence during feast period, meaning that aeration velocities can be lowered during famine periods without affecting the settleability of the granular sludge.

2.4.2. Aerobic Granular Sludge and Biogas Production

Producing biogas from waste aerobic granular sludge is an opportunity for facilities to cover technology costs and to handle the sludge in a sustainable way (Kehrein et al., 2020). Previous studies have been made with both synthetic and municipal wastewater to assess the biogas potential of granules. Most of the results from previous studies show that AGS has a lower biogas potential than WAS, however, some studies show contrary results.

(Bernat et al., 2017) investigated and compared the BMP of WAGS-sludge and WAS. The results show that the BMP of WAGS-sludge was 1.8 times lower than that of WAS, due to the high lignin content and low lipid content in the AGS-sludge. Different organic loading rates (OLRs) were tested (2, 4, and 6 kg VS/m3 d) to measure their effect on biogas production, which decreased with an increase in the OLR. This could be because the biogas reactors were overloaded with organics. It is suggested that commercial facilities interested in producing biogas mixed AGS-sludge with PS, as the results indicated that biogas with higher methane content and at a higher rate was produced with this mixture.

(Guo et al., 2020) tested waste aerobic granular sludge (sludge that is removed every cycle), mixed aerobic granular sludge (excess AGS-sludge that originates from biomass growth), primary sludge, and waste activated sludge in an anaerobic batch BMP test. The results indicated that the BMP of the mixed AGS-sludge was 80% of that of WAS. The BMP of the WAGS-sludge was 1.5 times higher than that of mixed AGS-sludge, due to the slow settling properties of highly biodegradable cellulose fibers that end up in the waste aerobic granular sludge. Primary sludge obtained the highest BMP value followed by the BMP of WAGS-sludge which was just slightly lower. During this study the mixed AGS granules were crushed, however this did not affect the total amount of biomethane produced but did increase the production rate.

A study made by (Jahn et al., 2019) provided results contrary to the two studies described above. Anaerobic batch tests were performed to compare the methane yield of AGS's separated fractions (large granules and sludge flocs) and suspended activated sludge. The different AGS fractions were obtained after sieving with a 500 µm mesh. The methane production of the pure granules was 1.4 times higher than the flocculent fraction, and the methane yield of mixed AGS-sludge was slightly higher than that of suspended activated sludge.

Previous studies have been made to measure how pretreatment can enhance biogas production from AGS. In a study made by (Liu et al., 2019) steam explosion at 170 °C and normal thermal treatment at an autoclave with 70, 100, and 125 °C were tested on AS, and AGS with two different calcium levels: 25 mg/l and 100 mg/l. The results showed that biogas production depends on the granules' mineral content as it lowered with higher mineral contents. A linear relationship was found between biogas production of aerobic granules and thermal treatment temperature in an autoclave. Additionally, steam explosion improved methane production of aerobic granules more than that of activated sludge. In a study made by (Val Del Río et al., 2014) the anaerobic digestion of both raw and pre-treated at 133 °C in an autoclave AGS and AS for 20 minutes was tested in a continuously fed-batch stirred tank reactor. The results showed that the anaerobic biodegradability of AGS was similar to that of AS. The thermal pretreatment of AGS at 133 °C improved the performance of the anaerobic digester by enhancing the biodegradability of the sludge and solids reduction. The thermal pretreatment performed better on the AGS than on the AS, improving its production by 48%. In a study performed by (Cydzik-Kwiatkowska et al., 2022) biogas potential of pretreated AGS with ultrasound and untreated AGS were tested in batch

assays with three different OLRs (1, 2, and 3 kg VS/m3 d). The different OLRs did not influence the biogas yield and methane content. After pretreatment the digestion time was reduced by 25% in comparison to that of untreated AGS. Ultrasound enhanced the solubility of organic matter and a higher methane content in the biogas produced was observed.

Table 2. BMP of previous studies.

Study	BMP AGS	BMP AS	Important Remarks
(Bernat et al., 2017)	**WAGS:** 480 – 600 ml/g VS (56 - 60% CH_4) **WAGS + PS:** 518 – 560 ml/gVS (59-62% CH_4)	**WAS:** 731 – 1115 ml/g VS (60 – 63% CH_4) **WAS + PS:** 982 -1032 ml/gVS (61-65% CH_4)	AGS-Sludge taken from a 3-column laboratory-scale aerobic granular-sludge batch reactors (GSBR) No information on primary clarifiers Cycle of 6 – 12 h
(Guo et al., 2020)	**WAGS:** 296 ± 15 ml CH_4/gVS **Mixed AGS:** 194 ± 10 ml CH_4/gVS	**WAS:** 232 ± 11 ml CH_4/gVS **PS:** 313 ± 11 ml CH_4/gVS	AGS-Sludge taken from a full-scale municipal WWTP fed with influent, crude water Cycle of 6 h SRT of AGS plant was of 28 days 5% VS
(Jahn et al., 2019)	**Mixed AGS:** 260 ml CH_4/gVSS with SRT 25 days **Mixed AGS:** 169 ml CH_4/gVSS with SRT >40 days	**WAS:** 240 ml CH_4/gVSS	AGS-Sludge taken from laboratory-scale SBRs fed with sewage from a municipal WWTP 4-5% TSS Cycle of 3 – 6 h SRT of AGS varied from 25 to more than 40 days No information on primary clarifiers

(Liu et al., 2019)	**Mixed AGS with calcium level 25 mg/l:** 298 ml CH_4/gVS **Mixed AGS with calcium level 100 mg/l:** 225 ml CH_4/gVS **With steam explosion at 170 °C:** **Mixed AGS with calcium level 25 mg/l:** 380 ml CH_4/gVS **Mixed AGS with calcium level 100 mg/l:** 344 ml CH_4/gVS	**WAS:** 266 ml CH_4/gVS **WAS with steam explosion 170 °C:** 316 ml CH_4/gVS	AGS-sludge taken from two SBR fed with synthetic wastewater at two different calcium levels (25 mg/l & 100 mg/l) Cycle of 3 h No information on primary clarifiers
(Val Del Río et al., 2014)	**Untreated WAGS:** 208 ± 51 mLCH4/gVS **With thermal pretreatment in an autoclave for 133 °C:** 309 ± 58 ml CH4/gVS **PS + thermally treated WAGS:** 343 ml CH4/ gVS	**Untreated WAS:** 254 ± 31 mLCH4/gVS **With thermal pretreatment in an autoclave for 133 °C:** 285 ± 22 mlCH4/gVS	AGS-sludge collected from a pilot plant SBR Reactor fed with liquid fraction of pig slurry SRT of 20 days No information on primary clarifiers
(Cydzik-Kwiatkowska et al., 2022)	**Untreated WAGS:** 375 ml CH4/gVS with CH4 content of approximately 60% **WAGS with ultrasound pretreatment:** 455 ml CH4/g VS with CH4 content of approximately 66%	NA	AGS-sludge taken from a full-scale plant Cycle of 4.8 h Sludge age of 30 days No primary clarifier

2.5. Anaerobic digestion

Anaerobic digestion is one of the most common technologies used by WWTPs to stabilize sludge due to its several advantages. It destroys most of the pathogens present in the sludge, reduces the amount of final sludge solids, and limits odor problems. Additionally, it can transform organic matter into biogas, which typically contains 60% methane and 40% carbon dioxide (Appels et al., 2008). Biogas is a renewable energy source for combined heat and power generation (CHP). This biogas can be upgraded to biomethane by removing CO_2 content (Adnan et al., 2019). The upgraded biogas can be injected into the gas grid and converted to compressed or liquefied renewable natural gas to serve as a transport fuel (Hakawati et al., 2017).

Anaerobic digestion is a complex process. The formation of methane is accomplished in four different steps: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis.

Hydrolysis is the first step of the anaerobic digestion process and it can be regarded as a biological pretreatment of the substrate (Menzel et al., 2020). During this step insoluble organic materials and higher molecular mass compounds are transformed into soluble organic materials. Lipids, polysaccharides, proteins, fat, and nucleic acids are transformed into simple organic compounds such as monosaccharides and amino acids. Biodegradation is performed by extracellular enzymes which split the large molecules into smaller ones that microorganisms take into their cell and use it as a form of energy (Merlin Christy et al., 2014; Adekunle & Okolie, 2015).The rate of bioconversion of the substrate depends on different parameters such as particle size, pH, production of enzymes, diffusion and adsorption of enzymes to particles (Gavala et al., 2003)

Acidogenesis, also referred to as fermentation, is the second step of the anaerobic process, during which anaerobic bacteria degrade the monomers produced during the hydrolysis process into short chain organic acids such as volatile fatty acids, hydrogen, and alcohols (Adekunle & Okolie, 2015;Córdova & Chamy, 2020). To maintain a stable process, the concentrations of the generated products must be balanced. If the hydrogen pressure is too high, this could diminish the number of compounds in the reactor (Córdova & Chamy, 2020).

Acidogenesis is followed by the acetogenesis process. In this third phase, different microorganisms consume acids and alcohols, degrading them into carbon dioxide, hydrogen, and acetic acid (Córdova & Chamy, 2020). This conversion is controlled by the partial pressure of hydrogen in the mixture (Appels et al., 2008), and syntrophic activity between microorganisms is fundamental for them to consume hydrogen and produce methane (Córdova & Chamy, 2020)

Methanogenesis is the final phase of the anaerobic digestion process. It is the most critical and slowest phase since the microorganisms that perform it are sensitive to environmental factors and their growth is slow (Córdova & Chamy, 2020). Two groups of methanogenic archaea produce methane by splitting acetate into methane and carbon dioxide, and by using hydrogen as an electron donor and carbon dioxide as an electron acceptor (Appels et al., 2008).

