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Systematic Quantification of Biogas Potential in Urban Organic Waste

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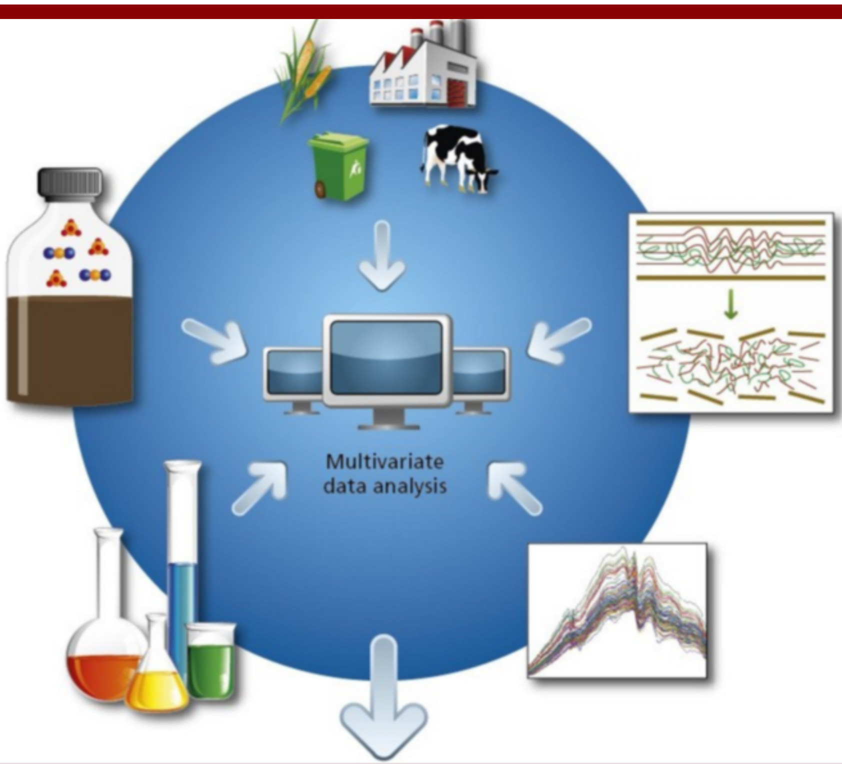

Temesgen Mathewos Fitamo

PhD Thesis
April 2017

DTU Environment
Department of Environmental Engineering

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The synopsis part of this thesis is available as a PDF file for download from the DTU research database ORBIT: http://www.orbit.dtu.dk.

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Preface

The PhD thesis was carried out at the Department of Environmental Engineering, Technical University of Denmark under the supervision of Professor Charlotte Scheutz and co-supervision of Senior Researcher Alessio Boldrin from November 15,2013 to February 28,2017.

The thesis is organised into two parts. The first places into context the findings of the PhD in an introductive review, whilst the second part consists of the papers listed below. These will be referred to in the text by their paper number, written in the Roman numerals **I-IV**.

I Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C. 2016. Co-digestion of food and garden waste with mixed sludge from wastewater treatment in continuously stirred tank reactors, *Bioresource Technology* 206, 245–254.

II Fitamo, T., Boldrin, A., Dorini, G., Boe, K., Angelidaki, I., Scheutz, C. 2016. Optimising the anaerobic co-digestion of urban organic waste using dynamic bioconversion mathematical modelling, *Water Research* 106, 283-294.

III Fitamo, T., Treu, L., Boldrin, A., Sartori, C., Angelidaki, I., Scheutz, C. Microbial population dynamics in urban organic waste co-digestion during a change in feedstock composition and different hydraulic retention times. *Water Research.* Accepted with revisions. February 2017.

IV Fitamo, T., Triolo, J.M, Boldrin, A., Scheutz, C. Rapid biochemical methane potential prediction of urban organic waste with Near Infrared Reflectance Spectroscopy. Submitted to *Bioresource Technology*, February 2017.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study:

Boldrin, A., Baral, K.R., Fitamo, T.M., Vazifehkhoran, A.H., Jensen, I.G., Kjærgaard, I., Lyng, K-A., van Nguyen, Q., Nielsen, L.S., Triolo, J.M. (2016) Optimised biogas production from the co-digestion of sugar beet with pig slurry: integrating energy, GHG and economic accounting. *Energy*, 112, 606-617.

Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C. (2015). Combined anaerobic digestion of green waste with wastewater treatment plant mixed sludge in continuous stirred tank reactor (CSTR). Proceedings Sardinia symposium 2015, 15th International Waste Management and Landfill Symposium.

Fitamo, T., Boldrin, A., Boe, K., Angelidaki, I., Scheutz, C. (2015). Co-digestion of food waste and garden waste with wastewater treatment plant mixed sludge in CSTR. Proceedings AD World Congress Series, 14th World Congress on Anaerobic Digestion.

Fitamo, T., Boldrin, A., Baral, K. R., Vazifehkhoran, A. H., Jensen, I. G., Kjærgaard, I., Lyng, K-A., Van Nguyen, Q., Nielsen, L. S. & Triolo, J. M. (2015). Integration of Energy, GHG and Economic accounting to optimise biogas production based on co-digestion. Proceedings DTU-Sustain 2015, DTU Conference.

Fitamo, T., Boldrin, A., Dorini, G., Boe, K., Angelidaki, I., Scheutz, C. (2016). Dynamic bioconversion mathematical modelling and simulation of urban organic waste co-digestion in continuously stirred tank reactor. Proceedings BioCycle REFOR16, "Renewable Energy From Organics Recycling" 2016, 16th Annual Conference.

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Summary

Organic household waste can be treated in various ways, depending on the preferred choice of waste management hierarchy and European member state and individual country legislation. Currently, there is renewed interest in gradually introducing the compulsory, separate collection of biowaste from households, restaurants and commercial and industrial sources by 2020 in Denmark and throughout the European Union. In Denmark, conventionally organic household waste has been incinerated, so the biological treatment of organic household waste is very limited. Current policies promote moving away from incineration and increasing the recycling of household waste by 50%, according to Danish and EU targets. The Danish government has opted for the production of biogas from recycled organic household waste whilst decreasing the amount sent to incineration plants, while the EU favours composting and biogas production in comparison to incinerating and landfilling organic household waste, in order to reduce environmental impacts. Biogas production from urban organic waste (UOW) could also contribute to achieving the EU's renewable energy directive targets by producing 20% of overall energy and 10% of vehicle fuel from renewable sources. Moreover, biogas could play a vital role, together with wind energy, in accomplishing the ambitious Danish government's energy strategy of becoming a 100% fossil fuel-free nation by 2050.

This PhD research was carried out as part of the BioChain project, seeking to optimise the biogas production value chain in Denmark. The BioChain project focuses on identifying technical, economic and legislative barriers and challenges throughout the biogas production value chain and aims at providing decision support tools to produce scientifically-based and sound solutions.

The main objective of this PhD study was systematic quantification of biogas production and biochemical transformation of urban organic waste comprising organic household waste, garden waste and industrial organic waste. The overall objective of this PhD research has been carried out in four phases: (i) Developing a near-infrared reflectance spectroscopy (NIRS)-based computational model for predicting the methane potential of urban organic waste, (ii) Performance of co-digestion of urban organic waste with mixed sludge from wastewater treatment plants in a continuous reactor operation, (iii) Analysis of microbial population dynamics during the co-digestion of

urban organic waste in a continuous reactor and (iv) Identification of optimal co-digestion scenarios of urban organic waste.

The biochemical methane potential (BMP) of organic waste is normally used to quantify the energy content of various biomasses. However, performing BMP measurements is very time-consuming and can take several weeks to complete, depending on the degradability of the biomasses being tested, which challenges the optimisation of biogas production and feedstock management at biogas plants. Therefore, there is a need to develop new methods for rapid determination of urban organic waste BMP. In this study, a BMP prediction method, using NIRS, was developed. A total of 87 samples consisting organic household waste, garden waste and industrial organic waste (e.g. cheese and milk) were collected, prepared and analysed for BMP and NIR. Partial least square (PLS) models were built based on measured BMPs and NIRS spectra. The root mean square error of prediction ($RMSE_P$) and the coefficient of determination (R^2) of the best PLS model for predicting the BMP of urban organic waste was 44 mL CH_4/g VS and 0.88, respectively with a relative root mean square error (rRMSE) of 9%. In addition, 175 samples were considered, in order to develop a joint UOW and plant biomass BMP prediction model. The model predicting the BMP of joint UOW and plant biomasses had an $RMSE_P$ of 50 mL CH_4/g VS, a rRMSE of 16% and an R^2 value of 0.89. The NIRS-based prediction of BMP was satisfactory and moderately successful. The models can be used for quantifying the BMP of UOW and plant biomasses. Traditional BMP measurements can thus be replaced with NIRS-based BMP measurement for first hand estimation of the BMP.

The co-digestion of urban organic waste with sewage sludge was studied in continuous stirred tank reactors, R1 and R2, each with a working volume of 7.5 L operated in thermophilic conditions. Both R1 and R2 was fed with co-substrates of sewage sludge, food waste, grass clippings and garden waste. The amount of mixed sewage sludge was fixed at 10% of the total VS of co-substrates and the remaining 90% consisted of food and green waste with a corresponding VS mixing ratio of 75:25 and 50:50 in R1 and R2 respectively. The green waste was a mixture of 70% grass clippings and 30% garden waste on VS basis. Accordingly, the reactors were fed with co-substrates of sludge and food waste, grass clippings and garden waste with a corresponding percentage VS of 10:67.5:15.75:6.75 (R1) and 10:45:31.5:13.5 (R2). The effects of the co-substrate mixing ratio and hydraulic retention time (HRT) on reactor performance and operational parameters were investigated. The methane yield of mixed sewage sludge was 287 mL CH_4/g VS, whereas it was 424 and 359 mL CH_4/g VS for R1 and R2 respectively at HRT of 30 days. The co-digestion of food and green waste with sludge thus improved the specific methane yield by about 48% in the reactor fed with a high proportion of food waste (R1) and by 35% in the reactor fed with a high share of green waste (R2) in comparison to the anaerobic digestion (AD) of 100% mixed sludge. Methane productivity increased in line with decreasing HRTs (30, 20 and 15 days), whereas methane yield remained almost constant. However, the specific methane yield dropped considerably when reducing the HRT to 10 days. The anaerobic digestion of mixed sludge with UOW at low HRTs (<10 days) is problematic, due to microbial washout and overloading. In conclusion, the addition of UOW to sewage sludge digesters enhanced biogas production significantly.

Analysis of microbial population dynamics was conducted with samples taken during the co-digestion of urban organic waste with mixed sludge in a continuous reactor in a steady state condition. DNA was extracted via the PowerSoil® DNA Isolation Kit protocol, and sequencing was done with an Illumina MiSeq 16S ribosomal RNA. A shift in microbial community diversity was observed during the co-digestion of urban organic waste compared to the mono-digestion of sludge. During the AD of 100% mixed sewage sludge, *Proteobacteria* was the dominant bacteria in the microbial community, though this decreased considerably during the co-digestion of UOW. In contrast, a new, predominant community of *Thermonema* increased during the anaerobic co-digestion of UOW. The most prevalent methane formation occurred through syntrophic acetate oxidation, followed by hydrogenotrophic methanogenesis (*Methanothermobacter*). At a HRT of 10 days, the relative abundance of *Methanothermobacter* decreased, while the abundance of *Methanosarcina* increased in the archaeal community. Hydrolytic microbes were found to be correlated with the concentration of acetate, methane productivity and methane yield. In conclusion, this study showed that the composition of microbial diversity is linked to feedstock composition and operational process parameters, whilst biogas production process parameters are associated with the relative abundance of particular microbes.

The identification of optimal co-digestion scenarios for urban organic waste with wastewater sludge was achieved using a dynamic mathematical bioconversion model (BioModel). The BioModel was applied to simulate the co-digestion of urban organic waste with sludge at various mixing ratios of co-substrates and HRTs, in order to identify optimal biogas production scenarios. Reactor performance and operational parameters obtained by BioModel simulations were in agreement with the experimental results obtained in the co-digestion of urban organic waste conducted in a continuous reactor. The simulation scenario analysis showed that increasing the amount of sludge in the co-substrate had a marginal effect on reactor performance, whereas increasing the amount of food and garden waste improved methane productivity and yield.

The maximum methane productivity for optimal feedstock composition with a VS mixing ratio of 10% mixed sludge, 79% food waste, 8% grass clippings and 3% garden waste was 2557 mL CH_4/L·day, but the specific methane yield was 393 mL CH_4/g VS at a HRT of 12 days. On the contrary, the maximum specific methane yield of 418 mL CH_4/g VS was achieved at a HRT of 30 days, whereas productivity dropped twofold. Identifying the optimal mixing of substrates (sludge, food waste and green waste), to achieve maximum biogas production, should be based on trade-off between methane productivity, specific methane yield and stable microbial process operation. The bioconversion model can be used for the quantification of biogas production from UOW. Moreover, the model can provide support for quantifying the biochemical transformation of UOW, by controlling, monitoring and running the AD of UOW at its full potential.

Dansk sammenfatning

Organisk husholdningsaffald kan behandles på forskellige måder alt efter hvad foretrækkes på baggrund af affaldshierarkiet og lovgivningen i europæiske medlemslande eller individuelle lande. I øjeblikket er der fornyet interesse for gradvist at introducere tvungen separat indsamling af bioaffald fra husstande, restauranter samt kommercielle og industrielle kilder inden 2020 både i Danmark og resten af EU. I Danmark er organisk husholdningsaffald traditionelt blevet håndteret via affaldsforbrænding, hvorfor biologisk behandling af denne fraktion er begrænset. De nuværende politikker i Danmark og EU taler for at gå væk fra forbrænding af organisk husholdningsaffald, og i Danmark er det besluttet at 50 % af husholdningsaffaldet skal genanvendes. Den danske regering vil bruge indsamlet organisk husholdningsaffald til produktion af biogas og reducere affaldsmængden til forbrænding, mens EU foretrækker kompostering og biogasproduktion frem for forbrænding og deponering for at reducere de relaterede miljøpåvirkninger. Biogasproduktion fra bioaffald af urban oprindelse kan desuden hjælpe til at opnå EU's mål om at vedvarende energikilder skal stå for 20 % af den samlede energiproduktion og 10 % af energien til transportområdet, som det er beskrevet i EU-direktivet om vedvarende energikilder. Desuden kan biogas spille en vigtig rolle sammen med vindenergi i bestræbelserne på at opnå den danske regerings ambitiøse energistrategi om at blive 100 % uafhængig af fossile brændsler inden 2050.

Dette Ph.d.-studie blev udført som en del af BioChain-projektet: Optimering af værdikæden for biogasproduktion i Danmark. BioChain-projektet fokuserer på at identificere tekniske, økonomiske og lovgivningsmæssige barrierer og udfordringer i værdikæden for biogasproduktion, og sigter efter at fremsætte beslutningsværktøjer for at skabe videnskabeligt baserede og velfunderede løsninger.

Hovedformålet ved dette Ph.d.-studie var at systematisk kvantificere biogasproduktionen ved biokemisk omdannelse af organisk affald af urban oprindelse herunder organisk husholdningsaffald, haveaffald og organisk industriaffald. Ph.d.-studiet er blevet udført i fire faser: (i) Udvikling af en computermodel til forudsigelse af metanpotentialet af organisk affald af urban oprindelse baseret på nær infrarød reflektans spektroskopi (NIRS), (ii) Undersøgelse af udbyttet ved samudrådning af organisk affald af urban oprindelse iblandet slam fra renseanlæg i anaerobe reaktorer, (iii) Analyse af dynamikker i populationer af mikroorganismer under samudrådning af organisk affald af urban oprindelse i anaerobe reaktorer, og (iv) Identifikation af optimale samudrådnings-scenarier for organisk affald af urban oprindelse.

