KTH ROYAL INSTITUTE
OF TECHNOLOGY

Doctoral Thesis in Chemical Engineering

Bio-based recovery of organic carbon from municipal waste streams

Process optimization and microbial community dynamics

ISAAC OWUSU-AGYEMAN

Stockholm, Sweden 2022

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Academic Dissertation which, with due permission of the KTH Royal Institute of Technology, is submitted for public defence for the Degree of Doctor of Philosophy on Wednesday the 6th of April 2022 at 10:00 am in F3 Lindstedtsvägen 26, Stockholm.

Doctoral Thesis in Chemical Engineering
Department of Chemical Engineering
In co-operation with
Department of Sustainable Development, Environmental Science and Engineering
KTH Royal Institute of Technology
Stockholm, Sweden 2022

TRITA-CBH-FOU-2022:17
ISBN: 978-91-8040-154-8

Printed by: Universitetsservice US-AB, Sweden 2022

Summary

Resource recovery from waste contributes to the transition to a sustainable society. Municipal organic wastes have enormous potential for resource recovery due to the inherent organic content which makes it possible to obtain bio-based chemicals and bioenergy. In view of this, the focus of the current study was on the bio-based recovery of carbon from municipal organic wastes by exploring process optimization and microbial community dynamics of existing and new technologies for the recovery of bio-based products. The study involved two parts: 1) biogas production through direct anaerobic granule-based treatment of mainstream municipal wastewater; and 2) production of bio-based platform chemicals in the form of volatile fatty acids (VFAs) from sewage sludge and other municipal organic wastes through mixed microbiome co-fermentation.

Part 1 aimed to evaluate biogas recovery from municipal wastewater with anaerobic granule-based technology. The link between the structure of anaerobic granules and their methane-producing pathways was elucidated together with the impact of operating parameters on the microbial community structure of different granules. This part of the study employed two identical upflow anaerobic sludge blanket (UASB) reactors with varied granule size distribution, each one with a working volume of 2.5 m^3. The reactors were operated for a total of 456 days under different conditions of temperature (20 – 28 ºC) and hydraulic retention time (HRT, 3 – 5 h).

One of the reactors (UASB1) had granules with larger sizes (3 – 4 mm) while the granules of the other reactor (UASB2) had smaller sizes (1– 2 mm). The granules of UASB1 were characterised by multi-layered internal structures while the granules from UASB2 were without layered internal structures. The microbial community of UASB1 granules was dominated by *Methanosaetaceae* which are strictly acetoclastic whereas hydrogenotrophic methanogens and formate users, *Methanomicrobiales* dominated the UASB2 granules. Moreover, the acetoclastic methanogenic activities of UASB1 were higher than UASB2, confirming that acetoclastic methanogenesis was more prominent in UASB1 than UASB2.

An increase in temperature from 20˚C to 28˚C led to an increase in biogas production for UASB1. There was also a stable and higher biogas production rate for UASB2 at 28˚C. The increase in biogas production was attributed to the reduction in the methane solubility in the effluent and stable activity of methanogens. Chemical oxygen demand (COD) removal efficiency and biogas production increased with HRT with the best performance at HRT of 5 h and temperature of 20 ˚C, where COD removal was up to 85% for both reactors. The biogas production at HRT 5 was up to 102 L/($m^3 \cdot d$) and 90 L/($m^3 \cdot d$) for UASB1 and UASB2, respectively. This was attributed to the increase in contact time between the substrate and microbial consortium. The study has demonstrated that the application of granule-based technology for the treatment of municipal wastewater is feasible under mesophilic temperatures with very good performance.

Part 2 aimed to explore the practicability and process optimization of fermentation systems to recover VFAs as platform chemicals from municipal organic wastes. The study focused on the impact of substrate ratio of primary sludge and external organic waste (OW) and assessed the robustness and the microbial community dynamics of the VFA system in the long-term operation on an up-scale. The impacts of substrate proportions on alkaline co-fermentation were elucidated using short-term batch reactors with different substrates proportions at initial pH 10 and pH 5. Subsequently, there was a long-term study with three semi-continuous reactors under alkaline (pH 10 and 9), acidic (pH 5), and no pH control and operated for up to 315 days. The co-fermentation system was upscaled in a 2 m^3 continuous reactor with different kinds of OW for 264 days to ascertain the implication of substrate variability on the VFA system in the long term. The results revealed that there was an increase in VFA production with an increase in OW proportion due to the availability of higher biodegradable organics.

Acetic and propionic acids were the most dominant VFA types under alkaline conditions (pH 10 and 9), whereas caproic acid dominated under acidic (pH 5) conditions and no pH control conditions. The mixed microbiome under alkaline pH was dominant by *Bacillaceae* and *Dysgonomonadaceae* with the latter as the main actor to restore production in the long term. Without pH control, the average pH was 5.3 ± 3 and the microbial community was dominated by *Lachnospiraceae* which correlated with caproic acid and other VFAs production. The 2 m^3 pilot reactor showed robustness in response to substrate variability. VFA-rich fermentation liquids showed promising potential as carbon sources for the denitrification process and production of polyhydroxyalkanoates (PHAs).

This Ph.D. project demonstrated a waste-to-value approach to shifting wastewater treatment plants to biorefineries for recovering valuable carbon resources through both direct anaerobic treatment of municipal wastewater and co-fermentation of municipal organic waste. The application of VFAs for other processes could lead to a bio-based production platform as an alternative to fossil-based processes.

Keywords: Biogas, Volatile fatty acid, Anaerobic granules, Municipal organic waste, Mixed microbiome

Sammanfattning

Resursåtervinning från avfall bidrar till omställningen till ett hållbart samhälle. Kommunalt organiskt avfall har en enorm potential för resursåtervinning på grund av det inneboende organiska innehållet som gör det möjligt att få fram biobaserade kemikalier och bioenergi. Med tanke på detta låg fokus för den aktuella studien på biobaserad återvinning av kol från kommunalt organiskt avfall genom att utforska processoptimering och dynamik hos mikrobiella samhällen hos befintliga och nya teknologier för återvinning av biobaserade produkter. Studien omfattade två delar: 1) biogasproduktion genom direkt anaerob granulatbaserad rening av kommunalt avloppsvatten; och 2) produktion av nya plattformskemikalier i form av flyktiga fettsyror (VFA) från avloppsslam och annat kommunalt organiskt avfall genom samfermentering av blandad mikrobiom.

Del 1 syftade till att utvärdera biogasutvinning från kommunalt avloppsvatten med anaerob granulatbaserad teknik. Kopplingen mellan strukturen hos anaeroba granuler och deras metanproducerande vägar klargjordes tillsammans med påverkan av driftsparametrar för den gemensamma mikrobiella strukturen hos olika granuler. Denna del av studien använde två identiska uppflödesreaktorer för anaerob slamfilm (UASB) med varierande granulstorleksfördelning, var och en med en arbetsvolym på 2,5 m^3. Reaktorerna drevs i totalt 456 dagar under olika temperaturförhållanden (20 - 28 ºC) och hydraulisk uppehållstid (HRT, 3 - 5 timmar).

En av reaktorerna (UASB1) hade granulat med större storlekar (3–4 mm) medan granulerna i den andra reaktorn (UASB2) hade mindre storlekar (1–2 mm). Granulerna av UASB1 karakteriserades av flerskiktig inre struktur medan granulerna från UASB2 var utan skiktad inre struktur. Det mikrobiella samhället för UASB1-granuler dominerades av Methanosaetaceae som är strikt acetoklastiska medan hydrogenotrofa metanogener och formiatanvändare, Methanomicrobiales dominerade UASB2-granulerna. Dessutom var de acetoklastiska metanogena aktiviteterna hos UASB1 högre än hos UASB2, vilket bekräftar att acetoklastisk metanogenes var mer framträdande i UASB1 än UASB2.

En ökning av temperaturen från 20 ºC till 28ºC ledde till en ökning av biogasproduktionen för UASB1. Det fanns också en stabil och högre biogasproduktionstakt för UASB2 vid 28ºC. Ökningen av biogasproduktionen tillskrevs minskningen av metanlösligheten i avloppsvattnet och stabil aktivitet av metanogener. Effektiviteten för avlägsnande av kemisk syreförbrukning (COD) och biogasproduktion ökade med HRT med bästa prestanda vid HRT på 5 timmar och en temperatur på 20 ºC, där COD-borttagning upp till 85 % erhölls för båda reaktorerna. Biogasproduktionen vid HRT 5 var upp till 102 L/($m^3 \cdot d$) och 90 L/($m^3 \cdot d$) för UASB1 respektive UASB2. Detta tillskrevs ökad kontakttid mellan substratet och mikrobiellt material. Studien har visat att tillämpningen av granulatbaserad teknik för rening av kommunalt avloppsvatten är genomförbar under mesofila temperaturer med mycket god prestanda.

Del 2 syftade till att utforska genomförbarheten och processoptimeringen av fermenteringssystem för att återvinna VFAs som plattformskemikalier från kommunalt organiskt avfall. Studien fokuserade på effekterna av substratförhållandet mellan primärt slam och externt organiskt avfall (OW) och bedömde robustheten och dynamik hos det mikrobiella samhället hos VFA-systemet i den långsiktiga driften i uppskala.

Substratproportionernas inverkan på alkalisk samjäsning klargjordes med korttidssatsreaktorer med olika substratproportioner vid initialt pH 10 och pH 5. Därefter gjordes en långtidsstudie med tre halvkontinuerliga reaktorer under alkaliska (pH 10) och 9), sura (pH 5) och utan pH-kontroll som arbetade i upp till 315 dagar. Samfermenteringssystemet uppskalades i en 2 m^3 kontinuerlig reaktor med olika typer av OW under 264 dagar för att fastställa implikationen av substratvariabilitet på VFA-systemet på lång sikt. Resultaten avslöjade att det var en ökning av VFA-produktionen med en ökning av OW-andelen på grund av tillgången på högre biologiskt nedbrytbara organiska ämnen.

Ättik- och propionsyror var de mest dominerande VFA-typerna under alkaliska betingelser (pH 10 och 9), medan kapronsyra dominerade under sura (pH 5) betingelser och utan pH-kontroll. Det blandade mikrobiomet under alkaliskt pH dominerades av *Bacillaceae* och *Dysgonomonadaceae* med de senare som huvudaktör för att återställa produktionen på lång sikt. Utan pH-kontroll var det genomsnittliga pH-värdet 5,3 ± 3, och det mikrobiella samhället dominerades av *Lachnospiraceae* som korrelerade med produktion av kapronsyra och annan VFA. Pilotreaktorn på 2 m^3 visade robusthet som svar på substratvariabilitet. VFA-rika jäsningsvätskor visade lovande potential som kolkällor för denitrifieringsprocessen och produktionen av polyhydroxialkanoater (PHA).

Detta Ph.D. projekt visade på ett avfall till värde synsätt på att förvandla avloppsreningsverk till bioraffinaderier för att återvinna värdefulla kolresurser genom både direkt anaerob behandling av kommunalt avloppsvatten och samjäsning med kommunalt organiskt avfall. Tillämpningen av VFA för andra processer skulle kunna leda till en biobaserad produktionsplattform som ett alternativ till fossilbaserade processer.

Nyckelord: Biogas, Flyktiga fettsyror, Anaeroba granulat, Kommunalt organiskt avfall, Blandmikrobiom

Acknowledgements

This Ph.D. project was done in a collaboration between the Department of Chemical Engineering (School of Engineering Sciences in Chemistry, Biotechnology and Health) and the Department of Sustainable Development, Environmental Science and Engineering (School of Architecture and the Built Environment). Financial support from KTH for this co-operation is highly appreciated.

The degree project was part of the Pioneer_STP project (Project No: FR-2016/0002) and CarbonNextGen project (Project No: 46119-1) financed by Formas within the WaterWorks2014 Co-funded Call (Water JPI/Horizon 2020) and the Swedish Energy Agency in the scope of RE: Source Strategic Innovation Programme, respectively. A major part of the experimental work was performed at Hammarby Sjöstadverket in collaboration with IVL Swedish Environmental Research Institute. Water Research School is thanked for organizing valuable courses related to my Ph.D. studies and for travel support.

First, I would like to thank my supervisors, Assoc. Prof. Zeynep Cetecioglu and Prof. Elzbieta Plaza, for their immense support during the study. Special thanks go to Dr. Andriy Malovanyy, Jesper Karlsson, Dr. Jingjing Yang, Mayumi Narongin, Dr. Christian Baresel, Niclas Bornold and Andrea Carranza Munoz all from IVL for their technical support and discussions during the operation of the pilot plants. I would like to appreciate the Masters' students, Elli Isaakidou, Karolina Los, Binyam Bedaso, Chengyang Pan and Cora Doehler, and intern Erol Orhun for their help with experimental works.

I would like to thank my current and former colleagues from the Resource Recovery Division and the Chemical Engineering Department of KTH. Special thanks go to Dr. Nilay Elginöz Kanat, Dr. Merve Atasoy, Dr. Mariel Perez Zabaleta, Kasra Khatami Mashhadi, Dr. Ece Kendir Cakmak and Fengyi Zhu for the friendly atmosphere they created to make life bearable and for the many fruitful discussions. I would also like to thank Prof Gustaf Olsson of Lund University for his mentorship and the valuable advice I received from him during the Ph.D. period. Many thanks to Dr. Gunaratna Kuttuva Rajarao for serving as an Internal reviewer and giving valuable comments to improve the thesis. Lastly, I would like to thank my family and friends especially my wife for the love, friendship and encouragement.

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Appended Papers

This thesis is based on the results presented in the following papers, which are appended to this thesis:

Paper I

Owusu-Agyeman I., Eyice Ö., Cetecioglu Z., & Plaza E. (2019). The study of structure of anaerobic granules and methane producing pathways of pilot-scale UASB reactors treating municipal wastewater under sub-mesophilic conditions. Bioresource Technology, 290, 121733.

Paper II

Owusu-Agyeman I., Plaza E., & Cetecioglu Z. (2021). A pilot-scale study of granule-based anaerobic reactors for biogas recovery from municipal wastewater under sub-mesophilic conditions. Bioresource Technology, 337, 125431.

Paper III

Owusu-Agyeman I., Plaza E., & Cetecioglu Z. (2020). Production of volatile fatty acids through co-digestion of sewage sludge and external organic waste: effect of substrate proportions and long-term operation. Waste Management, 112, 30–39.

Paper IV

Owusu-Agyeman I., Plaza E., & Cetecioglu Z. (2022). Long-term alkaline volatile fatty acids production from waste streams: Impact of pH and dominance of *Dysgonomonadaceae*. Bioresource Technology, 346, 126621.

Paper V

Owusu-Agyeman I., Bedaso B., Döhler C., Pan C., Malovanyy A., Baresel C., Plaza E., & Cetecioglu Z. (2022). Volatile fatty acids production from municipal waste streams and use as a carbon source for denitrification: The journey towards full-scale application and revealing key microbial players. Submitted to Water Research

Contributions to the appended papers

Paper I

I planned the study together with Zeynep Cetecioglu and Elzbieta Plaza, performed the experimental work and analysed the results. I wrote the manuscript with contributions from all co-authors.

Paper II

I planned the study together with Zeynep Cetecioglu and Elzbieta Plaza, performed the experimental work and analysed the results. I wrote the manuscript with contributions from all co-authors.

Paper III

I planned the experiments together with co-authors, performed the experimental work with contribution from master thesis students and analysed the results. I wrote the manuscript with the support from co-authors.

Paper IV

I planned the experiments together with co-authors, performed the experimental work with contribution from master thesis students and analysed the results. I wrote the manuscript with the support from co-authors.

Paper V

I planned the experiments, performed the part of the experiments, supervised master students Binyam Bedaso, Cora Döhler and Pan Chengyang to perform other parts of the experiment and analysed the results. I wrote the manuscript with the support of co-authors.

Other contributions

Papers

Owusu-Agyeman I., Balachandran S., Plaza E., Cetecioglu Z. (2021). Co-fermentation of municipal waste streams: Effects of pretreatment methods on volatile fatty acids production. Biomass and Bioenergy, 145, 105950.

Khatami K., Perez-Zabaleta M., **Owusu-Agyeman I**., Cetecioglu Z. (2021). Waste to bioplastics: How close are we to sustainable polyhydroxyalkanoates production? Waste Management, 119, 374-388.

Elginoz N., Khatami K., **Owusu-Agyeman I**., Cetecioglu Z. (2020). Life Cycle Assessment of an innovative food waste management system. Frontiers in Sustainable Food Systems, 4, 23.

Atasoy M.*, **Owusu-Agyeman** I.*, Plaza E., Cetecioglu Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. Bioresource technology, 268, 773-786.

*First co-authors

Thesis

Owusu-Agyeman I. (2020). Recovery of organic carbon from municipal waste streams. Licentiate Thesis, KTH Royal Institute of Technology, TRITA-CBH-FOU-2020:23.

Book Chapters

Kendir C. E., Atasoy M., **Owusu-Agyeman I.**, Khatami K., Cetecioglu Z. (2021). 19. Circular city concept for future biorefineries. In Tyagi V., Kumar M., An A., Cetecioglu Z (Eds.), Clean Energy and Resource Recovery Wastewater Treatment Plants as Biorefineries, Volume 2, 1-482, Elsevier Science, Oxford/Amsterdam.

Owusu-Agyeman I., Plaza E., Cetecioglu Z. (2020). Wastewater to energy: Relating granule size and biogas production of UASB reactors treating diluted municipal wastewater. In V. Naddeo, M. Balakrishnan, & K.-H. Choo (Eds.), Frontiers in Water-Energy-Nexus – Nature-based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability: Proceedings of WaterEnergyNEXUS Conference, November 2018, Salerno, Italy (1st Ed., p. 320). Switzerland: Springer International Publishing.

Oral Presentations

Owusu-Agyeman I., Plaza E., Cetecioglu Z. (2021). Closing the gap between lab-scale and full-scale production of volatile fatty acids from wastes: The impact of substrate variability.

IWA 4th Resource Recovery Conference 2021 – September 5-8, 2021 Turkey. (Oral presentation).

Owusu-Agyeman I., Malovanyy A., Baresel C., Plaza E., Cetecioglu Z. (2021). Optimization of volatile fatty acid production from sewage sludge and external organic waste: pH effect and microbial community dynamics. 5th IWA Specialized International Conference 'Ecotechnologies for Wastewater Treatment (ecoSTP) 2021' - June 21-25, 2021. Milan, Italy (Oral presentation).

Owusu-Agyeman I., Cetecioglu Z., Plaza E. (2021). Direct energy recovery from municipal wastewater with UASB reactors: effects of operation parameters. 5th IWA Specialized International Conference. Ecotechnologies for Wastewater Treatment (ecoSTP) June 21-25, 2021. Milan, Italy (Oral presentation).

Owusu-Agyeman I., Malovanyy A., Baresel C., Plaza E., Cetecioglu. Z. Production of volatile fatty acids from sewage sludge and food waste for denitrification: closing the loop for wastewater treatment plants. IWA Nutrient Removal and Recovery Conference (Virtual), September 1-3, 2020. Helsinki, Finland (Oral Presentation).

Owusu-Agyeman I., Cetecioglu Z., Plaza E. Energy recovery from municipal wastewater through anaerobic treatment with UASB reactors. 2020 Closed Cycles and Circular Society Symposium. September 2-4, 2020. Waedenswil, Switzerland (Workshop).

Owusu-Agyeman I., Plaza E., Cetecioglu Z. (2019). Anaerobic co-digestion of sewage sludge and external organic waste: strategy to shift production from biogas to volatile fatty acids. 3rd IWA Resource Recovery Conference. September 8-12, 2019. Venice, Italy (Oral presentation).

Owusu-Agyeman I., Plaza E., Cetecioglu Z. (2018). Wastewater to energy: Relating granule size and biogas production of UASB reactors treating diluted municipal wastewater. WaterEnergyNEXUS Conference, November 14-17, 2018. Salerno, Italy (Oral presentation).

Poster presentations

Owusu-Agyeman I., Malovanyy A., Baresel C., Plaza E., Cetecioglu. Z. Optimization of volatile fatty acids production from sewage sludge and food waste for up-scaling purposes: closing the loop for treatment plants. 2020 Closed Cycles and Circular Society Symposium. September 2-4, 2020. Waedenswil, Switzerland (Poster).

Owusu-Agyeman I., Eyice Ö., Cetecioglu Z., Plaza E. (2019). The dependency of methanogenic pathway of anaerobic granules on their characteristics. 3rd IWA Resource Recovery Conference, September 8-12, 2019. Venice Italy (Poster).

Abbreviations

ABR	Anaerobic baffled reactor
AD	Anaerobic digestion
AnMBR	Anaerobic membrane bioreactor
ANOVA	Analysis of variance
ASBR	Anaerobic sequencing batch reactor
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EGSB	Expanded granular sludge bed
EU	European Union
FB	Fluidized bed
FID	Flame ionization detector
GHG	Greenhouse gas
HRT	Hydraulic retention time
LCFA	Long-chain fatty acids
OW	Organic waste
OW1	Homogenized organic waste from Himmerfjärden WWTP
OW2	Non-homogenized organic waste from Himmerfjärden WWTP
OW3	Homogenized organic waste from Scandinavian Biogas
PC1	First principal component
PC2	Second principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PHA	Polyhydroxyalkanoate
PMP	Potential methane production
PS	Primary sewage sludge
SEM	Scanning electronic microscope
SDG	Sustainable Development Goal
SMA	Specific methanogenic activity
TS	Total solids
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
UASB1	The UASB reactor with larger granule size distribution
UASB2	The UASB reactor with smaller granule size distribution
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

1 Introduction

An increase in the world's population has reinforced the demand for materials to meet the needs of people. Since petroleum-based production is finite and poses a serious threat to the environment, it is now of utmost importance to ensure sustainable production in the context of a circular economy. In addition, a large quantity of waste is produced that leads to the emission of greenhouse gases (GHGs) and plays a role in the change of the global climate. Nations are now putting measures in place to address the issue of climate change. Notable among such measures is the United Nations sustainable development goals (SDGs) established in 2015 and has been adopted by all member states to ensure that the needs of both present and future generations will be met sustainably. Moreover, the United Nations SDG 6 (Clean Water and Sanitation) and SDG 11 (Sustainable Cities and Communities) motivate major research, further boosted by the alarming increase in worldwide pollution levels and climate change, primarily caused by the overuse of fossil fuels.

To achieve the SDGs, regional bodies such as the European Union (EU) and individual countries like Sweden have a new climate policy framework to curb GHG emissions by increasing the use of renewable feedstocks through a transition into bio-based products. Typical examples are the new "*EU Circular Economy Action Plan*"(European Union, 2020) and "*Swedish National Strategy for a Circular Economy*" (Government Offices of Sweden, 2020) which advocate for the replacement of fossil-based products with bio-based products as well as resource-efficient feedstock and energy recovery. Innovative waste management is a major contributor to achieving the SDGs and targets set by nations and regional bodies. In view of this, the concept of waste handling is being shifted from just the removal of contaminants to the recovery of valuable resources. There are several resources that can be recovered from various waste streams. Waste streams available for resource recovery include municipal waste streams, agricultural waste, construction waste, commercial waste, and industrial wastes.

There have been efforts to recover bioenergy, nutrients, organic chemicals, metals with different biological and physiochemical technologies from municipal, industrial, and agricultural waste streams (Govindarajan, 2018; Puyol et al., 2017; Solon et al., 2019). The International Energy Agency stipulates in its recent report, *Roadmap for the Global Energy Sector for Net Zero by 2050* that major clean energy innovation efforts must take place to bring global carbon dioxide emissions to net-zero by 2050 (International Energy Agency, 2021). In addressing this challenge, there is the need to make use of all available technologies including available clean energy technologies as well as the development of next-generation carbon-neutral wastewater treatment technologies to turn waste streams into bioenergy and bio-based chemicals and materials.

The focus of the current study is on the recovery of bio-based products from municipal waste streams. The Ph.D. project evaluated existing and up-and-coming technologies to recover bio-based products and energy from organic-rich municipal waste streams. The study has specifically emphasized on the recovery of carbon from mainstream municipal wastewater as well as sewage sludge and external organic waste (including food waste) by investigating new approaches that can shift waste treatment plants to resource recovery facilities.

The Ph.D. project has both direct and indirect impacts on some of the 17 UN SDGs. This project has a direct impact on SDG 6 (Clean Water and Sanitation) because the study involves treatment techniques for municipal organic wastes. Thus, the study contributes to wastewater treatment and consequently leads to the improvement of water quality by reducing the discharge of contaminants to the water resources. Moreover, proper management of municipal waste streams which is the focus of this study will contribute to sanitation. The current study also imparts SDG 7 (Affordable and Clean Energy) through the production of biogas from municipal wastewater. The main focus of the Ph.D. study is resource recovery. Recovery resource from waste streams is a responsible way of waste management and could eventually lead to the reduction of waste. Consequently, this project will directly impact SDG 12 (Responsible Production and Consumption). The approach of the current study is the biobased production of energy and material from waste streams. Implementation of this strategy will allow cities and towns to become future biorefinery hubs through biobased transformation of their organic wastes into valuable biobased products. Therefore, this Ph.D. project has an indirect impact on SDG 11 (Sustainable Cities and Communities).

1.1 Municipal waste streams

Municipal waste streams, specifically municipal wastewater and municipal solid waste are the inevitable by-products of modern society. Municipal waste streams are promising for resource recovery due to their high generation rate and composition.

1.1.1 Municipal wastewater

Municipal wastewater consists of domestic, commercial, and pre-treated industrial wastewaters. It is estimated that over 330 km^3 of municipal wastewater is generated annually in the world (Mateo-Sagasta et al., 2015). The constituents of municipal wastewater vary with location and time. These waste streams contain organic carbon and nutrients which when released into the environment can have severe consequences on the receiving environment and the climate. Municipal waste streams are comprised of wastewater and solid wastes. Besides water, wastewater contains microorganisms, organic matter, nutrients (nitrogen, phosphorus), metals, pharmaceuticals, and other pollutants. While it is important to treat the wastewater to remove these contaminants to prevent harmful environmental impacts, there are also avenues to recover resources.

The typical composition of raw municipal wastewater is shown in Table 1. Organic carbon (often measured as chemical oxygen demand (COD)) is the major contaminant in municipal wastewater. It is estimated that municipal wastewater has a total COD concentration of 500-1200 mg/L (Henze and Comeau, 2008). The main sources of organic carbonaceous materials in wastewater are carbohydrates (25-50%), proteins (40-60%) and fats and oil (10%) (Tomei and Mosca Angelucci, 2017). Volatile acids, detergents, uric acid, and creatine are carbon compounds that contribute less to the total organic carbon in municipal wastewater. The Swedish Environmental Protection Agency reported that in 2016 about 35,300 tonnes of organic carbon (as COD), 15,400 tonnes of nitrogen, and 240 tonnes of phosphorus were discharged through municipal wastewater (Statistical Agency, 2018).