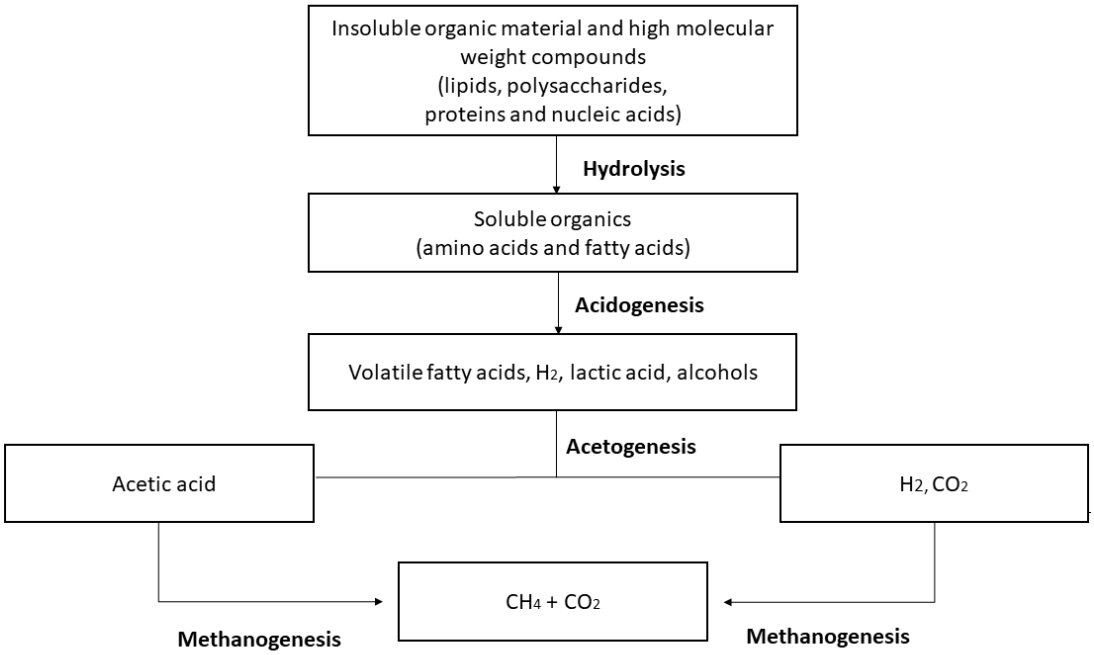

Figure 2. Phases of the anaerobic digestion process (Adapted from Appels et al., 2008).

2.6. Biogas Production at the Rya WWTP

In the year of 2022, Gryaab produced 10,670,363 m^3 biogas from sludge generated at the treatment plant, grease, and food waste from restaurants and schools in the Gothenburg region. The biogas produced at Gryaab is ISCC (International Sustainability Carbon Certification) certified. The certification means that the biogas produced meets the European Union's requirements on promoting the use of renewable energy sources (Gryaab AB, 2022a).

The anaerobic digestion of biomass at the Rya WWTP is performed in two digestion chambers with a height of 30 m and a volume of 11,400 m^3 each where sludge level is maintained constant. They are followed by one unheated digester with a height of 20 m and a volume of 4,260 m^3 where the sludge level can vary depending on the amount of sludge that can be sent to the dewatering units. The sludge is first conditioned with the addition of a polymer and dewatered in gravity belt thickeners to increase its TS % content. The digesters run in series and the digestion time is around three weeks. The first and second digesters are heated to 35°C. The third digester has no external heating and from here the sludge is pumped to a screw press where the sludge is dewatered to a TS content of 25%-30%. The reject water produced by the dewatering process is filtered and led either to the reject water treatment, trickling filters, activated sludge process, or mixed with decant water from the thickener and returned to the water canal after pre-sedimentation. The dewatered sludge is later sanitized to be used as a fertilizer for agricultural land or composted to be used in soil products (Gryaab AB, 2020). Gryaab is under the REVAQ certification system, meaning the sludge spread on Swedish arable land is of good quality and creates a strong partnership between the water industry, agriculture, and food industry (Svenskt Vatten, 2023).

The biogas produced is kept under pressure and later sold to Göteborg Energi, the company owned by the City of Gothenburg in charge of delivering and developing solutions to energy and urban fiber. They upgrade the biogas to 99% methane and let it out to the local natural gas grid (Gryaab AB, 2021) The profit Gryaab gains from biogas production is used to cover up to 25% of the cost of the total cost of running the wastewater treatment (Gryaab AB, 2022a).

3. Methods

In this section the methods used to address the aim of the master thesis are presented.

3.1. Literature Review

A literature review was first conducted by using different databases such as Scopus, Google Scholar, Web of Science, and reports produced by Gryaab AB. The literature review focused on the following themes:

- Biogas production from aerobic granular sludge with anaerobic digestion
- Characteristics of aerobic granular sludge and activated sludge
- Biomethane potential tests and AMPTS's functionality

3.2. Experimental Work

The experimental work of this master thesis consisted of experiment design, sludge sampling, TS and VS analysis, thickening of sludge samples, and experiment setup by using AMPTS II equipment.

3.2.1. AGS pilot plant at the Rya WWTP

The pilot plant used for sampling AGS in this project was installed at the Rya WWTP to gain experience and build confidence on the AGS technology. The main objectives were to find out what effluent quality can be expected from running the process with pre-settled and influent wastewater and to elucidate the effect of methanol dosing on effluent concentrations when operating with pre-settled effluent.

The pilot consists of one reactor, an influent buffer, a waste buffer, and an effluent buffer. It is fully equipped with instruments, samplers, SCADA, and Nereda® controller for process control. The flow to the reactor has been scaled to the size of the pilot but is representative to the flow of the full-scale plant.

Table 3. AGS Pilot Plant Process Information.

Parameter	Unit	Value
Average N-Load	g/m^3	59
Average volumetric Load	$m^3/m^3/d$	2.1
Average cycle time	hr	4
Dates of Pre-settled wastewater influent		14/9/23- 16/2/23
Dates of Crude wastewater influent		17/2/23 – 21/4/23
Pre-settled influent + methanol dosing		22/4/23 – 20/6/23

Figure 3. AGS pilot plant at the Rya WWTP.

3.2.2. Design of the experiments

Since one of the objectives of the master thesis was to determine if primary sedimentation affected the biogas potential of the AGS, two different set-ups were proposed.

For Setup I the pilot plant was fed with pre-clarified water. It consisted of Mixed and Waste AGS samples, WAS, inoculum, and cellulose as a control substrate. For Setup II the pilot plant was fed with crude, influent water. This group of samples consisted of mixed and waste AGS, primary sludge, inoculum, and cellulose as a control substrate.

The inoculum alone (digested sludge) was subject to anaerobic conditions in the AMPTS II to be able to find the BMP of the substrate by subtracting its BMP from the BMP produced by the sludge and inoculum mixture. A control, in this case powdered cellulose mixed with distilled water, was put under anaerobic digestion to compare its biogas potential to literature values and test the quality of the inoculum.

For Setup I, WAS and Cellulose were tested in duplicate and not in triplicate. This is because two WAGS-sludge bottle samples were added one week after the start of the experiment due to time constraints with N2 flushing on the day of the experiment. This made the sampling and testing of the inoculum used for these two new bottles necessary as well. Additionally, the AMPTS II at the Rya WWTP consists of only 14 cells, therefore cellulose was tested in duplicate for Setup II too.

Table 4. Types of sludge tested.

Sample	Definition
Waste Activated Sludge (WAS)	Sludge produced from secondary sedimentation and composed of microorganisms (Xu et al., 2020)
Primary Sludge (PS)	Sludge produced from primary sedimentation with a high content of organic matter (Bernat et al., 2017)
Waste Aerobic Granular Sludge (WAGS)	Flocculent sludge that is removed every cycle, with a lower settling velocity than the granules (Guo et al., 2020)
Mixed Aerobic Granular Sludge (Mixed AGS)	Excess that originates from biomass growth and is removed to avoid too high concentrations of biomass in the reactor (Guo et al., 2020)
Cellulose	Standard substrate used to test the quality of the inoculum (BPC Instruments, 2022)
Inoculum	Digested sludge used to enhance anaerobic biodegradability (Quintero et al., 2012)

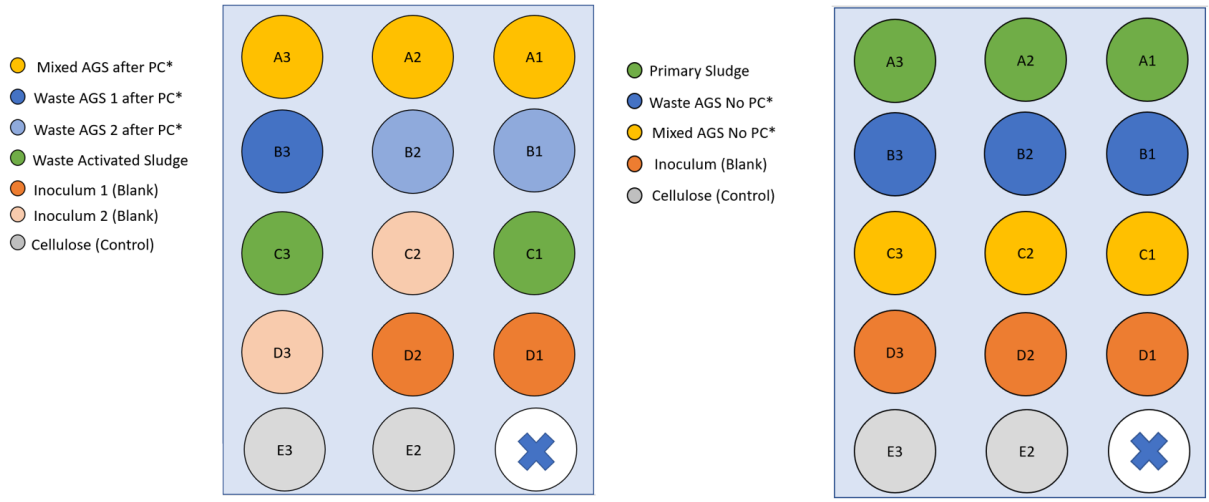

*Figure 4. Setup I (Left) and Setup II (Right). *PC is referred to as Primary Clarification.*

3.2.3. Sludge Sampling

Waste AGS-sludge, mixed AGS-sludge, PS, and WAS were sampled to compare the biogas potential of both AGS and AS technologies. The AGS samples were collected from the AGS pilot plant located at the Rya WWTP. The mixed AGS-sludge samples were taken from the reactor ten to fifteen minutes after the aeration phase had started to ensure fully mixed conditions. The WAGS-sludge samples were collected from the WAGS-sludge tank located next to the reactor in the pilot plant. The WAS and PS samples were collected from the sludge pipes exiting the respective processes at the Rya WWTP. The sludge age of the WAS was 6 days. Inoculum samples were collected from digester 3 which is not heated and acts as a buffer in the anaerobic digestion plant. The samples were taken from this digester since the inoculum was expected to have a lower background BMP as the digesters run in series.

