International Masters of Science in Environmental Technology and Engineering

Master's dissertation submitted in partial fulfilment of the requirements for the joint degree of
EU Erasmus+ Master course organized by
University of Chemistry and Technology, Prague, the Czech Republic
IHE Delft Institute for Water Education, Delft, the Netherlands
Ghent University, Ghent, Belgium

Academic year 2020-2021

Process optimization of a dry, thermophilic anaerobic digestion plant treating household food waste in southern Belgium: A case study.

Ghent University, Ghent, Belgium

Sarah Moreno Sayavedra

Promotor: Erik Meers

Co-funded by the Erasmus+ Programme of the European Union

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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

UNESCO IHE DELFT

GHENT UNIVERSITY

ABSTRACT

Collaboration between the academic and industrial sectors for the optimization of the anaerobic digestion (AD) process can help accelerate the implementation of AD technology so it can approach its estimated energetic potential of ~14 000 TWh. Currently, there is much academic research on the effect of different parameters on the AD of the organic fraction of municipal solid waste (OFMSW), with the purpose of optimizing biogas production. However, there is little information confirming whether the reported optimal parameters are truly scalable to industrial plants. Therefore, this study aims to explore the technical feasibility of optimizing reactor performance in an existing AD plant via pilot-scale simulations of the full-scale process. To accomplish this, a dry, thermophilic plant treating OFMSW at 63 % of its designed capacity (at an SRT of 48 d) was chosen as case study. In the first pilot simulation, two pilot reactors were run to test the effect of the plant's OFMSW on reactor performance when the plant's target solids retention time (SRT) of 30 d was applied. One reactor was inoculated with digestate from the studied plant (TR-OF), the other with a good-quality thermophilic digestate (TR-MF). The simulation showed that ammonia toxicity was triggered under these conditions (with NH_3 levels between 1.44-1.59 g/kg). TR-OF performed poorly from the beginning, achieving only 37 % of the substrate's specific methane yield (SMY) (62.5 ± 7.2 Nm^3/t). TR-MF achieved 99% of the SMY (153.9 ± 17.7 Nm^3/ton) before inhibition, after which it began performing similarly to TR-OF. Out of the strategies tested to mitigate ammonia toxicity, feeding of hydrolysed OFMSW under mesophilic conditions at an SRT of 34 d was found to be the best option, given it achieved 76% of the SMY and improved process stability. This study demonstrated that pilot simulations are useful for anticipating issues that can arise in the full-scale process when new operational parameters are applied.

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AD	Anaerobic digestion	**HI-Val**	Isovaleric acid	**S-digestate**	NH3-stripped digestate
AR	Aqua regia	**HRT**	Hydraulic retention time	**SMY**	Specific methane yield
AM	Acetoclastic methanogen	**I:S**	Inoculum-substrate ratio	**SRT**	Solids retention time
BB-CCD	Box-Behnken central composite experimental design	**LL**	Landfill leachate	**SS**	Sewage sludge
BDI	Bottled fruit drinks industry	**LN-digestate**	Low nitrogen digestate	**ss-OFMSW**	Source-sorted OFMSW
BMP	Biochemical methane potential	**LFD**	Liquid fraction of digestate	**TAC**	Total inorganic carbon (in mg CaCO3/L)
C/N	Carbon-Nitrogen ratio	**MARS**	Multivariate adaptive regression splines	**TAN**	Total ammonia nitrogen
CHP	Combined heat and power	**MFW**	Mixed food waste	**TBD**	To be determined
CIR	Cocoa industry residue	**MR**	Pilot, mesophilic reactor inoculated with DMFA	**TBP**	Theoretical biogas potential
D1	Full-scale digester 1	**MR-HL**	Pilot, mesophilic reactor inoculated with DMFA and fed with hydrolysed OFMSW	**TKN**	Total Kjeldahl nitrogen
D2	Full-scale digester 2	**MS**	Mixed sludge	**TPAD**	Temperature phased anaerobic digestion
DMF	Digestate derived from manure, maize and food waste treatment	**ms-OFMSW**	Mechanically sorted OFMSW	**TR-MF**	Pilot, thermophilic reactor inoculated with DMF
DMFA	Digestate derived from manure and food and agricultural waste treatment	**MTPAD**	TPAD phased from mesophilic to thermophilic conditions	**TR-OF**	Pilot, thermophilic reactor inoculated with DOF
DOF	Digestate derived from OFMSW treatment	**NA**	Not applicable	**TS**	Total solids
FAN	Free ammonia nitrogen	**NS**	Not specified	**tVFA**	Total volatile fatty acids
FOS	VFA (in mg HAc/L)	**OFMSW**	Organic fraction of solid waste	**VFA**	Volatile fatty acid
FOS/TAC	VFA to alkaline buffer capacity ratio	**OLR**	Organic loading rate	**VS**	Volatile solids
FW	Food waste	**PCW**	Paper and cardboard waste	**WAS**	Waste activated sludge
GHG	Greenhouse gas	**PLC**	Programmable logic controller		
GW	Green/garden waste	**PM**	Pig manure		
HAc	Acetic acid	**PS**	Primary sludge		
HPr	Propionic acid	**PW**	Paper waste		
HBu	Butyric acid	**R&D**	Research and development		

CHAPTER 1. INTRODUCTION

1.1. Context

Anaerobic digestion (AD) is a biological process used for waste treatment that generates biogas and a stabilized waste residue (Deublein and Steinhauser, 2008). The residual waste fraction, called digestate, is more stable than the incoming waste and it is also rich in nutrients that can be recycled into crop cultivation in different ways (Pigoli et al., 2021). Biogas is generated via the anaerobic decomposition of volatile solids (VS) in the waste by different microorganisms, via 4 sequential steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Due to the methanogenesis step, the main components of biogas are methane (CH_4) and carbon dioxide (Deublein and Steinhauser, 2008). Methane provides biogas with a high energy content, which can be harnessed to produce heat and electricity (Clementson, 2007).

<u>1.1.1 Biogas potential</u>

Biogas production from organic wastes in AD systems can greatly contribute to the transition into a climate-neutral and circular economy in Europe, as well as to leapfrogging fossil energy sources in rural areas in developing economies (Terlouw et al., 2019; The World Biogas Association, 2019). In virtue of its high calorific value, biogas can be used instead of coal, oil, and natural gas for energy purposes, reducing fossil fuel emissions (Nevzorova and Kutcherov, 2019). Since it has the same energy flexibility as natural gas, such renewable fuel can also be used in sectors which are difficult to electrify, such as marine and heavy-duty transport. Biogas can also play an important role in complementing renewable electricity produced from intermittent sources (e.g., solar and wind) at times of low output (Kampman et al., 2016). The global AD energy potential (10 100-14 000 TWh) could, in fact, meet 6-9 % of the world's primary energy consumption (or 16-22 % of the electricity consumption). Even though there are currently millions of digesters operating globally (about 50 million micro-digesters, 132 000 small, medium and large- scale digesters and 700 upgrading plants), their energy generation (87 TWh) is only 1.6-2.2 % of the defined AD potential (The World Biogas Association, 2019). Therefore, there is still much room for growth in the AD sector in order to significantly contribute to regional transitions to sustainable energy.

A controlled AD process can also reduce CH_4 emissions from the decomposition of mismanaged organic waste. Anthropogenic emissions make up 51 % of total CH_4 emissions, contributing to climate change and production of tropospheric ozone. In response to this, the European Commission has put forward a new Methane Strategy to boost CH_4 emission reductions, as emissions from the agriculture and waste sectors have only fallen by a fifth and a third relative to 1990 levels (European Commission, 2020). In the EU, emissions from the waste sector are important to address given that bio-waste (i.e., food and garden waste) accounts for more than 34 % of the municipal solid waste (MSW) generated (van der Linden and Reichel, 2020). With AD, organic human and agricultural waste can be treated while recovering the methane for energy purposes (European Commission, 2020; Kampman et al., 2016).

Since AD can help decrease greenhouse gas (GHG) emissions in various sectors (energy and waste), it has the potential to reduce global GHG emissions by 3290 to 4360 Mt CO_2 eq. per year, which is equivalent to 10-13% of the world's current emissions (The World Biogas Association, 2019). Therefore, investment in research to optimize the AD of organic wastes for stable and maximized biogas production is worthwhile. AD technology provides the opportunity to mitigate GHG emissions and accelerate the energy transition via a fundamentally circular value-chain: food and agricultural wastes are treated while simultaneously being converted into energy and other added-value products that serve as nutrients to be reinjected into agriculture.

1.1.2 Barriers to the implementation of anaerobic digestion

Barriers to the wide-spread implementation of the AD process are related to technical, economic, market, institutional, socio-cultural and environmental factors. In developed economies, one of the main barriers to the wider implementation of biogas reported in scientific literature is of technical nature: the specific characteristics of biogas, namely its calorific value, and availability of biogas vehicles and filling stations (Nevzorova and Kutcherov, 2019). The calorific value is dependent on operating parameters (i.e., feedstock, temperature, retention time, total suspended solids and organic loading rate) that lead to different biogas compositions, influencing the final CH_4 content. Biogas composition can also be affected by undesirable impurities generated during the process (H_2S, NH_3, N_2, O_2, H_2) (Nevzorova and Kutcherov, 2019). Other points to be addressed are the management of the large volumes of digestate generated by centralized plants and the need for political support and clear policies to sustain the biogas industry (Nevzorova and Kutcherov, 2019; Stiles et al., 2018). Despite these issues, improving CH_4 production from AD remains of interest, given there is extensive infrastructure for natural gas already in place in Europe, which can be used for storage and transport of biogas when it has been upgraded to biomethane (European Biogas Association, 2019). In fact, between 2010-2015, there was a 50 % increase in the AD capacity in Europe (European Bioplastics, 2015). Still, AD is far from reaching its potential in the EU, having treated only ~900 kt of organic waste in 2015, while 86 Mt of bio-waste alone were produced in 2017 (European Bioplastics, 2015; van der Linden and Reichel, 2020).

Developing economies currently have a more barriers for AD implementation than developed ones (Nevzorova and Kutcherov, 2019). Regarding technical barriers, they lack the expertise to manage and operate biogas plants and households have insufficient resources (manure and water) to produce enough biogas to meet their needs. To overcome these barriers, there must be more research and development (R&D) to produce technologies that are better adapted to the living conditions in developing economies and also more economically accessible. There are also many political barriers related to lack of national ambitions for sustainable development, political instability and inefficient networks between institutions and other stakeholders. It is suggested that policymakers and entrepreneurs in developing economies could be inspired from experiences of developed economies with more mature biogas markets (Nevzorova and Kutcherov, 2019). In order to overcome barriers in both developed and developing economies, these authors highlighted the need for collaboration between all stakeholders (private sector, government, financial institutions, academia, lobbies, the media and local communities).

1.2. Literature review and state of the art: Anaerobic digestion of the organic fraction of municipal solid waste

The object of this section is to (i) review current trends in the AD of the organic fraction of municipal solid waste (OFMSW), (ii) discuss the different operational parameters recently studied to optimize biogas production from AD and provide an overview of the methods used to identify the optimum values of said operational parameters, (iii) compare optimal parameters proposed by lab and pilot scale studies to parameters currently applied in the full-scale and (iv) identify challenges and perspectives in the field of the AD of OFMSW.

1.2.1 Current trends in the anaerobic digestion of OFMSW

Anaerobic digestion has been a common waste treatment option since the 1970s energy crisis in western countries caused by geopolitical issues and their high dependence on oil imports for energy (Energy Information Administration, 1998; Franca and Bassin, 2020). Since then, different process conditions have been tested to treat a variety of wastes for improved biogas production. The dry AD of OFMSW has mainly been researched from the year 2007 (Franca and Bassin, 2020). Dry AD treats waste with high total solids (TS) content (>20 %), while wet AD treats substrates with a TS content of 15 % or less. Recent studies continue to use both conditions for their experimental setup, but dry AD is applied more frequently for OFMSW (see **Annex A.1-A.3**). This might be due to the advantages of dry over wet AD: i) reduced water footprint, with less wastewater generation, ii) simpler digestate management, iii) capacity of working at a higher concentration of volatile fatty acids (VFA) and total ammonia nitrogen (TAN) concentrations (since these inhibitors might be localised due to waste heterogeneity) and iv) higher methane production (2 to 10 times) for the same footprint, given the higher organic loading rates (OLRs) (Rocamora et al., 2020). However, various studies do not specify whether dry or wet conditions were employed (de Sousa et al., 2021; Mosquera et al., 2020; Nguyen et al., 2021).