Table 1: Composition of typical municipal wastewater with the range of concentrations as modified from (Henze and Comeau, 2008).

Parameter	Concentration range (mg/L)
Total COD	500-1200
Soluble COD	200-480
Suspended COD	300-720
BOD	230-560
VFA (as acetate)	10-80
Total N	30-100
NH_4-N	20-75
Total P	6-25
PO_4-P	4-15
TSS	250-600
VSS	200-480

The compositions show that municipal waste streams have a huge potential for resource recovery and different bio-based products can be valorised from them (Khoshnevisan et al., 2020). It is estimated that municipal wastewater with a COD concentration of 400-500 mg/L has the potential to produce energy of 1.5-1.9 kWh/m^3 and with nutrient and energy recovery strategy in place, there is a possibility to obtain a net energy yield of 0.24 kWh/m^3 of wastewater and to cut CO_2 emissions by 35% for municipal wastewater treatment plants (WWTPs) (Khiewwijit et al., 2015). Moreover, it is estimated that 1 kg of COD in wastewater is equivalent to about 3.5-4 kWh of chemical energy (Kaless et al., 2017; Svardal and Kroiss, 2011). The energy potential of a WWTP in the US was experimentally determined and it was estimated that the energy content of the raw wastewater was approximately nine (9) times higher than the required energy for the plant operation (Shizas and Bagley, 2004).

1.1.2 Sewage Sludge and other external organic wastes

The conventional method for the treatment of municipal wastewater gives rise to the production of sewage sludge, a semi-solid material that needs to be stabilized before disposal. Sewage sludges are categorised into two kinds, depending on which stage of the mainstream wastewater treatment process they are produced. Primary sewage sludge (PS) is produced after primary sedimentation whereas secondary (waste activated) sludge is obtained after the biological treatment stage followed by secondary clarifier (Figure 1).

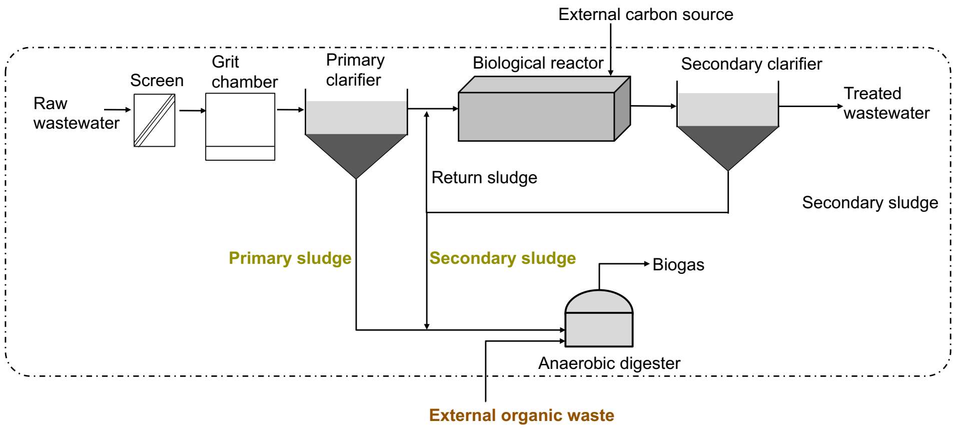

Figure 1: Schematic of a typical wastewater treatment plant showing sewage sludges and external organic wastes.

Sewage sludge is produced in large quantities in the world. In 2018, it was estimated that about 5.6 million tonnes of sewage sludge were produced in the EU, of which Sweden produced 211 thousand tonnes (Eurostats, 2018). In the case of the United States, it is estimated that 12.5 million tonnes of dry solids of sewage sludge are produced from WWTPs of which 50% is not beneficially utilized (Seiple et al., 2017). If not handled well, sewage sludge can contribute negatively to carbon dioxide emissions. It is estimated that treatment and disposal of sludge account for up to 40% of the total GHG emissions from WWTPs (Brown et al., 2010). On the other hand, sewage sludge has inherent properties which offer an opportunity for energy and material recovery. It is estimated that the energy contents of primary and secondary sludge are 6.4-8.1 and 5.2-6.4 kWh/kg, respectively (Nazari et al., 2018; Weemaes and Verstraete, 1998).

Other external organic wastes (OW) that are not generated in the WWTPs also have the inherent potential for resource recovery. These external organic wastes include food waste, yard waste, agricultural waste, organic wastes from food processing industries, etc. These wastes are often easily biodegradable with inherent value and can be combined with sewage sludge in bioprocesses to ensure simultaneous waste management and resource recovery. A chunk of the external organic wastes is from municipal sources. Municipal solid waste is the solid waste material that is often referred to as garbage and it originates from households and commercial activities.

Municipal waste often consists of papers, garden wastes, food wastes, plastics (Artiola, 2019). The organic fraction of municipal solid waste is known to consist of a mixture of paper, garden, and food wastes. It is composed of carbohydrates, protein, fat, oil, and grease (Campuzano and González-Martínez, 2016). Globally about 1.3 billion tonnes of municipal solid waste is produced yearly with about 46% organic contents (Campuzano and González-Martínez, 2016). Besides, the EU estimates that about two-thirds of its organic waste originates from municipal waste streams (Bourguignon, 2015). The annual production of municipal solid organic waste in the EU is 88 million tonnes (European commission, 2010). Large quantities of organic fraction of municipal solid waste are food wastes. Globally , one-third of all edible parts of food produced for human consumption is lost or wasted, according to the Food and Agricultural Organisation of the United Nations (FAO, 2019). This amounts to about 1.3 billion tonnes of food waste annually. The food loss and waste results in a 4.4 Gt CO_2 equivalent of GHG emission that contributes to about 6-8% of the total anthropogenic GHG emissions (FAO, 2011; Ritchie, 2020). However, these organic-rich waste streams can be handled sustainably to recover energy and feedstock to replace petroleum-based production. It is estimated that recovered bio-based products from organic waste streams currently have market values between €0.5 and €2/kg which could increase in the future (Eswari et al., 2020). This points to a positive outlook that needs to be exploited sustainably.

1.2 Setting the scene

The most common handling option for municipal solid waste is landfilling, whereby the organic content leads to the production of greenhouse gases. Likewise, traditionally, organic carbon is removed from wastewater through the activated sludge treatment process which uses microorganisms in the presence of air to biologically oxidize the organic compounds. However, the inherent characteristics of municipal waste streams present an opportunity to recover resources. Moreover, recoverable carbon resources such as volatile fatty acids (VFAs), polyhydroxyalkanoates (PHA), cellulose fibres, extracellular polymers can be obtained from municipal waste streams.

Anaerobic treatment is currently the most proven and widely used technology all over the world for carbon recovery, usually in the form of biogas from municipal waste (Kehrein et al., 2020; van Lier et al., 2001). The anaerobic process has been altered to produce not only end-products but other intermediate bio-based products such as VFAs, and hydrogen gas. Enhancement of bioenergy production and/or recovery of carbon in more valuable forms from municipal wastes with an anaerobic treatment process will increase resource recovery efficiency from waste. This will augment the recent advances attained in changing waste treatment plants to resource recovery facilities. Bio-based recovery of organic carbon from municipal waste streams through evaluation of existing and emerging innovative technologies will lead to maximization of resource recovery from waste streams. This will result in a shift from a linear to a circular economy with the production of new bio-based products from waste streams.

The scope of this Ph.D. work included biogas recovery from municipal wastewater through direct treatment of mainstream wastewater with anaerobic granule-based treatment technology and VFA production from co-fermentation of sewage sludge and external organic wastes. Optimization of the processes has been evaluated by elucidating the effect of operating parameters such as pH, temperature, hydraulic retention time (HRT) on the performances of the system as well as microbial community dynamics. The overview of the current Ph.D. work coupled with the papers appended to the thesis, has been shown in Figure 2.

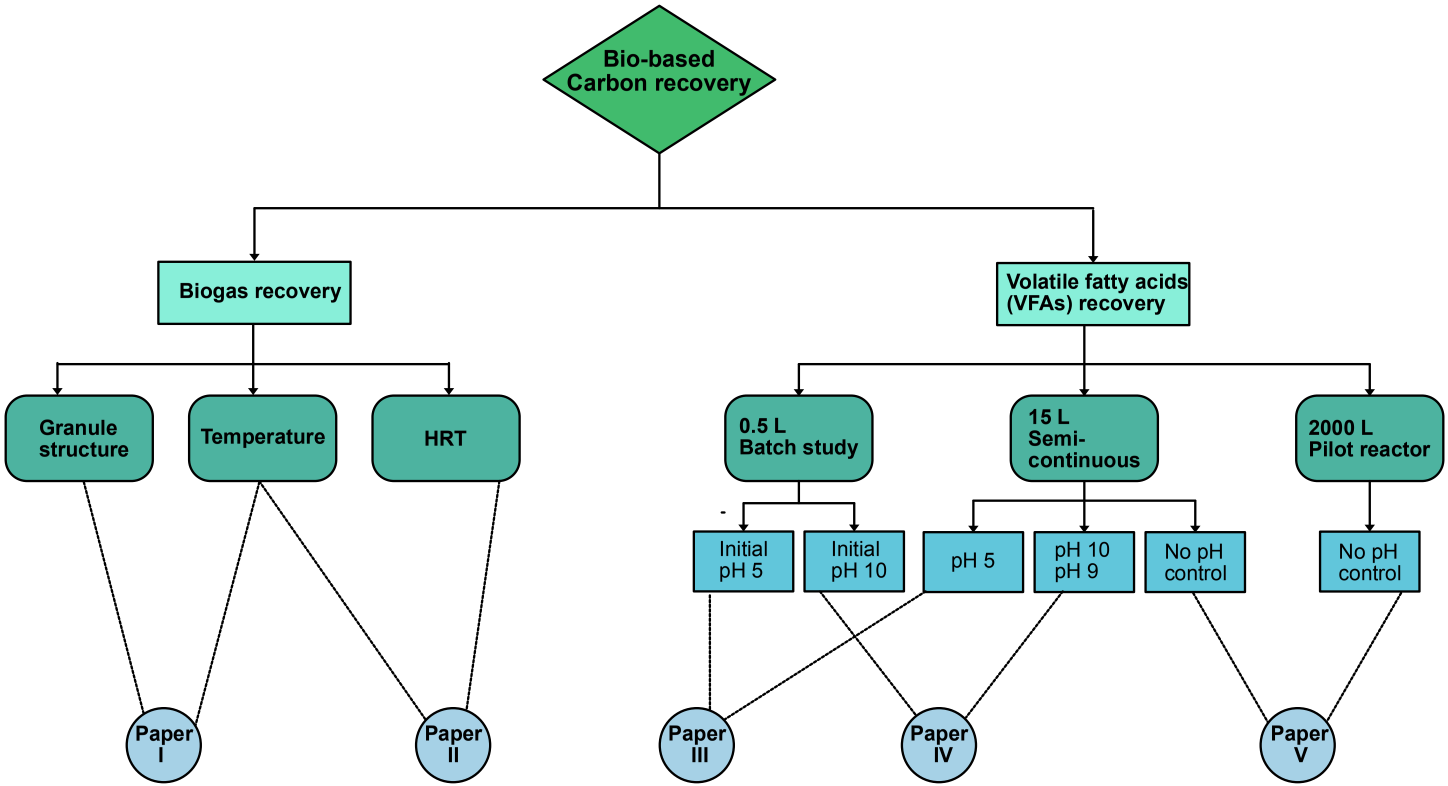

Figure 2: Overview of the content of the Ph.D. thesis.

2 Anaerobic treatment of municipal waste streams

This chapter provides the background on the anaerobic digestion process and its application in the treatment of municipal waste streams. The chapter also reviews the various techniques employed for direct anaerobic treatment of municipal wastewater. The production of VFA from waste streams and operating parameters that influence the process are outlined.

2.1 Anaerobic treatment process

The anaerobic treatment process is a nature-inspired engineered process involving the use of different stages in which organic matter is broken down by a consortium of microorganisms in the absence of oxygen. It leads to the formation of biogas (methane, carbon dioxide, and some trace gases). The stoichiometric equations of the anaerobic digestion (AD) processes depending on the elemental composition of the substrates are given in equation 1 and 2 (Boyle, 1977; Buswell and Mueller, 1952). The equation 1 shows the reactants and products of AD process with organic matter ($C_aH_bO_c$) containing carbon, hydrogen, oxygen while equation 2 is for organic matter ($C_aH_bO_cN_dS_e$) containing carbon, hydrogen, oxygen, nitrogen, and sulphur.

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right) CO_2 \qquad 1)$$

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{4} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S \qquad 2)$$

Table 2: Typical composition of biogas (adapted from Gould, 2015; Lackey et al., 2015).

Biogas component	Formula	Content (%)
Methane	CH_4	50-75
Carbon dioxide	CO_2	25-50
Hydrogen sulphide	H_2S	0-3
Ammonia	NH_3	0-1
Moisture	H_20	0-10
Nitrogen	N_2	0-10
Hydrogen	H_2	0-1

Biogas is composed mainly of CH_4 and CO_2. There are other gases in biogas with relatively low percentages, including hydrogen sulphide, ammonia, nitrogen, moisture, and hydrogen. Table 2 presents the typical percentages of various gases that make up biogas from the AD of waste streams.

Four main stages are involved in the AD process which include hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Bajpai, 2017), as illustrated in Figure 3. These four stages of the AD process are further explained below:

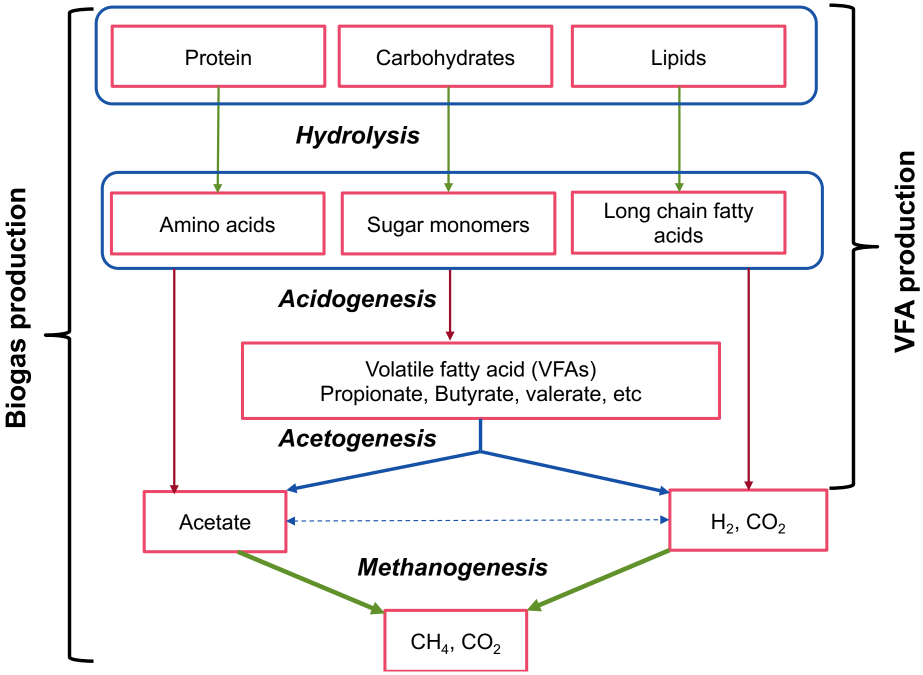

Figure 3: Stages in anaerobic digestion adapted from (Bajpai, 2017; Ersahin et al., 2011; Gould, 2015; Tezel et al., 2011).

In the first stage of the anaerobic process-*hydrolysis*, large organic polymers such as carbohydrates, proteins, and lipids are broken down to sugars monomers, amino acids, and long-chain fatty acids by hydrolytic enzymes produced by the anaerobes. Carbohydrates such as cellulose are hydrolysed by cellulase to glucose and proteins are hydrolysed by protease to amino acids. Moreover, the lipase enzyme breakdown lipids to long-chain fatty acids (LCFAs) and glycerol (Pavlostathis, 2011). Equations 3 and 4 show a typical equation of how polysaccharides (Cyclotetraglucose; $C_{24}H_{40}O_{20}$) and lipids (Triolein; $C_{57}H_{104}O_6$) are hydrolysed into glucose and LCFA (oleate, $C_{18}H_{34}O_2$) and glycerol ($C_3H_8O_3$), respectively (Abdelgadir et al., 2014; Angelidaki et al., 1999). Hydrolysis is a rate-limiting step of the AD process and therefore in some cases, to speed up the process, other pretreatment techniques, including thermal, chemical, and physical methods are used to facilitate solubilization of particulate organic matters (Ariunbaatar et al., 2014). The pretreatment methods often employed for waste treatment include hydrothermal, ultrasonic, alkaline techniques and use of chemicals like sodium dodecylbenzene sulfonate (Guo et al., 2014; Owusu-Agyeman et al., 2021a; Wan et al., 2020)

$$C_{24}H_{40}O_{20} + 4H_2O \rightarrow 4\,C_6H_{12}O_6 \quad 3)$$

$$C_{57}H_{104}O_6 + 3H_2O \rightarrow 3C_{18}H_{34}O_2 + C_3H_8O_3 \quad 4)$$

The second stage is *acidogenesis (fermentation),* where the simpler organics are anaerobically oxidised by acid-forming bacteria (acidogens) to VFAs (such as propionic acid, butyric acid to acetic acid) alcohols, hydrogen (H_2), and carbon dioxide (CO_2). Acidogenesis can be carried out by both obligatory and facultative anaerobic bacteria (Manchala et al., 2017). Equation 5 shows the stoichiometry of acidogenesis of sugar monomer (unit of polymers of glucose) to VFAs (acetic acid ($C_2H_4O_2$), propionic acid- ($C_3H_6O_2$) and butyric acid ($C_4H_8O_2$)). In the course of acidogenesis, biomass ($C_5H_7NO_2$) is usually formed (Yu and Wensel, 2013). Acidogenic conversion of Gelatin ($CH_{2.03}O_{0.6}N_{0.3}S_{0.001}$) to acetic, propionic, butyric, valeric acids($C_5H_{10}O_2$) is shown in equation 6 (Angelidaki et al., 1999). Equations 7 and 8 show the stoichiometry of acidogenesis of an LCFA($C_{18}H_{34}O_2$, oleate) and glycerol ($C_3H_8O_3$) to VFAs and H_2 (Angelidaki et al., 1999; Manchala et al., 2017). The equations show that the kind of acidogenic product depends very much on the composition of the substrate being digested.

$$C_6H_{10}O_5 + 0.11115NH_3 \rightarrow 0.1115C_5H_7NO_2 + 0.744C_2H_4O_2 + 0.5C_3H_6O_2 + 0.4409C_4H_8O_2 + 0.6909CO_2 + 0.0254H_2O \quad 5)$$

$$CH_{2.03}O_{0.6}N_{0.3}S_{0.001} + 0.3006H_3O \rightarrow 0.017013C_5H_7NO_2 + 0.29742C_2H_4O_2 + 0.02904C_3H_6O_2 + 0.022826C_4H_8O_2 + 0.013202C_5H_{10}O_2 + 0.07527CO_2 + 0.28298NH_3 + 0.001H_2S \quad 6)$$

$$C_{18}H_{34}O_2 + 15.2398\ H_2O + 0.2500CO_2 + 0.1701NH_3 \rightarrow 0.1701C_5H_7NO_2 + 8.6998C_2H_4O_2 + 14.500H_2 \quad 7)$$

$$C_3H_8O_3 + 0.0291CO_2 + 0.04071NH_3 \rightarrow 0.04071C_5H_7NO_2 + 0.9418C_3H_6O_2 + 1.09305H_2O \quad 8)$$

The third stage is *acetogenesis.* Acetogenesis involves the production of acetate, hydrogen, and carbon dioxide from VFAs and LCFAs by acetogenic bacteria. Moreover, H_2 and CO_2 can also be converted to acetate in a process referred to as Homoacetogenesis by acetogens such as *Acetobacterium woodii*, *Clostridium aceticum*, *Clostridium thermoaceticum* (Diekert and Wohlfarth, 1994; Pavlostathis, 2011). Equation 9-12 show stoichiometric conversions of propionic acid, butyric acid, valeric acid, and oleate, respectively, to acetic acid, H_2 and CO_2 (Angelidaki et al., 1999; Manchala et al., 2017; Yu and Wensel, 2013). Homoacetogenic reaction is shown in Equation 13 (Diekert and Wohlfarth, 1994). This shows a complex system, and it is not straightforward to know the exact product that was produced at the acetogenesis stage.

$$C_3H_6O_2 + 1.764\ H_2O + 0.0458\ NH_3 \rightarrow 0.0458\ C_5H_7NO_2 + 0.9345\ C_2H_4O_2 + 2.804\ H_2O + 0.902\ CO_2 \quad 9)$$

$$C_4H_8O_2 + 1.7818\ H_2O + 0.0544\ CO_2 + 0.0544\ NH_3 \rightarrow 0.0544\ C_5H_7NO_2 + 1.8909\ C_2H_4O_2 + 1.8909\ H_2 \quad 10)$$

$$C_5H_{10}O_2 + 0.8045\ H_2O + 0.5543\ CO_2 + 0.0653\ NH_3 \rightarrow 0.0653\ C_5H_7NO_2 + 0.8912\ C_2H_4O_2 + 0.02904\ C_3H_6O_2 + 0.4454\ CH_4 \quad 11)$$

$$C_{18}H_{34}O_2 + 15.2398\ H_2O + 0.25\ CO_2 + 0.1701\ NH_3 \rightarrow 0.1701\ C_5H_7NO_2 + 8.6998\ C_2H_4O_2 + 14.5\ H_2 \quad 12)$$

$$4\,H_2 + 2\,CO_2 \rightarrow C_2H_4O_2 + 2\,H_2O \qquad 13)$$

The final stage of the anaerobic process is *methanogenesis*. This stage involves the use of acetate and hydrogen as substrates to produce methane (CH_4) and CO_2 by methane-producing microorganisms (methanogens). There are two main methanogenic pathways: 1) Acetoclastic methanogenesis (equation 14) during which acetoclastic methanogens produce CH_4 from acetate, and 2) Hydrogenotrophic methanogenesis (equation 15) which involves the production of CH_4 from H_2 and CO_2 (Manchala et al., 2017). Besides hydrogenotrophic and acetoclastic archaea, some methanogens can utilize methylated substances (such as methanol, methylamines, and methyl sulphides) and CO_2 to produce CH_4. These methanogens are termed methylotrophic methanogens (Holmes and Smith, 2016). An example of a methylotrophic methanogenic reaction with methanol (CH_3OH) as the substrate is shown on equation 16 (Hippe et al., 1979). Most of the known methanogens use a hydrogenotrophic pathway to produce CH_4, while very few methanogens take the acetoclastic pathway. The only known acetoclastic methanogens are from the families *Methanosaetaceae* and *Methanosarcinaceae* – all from order *Methanosarcinales* (Fournier and Gogarten, 2008). The Genus *Methanosarcina* under the family *Methanosarcinaceae* is said to be the most diverse genus because it has distinctive species which produce CH_4 from all the three methanogenic pathways (Holmes and Smith, 2016). In an anaerobic digester, methane could be produced from different methanogenic pathways depending on the dynamics of the archaea community.

$$C_2H_4O_2 + 0.022\,NH_3 \rightarrow 0.022\,C_5H_7NO_2 + 0.945\,CH_4 + 0.945\,CO_2 + 0.066\,H_2O \qquad 14)$$

$$2.804\,H_2 + 0.7413\,CO_2 + 0.01618\,NH_3 \rightarrow 0.001618\,C_5H_7NO_2 + 0.6604\,CH_4 + 1.45\,H_2O \qquad 15)$$

$$4\,CH_3OH \rightarrow 3\,CH_4 + CO_2 + 2\,H_2O \qquad 16)$$

As can be seen from the equations above, biogas, mainly CH_4 and CO_2, is the end-product of the AD process. Because of this, biogas (methane) is the main form through which carbon is recovered from waste streams. Besides biogas, VFAs produced in acidogenesis and acetogenesis steps are important renewable carbon sources, with various applications such as bioplastics, hydrogen, biodiesel, bioelectricity, biogas productions, and biological nutrient removal from wastewater (Lee et al., 2014; Zhou et al., 2018). In an AD process, VFA production can be promoted by shortening the reaction time to prevent methanogenesis. Adjusting pH above 8.0 or below 6.0 can also promote VFA production and inhibit the growth of methanogens. Adding a methanogenic inhibitor could also be used to inhibit methanogens and promote VFAs production.

AD operation has been applied in municipal wastewater treatment for many years. In municipal WWTPs, AD can be applied for the treatment of mainstream wastewater or the treatment of side stream sewage sludge. The stabilization of sewage sludge (sidestream) with AD to produce biogas is the common practice in many WWTPs. On the other hand, mainstream municipal wastewater is often treated by employing the traditional activated sludge method which requires air. Nonetheless, municipal wastewater can be anaerobically treated directly. An example of anaerobic technologies for direct wastewater treatment is the upflow anaerobic sludge blanket reactors (UASB). Treating mainstream municipal wastewater anaerobically will cut the cost associated with aeration and produce biogas.

2.2 Direct anaerobic treatment of municipal wastewater

Direct anaerobic treatment involves the application of AD in mainstream municipal wastewater. Thus, the anaerobic digestion process becomes the core of the WWTP. Biogas is therefore produced directly and energy that would have been used for aeration in the case of conventional wastewater treatment can be saved. Mainstream anaerobic treatment also leads to low excess sludge production which is usually already well stabilized (van Lier et al., 2008). Moreover, nutrients (N and P) are not removed during direct anaerobic treatment of municipal wastewater, making it a good candidate for nutrient recovery or application directly to farmlands. However, in large WWTPs, this can be a bottleneck, and post-treatment may be required before releasing the wastewater into the environment due to national and regional standards to be met. Another issue with the direct anaerobic treatment of municipal wastewater is low efficiency, especially at low temperatures. In addition, due to the generally low COD removal rate of anaerobic treatment of wastewater in comparison with conventional activated sludge, larger reactor volumes are required which increases the footprint.