Sludge sampling was performed for different purposes. For Setup I, the first samples were taken on January 31st, to determine with TS and VS analysis if the organic content of the different samples was in the recommended range of 20-60 gvs/L (Holliger et al., 2016). On February 2nd, sludge samples were taken to test if decanting and centrifugation would increase the TS and VS content of the samples. For Setup II, the first samples were taken on March 6th to measure their VS content and on March 10 to test decanting and centrifugation to increase their VS% content. The procedure used to calculate TS and VS is further explained in Section 3.3

For the official experiment, the sampling for Setup I was performed on February 9th and February 14th whereas the sampling for Setup II was performed on April 11th. All samples were collected and used to prepare the bottles on the same day of sampling. The weather conditions of the two sampling dates were different as higher precipitation was obtained the days before sampling in February for Setup I than the days prior the sampling in April for Setup II as can be seen in Appendix B (SMHI, 2023).

Prior the sampling of AGS-sludge for Setup I, the pilot plant had been running for 159 days straight. Whereas for Setup II, the pilot plant had been running for 54 days straight. From March 17th to March 27th the pilot stopped operating due to a faulty compressor and delayed the sampling for Setup II.

The characteristics of the AGS samples are detailed in Table 5.

Table 5. AGS parameters on sampling days.

Parameter	Unit	09-Feb	14-Feb	11-Apr
Temperature	°C	11.0	12.0	13.4
MLSS	g/L	4.91	4.91	5.75
Volumetric Load	$m^3/m^3/d$	2.80	2.04	1.51
Sludge production	l/d	240	199	198
COD load	kg $COD/m^3/d$	0.48	0.57	0.30
Share granules > 200 µm	%	88	88	79
Sludge Age	d	41	46	17
WAGS concentration	mg/l	750	800	2600

Figure 5. AGS samples taken for centrifugation test.

3.2.4. TS and VS Analysis

The Total Solids (TS) and Volatile Solids (VS) analysis was conducted at the laboratory at the Rya WWTP for all the different samples before and after digestion, including the inoculum from digester 3.

TS or total solids is defined as all the organic and inorganic compounds in wastewater, whereas Volatile Solids is defined as the organic compounds in wastewater. The VS can be represented as a % of TS (BPC Instruments, 2022).

The process to calculate TS included the following steps:

1. Weigh the dish and record its value.
2. Add 10-12 g of representative sample on the dish.
3. Weigh the dish + representative sample.
4. Add the dish to a 105°C oven for 20 h so that the volatiles evaporate.
5. Take the dish out of the oven, weigh it, and record this value.

The process to calculate VS included the following steps:

1. Transfer the dish into the ignition oven, preheated at 550 °C.
2. Take the dish out of the ignition oven after 2 h.
3. Weigh the dish and record this value.

The TS analysis performed to find out if the samples were in the range of 20 – 60 gVS/l were performed with an IR moisture analyzer at 130 °C due to its rapidness, as TS% can be obtained after 30 minutes. This provided the time to plan for the sludge thickening process before setting up the experiments.

The TS% was calculated with the following formula:

Equation 1

$$TS\ (\%) = \frac{m_{Dried}}{m_{Wet}} \times 100$$

The VS (%) was calculated with the following formula:

Equation 2

$$VS\ (\%) = \frac{m_{Dried} - m_{Burned}}{m_{Wet}} \times 100$$

Equation 3

$$VS\ (\%)\ of\ TS\ = \frac{VS\ (\%)}{TS\ (\%)} \times 100$$

Where m_{Dried} – is the weight of dried sample
m_{Wet} – is the initial weight of wet sample
m_{Burned} –is the weight of the sample after drying and burning

3.2.5. Thickening of sludge samples

To increase the VS content of the sludge samples to 2.5%, they were centrifuged with a Hermle Z 510 centrifuge at a velocity of 3,700 rev/min for ten minutes. Since the VS content was very low in the AGS samples, it was necessary to decant the samples first before centrifuging. Following the centrifugation process, a part of the supernatant was removed in each of the bottles and the pellet was mixed with the remaining supernatant to achieve a minimum VS of 2.5%.

Figure 6. Decanting of WAGS-sludge sample.

Figure 7. Centrifugator Hermle Z 510 and sludge samples after centrifugation.

Figure 8. Mixed AGS-sludge samples after centrifugation.

Figure 9. Mixed AGS-sludge thickened sample.

3.2.6. Preparation of bottles

The AMPTS II equipment at the Rya WWTP has fourteen cells or bottles that can be used for experimentation. A mixture of inoculum and substrate of 400 g was added to each of the bottles as recommended by BPC instruments when using 500 ml bottles. The inoculum was added to enhance anaerobic biodegradability and make the digestion process more stable (Quintero et al., 2012).

The mass of inoculum and substrate added to each bottle was calculated with an inoculum to substrate ratio of 2:1 and 400 g of substrate added to each bottle, leading to Equation 4 and Equation 5 (BPC Instruments, 2022). It is recommended that the portion of VS from the inoculum is greater than that of the substrate to minimize acidification or inhibition problems (Holliger et al., 2016). A 2.5% VS was assumed for each of the substrates and a 2.0% VS was used for the inoculum. This value was chosen to comply with the 20 – 60 gVS/l range stated by (Holliger et al., 2016) to prevent underestimations in the methane potential or overloaded situations which could result in a slow AD process inhibition (Wang et al., 2015). However, the actual obtained VS% values after thickening the samples by decanting and centrifugation deviated slightly from the desired 2.5% (See Table 6). Therefore, the inoculum to substrate ratio of 2:1 could have been affected in some samples.

The calculation resulted in a mass of 115 g of substrate and 285 g of inoculum. For the control samples, 2.90 g of powdered microcrystalline cellulose was added to 115 ml of distilled water and the blank samples were filled with 400 g of only inoculum. In Appendix A, the amount of inoculum and substrate added to each of the bottles for Setup I and II can be found.

After adding the same mass of substrate and inoculum to each of the bottles, they were flushed for 60 seconds at the Rya WWTP laboratory with pure nitrogen gas to create fully anaerobic conditions as recommended by (Holliger et al., 2016).

Equation 4

$$m_{IS} = \frac{800 \cdot VS_S}{(VS_I + 2 \cdot VSs)}$$

Equation 5

$$m_{sS} = 400 - m_{IS}$$

Where - m_{IS} - Mass of inoculum added to the sample
m_{sS} - Mass of substrate added to the sample
VS_S - VS of substrate
VS_I - VS of inoculum

3.2.7. Start of the AMPTS II

The AMPTS II equipment is an analytical tool used to determine the anaerobic biodegradability and potential of waste and biomass. It consists of three different units: "A", "B", and "C". Unit "A" is the Sample Incubation Unit where fourteen vials containing inoculum and sample are incubated at a chosen temperature with a water bath. The samples are mixed with a slow rotating agitator at a chosen speed. Biogas is produced and channeled to Unit "B", known as the CO_2-Absorbing Unit. Here, fourteen different bottles containing NaOH allow only CH_4 to pass through to Unit "C", known as the Gas Volume Measuring Device. In Unit "C", the volume of CH_4 released from Unit B is quantified with a wet gas flow measuring device which works according to the principle of liquid displacement and buoyancy and can monitor ultra-low gas flows. When a defined volume of gas flows to the device a digital pulse is generated. An embedded data acquisition system is used to record, display, and analyze results (BPC Instruments, 2022).

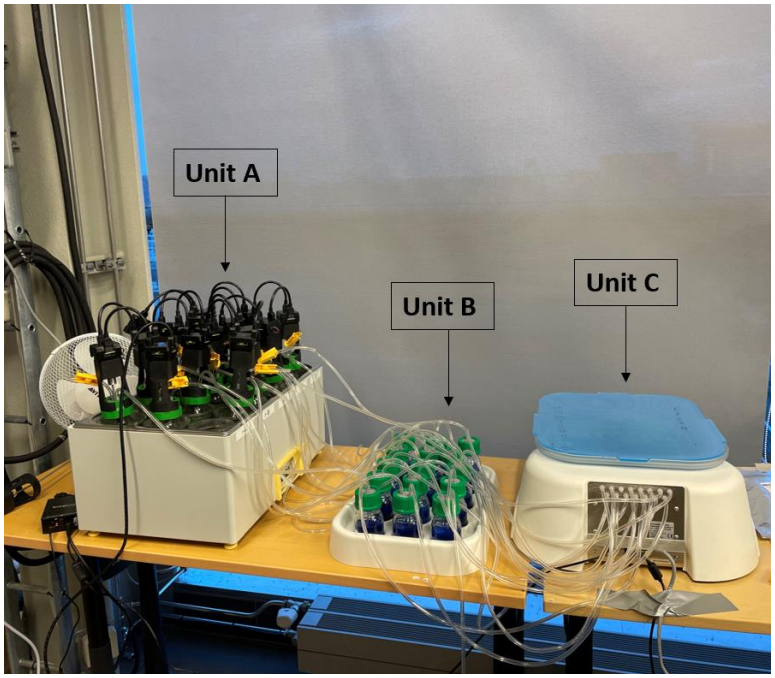

Figure 10. AMPTS II equipment used for experimentation.

After adding the respective volume of sludge and inoculum to the bottles, these were connected to their individual motor. Water was added to the sample incubation unit and the temperature was set. (Holliger et al., 2016) recommends that the BMP tests should be performed in the range of 37°C to 55°C. However, if the inoculum is taken from a digester at another temperature than this, the BMP should be carried out at this temperature as well. The sample incubation unit was consequently set to 35°C to simulate the digestion conditions at the Rya WWTP. Each of the bottles was connected to a bottle filled with Sodium hydroxide (NaOH) in the CO_2 fixing unit where CO_2 was absorbed by the alkaline solution, allowing only CH_4 to reach the monitoring unit.