Man bruger oftest det biokemiske metanpotentiale (BMP) af organisk affald til at kvantificere energiindholdet af forskellige biomasser. Udførelsen af BMP-målinger er dog ganske tidskrævende og kan, afhængig af nedbrydeligheden af den testede biomasse, tage adskillige uger, hvilket besværliggør optimeringen af biogasproduktionen og håndtering af råmaterialer ved biogasanlæg. Derfor er der brug for udvikling af nye metoder, der hurtigt kan bestemme BMP af organisk affald af urban oprindelse. I dette studie blev der udviklet en metode til at estimere BMP ved hjælp af NIRS. 87 organiske affaldsprøver af urban oprindelse blev indsamlet, behandlet og analyseret for BMP og NIR. De 87 prøver indbefattede prøver af organisk husholdningsaffald, haveaffald og industriaffald (bl.a. brie og mælk). På baggrund af målte BMP-værdier og NIRS-spektre blev der udviklet en ”partial least square” (PLS)-model. ”Root mean square error of prediction” ($RMSE_P$) og korrelationskoefficienten (R^2) af den bedste model til forudbestemmelse af BMP of organisk affald af urban oprindelse var henholdsvis 44 mL CH_4/g VS og 0,88 med en relativ RMSE på 9 %. Desuden blev 175 prøver brugt til at udvikle en model til forudbestemmelse af BMP fra bioaffald af urban oprindelse samt plantebaseret biomasse. Modellen havde en $RMSE_P$ på 50 mL CH_4/g VS, og en relativ RMSE på 16 % og en R^2-værdi på 0,89. Det NIRS-baserede estimat af BMP var tilfredsstillende og klassificeres som moderat succesfuld. Modellerne kan bruges til kvantificering af BMP af bioaffald af urban oprindelse og plantebaserede biomasser. De traditionelle BMP-målinger kan derfor udskiftes med NIRS-baseret BMP-måling til et første estimat af metanpotentiale.

Samudrådning af organisk affald af urban oprindelse og spildevandsslam blev undersøgt i kontinuert omrørte reaktorer med et arbejdsvolumen på 7,5 L under termofil temperatur. De to reaktorer, i det følgende betegnet R1 og R2, blev tilført biomasse bestående af spildevandsslam og madaffald, afklippet græs og haveaffald. Mængden af spildevandsslam i samudrådnings-blandingen var fastsat til 10 % af det totale organiske tørstof (VS), og de resterende 90 % bestod af madaffald og haveaffald i et blandingsforhold på henholdsvis 75:25 og 50:50 for R1 og R2. Haveaffaldet bestod af en blanding af afklippet græs og andet haveaffald i et forhold på henholdvis 70 % og 30 % af VS. Dette betyder at det endelige procentvise blandingsforhold for spildevandsslam, madaffald, afklippet græs og haveaffald var på

10:67,5:15,75:6,75 (R1) og 10:45:31,5:13,5 (R2). Det blev undersøgt, hvordan forholdet mellem de forskellige biomassers blandingsforhold og den hydrauliske retentionstid (HRT) påvirkede reaktorens ydeevne og driftsparametre. Det specifikke metanudbytte fra blandet spildevandsslam var 287 mL CH_4/g VS, mens det for R1 og R2 var henholdsvis 424 og 359 mL CH_4/g VS (HRT = 30 days). Samudrådning af madaffald og grønt affald med slam forøgede derved det specifikke metanudbytte med ca. 48 % i reaktoren med høj tilførsel af madaffald (R1) og med 35 % i reaktoren med høj tilførsel af grønt affald (R2), i forhold til anaerob udrådning af 100 % blandet slam. Produktiviteten i forhold til metandannelse steg med en faldende HRT (30, 20 og 15 dage), hvorimod metanudbyttet stort set forblev konstant. Dog faldt det specifikke metanudbytte betydeligt ved reduktion af den HRT til 10 dage. Det blev observeret, at anaerob udrådning af blandet slam med organisk affald af urban oprindelse ved lave HRTer (<10 dage) er problematisk grundet mikrobiel udvaskning og overbelastning. Det kan heraf konkluderes at tilførsel af organisk affald af urban oprindelse til rådnetanke for spildevandsslam vil forøge biogasproduktionen betydeligt.

Analyse af udviklingen i sammensætningen af den mikrobielle population blev udført ved udtag af prøver fra samudrådning af organisk affald af urban oprindelse og slam i anaerobe reaktorer ved stabile forhold. DNA blev udtrukket med PowerSoil ® DNA Isolation Kit protokollen og sekvenseringen blev foretaget med Illumina MiSeq 16S ribosomal RNA. En ændring i den mikrobielle sammensætning blev observeret under samudrådning af organisk affald af urban oprindelse i forhold til udrådning af slam alene. Under anaerob udrådning af 100 % blandet spildevandsslam var *Proteobacteria* den dominerende bakterie i det mikrobielle samfund, men forekomsten faldt drastisk under samudrådning med organisk affald af urban oprindelse. I modsætning til under anaerob udrådning af 100 % blandet spildevandsslam, steg den nye dominerende bakterie *Thermonema* i det mikrobielle samfund under samudrådning med organisk affald af urban oprindelse. Metandannelse forekom primært gennem syntrofisk acetatoxidering efterfulgt af hydrogenotrof metanogenese (*Methanothermobacter*). Ved en HRT på 10 dage faldt den relative andel af *Methanothermobacter*, mens forekomsten af *Methanosarcina* steg. Det sås, at hydrolytiske mikrober korrelerede med koncentrationen af acetat, metanproduktion og metanudbytte. Dette studie viste, at sammensætningen af den mikrobielle diversitet hænger sammen med sammensætningen af biomasser og de operationelle procesparametre. Procesparametre for biogasproduktion hænger sammen med den relative forekomst af særlige mikrober.

Bestemmelsen af optimale scenarier for samudrådning af organisk affald af urban oprindelse og spildevandsslam blev udført ved hjælp af en dynamisk matematisk bio-omdannelsesmodel (BioModel). BioModellen blev anvendt til at simulere samudrådning af organisk affald af urban oprindelse og slam ved forskellige blandingsforhold af biomasse og forskellige HRT'er for at identificere scenarierne for den optimale biogasproduktion. Reaktorens ydeevne samt operationelle parametre som blev fundet ved BioModel-simuleringer stemte overens med de eksperimentelle resultater fundet ved samudrådning af organisk affald af urban oprindelse i anaerobe reaktorer. Simuleringerne viste, at forøgelse af slammængden kun har en marginal effekt på reaktorens ydeevne, hvorimod en forøgelse af mængden af madaffald og haveaffald både øger metanproduktionen og metanudbyttet.

Den optimale sammensætning af indfødningsmaterialet til samudrådning blev fundet til at have et VS-blandingsforhold på 10 % slam, 79 % madaffald, 8 % afklippet græs og 3 % haveaffald. Den maksimale metanproduktivitet for denne blanding af biomasser var 2557 mL CH_4/L·dag, og det specifikke metanudbytte var 393 mL CH_4/g VS ved en HRT på 12 dage. Derimod blev det maksimale specifikke metan udbytte på 418 mL CH_4/g VS opnået ved en HRT på 30 dage, hvilket dog betød en halvering af metanproduktiviteten. For at opnå den maximale biogasproduktion bør sammensætningen af indfødningsmaterialer (slam, madaffald og grønt affald) baseres på en afvejning mellem metanproduktivitet, specifikt metanudbytte og en stabil mikrobiel proces. Bio-omdannelsesmodellen kan bruges til at kvantificere biogasproduktionen fra organisk affald af urban oprindelse, Desuden kan modellen bruges som support til at kvantificere den biokemiske omdannelse, ved at kontrollere, monitorere og operere den anaerobe udrådning for at opnå det fulde potentiale.

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Abbreviations

AD	Anaerobic digestion
BMP	Biochemical methane potential
CSTR	Continuous stirred tank reactor
DTU	Technical University of Denmark
EU	European Union
FID	Flame ionisation detector
GC	Gas chromatography
HRT	Hydraulic retention time
MSW	Municipal solid waste
NIRS	Near infrared reflectance spectroscopy
OLR	Organic loading rate
OTU	Operational taxonomical unit
PLS	Partial least square
RED	Renewable energy directives
RMSE	Root mean square error
RPD	Ratio of performance to deviation
SSOW	Source segregated organic waste
T-BMP	Theoretical biochemical methane potential
TCD	Thermal conductivity detector
TS	Total solids
UOW	Urban organic waste
VFA	Volatile fatty acid
VS	Volatile solids
WWTP	Wastewater treatment plant

1 Introduction

1.1 Background

Globally, there is increasing interest in substituting conventional fossil fuels with clean, renewable energy systems to mitigate the accumulation of greenhouse gases in the atmosphere which result in climate change. For this reason, the share of renewable energy production is expected to increase in future energy systems in many European Union (EU) countries (European Union, 2016). The EU Parliament recently adopted the renewable energy directives (REDs), which include targets generating 20% of overall energy and 10% of vehicle fuel from renewable energy sources (European Parliament, 2009) by 2020. Similar initiatives in China involve the construction of several rural household biogas digesters and the provision of improved service systems such as raw material supply, technology, policy and regulations (Peidong et al., 2009). In addition, the USA adopted the 2007 Energy Independence and Security Act, which paved the way for an increase in advanced biofuel production (U.S. Congress, 2007). The EU has also set a target of an 80 - 90% greenhouse gas emissions reduction, below the 1990 level, by 2050 (European Comission, 2012).

Meanwhile, Denmark has adopted a very ambitious energy strategy to become a fossil-free nation in 2050, whereby all energy demands will be serviced by renewable energy sources. If the strategy is realised, Denmark will be the first nation to be 100 % independent of fossil fuels (Energinet.dk, 2010). This could be achieved mainly through the production of renewable energy from wind sources; however, when wind energy is in short supply, biogas could meet demand, whilst if there is a surplus of wind energy, it could be used to produce hydrogen (H_2) via electrolysis, which could then be used to upgrade biogas to biomethane.

Biogas is a versatile form of renewable energy and can be used to produce natural gas-grade biomethane, vehicle fuel, heat and electricity in a combined heat and power plant. Biomethane can be supplied to the existing natural gas grid infrastructure, or the heat produced can be utilised in district heating systems. In Denmark, there are 65 sewage sludge-based biogas plants, 82 agricultural plants (primarily manure-based) and five industrial biogas facilities (Biogas, 2014). Several new large-scale and centralised biogas plants are being built or are at the time of writing in the planning phase. In addition, there are six biogas upgrading plants and eight gas filling stations

(IMF, 2014). In Denmark, currently 3.8 PJ of biogas is produced, with the majority found in the agricultural plant (2.88 PJ), sewage sludge (0.79 PJ) and industrial (0.41 PJ) fields (Biogas, 2014). Currently, only 7% of the animal manure produced in agriculture (Energistyrelsen, 2014) is exploited for biogas production. By 2020, 50 % of the manure should be treated by anaerobic digestion (AD). Combining the AD of animal manure and industrial and household organic waste and sewage sludge could potentially contribute significantly, increasing the production of biogas by up to 40 PJ (Biogas, 2014). This shows that there is huge untapped potential for biogas production from organic waste in Denmark. Accordingly, the Danish Energy Agency has predicted a fourfold increase (up to 16 PJ) of total biogas production by 2020, which could be accomplished through utilising various kinds of feedstock such as animal manure, plant biomass, agricultural crop residues, industrial organic waste and urban organic waste (UOW) from households. The energy strategy, however, has set a cap of 25% on the maximum share of energy crops that can be used in biogas production (2015-2017). Between 2018-2020, this cap will be reduced further to 12% and gradually diminish the utilisation of energy crops for bioenergy production after 2021 (Styrelsen, 2012). Hence, introducing and utilising urban organic waste could play a vital role in increasing the share of biogas in the Danish energy system.

The EU promotes the separate collection of biowaste and favours composting and biogas production from biowaste compared to incineration and landfilling (European Commission, 2008). As of 2010, between 118 and 138 million tonnes of biowaste were generated annually in the EU (European Commission, 2008), which could be a huge input potential for biogas production. By 2020, the generation of biowaste in the EU is projected to increase by 10% (European Commission, 2008). In addition, the Danish government is promoting new policies, with the view of gradually introducing compulsory source separation and the collection of biowaste from households, restaurants and commercial and industrial sources, as well as treatment by anaerobic digestion, thereby reducing the amount of organic waste sent to incineration plants (The Danish Government, 2013). The amount of residual household waste generated in Denmark in 2014 was 1.1 million tonnes (417, 000 tonnes from single-family and 687,000 tonnes from multi-family houses), consisting of about 25% food waste (Miljøministeriet, 2014). The potential for biogas production from available feedstock materials (animal manure, energy crops, sewage sludge or industrial waste and green waste), if fully exploited, could reach up to 80 PJ (Biogas, 2014), which is about 12 % of current national energy consumption.

1.2 Anaerobic digestion and quantification of biogas production

The conversion of organic materials into biogas occurs through an anaerobic digestion (AD) process. AD bioconversion involves complex biochemical reactions such as hydrolysis, acidogenesis and acetogenesis fermentation as well as methanogenesis. Organic polymers, for instance lipids, carbohydrates and proteins, are hydrolysed to monomers of fatty acids, glucose and amino acids by faculitative or strictly anaerobic bacteria during the hydrolysis process (Treu et al., 2016; Yu et al., 2010). The monomers are then converted into intermediate products, mostly volatile fatty acids (VFAs), during the acidogenesis stage (Ali Shah et al., 2014). VFAs undergo biochemical transformation into acetate, carbon dioxide (CO_2) and methane (CH_4) through acetogenesis. These products are then converted to methane via methanogenesis. The production of methane proceeds through acetoclastic and/or syntrophic acetate oxidation (SOA). During acetoclastic methanogenesis, acetate is cleaved into methyl and carboxyl groups, which are converted later to CH_4 and CO_2, respectively, by *Methanosarcinaceae* or *Methanosaetaceae* (Ferry, 1993). Meanwhile, in the second methanogenic pathway, the acetate is oxidised to a H_2 and a carboxyl group (converted to CO_2) by SAO, followed by the syntrophic association of hydrogenotrophic methanogenesis reducing the CO_2 to CH_4 by H_2. This process is facilitated by *Methanomicrobiales* or *Methanobacteriales* (Hattori et al., 2000; Petersen and Ahring, 1991; Zinder and Koch, 1984). Due to technological advancements in sequencing techniques, the role of bacteria and archea in bioconversion processes, along with community composition in the AD process, has been established (Campanaro et al., 2016; Eikmeyer et al., 2013). Microorganisms act as the main engine in the biogas production process. The AD process can be influenced or enhanced by microorganisms. Fotidis et al. (2013) reported that ammonia inhibition can be overcome through the bio-augmentation of hydrogenotrophic methanogens, while Kougias et al. (2014) reported that foaming incidents in biogas reactors have been associated with *Microthrix* or *Nocardia* bacteria. However, the effects of microbial composition on process performance, and the correlation of biochemical parameters with the relative abundance of microorganisms, have not been established yet.

Methane, which potentially can be produced from various feedstock materials involving complex biochemical reactions, is conventionally measured with a biochemical methane potential (BMP) assay, in order to understand the extent of biodegradability. The BMP of organic materials is measured conventionally in a batch reactor incubated either in mesophilic (37°C) or thermophilic (55°C) conditions with proper inoculum (Hansen et al., 2004). However, the experimental BMP measurement technique is very costly and time-consuming, as it can take between 30 and 90 days to complete, depending on the degradability of the feedstock. Hence, estimating the theoretical biochemical methane potential (T-BMP) of various feedstocks based on elemental composition (C,H,O,N) and chemical components (carbohydrates, lipids and proteins) has been proposed.

The T-BMP is calculated with the Bushwell formula, by taking into consideration the elemental chemical composition (Symons and Buswell, 1933). Nevertheless, this BMP estimation technique overestimates the methane potential of organic materials, since it takes both degradable and non-degradable matter into account (Davidsson et al., 2007). Determining maximum methane potential based on chemical components provides more realistic estimations compared to elemental composition (Davidsson et al., 2007); nevertheless, this method involves rigorous sample preparation and analytical methods to measure the physicochemical parameters of the feedstock, which may lead to high uncertainty, particularly for very heterogeneous and solid waste samples. Furthermore, this technique is costly, time-consuming and may involve the use of chemicals during analytical analysis.

Currently, a new rapid and reliable methane potential prediction technique based on near-infrared reflectance spectroscopy (NIRS) and measured BMP is gaining increased interest and attention. NIRS has been applied moderately successfully to estimate the BMP of municipal solid waste (MSW) (Lesteur et al., 2011), meadow grass (Raju et al., 2011), MSW and agro-industrial waste (Doublet et al., 2013) and plant biomass (Triolo et al., 2014). BMP prediction based on the NIRS method supports biogas plant operators making decisions regarding substrate feeding into the digester as well as feedstock inventory management, and it also improves the efficiency of the overall biogas production value chain. BMP prediction using NIRS is still in the early stages, but it seems a promising method. Up to this point in time, there has been a lack of knowledge on a dedicated model for predicting the BMP of urban organic waste. Furthermore, there is also interest in developing a robust

NIRS model for the BMP prediction of various organic waste fractions. Normally, BMP values differ between laboratories, due to the biological nature of the experiment, such as the source and activity of inoculum, so the quality of the reference data is important, in order to develop rapid and reliable BMP prediction models.