One prominent challenge of anaerobic process application for the treatment of mainstream municipal wastewater is difficulty in retaining anaerobic microorganisms (slow growth rate) due to the low concentration of substrates in the wastewater. Because of this, there are several configurations of anaerobic reactors for the treatment of municipal wastewater to which ensure high efficiency by retaining the biomass regardless of the hydraulic retention time (Ince et al., 2017). These include anaerobic baffled reactors, anaerobic filter, anaerobic sequencing batch reactor, anaerobic fixed film, anaerobic membrane bioreactor, and anaerobic granular-based reactors. Some of the high-rate anaerobic processes for direct municipal wastewater treatment are elaborated below:

Anaerobic baffled reactor (ABR) design is one of the direct anaerobic treatment technologies that have been applied for the treatment of municipal wastewater. It is a high-rate anaerobic treatment technology that was developed in the 1980s by Bachmann et al. (1982). ABR is an anaerobic design where influent wastewater passes through a series of compartments connected employing baffles within a downflow or upflow mode in an anaerobic filter system (Figure 4) (Bachmann et al., 1985). Biomass is either attached to filter media or form microbial mass due to the upflow process which gives stability and reliability. Microorganisms with different functions are separated into different compartments along the reactor resulting in the occurrence of different stages of the anaerobic process in different compartments. There are different configurations of ABR depending on the type of wastewater to be treated. Studies have shown that ABR has been successfully being applied in the treatment of municipal and domestic wastewater with COD removal of 72-93% (Zhu et al., 2015). However, application on large scale is limited due to hydrodynamic constraints which result in high apparent liquid velocity which lowers the sludge retention time by moving sludge with the water through different compartments (van Lier et al., 2015). This limitation clearly explains why ABR has not attracted high attention and has not been further developed in a full-scale application.

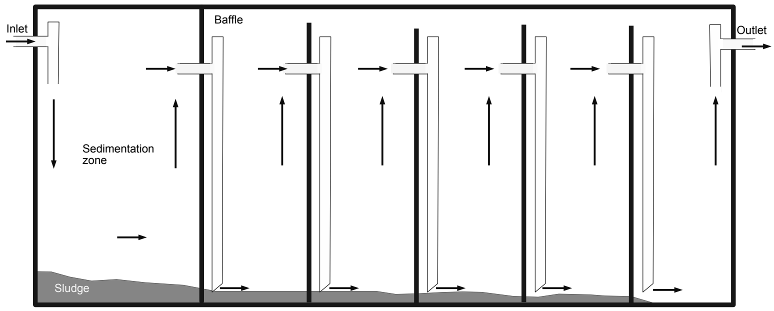

Figure 4: Schematic diagram of an anaerobic baffled reactor.

Anaerobic sequencing batch reactor (ASBR) is a suspended growth system that operates in four sequencing steps including feeding, reaction, settling, and discharging (Figure 5). During the feeding step, influent wastewater is added to the reactor while the reactor is continuously mixed. In the reaction step, there is continuous or intermitted mixing to allow organic matter conversion to biogas. During the settling step, there is no mixing and the biomass in the reactor is allowed to settle to the bottom of the reactor. After settling, the treated waste is decanted at the discharge stage leaving the biomass in the reactor (Zaiat et al., 2001). ASBR, at the start of the operation, usually has a high concentration of substrate and decreases towards the end of the reaction step resulting in variation in the food/microorganism (F/M) ratio. It is said that this variation is an important characteristic that gives ASBR a unique feature to achieve high removal efficiency of organic matter, shock load tolerance, and good microbial selection (Ndon, 1995). Although there have been studies on the application of ASBR for treatment of low strength wastewater (Donoso-Bravo et al., 2009; Ndon and Dague, 1997), its full-scale application for municipal wastewater treatment is very limited, probably due to the bottleneck of high reactor volume requirement (Ince et al., 2017).

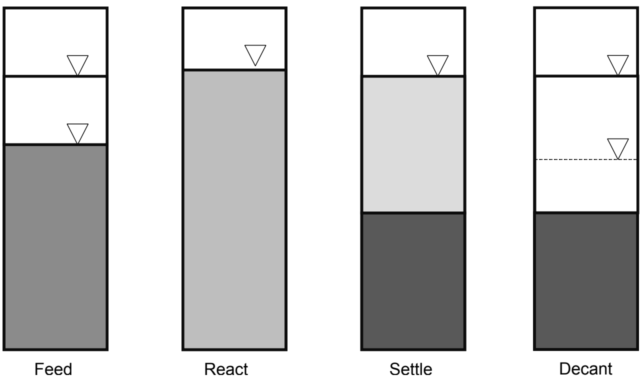

Figure 5: Schematic diagram of how an ASBR works.

A more compact anaerobic system for mainstream municipal wastewater treatment is the *anaerobic membrane bioreactor (AnMBR)* which incorporates solid-liquid separation by membrane filtration into the anaerobic treatment process. There are two main configurations of AnMBR: side stream and submerged. With the side stream configuration, the membrane system is externally connected to the anaerobic bioreactor while for the submerged configuration, the membrane is directly immersed in the anaerobic reactor or submerged in a separate chamber connected to the anaerobic bioreactor (Dvořák et al., 2016). As an emerging technology, AnMBR has a couple of hurdles to overcome before its full-scale application for municipal wastewater can be widely accepted by WWTPs. Notable among the challenges is membrane fouling and cleaning which lead to high operational costs making it unattractive for WWTPs. Integration with fluidized activated carbon sludge is seen as one of the ways to reduce fouling and energy demands of AnMBR (Seib et al., 2016). It is opined AnMBR can be a promising treatment option for municipal wastewater treatment to produce quality treated effluent for reuse if the membrane system is coupled with high rate granular based bioreactors such as UASB (Ozgun et al., 2013).

Anaerobic granular-based treatment techniques are high-rate processes that use biomass immobilized in a granular form as the core of the wastewater treatment technology. When compared with other technologies for direct wastewater treatment, anaerobic granular-based reactors are seen as suitable for the treatment of municipal wastewater due to many advantages of granular sludge which include good settling properties. The settling properties of anaerobic granules prevent biomass washout and maintain a very high number of microorganisms in the reactor (Lim and Kim, 2014; Liu et al., 2002). In anaerobic granular systems, there is sufficient contact between the substrate and microorganisms due to natural turbulence resulting from gas bubbles released by granules and influent flow (Subramanyam and Mishra, 2013). Another unique characteristic of anaerobic granules is their high tolerance level against fluctuation in temperature, pH, substrate concentration, and high salinity (Sudmalis et al., 2018). Anaerobic granular-based treatment processes include UASB, fluidized bed (FB) reactor, expanded granular sludge bed (EGSB) reactor, internal circulation (IC) reactor. FB, EGSB, and IC are seen as advancements in the UASB system (van Lier et al., 2015). Even so, UASB is still the most applied high-rate anaerobic process for mainstream municipal wastewater treatment showing the robustness and feasibility of the UASB system.

2.2.1 Upflow anaerobic sludge blanket reactor

UASB was developed in the 1970s with the first full-scale plant in 1977 in Halfweg, the Netherlands (Lettinga et al., 1980). UASB operates in such a way that influent wastewater flows upwards from the bottom of the bioreactor upwards (Figure 6). The combination of the upward flow and gravity results in a suspended sludge blanket. The hydrodynamics of the reactor results in the formation of compact biomass aggregates with anaerobic microorganisms that degrade organic matter resulting in the formation of biogas. The granular sludge is suspended throughout operation with help of influent upward flow and gas bubbles formed during degradation (Rodríguez-Gómez et al., 2014; Rodriguez and Moreno, 2010). UASB reactors have three-phase separators located above the suspended sludge blanket to separate the mixture of biogas, water and granular sludge after treatment.

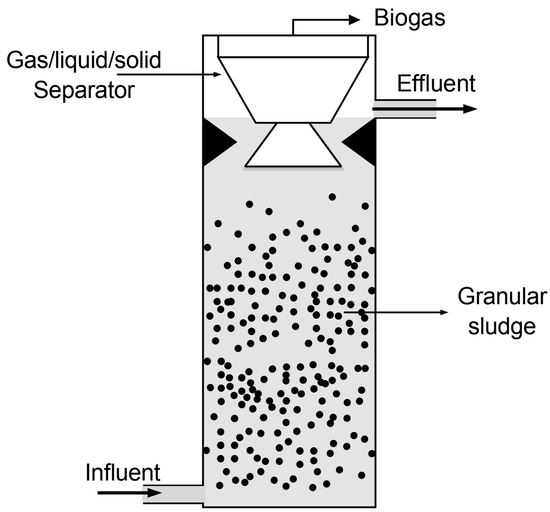

Figure 6: Schematic diagram of an UASB reactor.

UASB has proven to be a robust system which has been applied mainly for the treatment of high-strength wastewater with higher COD concentrations such as food industrial wastewater. Moreover, there are several applications of UASB for the treatment of low-strength wastewaters such as domestic and municipal wastewaters, especially in tropical climates. The application of UASB for the treatment of low-strength wastewater in temperate regions is yet to be materialised due to the low temperatures of the wastewater in such regions. Besides temperature, the performance of the UASB reactor is influenced by several other factors including pH, COD loading, hydraulic retention time (HRT), upflow velocity, and granulation. The factors influencing the efficiency of UASB reactors are detailed below.

2.2.2 Parameters affecting UASB reactor performance

Temperature

Most anaerobic bacteria and archaea have optimal growth in the mesophilic temperature range of 35-38°C. At this range, metabolic activities of the microorganism are optimum and therefore the performance of the UASB reactor is high. Decreasing the temperature can drastically decrease the activities of anaerobic microbiomes. In some recent studies, UASB has also been examined for the treatment of municipal wastewater at low temperatures (Bandara et al., 2012; Petropoulos et al., 2019; Ribera-Pi et al., 2020; Serrano León et al., 2018; Zhang et al., 2013). A study obtained a COD removal efficiency of 79 % by operating at a low temperature of 15 °C and energy balance showed that the UASB system was energy positive with a net energy gain of 41 W h per m^3 of treated wastewater (Petropoulos et al., 2019).

pH

Anaerobic microorganisms responsible for the breakdown of organic matter in UASB reactors can function in a certain pH range. pH values out of the optimal range for the bacteria or archaea could drastically reduce their activities which will adversely affect the performance of the UASB reactor. It has been shown that the UASB reactor could handle influent wastewater of pH 5.5–8.5, however, the reactor performed best at neutral pH (Majumder and Gupta, 2009). At high pH values, ammonium nitrogen could dissociate and increase the ammonia concentration thereby inhibiting methanogenic and acidogenic activities (Kadam and Boone, 1996; Park et al., 2018)

Influent COD

Municipal wastewaters are usually low strength wastewater. The lower the concentration of the influent COD, the lower the UASB reactor efficiency. This is because there is a mass transfer limitation at lower dissolved COD concentrations (Leitao, 2004).

HRT

The efficiency of the UASB reactor increases with an increase in the HRT (Bhatti et al., 2014). The decrease in organic carbon removal with a decrease in the HRT can be attributed to sludge washout and limited contact time between the wastewater and the microorganisms (Leitao, 2004). HRTs of 4-10 h have been used in full-scale UASB plants treating municipal wastewaters (van Lier et al., 2010).

Effect of granulation

Granular sludge is the core of the UASB reactor. The quality of granulation therefore greatly determines the performance of the UASB reactor. The characteristics of the anaerobic granules such as size and internal structure influence the efficiency of UASB reactors. Bigger granules give higher organic removal and biogas production (Jijai et al., 2015). The internal structures of granules also influence the efficiency of UASB reactors since the mass transfer of substrates and produced biogas bubbles are influenced by internal microstructure (Jiang et al., 2016).

2.3 Anaerobic digestion of sewage sludge and other municipal organic solid

Anaerobic digestion is by far the most proven and widely used technology for stabilizing and treating sewage sludge and other organic waste with recovery of resource possibilities. AD reduces the amount of waste to be disposed of, kills pathogens, and reduces odour. The main potential for renewable feedstock and energy from wastewater is sewage sludge. AD provides both economic and environmental benefits. AD of sewage sludge with other organic waste maximize biogas production and WWTP energy self-sufficient (Nghiem et al., 2017).

Treatment of sewage sludge and external organic waste such as food and biodegradable garden waste offers an opportunity for energy and feedstock recovery from municipal waste streams. Recovery of resources such as biogas and VFAs from sludge and external organic waste can be utilized as fuel and raw material to offset heat, electricity, and raw material requirements of the wastewater treatment sector. The end-product of the anaerobic process is biogas. Biogas is often upgraded to biomethane and used in applications such as electricity, combined heat and power and fuel for transport. Besides biogas, VFA is one of the renewable energy resources that can be produced from organic wastes through anaerobic digestion.

2.4 Volatile fatty acids

Volatile fatty acids are the intermediate products of the AD process.These important building block chemicals are produced at the acidogenesis and acetogenesis stages of the AD process. To achieve VFA production in the AD process, there is suppression of the methanogens through what is referred to as arrested methanogenesis (Bhatt et al., 2020). Thus, VFA can be produced through the AD process by cutting the time of the process to prevent methanogenesis from taking place. Moreover, pH adjustment is one of the common ways to shift the production of anaerobic digester from biogas to VFA. Methanogens which are responsible for biogas production can be inhibited by altering the pH of the reactor above 8.0 or below 6.0 (Zhou et al., 2018). Methanogenic inhibitors can also be added to reduce the activities of methanogens, but this will add to the costs of operation and may not be cost-effective full-scale application. periodical heat shock is another way to promote VFA production in the AD system (Atasoy et al., 2018; Wu et al., 2021). Although methane-biogas is an energy and carbon carrier that can be used in various applications, the value of methane is relatively low in comparison with VFAs. The prices of VFAs are high and the market size is significantly increasing due to unique properties and the wide range of application areas. Table 3 shows the properties and prices of biogas and VFAs.

Table 3: Chemical properties and prices of bioproducts from AD processes.

VFAs	Chemical Formula	Molar mass (g/mol)	Density at 25°C (kg/m^3)	Boiling point (°C)	pKa	Price* ($/kg)
Methane	CH_4	16	657	-161.5		0.15
Acetic acid	CH_3COOH	60	1049	118	4.79	0.6-1
Propionic acid	CH_3CH_2COOH	74	993	141	4.87	1.5-4.0
Butyric acid	$CH_3(CH_2)_2COOH$	88	964	162	4.82	2.0-4.0
Iso-butyric acid	$(CH_3)_2CHCOOH$	88	950	154	4.86	
Valeric acid	$CH_3(CH_2)_3COOH$	102	939	185	4.82	2
Iso-valeric acid	$(CH_3)_2CHCH_2COOH$	102	926	176	4.78	6
Caproic acid	$CH_3(CH_2)_4COOH$	116	927	204	4.88	2.2-2.5
Iso-caproic acid	$(CH_3)_2CHCH_2CH_2COOH$	116	923	200	5.09	

*Prices are from (Zacharof and Lovitt, 2013) and (LePro PharmaCompass, 2018)

2.4.1 Biogas versus VFA

Now the question is which of the renewable energy sources, biogas and VFA, is more valuable and will produce the net economic gain? As it has been shown in Figure 3, generating biogas from sludge will take a longer time than producing VFA. This means that for the same amount of sludge to be digested, the size of a biogas reactor must be bigger than that of a VFA reactor. The reaction time of sludge is about 25 days for biogas production (Duan et al., 2012), but about 7 days for VFA (Liu et al., 2018).

VFAs have a higher value than biogas. Calt (2015) has shown that by allowing the conversion of VFAs to methane during the AD process, a significant economic opportunity is lost, as illustrated in Table 4. Biogas needs to be purified as it contains not only methane but also carbon dioxide and impurities such as water vapour and hydrogen sulphide. Carbon dioxide, which is a greenhouse gas, may end up in the atmosphere. If accidentally released into the atmosphere, methane has about 80 times more greenhouse potential (during 20 years) than carbon dioxide (Myhre et al., 2013).

Table 4: Economic loss in converting acetate (VFA) to biogas (modified from Calt (2015)).

Description	Acetate (VFA) production	Biogas production
Formula	$C_2H_4O_2$	$CH_4 + CO_2$
Carbon available for sale (%)	100	50
Price ($/ton)	600	150
Time to produce (days)	1-7	>25

Before VFA production from sludge, there may be the need for pre-treatment of sludge including pH adjustment, which may add to the cost of production. Like biogas upgrades, recovery can be an important step for the VFA production process. Depending on the intended use and the required level of purity, the cost for purification of VFA produced from the digestion of sludge can be high since methods such as electrodialysis and pressure-driven membrane technology are used (Tao et al., 2016; Zhou et al., 2013). In a recent study, biogas and VFA production from sludge were compared and it was concluded that the net profits for VFAs and biogas productions are 9.12 and 3.71 USD/m^3 sludge (Liu et al., 2018). In that study, VFA produced was used to improve biological nutrients removal in domestic wastewater and therefore there was no VFA recovery or purification cost.

2.4.2 Factors affecting VFA production

VFA production from waste streams is influenced by several factors including pH, temperature, organic loading rate, substrate characteristics, seed sludge, etc (Atasoy et al., 2018). These factors affect not only the VFA yield but also the composition. There have been efforts from various studies to understand how each factor influence VFA production and composition through mixed culture fermentation. However, the outcomes point to the fact that there are no clear-cut conclusions. It is important to note that the factors don't act in isolation but through a complex network to influence the quantity and quality of VFA production (Atasoy et al., 2019). More importantly, the kind of waste stream under question is a key factor when considering operation parameters. The issue can be even more complex when a mixture of different waste streams is to be treated through co-fermentation. A comprehensive explanation of the factors affecting VFA production can be found in Atasoy et al., (2018), a review article written during this Ph.D. study. Some of the factors affecting VFA production from waste streams are discussed:

pH

pH is an important factor that affects both VFA yield and composition due to its effect on the microbial community structure and the metabolic pathway (Chen et al., 2017; Liu et al., 2012). Moreover, pH influences both solubilization and hydrolysis of complex organic compounds that precede the VFA production (fermentation) process. Solubilization and hydrolysis of complex organic matter into smaller and soluble molecules are rate-limiting steps of the AD process that are crucial for VFA production. Increasing the pH enhances the breakdown of complex substrates into simpler molecules (Atasoy et al., 2019; Garcia-Aguirre et al., 2017a). Nonetheless, the enzymatic activities of hydrolytic bacteria can be influenced by pH. The optimum pH range can vary and the suitable pH for hydrolysis will depend on the enzymes involved. Moreover, both extracellular pH and intracellular pH can influence the metabolic pathways of a VFA production process which will intend to determine the VFA type produce (Mohd-Zaki et al., 2016). In a mixed culture system for VFA production, pH can also influence the microbial population which subsequently influences the VFA yield and composition (Atasoy et al., 2019). Therefore, understanding how pH and the microbial community is important to ensure stable VFA production. pH affects not only the amount of VFA produced but also the VFA type. For instance, alkaline pH promotes acetic acid production through the phosphoroclastic degradation pathway (Dahiya et al., 2015; Regueira et al., 2021).

Substrate characteristics

VFA production is influenced by the properties of substrates or organic wastes being treated. The biodegradability of the organic substrates and the readily fermentable organic fraction of the substrate will determine the VFA yield of any given substrate. Different waste streams may have different characteristics and could have varying degrees of acidification as substrates for VFA production (Ucisik and Henze, 2008). In a study with 8 different organic waste streams but almost the same initial substrate concentration, the highest VFA productions of 2700–3400 COD mg/L were achieved with Cheese whey, molasses, and organic fraction of municipal solid waste (OFMSW), followed by Glycerol, olive mill effluent and winery effluent with VFA production of 930–1500 COD mg/L whereas soapy slurry waste and landfill leachate produced lowest 240–630 COD mg/L (Silva et al., 2013). Substrate characteristics do not only influence the amount of VFA but also the type of VFA produced. For instance, carbohydrate-rich waste streams usually can lead to acetic, propionic, and butyric acid production while protein-rich substrates are usually fermented to VFAs with high valeric and iso-valeric acids proportions (Garcia-Aguirre et al., 2017b; Shen et al., 2017, 2014)

Retention time

VFA production is influenced by the time given by the substrate to ferment. While enough time is required to ensure a complete fermentation process, higher retention time may lead to the conversion of acid into biogas; particularly, in a system whereby the methanogenesis is not inhibited. Moreover, the optimum retention time will depend on the characteristics of the substrate. Depending on the type of substrate, more time may be required to ensure complete hydrolysis. Other operating parameters such as pH and temperature may influence the optimal retention time to ensure enough VFA production (Jankowska et al., 2015). In addition, retention time could determine the type of VFA produced. In a study of anaerobic fermentation of vegetable and salad waste at retention times of 10, 20, and 30 days, butyrate and acetate were the dominant VFA types, however, caproic acid was detected as the second dominant VFA on retention times 20 and 30 days (Bolaji and Dionisi, 2017).

Temperature

Temperature influences VFA production because it can influence many other factors such as the growth of microorganisms, enzyme activity, and hydrolysis rate (Zhou et al., 2018). In a mixed culture environment, different VFA producers are involved. These microorganisms have different optimal temperature ranges for their growth and activities. Thus, the temperature will affect the bacterial population and subsequently the VFA production. Most fermentation bacteria have the highest activity in the mesophilic temperature range and therefore this is a favourable condition and economical for VFA production as the VFAs yields (Gruhn et al., 2016; Jiang et al., 2013). Nonetheless, thermophilic conditions can enhance hydrolysis which could result in higher VFA production (Hao and Wang, 2015). Temperature can also influence the VFA composition. In a study with food waste, it was reported that acetic acid (36%) and propionic acid (32) were the main VFA types at an operating temperature of 35°C whereas butyric acid (81%) dominated at 55°C (Jiang et al., 2013).

2.4.3 Co-fermentation of sewage sludge and external organic waste

Alongside sewage sludge from municipal WWTPs, there are other kinds of organic wastes which need to be treated. External organic wastes (OW) such as food waste, beverage waste, dairy waste, which are not generated within the WWTPs, usually are landfilled and eventually lead to the release of greenhouse gases, increasing the carbon footprint of waste management. It is estimated that about 88 Mt of food waste is produced in the EU countries. The production and disposal of this amount of food waste can release about 170 Mt of CO_2 equivalent of greenhouse gases into the atmosphere contributing immensely to climate change (European Parliament, 2017). External organic wastes such as food waste usually have a higher content of readily biodegradable organic matter than sewage sludge with enormous inherent energy and bioproduct potential. Moreover, it is established that many existing sewage sludge anaerobic digesters of various WWTPs have excess capacities (Ely and Rock, 2014; Nghiem et al., 2017). This scenario presents a great opportunity to practice co-digestion of sewage sludge together with external organic waste like food waste. Since co-digestion will divert other waste streams from landfills, it is seen as an innovative waste management system with resource recovery potential (Xie et al., 2018). There have been several studies and full-scale applications on co-digestion of sewage sludge and external organic waste for biogas production with very positive outcomes. However, there are only a few studies on VFA production from sewage sludge and external organic wastes. To shift production from biogas to VFA, co-fermentation can be an ultimate option.

2.4.4 Application of waste-derived VFAs

In general, VFA can be used in various applications including food preservatives, flavours, supplement antibiotics, fragrances, emulsions. However, waste-derived VFAs are used in areas such as bioplastics, hydrogen, biodiesel, bioelectricity, biogas, single-cell protein productions, and biological nutrient removal from wastewater (Bhatt et al., 2020; Lee et al., 2014; Wainaina et al., 2020). Here, three applications including biological nutrient removal, bioplastics, and biodiesel, are discussed as found in Atasoy et al., (2018).

Biological nutrient removal

Biological nitrogen and phosphorus removal from wastewater processes require an external carbon source. Usually, petroleum-based acetate, methanol, ethanol, and glucose are used as carbon sources. These are usually expensive and have environmental consequences. Waste-derived VFAs which are easily assimilated by nutrient-removing microorganisms present sustainable and economically viable alternatives to petroleum-based conventional carbon sources. Several studies have shown that waste-derived VFAs performed better as carbon sources than conventional petroleum-based carbon sources (Kim et al., 2016; Liu et al., 2016; Zhang et al., 2016; Zhang et al., 2016). Moreover, the application of waste-derived VFAs for biological nutrient removal in full scale has been proven to be economically feasible (Liu et al., 2018).

Bioplastics (Polyhydroxyalkanoates)

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters that can be produced from waste-derived VFAs. Usually, PHAs are produced from pure cultures with pure substrates which makes the production cost high (Khatami et al., 2020). There have been many studies aiming at producing PHA from mixed microbial cultures using waste-derived VFAs as the main substrates (Bengtsson et al., 2017; Burniol-Figols et al., 2018a; Valentino et al., 2017). There have been promising results from PHA production from waste-derived VFAs with PHA accumulation as high as 76% dry cell weight according to (Burniol-Figols et al., 2018b).

Biodiesel

Waste-derived VFAs can be converted to microbial lipids for producing biodiesel with the use of oleaginous microorganisms (Chi et al., 2011; Fei et al., 2011; Ryu et al., 2013). This can be a sustainable alternative to fossil fuel and prevent the use of food crops for fuel production as well as growing fuel crops on arable lands. Oleaginous microorganisms can build up about 70% lipid of the cell biomass (Ratledge, 2004; Ratledge and Wynn, 2002).

3 Motivations and objectives

Enhancing the resources recovered from municipal WWTPs by producing new bio-based products and energy from carbon-rich waste streams will help meet the growing world population and associated material needs. Thus, the development of innovative bio-based technologies to recover resources from waste streams are crucial to curb the reliance on fossil-based materials to obtain a sustainable society. This has therefore warranted increased investigations on the development of next-generation wastewater treatment technologies to recover feedstock and energy. Because of this, the focus of the thesis has been to evaluate existing and up-and-coming biological carbon recovery systems. The study was divided into two main parts:
1) Biogas production through direct anaerobic treatment of municipal wastewater (3.1).
2) VFA production through co-fermentation of municipal sewage sludge and external organic waste (3.2).

3.1 Biogas production from municipal wastewater

The conventional activated sludge treatment system which is usually used for municipal wastewater treatment requires aeration which increases immensely the cost of operation. Moreover, organics are converted to carbon dioxide which is usually released into the atmosphere thereby increasing the carbon footprints of WWTPs. Direct anaerobic treatment of municipal wastewater is a more sustainable option that can transform WWTPs from energy-consuming to energy-producing systems. The use of granule-based systems such as UASB for direct anaerobic treatment is advantageous due to the robustness of anaerobic granules, good setting properties, and good substrate transfer. However, the application of direct anaerobic treatment of mainstream municipal wastewaters under psychrophilic and sub-mesophilic temperatures is limited due to low efficiency and inadequate understanding of in the long run (Lucas et al., 2018). To ensure an efficient system, there is the need for an in-depth understanding of the properties of anaerobic granules and their biochemical pathway and how they are coupled together with operation parameters to influence process performance. There have been studies attempting to relate the mass transfer process and structure of anaerobic granules, however, these studies have not focused on the relationship between the structure of granules and the microbial community. Even though the methanogenic pathway of granule-based reactors is determined mainly by the microbial community structure of the anaerobic granular sludge, only a few studies have linked the physiology of matured anaerobic granules to the microbial population and methanogenic pathway at specific operating temperatures.