Several settings were adjusted in the AMPTS II system. For example, the total amount of the sample was set to 400 ml, the temperature was set to mesophilic condition with a temperature of 35 °C, the percentage of CO_2 in the flush gas was set to 0, and the overestimation that was expected to occur due to the flushing with N2 gas was automatically eliminated by the AMPTS II.

For the motors, the speed adjustment was set to 30%, the mixers on-time was set to 10 min, and the mixers off-time was 0 min.

3.2.7. Maintenance of the AMPTS II during digestion

The AMPTS II equipment was checked daily during digestion (except on the weekends), to download daily results and provide proper maintenance to the equipment. Due to the mesophilic conditions of Unit "A" or the Sample Incubation Unit, water evaporated and was refilled every day. Distilled water was added to the gas volume measuring device whenever the water level lowered from the recommended level. The NaOH on each of the CO2 fixation bottles was replaced whenever needed. It was also monitored that the motors placed on each of the bottles were turned on and correctly mixing the samples.

Setup I was digested for 32 days due to the addition of two WAGS-sludge bottles after one week of the start of the experiment as explained in Section 1.2 Limitations and Setup II was digested for 21 days to provide enough time for the degradation of organic matter.

3.4. Degree of Digestion

The degree of digestion was calculated for each of the five mixtures of inoculum and substrate (WAS, PS, WAGS-sludge, mixed AGS-sludge, microcrystalline cellulose). It is defined as the percentage of organic material broken down and converted into biogas during the anaerobic digestion process (Schnürer, 2018).

The degree of degradation was calculated with the following formula (Schnürer & Jarvis, 2009):

Equation 6

$$Degree\ of\ Digestion\ (\%) = \left(\frac{TS_{IN} \times VS_{IN} - TS_{OUT} \times VS_{OUT}}{TS_{IN} \times VS_{IN}}\right) \times 100$$

Where TS_{IN} = TS% content before the start of the experiment

TS_{OUT} = TS% content after the experiment

VS_{IN} = VS% content of TS% before the start of the experiment

VS_{OUT} = VS% content of TS% after the experiment

3.5. Biomethane Potential Calculation

The AMPTS II software started producing data immediately after setting up the experiment. It provides the user with an Excel document with the hourly volume in $NmlCH_4$ of the biomethane produced per bottle.

To calculate the final biomethane potential of each substrate, the following formula was used ((BPC Instruments, 2022):

Equation 7

$$BMP = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,sS}}$$

V_S – accumulated volume of methane produced from the reactor with sample (i.e., inoculum and substrate)

V_B – mean value of the accumulated volume of methane produced by the three blanks

m_{IS} – total amount of inoculum in the sample

m_{IB} – total amount of inoculum in the blank

$m_{VS,sS}$ – amount of organic material (i.e., volatile solids) of substrate contained in the sample bottle

4. Results and Discussion

The findings from the experiments are discussed in this section. This includes TS and VS analyses, degree of digestion, accumulated BMP, and daily biomethane production.

4.1. TS and VS Analyses

As mentioned in Section 3.2.4, TS and VS Analyses were performed on representative samples to determine the amount of inorganic and organic material in each sludge sample. As seen in Table 6, the VS% values for each of the samples before centrifugation were below the recommended range of 20 – 60 g_{vs} L-1 and the desired VS% of 2.5% for the experiment. Therefore, it was necessary to thicken the samples with the detailed procedure in Section 3.2.5. The results of the TS% and VS% obtained after centrifugation are described in Table 6.

Table 6. TS% and VS% Before and After Centrifugation.

TS% and VS%					
SETUP 1					
	Before Centrifugation		**After Centrifugation**		
Sample	**TS (%)**	**VS (%) of TS**	**TS (%)**	**VS (%) of TS**	**VS (%)**
WAS	0.8	-	4.07	69.29	2.82
WAGS	0.27	66.67	2.95	74.24	2.19
Mixed AGS	0.25	64	3.17	76.34	2.42
SETUP 2					
PS	2.01	79.10	3.55	82.53	2.93
WAGS	0.41	53.66	3.42	77.19	2.64
Mixed AGS	0.76	77.63	4.10	80.73	3.31

4.2. Degree of Digestion

The degree of digestion was calculated as explained in Section 3.4 for each of the samples to determine their biodegradability. The required data for this calculation was the TS% and VS% of each mixture before and after digestion. The TS% and VS% of each mixture before and after digestion can be found in Table 7. As expected, the inorganic and organic content lowered for each mixture after digestion.

Table 7. TS% and VS% before and after digestion.

Setup I				
	Before Digestion		**After Digestion**	
Sample Mixture	**TS (%)**	**VS(%)**	**TS (%)**	**VS(%)**
WAS + Inoculum	3.23	62.54	2.80	50
WAGS + Inoculum	3.07	62.87	2.60	54.62
Mixed AGS + Inoculum	2.97	64.31	2.63	54.37
Cellulose + Inoculum	2.78	68.35	2.05	54.63
Inoculum	3.12	58.65	2.88	53.47

Setup II				
	Before Digestion		After Digestion	
Sample Mixture	**TS (%)**	**VS(%)**	**TS (%)**	**VS(%)**
PS + Inoculum	3.24	65.43	2.33	54.08
WAGS + Inoculum	3.21	63.24	2.54	53.93
Mixed AGS + Inoculum	3.40	65.59	2.64	57.95
Cellulose + Inoculum	2.94	67.01	2.03	53.69
Inoculum	3.12	57.37	2.62	53.82

In Figure 11, the results of the degree of digestion are presented. For both Setup I and Setup II, the cellulose + inoculum mixture presented the highest degradation as expected, followed by the PS + inoculum mixture. The inoculums used in both setups showed different biodegradation properties, as the one used in Setup II had a higher biodegradability. This could be the reason why the AGS + inoculum mixtures in Setup II presented similar biodegradability values to the WAS in Setup I. However, when calculating the degree of digestion for each individual substrate, the WAS had a higher degree of digestion than the AGS fractions.

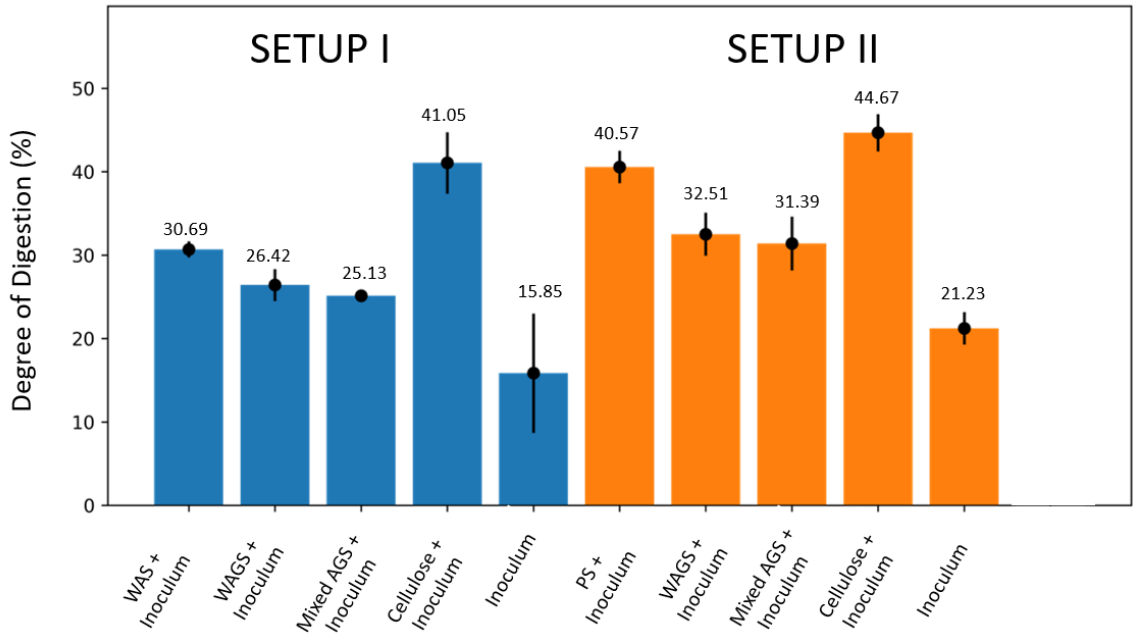

Figure 11. Degree of Digestion of Mixtures (%).

Table 8. Degree of Digestion of Mixtures (%).

Setup I			
Sample Mixture	**Degree of digestion of mixtures (%)**	**Substrate**	***Degree of digestion of substrate (%)**
WAS + Inoculum	30.69	WAS	*67.49
WAGS + Inoculum	26.42	WAGS	*52.64
Mixed AGS + Inoculum	25.13	Mixed AGS	*48.14
Cellulose + Inoculum	41.05	Cellulose	*103.52
Inoculum	15.85	Inoculum	15.85
Setup II			
Sample Mixture	**Degree of digestion of mixtures (%)**	**Substrate**	***Degree of digestion of substrate (%)**
PS + Inoculum	40.57	PS	*88.49
WAGS + Inoculum	32.51	WAGS	*60.48
Mixed AGS + Inoculum	31.39	Mixed AGS	*56.57
Cellulose + Inoculum	44.67	Cellulose	*102.76
Inoculum	21.23	Inoculum	21.23

*The degree of digestion (%) of the individual substrate was obtained from an estimate of the mixtures, therefore the total may exceed 100%.