The batch BMP value provides an estimate about the anaerobic biodegradation of organic waste. However, normally in biogas plants, the AD process proceeds under less optimal conditions in comparison to short-term laboratory incubation test assays. Mostly, continuously stirred tank reactors (CSTRs) are used on a laboratory scale to simulate full-scale biogas plants. The continuous reactors can be operated in mesophilic or thermophilic conditions. AD in thermophilic conditions provides improved yields, better organic matter reductions and biological and chemical reactions, reduced costs for digestate disposal and better hygienisation compared to mesophilic conditions (Angelidaki et al., 2006). The thermophilic AD of single substrates has been studied extensively for sewage sludge (Astals et al., 2012; Ferrer et al., 2008; Gavala et al., 2003) and food waste (Climenhaga and Banks, 2008; Forster-Carneiro et al., 2008) as mono-digestion. However, food waste mono-digestion does exhibit technical challenges, due to high protein content which leads to the accumulation of volatile fatty acids (VFAs) because of ammonia inhibition. For this reason, the co-digestion of substrates at optimal mixing ratios could possibility avoid process instability. Primary and secondary sludge generated in wastewater treatment plants (WWTPs) is normally treated and stabilised through anaerobic digestion (AD) to produce biogas. The energy produced during the bioconversion of sludge is mostly utilised to meet onsite demand, but sometimes it is delivered to energy utility companies. However, the addition of food and green waste as a co-substrate could boost biogas production in WWTP facilities. In pilot-scale and full-scale plants, the co-digestion of activated sludge with food waste has improved performance relative to mono-digestion (Bolzonella et al., 2006; Cecchi et al., 1988). Nowadays, the addition of new feedstocks such as food waste and green waste is attractive for biogas plants looking to boost biogas production. A few studies have reported on the co-digestion of food and green waste, but experiments were limited to batch tests. Chen et al. (2014) reported that an increased amount of food waste in the co-substrate did indeed improve methane yield. The thermophilic AD of food and garden waste is reported to provide enhanced reactor performance compared to mesophilic conditions (Liu et al., 2009). While several studies considering sludge as a main substrate are available in the literature, there is generally a lack of information on the co-digestion of food and green waste as the main substrate with sludge to improve the yield and productivity of biogas production (Mata-Alvarez et al., 2014).

The continuous AD process is prone to process disturbances such as changes in pH, the accumulation of VFAs and ammonia inhibition unless monitored thoroughly. The operation may take several weeks or months until it reaches a steady-state condition. Operating the reactors at optimal conditions is also very important to maximise gas production. Unforeseen process disturbance could be detrimental to microorganisms, and it could actually lead to process instability and breakdown, which could be very costly to biogas plant operators. Mathematical modelling of the AD process could provide a great support to biogas plant operators when making decisions regarding process monitoring, simulation, optimisation and stability. Kaspar and Wuhrmann (1978) proposed the mathematical modelling of sewage sludge AD based on the chemical oxygen demand (COD) of the substrate, whereas Angelidaki et al. (1999) proposed a comprehensive dynamic bioconversion model (BioModel) based on the composition of feedstock to simulate the AD of complex organic materials. The BioModel has been applied for simulating, monitoring and controlling cattle manure AD to analyse the effect of ammonia, pH and temperature (Angelidaki et al., 1993), as well as olive oil mill effluent co-digestion with manure. In response to the need for an internationally generic AD model, the IWA (the International Water Association) task group for the mathematical modelling of AD proposed "AD Model No.1" (ADM1). ADM1 expresses the concentration of substrates in terms of COD (Batstone et al., 2002) and has been applied mainly to simulate the AD of sludge in WWTPs (Batstone, 2006; Parker, 2005). The application of ADM1 to model the co-digestion of sludge with biowaste is reported to be relatively limited (Derbal et al., 2009). However, the application of the BioModel is convenient for modelling and simulating AD when the characterisation of COD is challenging, particularly for solid waste.

1.3 Research Objectives

The overall objective of this PhD study was to systematically quantify the biogas production and biochemical transformation of urban organic waste. The specific objectives of the research included:

- Assessing and quantifying the production of biogas during the co-digestion of urban organic waste (UOW) with sewage sludge by varying the mixing ratio of co-substrates and hydraulic retention times (HRTs)
- Identifying important anaerobic co-digestion scenarios for optimal biogas production from urban organic waste using a bioconversion model
- Analysing the association of biogas production from co-digestion of urban organic waste at various feedstock compositions and HRTs with changes in microbial communities
- Developing a systematic method for the quantification of biogas production from urban organic waste.

The specific objectives of the PhD thesis are provided in four papers. The assessment and quantification of biogas production from the co-digestion of urban organic waste is presented in Fitamo et al. **I**. The identification of key anaerobic co-digestion scenarios for urban organic waste is described in Fitamo et al. **II**. The dynamicity of microbial communities with the production of biogas from the co-digestion of urban organic waste is described in Fitamo et al. **III**, and the systematic quantification of biogas production from urban organic waste is reported in Fitamo et al. **IV**.

The PhD thesis is structured in **five** sections. The methods are described in section **2**, while the results and discussion of the main findings of the research are presented in section **3**. Conclusions and suggestions for further research are provided in sections **4** and **5**, respectively.

1.4 BioChain Project

This PhD study has been part of the BioChain project, supported by the Danish Innovation Fund, focusing on identifying technical, economic and legislative barriers and challenges in the biogas production value chain and aiming at providing decision support tools to make scientifically-based and sound solutions. The BioChain research project involves value chain analysis of biomass production/collection, transportation, pre-treatment, energy conversion, energy carrier substitution and the application of digestate as a fertiliser. AD is going through a number of technological advancement; however, the process is not yet fully optimised, efficient or economic. The composition of biomass and the value chain management of biomass production (agricultural or household waste) up to delivering electricity and heat to customers, and the application of digestate as fertiliser, are customarily related to biogas production and the reduction of greenhouse gas emissions. Computational models and decision support tools that could take up the analysis of a comprehensive biogas production value chain framework is crucial for supporting politicians, investors, farmers and biogas companies seeking to make scientifically-based decisions. The holistic approach is vital to identifying investment barriers, improving the efficiency of digesters and also exploring new alternative biomasses.

BioChain research consists of the value chain optimisation, integration and validation of models, biomass analysis, environmental impacts, logistics and economics. The biomass analysis part looks at developing methods that could enable the systematic quantification of biogas production from agricultural and household sources. This PhD study focuses mainly on the quantification of biogas production from urban organic waste (UOW), i.e. characterisation, methane potential, computation prediction of methane potential, co-digestion of urban organic waste, mathematical modelling of the anaerobic co-digestion of UOW and the dynamics of the microbial population in the AD of UOW. The data generated in this research will be used in the plant- and national-level optimisation of biogas production in Denmark, while digestate produced during the co-digestion process will be analysed further, to assess the environmental impact and define how digestate quality could affect carbon sequestration and greenhouse gas emissions when applied to agricultural land.

2 Methodology

The methods and materials used in this PhD study are described in this section regardless of the order of the papers. Section 2.1 explains the overall sample collection, sampling technique, sample preparation methods and sample analysis (Fitamo et al., **I, II, III, IV**). The BMP assay methodology, including the gas chromatographic analysis used to analyse the methane potential of organic waste, is described in section 2.2 (Fitamo et al., **I** and **IV**). Section 2.3 describes the methods and materials used in BMP, predicting partial least square (PLS) computational model development (Fitamo et al., **IV**). The co-digestion of urban organic waste reactor configuration, experimental set up and monitoring, including the methods used for microbial analysis during the co-digestion of UOW in CSTR, are provided in section 2.4 (Fitamo et al., **I, II,** and **III**). In section 2.5, the mathematical modelling of the anaerobic co-digestion of UOW, using BioModel, is described (Fitamo et al., **II)**.

2.1 Preparation and characterisation of waste

2.1.1 Sample collection and preparation

The samples were collected mainly from municipalities and private households in Copenhagen, Odense Kommune, Frederica wastewater treatment plant (WWTP), Econet and Ecogi A/S. In Fitamo et al., **I**, during the co-digestion of UOW, primary and secondary sludge (mixed at a volume ratio of 1:1) was obtained from Avedøre WWTP, garden waste was collected from Borgervænget recycling station (in Copenhagen municipality) and grass clippings and food waste collected from private gardens and the main canteen at Technical University of Denmark, respectively. These samples also served as the basis for developing a mathematical UOW co-digestion process model in Fitamo et al., **II**, as well as when investigating the dynamics of microbial composition in Fitamo et al., **III**. A large sample database was essential to create a dedicated computational UOW methane potential-predicting PLS model (Fitamo et al., **IV**). For this reason, source-segregated organic household waste from private households, Econet A/S (Copenhagen area) and Odense Kommune was collected, while biopulp (a mixture of food and green waste) was obtained from Ecogi A/S and industrial organic waste from Frederica WWTP.

A series of mechanical pre-treatments was used to reduce the particle size of course solid waste materials, in order to obtain representative samples for analytical and experimental tests (Fitamo et al., **I** and **II**). The samples were shredded with a shear shredder (ARP SC 2000) down to 16 mm particles. The required amount of representative sample was obtained after laying the shredded waste sample on elongated 1-D multilayer piles divided equally into portions to be accepted or rejected. Due to technical problems such as laboratory reactor piping and pumping issues, the particle size of the waste was reduced further to 4 mm in diameter with a comminutor (Fitzmill model D,Daso-6) and a cutter knife mill (Wiencken 19225). The pre-treated samples were then transferred into small containers and stored at a temperature of -20^{o}C. The required amount of sample was prepared once (Fitamo et al., **I)** and then used throughout the co-digestion experimental period and analytical testing. However, the samples were further freeze-dried and ground down to a maximum particle size of 1 mm for NIRS analysis (Fitamo et al., **IV)**.

2.1.2 Physicochemical characterisation

The characterisation of the waste samples was conducted according to the APHA standard methods for the examination of water and wastewater (APHA, 2005), in order to determine total and volatile solid content, pH, total concentration of ammonia and total Kjeldahl nitrogen (TKN). The determination of fat content, total nitrogen and total carbon was carried out in a commercial laboratory (Eurofins, DK) under the corresponding DHF 42, ISO 13878 and DS/EN 13137 standards. Gas chromatography (GC) (Shimadzu GC-2010AF, Kyoto, Japan), fitted with a flame ionisation detector (FID), was used to analyse the amount of VFAs and alcohol.

2.2 Biochemical methane potential (BMP) assay

2.2.1 Batch BMP set up

The determination of substrate BMP was performed in triplicate in 1 L batch reactors with a working volume of 0.3 L incubated in mesophilic conditions (Fitamo et al., **I** and **IV**). The inoculum-to-substrate ratio (ISR) was 2, while the organic loading rate (OLR) was 2.7 g VS/L (Hansen et al., 2004). Accordingly, the required amount of substrate and inoculum obtained from Va Syd Sjölunda WWTP (Malmö, SE) was transferred into the batch assay reactors. Finally, the batch reactors were purged with 100% N_2 to remove trace amounts of oxygen from the headspace of the bottle and to create anaerobically favourable conditions for microorganisms.

Generally, the batch BMP set up consists of blank and control experiments besides test substrates under consideration. Blank reactors were set up to determine the gas produced from the inoculum, whereas the control test checks and certifies if the BMP test has been conducted correctly and is used as a tool to validate the experiment. The standard substrate, Avicel (Fluka, DK), was used as a control in all BMP batch set ups. Methane production from the substrate assays was determined after subtracting the background contribution of gas produced from the inoculum (Angelidaki et al., 2009).

The theoretical methane potential (T-BMPs) of substrates considered in the co-digestion of urban organic waste in CSTR (Fitamo et al., **I)** was determined based on the chemical composition of carbohydrates, proteins and lipids, as reported in Møller et al. (2004) and Triolo et al. (2011). The maximum methane yield of the substrates was estimated by considering analytically determined chemical components and the respective T-BMP of the components. The theoretical methane potential of VFA (acetic acid), carbohydrate, protein and lipid gave the corresponding values of 370, 415, 496 and 1014 mL CH_4/g VS (Møller et al., 2004).

2.2.2 Gas Chromatography Analysis

Methane production over time, during the batch BMP test period, was analysed by taking gas samples followed with a manual injection into a thermo-scientific trace gas chromatograph (TRACE 1310 GC) equipped with a thermal flame ionisation detector (FID) in a 160°C oven (Fitamo et al., **I** and **IV)**. The method utilised by the instrument was selected as a standard operating procedure (SOP) split/splitless (SSL) injector FID, and a CO_2/CH_4 method was used for data treatment. Gas sampling and analysis were performed regularly until methane generation reached a steady-state condition (Fitamo et al., **I** and **IV)**.

During the co-digestion of UOW in CSTR, biogas composition (the content CH_4 and CO_2) was measured through the manual injection of gas samples into a thermal conductivity detector gas chromatograph (TCD-GC) (GC82 MikroLab, Aarhus A/S, DK) equipped with a packed column for compound separation (main column: 1.1 m × 1/16'' Molsive 137 + 0.7 m × ¼'' Lithiumsorb K8) (Fitamo et al., **I**). The oven temperature, detector and TCD-GC injector were all set to 50°C, while the flow of carrier gas, namely hydrogen in this instance, over the column was set at 40 mL/min (10 mL/15 s on a flow meter).

2.3 Computational BMP-Predicting Model

This section describes the methods and materials used to develop a rapid and reliable model for BMP prediction (Fitamo et al., **IV**). Experimentally measured BMP was used as a reference dataset together with corresponding NIR spectral data to build a dedicated UOW BMP-predicting model.

2.3.1 NIR spectroscopic analysis

NIR spectral analyses of the waste samples were performed with a Bomem QFA Flex Fourier Transform spectrometer, fitted with an InAs detector (Q-interline A/S, Copenhagen, Denmark), was used to obtain reflectance spectral data from the samples. Initially, the samples were prepared (maximum particle size of 1 mm) as described in section **2.1.1** of the methodology section. A glass rotating powder sampler (120 mL), filled up to 70% capacity, was used as a sampling test tube. The entire NIR spectral region of the samples was scanned 200 times at a resolution of 32 cm^{-1}, and average spectra were attained to ensure representative spectra were available, due to the heterogeneity of the waste samples. The spectral data collections took less than 2 mins per sample.

2.3.2 Data processing and statistical analysis of the PLS model

Spectral data analysis and computational BMP-predicting model development were carried out with Eigenvector Research Inc. Stand Alone Chemometrics Software Solo 8.0 (R8.0.1). The pre-processing of spectral data was done with common techniques to remove artefacts, make baseline corrections and remove background noise with a standard normal variate (SNV) (Barnes et al., 1989), de-trend (DT), first and second derivative Savistsky-Golay (SG) algorithm with smoothing (Rinnan, 2014; Rinnan et al., 2009). In order to analyse the input spectral data, and to understand if there were any unusual samples, principal component analysis was carried out.

Two PLS regression models for BMP prediction, based on transformed spectral data and reference BMP, were built. The first model was based on samples of UOW, whereas the second model was developed based on both UOW samples and samples of plant biomass, as reported by Triolo et al. (2014).

In the dedicated UOW BMP-predicting model development, a total of 87 samples were considered. The total sample dataset was separated into calibration (66 samples) and prediction sets (21 samples) at ratios of 3:4 and 1:4, respectively, using the Kennard-Stone (KNS) algorithm at a latent variable (LV) of 7 with SNV + DT + SG (9,2,1) data transformation. A PLS regression model with leave one out (LOO) cross validation (CV) was built on the calibration dataset (66 samples). Samples with high Q residuals and high Hoteling T^2 (a mixture of edible food waste, rhubarb pulp and mixed dairy and meat products), either due to a variance in the spectral variable or extreme variations, were excluded from the calibration set based on the influence plot. Finally, the calibration model was tested with a prediction set.