The main aim of this part of the study was to demonstrate the feasibility of integrating a direct anaerobic treatment system to WWTP and increase understanding of efficiency and biochemical processes. The study explored the relationship between the methane-producing pathways and the characteristics of anaerobic granules as well as microbial community structure and their response to alterations in the operating conditions. Consequently, the specific objectives of this part of the thesis included:

i. to investigate the effect of the structure of anaerobic granules on the methane-producing pathway of UASB reactors.

ii. to explore the microbial community dynamics at different levels of UASB reactors treating municipal wastewater.
iii. to determine the influence of operating parameters on the microbial community structure and performance of UASB reactors with different granular size distributions.
iv. to ascertain how the microbial community of different kinds of anaerobic granules responds to changes in operating conditions.

3.2 VFA production from municipal waste streams

While direct energy recovery from municipal wastewater is an interesting option, sewage sludge is seen as the main potential for bioproduct and energy recovery from municipal WWTPs. Moreover, external organic waste such as food waste with high readily biodegradable content can increase the potential of sewage sludge and ensure sustainable waste management. However, these valuable resources are often deposited in landfills with associated climate effects and other environmental impacts. Recovery of carbon from these organic-rich wastes is a way to ensure sustainable and innovative waste management. There are several studies on co-digestion of sewage sludge and external organic waste for biogas production with laudable outcomes. VFAs, which are intermediate products, are seen as an alternative to biogas due to their high value and lower carbon footprint. Unlike biogas, the number of studies on co-fermentation of sewage sludge and external organic waste for VFAs production is limited. The long-term operation of the VFA production system and resilience of such a system are yet to be assessed. Moreover, the long-term study of mixed microbiome dynamics of the VFA production system and how parameters such as pH and substrate proportion influence VFA yield, and composition is important for up-scaling purposes. Since substrate variability is inevitable in full-scale application due to different consignments, there is the need to have an in-depth understanding of responses of VFA production to such changes. Consequently, the main aim of this part of the thesis was to explore the feasibility and process optimization of the VFA fermentation system for the recovery of more valuable bioproducts instead of biogas from municipal organic wastes as platform functional chemicals for post-stream bioprocess.
The specific objectives were to:

v. determine the influence of substrate proportion and pH on VFA yield and composition.
vi. study the effect of the long-term semi-continuous operation on the VFA production and composition.
vii. elucidate the interlink between VFA production and the microbial community dynamics as well as the resilience of the system, in the long run.
viii. elucidate the feasibility for upscaling and how substrate variability will influence VFA production and composition.
ix. explore the potential of using VFA-rich effluent as a carbon source for denitrification in comparison with conventional carbon sources.

4 Materials and methods

This chapter describes the materials used in the Ph.D. project including the waste streams and reactor description. Moreover, the experimental design strategies used for both the biogas and VFA production parts of the study have been descried.

4.1 Characteristics of waste streams used in the study

The wastes employed in the study were mainstream municipal wastewater, sewage sludge, and external organic waste which is not generated within the WWTPs.

4.1.1 Municipal wastewater for biogas production

Pre-settled municipal wastewater was used in the first part of the study that involves biogas recovery through direct anaerobic treatment of wastewater. The municipal wastewater was obtained from the Henriksdal WWTP (Stockholm) which treats wastewater from central and southern parts of Stockholm city as well as wastewater from Tyresö, Haninge, Huddinge, and Nacka municipalities in the Stockholm County. In Henrisksdal WWTP, the wastewater went through screens and grit chamber before it was pumped to the Hammarby Sjöstadsverket research facility (Stockholm, Sweden), a research and development plant for wastewater purification operated by a consortium led by the KTH Royal Institute of Technology and IVL Swedish Environmental Research Institute located in Stockholm. The wastewater was then pre-treated with a sedimentation tank before feeding to the UASB reactors. Table 5 gives the characteristics of the pre-settled wastewater.

Table 5: Characteristics of pre-settled municipal wastewater.

Parameters	Unit	Value
Total COD	mg/L	320 ± 80
Soluble COD	mg/L	190 ± 50
pH		7.5 ± 0.2
Alkalinity	$mgCaCO_3/L$	210 ± 40
TSS	mg/L	110 ± 50
VSS	mg/L	90 ± 40
NH_4-N	mg/L	32 ± 5

4.1.2 Sewage sludge and external organic wastes for VFA production

For the VFA production through co-fermentation, primary sludge (PS) and external organic wastes (OW) were used as substrates. The PS was from primary a sedimentation tank situated at the Hammarby Sjöstadsverk. In the present study, 3 different types of OW with similar composition were used. The three types of organic wastes are designated as OW1, OW2, and OW3. OW1 and OW2 are homogenized organic waste and non-homogenized organic waste, respectively, from the Himmerfjärden WWTP (SYVAB, Grödinge). OW3 is homogenized organic waste from the Scandinavian Biogas plant (Södertörn, Sweden). The OW is said to be homogenized if it was heated at 71 °C for 61 min. OW1 and OW2 from Himmerfjärden WWTP were composed of alcohol and soda beverage, food, dairy, fruit, fat, and oil wastes, whereas OW3 from the Scandinavian Biogas consisted of food waste from households, school kitchens, restaurants, the grocery trade, and the food industry and other organic waste from commerce and industries. Seed sludge used as inoculum in both lab-scale and pilot-scale studies of VFA production was collected from a full-scale anaerobic digestor in Henriksdal WWTP. The characteristics of the waste streams used in the study are shown in Table 6.

Table 6: Characteristics of the primary sludge and external organic wastes used as substrates (Paper V).

Parameter	**Unit**	**Primary sludge**	**External organic wastes [a]**		
			OW1	**OW2**	**OW3**
Total COD	g/L	30 ± 15	190 ± 40	160 ± 20	200 ± 30
Soluble COD	g/L	1.3 ± 0.5	70 ± 10	70 ± 10	80 ± 10
TS	g/L	25 ± 10	120 ± 10	140 ± 20	130±10
VS	g/L	20 ± 10	110 ± 10	120 ±10	120 ±10
pH		6.1 ± 0.6	4.4 ± 0.2	3.6 ± 0.2	4.1 ± 0.2
Total N	g/L	0.7 ± 0.6	5.1 ± 1		3.4 ± 0.5
NH_4-N	mg/L	40 ± 10	510 ±180	180 ± 20	540 ± 100
VFA	mgCOD/L	870 ± 300	6000 ± 800	7000 ± 1000	7400 ± 260
Alkalinity	mg$CaCO_3$/L	340 ± 160	60 ± 10	150 ± 20	50 ± 40
Total P	g/L	0.1 ± 0.1	0.7 ± 0.1		0.6±0.2
PO_4-P	mg/L	16 ± 16	370 ± 70	160 ± 20	450 ±170
Carbohydrates	g/100g	2	5.6	6.4	7.4
Protein	g/100g	0.5	1.9	2.3	2.2
Fat	g/100g	< 0.5	1.2	4.4	2.1
Water content	g/100g	97.3	89.9	85.8	87.1
Dry substance (DS)	%	2.4	10.8	16.3	13.9
Ethanol	mg/kg DS	< 54	27 000	17 000	26 000
Methanol	mg/kg DS	250	1900	1500	1600
1-propanol	mg/kg DS	<63	5100	4100	6900
2-propanol	mg/kg DS	300	72	27	59
1-butanol	mg/kg DS	<54	78	30	68
2-butanol	mg/kg DS	<45	430	480	840

[a] OW1: homogenized organic waste from Himmerfjärden WWTP
OW2: non-homogenized organic waste from Himmerfjärden WWTP
OW3: homogenized organic waste from Scandinavian Biogas

4.2 Direct biogas recovery from municipal wastewater (UASB operation)

A granule-based technique using UASB reactors was employed for the study of direct anaerobic treatment of mainstream wastewater. The description of the UASB systems and the experimental strategy have been outlined in the subsequent sub-sections

4.2.1 System description

Two identical pilot-scale UASB reactors having different size distribution of granules and located at the Hammarby Sjöstadsverket were studied. Each of the reactors had a working volume of 2.5 m^3 with a height of 3.85 m. The two UASB reactors had different granule size distributions due to the operation strategy before the current study. The first reactor (UASB1) had larger granules while the second reactor had small granules. Previously, the reactors were operated in series whereby the UASB1 received the influent raw wastewater, whereas UASB2 received the effluent of UASB1 as influent. The reactors were connected in series for about two years before changing to parallel operation in March 2016.

The current study period was from February 2018 to June 2019. During the study, both UASB1 and UASB2 were fed parallelly with the same primary settled municipal wastewater from the Henriksdal WWTP. The schematic of the UASB system is shown in Figure 7.

4.2.2 Operation of the pilot-scale UASB reactors

The study was twofold. The first part of the study focused on the effect of temperature on the reactor performance of anaerobic granule systems. This spans between February and October 2018. During the temperature study, the HRT was set constant at 3 hours for each of the reactors and the temperature of the reactors was controlled at 20 °C and 28 °C from Mid-February to Mid-May, and Mid-May to October 2018, respectively. The study lasted for 247 days. Since the UASB reactors were already operated at 20 °C before the current study, the reactors run for 88 days at 20 °C and changed to 28 days for the rest of the first part of the study.

In the second part of the study, the UASB reactors were operated at different HRTs of 3, 4, and 5 hours between October 2018 and June 2019 to study the influence of retention time on the system performance. The second part of the study lasted for 217 days. The COD removal efficiency was used as criteria for changing HRT. Thus, HRT change only after COD removal was stable over a period. Since the reactors were previously operated at HRT 3 h, they were operated for further only 35 days and then changed to HRT 4 h for 109 days before changing to HRT 5 h for 73 days. At the HRT 3 h, the operating temperature operating were set at 25 °C (actual: 26 ± 1°C) whereas operating temperature was set at 20°C at HRT 4 h and 5 h with actual temperatures of 22 ± 2°C and 21 ± 2°C.
The pH was not controlled but monitored.

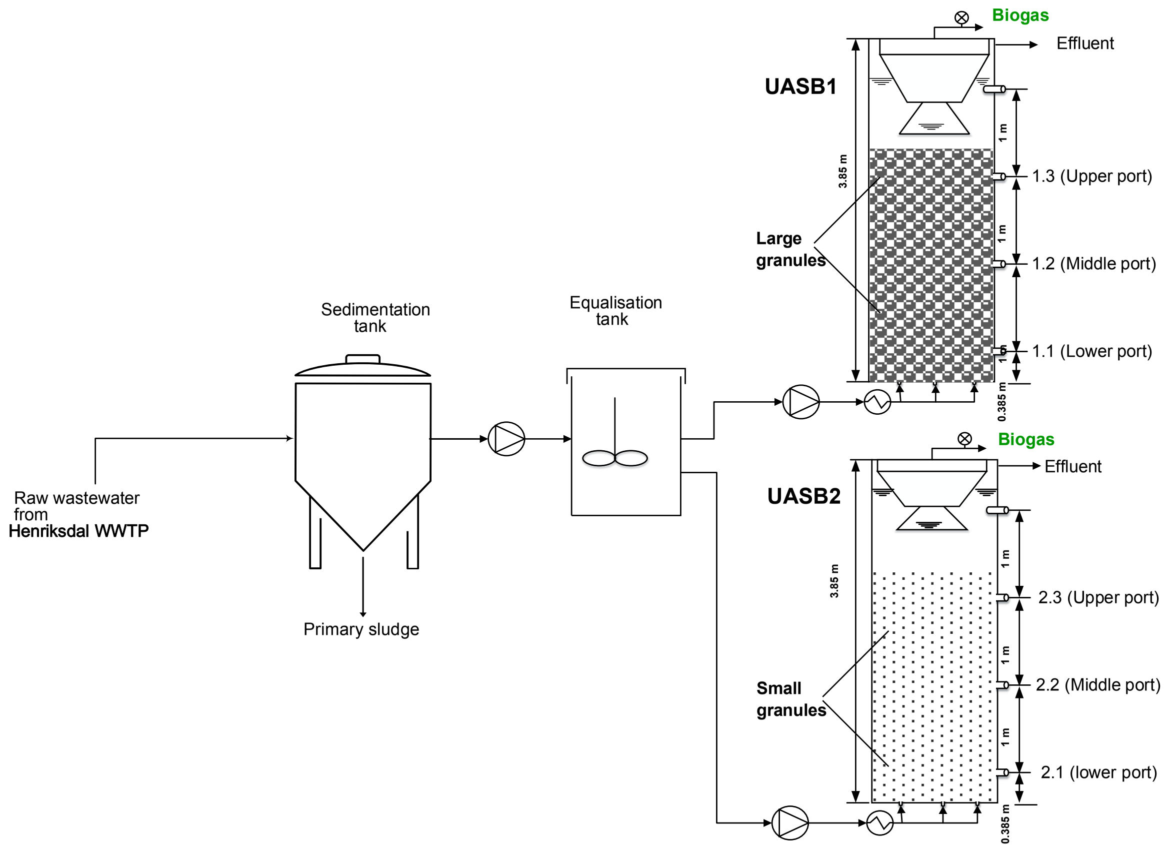

Figure 7: The schematic of the UASB system used for direct anaerobic treatment of municipal wastewater. 1.1-UASB1 lower port, 1.2-UASB1 middle port, 1.3-UASB1 upper port; 2.1-UASB2 lower port, 2.2-UASB2 middle port, 2.3-UASB2 upper port (Modified from PAPER 1).

4.2.3 Size distribution and internal structure of the anaerobic granules

Size distribution

To determine the size distribution of the granules in the UASB reactors, digital images of the granules were taken in triplicate with Huawei P20 dual camera which has Leica optics with 20 MP Monochrome (f/1.6, 1/2.3", 1.55 µm, OIS) and 12 MP RGB (f/1.8, 27mm). The image was analysed for the size distribution using ImageJ software (v 1.51, National Institutes of Health, USA) to do particles analysis.

Scanning electronic microscope (SEM)

SEM was used to determine the internal microstructure of the anaerobic granules. Before the SEM analysis, the samples were prepared according to the protocol described by Araujo et al. (2003). Thus, the anaerobic granules were firstly fixed with 2.5% glutaraldehyde (Sigma Aldrich, Germany) in 0.1 M phosphate buffer of pH 7.4 for up to 12 hours at 4°C and then rinsed in only the phosphate buffer three times for 10 minutes each. The samples were then graded dehydrated in an ethanol/water mixture of 50%, 70%, 80%, 90%, 95%, and 3 times in 100% of ethanol for 10 minutes each. Afterward, the samples were dried in hexamethyldisilazane (Sigma Aldrich, Germany) for 30 seconds before being examined with Benchtop SEM (TM 1000, Hitachi, Japan).

4.2.4 Specific methanogenic activity (SMA) test

SMA test was performed to determine the methanogenic activities of the anaerobic granules in the UASB reactors. An automatic methane potential test system (AMPTS II, Bioprocess Control, Sweden) fitted with a gas volume measuring device was used for the SMA test. The AMPTS II unit had fifteen 6 glass reactors of each of working volume 400 mL fitted in a water bath with a CO_2-capturing unit (3 M NaOH with 0.4% Thymolphthalein pH-indicator), so that only methane was measured. The temperature of the test was set at 35° C. The SMA test was done on granules samples taken after each operation period before changing the operating conditions.

The SMA test was carried out with 3000 mg/L of sodium acetate and granular sludge concentration was adjusted to 2000 mg VS/L by diluting with a medium solution. The acetate concentration of 3000 mg/L was chosen based on a preliminary study with different concentrations. Thus, a range of acetate concentrations from 1000 to 5000 mg/L were initially tested with 2000 mg VS/L of granular sludge for both UASB1 and UASB2. The preliminary results showed that the maximum potential methane production (PMP) was obtained at an acetate concentration of 3000 mg/L and therefore was chosen as the concentration for subsequent tests. The growth medium used for the SMA test was adapted from OECD, 2006 with trace elements and vitamins as adapted from Cetecioglu et al. 2013. The composition of the medium solution is given in Table 7.

Table 7: Composition of growth medium used for the SMA tests together with trace element and vitamins stock (adapted from OECD, 2006 and Cetecioglu et al. 2013).

Solution	Composition
Growth medium	270 mg/L KH_2PO_4, 1120 mg/L Na_2HPO_4, 530 mg/L NH_4Cl, 79 mg/L $CaCl_2.2H_2O$, 100 mg/L $MgCl_2.6H_2O$, 20 mg/L $FeCl_2.4H_2O$, 1 mg/L Resazurin (oxygen indicator), 10 mL/L trace element stock solution and 10 mL/L vitamin stock solution
Trace element stock	50 mg/L $FeCl_2.4H_2O$, 50 mg/L $CoCl_2.6H_2O$, 2; 25 mg/L $MnCl_2$, 1.5 mg/L $CuCl_2$, 2.5 mg/L $ZnCl_2$, 2.5 mg/L H_3BO_3, 0.5 mg/L NH_4 $Mo_7.4H_2O$, 2.5 mg/L Na_2SeO_3, 5 mg/L $NiCl_2.6H_2O$, 29 mg/L EDTA and 0.001 mL 36% HCl
Vitamin stock	2 mg/L biotin, 2 mg/L folic acid, 10 mg/L pyroxidine dihydrochloride, 5 mg/L riboflavin; 5 mg/L thiamine, 5 mg/L nicotinic acid, 5 mg/L calcium D (+)-pantothenate, 0.1 mg/L vitamin B12, 5 mg/L P-aminobenzoic acid, 5 mg/L lipoic acid and 20 mL NaP buffer (10 mM, pH 7.1)

4.3 VFA production by co-fermentation of sewage sludge and external organic waste

The VFA production was carried out first in small laboratory reactors and then in bench-scale reactors with semi-continuous feeding strategy. In the final stage, there was an operation of pilot-scale reactor for VFA production.

4.3.1 Laboratory-scale batch reactors

Firstly, lab-scale experiments were carried out to investigate the influence of substrate composition on VFA production under both acidic and alkaline conditions. The lab-scale experiments were performed in batch mode with a working volume of 450 mL using an automatic methane potential test system (AMPTS II, Bioprocess Control, Sweden) with a biogas measurement unit (Figure 8). The same set-up was used for the SMA test as described in section 4.2.4. The experiment was carried out with PS and OW1 (i.e., homogenized organic waste from Himmerfjärden WWTP). The concentration of substrate (PS and OW) in each reactor was maintained at initial total COD of 15 g/L, whereas the seed sludge used as inoculum was set at VS of 7.5 g/L to achieve a substrate/inoculum ratio of 2 gCOD/g VS. Substrate/inoculum ratio of 2 gCOD/g VS was chosen based on literature (Silva et al., 2013). Co-fermentation experiments were carried with OW proportion of 0%, 25%, 50%, 75%, 100% as total COD. Thus, 0% OW and 100% OW represent mono-digestion of PS and OW, respectively. To achieve the same concentration of substrate and inoculum for all different proportions, reactors' contents were diluted with tap water that is left overnight in a fume hood. Two sets of experiments with initial pH at either 5 or 10 were carried out. The reactors were operated at a temperature of 35 °C for 20 days. For each experiment, 5 mL samples were taken at 9 different retention times for analysis.

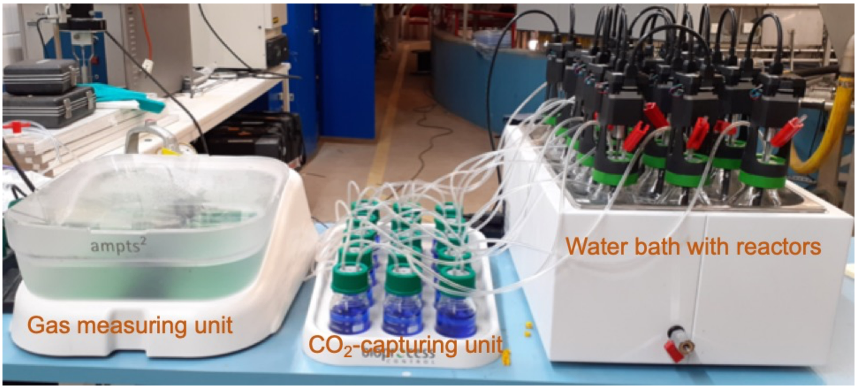

Figure 8: Lab-scale set-up for the batch study of VFA production (Photo: Isaac Owusu-Agyeman).

4.3.2 Bench-scale semi-continuous reactors

Based on the results of the lab-scale study, there were up-scaling experiments with 50% OW, which was 70% and 30% by volume of PS and OW, respectively. The semi-continuous experiments were carried out with three identical reactors set at acidic conditions (pH 5), alkaline conditions (pH 10 and 9), and no pH control. Each of the three reactors had a total volume of 15 L with a working volume of 10 L. The reactors were two walled glass reactors with stirrers that were driven by brushless DC motors (N3858, Bodine Electric Integramotor,) for mixing the reactors' contents. The reactors were connected to gas meters (MILLIGASCOUNTER, Type MGC-10, Ritter) and CH_4 sensor (TDS0068, Dynament) and CO_2 (TDS0054, Dynament). All sensors and control units were connected to the process control and data acquisition system, DAQFactory (AzeoTech) controlling and recording data online continuously.

The reactors (except no pH control reactor) had pH control units (986214, DULCOMETER, Prominent) with pH electrodes (PHEX 112 SE, Prominent) and pump (Ecoline VC-MS/CA8-6, Ismatec) connected to 5 M NaOH and/or 2 M HCl as dosing solutions to maintain the pH at the required value (Figure 9). With the reactor for the no-pH control experiment, the pH was only monitored with a pH electrode. The acidic (pH 5) reactor was operated for 184 days while the other reactors were operated for 315 days to study the effect of long-term operation on system resilience and performance. The operating temperature of all experiments was maintained at 35 ± 2^{o}C, and the retention time was 7 days except days 123-154 when the retention time of the no pH control experiment was 10 days. The retention time of 7 days was chosen based on the results of batch experiments. At the beginning of the experiments, the reactors were inoculated with digested sludge from Henriksdal WWTP as a seed sludge at a substrate/inoculum ratio of 2 gCOD /g VS.

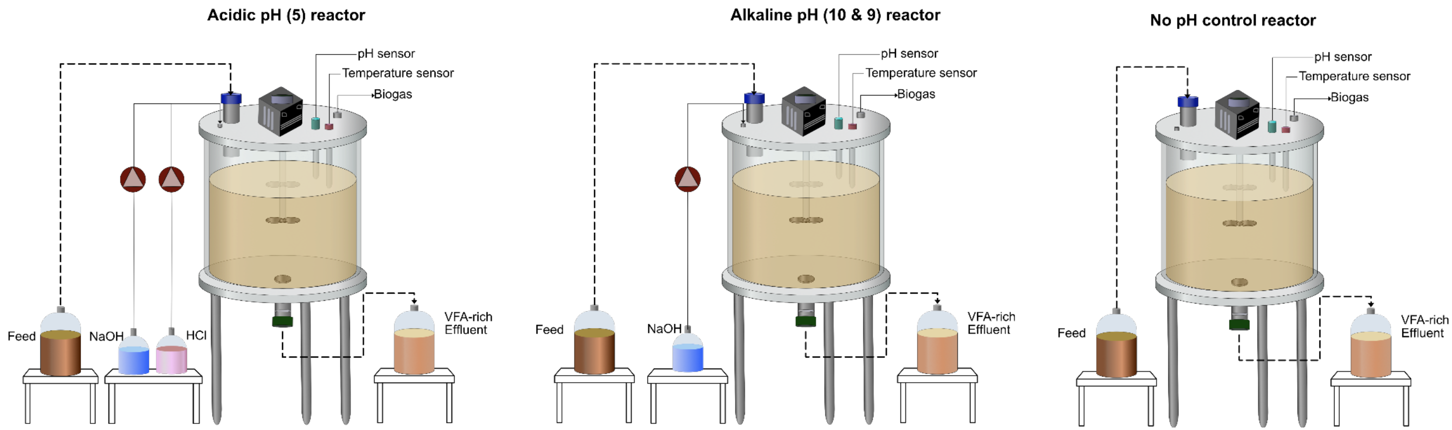

Figure 9: Schematic of the two identical reactors used for the semi-continuous VFA production experiments.

4.3.3 Pilot-scale continuous reactor operation

VFA production was scaled up in a $2m^3$ reactor with conditions similar to the no pH control semi-continuous reactor to study the effect of substrate variability on the VFA production system was assessed by using different organic waste in the feed. However, the pilot-scale reactor operated in a continuous mode of operation. A picture of the pilot-scale reactor is shown in Figure 10. The working volume was 1.5 m^3 and it was operated in continuous mode with a retention time of 7 days. The substrate ratio was similar to the semi-continuous reactors, i.e 70% v/v PS and 30% v/v OW. Importantly, the types of OW in the substrate were varied at different periods to study the influence of substrate variability on VFA yield and composition. The operation of the reactor was carried out for 264 days. Table 8 shows the various periods and the different substrates used. pH was not controlled but monitored throughout the experimental periods, while the temperature was regulated at 35 ± 2°C.

Table 8: The operational periods and the corresponding substrates used.

Period	Substrate [a]	Duration (days)
I	PS+OW1	82
II	PS+OW2	94
III	PS only	19
IV	PS+OW3	69

[a] OW1: homogenized organic waste from Himmerfjärden WWTP
OW2: non-homogenized organic waste from Himmerfjärden WWTP
OW3: homogenized organic waste from Scandinavian Biogas

Figure 10:The pilot-scale reactor for VFA production (Photo: Isaac Owusu-Agyeman).