Current research in the AD of OFMSW examines different operational parameters in the lab and pilot scales with the purpose of optimizing biogas production and quality (namely methane production). These optimization studies are mainly carried out for the dry mono-digestion of OFMSW, but some studies focus on optimizing parameters for its wet digestion (TS content) or co-digestion (type of co-substrate and ratio) (Ahmed et al., 2020; Mosquera et al., 2020; Pastor-Poquet et al., 2019; Shahbaz et al., 2020; Ziaee et al., 2021). The systematic review by Franca and Bassin, 2020 confirms that most AD studies deal with mono-digestion (65.3%), with only 33.5% of the papers dealing with co-digestion. Temperature-phased AD also figures into some optimization studies (Amodeo et al., 2021; Fernández-Rodríguez et al., 2016; Jiang et al., 2018; Kim et al., 2004; Nguyen et al., 2021), but single-stage AD studies still predominate (see **Annex A.1** and **A.2**). Strategies to deal with foaming, ammonia toxicity and other inhibitors in the AD of OFMSW are currently researched to a lesser extent (Liu et al., 2021; Moeller and Zehnsdorf, 2016; Zhu et al., 2021).

Authors in different countries are also delving into research on the feasibility of the AD of OFMSW in a specific city or state. Some authors explored the feasibility of installing small, decentralized plants in Europe and the USA and demonstrated that small-scale AD plants (~100 kW capacity) could treat OFMSW for mid-size towns at an acceptable cost (Chinellato et al., 2021). Along the same line, other authors found that the application of small-scale AD was more feasible than centralized AD in Brazil, in terms of investment and energy production (de Brito et al., 2021; de Sousa et al., 2021). The technical feasibility and energy potential form treating district- or region-specific OFMSW is also explored. Case-studies showed that the AD of OFMSW was a worthwhile investment in terms of its energy potential for both one district in Vietnam (201 GWh/y) and the whole of South Korea (4920 GWh/year) (Nguyen et al., 2017, 2021). Studies carried out in countries with developing economies also looked into synergies between landfilling and AD, with one study finding that capturing methane from landfills was more practical for the region than investing in AD systems (de Brito et al., 2021) and another finding that co-digestion of OFMSW and landfill leachate (LL) was technically and economically feasible (T.a.s et al., 2020).

1.2.2 Optimization of biogas production: Operational parameters and methods

The conditions and configuration of the AD process can be modified to optimize CH_4 production in an AD plant (Rocamora et al., 2020). There are advantages and disadvantages associated to modifications of these parameters, for example, they can lead to improved biogas production, but negatively impact system stability, or vice versa (Pastor-Poquet et al., 2019; Seruga et al., 2018; Shahbaz et al., 2020). Recent operational parameters investigated in literature regarding the optimization of biogas and CH_4 production in the AD of OFMSW, include: temperature, TS, co-substrate type and ratio, substrate pre-treatment and its conditions, process configuration and techniques to reduce inhibition. The most commonly tested parameters are temperature and co-substrate type (see **Annex A.3**)**.** Often, 2 or more operational parameters are investigated in the same study, to propose optimum values that can be applied together for the best biogas/CH_4 production (Ahmed et al., 2020; Mosquera et al., 2020; Ziaee et al., 2021). **Annex A.1** compiles recent studies carried out in the optimization of biogas and CH_4 production, summarizing the parameters tested and optimum conditions identified in the literature discussed below.

1.2.2.1 Temperature conditions

In order to optimize CH_4 production, some authors tested reactor performance under 3 different temperature ranges (20, 35 and 55°C), finding that the highest average CH_4 production was achieved under mesophilic conditions (Castellón-Zelaya and González-Martínez, 2021; Ossa-Arias and González-Martínez, 2021). Despite differences in inoculum (un-adapted or adapted to all temperature conditions), feeding mode (batch and semi-continuous) and OFMSW pre-treatment, both studies achieved a similar average CH_4 production (**Annex A.1**). Other authors compared process temperatures of 38, 40 and 55°C, with thermophilic conditions yielding the highest average CH_4 production (Basinas et al., 2021; Nguyen et al., 2017). However, average CH_4 production greatly differed between the studies (0.176 and 0.44 m^3/kg VS). Another study concluded that optimal CH_4 production (0.364 ± 0.025 m^3/kg VS) could be achieved under psychrophilic conditions (25°C) when testing AD at 23, 25, 35 and 40 °C (Sailer et al., 2021). These results show that optimal CH_4 production can be achieved under a variety of temperature conditions. The variations in CH_4 production might be due to differences in applied operational parameters or in substrate chemistry, so optimal temperature should be determined on a case-by-case basis.

The possibility of running the AD process at low operating temperatures, mimicking ambient conditions, was also explored. Generally, CH_4 production was much lower at 20°C than in mesophilic and thermophilic ranges (Castellón-Zelaya and González-Martínez, 2021; Ossa-Arias and González-Martínez, 2021). Nevertheless, other studies showed that it might remain feasible to operate the AD process at temperatures <35 °C. BMP tests for OFMSW with inoculum from digested sewage sludge (SS) showed that CH_4 content in biogas increased at lower operating temperatures (23 and 25 °C), and that a larger hydraulic retention time (HRT) could be applied for acceptable biogas generation (Sailer et al., 2021). Additionally, in India, it was possible to run full-scale air-lift reactors without temperature control (ambient temperature, 24-39 °C) for a 47-week period without significant changes in biogas production. A lower OLR was applied during the period of the lowest temperatures, which might have aided in maintaining a stable biogas production (T.a.s et al., 2020). Based on these results, it might be worthwhile to evaluate whether eliminating temperature control can translate into profits due to a reduced heat demand, especially in countries with high average temperature.

Other recent optimization studies that tested the influence of parameters other than temperature on biogas production from OFMSW, generally did so under mesophilic conditions (**Annexes A.1-A.3**). This is consistent with findings showing that between 2007-2018 most dry AD studies for OFMSW were carried out in mesophilic conditions (Franca and Bassin, 2020). Thus, mesophilic conditions remain the most common process temperature used for

AD studies, even though previous studies have shown that thermophilic conditions can provide higher gas production and other benefits (Rocamora et al., 2020).

1.2.2.2 Organic loading rate (OLR) and inoculum to substrate (I:S) ratio

Recent studies have continued to investigate the effect of OLR and I:S on CH_4 production under different conditions (e.g., temperature, waste pre-treatment and co-digestion. These parameters should be carefully chosen to maximize treatment capacity without compromising stable operation. The effect of OLR on reactor stability is monitored by measuring VFA concentrations in digestate (Ossa-Arias and González-Martínez, 2021). Reactor overloading can take place at high OLRs, causing an accumulation of VFAs that can lead to the acidification of the reactor, decreasing pH to levels toxic to methanogens or that trigger VFA toxicity (Deublein and Steinhauser, 2008; Alavi-Borazjani et al., 2020).

In semi-continuous experiments, the OLRs tested fell within the range of 2-8.62 kg VS/m^3.d. Optimal OLRs were generally found at the lower end of this range for reactors operated under mesophilic conditions (Nguyen et al., 2021; Ossa-Arias and González-Martínez, 2021; Shahbaz et al., 2020). However, Nguyen et al. (2017) found that, under thermophilic conditions, the OLR of 8.62 kg VS/m^3.d led to the highest CH_4 production. Ossa-Arias and González-Martínez (2021) also tested different OLRs under thermophilic conditions, but a maximum OLR of 1.0 kg VS/m^3.d was achieved without causing reactor instability. This was due to the fact that mesophilic inoculum was used to inoculate the thermophilic reactor, without providing a period for acclimation. To have more significant results about the effects of OLR on thermophilic AD, mesophilic inoculants should be adapted to thermophilic temperatures by small step increases in temperature (1°C) (Nguyen et al., 2017). Thermophilic inoculum could also be employed instead, if it is accessible. Results from previous studies support the fact that CH_4 production under thermophilic conditions is optimized with increasing OLRs (8.8-13 kg VS/m^3.d), for certain OFMSWs (Fdez.-Güelfo et al., 2011; Fernández-Rodríguez et al., 2014). This reveals that optimum OLRs larger than 8 kg VS/m^3.d (for maximized CH_4 production) are more likely to be achieved under thermophilic conditions than under mesophilic ones.

In recent studies, the effects of I:S ratios between 1:4-2:1 have been tested under different temperature and substrate conditions, TS content and configurations, generally having an optimal ratio of 2:1 (Castellón-Zelaya and González-Martínez, 2021; Mosquera et al., 2020; Ziaee et al., 2021). This ratio might offer a good starting point when carrying out batch optimization studies. OLR and I:S values applied when testing other operational parameters usually fall within the optimal ranges identified in previous literature (2.5-19 kg VS/m^3.d and 1:4 – 2.5, respectively) (Rocamora et al., 2020), with a few exceptions that used lower OLRs (Basinas et al., 2021; de Sousa et al., 2021).

1.2.2.3 Substrate characteristics

The theoretical biogas potential (TBP) and specific methane yield (SMY) of OFMSW has been extensively studied (Campuzano and González-Martínez, 2016; Nwokolo et al., 2020; Rocamora et al., 2020). TBP and SMY for the dry AD of OFMSW are reported to be in the range of 60-200 m^3 biogas/t of waste (Li et al., 2011) and 0.137-0.631 m^3 CH_4/ kg VS (Rocamora et al., 2020). Some reported OFMSW methane yields reach larger values than those reported for agricultural waste (0.013-0.331 m3/kg VS), green waste (0.049-0.48 m3/kg VS), cattle manure (157.0-395.0 m^3/kg VS) and pig manure (204-438.4 m^3/kg VS) (Nwokolo et al., 2020; Rocamora et al., 2020). The large range of values is due to differences in its composition from region to region, related to differences in eating habits, and also to variations of process temperature and type of sorting (Campuzano and González-Martínez, 2016;

Rocamora et al., 2020). For example, Seruga et al. (2020) demonstrated that at the industrial scale, source-sorted OFMSW (ss-OFMSW) resulted in a larger biogas production than mechanically sorted OFMSW (ms-OFMSW).

The actual CH_4 production of OFMSW could be much lower than its TBP and SMY due to the presence of inhibitors or other physicochemical characteristics (Rocamora et al., 2020). Different pre-treatment methods have been attempted by authors to improve OFMSW characteristics and optimize CH_4 production. The hydrolysis of OFMSW under alkali conditions (pH 8-13) or via silage improved CH_4 production, compared to the production from OFMSW that did not undergo pre-treatment. For the alkali pre-treatment, hydrolysis of OFMSW at pH 10 (neutralized before feeding to the digester), yielded optimal CH_4 production; however, cost assessments showed that hydrolysis at pH 9 was the most cost-efficient (Dasgupta and Chandel, 2020). OFMSW silage was performed by incubating the OFMSW at 20, 35 or 55°C for 15 days (with no inoculum). Ensiled OFMSW performed better than fresh OFMSW when the AD process temperature was 20 and 35°C. However, under thermophilic conditions and at high TS, fresh OFMSW performed better (Castellón-Zelaya and González-Martínez, 2021).

Mechanical pre-treatment can also improve the AD process (Rocamora et al., 2020). Basinas et al. (2021) selected for 2 different fine particle fractions by sieving: 3.5-15 mm and 15-24 mm. They found that the finer fraction led to an improvement in the rate and stability of CH_4 production (0.148 m3/kg VS), which was 1.3 times higher than production from the coarser fraction. However, OLR and TS were not the same between the experiments, while these parameters can also affect CH_4 production. Other forms of pre-treatment applied in the studies reviewed include thermal pre-treatment by incubation at 55°C for 2.5 h and sun-drying of the OFMSW to prevent microbial activity and further decomposition of the waste (Nguyen et al., 2021; Ziaee et al., 2021).

Another type of pre-treatment often tested by authors is dilution, to modify the total solids (TS) content of OFMSW. Various authors have tested the effect of TS content on CH_4 production in both wet and dry conditions. For wet AD, CH_4 production improved at a TS content of 8-9 % (Ahmed et al., 2020; Liu et al., 2021). When comparing between wet and dry conditions (10-30 %), wet conditions led to optimal CH_4 production, at TS of 10 and 15 % (Castellón-Zelaya and González-Martínez, 2021; Ziaee et al., 2021). Nevertheless, CH_4 production might not be the best indicator to use when choosing an optimal TS. Despite a high CH_4 production, a low TS content could imply the generation of low volumes of biogas and energy due lower possible OLRs and would generate large volumes of wastewater. The benefit of applying dry conditions is that the water footprint is reduced and larger amounts of waste can be treated at one time (Rocamora et al., 2020). In the case of TS, using reactor productivity (m^3 biogas/m^3 reactor.d) and methane content as indicators for optimization might be more interesting from an energy and waste treatment point of view.

1.2.2.3.1 Ammonia Toxicity

Regarding inhibitors, high protein feedstocks like OFMSW contain high levels of nitrogen that can lead to ammonia toxicity (Jiang et al., 2019; Kim et al., 2021). Common strategies to mitigate ammonia toxicity include acclimation, decreasing OLR (or increasing solids retention time [SRT]), adjusting C/N ratio between 27-32 and diluting the feed to reduce total ammonia nitrogen (TAN) concentration in the digestate (Jiang et al., 2019). Recent studies have investigated the effects of ammonia concentration in digestate and C/N ratio on the CH_4 production from OFMSW. The effect of process temperature, additives and illumination on ammonia toxicity and inhibition in the AD of OFMSW have been studied as well. Adjustment of C/N ratio is often related to co-digestion, and relevant literature will be discussed in the following section (**Section 2.2.4**).