In the combined PLS model, a total of 175 samples (87 UOW and 88 plant biomass) were used to develop the BMP-predicting model. The combined dataset was split into calibration and prediction sets at ratio of 2:3 and 1:3, respectively, using transformed data at an LV of 16 with the KNS algorithm. The distribution of the measured BMP was presented in Fitamo et al., **IV**. The PLS regression calibration model of the combined data was developed based on transformed data with a cross-validation of LOO. Samples that showed high Q residuals and high Hoteling T^2 in the specific UOW and plant biomass PLS model appeared to have high Q residuals and high Hoteling T^2 in the combined calibration PLS model, too. In addition, three samples (meat, unavoidable food waste and straw) were excluded based on the influence plot, due to either the uniqueness of the spectral variable or a reference data error – or the combination of both effects. The combined calibration model was built without outliers and then tested with a prediction dataset.

The performance of the models was evaluated with the coefficient of determination (R^2) and root mean square error (RMSE) of the model to assess its quality. The lower RMSE and closer R^2 are to a value of 1 means that the prediction and measured BMP values are correlated well. In order to compare the current models with previously reported examples, and then to analyse the degree of prediction success, the relative root mean square error (*r*RMSE) and the ratio of performance to deviation (RPD) were determined (Fitamo et al., **IV**).

2.4 Co-digestion of UOW in CSTR

The co-digestion of food and garden waste with mixed sludge from a WWTP was carried out in a continuously stirred tank reactor (CSTR) (Fitamo et al., **I**). The same experimental set up was used to analyse microbial population dynamics in respective co-digestion process conditions (Fitamo et al., **III**).

2.4.1 CSTR experimental set-up and monitoring

The co-digestion of UOW was performed in two CSTR reactors, each with a 7.5 L working volume, R1 and R2, in thermophilic process conditions. Both R1 and R2 were fed via an automated feeding system (a timer connected to the feeding pump). An automated stirring system, set at 150 rpm every 2-minute on/off interval, was used to continuously mix the reactor mixture throughout the experimental period. Reactor temperature was maintained at a constant by circulating hot water through the outer glass chamber of the reactors. The experimental set up is provided in Figure 1. Both reactors, R1 and R2, were equipped with an automatic stirring control unit, a liquid samples port, a temperature control unit and a gas sampling port. A liquid displacement gas metering system (Angelidaki et al., 1992) was used to measure the volume of gas produced on a daily basis, whilst gas samples and digestate were taken twice a week via a gas and a liquid sampling port to analyse the corresponding composition of biogas and the concentration of VFAs, ammonia and pH to monitor the co-digestion process. Biogas composition was analysed with TCD-GC, as described in Section **2.2.2**.

Figure 1. CSTR experimental set up used for the anaerobic co-digestion of urban organic waste (Fitamo et al. **I**, Supplementary Information).

The co-digestion of UOW in CSTR involved five phases spanning over a total experimental duration of 230 days to investigate the effect of co-substrate mixing ratios and hydraulic retention time (HRT) on reactor performance. In Phase I, the mono-digestion of sludge (primary and secondary sludge blended at 1:1 v/v%) was conducted to achieve baseline biogas production without the addition of co-substrates. During Phase II and up to Phase V, R1 and R2 were fed with different VS basis mixing co-substrate ratios (food and garden waste, grass clippings and sludge). The characteristics of the co-substrates and an overview of the process parameters are given in Table 1. The mixing ratio of the co-substrates was chosen by considering the current state of existing WWTPs, where the mono-digestion of sludge occurs in comparison to exploring the potential of sludge co-digestion and UOW to maximise biogas production with small changes in infrastructure to accommodate the new co-substrates. The amount of mixed sludge in the co-substrates was set at 10% of the total VS whereas the remaining 90% of the VS was food waste:green waste with a mixing ratio of 75:25 in R1 and 50:50 in R2.The green waste consisted a fixed ratio of 70% grass clippings and 30% garden waste on VS basis. According to the mixing conditions for co-substrates above, in Phase II up to Phase V, the corresponding mixing ratio of sludge, food waste, grass clippings and garden waste for R1 was 10:67.5:15.75:6.75, whereas for R2 it was 10:45:31.5:13.5 on a VS basis. This experimental plan helps compare the effect of sludge mono-digestion in Phase I relative to the effect of sludge co-digestion with UOW in Phase II up to Phase V in both R1 and R2. The composition of the co-substrate in the individual reactors R1 and R2 remained the same throughout the experiment, whereas HRT was changed to investigate the effect of retention time on reactor performance; during Phases I and II, III, IV and V the HRT was set at 30, 20, 15 and 10 days, respectively. The accumulation of soil and sediment build-up inside the reactor was quantified as 12% of the working volume, due to the content of particulate and soil in the co-substrates, particularly in grass clippings and garden waste. Consequently, HRT time was corrected, as seen in Table 1, based on the actual working volume and the average feed rate assuming a linear build-up of sediments inside the reactor. Additionally, a correction was made to the OLR, based on the measured TS/VS of the co-substrates and the actual daily feed pumping rate.

Table 1. Overview of the process parameters for the anaerobic co-digestion of food and green waste with mixed sludge in CSTR, the VS mixing ratio of substrates and the characteristics of the substrates. The standard deviation is given in brackets. OLR and HRT were computed in steady-state conditions, whereas TS and VS were analysed in duplicate (Fitamo et al., **I**).

Parameter	Unit	Phase 1 (days 0-74)		Phase 2 (days 75-130)		Phase 3 (days 131-164)		Phase 4 (days 165-204)		Phase 5 (days 205-230)	
		R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
HRT	Days	30	30	30	30	20	20	15	15	10	10
Temperature	°C	55	55	55	55	55	55	55	55	55	55
Corrected working volume	L	7.0	7.4	7.0	7.4	7.0	7.4	7.0	7.4	7.0	7.4
Feed	$L\ d^{-1}$	~0.250	~0.250	0.262	0.248	0.381	0.388	0.508	0.544	0.789	0.804
HRT *	D	~28	~29.60	26.72	29.84	18.37	19.07	13.78	13.60	8.9	9.2
OLR *	g VS $L^{-1}\ d^{-1}$	0.65 (0.03)	0.62 (0.04)	2.55 (0.21)	2.25 (0.15)	3.91 (0.08)	3.74 (0.30)	5.04 (0.11)	4.99 (0.26)	7.79 (0.28)	7.57 (0.39)
TS	% ww	2.85 (0.1)	2.85 (0.1)	7.97 (0.1)	8.19 (0.3)	8.33 (0.1)	8.52 (0.1)	7.94 (0.2)	8.21 (0.1)	7.89 (0.2)	7.93 (0.4)
VS	% ww	2.02 (0.1)	2.02 (0.1)	7.06 (0.1)	6.92 (0.2)	7.3 (0.1)	7.3 (0.1)	6.93 (0.1)	6.77 (0.1)	6.91 (0.1)	6.70 (0.3)
VS/TS	% TS	70.93 (4)	70.93 (4)	88.50 (2)	84.25 (4)	87.19 (2)	84.32 (2)	86.37 (3)	82.57 (2)	87.54 (3)	84.25 (6)
Mixed sludge	% VS	100.00	100.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Food waste	% VS	0	0	67.50	45.00	67.50	45.00	67.50	45.00	67.50	45.00
Grass clippings	% VS	0	0	15.75	31.50	15.75	31.50	15.75	31.50	15.75	31.50
Garden waste	% VS	0	0	6.75	13.50	6.75	13.50	6.75	13.50	6.75	13.50

The volume of sediments accumulated in the CSTR in the final phase of the reactor operation was found to be 0.9 L.
*: values based on corrected working volume, due to accumulations of soil, sand and sediments in a steady state.

2.4.2 Microbial population analysis in CSTR

Microbial composition and relative abundance variation, along with a change in feedstock composition and HRT time, is described in Fitamo et al., **III**. The reactor configuration and experimental set up are described in Section **2.4.1**.

2.4.2.1 DNA extraction and 16S rRNA gene sequencing

Samples for DNA extraction were taken from both R1 and R2 at each operational phase (Phase I to Phase V) when the co-digestion of UOW in CSTR reached a steady-state condition, as provided in Table 1. The samples were filtered with a 100 µm nylon cell strainer to remove residual plant particles. Subsequently, 1.5 g of cell pellets were obtained through centrifugation set at 10,000 rpm for 10 minutes. The extraction of total microbial DNA was performed with the PowerSoil® DNA Isolation Kit protocol (MO BIO Laboratories, Carlsbad, 170 CA), with slight modifications, to isolate and purify the DNA. The concentration of the extracted DNA was examined with a NanoDrop 2000 (ThermoFisher 172 Scientific, Waltham, MA), while quality was assessed with gel electrophoresis.

DNA sequences were obtained through the Illumina MiSeq platform at the Ramaciotti Centre for Gene Function Analysis, University of New South Wales (Sydney, Australia). The V4 hypervariable region of the 16S ribosomal gene RNA was amplified using 515f-806r primers and according to the Earth Microbiome Project (Earth Microbiome, 2011). The raw sequences were submitted to the National Centre for Biotechnology Information's (NCBI) sequence read archive database (SRP078424) under bio-project number (PRJNA328964). CLC Genomic Workbench Software (V.8.0.2), 181, equipped with a microbial genomics module plug-in, as presented in Kougias et al. (2016), was used to analyse the sequences. The alignment of the operational taxonomical unit (OTU) was carried out using MUSCLE software (Edgar, 2004). Computation of the Maximum Likelihood Phylogenetic tree and the alpha and beta diversity index were determined as reported in Kougias et al. (2016b). The number of sequence reads and total OTUs with respective taxonomic assignments were presented in Fitamo et al., **III**. OTUs with 10 or lower sequence reads were deemed to be extremely rare and excluded. Relative abundance was computed for each sample as a percentage of the total community. OTU classification was done according to the percentage of relative abundance – highly abundant (> 0.5% relative abundance) and lowly abundant (between 0.01% - 0.5% of relative abundance) OTUs – whereas OTUs lower than 0.01% were excluded. Variations in relative abundance were explained with heat maps, done using a Multiexperiment viewer (MeV 4.9.0) (Saeed et al., 2003).

2.4.2.2 Statistical analysis of microbial dynamics

Variations in the percentage of microbial relative abundance, due to changes in co-substrate composition (R1 and R2) and HRT (Phase I - Phase V), were computed using a general linear model (GLM Procedure, SAS Institute, 2009) in a series of single-train analyses, as described in Fitamo et al., **III**, based on duplicate samples obtained in each experimental condition – as shown in Table 1. The reactor and phase were included as effects while the abundance of each microbe was considered as a trait.

In addition, overall trends (linear, quadratic and cubic) of the microbial relative abundance variation with respect to change in retention times was analysed. Phase effect was included in the analysis as a linear, quadratic or cubic covariate, in order to explore microbial abundance trends. For each microbe, the most significant model describing the shape of variation was chosen (linear, quadratic or cubic; $P \leq 0.05$).

The correlation between the relative abundance of OTUs and biochemical parameters was also computed, using the GLM, to estimate the coefficient of linear regression. The relative abundance of each microorganism was treated as a linear covariate, whereas the reactors (R1 and R2) and phases (Phase I to V) were treated as fixed effects to analyse the correlation of abundance and the biochemical process parameter as a trait, by considering changes in all operation process parameters. Confounding and over parameterisation were avoided by running a number of models, each one based on the relative abundance of one microorganism and a single biochemical parameter.

2.5 Mathematical Modelling of UOW AD in CSTR

The mathematical bioconversion modelling of food and garden waste, grass clippings and sludge from WWTP co-digestion was conducted using BioModel, as reported by Angelidaki et al. (1999). The model results were validated with experimental results obtained during the co-digestion of UOW in CSTR, as presented in Section **2.4.1**.

2.5.1 Description of BioModel

BioModel can be used to simulate the bioconversion of various mono- and co-digestion substrates regardless of the substrate type, rather than based on the compositions of substrates such as carbohydrates, proteins and lipids. Simulation of the bioconversion of organic waste, using BioModel, is convenient particularly when the determination of COD is challenging. Figure 2 shows the material flow diagram of the BioModel bioconversion process, which is similar to the biochemical pathways of biogas productions discussed in **Section 1.2**. Stoichiometric calculations and corresponding yield coefficients, kinetics and inhibition equations for the built-in BioModel are presented in Angelidaki et al. (1999). The model predicts the amount of biogas produced and the operational process parameters based on the composition input materials insoluble carbohydrates, proteins and lipids. BioModel's biochemical process involves two enzymatic hydrolysis (insoluble carbohydrate and protein hydrolysis) and eight microbiological bioconversion stages (glucose degraders, aminoacids degraders, glycerol trioleate degraders, lipolytic bacteria, intermediate VFA products such as butyrate, valerate and propionate degraders, acetogens, degrading long chain fatty acids, and CH_4 producing acetoclastic methanogenic bacteria), as shown in Figure 2. Enzymatic hydrolysis processes are described along with first-order kinetic reactions, while all the microbiological processes are modelled with Monod-type kinetics. BioModel includes the inhibition of hydrolysis, acetogenesis (conversion of intermediate VFAs to acetate) and acetoclastic bacteria through the accumulation of VFA, acetate and ammonia, respectively; however, long chain fatty acid is assumed to inhibit all processes. Cell mass decay was considered as 5% of the maximum growth rate, while the cell mass synthesis was achieved by utilising ammonia-N as a nitrogen source.

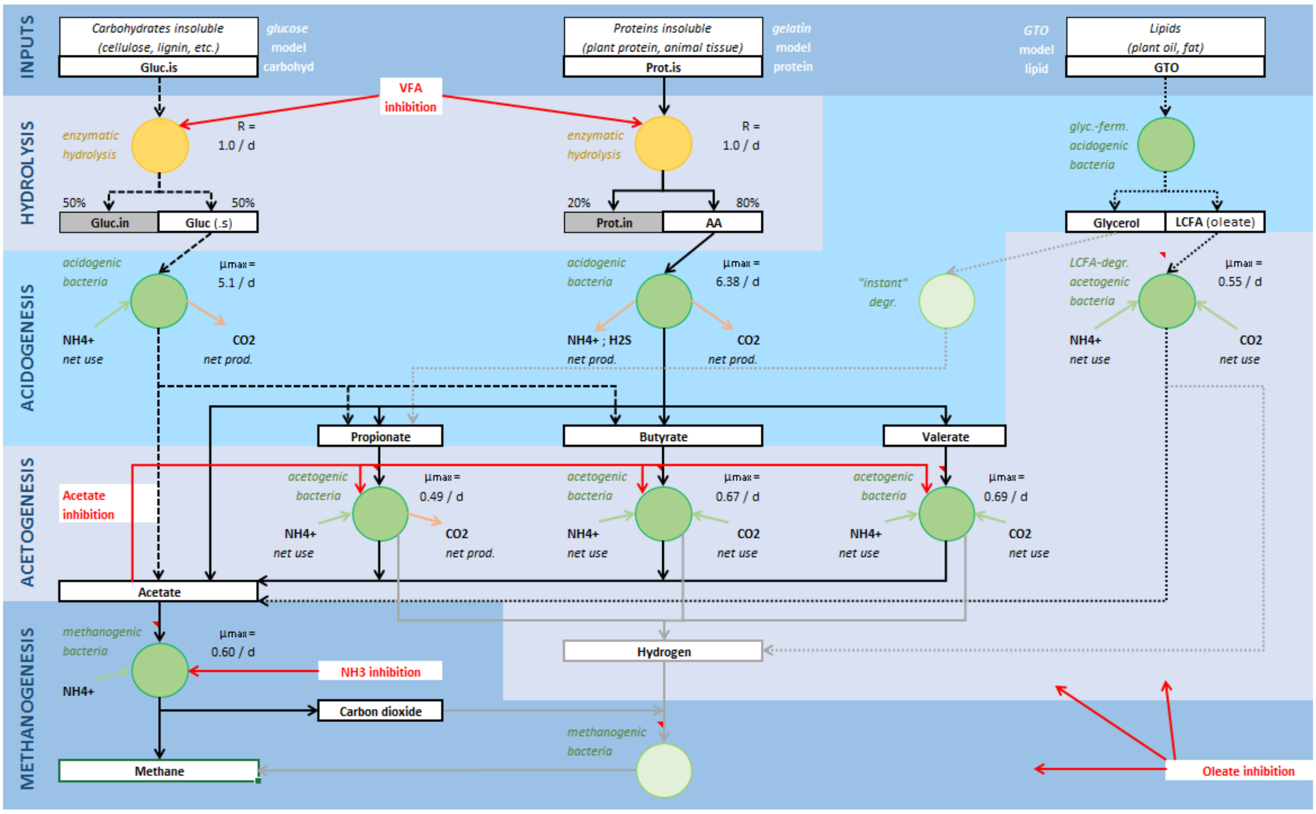

Figure 2. Biochemical pathways and material flow diagram for the bioconversion process of complex organic matter in the AD BioModel (DTU-Environment, Bioenergy research group achieve).