4.3. Accumulated BMP of Setup I and Setup II

As explained in Section 3.2.7, Setup I was anaerobically digested for 32 days, and Setup II was anaerobically digested for 21 days. The results of the different BMPs obtained will be presented on day 21st for both setups since it is the average digestion time at the Rya WWTP. In Figure 11 the accumulated BMP results of each of the different substrates for 32 days in Setup I are presented. Microcrystalline cellulose, which was used as a model substrate to test the quality of the inoculum obtained the highest BMP from all samples as expected. Its BMP after 21 days of anaerobic digestion was 314 ± 11 mL CH_4/gVS, which was lower than the theoretical BMP of 350 ± 29 ml CH_4/gVS proposed by (Raposo et al., 2011). It is uncertain why the BMP was lower than literature values, but it could be related to the fact that the BMP of anaerobic inoculum is variable and was sampled from the third digester at the Rya WWTP which is not heated and possesses no constant level. At 21 days, the WAS presented a BMP of 287 ± 2 mLCH_4/gVS which is higher than the BMP presented in previous studies (see Table 10). This could be due to the short sludge age of WAS at the Rya WWTP, which is around 6 days, making more organic carbon available for energy recovery (Ge et al., 2017). It has been determined that with extended sludge ages the biodegradability of protein, polysaccharides, and lipids in sludge decline (Chen et al., 2020). The WAS's BMP was 1.6 times higher than the WAGS-sludge's BMP and 1.3 times higher than the mixed AGS-sludge's BMP. The Mixed AGS-sludge presented a higher BMP (213 ± 29 mL CH_4/gVS) in comparison to the Waste AGS-sludge's BMP (185 ± 10 mL CH_4/gVS), which was not expected as the mixed AGS-sludge had a bigger sludge age than the WAGS-sludge. However, it is shown in Figure 12 that the mixed AGS-sludge had a higher standard deviation which could be an error source in these bottles.

As can be seen in Figure 12 the WAGS-sludge and mixed AGS-sludge results are shorter than 32 days. This is because two WAGS-sludge bottles were added one week after the start of the experiments due

to time constraints as explained in Section 3.2.2 and for the mixed AGS-sludge bottle A3 stopped registering values for the last 36 hours of digestion. This can be attributed to the fact that the AMPTS II equipment records data once a defined volume of gas passes through the device meaning the production was too low at this time (BPC Instruments, 2022).

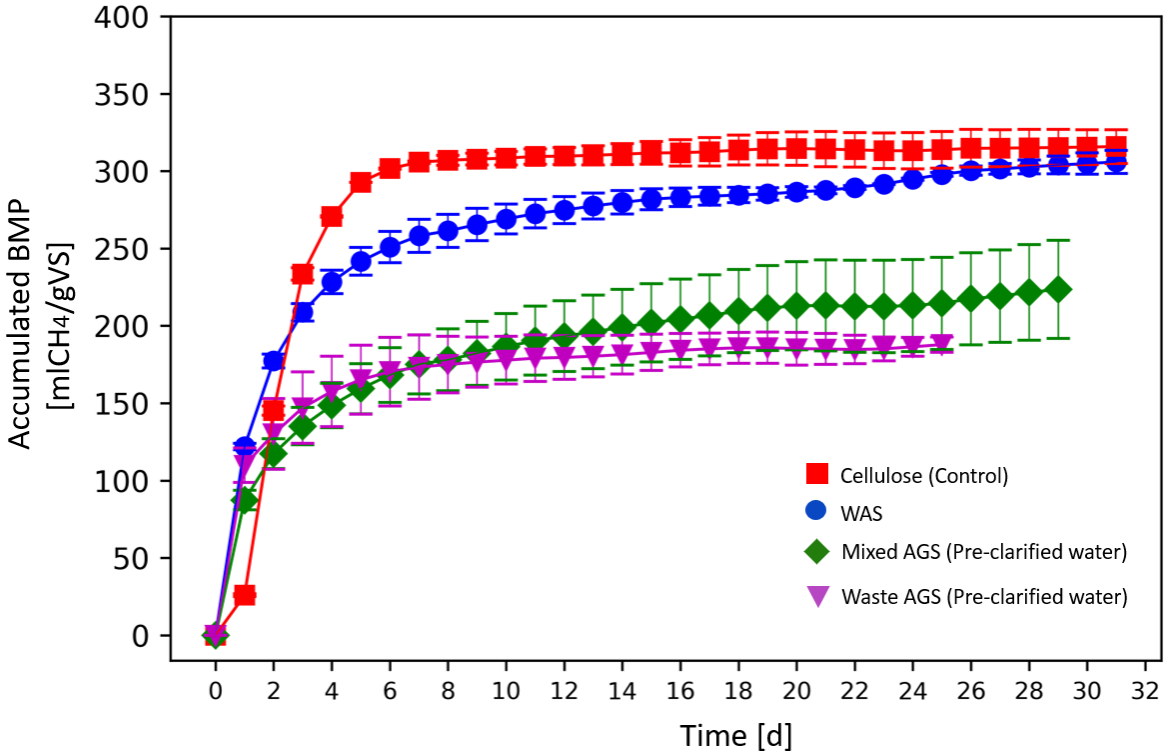

Figure 12. Setup I Accumulated BMP.

In Figure 13 the results from Setup II are presented. The cellulose presented a final BMP value of 352 ± 20 ml CH_4/gVS which is the range of the theoretical BMP of 350 ± 29 ml CH_4/gVS proposed by (Raposo et al., 2011). This indicated that the inoculum used for Setup II was of better quality than the one used for Setup I. This could be because the inoculum is variable and was sampled with a difference of two months (2023-02-09 for Setup I and 2023-04-11 for Setup II). PS presented the highest BMP value from both groups of samples, with 365 ± 7 ml CH_4/gVS. This is attributed to lack of cell walls PS as it is formed of colloidal organic matter ready to be converted to methane by anaerobes whereas WAS consists of cells of microorganisms that multiply during wastewater treatment (Bernat et al., 2017). This value is higher than the BMP obtained at the (Guo et al., 2020) study which was of 313 ± 11 ml CH_4/gVS. WAGS-sludge presented a BMP value of 223 ± 19 ml CH_4/gVS which was higher than the mixed AGS-sludge with 208 ± 15 ml CH4/gVS, however this difference is small, showing similar potential between both AGS-sludge fractions.

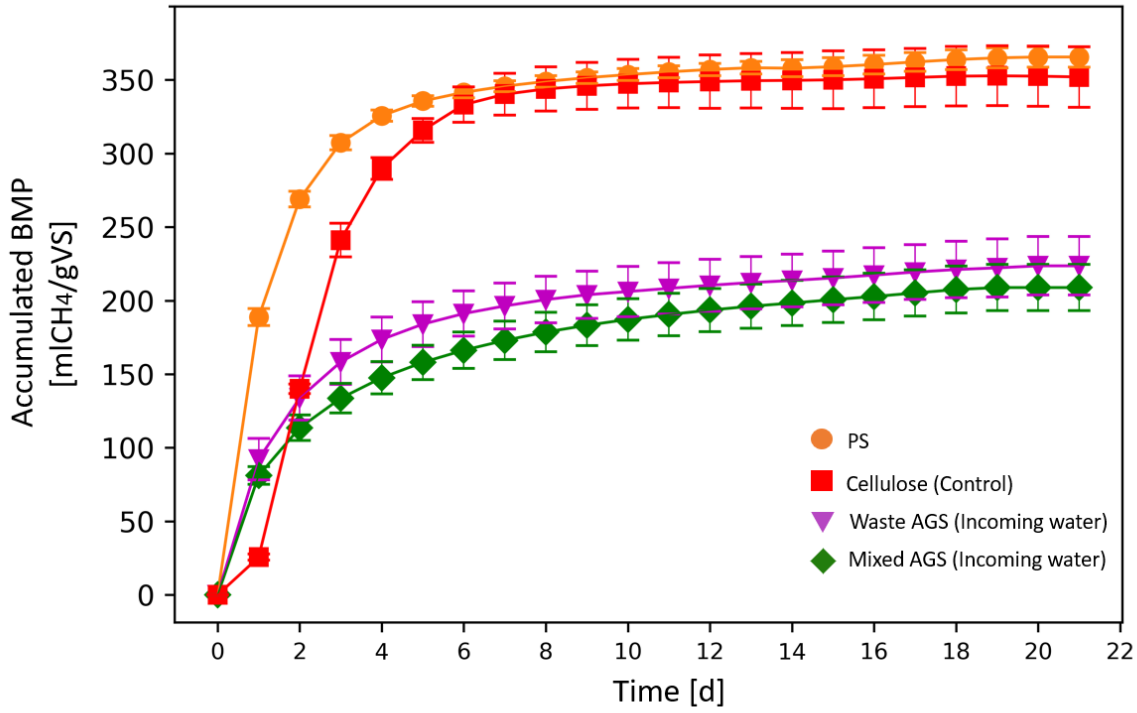

Figure 13. Setup II Accumulated BMP.

Table 9. BMP of Setup I and Setup II after 21 days of digestion

BMP (ml CH_4/gVS) after 21 days of digestion		
Substrate	**Setup I (pre-clarified AGS samples)**	**Setup II (crude AGS samples)**
Waste Activated Sludge	287 ± 2	NA
Primary Sludge	NA	365 ± 7
Waste AGS	185 ± 10	223 ± 19
Mixed AGS	213 ± 29	208 ± 15
Cellulose	314 ± 11	352 ± 20

In Table 10 below a comparison between the results obtained in this study and previous studies is presented. As it can be seen PS presented the highest BMP in both this study and the (Guo et al., 2020) study. As mentioned before, the BMP of the WAS of the Rya WWTP is higher than the BMP results obtained in previous studies. The BMP of the WAGS-sludge obtained in this study was lower than the values obtained in previous studies. This could be attributed to the long sludge ages when sampling the AGS-sludges from the pilot plant which varied from 17 – 46 days. The BMP of the mixed AGS-sludge in this study presented similar results to the results obtained in previous studies.

Table 10. Comparison of test results with previous literature.