An interesting approach tested to mitigate NH_3 toxicity is light intensity (Zhu et al., 2021). The number of photons received by a bioreactor per day per unit volume (μmol/L.d) was demonstrated to have an effect on CH_4 production from high-TAN (2-8 g/kg) synthetic medium under thermophilic conditions. The proposed mechanism for the apparent activation of methanogenesis by illumination is that light exposure activates CH_3-S-CoM reductase, an enzyme that catalyses the formation of CH_4 from its precursors (Olson et al., 1991). In turn, the enhanced bioactivity might lead to better NH_3 resistance. A photon number of 1.25E+4 μmol/L.d led to a markedly reduced inhibition of CH_4 production in comparison to non-illuminated reactors, both for different scales (250-1000 L) and under a wide range of TAN concentrations. CH_4 production was consistently 2-fold higher for illuminated AD. Additionally, it was also determined that retention time had an effect on CH_4 production, with an HRT of 20 d yielding better production than shorter HRTs (10 and 15 d) (Zhu et al., 2021). This is consistent with the fact that an increasing retention time has been shown to have a mitigating effect on ammonia toxicity. Giménez-Lorang et al. (2021) also recently showed that ammonia toxicity decreased with increasing SRT, via a simplified dynamic model of their AD process. The model was built on the AD of the liquid fraction of digestate derived from OFMSW, containing 3.6 ± 1.0 g NH_4^+-N/L.

Puig-Castellví et al. (2020) studied the effect of ammonium loading (as NH_4Cl) on the acclimation of mesophilic reactors (fed with industrial food waste [FW]) to a final NH_3 concentration of 183 mg/L. They concluded that higher NH_3 loading rates resulted in lowered biogas production and in VFA accumulation, leading to a decline in acetoclastic methanogens (AMs) in digestate and favouring the predominance of hydrogenotrophic methanogens and acetate-consuming microorganisms. Lower NH_3 loading rates allowed for better acclimation as the reactor showed a faster recovery in terms of biogas production and VFA content when the targeted ammonia concentration was reached and maintained. Increasing NH_3 concentration correlated with increasing VFA concentration and decreasing biogas production, but had no effect on the CH_4 content in biogas.

The use of additives for ammonia toxicity mitigation has also been explored. Biochar amendments (10 g/L), made from mixed food waste (MFW) or pinewood can reduce TAN concentration in a thermophilic rector fed with a co-substrate mixture of mock high-N FW (chicken) and waste activated sludge (WAS) (Leininger and Ren, 2021). The unamended co-digestion of FW and WAS had an average TAN of 527 mg/L, while the AD amended with biochar derived from MFW and pinewood had an average TAN of 472 and 461 mg/L, respectively. MFW biochar proved to be a superior amendment to other sources of biochar (pinewood, cattle bone and wood), allowing for a higher CH_4 production and a shorter lag phase. This opens more opportunities for the circular use of food waste. Prussian blue analogue nanoparticles (PNPs) [$K_2ZnFe(CN_6)_6$] have also been tested as additives to reduce NH_3 concentration in AD via BMP tests with high-ammonia rural household waste (Liu et al., 2021). PNPs showed a high adsorption capacity for TAN between pH 6-9 (71.09 mg/g), reducing its concentration down to 1.7 g/L, compared to the 2.7 g/L concentration in the unamended control.

Giwa et al. (2021) tested the effects of reactor configuration (single vs. 2-stage) on ammonia concentration in digestate and on biogas production from the mesophilic co-digestion of household food waste and blackwater. TAN was maintained at lower levels in each reactor of the 2-stage system (900 ± 2.5 mg/L in the first stage of 600 mL and 415 ± 2.1 mg/L in the second stage of 200 mL), compared to the single stage system (1100 ± 5.7 mg/L of 800 mL). Daily biogas volume was also higher in the 2-stage system, but methane content was not reported. As part of their biogas production optimization experiment, Nikulina et al. (2021) also monitored the effects of pH and ammonia on reactor stability and performance.

They concluded that keeping the pH in the optimal range of 6.5-8.0 was important for reactor start-up, with high N substrates (like chicken manure) having a positive effect on digestates with low pH under mesophilic conditions during start-up. Finally, Yirong et al. (2017) studied the effect of temperature (37 and 55 °C) on ammonia inhibition. Their results show that under high-N conditions, optimal CH_4 production is attained under mesophilic conditions (0.460 m^3/kg VS, or 97% of SMY), rather than thermophilic ones. Mesophilic digestion did not show signs of inhibition up to the steady-state TAN of 4.5 g/L, with no VFA accumulation (<0.2 g VFA/L). On the contrary, thermophilic reactors fed with high-N FW, or urea, accumulated VFAs from a TAN concentration >2.5 g/L, and eventually failed at a TAN concentration >5.0 g/L. After 383 days of reactor operation, acclimation to high TAN content was not observed.

1.2.2.4 Co-digestion

Co-digestion has mostly been researched to explore possible synergies between locally available wastes for enhancing CH_4 production and process stability (i.e., ammonia toxicity mitigation), or for proposing new waste management strategies. Co-digestion of OFMSW with high-C waste, such as sawdust, paper waste (PW) and residues from the cocoa and bottled fruit drinks industries (CIR and BDI, respectively), has been recently tested to adjust the C/N ratio of OFMSW. Co-digestion with sawdust led to increases in TS content in digestate due to its low degradability, but resulted in improved CH_4 production at ratios of 1:4 and 1:2 (OFMSW/sawdust) at C/N of 25 and 30 (respectively), compared to the mono-digestion of OFMSW at C/N 20 (Ziaee et al., 2021). Co-digestion of restaurant FW with PW was demonstrated to perform better than co-digestion with cardboard or tissue waste. Ammonia accumulation was observed for all substrates, but only led to a significant pH decrease in the co-digestion of cardboard and tissue waste (due to VFA accumulation). At higher OLRs (3 and 5 kg VS/m^3.d), PW showed lower CH_4 production even though TAN concentration was lower. This indicated that at these OLRs there were insufficient hydrolysis and nutrients for microbial growth (Shahbaz et al., 2020).

CIR and BDI were tested for co-digestion with OFMSW by Mosquera et al. (2020) given these wastes are readily available in Colombia. They tested the effect of C/N ratio (25, 35 and 45) on CH_4 production under mesophilic conditions using SS and pig manure as N sources. Modelling with Box-Behnken central composite experimental design (BB-CCD) multivariate adaptive regression splines (MARS) showed that the highest maximized CH_4 production (0.382 and 0.350 m^3/kg VS, respectively) was obtained for a mixture of OFMSW and BDI, with SS as N source at a C/N of 40. Ahmed et al. (2020) also tested SS as a potential co-substrate for the AD of OFMSW. They evaluated the differences in biogas production when using primary sludge (PS), waste activated sludge (WAS) and a mixture of both (MS) (in a 1:1 ratio). Under mesophilic, batch conditions, co-digestion with MS resulted in higher biogas yields when compared to the mono-digestion of OFMSW (19 % TS) and to the wet co-digestion with PS or WAS (at 1:1 ratio, <15% TS). The optimal co-substrate ratio was determined to be 80:20 (OFMSW:MS) at a TS of 9%, yielding 0.967 m^3 CH_4/kg VS.

1.2.3 Operational parameters in full-scale OFMSW AD plants

Parameters applied in pilot-scale proof of concept studies and full-scale plants, as reported in scientific literature, are compiled in **Annex A.2**. Comparing these parameters to optimal conditions identified in AD optimization studies, it can be concluded that optimal values proposed in recent studies are not largely applied at the moment in full-scale plants (see **Annex A.3**). However, for the AD of OFMSW and FW, only 8 studies performed with large scale reactors (pilot and industrial, >5m^3 capacity) could be identified by this survey (**Annex**

A.2). Therefore, it is difficult to make accurate conclusions from such a small sample size. From the available information, it is more common to find full-scale plants working in the mesophilic temperature range (Gimenez-Lorang et al., 2021; Moeller & Görsch et al., 2015; T.A.S. et al., 2020). Most optimal temperatures found in recent literature fall within the mesophilic range as well, but there are also certain cases where thermophilic temperatures, or even psychrophilic (25°C) have been reported. In terms of HRT, full-scale plants operate between 26-31 d, while optimal values are <26 or >54 d (based on OLR or TS loading). Additionally, when determining optimal values for parameters other than OLR and HRT, lab-scale experiments have often applied very long HRTs, thus these values might not result in optimal biogas production when applied using a shorter HRT.

There are systems available in the market that allow for both continuous (DRANCO, VALORGA, KOMPOGAS, LARAN) and batch (BEKON, BIOFerm, SEBAC) operation in the full-scale (Franca and Bassin, 2020; Rocamora et al., 2020). Still, more than half of the optimization studies were carried out in batch mode. This might be due to the fact that batch operation remains the most used operation mode in industrial bioprocesses due to its simplicity and stability (Croughan et al., 2015). However, all 8 studies on pilot and full-scale AD reported that the reactors were operated (semi)continuously and an equal number of optimization studies determined the effect of OLR and I/S on biogas production.

While many optimization studies dealt with co-digestion, only 3 large-scale plants reviewed in this study employed co-digestion (Moeller and Görsch, 2015; Pigoli et al., 2021; T.a.s et al., 2020). Few optimization studies, and full-scale plants focused on the effects of C/N ratio on reactor stability and CH_4 production. Reported optimal C/N ratios differed in the full-scale (T.a.s et al., 2020) and in the lab scale (Mosquera et al., 2020). Regarding TS of the feedstock, optimal values have been mostly explored in the wet spectrum (≤15 %). However, most large-scale studies report feed TS to be 21 % or higher, or do not specify the TS of the feedstock; and even feedstock used for lab-sale studies is usually >20 %. Finally, no additives or illumination was reported to be provided in the pilot and full-scale studies and a common pre-treatment technique in these scales, also identified as optimal by one optimization study (Giwa et al., 2021), is hydrolysis using a 2-stage reactor system. All the conclusions made in this section were drawn from the comparison between optimal and applied values for different parameters carried out in **Annex A.3**, based on the reviewed literature compiled in **Annexes A.1** and **A.2**.

<u>1.2.4 Challenges and Opportunities</u>

Results from the reviewed literature show that maximized CH_4 production values, as well as the conditions under which these values are reached (pH, TS, OLR, I:S, temperature), mostly differ from study to study, which is expected given the differences in applied parameters and substrates employed in the studies (mode of operation, HRT, etc.) (**Annex A.1-A.2**). From the systematic comparison of the literature reviewed (**Annex A.1-A.3**), it can be concluded that optimal values are highly dependent on OFMSW characteristics (Seruga et al., 2020; Yirong et al., 2017), the cumulative effect of different operational parameters on biogas production and system stability (e.g. pH, TS load, OLR, I:S), and choice of operational parameters to be controlled at a constant value. It is thus challenging to generalize optimum values for the AD of OFMSW, given the large number of operational parameters to be accounted for. Therefore, an interesting research direction to pursue is the optimization of existing AD plants, or those under construction, which already have defined operating parameters given permit restrictions and plant design choices. For example, full-scale plants must pre-determine reactor volume based on a desired waste capacity and SRT.

As follows, process optimization research could then consider the limitations of full-scale plants and eventually test the effects of different parameters on the full-scale process. This would yield results that could not only enhance the performance of many full-scale plants and boost biogas upgrading opportunities, but also better inform the design of future AD plants. Recent lab and pilot-scale optimization study results could be used as a starting point to achieve this.

Another interesting direction is to gather information on existing AD plants and compile pertinent operational parameters and then compare these to optimal values reported between 2005-2016 to see if these are reflected in the surveyed functioning AD plants. Results from older studies could also be compared to results from optimization studies carried out after 2016. In this way, gaps and opportunities for strategic collaboration between industry and academia could be identified, to continue improving AD circularity and enhance biomethane contributions to the energy matrix. For example, reporting in literature could be adapted to include units more commonly used in industry: in one case study of a full-scale plant in Poland, reactor loading is not reported in terms of OLR, but rather in terms of ton of waste load added per day, with a known TS content and unspecified VS (Seruga et al., 2020). More importantly, it could aid in adapting the experimental setup of lab-scale optimization studies to render their results more readily scalable.

1.3. Research Goals

1.3.1 Rationale

There is a large amount of research on the effect of different parameters on the AD of OFMSW, with the purpose of optimizing CH_4 production (discussed in **Section 2** of this chapter). To the author's best knowledge, there is no literature available that reports the application of optimal parameters identified in the lab or pilot scales for the design or optimization of full-scale plants. Thus, there is little information confirming (i) whether these optimal parameters are scalable to full-scale plants without compromising reactor stability and productivity ([volume CH_4 or biogas]/ volume reactor.d) or methane production (volume CH_4/mass VS) and (ii) whether the outcomes of these studies are useful for or applied in full-scale plants. Additionally, studies that are carried out in the laboratory or pilot scale cannot be generalized to all OFMSW, but at best to the OFMSW of a certain district. This is due to the fact that OFMSW and FW characterization shows that the properties of these substrates vary with the region of collection. Therefore, optimal parameters will be specific to the waste in the waste treatment or transfer facility from which samples are taken for a given study (Alibardi and Cossu, 2015; Campuzano and González-Martínez, 2016; Seruga et al., 2020).