The simulation was performed using model parameters, the input characteristics of the substrates and inoculum. The physicochemical characteristics of the substrates were determined according to the methods described in **Section 2.1.2**, with the exception being the content of carbohydrate (computed based on the VS mass balance and the content of proteins, lipids and VFAs) provided in Fitamo et al., **II** (Table 1). The content of inorganic components and ions was estimated based the data for manure reported by Angelidaki et al. (1999). The concentration of ammonia was reported as total ammoniacal nitrogen (TAN), unless stated as free ammonia nitrogen (FAN). Stoichiometric yield coefficients remained the same as the previous BioModel, though kinetic parameters were slightly modified. The only changes were the half-saturation and inhibition constants for VFA degrading microbials, estimated through manual iteration until a favourable result was attained between the simulation and experimentally measured values, by considering sludge and co-substrates in terms of mono- and co-digestion. The input data for inoculum were obtained using the AD simulation of manure as a starting point and gradually feeding and running the reactors with sludge and co-substrates for a year to mimic actual process conditions, with the aim of acquiring stable and adapted microorganisms.

2.5.2 Simulation and co-digestion scenarios

The effects of various feedstock compositions of food waste, green waste and mixed sludge at different HRTs (30, 20, 15 and 10 days) on reactor performance and operational process parameters were investigated using BioModel, as described in **Section 2.5.1**. The AD simulation scenarios comprised: i) mono-digestion of single substrates ii) co-digestion of substrates with a fixed amount of sludge at 10% VS of the total feedstock, while the VS ratio for food and green waste varied, and iii) the co-digestion of substrates where the amount of sludge varied from 5, 10, 12.5 and 15% VS while the VS ratio for food and green waste was fixed at 75:25 and 50:50% VS. Table 2 shows the summary of the simulation scenarios published in Fitamo et al., **II** (Table 3).

Table 2. Overview of the urban organic waste mono- and co-digestion simulation scenarios in thermophilic conditions based on VS mixing ratio and hydraulic retention time (HRT) (Fitamo et al., **II)**.

Scenarios	ID	Mixing ratio (% VS)				HRT (days)	Temperature (°C)
		MS	FW	GC	GW		
I Single substrate	MS 100	100	0	0	0	30,20,15,10	Thermophilic
	FW 100	0	100	0	0		
	GW 100	0	0	70	30		
II Co-substrate Fixed amount of mixed sludge	FW 12.5	10	11.25	55.13	23.63	30,20,15,10	Thermophilic
	FW 25	10	22.50	47.25	20.25		
	FW 37.5	10	33.75	39.38	16.88		
	FW 50	10	45.00	31.50	13.50		
	FW 62.5	10	56.25	23.63	10.13		
	FW 75	10	67.50	15.75	6.75		
	FW 87.5	10	78.75	7.88	3.38		
III Co-substrates Fixed amount of co-substrates	MS 5/50	5	47.50	33.25	14.25	30,20,15,10	Thermophilic
	MS 10/50	10	45.00	31.50	13.50		
	MS 12.5/50	12.5	43.75	30.63	13.13		
	MS 15/50	15	42.50	29.75	12.75		
	MS 5/75	5	71.25	16.63	7.13		
	MS 10/75	10	67.50	15.75	6.75		
	MS 12.5/75	12.5	65.63	15.31	6.56		
	MS 15/75	15	63.75	14.88	6.38		

2.5.3 Identification of optimal UOW co-digestion

The BioModel simulation of the co-digestion process resulted in several process performance and operational model output variables. Identification of the optimal combination was done via a multi-objective optimisation problem with no best overall option. However, increasing the number of variables increases the complexity of the optimisation procedure, which in turn could result in improvements to some of the co-digestion options whilst others may worsen. The assumptions of the optimisation framework included: i) the only decision variable available for the operator is retention time t $t \in [t_0, t_f]$ and ii) dependence between the retention time and output variables is strictly monotonic and either increases or decreases. A detailed description is provided in Fitamo et al., **II**. The output variables were categorised into two groups: the first group was named "waste products" (to be minimised, such as the accumulation of VFA concentrations), and the second group was designated as "products" (to be maximised, such as specific methane yield). The maximisation and minimisation objective for several outputs creates conflicting interests, resulting in no globally optimal solution. Accordingly, this provides a decision domain of Pareto efficient solutions, which are optimal solutions with the corresponding multi-objective optimisation problem. The operator can choose from amongst any number of possible Pareto solutions, since the solutions are not preferable to each other and are uncertain. Constraints were added to reduce uncertainty and restrict the decision domain. Upper or lower bound constraints could be set depending on the objective of the optimisation, in order to exclude technically or economically unviable solutions. Decision makers could choose the optimal process condition based on the restricted Pareto efficient solution, which provides the best possible options.

3 Results and Discussion

The major outcomes and findings of this PhD research study are presented and discussed in this chapter. UOW characterisations are reported and described in Section **3.1** (Fitamo et al., **I**). The measured BMP and computational models, predicting the methane potential of UOW, are presented in Section **3.2** (Fitamo et al., **I** and **IV**). The co-digestion of UOW in CSTR and the corresponding analysis of microbial population dynamics and mathematical modelling of UOW co-digestion are reported and discussed in Section **3.3** (Fitamo et al., **I**, **II** and **III**).

3.1 Physicochemical characteristics

Table 3 shows the physicochemical characterisation of UOW, which formed the basis for the work on CSTR co-digestion experiments (Fitamo et al, **I**), microbial composition and abundance analysis (Fitamo et al, **III**) and the mathematical modelling (Fitamo et al, **II**) of the bioconversion of UOW in CSTR. TS, VS, total carbon, total nitrogen and lipids were determined in triplicate, while the rest of the parameters were analysed in duplicate. The waste fractions provided in Table 3 were also included, in order to develop the methane predicting PLS model (Fitamo et al., **IV**). Food waste and grass clippings had higher VS contents (% TS) compared to garden waste and sludge. This could be due to the relatively high content of recalcitrant components in sludge and inorganic material in garden waste (e.g. soil/sand particles) in comparison to food waste and grass clippings. The maximum content of fat and protein was obtained for food waste, which could be due to food products such as cheese, oil and meat. Food waste had a higher C/N ratio compared to mixed sludge (primary and secondary sludge). The high C/N ratio of food waste relative to mixed sludge was favourable to adjusting the C/N ratio of mixed substrates for the co-digestion of UOW in AD. The ammonia concentration of the input substrates was found to be insignificant, and the concentration of VFAs was less than 5% of wet weight (Table 3).

Table 3. Physicochemical characteristics of substrates used in the co-digestion of urban organic waste in a CSTR experiment. Standard deviation is provided in brackets (Fitamo et al. **I**).

Parameter	Unit	Food waste	Grass clippings	Garden waste	Primary sludge	Secondary sludge
DM	g/kg ww	160 (1.1)	211 (1.84)	369 (1.12)	38 (0.51)	17 (0.28)
VS	g/kg ww	149 (0.93)	184 (1.66)	249 (1.60)	28 (0.45)	12 (0.27)
VS	% DM	93.41 (0.13)	87.28 (0.11)	67.62 (0.37)	72.94 (0.22)	69.08 (0.49)
Carbon (total)	% DM	50 (10)	46 (9.2)	35 (7)	39 (7.8)	34 (6.8)
Nitrogen (total)	% DM	3.5 (0.7)	3.9 (0.78)	1.6 (0.32)	2.2 (0.44)	6 (1.2)
Lipids	g/kg ww	30.56 (2.14)	12.48 (0.87)	9.05 (0.63)	2.26 (0.16)	0.75 (0.05)
VFA (total)	g/kg ww	2.89 (0.002)	4.03 (0.97)	0.83 (0.04)	1.35 (0.03)	0.07 (0.004)
Alcohol	g/kg ww	5.3 (0.06)	1.07 (0.001)	0.02 (0.002)	0.024 (0.001)	0.002 (0.001)
TKN	g/kg ww	5.20 (0.38)	6.85 (0.31)	5.38 (0.23)	0.99 (0.05)	1.07 (0.17)
Protein	g/kg ww	44.80 (1,72)	35.20 (1.72)	23.60 (1.16)	4.93 (0.25)	8.14 (0.41)
NH_3-N	g/kg ww	0.56 (0.16)	0.54 (0.17)	0.43 (0.03)	0.13 (0.02)	0.20 (0.03)

3.2 Biochemical methane potential (BMP)

The methane potential of substrates given in Table 3, including co-substrates comprising the combination of respective single substrates, was determined experimentally in batch incubation experiments. The corresponding T-BMP of the substrates and mixed substrates was estimated based on the chemical composition of the substrates provided in Table 3. However, the BMP of additional UOW substrates was quantified to build the computational methane predicting model. Experimental BMP is reported in Section **3.2.1**, whereas the computational BMP predicting model is presented in Section **3.2.2**.

3.2.1 Experimental BMP of urban organic waste

The measured and theoretical BMP (mL CH_4/g VS) of single UOW substrates and mixed substrates is described in Fitamo et al., **I**. The cumulative methane yield reached a steady-state condition at day 15, when 80% of the methane was produced, though the incubation period lasted for 28 days. Complete degradation of the substrates' organic components, given in Table 3, was assumed to estimate the T-BMP. The ratio of actual BMP to theoretical BMP, multiplied by 100 %, is defined as biodegradability.

Food waste resulted in the highest BMP, whereas garden waste provided the lowest BMP with corresponding values of 579 and 160 mL CH_4/ g VS, respectively. Food waste showed better degradability and a higher BMP compared to the rest of the substrates, partly due to the high content of fats and proteins. The measured BMPs of garden waste, grass clippings and secondary sludge were significantly lower compared to the corresponding T-BMP (Fitamo et al., **I**), possibly due to the content of recalcitrant cell biomass in the secondary sludge and lignocellulosic components in plant materials. The measured methane potential values obtained in this study were comparable to values reported in the literature for food waste (500 – 700 mL CH_4/g VS), grass clippings and garden waste (160 – 390 mL CH_4/g VS) and primary sludge (up to 590 mL CH_4/g VS) (Chynoweth et al., 1993; Nallathambi Gunaseelan, 1997; Zhang et al., 2007). Browne and Murphy (2013) also reported BMP values between 467 and 529 mL CH_4/g VS for source-segregated food waste collected from a canteen. The BMPs of grass clipping and garden waste co-digestion at VS mixing ratios of 70:30, 50:50 and 30:70 were found to be 283, 249 and 201 mL CH_4/g VS, respectively (Fitamo et al., **I**). This indicates that BMP could provide important information regarding the effect of mixing ratios on the performance of co-digestion. When co-digesting grass and garden waste, a higher methane yield was achieved in line with an increasing amount of grass clippings compared to the amount of garden waste, probably due to higher soil and lignocellulosic woody material content in the garden waste in comparison to grass clippings (Fitamo et al., **I**). The BMP of co-substrates in R1 provided an enhanced specific methane yield compared to R2, possibly owing to the higher content of food waste in R1 rather than R2, as described in **Section 2.4.1**.

In addition to the investigation of substrate BMP considered for the co-digestion of UOW in CSTR, the methane potential of several substrates was determined to develop a dedicated methane potential BMP-predicting model (Fitamo et al. **IV**). The samples considered for continuous reactor operation mode were also included as reference data to build the prediction model. In total, 87 waste samples were used to develop the computational BMP prediction model. The UOW fractions were categorised into six classes: food waste, biopulp, fibre fraction, plant materials, industrial organic waste and mixed waste. The distribution of the VS (% TS) and measured BMP profile of the waste fractions are provided in Figure 3.

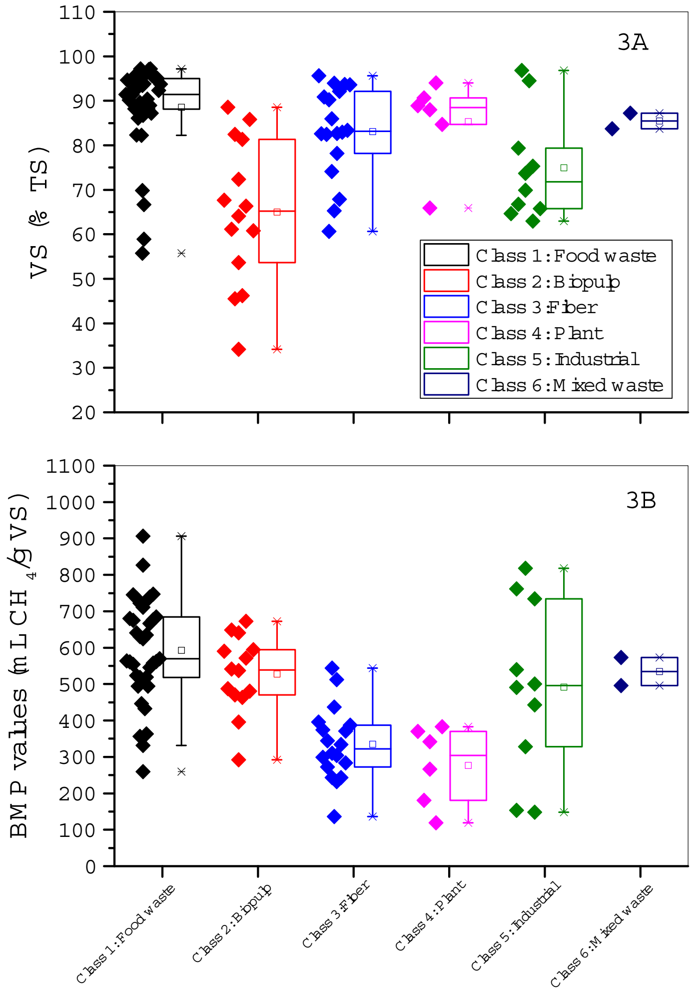

Figure 3. Biochemical methane potential and volatile solid content expressed as a percentage TS of urban organic waste fractions (Fitamo et al. **IV**).

Meat waste had the highest BMP (904 ± 49 mL CH_4/g VS), due to the high content of fat in meat products, whereas straw (119 ± 7 mL CH_4/g VS) used as animal bedding resulted in the lowest BMP, most likely due to the high content of lignocellulosic material. Food waste fractions can be classified as "avoidable" (food products that could have been edible but are thrown out as a waste) and "non-avoidable" food waste (non-edible parts of food products such as peel, egg shells and bones). The average BMP of the food waste fractions was calculated as 592 mL CH_4/g VS; however, the minimum methane yield was found to be 260 mL CH_4/g VS for unavoidable vegetable food waste. These results are comparable with the BMPs of fruit and vegetable solid waste (300 mL CH_4/g VS) reported by Gunaseelan (2004).

The BMP of biopulp fractions (a mixture of source-segregated organic waste (SSOW) and green waste, pre-treated using a pulping technique) resulted in higher yields when the amount of food waste in the biopulp was increased. The BMP of biopulp waste fractions composed of 100% SSOW resulted in 672 ± 75 mL CH_4/g VS, whereas biopulp composed of 75% green waste and 25% SSOW provided a BMP value of 292 ± 15 mL CH_4/g VS. The BMPs of fibre waste fractions, such as kitchen tissue contaminated with food waste, books and moulded fibre, were found to be 544 ± 6, 136 ± 25 and 232 ± 17 mL CH_4/g VS, respectively. Industrial waste fractions such as milk and a mix of dairy and meat product substrates had BMPs of 818 ± 24 mL CH_4/g VS and 762 ± 22 mL CH_4/g VS, respectively. Digested sludge before the final dewatering stage obtained from a WWTP provided a BMP of 148 ± 10 mL CH_4/g VS.

Figure 3A shows that the average VS content (% TS) of biopulps (65% TS) was lower in comparison to plant materials (83% TS) and fibre fractions (85%). Nevertheless, the average biopulp BMP (528 mL CH_4/g VS) was found to be higher compared to plant materials (277 mL CH_4/g VS) and fibre fractions (335 mL CH_4/g VS), as seen in Figure 3B. This indicates that the VS in biopulp was easily degradable in comparison to plant materials and fibre fractions.