4.3.4 Denitrification test with VFA-rich liquid as carbon source

The VFA-rich liquids were taken from the semi-continuous reactors and tested for their denitrification potential. The results were compared with traditional carbon sources; acetate and methanol that are often used in the denitrification process. Denitrification batch activity tests were performed using the manometric tracking technique. The manometric tests were executed by using air-tight apparatus that consisted of glass reactors that are sealed by rubber septa that are tightened in place by a plastic lid. Each of the glass reactors had an active volume of 320 mL with two top openings for gas flushing and discharge. The reactors were connected with a pressure measuring device (GMH 5150, Greisinger electronic GmbH, Regenstauf, Germany) which was fitted with a data logger. The denitrification batch activity tests were performed with activated sludge from a membrane bioreactor pilot plant situated at the Hammarby Sjöstadsverk as inoculum. The reactor design was in a way that 80 ml phosphate buffer solution of pH 7.8 and 200 mL of diluted activated sludge was added to the reactor to achieve volatile suspended solids (VSS) of 4000 ± 400 mg/L. The tests were performed at COD to nitrate (COD/N) ratio of 4.5 with an initial nitrate concentration of 28.5 mg NO_3-N/L. The test temperature was maintained at 25°C with the help of a water bath and stirred at speed of 200-300 rpm with a magnetic stirrer. Each experiment was done in duplicates. Before the start of each experiment, the reactors were purged with nitrogen gas to remove any residual dissolved oxygen and ensure anoxic conditions. Specific denitrification rate was calculated from equation 17:

$$K_N = \left(a \cdot \frac{M_{N_2} \cdot V_{HS}}{RT}\right) \cdot \left(\frac{1}{60 \cdot X_{VSS}}\right) \tag{17)}$$

Where:

K_N - Specific denitrification rate (mg NOx-N / g VSS·h)
a - Slope value of the curve calculated with linear regression method describing changes of pressure in the batch reactor as the function of time
M_{N_2} - Molecular weight of nitrogen gas
V_{HS} - Headspace volume in the batch reactor
R - Universal gas constant
T - Temperature
X_{VSS} - Amount of biomass in the batch reactor

4.4 Microbial community analysis

Sludge samples were taken from both biogas and VFA production experiments to analyse the archaeal and bacterial populations. The microbial analysis involved DNA extraction, polymerase chain reactions, sequencing, and bioinformatic analysis.

4.4.1 Total genomic DNA extraction

Granular sludge samples from the UASB reactors were centrifuged at a speed of 4200 rpm for 5 min whereas slurry sludge samples from the VFA production reactors were centrifuged at a speed of 8500 rpm for 11 minutes. The supernatants were discarded from the centrifuged samples and the decanted sludge samples were stored at -20°C until total genomic DNA extraction. All samples were extracted in triplicate. The DNA extractions were done using a NuceloSpin Soil DNA kit (Macherey-Nagel, Germany) and with 300 mg of the sludge by following the manufacturer's protocol. Fluorimetry method with Qubit dsDNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, USA) was used to measure the concentrations of the extracted DNA before further processing the DNA samples for high throughput sequencing.

4.4.2 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) amplification of DNA target of the bacterial and archaeal 16S rRNA genes was done using forward and reverse primers, 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT), respectively (Caporaso et al., 2011). The primers were extended on the DNA templates with PCR reagents, MyTaq Red DNA Polymerase (Bioline Reagents Ltd., London, UK) or Red Taq DNA Polymerase Master Mix (VWR international, Sweden) using Master cycler Pro thermal cycler (Eppendorf UK Ltd., Stevenage, UK) or Techne® Prime thermal cycler (Cole-Parma). PCR amplification conditions used consisted of initial denaturation at 95°C for 5 min, 35 (or 28) cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1.5 min, and a final elongation step at 72°C for 5 min. Libraries were prepared for sequencing by using the PCR products as a template and 2x300 bp Illumina index adapter primers (Illumina, Inc, San Diego, CA, USA) and PCR reagent with conditions for amplification of initial denaturation at 95 °C for 5 min, 5 cycles of 95 °C for 30 sec, 60 °C for 30 sec, 72 °C for 30 sec, a final elongation step at 72 °C for 5 min.

4.4.3 High-throughput 16S rRNA sequencing

High-throughput amplicon purification and normalization of the indexed PCR products were performed using SequalPrep TM Normalization Plate (96) Kit (Invitrogen, Life Technologies, Thermo Fisher, USA) to bind and elute ≈ 25 ng of each PCR amplicon by following manufacturers' protocol. The eluted PCR amplicons were subsequently pooled into one single sample. Sequencing of the pooled library was done on MiSeq (MSC 2.5.0.5/RTA 1.18.54) with a 2x301 setup using 'Version3' chemistry by the National Genomics Infrastructure, Science for Life Laboratory (Stockholm, Sweden). CASAVA software suite with bcl2fastq_v2.20.0.422 was used for converting the Bcl to FastQ. The quality scale used was Sanger / phred33 / Illumina 1.8+.

4.4.4 Bioinformatic analysis of the sequencing data

QIIME 2, a next-generation microbiome bioinformatics platform, was used for data analysis according to procedures outlined by Bolyen et al.,(2019). Taxonomic assignments for archaeal and bacterial populations were done using Greengenes (DeSantis et al., 2006) or SILVA database (Bokulich et al., 2020). Sequence datasets have been deposited into the National Center for Biotechnology Information (NCBI) Read Archive with the bioproject accession numbers PRJNA522972, PRJNA720425, and PRJNA752498.

4.5 Analytical methods

COD, NH_4-N, NO_3-N, NO_2-N, total nitrogen, and alkalinity were measured with respective cuvette test kits (WTW, Germany) and a spectrophotometer (PhotoLab 6600 UV Vis, WTW, Germany). Total suspended solids (TSS), VSS, total solids (TS), volatile solids (VS), and settling velocity were measured according to Standard Methods (APHA, 2005). The pH of samples was measured with pH electrode (InLab Expert Pro-ISM, Mettler Toledo) and meter (SevenCompact S220, Mettler Toledo) or pH electrode and meter (pH 330i, WTW, Germany).
Gas chromatography system (Intuvo 9000 GC System, Agilent) with a capillary column (DB-WAX Ultra Inert, 30 m x 250 µm x 0.25 µm, Agilent 122-7032UI) and coupled with a flame ionization detector (FID) was used to measure the concentration of the individual carboxylic acids, namely, acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, caproic acid, and iso-caproic acid. The analytical method consisted of a start oven temperature of 70°C and an equilibrium time of 1 minute with a ramp rate of 10°C/min and up to 200 °C. The FID was fixed at a temperature of 280°C. The carrier gas was helium with a flow rate of 2 mL/min. The VFA concentration was converted to COD units using factors of 1.0667 for acetic acid, 1.512 for propionic acid, 1.813 for butyric acid, 1.813 for iso-butyric acid, 2.036 for valeric acid, 2.036 for iso-valeric acid, 2.207 for caproic acid, and 2.207 for iso-caproic acid according to stoichiometry.

4.6 Statistical analysis

Paleontological statistics tools (PAST 3.20 and PAST 4.10, University of Oslo, Norway) and Prism 9 (Version 9.1.2, GraphPad Software) were used for Student's t-distribution (T-test) analysis, one-way analysis of variance (ANOVA), and correlation analysis. Paleontological statistics and SPSS Statistics 28.0 software (IBM Corp. USA) were used for principal component analysis (PCA).

5 Results and discussions

This thesis evaluated existing and emerging strategies for bio-based carbon recovery from municipal wastes. The first part of the study focused on biogas recovery from direct anaerobic treatment of wastewater by studying the coupling effect of operational conditions and properties of anaerobic granules on reactor performance and microbial community dynamics. The second part of the study aimed at recovery of a higher value VFA from co-fermentation of sewage sludge and external organic waste as a platform chemical for post-stream bioprocesses.

5.1 Biogas recovery from direct anaerobic treatment of municipal wastewater (Papers I & II)

This part of the study sought to validate the practicability of integrating a direct anaerobic granule-based treatment system under sub-mesophilic temperatures to WWTP by increasing the understanding of efficiency and biochemical processes in relation to the characteristics of the anaerobic granules. For this reason, two pilot-scale UASB reactors with different granule size distributions were employed to treat municipal wastewater. An in-depth study of the properties of the granules from the two pilot reactors was carried out. This included the determination of the size distribution of the granules and their internal structural layout. Moreover, the study also explored the microbial population dynamics and methanogenic activities of the granules and the impacts of operational parameters on the reactors' performance. Moreover, the responses of the microbial community of anaerobic granules with different structural characteristics to changes in HRT and temperature were explored.

5.1.1 The structural properties of the granular sludges from the UASB reactors (Paper I)

Determination of the size distribution of anaerobic granular sludge from the two UASB reactors was carried out to elucidate how the anaerobic size of anaerobic granule sludge can influence the performance. Figure 11 indicates the variations in the sizes of the sludges from UASB1 and UASB2 reactors. The sludge from UASB1 consisted of bigger granules with sizes often ranging between 3 and 4 mm. On the other hand, the sludge from UASB2 was characterized by granules with smaller sizes with a dominant size range of 1-2 mm. It is noteworthy to point out that anaerobic sludge from both UASB1 can be classified as good granules because a typical size of anaerobic granules must be in the range of 0.5-5 mm (Bhunia and Ghangrekar, 2007; Show et al., 2004). The dissimilarity in the granule size of the sludge from UASB1 and UASB2 was explained by the initial operation strategy of the UASB reactors that influenced the size of the anaerobic granules. During the initial stage of granulation, the UASB1 and UASB2 reactors were connected in a series in the order of influent → UASB1→ UASB2 → effluent. It means the raw wastewater was first received as an influent for UASB1, while UASB2 received the effluent of UASB1 as influent. For this reason, UASB1 received a relatively higher substrate concentration than UASB2. It has been shown that when higher substrate concentration with associated higher loading rate enhances metabolic activities of anaerobic microorganisms which in turn promote extracellular polymer production and consequently support granulation (Zhou et al., 2007). Granule sludge from UASB1 and UASB2 reactors obtained settling velocities of 86-225 and 27-180 m/h, respectively. The settling velocities of sludge of both reactors were higher than the minimum required settling velocity of 60 m/h of a typical methanogenic anaerobic granule (Yi et al., 2016). Moreover, the settling velocities obtained by the granule sludge for both reactors were faster than the highest upflow velocity (1.1 m/h) employed in the study. This explained why no obvious washout of granules was observed in the study. Thus, VSS of the influent and effluent samples were 50–300 and 0–100 mg/L, respectively.

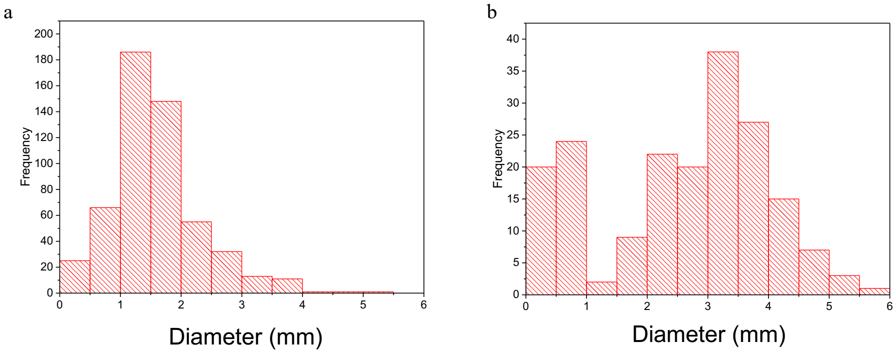

Figure 11: Size distribution of granular sludge in (a) UASB1 and (b) UASB2 reactors (Paper I).

The internal structures of the granules were elucidated by studying cross-sections of granules using SEM (Figure 12). The anaerobic granules of the sludge from UASB1 with relatively larger diameters had internal structures that were stratified. There were different layers and it can be said that each layer could represent distinct functional units of microorganisms that execute the various steps of the AD process (Abbasi and Abbasi, 2012). Thus, the layer closer to the surface of the granule can be said to be the stratum for hydrolysis/acidogenesis where complex organics are broken down into simpler molecules and organic acids by hydrolytic bacteria and acidogens. The next layer can be the unit for acetogenesis where the acetate and hydrogen are produced. The layer with the methanogenic function will be situated close to the central part, where methanogens user acetate or hydrogen produced in the other layers to produce methane. It is said that the connection of the different stages of the anaerobic process in granules improves the efficacy of substrate degradation and ensures efficient transfer of intermediate products between the different functional microorganisms (Agapakis et al., 2012).

While the internal structures of granules from the sludge of UASB1 reactors were multi-layered, the granules of UASB2 sludge with smaller sizes, had a more uniform internal structure with no obvious stratification. UASB2 had smaller anaerobic granules because during the granules' formation stage, the two reactors were operated in series and because of that, UASB2 received effluent of UASB1 as influent. It has been shown that low substrate concentration in the influent can result in the formation of granules with smaller sizes (Bhatti et al., 2014). In addition, because UASB2 received partially treated wastewater, substrates could have characterised by organics that are not easily biodegradable. Anaerobic granules which receive more complex substrates that require longer a rate-limiting hydrolytic step do not usually show internal layered functional units, but the microorganisms are rather interconnected and dispersed evenly (Fang, 2000). Unlike layered granules, in anaerobic granules with uniform internal microstructure, organic breakdown kinetics is through diffusion toward the granule interior without necessarily being hydrolysed near the surface.

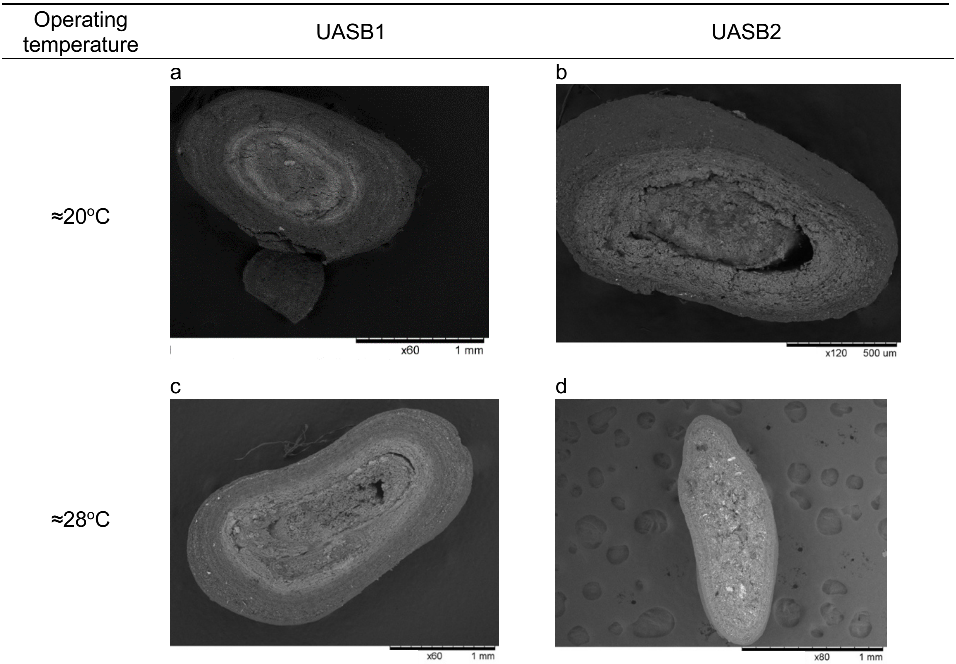

Figure 12: SEM images showing the cross-section of the granule from UASB reactors at operation temperature of 20°C (a) UASB1 and (b) UASB2; and at a temperature of 28°C (c) UASB1 and (d) UASB2.

5.1.2 Effects of temperature on the performances of the UASB reactors (Paper I)

The impact of the operating temperature on the reactors' performance of the two identical UASB reactors with different granular sludge characteristics was investigated. Both reactors received the same influent wastewater and were operated at 20°C and 28°C while maintaining the HRT at 3 hours. At an operating temperature of 20°C, the average COD removal efficiencies of UASB1 and UASB2 were 46 ± 14% and 40 ± 11% (Figure 13A). Statistical analysis showed that there was no significant difference between the COD removal efficiencies of both UASB1 and UASB2. Conversely, while production biogas at 20°C by UASB1 with large granule size distribution was steady, that of UASB2 with smaller granule size distribution was erratic at the period when the reactors were operated at 20°C (Figure 13B). The erraticism of the biogas production by the UASB2 reactor during the operating period of 20°C could be due to the effect of low temperature which might have influenced the biogas production and transfer into the gaseous phase. However, unlike the UASB2, the biogas produced by the UASB1 reactor was fairly stable even at a low temperature of 20°C. This could be explained by the larger size and well-layered internal structure of the UASB1 granules. A micro-scale study of the size effect of anaerobic granules on the mechanism of biogas production has shown that large anaerobic granules have higher bioactivity due to larger pore diameter, higher porosity, and quicker diffusion distances which stimulate enhanced substrate transport and advance biogas production (Wu et al., 2016). When the operating temperature of the reactors was elevated to 28°C, there was a rise in biogas production for the UASB1 reactor and a steady and higher biogas production rate for UASB2 with an average of 60 ± 10 and 58 ± 20 L/(m^3·d), respectively. The enhancement and steadiness achieved in the biogas production at 28°C was attributed to the reduction in the methane solubility and the increased and stable methanogenic activities at higher temperatures (Crone et al., 2016; Zhang et al., 2018). It would have been expected the biogas production and COD removal efficiency of UASB1 should have been much higher than UASB2, especially at the higher temperature. However, this was not the case since both reactors were operating below their capacity in terms of organic loading rate to volatile solids ratio.

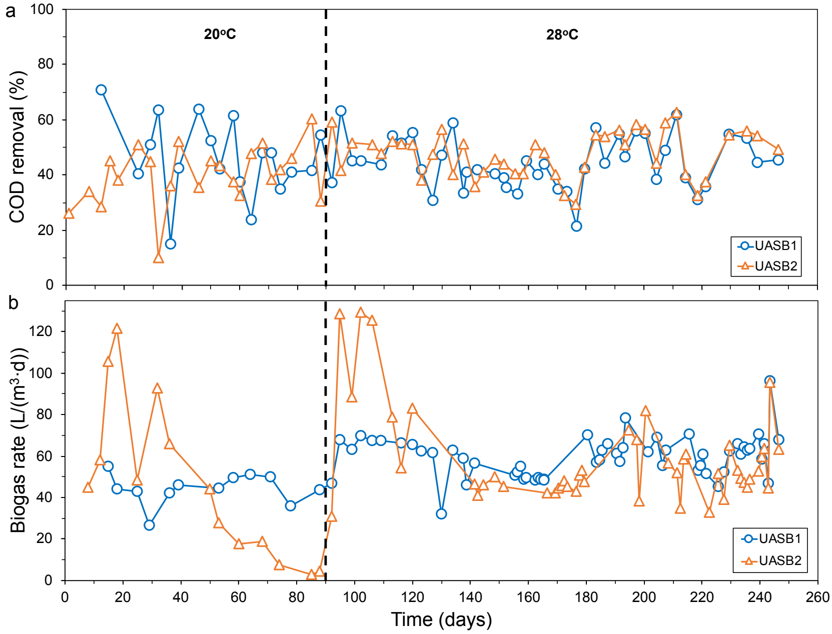

Figure 13: Impact of temperature on reactors' performance. (a) COD Removal efficiency and (b) biogas production of the UASB reactors (Modified from Paper I).

5.1.3 Effects of HRT on the performances of the UASB reactors (Paper II)

The effects of HRT on the performance of both UASB reactors were studied by operating at HRTs of 3, 4, and 5 hours (Figure 14). At HRT 3, the reactors were operating at a temperature of ≈25 ºC (actual average was 26 ± 1 ºC) and therefore the performance of the reactor was relatively high and the COD removal efficiency of UASB1 and UASB2 were 52 ± 7% and 56 ± 8%, respectively (Figure 14). The biogas production rates for UASB1 and UASB2 were 65 ± 7 and 65 ± 10 L/(m^3·d), respectively. These results were higher than what was achieved previously where average COD removal efficiency of 45 ± 9% and 48 ± 8% was obtained for UASB1 and UASB2, respectively even at a higher temperature of ≈28°C (Figure 13). The higher COD removal efficiency than the previous can be explained by the adaptation of the system over time. Increasing the HRT to 4 h and decreasing the temperature to ≈20 °C, led to a decrease in COD removal efficiency for both reactors with an average of 38 ± 10% and 40 ± 10% for UASB1 and UASB2, respectively. The low COD removal efficiency at the HRT 4 can be attributed to the lower temperature since temperature affects the kinetics of the anaerobic treatment process (Lew et al., 2003). The normalized biogas productions rates at HRT 4 were 61 ± 10 and 64 ± 9 L/(m^3·d) for UASB1 and UASB2, which were not very different from those obtained at HRT 3, although the COD removal efficiencies were different.

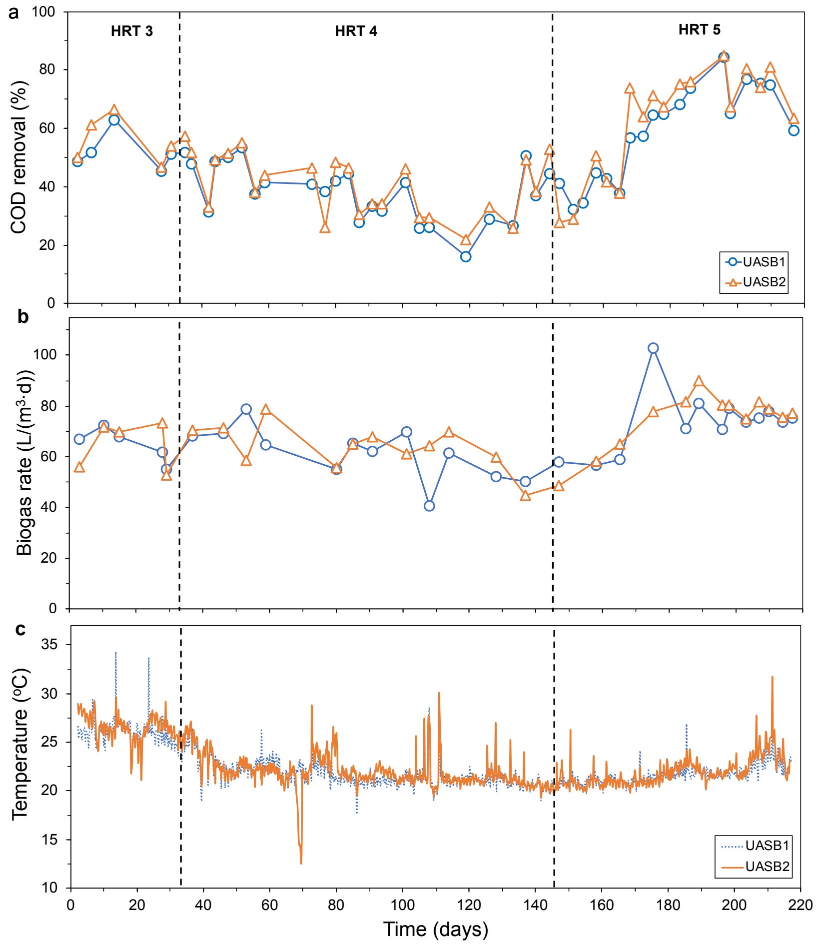

Figure 14: Impact of HRT on reactors' performance (a) COD removal efficiency, (b) production of biogas, and (c) operation temperature of the UASB reactors (Paper II).

When the HRT was raised from 4 to 5 h while maintaining the operating temperature at ≈ 20 ºC, the COD removal efficiencies of both reactors improved with a maximum of up to 85%. The average COD removal efficiencies at HRT 5 h were 59 ± 16% and 63 ± 16% for UASB1 and UASB2. The biogas also increased to 73 ± 9 L/(m^3·d) and 75 ± 9 L/(m^3·d) for UASB1 and UASB2, respectively. The improvement in the performance of the reactors at the longer HRT of 5 h can be attributed to the adequate contact time allowed for the microorganisms to degrade the substrate (Musa et al., 2019). Thus, the operation conditions influenced the performance of the UASB reactors. This was confirmed by a one-way ANOVA which showed that there was a statistically significant difference in the performance of the reactors with p-values of 2.033 x 10^{-5} and 3.359 x 10^{-5} for COD removal efficiency of UASB1 and UASB2, respectively.

5.1.4 Microbial community dynamics and methanogenic activities of the UASB reactors (Papers I & II)

The microbial structures of the granular sludge from the UASB reactors were analysed to understand the archaeal and bacterial populations and how they influence the functioning of the reactors. High-throughput sequencing was done with granular sludge taken from sampling ports of the UASB reactors and at different phases of operation to know how the microbial population dynamics changes. Moreover, specific methanogenic activity tests were carried out to evaluate the methane-producing capability of anaerobic granule sludge and how they change with operational conditions.

5.1.4.1 Effects of temperature on the microbial community structure (Paper I)

The microbial community analysis revealed that Methanoregulaceae (33.7%–52.5%) and Methanosaetaceae (14.2%–34.8%) were the most dominant families in the archaeal population of the granular sludge from the UASB reactors (Figure 15). The species affiliated to the family *Methanoregulaceae* are hydrogenotrophic methanogens, which produce methane from carbon dioxide and hydrogen, while *Methanosaetaceae* members strictly utilize acetate for their methane production (Holmes and Smith, 2016; Karakashev et al., 2006; Schnürer, 2016). Other archaeal families that were also present in the granular sludge from the UASB reactors with considerably higher relative abundances were *Methanobacteriales_WSA2* (10.7%–14.9%), *Methanospirillaceae* (2.6%–6.6%), *Methanomassiliicoccaceae* (2.4%–5.3%), and *Methanobacteriaceae* (1.9%–7.7%). Specifically, in the reactor with larger granules (i.e., UASB1), the relative abundance of Methanoregulaceae was 39.5 ± 1.2% at 20 °C, which was closely followed by Methanosaetaceae (30.5 ± 2.8%) (Figure 15). Since both *Methanoregulaceae* and *Methanosaetaceae* dominated, it is a signal that methane is produced through both acetoclastic and hydrogenotrophic pathways. The dominant genus for the family *Methanosaetaceae* was *Methanosaeta*. The species associated with the genus *Methanosaeta* are often strict acetoclastic methanogens and perform the architectural task in the granulation stage to reinforce anaerobic granules (Leclerc et al., 2004; Zinatizadeh et al., 2007). The dominancy of the family *Methanosaetaceae* found in the granular sludge of UASB1, therefore, rationalized the bigger granules with stratified internal structure. This assertion can be sustained by the fact that other studies have revealed that acetoclastic methanogenesis typically happened in the internal layer of anaerobic granules (Baloch et al., 2008; Cunha et al., 2018).

The sludge samples with smaller sizes from UASB2 had a relatively lower abundance of *Methanosaetaceae* with a percentage of 17.5 ± 3.3 at the operating temperature of 20 °C. This value is only about half of the relative abundance obtained for UASB1. Conversely, the family *Methanoregulaceae* was conspicuously higher in UASB2's granules with a relative abundance of 51 ± 0.5% (mainly genus *Candidatus Methanoregula* genus, 35.5 ± 1.2%). The elevated relative abundance of *Methanoregulaceae* infers that the hydrogenotrophic pathway for biomethanation of the UASB2 reactor. Furthermore, the relative percentage of

Methanobacteriales__WSA2 whose members are hydrogenotrophic was higher in UASB2 (11 ± 0.6%). Nevertheless, although lower than UASB1, the relative percentage of the family *Methanosaetaceae* was significant and therefore acetoclastic biomethanation also happened in the UASB1. The smaller sizes of granules of UASB2 than with no distinct internal layers could be attributed to the much lower abundance of the family *Methanosaetaceae* than UASB1.