Study	WAS	PS	Waste AGS-slduge	Mixed AGS-sludge
	BMP (ml CH_4/gVS)			
This study	287 ± 2 ml CH_4/gVS	365 ± 7 ml CH_4/gVS	185 ± 10 – 223 ± 19 ml CH_4/gVS	208 ± 15 – 213 ± 29 ml CH_4/gVS
(Bernat et al., 2017)	731 – 1115 ml/g VS (60 – 63% CH_4)	NA	480 – 600 ml/g VS (56 - 60% CH_4)	NA
(Guo et al., 2020)	232 ± 11 ml CH_4/gVS	313 ± 11 ml CH_4/gVS	296 ± 15 ml CH_4/gVS	194 ± 10 ml CH_4/gVS
(Jahn et al., 2019)	240 ml CH_4/gVSS	NA	NA	169 - 260 ml CH_4/gVSS
(Liu et al., 2019)	266 ml CH_4/gVS	NA	NA	225 ml CH_4/gVS – 298 ml CH_4/gVS
(Val Del Río et al., 2014)	254 ± 31 ml CH4/gVS	NA	208 ± 51 mLCH4/gVS	NA
(Cydzik-Kwiatkowska et al., 2022)	NA	NA	375 ml/gVS (60% CH4)	NA

4.4. BMP comparison of AGS fractions based on influent characteristics

In this section the effect of primary clarifiers on the BMP of both WAGS-sludge and mixed AGS-sludge will be elucidated. As mentioned in Section 3.2.2, for Setup I samples were taken when the AGS pilot plant was fed with pre-clarified water and for Setup II samples were taken when the AGS pilot plant was fed with crude, incoming water. As described in Table 2, most of the previous studies have tested AGS in plants fed with crude, incoming water. Therefore, it can be deducted that having primary clarifiers before the AGS process is not a common configuration. As explained in Section 3.2.3, the AGS pilot had been running for different times before sampling, 159 days for Setup I and 54 days for Setup II. Considering it takes time to reach stable granulation conditions (Nancharaiah & Kiran Kumar Reddy, 2018), this might have had an impact on the results.

In Figure 14, a comparison between the BMP of WAGS-sludge in Setup I and Setup II is presented. The BMP of the WAGS-sludge in Setup II was 1.15 times higher than the BMP in Setup II. This shows that pre-clarifying the water before entering the AGS process can lower the biomethane potential of the WAGS-sludge.

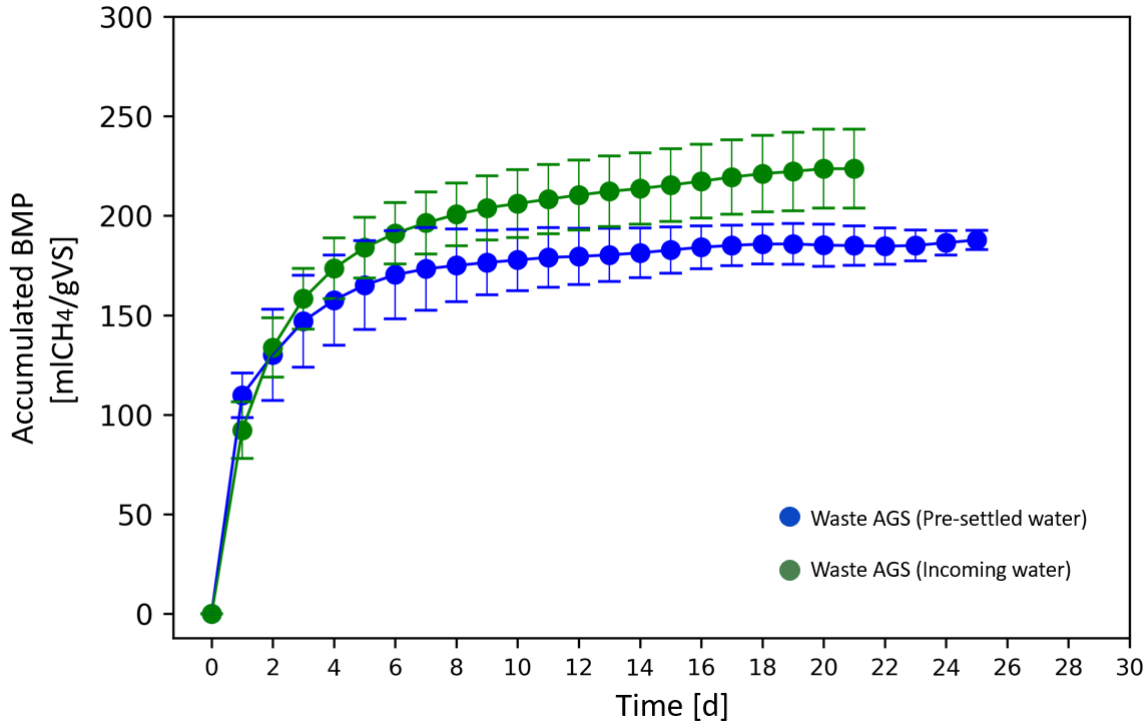

Figure 14. Comparison between WAGS in Setup I and Setup II.

Figure 15 a comparison between mixed AGS-sludge in Setup I and Setup II is presented. As can be seen, there was little to no variability between the BMP of both samples. Therefore, the BMP of this sludge fraction is not affected by feeding the AGS process with pre-clarified water. This is because the organic matter in the incoming, crude water which fed the AGS pilot plant presumably got attached to the flocs in the WAGS-sludge and not to the granules in the mixed AGS-sludge. When the pilot was fed with pre-clarified water, the most biodegradable parts of the incoming wastewater such as dissolved toilet paper, food scraps, and feces were removed in the PS lowering the biodegradability of the sludge produced in the AGS process.

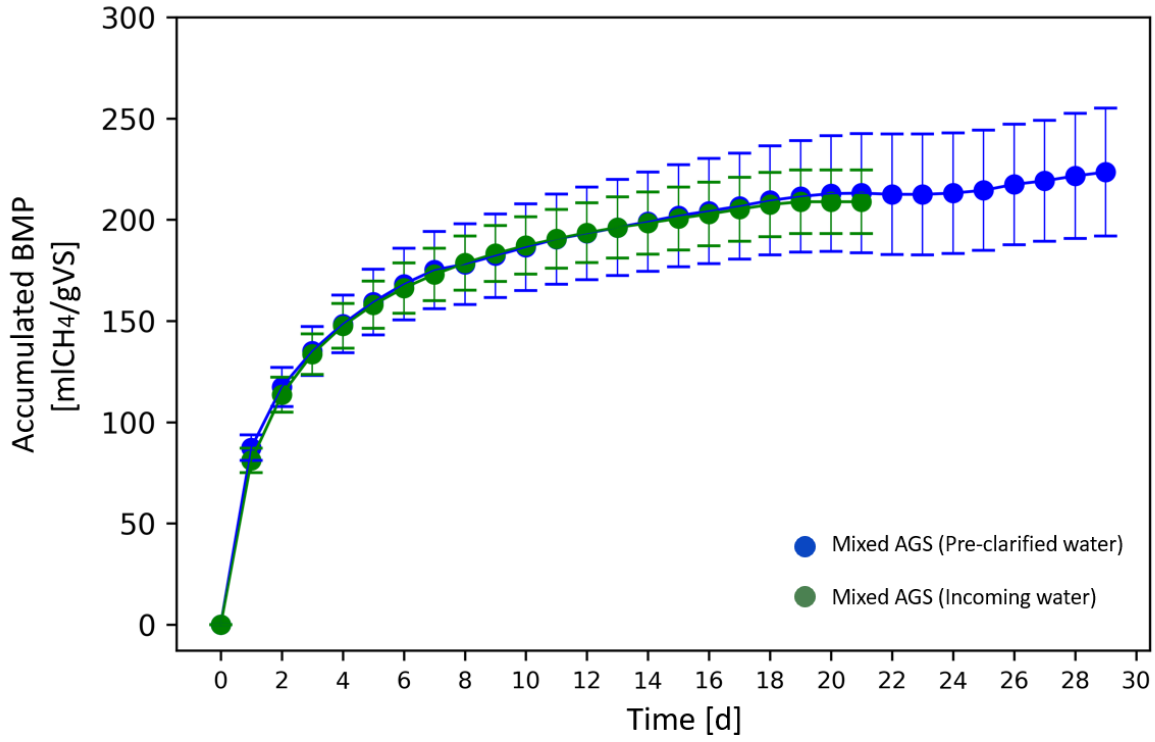

Figure 15. BMP Comparison between mixed AGS in Setup I and Setup II.

Table 11. BMP comparison between AGS samples in Setup I and Setup II.

BMP (mlCH4/gVS) after 21 days of digestion		
Substrate	**Setup I (pre-clarified AGS samples)**	**Setup II (crude AGS samples)**
Waste AGS	185 ± 10	223 ± 19
Mixed AGS	213 ± 29	208 ± 15

4.5. Daily BMP of Setup I and Setup II

In this section the daily production of each substrate from Setup I and Setup II will be discussed. The Daily BMP values were calculated to make sure that the biomethane production was low enough for digestion to stop. Since the data provided by the AMPTS II equipment is only of the accumulated BMP, the daily values were obtained by subtracting the accumulated BMP of a specific day by the BMP of the previous day.

As can be seen in Figure 16 and Figure 17, the highest production for all substrates was obtained during the first days of digestion. This means that the experiments could have been stopped earlier due to the low production observed with the passage of time. (Holliger et al., 2016) states that the BMP experiment should stop once the daily methane production for three consecutive days is <1% of the accumulated volume of methane. This criterion was achieved in Setup I for cellulose on day 9, for WAS at day 14, for WAGS-sludge (pilot fed with pre-clarified water) at day 10, and for mixed AGS-sludge (pilot fed with pre-clarified water) at day 21. This shows that mixed AGS-sludge fractions degrade at a slower rate than AS and WAGS-sludge. This can be attributed to the limited degradation capacity of proteins and carbohydrates in aerobic granules due to the structural differences in the EPS the sludges present (Guo et al., 2020). In previous studies such as the (Guo et al., 2020) the aerobic granules were crushed and this increased the degradation rate of samples, however, it was not performed for this thesis project.