3.2.2 Computation BMP prediction model

3.2.2.1 Local PLS model (BMP prediction of UOW)

A PLS model for predicting the BMPs of UOW, with and without pre-processing, was developed and is presented in Fitamo et al., **IV**. The R^2 and RMSE of the prediction set were found to be 0.82 and 61 mL CH_4/g VS, respectively, without spectral pre-processing (Fitamo et al., **IV**). The best prediction model, developed with transformed data using pre-processing technique of SNV, DT and SG first derivative with smoothing data points of 9, resulted in an R^2 and an RMSE of the prediction dataset with a corresponding value of 0.88 and 44 mL CH_4/g VS (Fitamo et al., **IV**). This shows that the model's performance improved following pre-processing the spectral data, which removes noise and background effects (SNV), baseline correction to remove tilting and offset (DT) and the removal of background noise whilst emphasising relevant chemical information (SG) (Agelet and Hurburgh, 2010). Based on data transformation, the numbers of PLS components obtained were in the range of 7-10. The combined data transformation provided lower PLS components. Figure 4 shows the BMP of measured and predicted calibration and prediction datasets. The model prediction error obtained in this study is comparable with previously reported values of 28 mL CH_4/g VS (Lesteur et al., 2011), 37 mL CH_4/g VS (Raju et al., 2011), 40 mL CH_4/g VS (Doublet et al., 2013) and 37 mL CH_4/g VS (Triolo et al., 2014) for a corresponding feedstock of MSW, meadow grass, combined MSW and agro-industrial waste and plant biomass. A comparison of model performance parameters for the present and previous studies is given in Table 4.

Table 4. Comparison of models predicting BMP using near-infrared reflectance spectroscopy (NIRS)

Substrates type	Pre-processing	Number of samples	Min	Max	Mean	R^2	$RMSE_p$	RPD
Meadow grass (Raju et al., 2011)	Mean normalisation	95	51	406	288	0.69	37	1.75
Municipal solid waste (Lesteur et al., 2011)	SNV + DT	74	23	400	234	0.76	28	2.36
Municipal solid waste and agro-industrial waste (Doublet et al., 2013)	SNV + DT+ SG (15, 2, 2)	243	0	1344	291	0.85	40	2.61
Plant biomasses (Triolo et al., 2014)	SNV + DT+ SG (11, 2, 2)	88	104	502	251	0.84	37	2.49
Urban organic waste (Current study, Fitamo et al. **IV**)	SNV + DT+ SG (9, 2, 1)	87	119	906	494	0.88	44	2.9
Combined urban organic waste and plant biomasses (Current study, Fitamo et al. **IV**)	SNV + DT+ SG (11, 2, 1)	175	104	906	372	0.89	50	2.98

The RPD and *r*RMSE of the best PLS prediction model were 2.9 and 9%, respectively (Fitamo et al., **IV**). The degree of prediction success is reported to be moderately successful if the RPD is in the range of 2.25 – 3.00, according to the model performance criteria set by Malley et al. (2005). This shows that the PLS model built for predicting the BMP of UOW is moderately successful. The model error of the best PLS model (9%) is comparable with model error values of 12.7% and 14.6% reported by Doublet et al. (2013) and Triolo et al. (2014), respectively. The repeatability standard deviation (RSD) of the reference method, determined as the ratio of standard deviation of triplicates (SDr) to the overall mean, resulted in a value of 6.2%, which corresponds to 30 mL CH_4/g VS. This shows the prediction model error, which is the sum of square error of reference method. In addition, the sum of square of the PLS model algorithm error is higher than the error of the reference method. The RSD is comparable to the value of 6.6% obtained by Triolo et al. (2014). Doublet et al. (2013) also reported an RSD of 19 mL CH_4/g VS for an entire dataset and 34 mL CH_4/g VS for samples with a BMP value of above 500 mL CH_4/g VS.

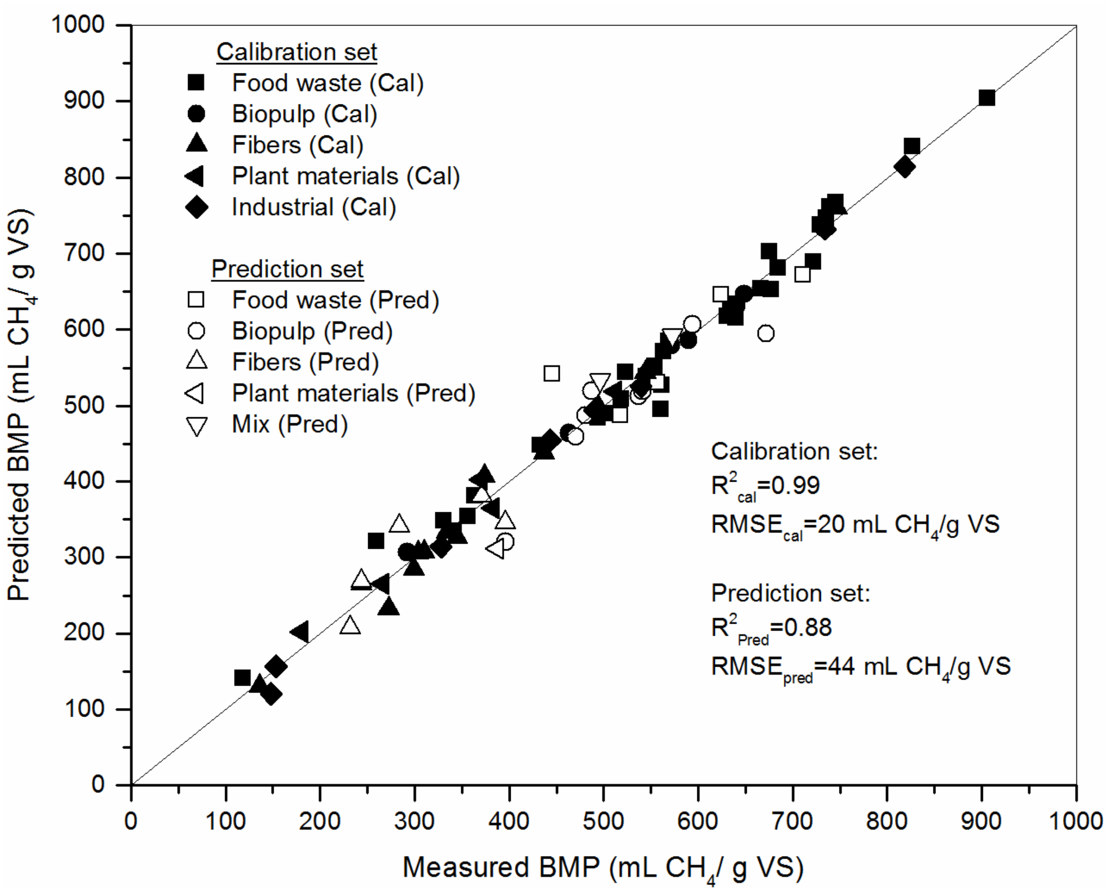

Figure 4. The measured and predicted biochemical methane potential values of the best PLS model developed for predicting the methane potential of urban organic waste (Fitamo et al. **IV**).

3.2.2.2 Combined PLS model (BMP prediction of UOW and plant biomass)

The computational PLS model for predicting the BMP of UOW and plant biomass, with and without transformed data, is reported in Fitamo et al., **IV**. The best prediction of the combined PLS model resulted in an R^2 and an RMSE of 0.89 and 50 mL CH_4/g VS for the prediction dataset (Fitamo et al.,

IV). The RMSE of the combined model is higher than the specific models built for the BMP prediction of UOW and plant biomass with a corresponding value of 44 and 37 mL CH_4/g VS. The performance of model prediction may be affected by the diversity of the samples, as presented in Doublet et al. (2013), and the reference method error (Ward, 2016). The measured and predicted BMP values were reported in Fitamo et al., **IV**. The RPD and rRMSE of the best PLS model for the BMP prediction of combined feedstock were 2.98 and 16.12%, whereas the RSD was 6.2%. The RPD of the combined PLS model improved slightly compared to the RPD of the specific models, but the predictive power of the model remained moderately successful. The specific models built for the BMP prediction of UOW and plant biomass provided lower model errors compared to the combined PLS model. The addition of more samples to the calibration dataset may decrease model errors.

3.2.2.3 Uncertainty of the BMP prediction model

Quantifying the BMP of organic waste is a complex biological process which involves microorganisms. The main source of this reference data uncertainty could be the variability and activity of inoculum, instrumental errors such as sampling and gas chromatographic analysis, human error or sample heterogeneity. The quality of the input reference data must be verified. Hence, a control (Avicel) with a theoretical BMP of 415 mL CH_4/g VS was used to examine the quality of the BMP data obtained during the batch test. Accordingly, the average BMP of the control was 392 ± 28 mL CH_4/g VS for eight batch BMP test set up performed in this study. This shows the results of the batch experiment was acceptable. The uncertainty of the PLS model predicting BMP arises mostly from the combined error of the reference method and the model algorithm. The prediction error is expected to be higher than the sum of the reference method and the model algorithm error. The estimation of BMP by means of elemental composition analysis tends to overestimate methane potential, while the chemical component based on BMP prediction is expensive and prone to uncertainty of analytical and human error due to analysis of several physicochemical parameters. Hence, NIRS-based PLS models for predicting the BMP of organic waste could be an alternative option. Furthermore, the uncertainty of the PLS model could be reduced if additional samples were added to the calibration dataset. However, the prediction results obtained in this study are satisfactory. The relevance of such a model is in its ability to support biogas plant operators, optimise stock and the bioconversion process and identify rapidly and slowly degradable substrates.

3.3 Co-digestion of urban organic waste

This section (**3.3.1**) presents the co-digestion of food and green waste with sludge from waste water in CSTR (Fitamo et al., **I**). During the co-digestion process, reactor broth was taken in a steady-state condition, to analyse microbial composition and study variations in relative abundance within each operational phase (Fitamo et al., **III**). The results are described in Section **3.3.2**. Finally, CSTR experimental data was used to validate the BioModel simulation output results (Fitamo et al., **II**), reported in Section **3.3.3**.

3.3.1 Co-digestion of urban organic waste in CSTR

The productivity and specific methane yield obtained during the co-digestion of food and green waste with sludge from a WWTP are provided in Figure 5. In both R1 and R2, methane productivity increased substantially when HRT was decreased. The addition of UOW co-substrates in Phase II improved methane productivity compared to the mono-digestion of sludge in Phase I. During Phase II, higher methane productivity (approximately 21%) was obtained in R1 in comparison to R2, due to the higher share of food waste in the mixed feedstock. In general, AD of co-substrates in R1 provided 10-20% higher methane productivity compared to R2. The content of methane was 70% in Phase I (AD of 100% sludge) but dropped considerably to 60% during the co-digestion of UOW in Phase II, as seen in Figure 6. However, in both R1 and R2, the content of methane in the biogas remained at approximately 60% during subsequent changes of HRT.

The co-digestion of substrates (in Phase II) resulted in a specific methane yield of 424 mL CH_4/g VS in R1 and 391 mL CH_4/g VS in R2 compared to the AD of only sludge (i.e. 287 mL CH_4/g VS). The results are comparable with previous studies. Jansen et al. (2004) reported a methane yield of 270 mL CH_4/g VS for the AD of WWTP sludge in Malmo. Co-digestion of sewage sludge with an organic fraction of municipal solid waste (75:25 v/v) in CSTR and UASB provided a methane yield in the range of 400- 600 mL CH_4/g VS, as reported by Sosnowski et al. (2003). In Phase II to Phase V, the yield in R1 was higher than R2 by 9, 13, 14 and 14%, respectively, with corresponding HRTs of 30 (Phase II), 20 (Phase III), 15 (Phase IV) and 10 days (Phase V). The results obtained in this study are comparable with previous co-digestion experiments on food and activated sludge (50:50) with a methane yield of 321 mL CH_4/g VS at an OLR of 2.43 g VS/(L·day) and

HRT of 13 days, as reported by Heo et al. (2004). The methane yield remained more or less constant at around 430 mL CH_4/g VS and 376 mL CH_4/g VS in R1 and R2, respectively, when HRT was decreased from 30 to 20 and then 15 days. Nevertheless, when HRT was changed to 10 days a significant drop in specific methane yield was observed in both reactors. This could be due to microbe washout and the overloading of the reactors. When HRT was changed from 15 (Phase IV) to 10 days (Phase V), the yield decreased from 430 to 359 mL CH_4/g VS in R1 (dropped by 17%) and 375 to 315 mL CH_4/g VS in R2 (dropped by 19%), respectively. The methane yield measured in the batch test and estimated through chemical compositions is comparable with the values obtained during co-digestion in CSTR (Fitamo et al., **I**). This could be due to indefinite biodegradation and nutrient supplement in batch testing compared to the continuous reactor mode experiment.

During the AD of co-substrates (Phase II), the concentration of total VFA and ammonia increased considerably compared to the AD of mixed sludge alone (Figure 6). However, the concentration of ammonia decreased slightly in Phases II to V, probably due to microbial adaptation to the newly added substrates and process conditions. The concentration of ammonia observed in R1 was higher by 4 -12% compared to R2, possibly due to the high share of food waste in the co-substrate of R1. In both R1 and R2, the concentration of total VFA and ammonia-N was, however, lower than the maximum inhibition limit of 3100 mg-N/L (Mata-Alvarez et al., 2000). The concentration of total VFA was similar to the acetate concentration in Phase I, since the amounts of butyrate, valerate and propionate were insignificant during the AD of sludge, as reported in Fitamo et al., **I**. However, the concentration of acetate increased during the AD of UOW (Phase II), and the concentration of propionate increased in subsequent phases. Overall, the concentration of valerate and butyrate remained insignificant during the AD of co-substrates in Phases II –V. Due to the higher fraction of food waste in the co-substrate composition, the VFA in R1 was higher compared to R2. The pH increased in Phase II compared to Phase I, but only very small changes were seen during Phases II-V, which could be due to the combined effect of the accumulation of VFA and ammonia.

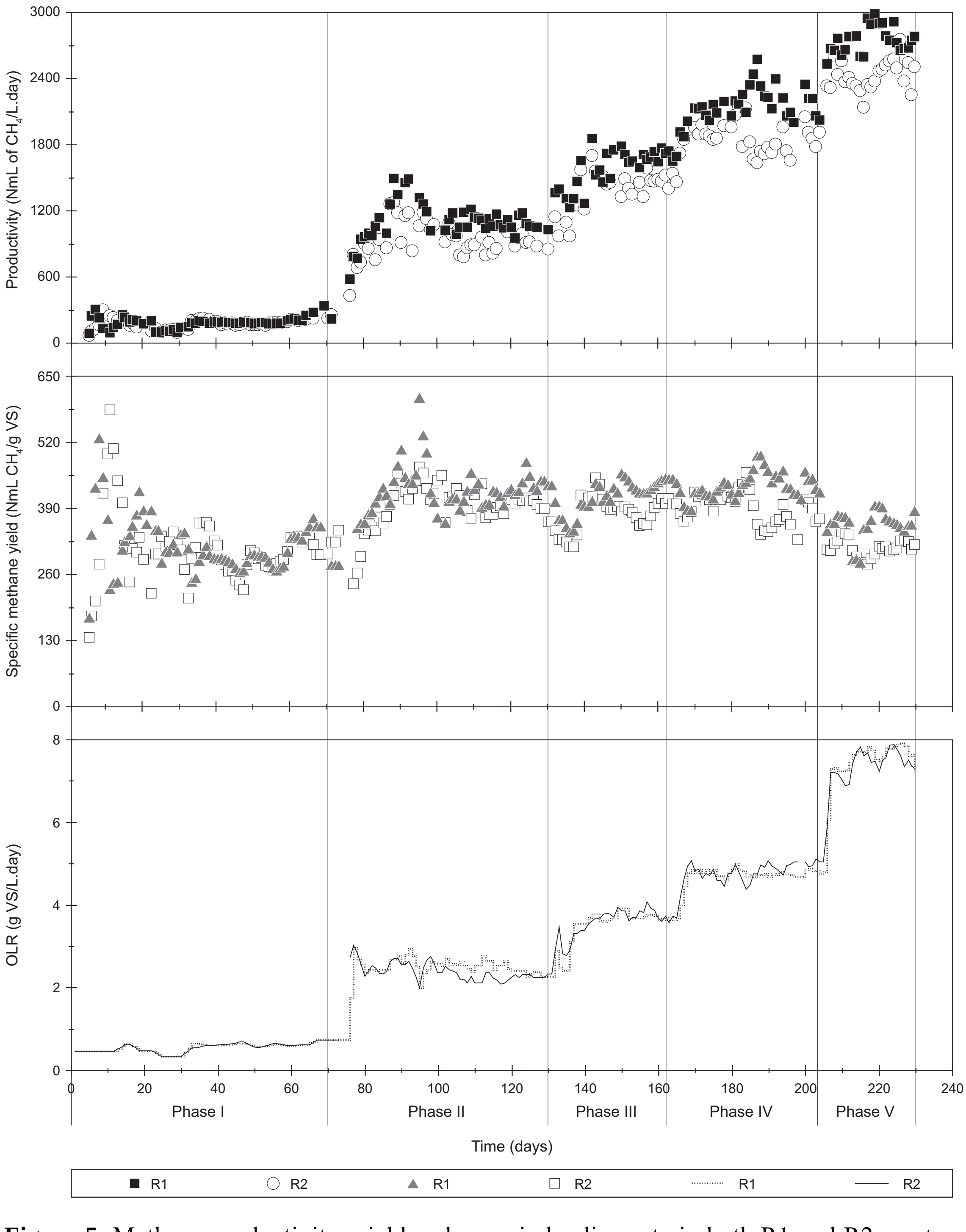

Figure 5. Methane productivity, yield and organic loading rate in both R1 and R2 reactors during the mono-digestion of sludge in Phase I (0 - 75 days) and co-digestion of food and green waste with sludge in Phase II (76-130 days), III (131-164 days), IV(165-203 days) and V (204-230 days) (Fitamo et al. **I**).