These conclusions point to the need of defining the best way to develop synergies between academic studies and the AD industry in order to i) enhance the performance of AD plants (in terms of bigas production, methane content and reactor productivity) and ii) identify best practices for studies so they can better inform the design and construction of new AD facilities. These actions can lead to the acceleration of the implementation of AD, helping it approach its global energetic potential (10 100-14 000 TWh). I hypothesise that an important step to achieve this is to carry out case studies of existing AD plants of different capacities and attempt to validate an optimization methodology via pilot-scale simulations, considering common permit restrictions of full-scale plants. Testing different operational parameters at a small scale (30-50L) rather than at full-scale facilities (Seruga et al., 2020, 2018) could limit problems related to reactor stability in the operating plant. This method could also allow for higher-throughput screening of different test conditions, out of which the most pertinent can be chosen to test at the full-scale.

Such research is significant because it would increase the availability of data from full-scale AD plants, allowing researchers to follow up on these results and monitor whether optimal parameters identified in the lab/pilot scale improve AD performance in the full-scale. For stakeholders in industry and green entrepreneurship, this type of case studies could facilitate their navigation of the state of the art. Pooled information and gained experience involving full-scale AD plants could help deploy more stable and efficient AD plants that can facilitate biogas upgrading in regions and countries where the technology is still emerging.

3.2 Objectives

The main aim of this master's thesis is to explore the technical feasibility of optimizing biogas production and methane productivity in an existing full-scale OFMSW biogas plant via pilot-scale simulations of the full-scale process. In order to fulfil this, the specific objectives defined for this study are the following:

1) Providing an overview of the configuration, operation and performance of the chosen full-scale AD plant
2) Identifying parameters that compromise optimal biogas production and reactor productivity in the chosen AD plant
3) Applying strategies that could resolve the identified issues (considering the chosen AD plant's constraints) and evaluating their effect on biogas production and reactor productivity and stability in the pilot-scale.
4) Comparing reactor performance (i.e, gas production, productivity and quality) and digestate quality in the full-scale plant and in the pilot scale simulations (33 L) under the applied operational conditions.
5) Discussing opportunities for collaboration between academia and industrial partners in the AD sector, based on experiences from this case study

CHAPTER 2. MATERIALS AND METHODS

2.1 Case study: Data gathering and analysis

An AD plant located in Liege, Belgium and operated by the company Uvélia was chosen for this case study (**Figure 2.1**). Uvélia's staff provided the following data relevant to the AD process: (i) daily reactor feeding (t/d) and volumetric biogas production (Nm^3/d) for the period 01/2020-05/2021; (ii) weekly or bi-weekly digestate analyses (VFA to alkalinity ratio [FOS/TAC], pH and total solids) for the period 06/2019-12/2020; (iii) a preliminary mass flow analysis report from 2017 and (iv) substrate analysis data for the period 01/2020-11/2020. Data on the plant's design and configuration was reported based on information from the mass flow analysis report and information gathered in meetings and communications with Uvélia's staff. The data provided was reviewed and analysed by calculating monthly and yearly averages and standard deviations for 2020 for the following parameters: daily feed, SRT, methane content, daily biogas/CH_4 quantity (Nm^3/d), production (Nm^3/ton) and productivity; and FOS/TAC, TS, VS and pH. Yearly and monthly totals (for 2020) were also calculated for feed load and biogas and methane generation. The averages for SRT and biogas and methane production were calculated using the following equations:

$$(Eq. 1) \qquad Average\ SRT_{month/year}(d) = \frac{V_{Reactor}\ (m^3)}{Average\ feed\ load_{month/year}\left(\frac{m^3}{d}\right)^{*}}$$

$$(Eq. 2) \qquad Average\ MP/BP_{month/year}(Nm^3/t) = \frac{\sum[V\ of\ biogas/CH_4]_{month/year}\ (Nm^3)}{\sum[Mass\ of\ feed]_{month/year}\ (ton)}$$

*Assuming the density of the feed is not significantly different from 1 ton/m^3.
MP: methane production, BP: biogas production.

Estimations of (i) dilution factor (**Eq. 3**) and digestate recycle (**Eq. 4**) applied in the full-scale, (ii) the effects of biogas production and dilution on TS content (**Eq. 3**), (iii) steady state TAN content (**Eq. 5**) and (iv) suggested recycle flow for ammonia-stripped digestate (**Eq. 5-7**) were calculated using the data available and the following mass balances (for yearly amounts):

$$(Eq. 3) \qquad \sum Mass\ feed_{in} - \sum Mass\ biogas_{out} - \sum Mass\ feed_{out}(digested) = 0$$

$$(Eq. 4) \qquad F_1TS_1 + F_2TS_2 - (F_1 + F_2)TS_{Mix} = 0$$

$$(Eq. 5) \qquad \sum\ Mass\ TKN_{in} - \sum Mass\ TKN_{out} = 0$$

$$(Eq. 6) \qquad Up - concentration\ factor = \frac{\sum\ Mass\ TKN_{out}}{\sum Mass\ feed_{out}(digested)} \times \frac{\sum Mass\ feed_{in}}{\sum Mass\ TKN_{in}}$$

$$(Eq. 7) \qquad F_{in}C_{TAN\ in} - F_R(C_{TAN\ R} - C_{TAN\ S}) = F_{out}C_{TAN\ out}$$

$$(Eq. 8) \qquad C_{TAN\ R} = C_{TAN\ in} \times Up\ concentration\ factor\ (\mathrm{TAN})$$

In **Eq. 4**, F_1 and F_2 are the feed load of different substrates in t/d and TS1 and TS2 are their corresponding TS content, TSmix is the TS of the mixture of the two substrates**. In Eq. 7**, F_{in} and F_{out} are the inflow of feed and outflow of digested feed in t/d, F_R is the recycle flow of digestate in t/d, $C_{TAN\ in}$ is the TKN content in feed that will be transformed to TAN (kg/t), $C_{TAN\ out}$ is the desired steady state TAN content leaving the reactor ($C_{TAN\ out}$); $C_{TAN\ R}$ is the TAN content in recycled digestate and $C_{TAN\ S}$ is the desired TAN content in digestate after it leaves an ammonia stripping unit. It is assumed that no TAN production or degradation takes place in the reactor (TAN used in microbial activity is not transformed, but released back into the ammonia pool upon cell death). All N in TKN was assumed to be organically bound, with only about 60 % of it converted to TAN (Sheikh et al., 2016).

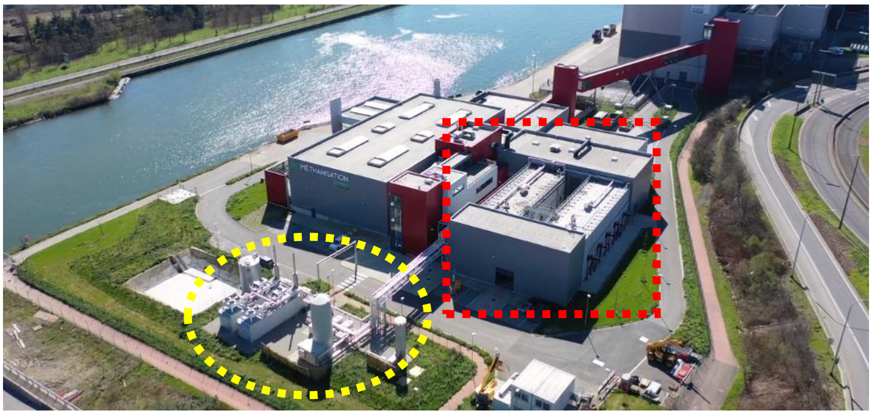

Figure 2. 1. Aerial view of the anaerobic digestion plant in Uvélia. The two digesters are outlined by the red square and the cogeneration engines are circled in yellow. **Source:** Image captured from a video created by the company Greg Bugni créateur d'images.

2.2 Substrate and inoculum characteristics

The OFMSW used in the pilot experiments was obtained from the AD plant under study. The samples were collected once on January and once more in April to replenish the feedstock. The first sampling (100 kg) was done by plant operators by collecting 20 kg OFMSW on 5 separate days, and the second sampling (150 kg), by collecting ~75 kg of OFMSW in 2 days. After collection, the raw OFMSW was transported to the laboratory where the pilot experiments were carried out. The OFMSW was grinded into a paste and stored in the lab refrigerator (4°C) until the start of the experiments. Before grinding, some materials in the OFMSW were removed manually (mainly visible hard plastic coffee containers and pens). The resulting OFMSW contained a non-negligible fraction of non-biodegradable contaminants: plastic bags, thick branches, oyster shells and small hard plastics and metals. The green waste (GW) used as co-substrate was obtained from the waste collection center Grace-Hollogne in Liege, receiving garden waste from households and entrepreneurs. It consisted of small branches, small leaves and grass clippings, without signs of contamination.

Fresh digestate was obtained from three different full-scale plants in Belgium and used to inoculate reactors for different experiments (**Section 3**). The digestate was either used to inoculate the reactors upon arrival or stored in the lab refrigerator (4°C) until use. The following inoculants were used in this study: (i) dry thermophilic digestate from the AD plant chosen as case study (DOF), (ii) wet thermophilic digestate from manure, maize and food waste treatment (DMF) and (iii) wet mesophilic digestate from manure and food and agricultural waste treatment (DMFA). The plants providing DMF and DMFA were chosen based on results from previous analyses showing that their digestate is of good quality (data not shown).

2.3. Pilot-simulations: Experimental setup

Pilot simulations were carried out using two plug-flow, 33 L (working volume) reactors (**Figure 2.2**). Each reactor is connected to its own gas meter and both are connected to a programmable logic controller (PLC) panel to control temperature and mixing (speed and frequency). The mixer consisted of 8 paddles along the length of the reactor.

In all experiments, the reactors were filled with 33 kg of inoculum and incubated for at least 1 day before initiating feeding. A period for acclimation to the targeted feed load was also provided. For acclimation, an initial OLR between 4.55-5.88 kg VS/m^3.d was applied. OLR was decreased whenever methane content fell below 50 % and increased in steps of 1-3.5 kg VS/m^3 (this depended on whether a resistance to acclimation was detected) if a methane content >50 % was maintained for at least 2 days for a given OLR. Stable feeding at the targeted OLR was then maintained until a decline in biogas production and methane content

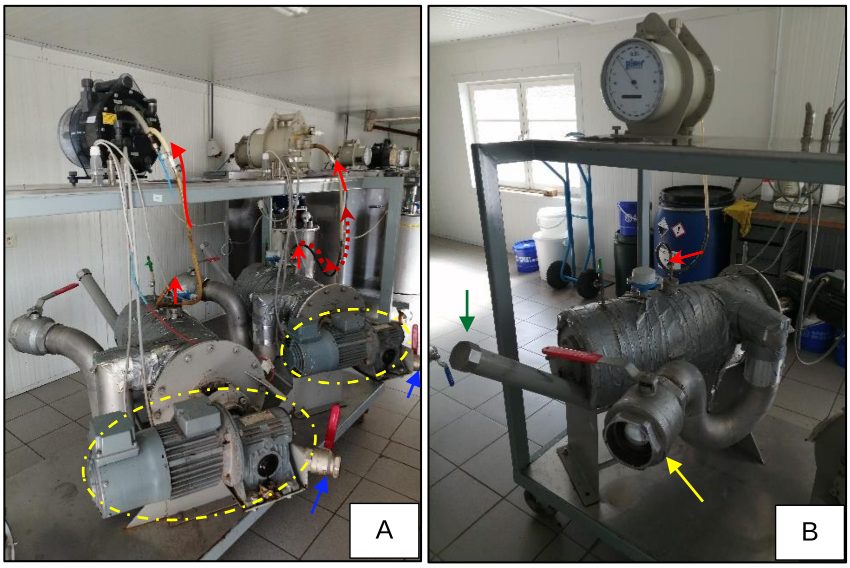

Figure 2. 2. Pilot reactor setup used for this study's pilot simulations. Panel A: Back view of the two pilot reactors used in this study. The sampling ports are shown by a blue arrow, the mixers are circled in yellow and the gas outlet connections to the gas meter are shown with red lines. Panel B: Front view of the pilot reactor and the gas meter. The green arrow points to the feed inlet, the red one to the gas outlet and the yellow one to the digestate outflow.

was observed. At this point, feeding was reduced to 50 or 80 % of the targeted OLR to prevent reactor failure.