In terms of the bacterial population, in UASB1 at 20°C, the family Anaerolinaceae was the most dominant with a relative abundance of 16.9±0.8%, followed by Syntrophaceae (9.0±1.1%) (Figure 15). Members associated with the family *Anaerolinaceae* are fermentative acidogens capable of producing acetate. Their presence gave a hint of a syntrophic relationship that could exist between *Anaerolinaceae* and *Methanosaetaceae*. Thus, the family *Methanosaetaceae* could have used the acetate produced by *Anaerolinaceae* to produce methane. This revealed that acetoclastic methanogenesis is an important methane-producing pathway in UASB1. However, *Syntrophaceae* dominated the bacterial population of the granular sludge from UASB2 with a relative percentage of 11.7±0.9% while the percentage of *Anaerolinaceae* was only ~4.0%. Species affiliated to the family *Syntrophaceae* often grow in syntrophic associations with H_2-utilizing methanogens (Kuever, 2014). Moreover, the genus *Syntrophus* dominated at the genus level with an abundance of ~6.5%. *Syntrophus spp.* produce H_2 from substrates in an anaerobic environment (McInerney et al., 2007). The bacterial community structure also supports the notion that hydrogenotrophic is the main methanogenic pathway for the UASB2.

Statistically, principal component analysis (PCA) of the microbial community revealed that there was an obvious dissimilarity between granular sludge taken from UASB1 and UASB2. The assemblage of samples on the basis of the kind of reactor is an indication of the differences in the microbial populations of the two UASB reactors. A one-way ANOVA, on the other hand, revealed that statistically, a significant difference did not exist in the microbial community of granular sludge samples from the different sampling ports of the same reactor. There was also no significant difference in the microbial community of the samples taken during the operation periods with different temperatures. Nevertheless, because the production of biogas was not stable at 20 °C in the UASB2 reactor, which was mostly dominated by hydrogenotrophic methanogens, it can be contemplated that the hydrogenotrophic methane-producing pathway is less stable at the lower temperature of 20°C than acetoclastic methanogenesis.

Specific methanogenic activities of the granular sludge from the UASB reactors were carried out with acetate as the substrate to elucidate the acetoclastic methanogenic activities of both UASB1 and UASB2. It was shown that while the SMA of granule sludge from UASB1 had a range of 625–1093 mg CH_4-COD/(gVS·d) (250–437 mL CH4 /(gVS·d)), the SMA results of UASB2 were only 375–650 mg CH_4-COD/(gVS·d) (150–260 mL CH4 /(gVS·d)), respectively. The greater SMA achieved for granular sludge from UASB1 than UASB2 reveals that acetoclastic activities are more prominent in the granular sludge from UASB1 than UASB2 granules, which backs the outcomes of the microbial community analysis that acetoclastic methanogens were more predominant in granular sludge from UASB1 than UASB2.

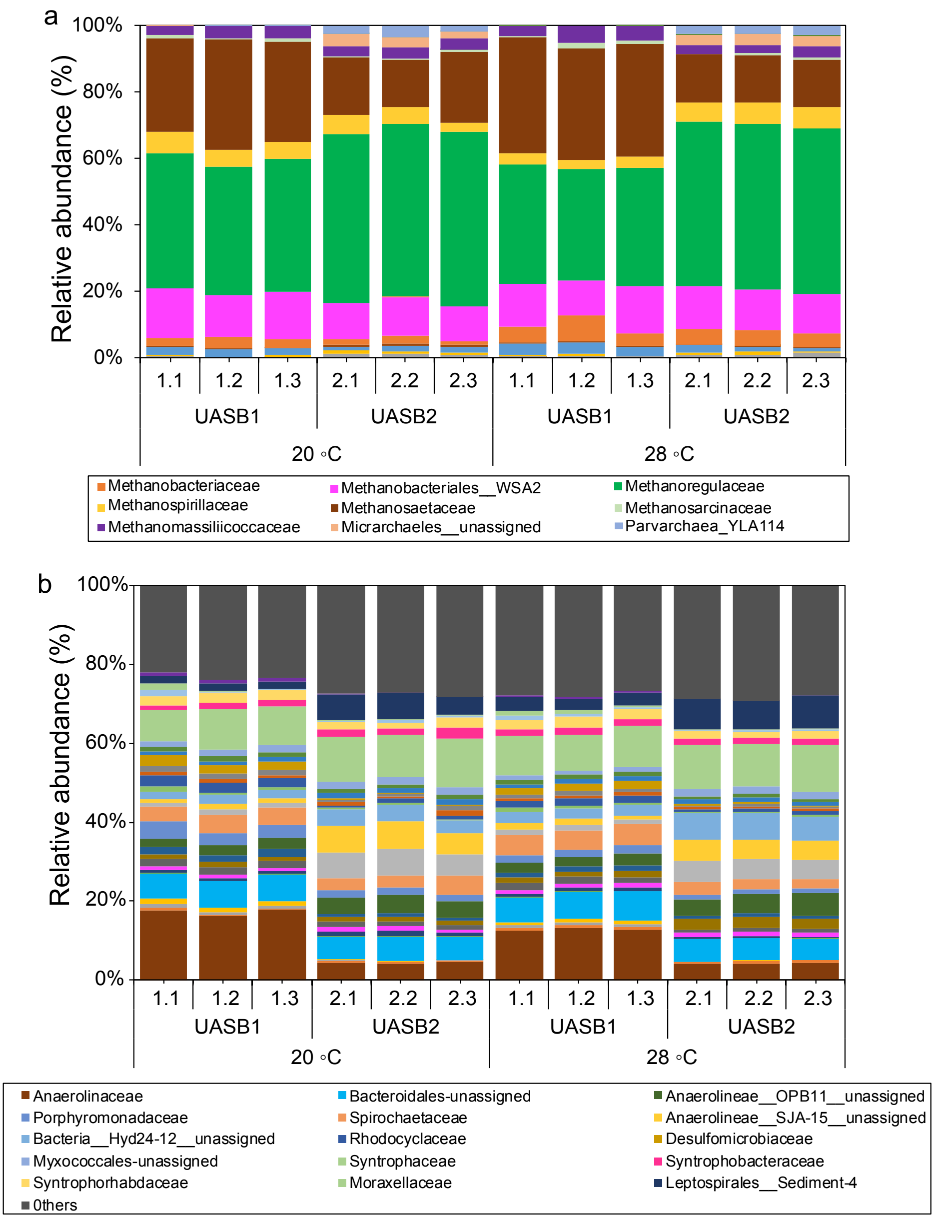

Figure 15: Microbial community showing the relative abundance of the (a) archaeal and (b) bacterial population at the family level at operating temperatures of 20 and 28 °C. 1.1-UASB1 lower port, 1.2-UASB1 middle port, 1.3-UASB1 upper port; 2.1-UASB2 lower port, 2.2-UASB2 middle port, 2.3-UASB2 upper port (Paper I).

To ratify this proposition statistically, a correlation analysis between the SMA results and the microbial community was carried out. For the microbial community, the scores of the first and second principal components (PC1 and PC2) from the PCA analysis were used. This is because the PC1 scores explained 92.9% and 88.8%, and PC2 explained 4.1% and 4.7% of variabilities of the archaeal and bacterial communities, respectively. The correlation analysis results revealed a strong positive correlations with a Pearson coefficient of 0.715, between SMA and archaeal PC1, which was statistically significant with a p-value of 0.0089. Moreover, the SMA correlated positively with the bacterial PC1 with a coefficient of 0.838 and a p-value of 0.00066. In addition, a significant positive correlation existed between PC1 of archaeal and bacterial populations. The results of the correlation analysis of the acetoclastic SMA and microbial community emphasises the suggestion that acetoclastic methanogenesis is more dominant in the granular sludge from UASB1 than UASB2.

5.1.4.2 Effects of HRT on the microbial community structure (Paper II)

The next-generation sequencing of granule sludge taken on days 31 (HRT 3), 59 (HRT 4), 144 (HRT 4), and 207 (HRT5) showed that the most dominant archaeal family of the UASB1 was Methanosaetaceae regardless of the HRT with relative abundance ranging between 58 and 69% (Figure 16). It is noteworthy to point out that the relative abundance of Methanosaetaceae obtained during the HRT study period is higher than what was previously observed in the temperature effect study (Figure 15). The difference can be attributed to the time intervals and adaptability. The time interval between those samples was 164 days after the last sample from the previous study. This difference in the dominant archaeal community could also explain the higher COD removal at HRT 3. Apart from the fact that Methanosaetaceae are acetoclastic methanogens, a study has shown that members of this family can reduce carbon dioxide to methane via direct interspecies electron transfer (Rotaru et al., 2014). The specific relative abundance of Methanosaetaceae shows that acetoclastic methanogenesis is the most dominant pathway for methane production. Methanosaetaceae in UASB1 samples on days 31 (HRT 3), 59 (HRT 4), 144 (HRT 4) and 207 (HRT 5) were 59 ± 1%, 65 ± 2%, 68 ± 1% and 62 ± 2%, respectively. The high relative abundance of Methanosaetaceae is also known to be robust and resilient to perturbation and found to be dominant in anaerobic treatment systems operating under sub-mesophilic temperatures (Amha et al., 2018; Chen and He, 2015; Petropoulos et al., 2017). The family Methanoregulaceae which is affiliated with the order Methanomicrobiales was the second most dominant archaea in granule sludge from UASB1 with a relative abundance of 16 ± 2%. Species of Methanoregulaceae use H_2/CO_2 and formate as substrates for methane production (Oren, 2014). The Methanoregulaceae family was found to be associated with Methanosaetaceae and both prevailed in anaerobic digesters when the operating temperature was reduced from 40 to 20°C (Vítěz et al., 2020). Thus, both families can strive well under sub-mesophilic conditions. The abundance of Methanoregulaceae suggests that besides acetoclastic methanogenesis, hydrogenotrophic methane-producing pathways also occur in UASB1. Moreover, other hydrogenotrophic archaeal families were found in UASB1 granules. These included Methanobacteriaceae, Methanofastidiosaceae, and Methanomicrobiales_unassigned, with an average relative abundances range of 4 ± 2, 4 ± 1, and 3 ± 1%, respectively.

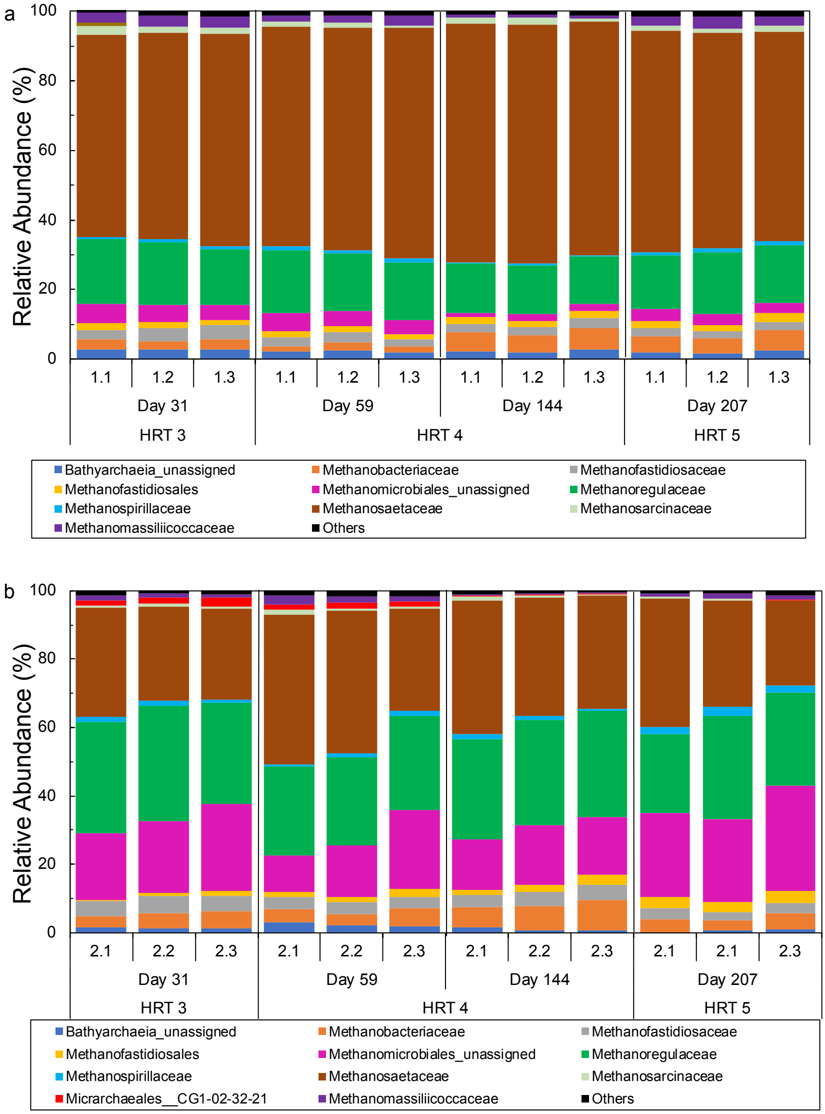

Figure 16:The archaeal community diversity in relation to HRT at the family level of granular sludge taken from different levels of (a) UASB1 and (b) UASB2. 1.1-UASB1 lower port, 1.2-UASB1 middle port, 1.3-UASB1 upper port; 2.1-UASB2 lower port, 2.2-UASB2 middle port, 2.3-UASB2 upper port (Paper-II).

The relative abundance of *Methanosaetaceae* in granule sludges taken from UASB2 was lower than UASB1. Thus, a relative abundance of *Methanosaetaceae* in UASB2 was approximately half of what was observed for UASB1 with an average value of 33 ± 6%. The lower relative abundance of *Methanosaetaceae* in UASB2 has been associated with the smaller size distribution of its granule sludge (Owusu-Agyeman et al., 2019). On the other hand, the relative abundance of the family *Methanoregulaceae* was higher in UASB2 granules with a relative abundance of 29 ± 3%. This was followed by *Methanomicrobiales_unassigned,* which had a relative abundance of 20 ± 6%. Both *Methanoregulaceae* and *Methanomicrobiales_unassigned* belong to the hydrogenotrophic order of *Methanomicrobiales* (Oren, 2014) and their higher abundance implies that hydrogenotrophic methanogenesis is a dominant pathway to produce methane in UASB2. Unlike UASB1, changes in the archaeal community with HRT were observed in UASB2. Particularly, the abundance of *Methanomicrobiales_unassigned* in UASB2 granules was lower during operation at HRT 4 where the abundance was only 16±6 and 16±1% on day 59 and day 144, respectively. The abundance during HRT 3 (day 31) and HRT 5 (day 207) were higher with averages of 22 ± 3% and 27 ± 4%, respectively. The relative abundance of *Methanomicrobiales_unassigned* was lowest during the period where UASB2 performance was lowest (HRT 4) probably because archaea from the family are sensitive and are not able to withstand unfavourable conditions. PCA tests were performed to elucidate how the archaeal population relates to the HRT of the reactors. Generally, the PCA results showed that samples were clustered based on HRT (Owusu-Agyeman et al., 2021b). However, a few of the samples from UASB2 overlapped. This was not observed in UASB1 which indicates that microbial communities in UASB1 granules were more well-structured.

The bacterial population in the UASB1 granules was dominated by *Anaerolineaceae*, *Fermentibacteraceae*, *Leptospiraceae*, *Bacteroidetes_vadinHA17,* and *Spirochaetaceae* with a relative abundance of 11 ± 1%, 11 ± 2%, 5.0 ± 0.4%, 4.3 ± 1%, and 3.4 ± 1, respectively (Figure 17a). As has been discussed above, species of *Anaerolineaceae* often have syntrophic relationships with the family *Methanosaetaceae* and contribute to the structure of anaerobic granules. The family *Anaerolineaceae* also produce acetate which can be used by acetoclastic methanogens showing that acetoclastic methanogenesis is a very important pathway on UASB1 The helical morphology with hooked ends of species of the family *Leptospiraceae* suggests they may also have a functional role in anaerobic granules (Picardeau, 2014; Zamorano-López et al., 2019). The family *Fermentibacteraceae* produce acetate and hydrogen from sugars (Kirkegaard et al., 2016) and their higher abundance shows that hydrogenotrophic methanogenesis is an important methane-producing pathway in UASB1. The dominant bacterial families in UASB1 did not show any major change with HRT alteration. However, the relative abundance of *Fermentibacteraceae* slightly reduced during operation at HRT 5 with an average abundance of 8%. The cause for this reduction is not easily understood and could have led to an increase in the abundance of other minority bacterial families.

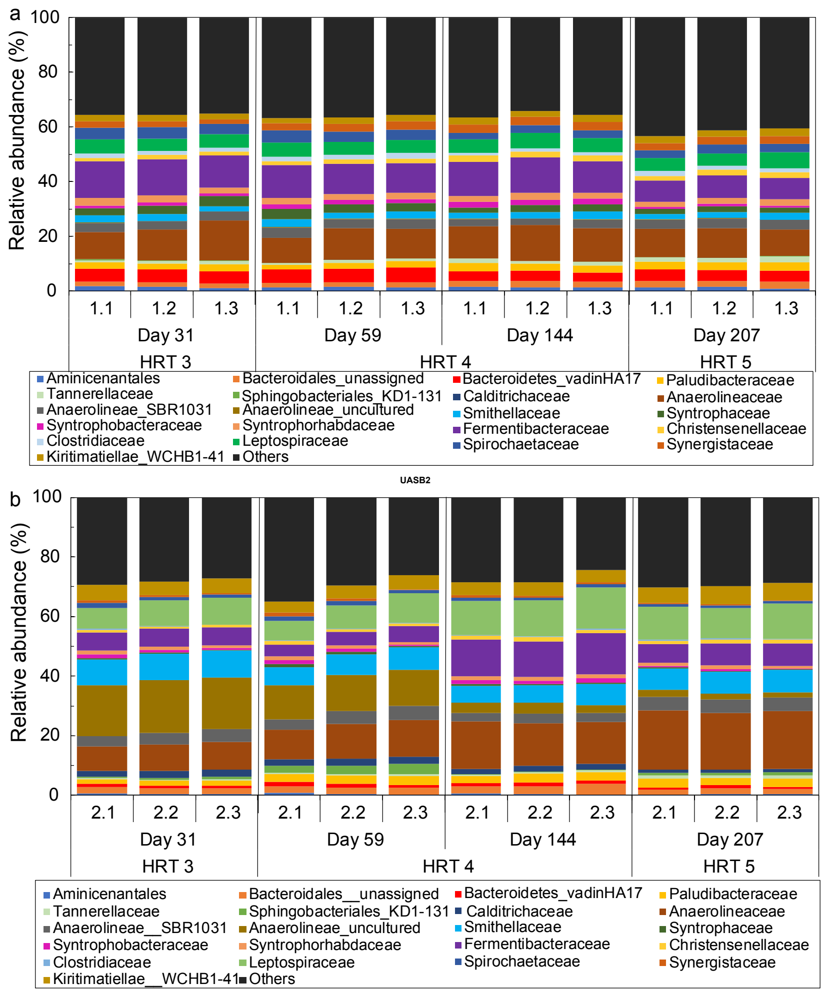

Figure 17:The bacterial community diversity in relation to HRT at the family level of granular sludge taken from different levels of (a) UASB1 and (b) UASB2. 1.1-UASB1 lower port, 1.2-UASB1 middle port, 1.3-UASB1 upper port; 2.1-UASB2 lower port, 2.2-UASB2 middle port, 2.3-UASB2 upper port (paper II).

The bacterial community of the UASB2 granules was also dominated by *Anaerolineaceae*, *Leptospiraceae*, *Anaerolineae_uncultured*, *Fermentibacteraceae*, *Smithellaceae,* and *Kiritimatiellae__WCHB1-41* with a relative abundance of 14 ± 4%, 10 ± 2%, 9 ± 7%, 8 ± 3%, 7 ± 1%, and 5 ± 0.6%, respectively (Figure 17b). Contrarily to the UASB1, the relative abundance of the dominant bacterial population in the granules of UASB2 changed with HRT. The relative abundance of the most dominant family, *Anaerolineaceae,* increased from 9 ± 1% during operation at HRT 3 to 20 ± 0.4% at HRT 5. On the other hand, the abundance of *Anaerolineae_uncultured* decreased from 17% at HRT 3 to 2% at HRT 5. This uncultured family belongs to the same class of *Anaerolineae* as *Anaerolineaceae*. The changes in the microbial community even among the same bacterial class of UASB2 granule sludge with

HRT are interesting. These obvious changes with HRT were not observed in UASB1. The results suggest that the size and structure of granules are important when it comes to the response of microbial community structure with alteration in operation conditions.

Another interesting observation was that the abundance of *Smithellaceae* was higher in UASB2 (7 ± 1%) than UASB1 (2 ± 0.3%). Members of the family *Smithellaceae* express genes for the production of formate but not for generation of hydrogen (Nobu et al., 2020). Their presence, therefore, suggests that methane production from formate could be a methanogenic pathway in both reactors but higher in UASB2. Moreover, an archaeal family with a higher rate of formate methanation, *Methanobacteriales* (Pan et al., 2016) was more dominant in UASB2 (5 ± 2%) than UASB1 (4 ± 2%). Additionally, the most dominant archaeal families in UASB2, *Methanoregulaceae,* and *Methanomicrobiales_unassigned* are well-known formate users (Oren, 2014). PCA results of the bacterial community showed that the samples were clustered according to HRT for both UASB1 and UASB2.

Like the temperature study, the SMAs of anaerobic granules in UASBB1 were higher than UASB2. Generally, UASB1 granule sludge obtained a maximum PMP of 195–635 mg CH_4-COD/(gVS·d) whereas UASB2 granules had SMA of 168–403 mg CH_4-COD/(g VS·d). This difference can be due to the fact that UASB1 is dominated by acetoclastic methanogens and therefore have higher acetoclastic activities than UASB2. Interestingly, the SMA of UASB1 was lowest with an average of 417 ± 172 mg CH_4-COD/(g VS·d) at the period with higher performance (HRT 5). This happened probably because the substrate (acetate) concentration of 3000 mg/L used for the test was below the optimum to achieve the maximum PMP. In the case of UASB2 which generally had low SMA, the highest result was obtained for granule sludge taken on day 207 (HRT 5). Moreover, it was observed that, generally, the SMA results in the HRT study were lower than what was obtained for the temperature study. This can be due to a lower concentration of substrate (acetate) than the optimum or because increasing HRT led to a reduction of the biological organic loading rate of the reactors.

5.2 VFA production (Papers III-V)

This part of the study sought to explore the VFA production potential from municipal waste streams by thoroughly studying the influence of substrate ratios of different organic waste streams on efficiency. Moreover, a long-term study with bench-scale reactors was carried out to elucidate the robustness of the VFA production system. Upscaling and application of VFA in other bioprocess applications were also exploited.

5.2.1 The impact of substrate ratio on VFA production and composition (Papers III and IV)

Lab-scale experiments in batch mode with different fractions of OW were carried out to determine the impact of OW fraction in the substrate on VFA production at the initial pH of 5 and 10 (Figure 18). The results showed that VFA production at initial pH 10 (maximum: 9300 mg COD/L) was higher than at initial pH 5 (maximum: 5370 mg COD/L). Other studies have reported that the production of waste-derived VFA is more favourable under alkaline conditions because of enhanced degradation and buffer capacity provided at the basic pH range (Atasoy et al., 2019; Cheah et al., 2019; Liu et al., 2012). Varying the fraction of OW in the feed with ratios of 0%, 25%, 50%, 75%, 100% OW showed that an increase in the fraction of OW led to an improved production of VFA at both initial pH 5 and 10 (Figure 18a & b).

At initial pH 5, VFA production of the experiments with 25%, 50%, 75% and 100% OW climaxed on day 8 with concentrations of 4100 ± 100, 4500 ± 200, 4800 ± 300 and 5300 ± 10 mg COD/L, respectively. However, the mono-fermentation of PS (i.e. 0% OW) obtained a VFA concentration of 3050 ± 120 mg COD/L on day 8 and whereas the maximum VFA concentration of 4350 ± 160 mg COD/L was obtained on day 18. Thus, OW helped to increase the rate of the fermentation process. Consequently, experiments with 0%, 25%, 50%, 75% and 100% OW attained VFA yield of 250 ± 10, 300 ± 10, 300 ± 10, 290 ± 10 and 310 ± 20 mg COD/g VS_{fed} on day 8 (Figure 18c). A similar trend was observed in the experiments performed at initial pH 10. The highest VFA productions for experiments with 75% and 100% OW at initial pH 10 were attained on day 8 with concentrations of 8830 ± 20 and 9300 ± 300 mg COD/L and yields of 580 ± 40 and 580 ± 30 mg COD/g VS_{fed}, respectively.

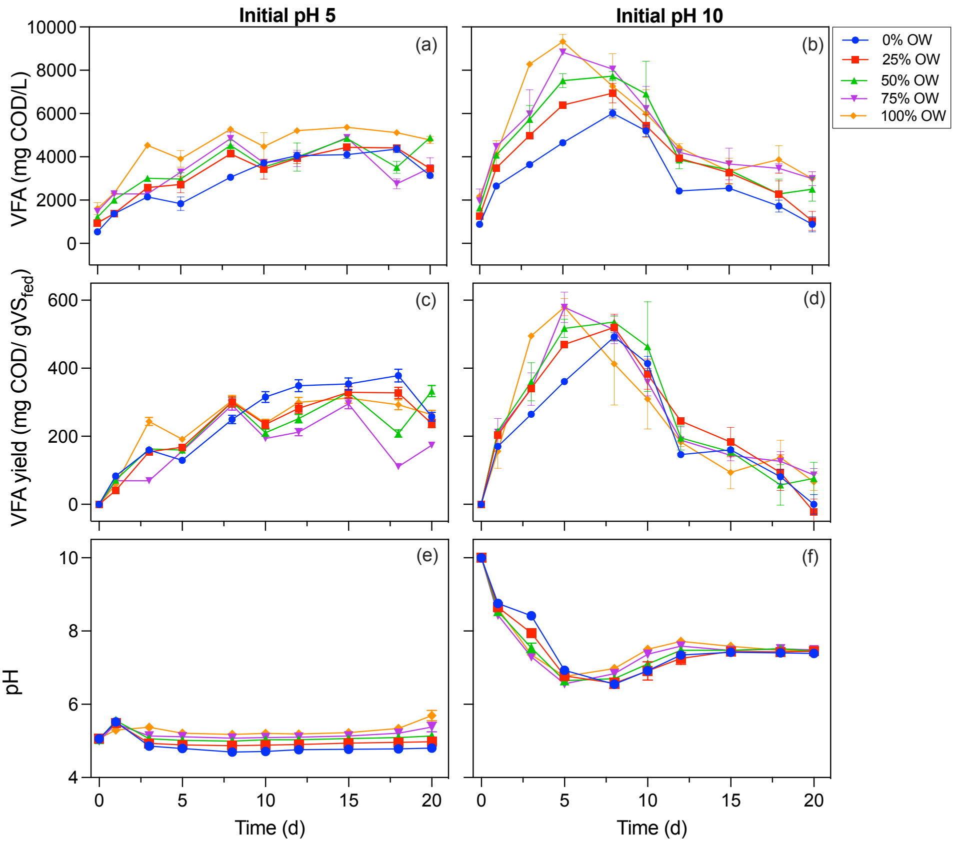

Figure 18: The results of the batch-scale study showing the impact of the ratio of OW on VFA production (a and b); VFA yield (c and d); and pH (e and f) at initial pH 5 and pH 10, respectively (Modified from Papers III and IV).