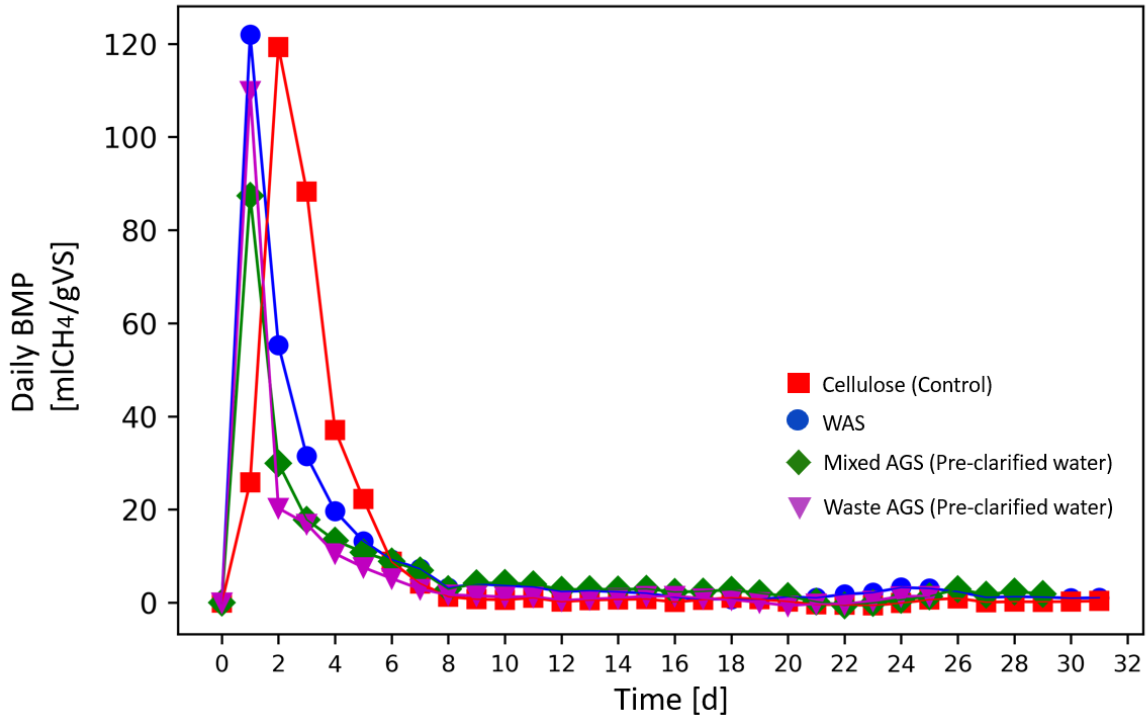

Figure 16. Daily BMP of Setup I.

In Figure 17 the Daily BMP of Setup II is presented. PS had the highest daily production rates, reaching a production of 188 ml CH_4/gVS on day 1. According to the criteria stated by (Holliger et al., 2016), the digestion of the cellulose sample could have stopped on day 11, for PS on day 10, for Waste AGS-sludge (pilot fed with crude, influent water) on day 14 and for mixed AGS-sludge (pilot fed with crude, influent water) on day 21.

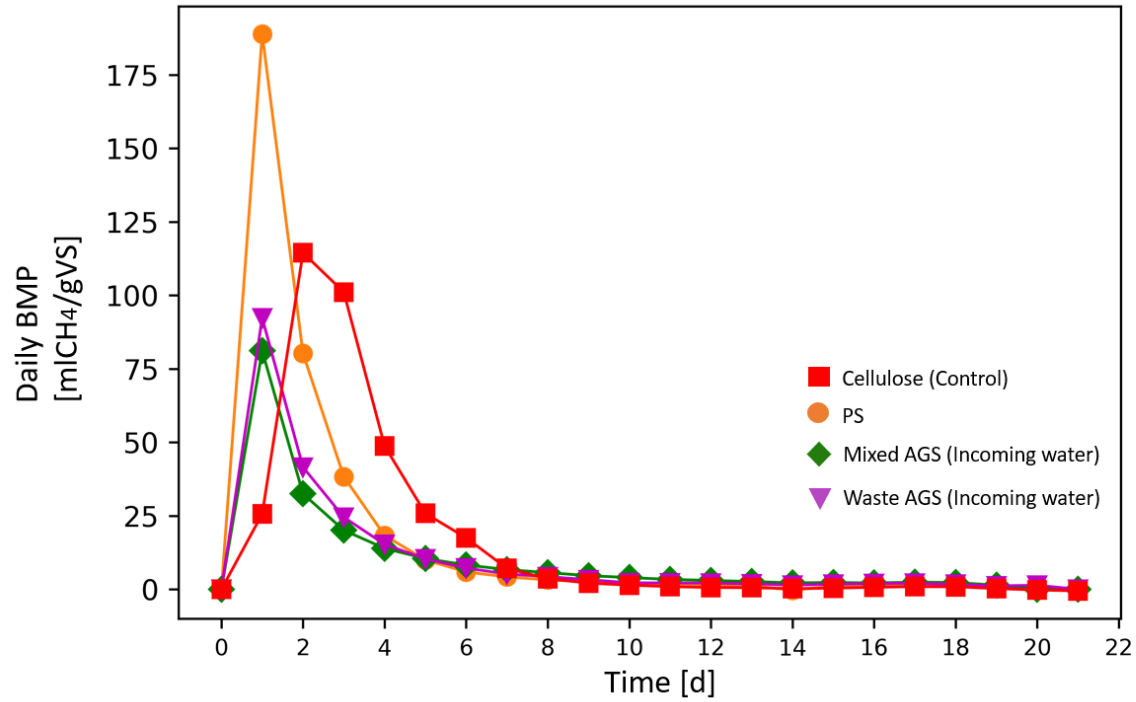

Figure 17. Daily BMP of Setup II.

The biomethane production of the AGS samples of the two different setups can be seen in Table 12. For the Waste AGS, it took 4 more days in Setup II to reach a daily production of less than <1% from the accumulated BMP. This means it degraded in a slower manner. It took the mixed AGS-sludge samples in both Setups 21 days to reach this criterion, therefore the production rate is not affected by the primary clarifiers and its digestion takes more time than for the WAGS-sludge. If the AGS process were to be fed with pre-clarified water, it would take less time for the WAGS-sludge to be digested.

Table 12. Highest daily BMP and Day of Biomethane Production <1%.

Substrate	Highest Daily BMP	Day of Highest Daily BMP	Day of Daily Production <1% from Accumulated
Waste AGS (Setup I)	109.84	1	10
Waste AGS (Setup II)	92.29	1	14
Mixed AGS (Setup I)	87.39	1	21
Mixed AGS (Setup II)	81.09	1	21

4.6. BMP of the inoculum

As mentioned in Section 3.2.3, the inoculum was sampled from digester 3 at the Rya WWTP. For the preparation of Setup I, the Inoculum 1 was tested on February 9th and the Inoculum 2 on February 15th for the two WAGS-sludge bottles which were added one week after the start of the experiment. For the preparation of Setup II, the inoculum was sampled on April 11th. The inoculum was used to create mixtures of inoculum to substrate in a 2:1 ratio to prevent inhibition (BPC Instruments, 2022).

The accumulated BMP of the three different inoculums used to prepare the mixtures can be seen in Figure 18. The two inoculums used in Setup I presented a higher BMP than the inoculum used in Setup II. The standard deviation of the Inoculum 2 in Setup I and the inoculum in Setup II is bigger than the standard deviation of Inoculum 1. Since the average volume of the blank reactors with the inoculum was subtracted from the volume of methane produced from each reactor with sample (inoculum + substrate) as in Equation 7 to find the BMP of each tested substrate, this could be considered as an error source.

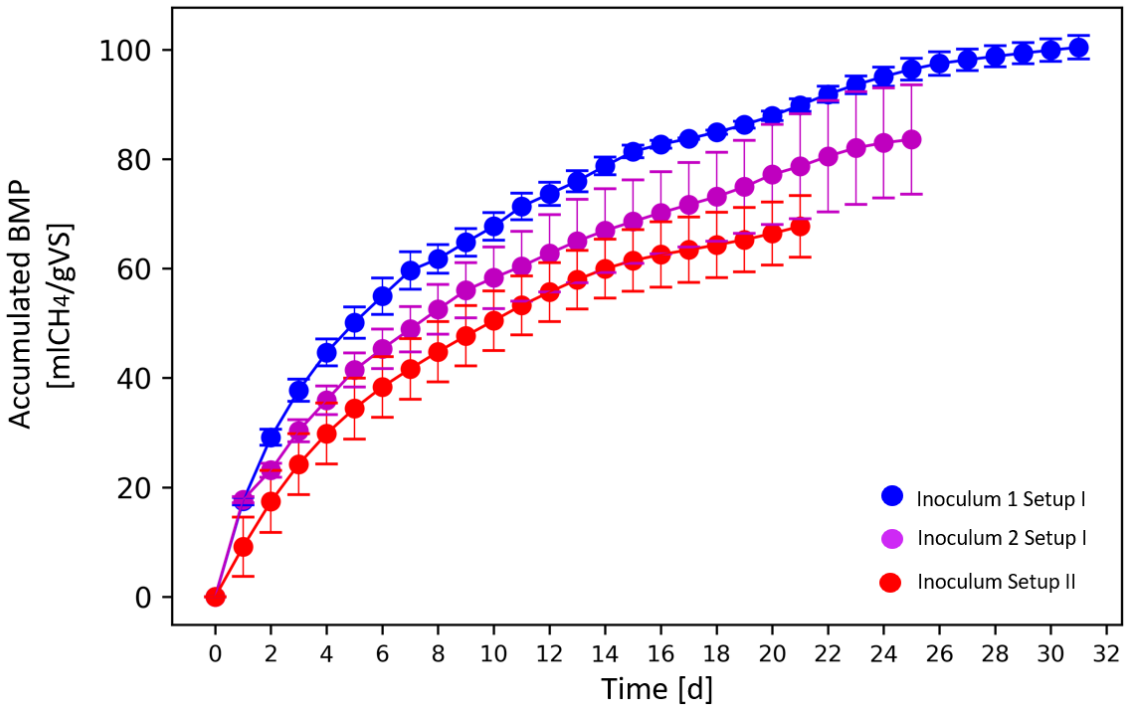

Figure 18. BMP of the different inoculums tested.

Table 13. BMP of the inoculum after 21 days of digestion.

Inoculum	**BMP (ml CH4/gVS) after 21 days of digestion**
Inoculum 1 Setup I	89 ± 1
Inoculum 2 Setup I	78 ± 9
Inoculum Setup II	67 ± 5

4.7. Total Biogas production at full-scale

During year 2022, a total volume of 10,670,363 m^3 of biogas was produced at the Rya WWTP. This biogas was later upgraded to 99% CH4 by Göteborg Energi. Since CH_4 represents approximately 60% of the biogas composition, the total volume produced was 6,402,218 m^3 CH_4. It is important to take into consideration that at the Rya WWTP, besides sludge, food waste and grease from restaurants is used, producing higher biogas amounts.