Reactor failure was determined to take place when a diminishing biogas production (in comparison to a previous steady state value) was observed, accompanied by a drop in methane content to levels below 50%. If reactor failure took place, feeding was stopped until a methane content in biogas of 60% was reached. At this point, feeding was resumed at 50% of the target OLR and increased stepwise up to the target value, as long as methane content remained above 50%. When severe foaming took place (i.e., creating overpressure in the reactor), the mixing program was changed so that mixing would be provided continuously.

Averages for feeding and reactor performance (biogas and CH_4 production [**Eq.2**] and productivity) were calculated for the periods of stable operation. Stable operation was considered to begin when the waste loading was kept at a stable rate (in g OFMSW/day) and methane content remained >50 %. The data used to calculate averages related to biogas production (CH_4 production, productivity, methane content and mass reduction) was taken from the period where gas production stabilized (which generally lagged behind the onset of stable feeding). Gas production on days where mixing was stopped due to technical issues, or when the gas line was blocked, was not taken into account to calculate biogas-related averages.

Four different simulation experiments were carried out within a six-month period (January-July 2021), as described in the following sections. Reactor performance (i.e., biogas quantity and quality and their derivations) and digestate quality was monitored throughout the reactors' operation. **Table 2.1** schematizes the parameters applied and tested in the different experiments.

2.3.1 Experiment 1

Two reactors were run in parallel under thermophilic conditions (52 ± 1 °C) and fed with undiluted OFMSW at an OLR of ~9.2 kg VS/m^3.d to achieve an SRT of 30 d. The tested parameter in this experiment was the target SRT of 30 d defined via the case study (**Chapter III**, **Section 1.1**), based on the plant's target treatment capacity. One reactor (TR-OF) was inoculated with DOF and the other (TR-MF) with DMF. TR-OF was run to simulate the

Table 2. 1. Target operational parameters of the four pilot experiments carried out in this study. Values are expressed as averages.

Parameters	Exp. 1			Exp. 2	Exp. 3	Exp. 4
			Dilution			
Reactor names	TR-OF	TR-MF	TR-MF	MR	MR-HL	CoTR-OF
Temperature (°C)	52	52	52	38	38	52
Target SRT (d)	30	30	30	30	30, then 38	30
OLR[a] (kg VS/m^3.d)	9.2	9.2	7.0	9.2	9 and 7.3	7.0
Inoculum (wet or dry)	DOF (dry)	DMF (wet)	DMF (wet)	DMFA (wet)	DMFA (wet)	DOF (dry)
Substrate	OFMSW	OFMSW	OFMSW	OFMSW	OFMSW	OFMSW+GW
Feed Pre-treatment	None	None	Dilution factor of 1.3	None	Hydrolysis	Dilution factor of 1.3

DOF: dry thermophilic digestate derived from the pre-treatment of OFMSW in the studied biogas plant, DMF: wet thermophilic digestate derived from the treatment of manure maize and food waste, DMFA: wet mesophilic digestate derived from the treatment of manure and food and agricultural waste, OLR: organic loading rate, SRT: solids loading rate
a. The OLR resulting from applying an SRT of 30 d.

behaviour of the full-scale digesters if a 30 d SRT is applied and TR-MF was run to determine the onset of inhibitory mechanisms (if any) and identify them. Both reactors were fed more or less the same amount of feed every day. The daily feed load associated to the target SRT was reached after a 22-day acclimation period. Initial mixing was provided to both reactors at 30 rpm for 1 minute every other minute. After its failure, TR-OF was provided with digestate from the overflow of TR-MF for an 11-day period. After acclimation, TR-OF was stably fed for 9 days (0.3 SRTs) and TR-MF for 29 days (0.96 SRTs). The experiments were ended when no signs of recovery were detected after failure.

<u>2.3.1.1 Dilution in TR-MF</u>

TR-MF was fed with diluted OFMSW two weeks after reactor failure, when no substantial recovery was observed. Dilution was applied to evaluate TR-MF reactor performance and VFA accumulation at a stable TAN concentration of 4.25 g/kg. This target TAN concentration was chosen because TR-MF failure took place between a TAN concentration of 4.29 and 5.03 g/kg. To quickly reduce TAN from 5.53 g/kg to 4.25 g/kg, a dilution factor of 1.5 was applied. When the target TAN concentration was reached, a dilution factor of 1.3 was applied to maintain the concentration constant (based on the estimated steady state TAN concentration of 5.6 g/kg). Target OLR was reduced to ~7 kg VS/m^3.d to apply a 1.3 dilution factor and keep an SRT of 30 d. The experiment was ended when no signs of recovery were observed, 2 weeks after having reached the target TAN.

2.3.2 Experiment 2

In Experiment 2, one reactor (MR) was run under mesophilic conditions (38 ± 1 °C) and fed with undiluted OFMSW at an OLR of ~9.2 kg VS/m^3.d to achieve an SRT of 30 d. The reactor was inoculated with the wet mesophilic digestate DMFA. This experiment was designed to assess the efficacy of mesophilic conditions in mitigating ammonia toxicity at an SRT of 30 d (equivalent to 9.2 kg VS/m^3.d). The daily feed load associated to the target SRT was reached after a 24-day acclimation period. Initial mixing was provided at 15 rpm for 1 minute every other minute. The speed was changed to 30 rpm when digestate reached dry conditions (TS >20%). After acclimation, MR was stably fed at the target OLR for 30 days (1 SRT). Twenty-three days after its first failure, MR was fed with hydrolysed and diluted feed (1.4 dilution factor) as an attempt to restore previous activity. The experiment was ended when no signs of recovery were detected after 15 days of feeding the hydrolysed and diluted OFMSW.

2.3.3 Experiment 3

In Experiment 3, one reactor (MR-HL) was run under mesophilic conditions (38 ± 1 °C) and fed with hydrolysed, undiluted OFMSW. The OFMSW was hydrolysed by incubation at 37°C with digestate from MR-HL, at a ratio of 2:1 (OFMSW to digestate). The reactor was inoculated with the wet mesophilic digestate DMFA. Initial mixing was provided at 30 rpm for 1 minute every other minute. This experiment was run to continue testing the efficacy of mesophilic conditions in mitigating ammonia toxicity and to test the efficacy of hydrolysis in mitigating severe foaming, both at an SRT of 30 d. The target OLR of ~9.2 kg VS/m^3.d was reached after a 5-day acclimation period. After 13 days of operation, target and applied OLR was decreased by 20 % (~7.32 kg VS/m^3.d). After acclimation, MR-HL was stably fed for 24 days (0.8 SRTs). Feeding was stopped due to the termination of the project period, not to reactor failure.

2.3.4 Experiment 4

In Experiment 4, one reactor (CoTR-OF) was run under thermophilic conditions (52 ± 1 °C) and fed with a diluted co-substrate mixture consisting of 3 parts OFMSW and 1 part GW (1.3 dilution factor). Initial mixing was provided at 30 rpm for 1 minute every other minute. The co-substrate ratio was chosen based on the assumption that C/N in GW was 50. The reactor was inoculated with the dry thermophilic digestate sourced from the plant under study (DOF). This experiment tested the potential of co-digestion with GW in improving DOF quality and activity. After the 5 days of the acclimation period, 2 L of water were added to the reactor to reduce its TAN concentration in an attempt to improve biogas quality (methane content >50 %) before initiating feeding. CoTR-OF was acclimated to an OLR of 5.21 kg VS/m^3 after 19 days. The target OLR of ~7 kg VS/m^3.d (for an SRT of 30 d at a 1.3 dilution factor) was not reached. The experiment had to be terminated during the acclimation period due to the end of the project period.

2.4. Analytical Methods

2.4.1 Substrate and inoculum analyses

A physicochemical analysis was done for each subsample of OFMSW received to determine average pH, TS, VS, TN, TC, protein, carbohydrate and lipid content, as well as biogas and methane potential (and their standard deviations). GW was analysed for TS, VS and TN content. All analyses were done in an accredited spin-off laboratory from Gent University (ISO 17025), except pH (in H_2O), TN, TC and metal analyses for OFMSW which were carried out in a CN analyser and ICP-OES in the Laboratory of Chemical Analysis in Gent University.

For the metal analysis, OFMSW (0.1 g) was digested in Aqua Regia (AR) (incubation overnight and 2 h boiling). Digested samples were analysed for heavy metals and micronutrients in AR diluted 10 times in 2 M HNO_3. Fresh TR-OF was also analysed once for TC, TN, metals and pH via the same methods. The C/N ratio of the digestate and feedstock was calculated using TKN and TOC, assuming that TN≈TKN (food waste will only have organically bound N) and that TOC equals 55.56 % VS (Iglesias Jiménez and Pérez García, 1992). Calculations for C/N in the co-substrate mixture (OFMSW+GW) for the chosen ratio (3:1) were carried out as described by (Shahbaz et al., 2020) and TS and VS content were calculated with (**Eq. 2.4**) and (**Eq. 2.8**).

$$(Eq. 2.8) \quad F_1TS_1VS_1 + F_2TS_2VS_2 - (F_1 + F_2)TS_{Mix}VS_{Mix} = 0$$

2.4.2 Reactor performance and digestate quality analyses in pilot experiments

The biogas volume generated by the pilot reactors and its methane content were measured daily using a RITTER drum-type (wet-test) gas meter and a Geotech BIOGAS 5000 gas monitor, respectively. Biogas volume was converted to normal values using the average reactor temperature and monthly averages for pressure in the town the laboratory was located (data retrieved from NCEP and the World Meteorological Organization as reported by World Weather Online).

Digestate from the pilot experiments was sampled and analysed once a week. The parameters analysed in this digestate were electrical conductivity, FOS/TAC, TS, VS, pH, TAN and the content of seven VFAs (acetic, propionic, [iso]butyric, [iso]valeric and caproic acids). Metals were analysed once in Experiment 1 during the dilution of TR-MF and in Experiment 2 for MR (after reactor failure). Analyses were carried out by the same accredited spin-off laboratory mentioned above (ISO 17025). FAN fractions in digestate were calculated from pH, TAN measurements and applied temperature conditions as described by (Puig-Castellví et al., 2020). VS removal efficiency (fresh weight basis) was calculated only when a steady state TS content was reached.

2.5. Comparisons in the pilot and full-scale

Reactor performance (related to biogas quantity and quality) within the pilot and full-scale was compared using two-tailed t-tests (alpha = 0.05). In the full-scale, data (including feed loads) was compared between the 2 digesters (D1 and D2) operated by Uvélia to determine whether they could be considered as one population. In the pilot scale, data was compared between the mesophilic reactors MR and MR-HL; and between the thermophilic reactors TR-OF and CoTR-OF, to determine whether the applied operational parameters led to a significant difference in reactor performance.

Analysis of variance (ANOVA) was used to compare reactor performance between TR-MF and D1 and D2 (alpha = 0.05). The purpose was to determine whether the means for biogas and methane productivity were significantly different between TR-MF and both D1 and D2, for the month in which the latter 2 were operated at the highest OLRs in 2020. Equal variance between the different data sets was confirmed either with the F-test or when the variance in the data set with the smallest variance was less than 4 times smaller than the variance in the data set with the largest variance (Ramachandran and Tsokos, 2015).

Statistical tests were not used to compare digestate quality within and between the pilot and full-scale, given the data sets in the pilot scale were not large enough for comparison. In the full-scale, only digestate quality information for D1 was available in electronic format.

CHAPTER 3. RESULTS

3.1 Case Study (Uvélia): Anaerobic digestion process performance

3.1.1 Plant configuration and target operational parameters

This section provides an overview of the configuration, operation and performance of the AD plant under study. The AD plant, run by Uvélia, was started up on July 2019. It was designed to treat 32 857 ton of OFMSW per year. The OFMSW is collected from 5 municipalities in the province of Liege, located in southern Belgium. The collected waste first undergoes size separation with a rotary screen. The coarse fraction of the waste is grinded and then sent back to the screen. The fine fraction (<60 mm) is continuously fed (every 20 min) to 2 thermophilic, plug-flow digesters (52 °C) with a volume of 1660 m^3 each (for 1320 m^3 working volume and 340 m^3 head space for biogas). Process water and waste percolate are mixed with the OFMSW before loading the digesters to ease its passage and slightly reduce its TS content so the TS in digestate can be maintained below 25 %. Uvélia's treatment permit requires that the OFMSW be treated a minimum of 21 days in a temperature larger than 50 °C for pasteurization.

The plug-flow digesters (D1 and D2) are running the whole year, 24 h a day. No additives or anti-foaming chemicals are added to them and they do not contain any special solids retention mechanism, so SRT and HRT are equal. Still, the AD plant studied expresses retention time as SRT, therefore results of this study are also reported with this term. Each digester is connected to a biogas desulfurization unit, from where the biogas is directed to 2 combined heat and power (CHP) engines with a capacity of 600 kW each. Of the electricity generated, 350 kW is reincorporated into the plant and the rest is injected into the grid. At the moment of writing this thesis, the plant is not yet working at full capacity and has not been commissioned due to technical issues with equipment. Mass flows and plant configuration (based on designed parameters) are illustrated in **Figure 3.1**. The designed operational parameters (for the AD plant are compiled in **Table 3.1**.