The experiments at initial pH 10 with 0%, 25%, 50% OW obtained maximum VFA production on day 8 with concentrations of 6000 ± 200, 6900 ± 450, 7700 ± 200 mg COD/L with yields of 490 ± 10, 520 ± 40, 540 ± 20 mg COD/ g VS_{fed}. This development was because an increase in the OW ratio increased the fraction of the substrates that were easily biodegradable and resulted in synergistic co-degradation by helping the fermentation process. Remarkably, the VFA production at initial pH 5 stayed fairly constant after reaching the maximum concentration until towards the end of the experiments when there was slight reduction in the VFA concentrations. On the other hand, VFA production of the experiments at initial pH 10 drastically reduced after the maximum VFA concentrations were attained. This can be explained by the conversion of VFAs to biogas. The pH values of the experiments at initial pH 5 range between 4.7 and 5.6 throughout the period (Figure 18e). At such a pH range, the activities of methanogens are greatly reduced (Atasoy et al., 2018). However, the pH of the experiments at initial pH 10 reduced to about 8.5 on day 1 and stayed in the neutral pH range throughout the experiment (Figure 18f). The near-neutral pH range is optimal for methanogenic activities (Zinder, 1993).

The compositions of the VFA mixture produced during the batch experiments are presented in Figure 19. In the experiments at initial pH 5, the VFA profile indicated that acetic acid was the dominant VFA type, followed by propionic acid with butyric or valeric acid as the third most dominant VFA species (Figure 19a). Remarkably, it is observed that increasing the OW fraction in the substrate led to an increase in the percentages of valeric and caproic acids. These VFAs have higher carbons (5C and 6C). The valeric and caproic acids percentages increased over time and they corresponded with a decrease in the fractions of low carbon VFAs like butyric and acetic acids. Valeric and caproic acids are produced through chain elongation of acetic acid and butyric acid using electron donors such as ethanol or lactic acid (de Araújo Cavalcante et al., 2017; Veras et al., 2020). Moreover, the production of these VFAs is favoured mostly under acidic pH.

At initial pH 10, the VFA mixture was equally dominated by acetic and propionic acids (Figure 19b). Unlike at initial pH 5, the percentage of acetic acid began to decrease after day 8 with propionic acid as the dominant VFA with a fraction of up to 93% in some cases. The decrease in acetic acids and the dominancy of propionic acid can be attributed to the conversion of acetic acids to biogas (Yin et al., 2021). Moreover, there could have been a transformation of acetic acid and other acids to propionic acid (Atasoy and Cetecioglu, 2021). Noticeably, iso-valeric acid was more dominant in the experiments at initial pH 10 than pH 5. Iso-valeric acid is usually produced from proteinaceous substances usually under alkaline conditions (Garcia-Aguirre et al., 2017a; Khatami et al., 2021; Liu et al., 2012). It was also observed that iso-caproic acid was produced in the experiments at pH 10. This was not the case for experiments at pH 5. Thus, while valeric and caproic acids were produced in experiments at initial pH 5, iso-valeric and iso-caproic acids were produced at initial pH 10. Both iso-valeric and iso-caproic acids are branched-chain alkyl carboxylic acids. The results suggest that the production of high-carbon branched-chain VFAs is more favoured under alkaline conditions.

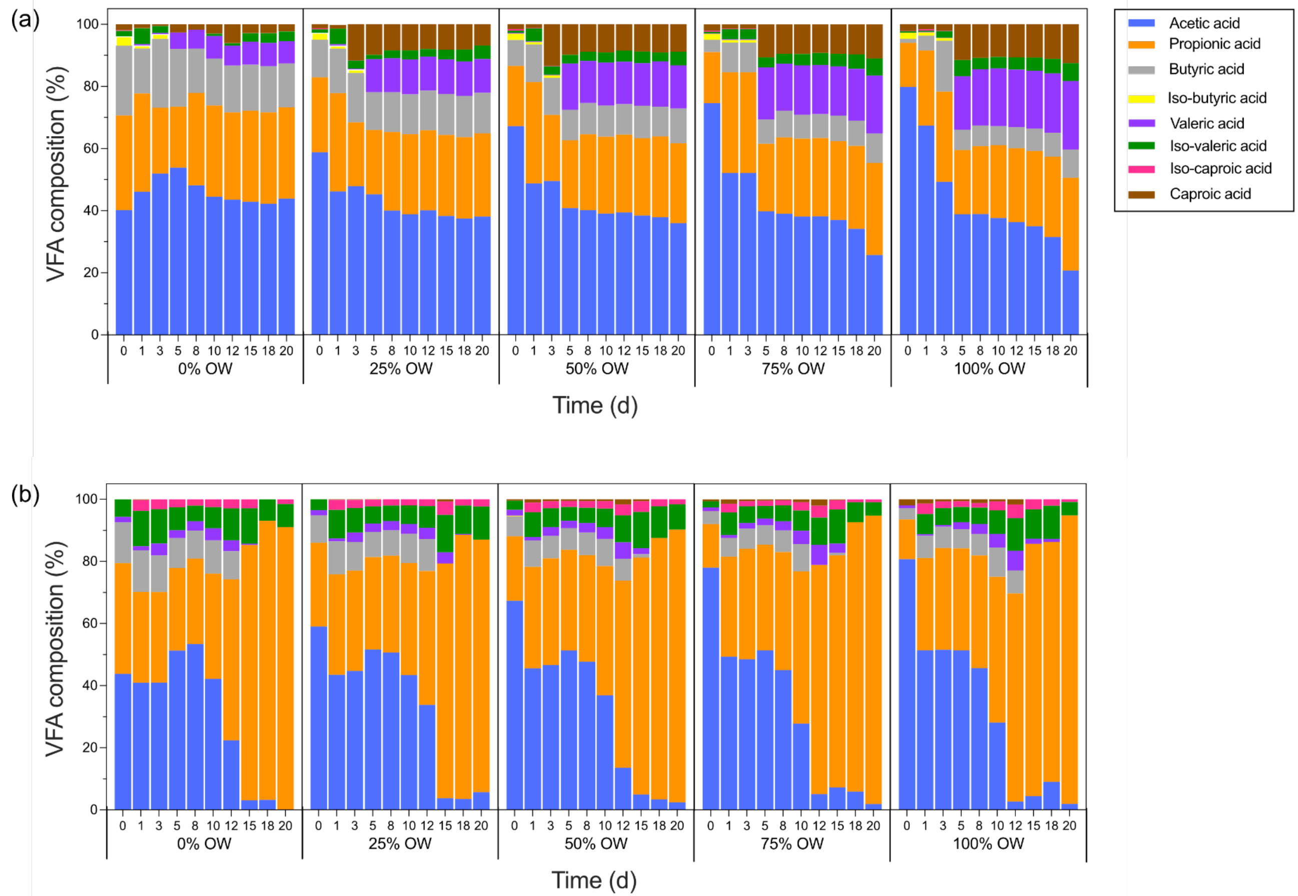

Figure 19:The composition of VFA for the batch experiments with different OW proportions at initial (a) pH 5 and (b) pH 10 (Paper III and IV).

5.2.2 Long-term VFA production in semi-continuous mode (Papers III, IV and V)

The semi-continuous reactors were operated for up to 315 days to ascertain the feasibility and resilience of the waste-derived VFA systems in the long term. The results of the VFA production including total VFA concentrations and VFA yield for the three parallel semi-continuous reactors under different pH conditions are presented in Figure 20. The VFA concentrations of the reactors operated at a controlled pH 5 and with no pH control increased in the initial stages of the experiments and peaked on days 16 and 14 with VFA concentrations of 19500 ± 100 and 18300 ± 300 mg COD/L, respectively. The corresponding VFA yields were 386 ± 20 and 360 ± 20 mg COD/g VS_{fed}, respectively. The VFA production then reduced after the initial peak concentrations. The reduction in the VFA production can be attributed to the accumulation of undissociated VFAs which could have affected the activities of acidogenic bacteria. Undissociated organic acids are capable of permeating the cell membrane of microorganisms and consequently acidifying the cell interior (Arslan et al., 2017; Roume et al., 2016). Remarkably, the reduction of the performance was more predominant in the reactor with no pH control where the lowest VFA concentration and yield of 2750 ± 500 mg COD/L and 5 ± 3 mg COD/g VS_{fed} on day 32 (Figure 20b). The worst performance of the reactor operated at controlled pH 5 was on day 28 when total VFA concentration and yield were 5300 ± 400 mg COD/L and 64 ± 3 mg COD/g VS_{fed} was obtained (Figure 20a). The effect was higher in the reactor without pH control because there was a reduction in pH below 4. The accumulation of undissociated VFAs which can reduce acidogenic activities are higher at such a low pH range (Xiao et al., 2016). The performances of both reactors were restored over time. The VFA concentration of the reactor controlled at pH 5 increased with almost stable production from day 49 with a maximum of 18000 ± 200 on day 113 and an average of 12000 ± 2000 mg COD/L. The VFA production pattern for the reactor with no pH control was similar to what was obtained at pH 5. The VFA concentration of the reactor with uncontrolled pH increased from day 46 and achieved a maximum of 24800 ± 500 mg COD/L on day 98 with an average of 16400 ± 3000. It is noteworthy that generally, the reactor without pH control performed slightly better than the reactor operated at pH 5, probably the initial drastic pH shock led to the development of a more robust microbial community. After the initial pH drop, the pH of the reactor without pH control reactor was around 5.3 ± 0.3, even without any chemical addition.

The reactor operated under alkaline conditions was initially operated at pH 10 for 275 days before lowering the pH to 9. Unlike the reactors operated at pH 5 and with uncontrolled pH, there was no observed reduction in the initial stages of experiments. This could be because of the fact VFAs at alkaline pH are not dissociated. At the operation at pH 10, the VFA concentration continuously increased with a maximum value of 30,300 ± 100 mg COD/L which is related to a yield of 630 ± 30 mg COD/g VS_{fed}. Thus, VFA production under alkaline conditions had a higher yield than acidic pH. Other studies have shown that fermentation of waste under alkaline conditions usually results in higher production than acid pH range because solubilisation and hydrolysis are often promoted (Atasoy et al., 2019; Cheah et al., 2019; Garcia-Aguirre et al., 2017a; Liu et al., 2012; Owusu-Agyeman et al., 2021a). According to these studies, an alkaline pH environment also creates a buffer condition that enhances the activities of acidogenic bacteria.

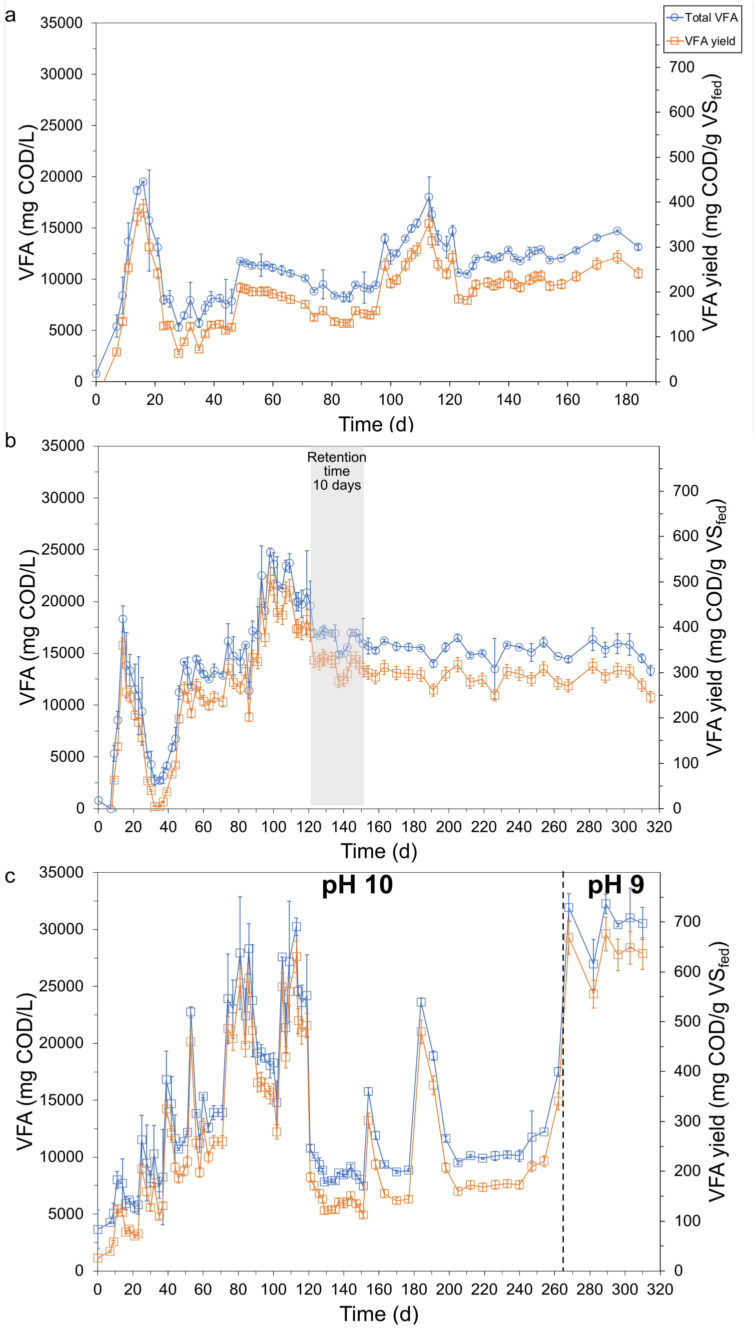

Figure 20: Total VFA and VFA yield of the semi-continuous experiments operated (a) under acidic pH 5, (b) with no pH control, and (c) under alkaline pH 10 and 9 (Modified from Papers III, IV, and V).

The distribution of the individual VFA produced is shown in Figure 21. The VFA composition of the fermented effluent from the semi-continuous reactors operated at controlled pH 5 was like the reactor with no pH control. VFA mixtures of both reactors were dominated by caproic acid. During the stable period of operation, caproic acids accounted for 53 ± 5% and 47 ± 6% of the total VFA produced from reactors operated at controlled pH 5 and with no pH control (Figure 21a & b). This result is remarkable because caproic acid is a higher-value carboxylic acid that is often produced at a post-stream step by a chain elongation process (Agler et al., 2012; Wu et al., 2018). Microbial production of caproic acids is getting the needed attention due to the higher inherent value and diverse usage area which includes antimicrobial extract for animal fodder and transport fuel precursor (Angenent et al., 2016). Caproic acid is produced microbially often by secondary chain elongation fermentation which uses β-oxidation of short-chain VFAs such as acetic and butyric acid with electron donors including lactic acid, ethanol, carbon monoxide, and hydrogen (Agler et al., 2012; Liu et al., 2020; Steinbusch et al., 2011; Wu et al., 2018). Equations 18 and 19 show typical reactions for caproic acid production. A pH of 5-6 is seen as optimal range for caproic acid production (de Araújo Cavalcante et al., 2017; Steinbusch et al., 2011). Hence, the production of caproic acid in both pH 5 and uncontrolled reactors could be attributed to the suitable pH (both had pH around 5) and the availability of electron acceptors like ethanol and lactic acid. Thus the formation of caproic acid was probably because the external OW1 contains ethanol and methanol (Table 6) which are well-known electron donors for microbial caproic acid production (Chen et al., 2016). Moreover, lactic acid and hydrogen could have been produced as intermediate products and used for chain elongation to produce caproic acid (Baleeiro et al., 2021; Kucek et al., 2016).

$$C_2H_3O_2^- + 2C_2H_6O \rightarrow C_6H_{11}O_2^- + 2H_2O \qquad 18)$$

$$3C_2H_3O_2^- + 2H^+ + 4H_2 \rightarrow C_6H_{11}O_2^- + 4H_2O \qquad 19)$$

Acetic and butyric acids are the other dominant VFA types after caproic acid that was produced in the reactors with controlled pH 5 and uncontrolled pH. During the time after stable VFA production, the average percentages of acetic acid in the VFA mixture of reactors controlled at pH 5 and with no pH control were 22 ± 2% and 23 ± 3%, followed by butyric acid with percentages of 14 ± 2% and 16 ± 3%, respectively. Other studies on VFA production from municipal waste streams have often found acetic as predominant VFA types (Garcia-Aguirre et al., 2019; Yu et al., 2021). Remarkably, heptanoic acid, another higher carbon carboxylic acid, was produced an insignificant amount. Heptanoic acid accounted for 4 ± 3% and 6 ± 3% of the total VFAs in the effluent of reactor operated at pH 5 and with no pH control, respectively. Bio-based production of heptanoic acids is through chain-elongation usually using propionic acid as electron acceptor (Kim et al., 2019). This can be one of the reasons why propionic acid was produced only in lower concentrations with an average percentage of ≈ 1% for both reactors.

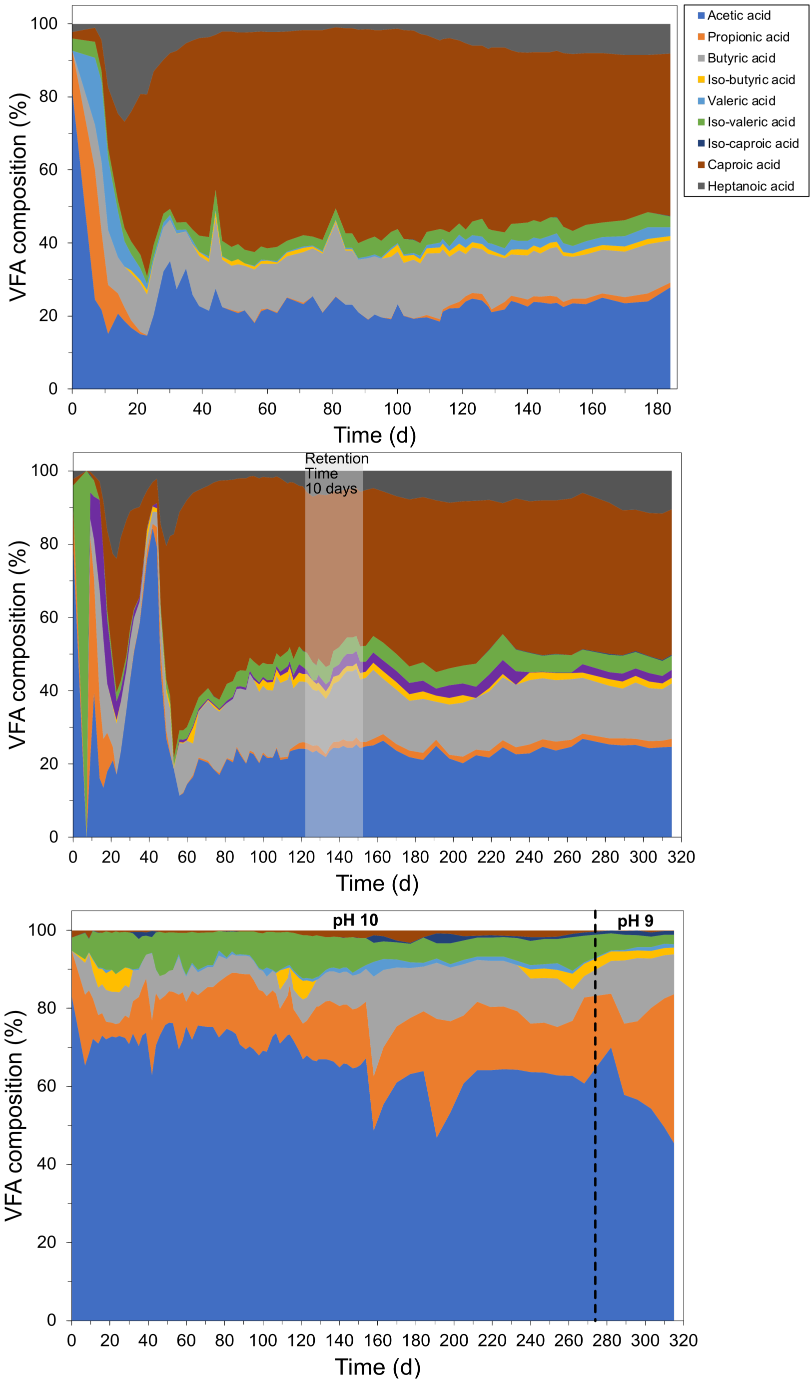

Figure 21: VFA distribution of the semi-continuous experiments operated (a) under acidic pH 5, (b) with no pH control, and (c) under alkaline pH 10 and 9 (Modified from Papers III, IV, and V).

Under alkaline pH conditions, the VFA distribution showed that acetic acid was the main VFA type, followed by propionic, butyric, and iso-valeric acids. Acetic acid accounted for 68 ± 6% of the VFAs produced during the period when the reactors were operated at pH 10. Acetic acid is often the predominant acid type under an alkaline pH environment because its production pathway (phosphoroclastic degradation), is promoted in the basic pH range (Dahiya et al., 2015; Regueira et al., 2021). The average percentages of propionic, butyric, and iso-valeric acids at operation pH of 10 were 13 ± 5%, 8 ± 4%, and 8 ± 2%, respectively. Noticeably, the relatively higher abundance of iso-valeric acid in the VFAs produced at pH 10 confirms the suggestion that alkaline pH conditions favour the production of branch-chained VFAs as discussed in section 5.2.1 above. Lowering the operation pH to 9 resulted in an increase in the percentage of propionic acid in the VFA mixture. At operation pH 9, the propionic acid fraction in the VFA mixture continually increased to a maximum of 38% with an average of 25 ± 9%. An increase in the percentage of propionic acids corresponded with a decrease in the abundance of acetic acid to 56 ± 8%. A study has also observed that lowering pH from 10 to 9 favours the production of propionic acid (Feng et al., 2009).

5.2.3 Production of VFA in Pilot-scale reactor with substrate variability (Paper V)

The reactor operated without pH control was scaled up to a 2 m^3 reactor which was operated in continuous mode with the same substrate proportion of 70% v/v PS and 30% v/v OW. The 2 m^3 pilot reactor was operated with three different kinds of OW, resulting in four periods of operation, to elucidate the impact of substrate inconsistency on the VFA production. In period I, the reactor was fed with PS and OW1 (homogenized organic waste from Himmerfjärden WWTP). The VFA production increased gradually initially achieving a maximum VFA concentration of 21,500 ± 500 mg COD/L and a yield of 410 ± 6 mg COD/g VS_{fed} on day 19 (Figure 22a). There was no drastic reduction in VFA production in the initial stage of operation, contrary to what was observed in the semi-continuous reactor without pH control (Figure 20b). The 2 m^3 pilot-scale reactor was operated continuously and the produced VFA was continuously removed which might have prevented acid accumulation as was observed in the semi-continuous reactor. Thus, unlike the 15 L semi-continuous reactor without pH control, there was no obvious reduction in the pH of the pilot reactor during the operation (Figure 22c). The average VFA concentration and yield during period I were 17500 ± 2000 mg COD/L and 320 ± 40 mg COD/g VS_{fed}, respectively. The average VFA productivity in period I was therefore 3.7 ± 0.4 kg COD/d. The VFA produced during period I was dominated by caproic acid with an average of 45 ± 5%. In terms of concentration, the average for caproic acid during period I was 7100 ± 2000 mg COD/L with a maximum of 9700 ± 900 mg COD/L. The result is interesting and higher than what has been obtained in a study of chain elongation from the fermentation of stillage with external ethanol and lactic acid as electron donors where the highest caproic acid was 6.8 g C_6 /L and 44.4% of the total acids (Carvajal-Arroyo et al., 2019).

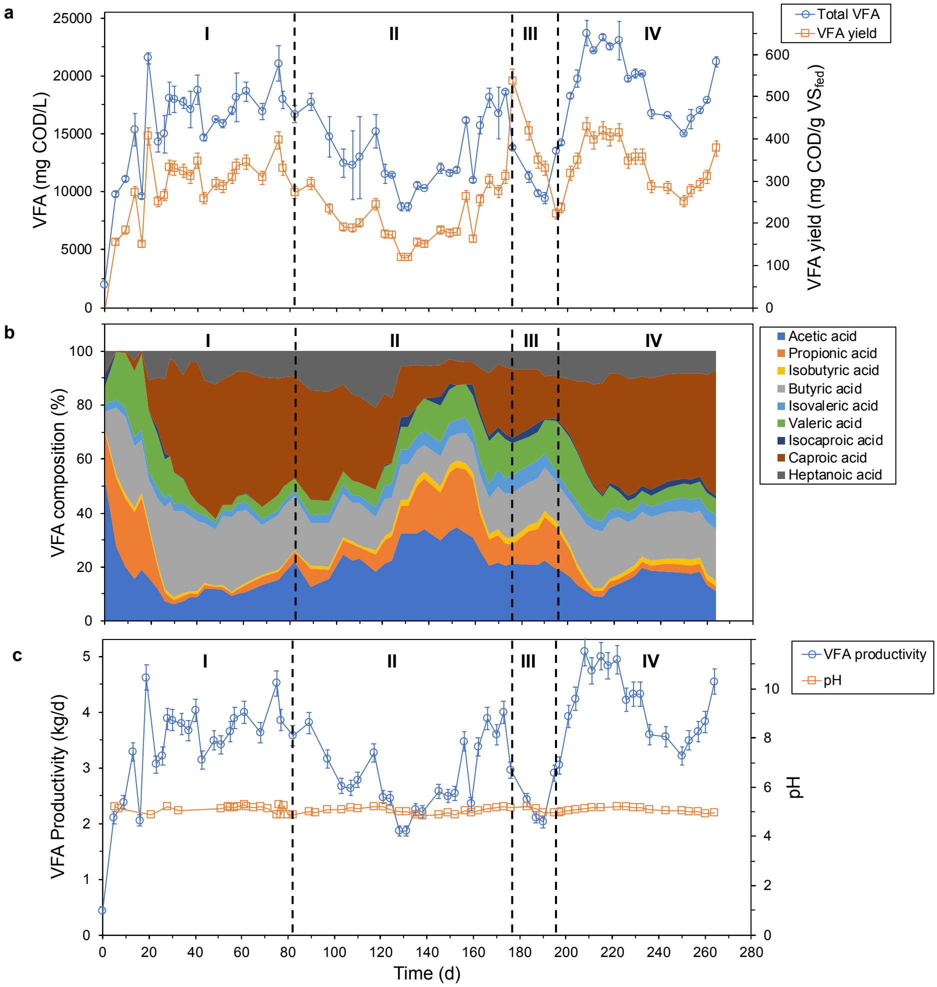

Figure 22:(a) Total VFA concentration and VFA yield; (b) Fraction of individual VFAs; (c) productivity and pH value, of the 2 m³ pilot reactor operated without pH control. I = PS + OW1 (homogenized organic waste from Himmerfjärden WWTP); II = PS+OW2 (non-homogenized organic waste from Himmerfjärden WWTP); III = PS only; and IV = PS+OW3 (homogenized organic waste from Scandinavian Biogas) (Paper V).