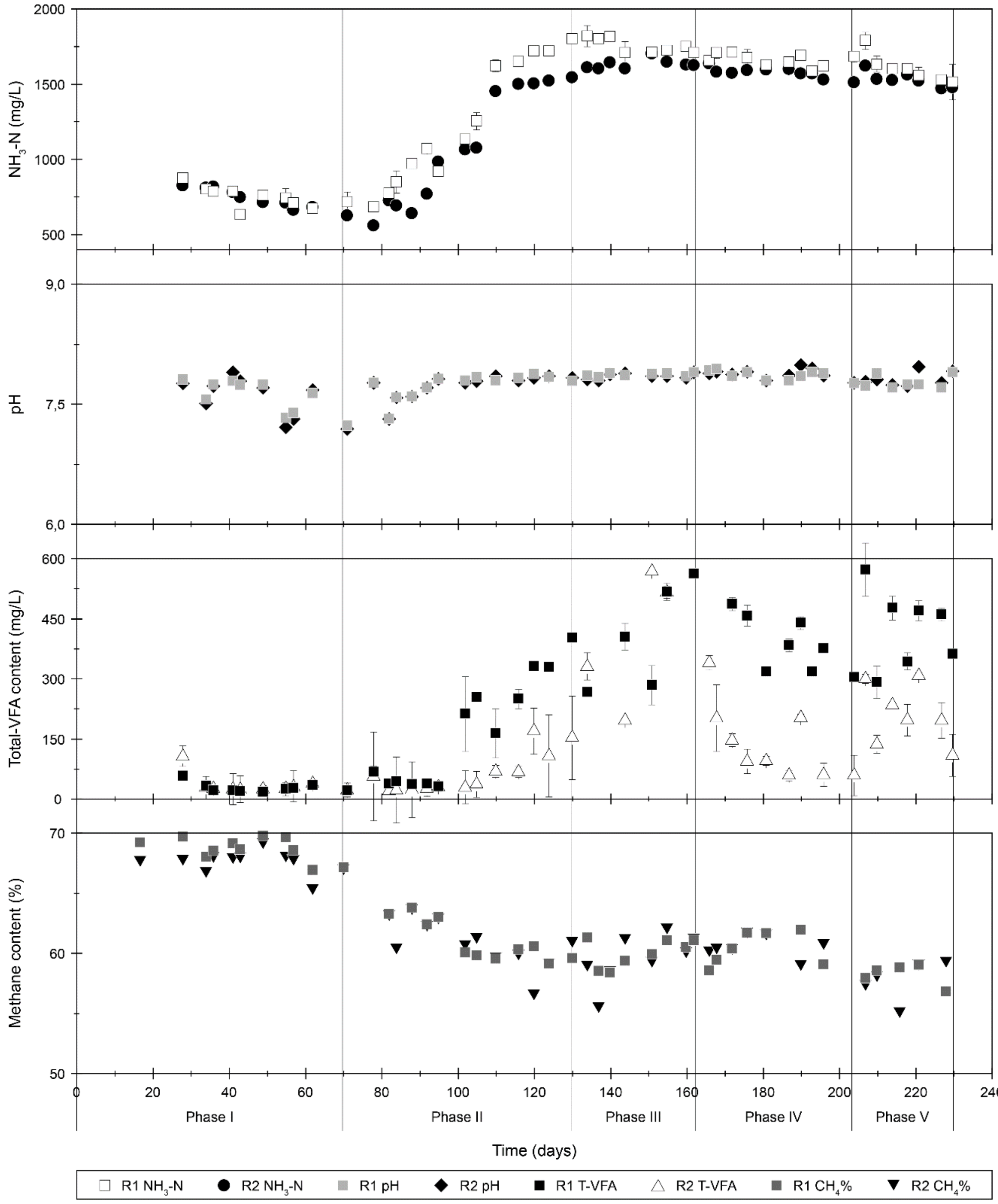

Figure 6. The concentration of ammonia, pH, total volatile fatty acid and the methane content in both R1 and R2 reactors during the mono-digestion of sludge in Phase I (0 - 75 days) and the co-digestion of food and green waste with sludge in Phases II (76-130 days), III (131-164 days), IV (165-203 days) and V (204-230 days) (Fitamo et al. **I**).

Figure **7** shows the trade-off between methane yield and productivity in a steady-state condition. Methane productivity increased when the co-substrates were introduced in Phase II and also increased with subsequent changes in HRT (30, 20, 15 and 10 days) in Phases II-V for both reactors (R1 and R2). On the contrary, methane yield increased between Phases I and II but remained nearly constant for R1, though it decreased slightly in R2 during Phases II-IV (HRT of 30, 20 and 15 days). Nevertheless, methane yield decreased considerably when HRT was changed from 15 to 10 days in both reactors, while methane productivity increased. Accordingly, optimum process conditions in terms of specific methane yield and methane productivity are achieved at a HRT of 15 days for both reactors, where process parameters such as ammonia, pH and VFA are also stable and below the maximum inhibition limits.

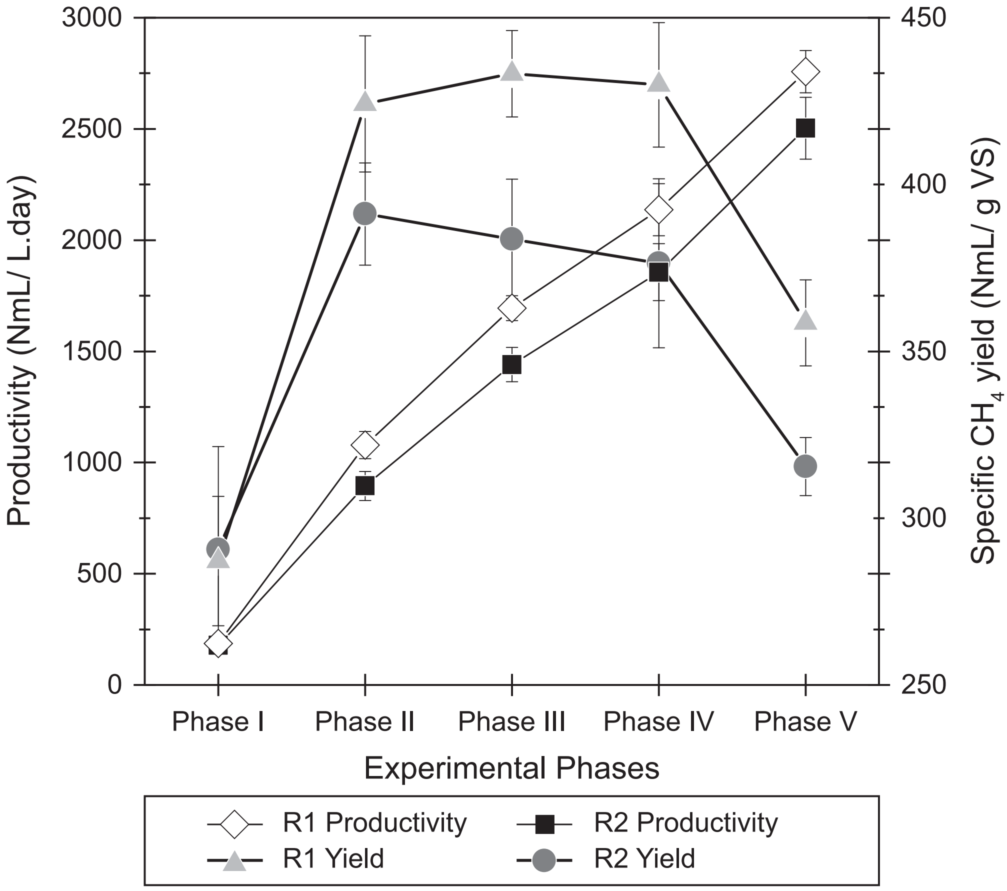

Figure 7. Specific methane yield versus methane productivity trade-off during the co-digestion of food and green waste with mixed sludge (Fitamo et al. **I**).

3.3.2 Microbial population dynamics of UOW co-digestion

In general, the main bacterial community observed at the phylum level in the CSTR reactors included *Firmicutes, Proteobacteria* and *Bacteroidetes, OP9, Synergistetes, Thermotogae, Dicyoglomi* and *Chrloroflexi* and the archaeal group of *Euryarchaeota*.

The similarities and differences between the microbial community for different Phases (I-V) during the AD of sludge and co-substrates in both reactors (R1 and R2) were evaluated using principal coordinate analysis, PCoA (Fitamo et al., **III**). A shift in microbial community diversity was observed when the feedstock was changed from the mono-digestion of sludge in Phase I to the AD of co-substrates in Phase II. Microbial community diversity decreased probably due to the increased content of lipids and proteins in the co-substrates, which may inhibit certain groups of microorganisms (De Francisci et al., 2015; Fotidis et al., 2013; Palatsi et al., 2010). In Phase II, R2 showed low microbial diversity compared to R1.

In both reactors, a shift in microbial community diversity during Phase III was observed compared to Phase II, which could possibly be due to the adaptation of microorganisms to the new process conditions and substrate. In Phases III and IV, R1 showed increased microbial diversity, but R2 developed a specialised community. In Phase V (HRT of 10 days), microbial diversity decreased in R1 (feed with more food waste), probably due to washout, but the microbial community in R2 was more resistant, which could be because of microbes adhering to the lignocellulosic materials from the green waste.

Figure 8 shows the percentage relative abundance of the microbial community in both reactors, R1 and R2, at the phylum and genus levels. *Proteobacteria* (11%) observed during AD of 100% sludge in Phase I became undetectable in Phase II (AD of co-substrates); however, a new community of *Bacteroidetes* (10.1%), absent in Phase I, was seen in Phase II. In both R1 and R2, the percentage relative abundance of *Thermotogae* increased in Phases II-V compared to Phase I. The role of *Thermotogae* bacteria is in the fermentation of organics into hydrogen, acetate and CO_2.

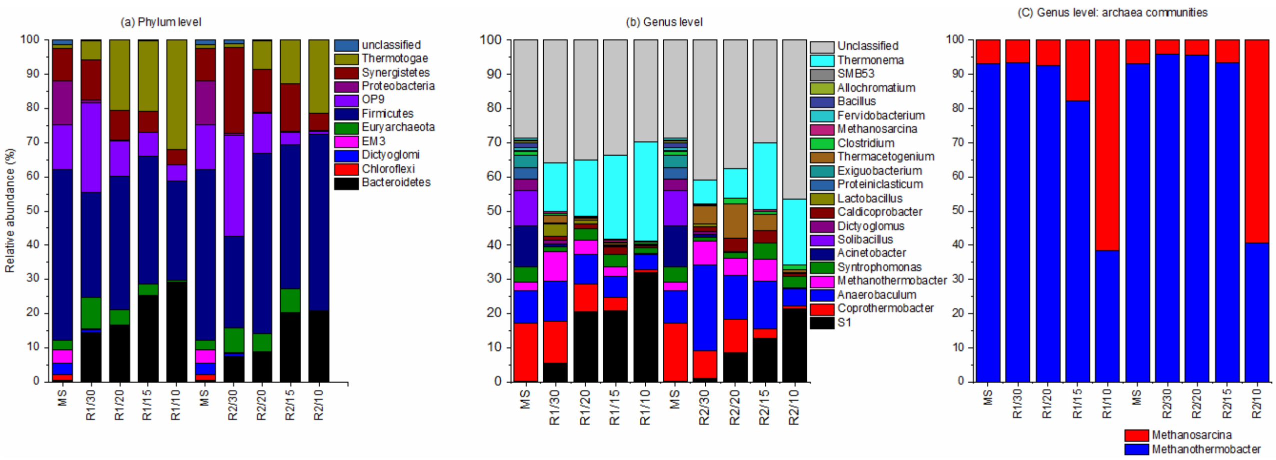

Figure 8. The variation in percentage relative abundance during the mono-digestion of sludge and the co-digestion of urban organic waste in both reactors (R1 and R2) in Phases I, II, III, IV and V based on a taxonomical classification of the microbial community: 8a) phylum level, 8b) at genus level (> 0.5% OTUs relative abundance) and 8c) community of archaeal at genus level (> 0.5% OTUs relative abundance).Unclassified represents all other unidentified OTUs (Fitamo et al. **III**).

The archaeal community found in both reactors involved in methane production included hydrogenotrophic *Methanothermobacter* and *Methanosarcina*. This indicated that archaeal community composition was similar regardless of the feedstock composition. When the AD of sludge in Phase I was changed to AD of co-substrates in Phase II, the relative abundance of *Euryarchaeota* increased from 2 to 9% in R1 and from 2 to 7% in R2. Conversely, the relative abundance of *Euryarchaeota* dropped from 3% to 0.5% (R1) and from 6% to 0.3% (R2) during the UOW co-digestion process when HRT was changed from 15 days (Phase IV) to 10 days (Phase V). At a HRT of 10 days, the abundance of *Methanothermobacter* decreased, but *Methanosarcina* increased. The acetoclastic and hydrogenotrophic *Methanosarcina* is favourable at elevated ammonia and VFA concentrations (Calli, 2005; De Vrieze et al., 2012; Staley et al., 2011).

The relative abundance of *S1* (*Thermotogales*, order) and *Thermonema* was found to be significantly higher during the AD of feedstock composition dominated by readily degradable food waste (R1) compared to the AD of feedstock composition composed of mostly slowly degradable lignocellulosic materials (R2) (Fitamo et al., **III**). However, the relative abundance of *Thermacetogenium*, *Anaerobaculum*, *Ruminococcaceae* (*Clostridia*), *Porphyromonadaceae (Bacteroidia)* and *Clostridium* in R2 (lignocellulosic feedstock) was significantly higher relative to R1 (readily degradable feedstock).

The relative abundance of *Coprothermobacter* and *Anaerobaculum* decreased when the HRTs were sequentially decreased (30, 20, 15 days), except for a HRT of 10 days. In contrast, the abundance of *S1* and *Thermonema* increased, as reported in Fitamo et al., **III**. The association of biochemical parameters with respective variations in the relative abundance of OTUs is described in Fitamo et al., **III**.

The correlation of the biochemical parameter with the relative abundance of OTUs showed that acetate, methane yield and methane productivity significantly increase ($P \leq 0.05$) when the relative abundance of *Proteobacteria* (*Acinetobacter iwoffii*, OTU: 532569; *Allochromatium*), *Firmicutes* (*Coprothermobacter*, *Syntrophomonas, clostridium*), *Thermotogae* (*S1*, *Fervidobacterium*, two OTUs) and Bacteroidetes (*Thermonema*) increase. This shows that the performance of the reactors was affected by microorganisms involved mainly in the hydrolysis stage. Acetate concentration was also significantly related ($P \leq 0.01$) to the relative abundance of *Methanosarcina*. Methane content was significantly associated ($P \leq 0.001$) with the relative abundance of *Chloroflexi*, class *Anaerolinea*, which was observed mainly during the AD of sludge in Phase I. The increase in ammonia concentration was associated with an increase in the relative abundance of *Syntrophomonas* (OUT 203894); however, the decrease in ammonia concentration was associated with *Anaerobaculum*. *Syntrophomonas* is known for reducing molecular N to ammonia (Sieber, 2010), while *Anaerobaculum* ferments organic acids (Menes and Muxí, 2002).