The target capacity defined by Uvélia for this study is 32 000 t/year, which is slightly lower than the designed capacity found in their preliminary mass flows report (2017). Calculations were done to define new target parameters based on the target treatment capacity of 32 000 t/y. The values for SRT and feed load calculated in this study differ from the original designed parameters to a greater degree than expected (based on the difference in the designed and targeted treatment capacities). For example, an SRT of 21 d is much lower than what is necessary to achieve a treatment capacity of 32 857 t/year. This might be due to initial design choices that are not currently applied, such as digestate recycle and water dilution (data not available). In **Table 3.1,** the initial design parameters are compared to the new target parameters that were defined. These target parameters were used to design the pilot simulations that were later carried out (i.e., feed load for a target SRT of 30 d, rather than 21 d), to better align with Uvélia's chosen waste treatment capacity.

3.1.2 Substrate and inoculum characteristics

Characteristics of the OFMSW treated by Uvélia and used in the pilot simulations are shown in **Annex B.1**. Uvélia analysed samples of their OFMSW throughout a 10-month period in 2019 through external analytical services. This study also characterised Uvélia's. The average values obtained for parameters analysed by this study are similar to those obtained by Uvélia. TKN content in the OFMSW samples used for the simulations were between 24.32-25.29 g/kg TS, which is at the higher end of the range of the data provided by Uvélia (17.4-30.2 g/kg TS). Average C/N content of OFMSW was found to be 16.6 by both this study and Uvélia's analyses. The substrate is also characterized by a low pH (4.80 ± 0.10) and an

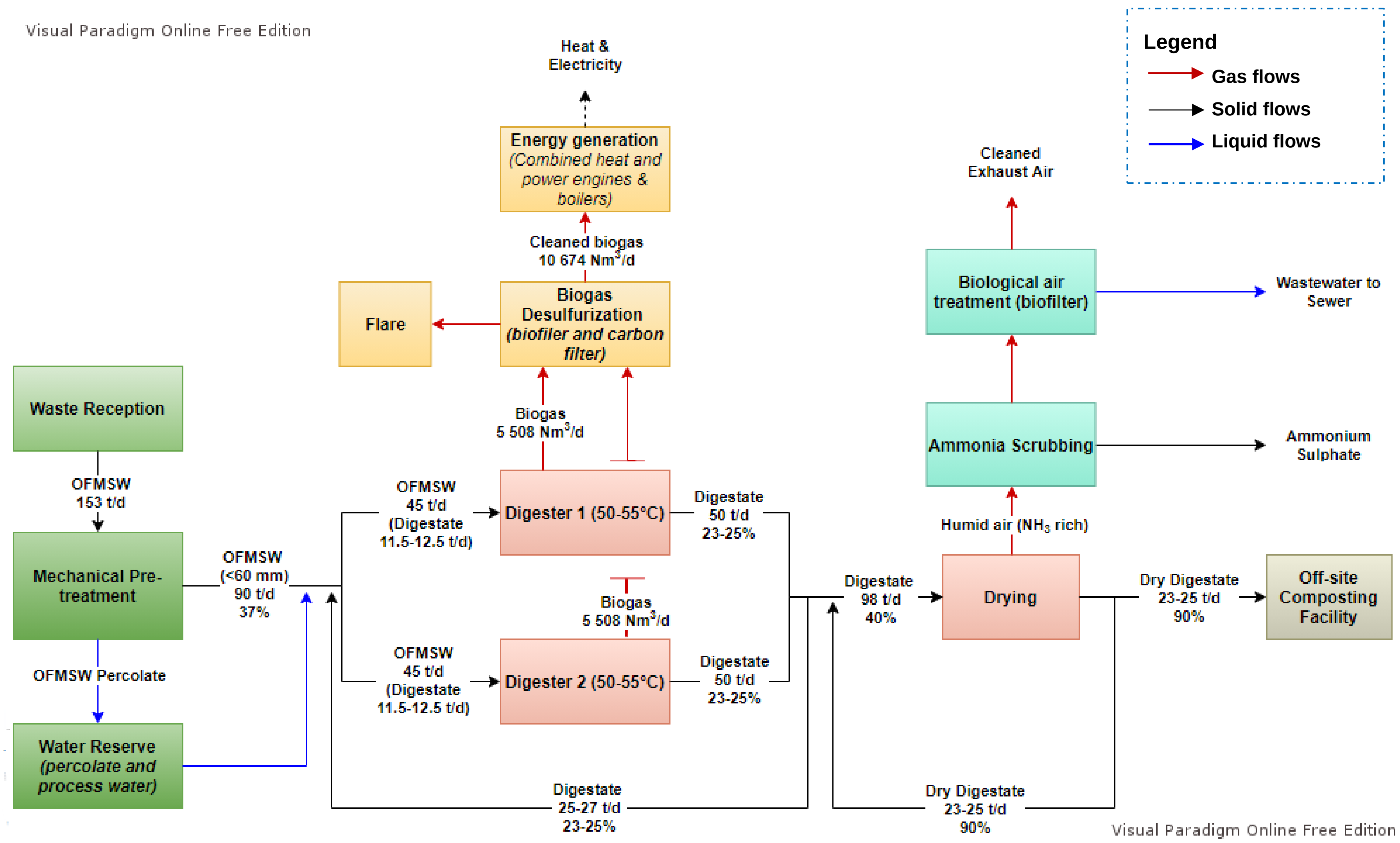

Figure 3. 1. General block flow diagram (with mass flows) of the anaerobic digestion plant chosen for this case study. The process configuration and estimated mass flows are based on the plant's mass flow analysis report (2017), information from meetings with Uvélia's staff and complementary calculations based on the available data. Mass flows are shown for OFMSW and digestate (in ton per day) and biogas (in normal, dry volume). The mass flows related to digestate drying are excluded. TS content of OFMSW and diestate is shown in % (of fresh matter).

Table 3. 1. Comparison between designed parameters and the calculated targeted parameters that were used to design the pilot experiments carried out in this study. The latter were determined based on the treatment capacity defined in meetings with Uvélia's staff and biogas potential data obtained in this study.

Parameters	Units	Designed	Targeted
Treatment Capacity	t/year	32 857	32 000
Hourly Feed load (per reactor)	t/h/reactor	2.5 (week) 1.3 (weekend)	2.05 (week) 1.3 (weekend)
Average feed load	t/d/reactor	51.8	44.1
Average OLR	kg VS/m^3.d	10.33	8.80
Digestate recycle	t/d	Yes (not specified, 0.25 estimated recycle rate)	No[a]
SRT	d	21	30
Biogas production[c]	Nm^3/ton	122 (90% of TBP)	140 (90% of TBP)[b]
TS content (digestate)	% FM	<25	NA

OLR: organic loading rate, FM: fresh matter, TBP: theoretical biogas potential of OFMSW.
a. The plant is not currently applying a digestate recycle, it is not envisioned to do so.
b. The biogas potential of the OFMSW samples used in this study was slightly higher than the average biogas potential calculated for the OFMSW

average biogas potential between 121-156 m^3/ton and SMY of 0.332 ± 0.015 m^3/kg VS, with a theoretical methane content in biogas of 59 ± 1%. Data on SMY, theoretical methane content and pH were not provided by Uvélia. The green waste (GW) employed for co-digestion in Experiment 4 (CoTR-OF) has a C/N ratio of 21.2. Therefore, the co-substrate mixture with OFMSW has a C/N ratio of 17.6.

Uvélia used wet, mesophilic digestate to inoculate their reactors (see **Annex B.2** for inoculum characteristics). The mesophilic inoculum was acclimated to thermophilic conditions by step-wise increases in temperature (data not available). Initial characteristics of the inoculants for each pilot simulation (Experiment 1: DOF & DMF; Experiment 2 & 3: DMFA; and Experiment 4: DOF) are shown in **Annex B.2** and discussed in the following sections.

Micronutrient and heavy metal contents in OFMSW and fresh digestate, and their up-concentration factors after AD are shown in **Annex B.3.** All metals analysed, except Cr and Ni, accumulated in digestate, compared to their content in OFMSW. Cr, Cd, Cu, Zn, Pb and Ni are all below their acceptable limits in digestate according to Belgian regulations (Saveyn et al., 2014). However, according to legislation in Wallonia, Zn, Cu, Pb and Cd contents are all over the limits imposed for using digestate as fertilizer (Heneffe, 2020). However, the digestate produced by Uvélia is composted to be used as soil amendment. Therefore, the high contents detected in the digestate might be due to the analysis of a sub-sample that had a high localised concentration of certain metals, which takes place due to the heterogeneity of dry wastes (Rocamora et al., 2020). Another explanation could be that the metal content in the digestate is diluted when it is mixed with other organic wastes in the composting plant.

3.1.3 Applied operational parameters and reactor performance

A comparison between targeted and applied operational parameters and reactor performance is shown in **Table 3.2**, for both D1 and D2 in 2020. The deficit in feed load was explained by the fact that the digestate drier is working only at half of its designed capacity due to technical issues, as explained by Uvélia. In general, less than 44 t/day (per digester) were extracted from the digester into the drier (data not shown). Even though a digestate

Table 3. 2. Comparison between target and observed parameters regarding operation and performance of the 2 digesters (D1 and D2) operated in the studied AD plant. Averages with standard deviations are shown for those calculated with the standard formula; stand-alone averages are used for those calculated with the sum of feed load and biogas quantity.

Parameters (per reactor)	**Units**	**Target**	**Digester 1**	**Digester 2**	**Deficit (D1)**	**Deficit (D2)**
Feed	ton/year	16 000	10 016	10 255	-5984	-5745
	ton/d	44	27.5 ± 12.8	28.2 ± 12.6	-16.5	-15.8
OLR	kg VS/m^3.d	8.77	5.47 ± 2.56	5.61 ± 2.52	-3.30	-3.15
Biogas Quantity	Nm³/year	1 825 000	1 323 203	1 355 362	-501 797	-469 638
	Nm³/d	5000	3640 ± 1221	3728 ± 1167	-1360	-1272
Biogas productivity	Nm^3/m^3.d	3.79	2.76 ± 0.93	2.82 ± 0.88	-1.03	-0.96
CH_4 productivity	Nm^3/m^3.d	2.12	1.60 ± 0.57	1.65 ± 0.54	-0.52	-0.47
CH_4 content	Vol.%	56	58.39 ± 5.95	58.86 ± 5.14	-0.60	-0.14
SRT	d	21	48.0	46.9	+27.0	+25.9
Biogas production [% TBP]	Nm³/ton [%]	137 [90]	132.1 [96.5]	132.2 [96.6]	-4.7 [+6.5]	-4.7 [+6.6]
CH_4 production [% TMP]	Nm^3/ton [%]	80.73 [90]	76.75 [100]	77.49 [101]	+0.13 [+10]	+0.87 [+11]
CH_4 production	Nm^3/kg VS	0.291	0.292	0.295	+0.013	+0.016

TBP: theoretical biogas potential, TMP: theoretical methane potential

recycle was foreseen (estimated to be around 0.25 by this study), this is not currently carried out. The applied operating temperature is 3 °C lower than the designed temperature because during reactor acclimation, a 1 °C step increase from 52 °C caused one of the digesters to overflow (information provided in a meeting with Uvélia's staff).

Only slight differences in operation and performance are observed from month to month between D1 and D2. Biogas volume (Nm^3/d), feed load (t/d) and methane content (%) data collected from both digesters showed normal distribution. All of the data, except for CH_4 content, had equal variance. The statistical t-tests (for equal and unequal variances, as necessary) performed to compare the digesters' means for the aforementioned parameters showed that there was no significant difference between them ($t<t_{critical}$, p >0.05). Thus, it is concluded that there is no significant difference in operation nor performance between the 2 digesters, for the year 2020.

Average biogas production in 2020 was 132 Nm^3/t for both D1 and D2, or 97% of the theoretical biogas potential (TBP) determined by Uvélia. Average methane production was ~100 % of SMY (based on the theoretical methane content in biogas determined by this study). However, the average daily feed load and SRT in 2020 was lower than the target resulting in a treatment deficit of 11 729 tons of waste. Furthermore, dynamic feeding (i.e, large differences in daily OFMSW load) was applied from the beginning of operation, which resulted in unstable daily biogas generation and production (following the feeding pattern). The daily changes in feed load (and OLR) and the resulting unstable biogas production are illustrated by the large standard deviations of the averages (see **Table 3.2** and **Annexes B.4-B.5**). This led to days in which the volume of biogas was only enough to run one of the two CHP engines, or too low to run them at all (so the biogas was flared). On the other hand, methane content in biogas was rather stable, so methane production followed fluctuations in biogas production.

Finally, there is an important positive correlation between increasing OLR and reactor productivity (R^2=0.82) between OLRs of 2.11-7.57 kg VS/m^3.d (**Annex B.6**). Monthly averages of reactor performance indicators and operational parameters for 2020 are illustrated in **Annexes B.4-B.5**.