VFA production in period II when the reactors were fed with PS and OW2 (non-homogenized organic waste from Himmerfjärden WWTP) was relatively lower than period I. The average VFA concentration and yield in period II was 13400 mg COD/L and 210 ± 60 mg COD/g VS. The VFA productivity was only 2.8 ± 0.7 kg COD/d. The reduction in the VFA production can be generally attributed to the fact that, unlike OW1, the OW2 was not homogenized at 71 °C for 61 minutes. Thus, homogenization helped the biodegradability of organic waste. Moreover, the VFAs produced during period II were dominated by acetic acid (25 ± 7%) while the percentage of caproic acid was only 22 ± 10%. The lower caproic acid percentage recorded during period II can be attributed to the relatively lower concentrations of reductive components such as ethanol and methanol (Table 6). As discussed earlier, the production of caproic acid is often from the chain elongation of low-carbon VFAs like acetic acid with electron donors such as ethanol. In period II when only PS was fed, the VFA production was only 11000 ± 2000 mg COD/L with VFA productivity of 2.4 ± 0.4 kg COD/d. There was a restoration of VFA production when the reactors were fed with PS and OW3 (a homogenized OW from a different source). The average VFA concentration and yield in period IV were 19300 ± 3000 mg COD/L and 340 ± 60 mg COD/L with VFA productivity of 4.1 ± 0.6 kg COD/d. Moreover, the caproic acid percentage during period IV was 41 ± 2%. The increase in VFA production and caproic acid percentage can be explained by the fact OW3 was homogenized and had a higher concentration of electron donors like ethanol.

5.2.4 Utilization of VFA-rich effluent as carbon sources in the denitrification process (Paper III and V)

In the denitrification process, carbon sources are needed as electron donors to convert nitrate (electron acceptor) to nitrogen gas by microorganisms. Often the organic matter in the wastewater is either non-biodegradable or insufficient and therefore the wastewater is usually supplemented with external carbon sources to complete the process (Grießmeier and Gescher, 2018). In the current study, VFA-rich effluents were taken from the three semi-continuous reactors assessed in terms of their potential to be used as organic carbon and electron source for the denitrification process. The VFA-rich liquids were also compared with conventional organic substances, acetate, and methanol which are often used as external carbon sources for the denitrification process. The results of the batch denitrification tests showed that all the VFA-rich liquids performed better as organic carbon and electron sources than both methanol and acetate (Figure 23). The specific denitrification achieved for VFA-rich liquids from reactors operated at acidic pH 5, alkaline pH 10 and with no pH control were 14 ± 2, 16 ± 3 and 15 ± 1 mg NOx-N/ g VSS·h, respectively. The specific denitrification rates with acetate and methanol as carbon and electron sources were 10 ± 1 and 5 ± 1 mg NOx-N/ g VSS·h. Methanol achieved the lowest specific denitrification rate among the organic carbon sources because methanol usage as a carbon source in the denitrification processes is limited to methanol-assimilating microorganisms (Liu et al., 2016). Although acetate is readily degradable, the higher denitrification rate accomplished for the VFA-rich fermentation liquids than acetate was credited to the fact that VFA-rich liquids contained other organic carbon such as butyric acid which has a higher electron donor capacity than acetate (Choi et al., 2021). Moreover, the VFA-rich broth may contain compounds such as amino acid which can diminish the poisonous effect of reactive nitrogen species produced during the denitrification process (Su et al., 2016). The results of the current study have therefore revealed that VFA production through co-fermentation of municipal waste streams does not only have the potential to generate revenue for WWTPs but also aid to close the material loop and reduce their carbon footprint.

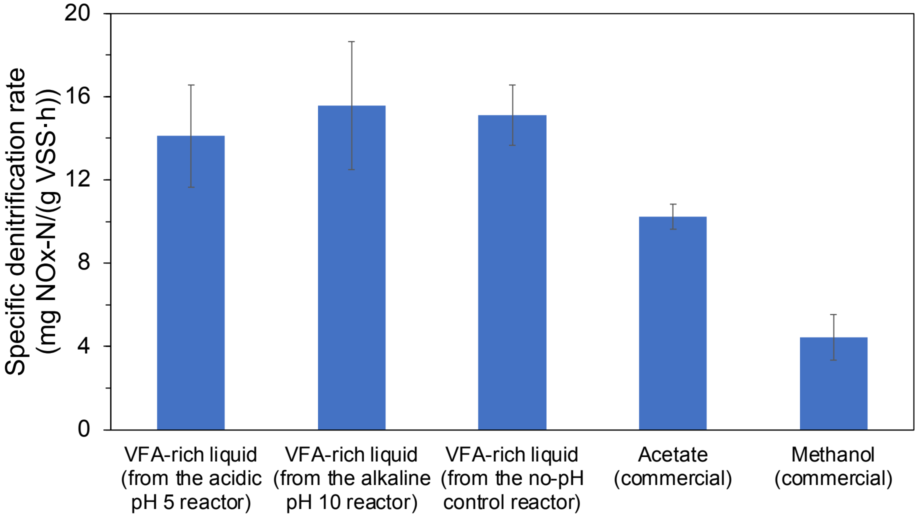

Figure 23: The use of the VFA-rich liquids from 15 L pilot-scale reactors in the denitrification process in comparison with acetate and methanol (The pH of all the tests were maintained at 7.3 ± 0.2 with the help of a buffer solution).

5.2.5 Microbial community dynamics of the VFA production systems (Papers IV and V)

The microbial community analysis of samples taken from the bench-scale semi-continuous reactors operated without pH control and under alkaline conditions. Microbial analysis of the pilot-scale reactor was also performed.

5.2.5.1 Microbial community structure of the semi-continuous reactors

Sludge samples were taken periodically from the semi-continuous reactors operated under alkaline conditions and without pH control to elucidate the microbial community dynamics during the operation and the link between the VFA production and the types of individual acids in the mixture. At the start of experiments, the microbial community which was mainly from seed sludge was dominated by Bacteroidetes (21 ± 1%), Proteobacteria (15 ± 1%), Firmicutes (15 ± 3%), and Chloroflexi (12 ± 1%) as the dominant phyla.

The semi-continuous reactor operated without pH control (Paper V)

In the reactor without pH control, the relative abundance of Bacteroidetes increased over time up to 38% till day 25. The abundance of Bacteroidetes began to reduce until day 46 (4%) and remained lower throughout the experimental period (Figure 24a). The initial increase and decrease in the relative abundance of Bacteroidetes coincided with the increase in VFA production. However, after day 46 there was no increase in the abundance of the phylum contrarily to the observed restoration of VFA production. The results suggest that Bacteroidetes contributed to VFA production in the beginning, but other phyla might have taken over time. Bacteroidetes have been observed to be the key players in anaerobic fermentation reactors for VFA production but they often show variation depending on the prevailing conditions (Magdalena et al., 2019). Moreover, the abundance of Chloroflexi phylum gradually decreased with time and eventually almost disappear after day 14. A study of VFA production from wastes has revealed that Chloroflexi negatively correlates with VFA production (Atasoy et al., 2019). After day 14, the phyla Firmicutes and Proteobacteria were enriched in the reactor without pH control with average abundances of 43 ± 10% and 31 ± 6%, respectively. On day 46, the Firmicutes abundance was relatively high (74 ± 2%). This day fell within the period with very low pH values (< 4). Thus, bacteria related to Firmicutes phylum can strive under harsh conditions like acidic environments (Filippidou et al., 2016). Actinobacteriota was the third most abundant bacterial phylum with an abundance of 9 ± 4%.

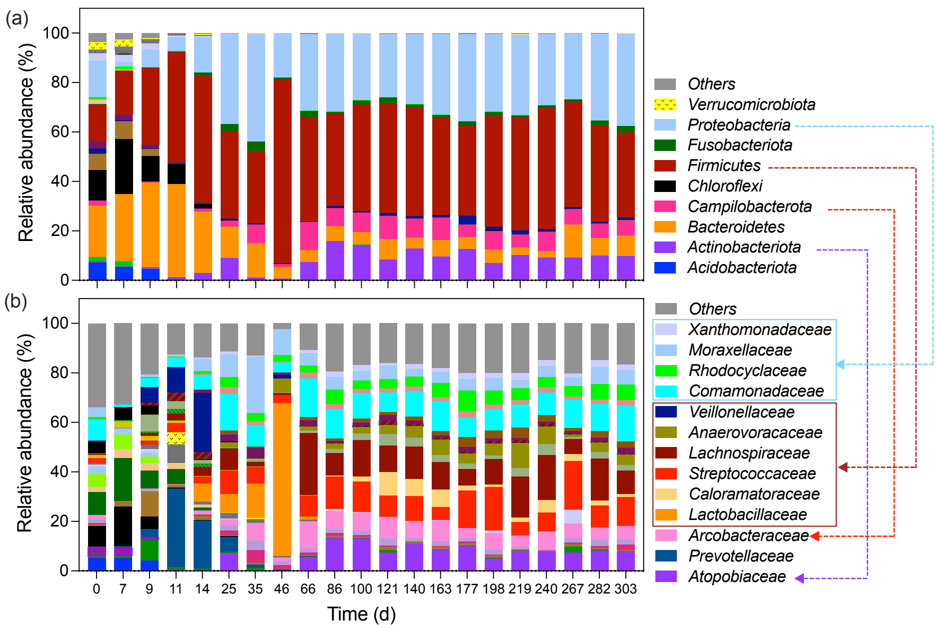

Figure 24: The bacterial community structure at (a) the phylum level and (b) the family level of the semi-continuous reactor operated without pH control (Paper V).

The mixed microbiome was further analyzed at the family level for the reactor without pH control. It was observed that the most dominant families were *Lachnospiraceae* and *Streptococcaceae* with an abundance of 11 ± 6 and 10 ± 4%, respectively (Figure 24b). These families belong to the phylum Firmicutes. *Lachnospiraceae* is capable of producing VFAs such as butyric acid and acetic acid from carbohydrates (Cotta and Forster, 2006). Moreover, a study has linked the production of caproic acid in a mixed microbial fermentation with the abundance of *Lachnospiraceae* (Blasco et al., 2020). *Streptococcaceae* bacteria are often associated with primary sludge and are known to produce from cellulosic sludge (Kirkegaard et al., 2017; Novaes, 1986). The next most abundant family was *Comamonadaceae* (10 ± 3%) which is related to the phylum Proteobacteria and is associated with caproic acid production (Kucek et al., 2016). Remarkably, *Atopobiaceae* was also a dominant family with a relative frequency of 7.5 ± 4%. There is limited information on *Atopobiaceae* in anaerobic fermentation reactors, but, species of this family have been isolated from the mammalian gut suggesting their fermentative abilities (Ndongo et al., 2019). Correlation analysis of the bacterial population with the VFA types resulted in a strong positive correlation between

Atopobiaceae and caproic acid with a statistically significant coefficient of 0.865 with a p-value of 4.28×10^{-7}. Moreover, there was a positive correlation between caproic acid and the bacterial families, *Lachnospiraceae* (0.765), *Comamonadaceae* (0.601), *Streptococcaceae* (0.687), *Arcobacteraceae* (0.775), and *Rhodocyclaceae* (0.678).

The semi-continuous reactor operated under alkaline conditions (Paper IV)

In the reactor operated under alkaline conditions, the phylum Firmicutes was highly enriched. The relative percentage of Firmicutes at the period when the reactor was operated at pH 10 was 86 ± 7%. Species belonging to Firmicutes have been known to strive well under extreme alkaline conditions (Kalwasińska et al., 2017). The second most dominant phylum at pH 10 was Proteobacteria with an average percentage of only 7 ± 3%. The results suggest that the bacterial community of the reactors under alkaline conditions was less diversified in the phylum level than the reactor operated without pH control. Bacteroidetes became the dominant phylum with an abundance of 58 ± 4% when the pH was lowered to 9. Thus, the relative abundance of Firmicutes was reduced to 38 ± 0%. This change in bacterial population could have resulted in the increase in VFA production at pH 9 because there are known acidogenic species affiliated to Bacteroidetes and they could have helped in the acidification process (Venkiteshwaran et al., 2016).

Furthermore, analysis of the bacterial population at the family level revealed that *Bacillaceae* (34 ± 10%), *Proteinivoracales_uncultured* (16 ± 11%), and *Peptostreptococcales-Tissierellales* (9 ± 2%) belonging to the phylum Firmicutes dominated at the period when the reactors were operated at pH 10 (Figure 25b). Several species associated with the most dominant family, *Bacillaceae*, are known to grow well at pH 10 and have acetic acid the main fermentative product (Logan and De Vos, 2015) (Park et al., 2015). This could explain the higher percentage of acetic acid in the VFA mixture produced at pH 10. Moreover, *Proteinivoracales_uncultured* belong to class Clostridia, which produce VFAs usually under alkaline conditions (Chen et al., 2017; Zhang et al., 2010). Again, lowering the pH to 9 changed the bacterial population with *Dysgonomonadaceae* (52 ± 8%) from the phylum Bacteroidetes as the most dominant bacterial family species. *Dysgonomonadaceae* usually produce acetic acid and propionic acid from protein and carbohydrates with an optimal pH range of 6.3–9.1 acids (Hahnke et al., 2016). The dominance of *Dysgonomonadaceae* could therefore explain the increased percentage of propionic acid at pH 9. Moreover, *Dysgonomonadaceae* correlated positively with propionic acid (0.763), acetic acid (0.625), and butyric acid (0.765).

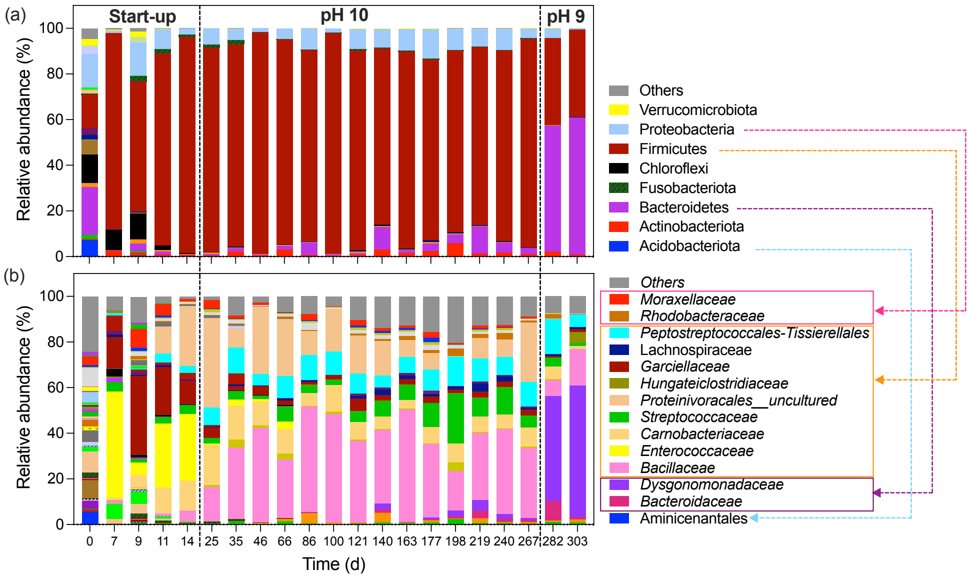

Figure 25: The bacterial community structure at (a) the phylum level and (b) the family level of the semi-continuous reactor operated under alkaline conditions (Paper IV).

5.2.5.2 <u>Microbial community structure of the 2 m³ Pilot reactor (Paper V)</u>

Sludge samples were taken selected times during the various operation period and microbially analyzed to elucidate how the dominant bacterial population changes with substrate variability. At the phylum level, the bacterial population was largely dominated by Firmicutes (47 ± 15%), Proteobacteria (27 ± 6%), and Bacteroidetes (16 ± 13%). A more detailed analysis of the microbial structure at the family level showed that *Prevotellaceae* from the phylum Bacteroidetes dominated in the initial stages of period I. Thus, the abundances of *Prevotellaceae* on days 16 and 19 were 16 ± 0.4% and 20 ± 2%. However, after day 19, the abundance of *Prevotellaceae* drastically reduced. *Prevotellaceae* bacteria are protein degraders which produce VFAs but their presence in mixed culture environment are often transient (Amaretti et al., 2019). During period I, *Lachnospiraceae* became the most dominant bacterial family after day 19 with an average relative abundance of 34 ± 12%. *Lachnospiraceae* bacteria have been found to be linked with the production of VFAs, particularly caproic and acetic acid (Blasco et al., 2020). *Lachnospiraceae* also dominated during the stable period of the semi-continuous reactor without pH control.

During period II, the relative abundance of *Lachnospiraceae* reduced to 18 ± 9%, while the *Lactobacillaceae* increased from 5 ± 0.3% to 9 ± 2% during periods I and II, respectively. Correlation analysis of the dominant microbial community with total VFA and the individual VFA types showed a strong positive correlation (0.802) between *Lachnospiraceae* and caproic acid at a 1% significant level. There was also a significant positive correlation between *Lachnospiraceae* and butyric acid (0.665) and total VFA (0.674). This confirmed the results of the semi-continuous reactor and revealed that *Lachnospiraceae* was a key microbial player to produce caproic acid and other VFAs in a mixed microbial microbiome during co-fermentation of municipal organic waste with pH control.

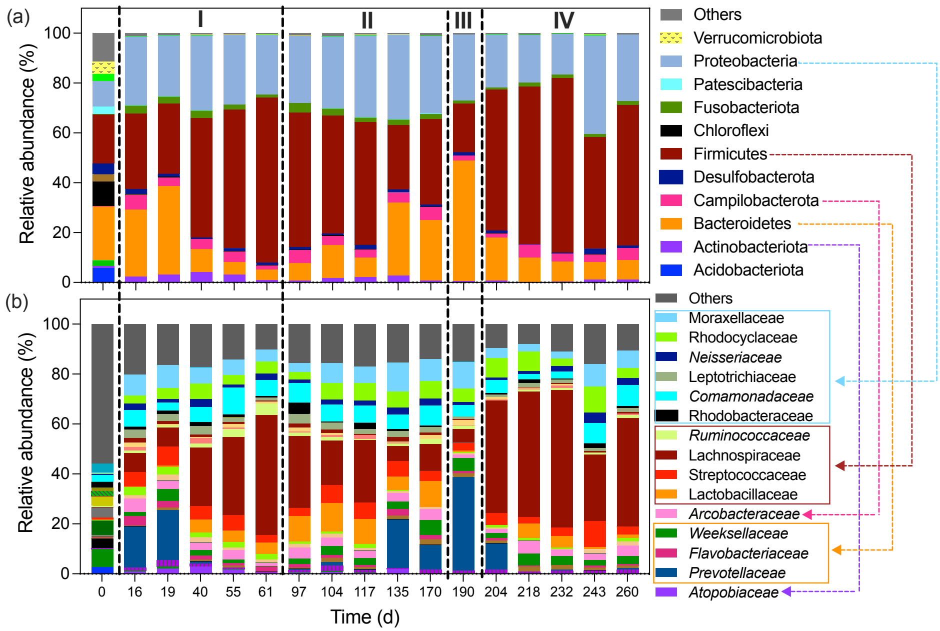

Figure 26: The bacterial community structure at (a) the phylum level and (b) the family level of the 2 m³ pilot-scale reactor (Paper V).

5.3 Significance of the study

This Ph.D. project has shown that direct anaerobic treatment of municipal wastewater is feasible at sub-mesophilic conditions with relatively low HRT. Applying anaerobic technologies such as UASB to directly treat wastewater could remove the cost associated with aeration in the conventional activated sludge system. The biogas produced from the direct anaerobic treatment of wastewater can be used within the WWTPs in areas such as heating. Whereas the technology of the granule-based is not new, the current study has given an insight into the link between the granule structure and the methanogenesis process. The study has revealed how the microbial community can be varied depending on the structure of the anaerobic granules. Moreover, the influence of operating conditions such as temperature and HRT on the microbial community structure of different granule size distribution have been explored. With this knowledge, a robust anaerobic granule-based system for the treatment of wastewater under sub-mesophilic conditions can be developed.

Production of VFA from the sewage sludge from the WWTPs and external organic wastes has shown that valuable chemicals can be recovered from the waste streams. The use of different proportions of sewage sludge and external organic waste has revealed how to combine these waste streams to achieved simultaneous waste management and resource recovery. The results from different pH values, retention times, and reactor types have revealed the strategy to maximize production and achieved the specific VFA product.

Moreover, the long-term studies carried out during the project have demonstrated the performance of the VFA production system in the long-term period. More importantly the strategy to restore production has been ascertained in the current study. The result of the current study, therefore, is an important future reference point for the implementation of the VFA production systems in full-scale applications. Scaling up to a 2 m^3 pilot reactor with real-world conditions has provided the outlook and served as a bridge between research and implementation in full-scale application. The usage of the VFA as a carbon source for the denitrification process has demonstrated an approach to close the material loop for the WWTPs and show these facilities can be self-sufficient. Moreover, the VFA produced in the project was used for PHA production using pure, co and mixed cultures in other studies with promising results (Khatami et al., 2022; Perez-Zabaleta et al., 2021). Thus, waste-derived VFAs can serve as a chemical platform for other post-stream bioprocesses. This will lead to a waste-to-value approach by recovering functional chemical products from waste streams.

In an integrated manner, this study has disclosed a carbon recovery system that can make wastewater treatment systems a self-sufficient system in terms of material and energy flows and turn WWTPs into biorefineries that can produce chemicals and energy to replace fossil-based products. This will contribute greatly to a sustainable society.

6 Conclusions and future recommendations

6.1 Conclusions

The first part of the study which was about the direct anaerobic treatment of mainstream municipal for biogas was performed to elucidate the relationship between the characteristics of anaerobic granules and the methane-production process. The outcomes include:

⇒ Biogas production through direct anaerobic treatment of municipal wastewater under sub-mesophilic conditions with granule-based reactor was found to be feasible with relative comparative performance.
⇒ The microbial community of anaerobic granules is a determining factor for the properties of the granules and the methane-producing pathways.
⇒ The UASB sludges with larger granules were characterized by multi-layered internal microstructure and had a relatively higher abundance of acetoclastic methanogens and associated bacterial population with higher specific methanogenic activities, pointing to the importance of acetoclastic methanogenesis as a significant methane-producing process.
⇒ Smaller granules had a uniform internal structure with no distinct strata with archaeal populations highly dominated by hydrogenotrophic methanogens lower specific methanogenic activities, implying that hydrogenotrophic methanogenesis was the main pathway for producing methane.
⇒ Increasing temperature to 28°C resulted in an increase in biogas production for UASB with larger anaerobic granules and a stable and higher biogas rate for UASB with smaller anaerobic granules.
⇒ An increase in HRT enhanced the COD removal efficiency and biogas production even at low temperatures due to increased contact time between substrate and microorganisms.
⇒ The extent of change in the microbial community with operating conditions (HRT and temperature) was higher in smaller granules than larger granules.

The second part of the study involved VFA production from the co-fermentation of municipal organic wastes. The findings of the study included:

⇒ An increase in the percentage of external organic waste in the substrate enhanced VFA production.
⇒ pH has shown to be an important factor to influence both the dominant VFA type and the microbial community.
⇒ Acetic acid and propionic acid were the dominant VFA type during the short-term batch study under both alkaline and acidic conditions.
⇒ VFA production in the semi-continuous mode was feasible even in the long-term period and showed resilience and the possibility to restore with pH change.
⇒ In the long-term operation under alkaline conditions, acetic acid was the dominant species at pH 10 whereas lowering to pH 9 resulted in an increase in the percentage of propionic acid.
⇒ Thus, changing operation pH from pH 10 to pH 9, resulted in the shift in the dominant microbial community from *Bacillaceae* to *Dysgonomonadaceae*.

⇒ Long-term semi-continuous operation under acidic pH and no pH control conditions obtained caproic acid as the dominant VFA type.
⇒ *Lachnospiraceae* was the dominant bacterial family and correlated with caproic acid production under no pH control conditions.
⇒ Upscaling of the VFA fermentation system to a 2 m^3 pilot reactor revealed the robustness in response to substrate variability.
⇒ The waste-derived VFA-rich liquids were proven to be a good carbon source for the denitrification process and PHA production.

6.2 Future recommendations

From the outcome of the current study, the following research directions are recommended for direct anaerobic treatment of municipal wastewater to ensure high efficiency:
⇒ Investigating the possibility of bioaugmentation with specific methanogens which are known to be more effective to increase conditions to favour extracellular polymer substances during granulation to enhance system efficiency.
⇒ Research on how the concentration of the influent can impact the structure of the granules and the process performance.
⇒ Research on using omics tools and visualization to understand the aerial distribution of microbial population in the microstructure of granules to better understand phenomena of substrate and biogas transport in the granules

With the VFA production, the following further research is recommended:
⇒ Elucidation of the active consortium of the mixed microbial using advanced tools such as bio-orthogonal non-canonical amino acid tagging (BONCAT) and fluorescence-activated cell sorting (FACS) to help determine which bacterial communities are active and producers of specific VFA type.
⇒ Study on the possibility of separating VFA mixture into lower value VFAs and higher value VFAs. The lower value VFAs can be used directly for denitrification whereas the higher value VFAs can be used for downstream processing.

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