In Table 14 an estimate of the annual production of biogas is presented. This was calculated by multiplying the tons of VS that would be produced at the Rya WWTP annually by the BMP obtained from each substrate. The highest production is achieved with the co-digestion of PS + WAGS whereas the lowest production would be obtained by digesting the WAGS alone.

Table 14. Annual production at full-scale.

Sludge Type	Annual production (m^3/year)
PS + WAS	6,305,310
PS + WAGS-sludge	4,288,409
WAGS-sludge	821,183

As it can be seen the results are lower than what is produced currently at the Rya WWTP. This could be due to the fact the results were obtained from the biomethane production of 400 ml bottles. It is known that in bigger digesters there is a maximum utilization of the surface area from the waste material for a greater biogas production (Nasir et al., 2015) and that decomposition factor levels are kept at optimum (Ogunwande & Akinjobi, 2017).

4.8. Validation of Test Results

The results were validated by using the criteria stated by (Holliger et al., 2016). The criteria suggests that Dixon's tests should be performed to eliminate single outliers. For Setup I, it was conducted for the mixed AGS-sludge and WAGS-sludge since they were the only samples performed in triplicate. For Setup II, it was conducted for all samples except the cellulose which was tested in duplicate. All the probabilities obtained from the test were higher than the recommended threshold value of 0.01, therefore no data was deleted as outlier.

The coefficient of variation (CV) was calculated for each of the sludge samples. (Holliger et al., 2016) states that the blank and the positive control should not have a CV higher than 5%. For Setup I, the cellulose had all CV's lower than 5%, however the Inoculum1 presented values higher than 5% (5.03-6.07%) on days 2 to 7. The inoculum used for bottles B2 and B1 for WAGS-sludge which were added one week after presented CVs that varied from 3% to 12%. The high CV for the inoculum can be attributed to the fact that only two data sets were evaluated, therefore the probability of having a normal distribution is low.

For heterogeneous substrates, a CV lower than 10% is recommended. The WAS had lower CV's during each day of the incubation period, whereas the WAGS-sludge presented lower CV's from day 10 until the last day of the incubation period. The mixed AGS-sludge presented CV's that varied from 7% to 14% during all the incubation period.

For Setup II, the cellulose presented CV's higher than 5% from day 11 to day 21. The inoculum for Setup II presented CVs higher than 5% during all digestion days. The PS had lower CV's than 10% during all days of the digestion period. For the WAGS-sludge, CV's higher than 10% were obtained during the first 3 days of digestion. For the mixed AGS-sludge, CV's were lower than 10% during all days of digestion.

5. Conclusion

The BMP results of the substrates indicated that WAGS-sludge sampled when the pilot plant was fed with incoming, crude water (Setup II) presented the highest BMP from all the AGS-sludge fractions. WAS was 1.29 times higher and PS was 1.64 times higher than this WAGS-sludge.

For Setup I, it was expected that the WAGS-sludge had a higher BMP than the mixed AGS-sludge. However, the mixed AGS-sludge was on average 1.15 times higher than the WAGS-sludge by the WAGS-sludge was within the standard deviation of the measurements. The results indicated that the BMP of the PS was significantly higher than that of other tested substrates.

The aim of the study was also to elucidate the effect of primary sedimentation on the BMP's of both WAGS-sludge and mixed AGS-sludge. The results showed that the WAGS had a higher BMP when the pilot was fed with incoming wastewater (Setup II) as it was 1.2 times higher than the BMP of the WAGS when it was fed with pre-clarified water (Setup I). The BMP of the mixed AGS-sludge was not affected by the characteristics of the influent wastewater. Considering the high BMP results obtained from the PS, it is recommended that at full-scale the incoming wastewater is pre-clarified before entering the aerobic granular sludge process to produce primary sludge rich in organic matter.

The biodegradability results of the different sludge samples obtained from TS and VS analyses conducted for each of the mixtures before and after digestion indicated that AGS-sludges have a lower biodegradability compared to WAS and PS. The degree of digestion of AGS-sludges ranged from 48-60% which was lower than the 67% obtained from WAS.

The mixed AGS-sludge had the lowest BMP in Setup II. This sludge presented low BMP's when compared to WAS and PS. It had the lowest production rate, meaning it takes more time to biodegrade its organic matter through anaerobic digestion.

5.1. Future studies and Recommendations

Considering there was a time frame to perform the literature review and experimentation for this study, some recommendations for future studies are provided below.

- Sampling should be performed at different times of the year for comparison between the different seasons.
- Sampling should be performed simultaneously for all samples tested to have the same conditions between samples.
- Conduct BMP tests to sludges from different regions and WWTPs for comparison.
- Identify the physical differences between the sludges by particle-size distribution.
- Characterize the sludges chemically to elucidate how the sludge composition relates to the BMP of each substrate.
- Conduct the BMP tests at both mesophilic and thermophilic temperatures.
- Test co-digestion of AGS-sludge with different substrates.
- Perform the mixtures of inoculum and substrate with an inoculum adapted to the specific substrate. Additionally, try different inoculum to substrate ratios.

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Appendix A

Table A.1 shows the amount of Inoculum and Substrate added to each of the bottles in Setup I.

Table A.1 Amount of Inoculum and Substrate per bottle Setup I

Setup I		
Bottle	**Inoculum (ml)**	**Substrate (ml)**
E3	284.8	115.5
E2	286.1	115.5
D3	405	-
D2	400.8	-
D1	404.2	-
C3	289.8	114.7
C2	401.8	-
C1	287.7	114.8
B3	283.0	114.8
B2	287.6	115.3
B1	286.1	114.9
A3	285.8	119.5
A2	287.7	115.7
A1	286.3	112.9

E3, E2= Cellulose (Positive control) + Inoculum
D3, D2, D1, C2= Inoculum (Blank)
C3, C1= Waste Activated Sludge after PS
B3, B2, B1= WAGS-sludge after PS
A3, A2, A1 = Mixed AGS-sludge after PS

Table A.2 shows the amount of Inoculum and Substrate added to each of the bottles in Setup II.

Table A.2 Amount of Inoculum and Substrate added per bottle Setup II

Setup II		
Bottle	**Inoculum (ml)**	**Substrate (ml)**
E3	285.3	118.0
E2	285.1	118.4
D3	400.0	-
D2	400.0	-
D1	400.0	-
C3	283.5	115.3
C2	284	115.2
C1	286.5	115.3
B3	287.3	116.9
B2	286.8	115.5
B1	288.5	115.2
A3	285	115.0
A2	295	115.7
A1	285	115.3

E3, E2 = Cellulose (Positive control) + Inoculum
D3, D2, D1 = Inoculum (Blank)
C3, C2, C1 = Mixed AGS-sludge without primary sedimentation
B3, B2, B1 = Waste AGS-sludge without primary sedimentation
A3, A2, A1 = Primary Sludge

Appendix B
Precipitation Data from SMHI

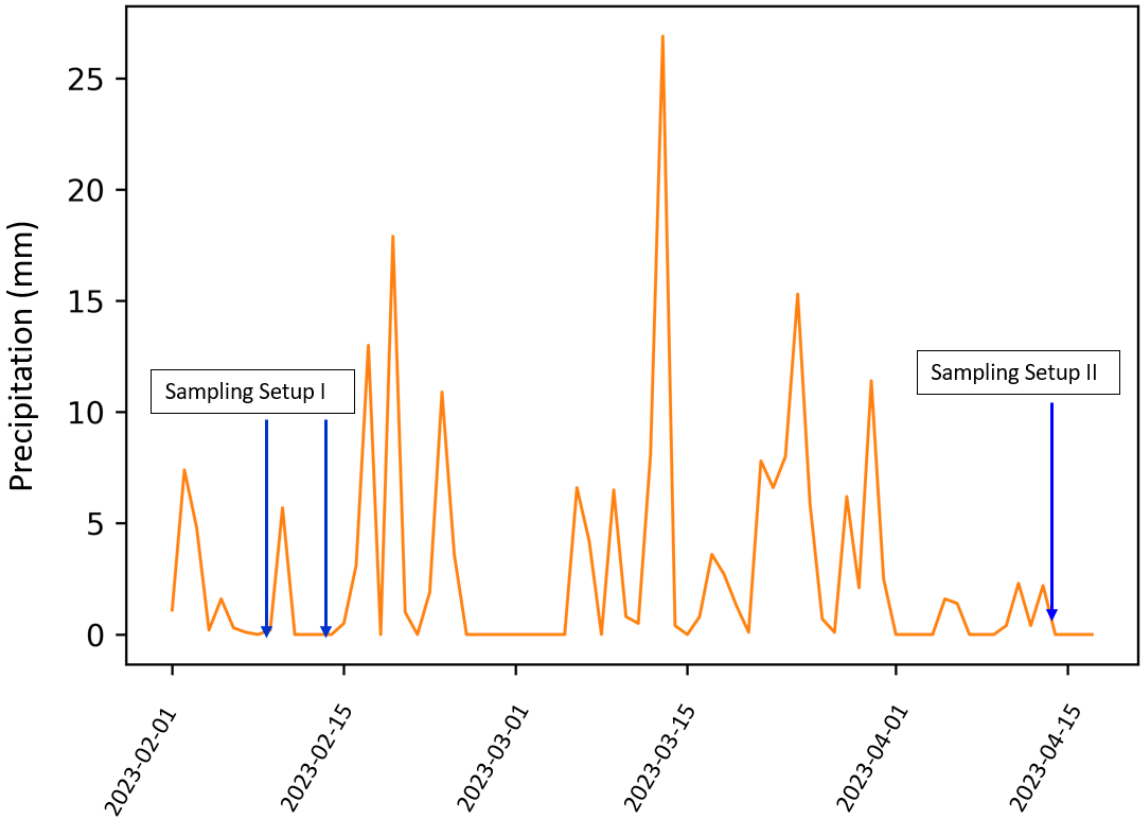

Figure B.1. Precipitation during the sampling periods (SMHI, 2023)

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