3.1.4 Digestate Quality

Uvélia monitors the following parameters in their reactors' digestate: pH, TS content and FOS/TAC. FOS/TAC is used as an indicator for digestate quality and the risk of acidification. The digestates' VS was only determined 3 times since the plant's start-up. Digestate quality was well-recorded from July 2019, when the plant was started up, in contrast to reactor performance and operating conditions, which were accurately recorded from December 2019. Given there was no significant difference in reactor performance between D1 and D2 (see **Section 1.3**), digestate quality will also be assumed to not be significantly different for both digesters. Therefore, in this section, only digestate quality of D1 was analysed and illustrated as it is assumed to be representative of both reactors.

Initial TS content in digestate was low (5.58%), and eventually stabilized to an average content of 24.03 ± 1.92 in the year 2020. Initial average FOS/TAC levels were below or within the defined optimal range of 0.3-0.4 for the first three months of operation. After this, FOS/TAC values steadily increased and stabilized around an average of 0.76 ± 0.14 in the year 2020. Initial average pH was between 7.69 ± 0.04 and 7.89 ± 0.23, and stabilized to an average of 8.0 ± 0.1 in 2020. Monthly averages of digestate quality parameters monitored by Uvélia for D1 in 2020 are shown in **Annex B.7.**

3.1.5 Opportunities for AD process optimization in Uvélia

There is room for process optimization of the AD plant under study, given their indicator for process stability, FOS/TAC, points to poor stability. Additionally, high N levels in feed and digestate had also been identified by Uvélia previous to this study. The impacts of these poor process conditions are difficult to determine given the plant is operating below its designed capacity and with a dynamic feed loading, due to technical issues concerning the digestate drying process. Pilot simulations would give insight into the reactors' potential behaviour when the designed operational parameters are applied.

3.2. Pilot simulation of the thermophilic digestion of OFMSW

In Experiment 1, the pilot reactors TR-OF and TR-MF were run in parallel to simulate the full-scale AD process when an SRT of 30 d was applied. This SRT was chosen based on the AD plant's target treatment capacity and feed load (**Table 3.1**). In this pilot experiment, the SRT of 30 d corresponded to an OLR of ~9.2 kg VS/m^3.d, based on the TS and VS content in the OFMSW samples collected from Uvélia for the simulation. The average OLR calculated for an OFMSW load of 44 t/d, based on the TS and VS data provided by Uvélia, is 8.8 ± 1.1 kg VS/m^3.d. Thus, the average OLR applied in the pilot simulation is within the range of the standard deviation in OLR expected for the OFMSW. Since the average OLR is not unrealistically high or low for the given SRT, it reflects loads that would be applied under real circumstances. The behaviour of TR-OF (reactor inoculated with DOF sourced from Uvélia) and TR-MF (reactor inoculated with the known good-quality wet thermophilic digestate, DMF) under this 30 d SRT are discussed in detail below. Initial inoculum characteristics for these pilot reactors are laid out in **Annex B.2**.

3.2.1. Reactor performance

The daily performance of TR-OF and TR-MF throughout their operation is shown in **Figures 3.2** and **3.3**. The TR-OF reactor showed signs of inhibition in terms of biogas production. A maximum of only 40 % of the TBP of 156 m^3/t was achieved from the beginning of feeding at OLR 9.17 ± 0.04 kg VS/m^3 (SRT 30 d). At this OLR, the average methane content in the biogas was also lower than the theoretical content (59 %), at 53.7 %. Stable feeding of undiluted organic waste at this OLR led to reactor failure and foaming after 10 days. Reactor failure was characterized by a diminishing biogas production and a drop in the methane content of biogas down to levels below 50%. The foaming accompanying the crash was severe, creating overpressure conditions in the reactor and causing digestate to spray out from the overflow (~5-10 L). Feeding TR-OF with digestate from the overflow of TR-MF during the subsequent starvation period (given TR-MF had a good performance at this point) did not

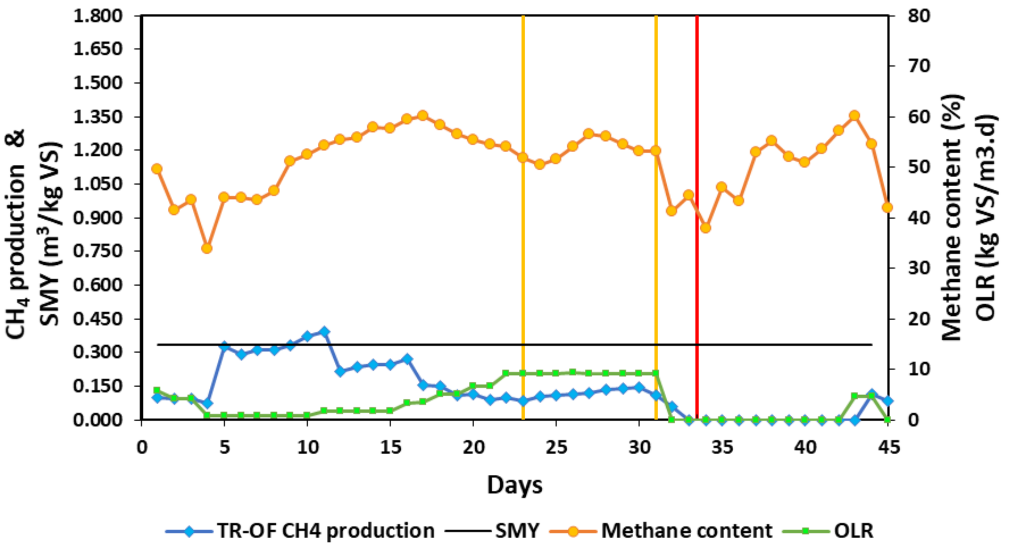

Figure 3. 2. Performance of TR-OF (thermophilic reactor inoculated with digestate sourced from the AD plant under study) during its operation time (45 days), in terms of methane content and production. Yellow bars delineate the period of stable feeding at 9.17 ± 0.04 kg VS/m^3.d. The days leading up to stable feeding are defined as the acclimation period. The red bar indicates when reactor failure took place. SMY: specific methne potential.

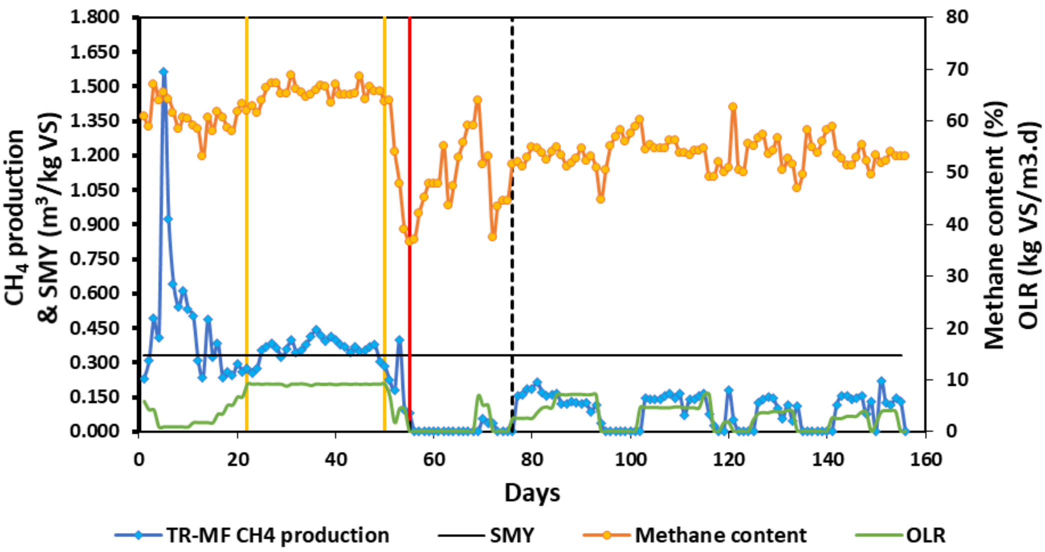

Figure 3. 3. Performance of TR-MF (thermophilic reactor inoculated with good-quality wet thermophilic digestate from an AD plant treating manure, maize and food waste) during its operation time (156 days), in terms of methane content and production. The end of the acclimation period is marked by the yellow bars, which delineate the period of stable feeding at 9.2 ± 0.09 kg VS/m^3.d. The dashed, black bar indicates the beginning of feed dilution. The red bar indicates when reactor failure took place. SMY: specific methane potential.

result in improved reactor performance. After TR-OF reached a methane content of 60.2 %, feeding was resumed at ~ 4.6 kg VS/m^3. This caused methane content to drop to 41.9 % in 2 days. Given reactor performance was not restored, the experiment was terminated.

The TR-MF reactor showed high performance in terms of average biogas production (99% of TBP), methane production (111% of SMY) and CH_4 content (65.8 %) at OLR 9.2 ± 0.09 kg VS/m^3 (SRT 30 d) and with biogas and methane productivities well over Uvélia's daily target [see **Table 3.2** and **Table 3.3,** TR-MF (E1)], until reactor failure took place. Stable feeding of undiluted organic waste at this OLR led to stable biogas production and methane content for 27 days. On day 28, a severe foaming event took place, and on the 29th (and last) day of stable feeding, process instability was observed in the form of decreasing biogas production and methane content and VFA accumulation. To counteract this, OLR was decreased by 50 % or more. However, the reactor failed within 6 days, with methane content dropping to 37 %. After reactor failure, methane content in TR-MF biogas reached 64.0% after 14 days of reactor starvation; however, when feeding was resumed, methane content fell to 31.7% after only 3 days of feeding.

Before its failure, TR-MF had a much better reactor performance than TR-OF and it acclimated better to the target OLR, in terms of methane content and biogas production. Stable feeding of TR-MF was possible for 3 times as long as TR-OF before reactor failure. TR-MF's methane production/productivity was larger than the SMY and much larger than the production/productivity achieved by TR-OF, which only reached around 37% of SMY (see **Table 3.3**). However, after TR-MF failed, its performance became more similar to that of TR-OF, with biogas production at OLR > 4.5 kg VS/m^3 generally being <50 % of the
SMY.

3.2.2 Digestate Quality

Initial digestate conditions in the TR-OF reactor were characterized by high acetic acid (HAc) (1.869 g/kg) and free ammonia nitrogen (FAN) concentration (1.43 g/kg). HAc concentration doubled after applying the OLR of 9.17 ± 0.04 kg VS/m^3 for 7 days. The reactor began acidifying from the beginning of stable feeding (pH drop from 8.07 to 7.63), due to the accumulation of HAc (4,214 – 15,134 g/kg). **Figure 3.4** [A & B] illustrates TR-OF digestate quality during its operation. The reactor's risk for acidification (measured by FOS/TAC) was high from the beginning of the experiment, with FOS/TAC values higher than the optimal ratio defined by Uvélia (0.3-0.4). With VFA accumulation and acidification, FOS/TAC increased, peaking at 1.64 after the reactor failed (**Annex B.8**). Even though TR-OF was inoculated with digestate adapted to the substrate, TAN levels stabilized at a concentration that was 0.6 units higher than the initial one, while TS content remained stable (see **Figure 3.4** [A & B] and **Annex B.8**). Higher TAN can be explained by variations in TKN content in the OFMSW (21.5 ± 4 g/kg) and/or by the fact that the OFMSW was undiluted. Average VS removal efficiency was 52.18 ± 1.68 % (**Annex B.8**). True stable operation was not possible given the reactor failed under the applied feed load.