3.3.3 Mathematical modelling of UOW co-digestion

3.3.3.1 Validation of experimental results

Optimisation of the co-digestion of urban organic waste was carried out with the BioModel mathematical bioconversion model. The performance and operational parameters of the BioModel simulation result in comparisons to the reactor experiment is reported in Fitamo et al., **II**. The simulation outputs were in agreement with the experimental results, as described in Fitamo et al., **II**. Exceptions were transition periods of unstable operational processes on days 1-20, 85-95, 136-152, 166-188 and 205-219 in Phases I, II, III, IV and V, respectively, due to start-ups (sudden changes in feedstock composition and HRT/OLR). The deviations between the simulation and experimental results on these particular days could possibly be due to technical problems encountered during the start-up of each phase, such as tuning and calibrating the feeding pumps and gas measuring devices. Other possible reasons for the observed deviations could be due to the adaptation of the microorganisms to the new process conditions (lag phase) or a delay in growth response to changed biochemical conditions (memory effect of microorganisms). The simulation outputs reach steady-state conditions quickly compared to the actual process (Fitamo et al., **II)**. Overall, a good fit was observed for predicting yield and methane productivity in the long run, when the process reached steady-state conditions, typically after two HRTs (Fitamo et al., **II**). Experimentally measured methane productivity for R1 in Phase I (0.19 ± 0.01 L CH_4/L·day), Phase II (1.08 ± 0.06 L CH_4/L·day), Phase III (1.70 ± 0.06 L CH_4/L·day), Phase IV (2.14 ± 0.12 L CH_4/L·day) and Phase V (2.77 ± 0.1 L CH_4/L·day) was comparable with the corresponding simulation output of Phase I (0.2 L CH_4/L·day), Phase II (1.0 L CH_4/L·day), Phase III (1.5 L CH_4/L·day), Phase IV (1.9 L CH_4/L·day) and Phase IV (2.8 L CH_4/L·day). This indicates that simulated methane productivity was in agreement with the experiment, and the residual error of the model prediction was insignificant. Similarly, the model predicting methane productivity in R2 resulted I a good fit.

The increased concentration of ammonia, from 0.72 g-N/L (AD of sludge in Phase I) to 1.72 g-N/L (AD of co-substrates in Phase II), was in agreement with the predicted BioModel simulation output, resulting in an insignificant model residual error, as described in Fitamo et al., **II**. The rapid increase in ammonia concentration on day 75 could be due to the sudden change in feedstock and inoculum composition. VFA concentration was in agreement with the simulation output in Phases I, II and III for R1, but the model value deviated from that measured in Phase IV, which may be due to an increased loading rate and the memory effect. However, the results were comparable in steady-state conditions except for Phase V (10 days HRT), where the process was unstable due to microbial washout and overloading. Components of the VFA simulation output, compared to the experimental results, are described in Fitamo et al., **II**. The model estimates for the VFA components in R2 deviate from the experimental values, particularly in Phases III to V. This could possibly be due to reactor pipes being clogged with grass and garden waste in the effluent, which causes loss of digestate because of overpressure resulting in an explosion via the sample port. Such technical problems were noted on days 132, 147 and 197 in Phases III, IV and V, respectively. The content of methane in the produced biogas was predicted accurately for all phases in both reactors, as reported in Fitamo et al., **II**. Methane content dropped from 70% in Phase I to 60% in Phase II and then remained constant, with subsequent changes in HRT in Phases III, IV and V being predicted well by the simulation BioModel.

3.3.3.2 Simulation scenarios of UOW co-digestion

According to the experimental plan provided in Section **2.5.2** (Table 2), simulation results for UOW (mono- and co-substrate) at various VS mixing ratios of substrates with HRT of 30, 20, 15 and 10 days are reported in Fitamo et al., **II**. Food waste resulted in improved yield and productivity compared to sludge and garden waste during the mono-digestion of UOW in scenario I. The concentration of VFA and ammonia was also found to be higher in the AD of food waste compared to the others. In reality, mono-digestion of food waste or garden waste is not practical, due to an imbalance of process nutrients, low buffering capacity, pumping problems and a risk of overloading, which may create an unstable process and eventually lead to failure. For this reason, co-digestion seems attractive to overcome these challenges.

Both methane productivity and yield increased when increasing the share of food waste comparatively to garden waste in scenario II, in which the VS of mixed sludge was fixed. Productivity increased when decreasing HRT for a given waste composition. When HRT was decreased from 30 to 15 days, methane productivity increased twofold for waste composition FW87.5 (79% of the total VS was food waste), but methane yield was affected only slightly (Fitamo et al., **II**). At HRT of 15 days, the highest methane yield and productivity were obtained for waste composition FW87.5 with a corresponding value of 2 L CH_4/L·day and 402 mL CH_4 g/VS, but the waste composition with a low share of food waste in the feed (FW12.5) resulted in a yield and productivity of 1.5 L CH_4/L·day and 290 mL CH_4 g/VS, respectively (Fitamo et al., **II**). This indicated that the yield and productivity improved in line with an increased proportion of food waste in the feed. Methane content increased slightly in line with increased food waste composition in the feed, but it was not affected at all by changes in HRT, which could be due to stable pH (ionisation degree of CO_2 to bicarbonate and finally to biogas). Concentrations of ammonia and VFA increased with decreased HRT but increased with a high share of food in the waste composition. The results obtained in scenario 3 show that increasing the amount of sludge in the waste composition at a fixed VS ratio of food and green waste in the co-substrate results in insignificant changes in methane productivity and yield at a given HRT. The reason could be due to low VS content in the mixed sludge. Concentrations of ammonia and VFA increased slightly in line with increasing the mixed sludge share for a given HRT and also increased when HRT was decreased for a given feed composition.

3.3.3.3 Optimisation scenarios

Generally, methane productivity increased but the yield dropped with decreasing retention times. The concentration of ammonia and VFA may also increase at low HRTs, which may cause process instability. Hence, a trade-off between the parameters must be considered to identify the best and most feasible solution to maximise yield and productivity while minimising the inhibition parameters of ammonia and VFA concentration. Figure 9 shows an example of optimal scenarios aimed at maximising productivity and yield while minimising ammonia concentration with a lower bound limit constraint of methane yield and productivity with a value of 382 mL CH_4/g VS and 2405 mL CH_4/L·day, respectively, with 1410 mg/L of ammonia as an upper bound constraint limit.

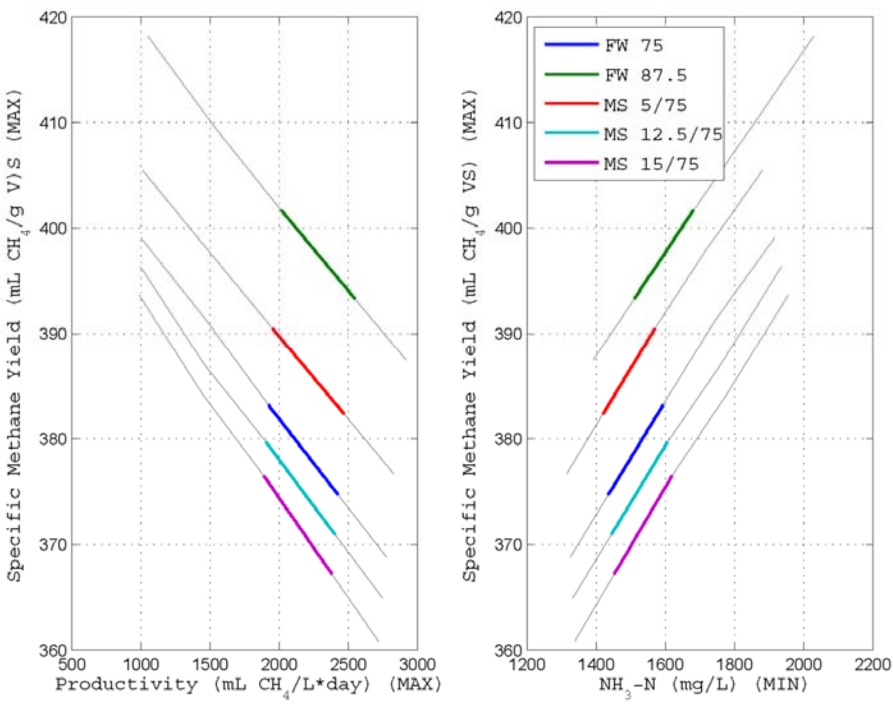

Figure 9. Identification of optimal urban organic waste co-digestion scenario with objective of methane productivity and yield maximisation and minimisation of ammonia concentration with lower bound constraint of methane productivity (> 2405 mL CH_4/L·day) and methane yield (> 382 mL CH_4/ g VS) and upper bound limit of 1410 mg/L of ammonia concentration at hydraulic retention time of 12 -15 days (Fitamo et al. **II**).

The optimal solution and process parameters are shown in Figure 9 for the objective of methane productivity and yield maximisation while minimising the concentration of ammonia. Methane productivity was inversely proportional to specific methane yield, as seen in Figure 9, whereas the concentration of ammonia was proportional to yield, but in this case the amount of ammonia was below the inhibition limit. The maximum methane productivity was 2557 mL CH_4/L·day for optimal feedstock composition of FW87.5 at HRT of 12 days however the maximum methane yield of 418 mL CH_4/g VS was obtained at HRT of 30 days (Fitamo et al., **II**). The operator or practitioner makes a decision based on retention time and optimal feedstock composition, to maximise yield and productivity while minimising the concentration of ammonia. For instance, improved productivity was obtained at a HRT of 12 days, where methane yield was lower compared to a HRT of 15 days and improved yield was attained but lower productivity for optimal feedstock compositions shown in Figure 9 between 12 and 15 HRT days. Hence, a trade-off should be considered to identify optimal feedstock composition at optimum process parameters to boost the recovery of energy while maintaining a stable process.

4 Conclusion

The focus of this PhD study was to develop and apply methods for the systematic quantification of biogas production from urban organic waste (UOW). This was done through the physicochemical characterisation of various waste fractions, assessing the biochemical methane potential (BMP) of organic waste, developing a computational BMP-predicting model and performing the co-digestion of organic waste in a continuously stirred tank reactor with corresponding microbial dynamics analysis and mathematical modelling of the co-digestion process, to identify optimal co-digestion scenarios. The major findings and conclusions of this PhD thesis are summarised below.

The addition of UOW to sewage sludge digesters enhanced both methane yield and productivity significantly. Specific methane yield improved by 48% and 35% when co-digesting sludge and food waste, grass clippings and garden waste, with a corresponding percentage VS ratio of 10:67.5:15.75:6.75 in R1 and 10:45:31.5:13.5 in R2, respectively, compared to the mono-digestion of mixed sludge in a continuously stirred tank reactor (CSTR). The differences in methane yield between R1 and R2 were attributed to the different shares of food/green waste in the feedstock. Methane yield remained constant as the hydraulic retention time (HRT) was subsequently decreased from 30, 20, 15 days, but it dropped considerably at a HRT of 10 days. The operation of digesters at low HRT (10 days) was found to be problematic, due to overloading and microbial washout.

Distinct differences in microbial community diversity were observed during the production of biogas from the co-digestion of UOW in comparison to the anaerobic digestion (AD) of mixed sewage sludge alone. The predominant microbial community of *Proteobacteria*, detected during the AD of 100 % mixed sludge, decreased considerably compared to the AD of UOW co-digestion. On the contrary, a new predominant community of *Thermonema* increased during the AD of UOW. The most prevalent pathway of methane formation was found to occur via syntrophic acetate oxidation, followed by hydrogenotrophic methanogenesis (*Methanothermobacter*). However, running the digesters at a HRT of 10 days, *Methanosarcina* became dominant. Methane productivity, methane yield and acetate concentration correlated with hydrolytic bacteria of *Proteobacteria*, *Thermotogae*, *Firmicutes* and *Bacteroidetes*, whereas the concentration of ammonia was associated with *Anaerobaculum* and *Syntrophomonas* and methane content was related to *Chloroflexi*. The relative abundance and diversity of microbes changed in line with the operational process parameters of biogas production, while the biochemical parameters of biogas production were correlated with the relative abundance of specific microbes.

The application of a comprehensive dynamic mathematical bioconversion model (BioModel), to simulate the anaerobic digestion (AD) of UOW, resulted in good correlation and reasonable predictions of reactor performances and operational parameters in steady-state conditions. Simulation scenario analysis revealed that an increasing the amount of mixed sludge in the co-substrate had only a marginal effect on methane productivity and yield. In contrast, reactor performance improved when the amount of UOW increased in the co-substrate at a given HRT. At a HRT of 12 days, the maximum methane productivity of 2557 mL CH_4/L·day was obtained for optimal feedstock composition with a VS mixing ratio of 10% mixed sludge, 79% food waste, 8% grass clippings and 3% garden waste, though specific methane yield was 393 mL CH_4/g VS. In contrast, a maximum specific methane yield of 418 mL CH_4/g VS was achieved at a HRT of 30 days, though methane productivity decreased by a factor of two. The trade-off between improved specific methane yield, methane productivity and stable operational process conditions should be considered when maximising biogas production with optimal process parameters and feedstock composition. The bioconversion model can be used to quantify biogas production, process control and monitoring during the biochemical transformation of sludge and the co-digestion of urban organic waste.

The best partial least square (PLS) model for predicting the BMP of UOW had a root mean square error of prediction ($RMSE_P$) of 44 mL CH_4/g VS, a coefficient of determination (R^2) of 0.88 and a ratio of performance to deviation (RPD) of 2.9, whereas the combined PLS model, comprising UOW and plant biomass, resulted in an $RMSE_P$ of 50 mL CH_4/g VS, an R^2 value of 0.89 and an RPD of 2.98. The model predicting the BMP of UOW had a relative root mean square error (rRMSE) of 9 % while the combined model had rRMSE of 16 %. The relative model error was slightly larger for the combined model due to variety of samples. The computational model for rapid analysis of the BMP of UOW, and a joint model based on near infrared reflectance spectroscopy (NIRS), was satisfactory with moderately successful prediction. Accordingly, future NIRS-based models for BMP measurements could replace traditional BMP measurements, thereby providing biogas plant operators with rapid decisions on improving biogas production, optimising feedstock management and identifying slowly degradable feedstocks prior to feeding them into the biogas digester.

5 Further Research

In this PhD thesis, the systematic quantification of biogas production from urban organic waste was presented and discussed, in order to enhance the performance of anaerobic digestion. However, based on the findings of the present study, the following further research work is suggested to improve the bioconversion of urban organic waste:

- The computational model used to predict the BMP of urban organic with near-infrared reflectance spectroscopy (NIRS) was moderately successful. Including additional samples in the model could improve its performance. Moreover, the model should be tested with an external dataset before industrial application. Hence, further study is recommended to perform external model validation.
- The interpretation of the NIRS spectral bands contributing to the prediction of the BMP model is complex, and it is hard to extract chemical information, due to overlapping overtones and combination bands. For this reason, further research is suggested to develop a computational model for predicting the BMP of organic waste, based on Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS).
- The addition of urban organic waste to mixed sludge improved biogas production. However, the experiment was carried out in a lab-scale continuous reactor operation mode. Hence, I recommend carrying out the co-digestion of urban organic waste in pilot-scale reactors before proceeding to a full-scale trial.
- A life cycle assessment (LCA) and a complete value chain analysis of the anaerobic co-digestion of urban organic waste at plant and national levels, taking into account waste collection, various pre-treatment techniques, bioconversion, several options of the utilisation of biogas and the application of digestate as an organic fertiliser on agricultural land are recommended before proceeding with a full-scale plant. This environmental impact assessment and economic optimisation will support the decision-making process for biogas companies, investors, politicians and consulting firms.

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The Department of Environmental Engineering (DTU Environment) conducts science based engineering research within six sections: Water Resources Engineering, Water Technology, Urban Water Systems, Residual Resource Engineering, Environmental Chemistry and Atmospheric Environment.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

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