For TR-MF, an OLR of 9.2 ± 0.09 04 kg VS/m^3 (SRT 30 d) was maintained for 22 days without an important accumulation of HAc (1.150-1.758 g/kg), but with an accumulation of propionic acid (HPr) and total VFAs (tVFA). TAN concentration increased from initial levels of 2.63 g/kg, up to 5.03 g/kg. Digestate pH remained between 8.02 and 8.26 during stable feeding. Between days 42 and 49 of reactor operation (third and fourth week of stable feeding), HAc concentration doubled. On the day the reactor crashed HAc concentration had increased by a factor of 7.3 (15.38 g/kg). After 1 week of reactor starvation, HAc concentration in

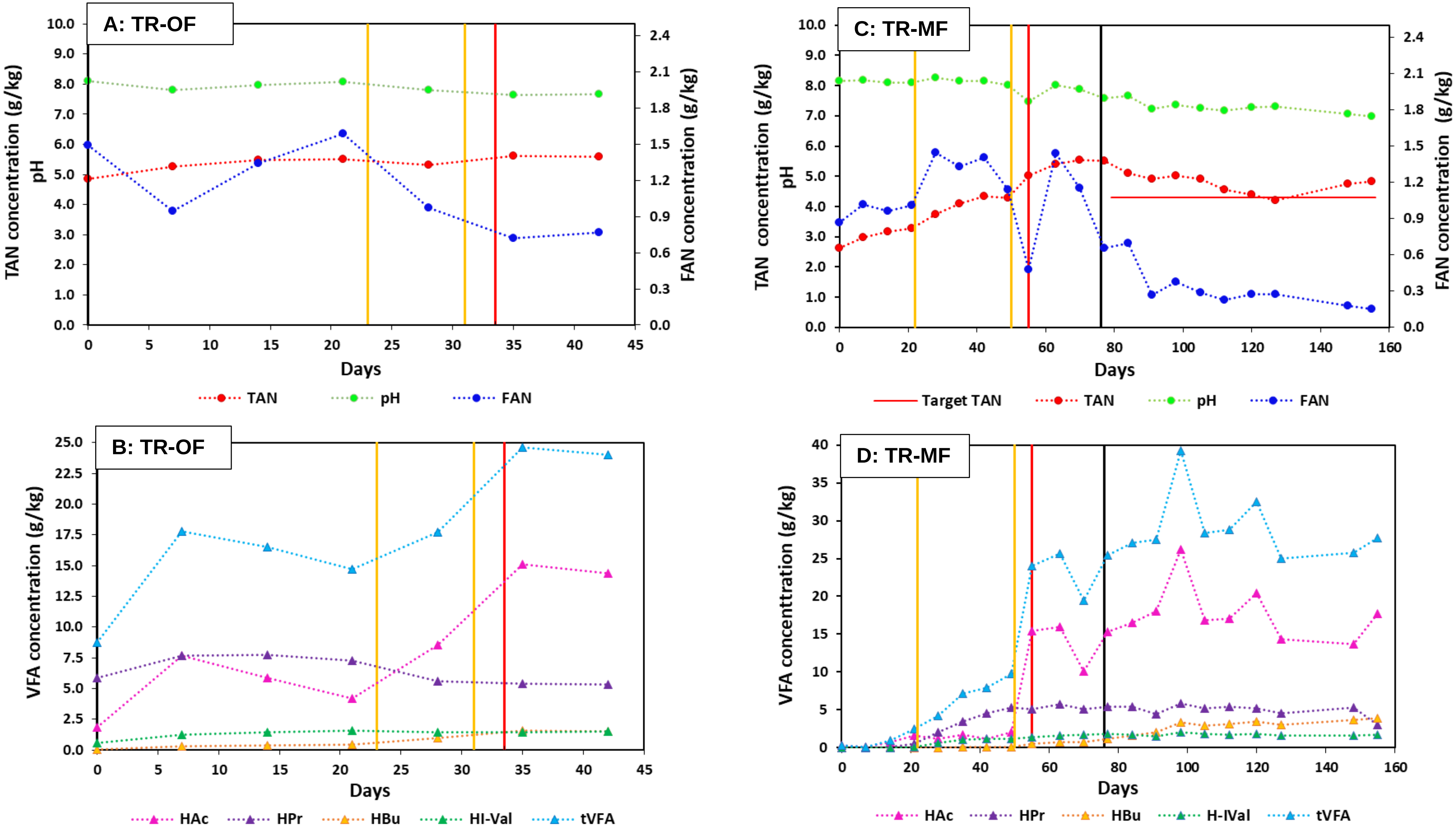

Figure 3. 4. Weekly digestate quality for the thermophilic reactors TR-OF (Panels A and B) and TR-MF (Panels C and D) throughout their operation time. TR-OF was inoculated with digestate from the plant under study (derived from organic household waste). TR-MF was inoculated with good-quality thermopihlic digestate sourced from a different plant. The end of the acclimation period of the reactors is marked by the yellow bars, which delineate the period of stable feeding, simulating an SRT of 30 d (OLR ~9.2 kg VS/m^{3}·d). The red bars indicate when reactor failure took place and the black bars the beginning of feed dilution (as an ammonia mitigation strategy). Panel A and C: Measured values for total ammonia nitrogen (TAN) and pH and calculated values for free ammonia nitrogen (FAN). Panels B and D: acetic acid (Hac), propionic aid (HPr), butyric acid (HBu), isovaleric acid (HI-VAL) and total VFA (tVFA) concentrations.

digestate was still extremely high. **Figure 3.4** [C & D] shows changes in digestate quality throughout the operation of TR-MF. TS content and TAN concentration only stabilized after the reactor failed, so a true stable operation was not achieved (**Figure 3.4** C and **Annex B.8**).

The risk of acidification in TR-MF increased after the first week of stable feeding, as indicated by an increase in FOS/TAC to 0.47 and above. Increases in FOS/TAC where consistent with the increasing concentration of HPr (**Figure 3.4** D, **Annex B.8**). There was then a marked increase in tVFA content (mainly caused by HAc accumulation) between the day stable feeding was stopped and the day the reactor failed, also reflected by a FOS/TAC ratio close to 1. During the starvation period, FOS/TAC and HPr plateaued while tVFA and HAc decreased and increased.

TR-MF had better initial digestate quality than TR-OF in terms of FOS/TAC and VFA and FAN concentration. Even though TR-MF had a lower TAN concentration than TR-OF throughout the acclimation and feeding periods, its FAN fraction increased quickly to levels similar to those in TR-OF (FAN between 1.00-1.50 g/kg). This was possible since TR-MF accumulated less VFAs and could then maintain a pH between 8.1-8.3 during acclimation and stable feeding, which was higher than the pH of TR-OF (7.6-8.1). After reactor failure, TR-MF digestate quality became more similar to that of TR-OF with (i) total VFA concentration > 10 g/kg, (ii) pH below 8.0 and (iii) poor FOS/TAC ratio.

3.3. Effects of optimization strategies on pilot reactor performance

3.3.1 Thermophilic mono-digestion of diluted OFMSW

Dilution as a mitigation strategy for NH_3 toxicity was tested on TR-MF (Experiment 1) about 3 weeks after its failure. This was done to determine whether TR-MF's previous activity could be restored via dilution. The target OLR of 7.1 kg VS/m^3.d was not reached when TAN levels were reduced to ~4.2 g/kg (the new target TAN concentration), given reactor performance did not stabilize at lower OLRs. Digestate quality was not successfully restored either by OFMSW dilution with tap water (**Figure 3.4** [C & D]). When the feeding of diluted organic waste was started, HAc levels were still very high (between 10-15 g/kg) and FAN concentration was 0.65 g/kg despite a peak in TAN concentration of 5.51 g/kg, given pH had dropped to 7.59. Despite dilution and the reduction of OLR to < 7.2 kg VS/m^3.d, the resumed feeding resulted in accumulation of butyric (2.890-3.393 g/kg), caproic (0.136-0.258 g/kg) and acetic acids (up to 26.181 g/kg), which in turn led to (i) an increase in FOS/TAC (>1.35), (ii) a steady drop in pH (7.59-6.98) and (iii) low FAN levels (<0.70 g/kg) until the end of the 11-week experiment.

3.3.2. Mesophilic mono-digestion of treated and untreated OFMSW

The effectiveness of mitigating ammonia toxicity by mesophilic digestion when applying an SRT of 30 d was tested in Experiment 2 with MR, the reactor inoculated with the wet mesophilic digestate DMFA. Due to issues with severe foaming in MR, another mesophilic run was designed (Experiment 3). In the second run a mesophilic reactor (MR-HL), once again inoculated with DMFA, was fed with hydrolysed OFMSW. Even though DMFA was used to inoculate the reactors in both experiments, it was sampled on different months from the AD plant producing it, so initial digestate quality differs between MR and MR-HL (see **Annex B.2**).

Table 3. 3. Operational, performance and digestate quality parameters during the stable feeding periods of the pilot reactors run in Experiments 1-4. For operational parameters and reactor performance, values are expressed as averages with standard deviation; for digestate quality, initial (left) and final (right) values for the stable feeding period are shown.

Parameters	TR-OF (E 1)	TR-MF (E 1)	TR-MF Dil.[b] (E 1)	MR (E 2)	MR-HL (E 3)	TR-OFCoD (E 4)
Operation time (d)	49	156	156	93	28	28
Substrate	OFMSW	OFMSW	Diluted OFMSW	OFMSW	Hydrolysed OFMSW	Diluted & hydrolysed OFMSW
Inoculum	Adapted	Non-adapted	Adapted	Non-adapted	Non-adapted	Adapted
Stable feeding period (d)[a]	22 - 31 (10)	22 - 50 (29)	85 - 93 (9)	24 - 53 (30)	5-28 (24)	20-28 (9)
Temperature (°C)	52 ± 1	52 ± 1	52 ± 1	38 ± 1	38 ± 1	52 ± 1
OLR (kg VS/m^3.d)	9.17 ± 0.04	9.2 ± 0.09	7.14 ± 0.04[b]	9.17 ± 0.13	7.76 ± 1.90	5.21 ± 2.39
SRT (d)	30	30	30	32	34	40
Biogas productivity [c] (Nm^3/m^3 reactor.d)	1.9 ± 0.4	5.1 ± 0.5	1.5 ± 0.4	3.6 ± 0.4	3.1 ± 0.4	1.2 ± 0.3
Methane productivity [c] (Nm^3/m^3 reactor.d)	1.0 ± 0.2	3.4 ± 0.3	0.8 ± 1.1	2.3 ± 0.3	1.8 ± 0.2	0.7 ± 0.2
Biogas production (Nm^3/t) [% TBP] [c]	62.5 ± 7.2 [40.2]	153.9 ± 17.7 [99.0]	57.4 ± 15.07 [36.9]	108.4 ± 9.2 [69.7]	112 ± 27 [71.9]	65.0 ± 12.9 [41.8]
SMY (Nm^3/kg VS) [% SMY] [c]	0.122 ± 0.016 [36.8]	0.369 ± 0.034 [111]	0.109 ± 0.030 [32.8]	0.248 ± 0.027 [74.6]	0.254 ± 0.065 [76.4]	0.140 ± 0.030 [42.2]
Methane content (%)[c]	53.7 ± 2.1	65.8 ± 1.4	51.7 ± 2.8	62.6 ± 3.0	62.3 ± 3.1	59.0 ± 1.6
Mass reduction (w.%)[c]	7.83 ± 0.91	19.28 ± 1.83	7.18 ± 1.89	10.67 ± 1.03	13.65 ± 1.68	7.64 ± 1.32
Dry matter (%)	25.1 - 24.24	9.8-15.7	15.84 - 16.91	10.88 - 19.83	9.12 - 18.6	19.82 - 20.8
TAN (g/kg)	5.50 - 5.31	3.73 - 4.29	5.1 - 4.9	3.93 - 5.07	3.81 - 5.18	5.24 - 5-07
FAN (g/kg)	1.59 - 0.97	1.44 - 1.14	0.66 - 0.25	0.36 - 0.48	0.30 - 0.55	1.03 - 1.02
pH	8.07 - 7.81	8.26 - 8.02	7.66 - 7.22	7.87 - 7.86	7.77 - 7.92	7.85 - 7.86
HAc (g/kg)	4.214 - 8.540	1.155 – 2.091	16.564 -18.002	3.315 - 3.625	4.313 - 2.980	6.496 - 9.111
HPr (g/kg)	7.278 - 5.618	2.021 – 5.369	5.386 - 4.447	3.119 - 7.939	2.101 - 10.168	6.209 - 6.340
P/A ratio	1.7 - 0.7	1.7 - 2.6	0.3 - 0.2	0.9 - 2.2	0.5 - 3.4	1 - 0.7
FOS/TAC	0.86 - 1.17	0.37 - 0.47	1.04 -1.35	0.66 - 0.88	0.44 - 0.8	0.97 - 1.15
tVFA (g/kg)	14.700 - 17.750	4.228 – 4.973	27.021 - 27.514	8.279 - 14.046	6.796 - 15.962	14.932 - 19.186

See next page for footnotes

Table 3.3 footnotes:

E: experiment, TR-OF: thermophilic reactor inoculated with digestate from the studied AD plant, TR-MF: thermophilic reactor inoculated with wet thermophilic digestate from maize, food and manure treatment, Dil.: with feed dilution, MR: mesophilic reactor inoculated with wet mesophilic digestate, MR-HL: mesophilic reactor inoculated with wet mesophilic digestate and fed with hydrolysed organic waste, TR-OFCoD: thermophilic reactor inoculated with digestate from the studied AD plant for co-digestion, OLR: organic loading rate, SMY: specific methane yield, SRT: solids retention time, TAN: total ammonia nitrogen, TBP: theoretical biogas potential, FAN: free ammonia nitrogen, P/A ratio: propionic to acetic acid ratio, tVFA: total VFA

a. In days of operation, duration in parenthesis

b. Dilution with tap water (by factor of 1.3)

c. Averages are calculated using data points from the period during stable feeding where biogas production was stable, and excluding outliers (caused by technical issues).

3.3.2.1 Reactor performance

The daily performance of MR and MR-HL throughout their operation is shown in **Figures 3.5** and **3.6**, respectively. During acclimation, MR showed low methane content in biogas. After supplementation with 5 kg of fresh digestate, methane content significantly increased and was successfully maintained. The MR reactor acclimated to OFMSW after 24 days, after which stable feeding was initiated. MR had an average biogas production of 69.7% of TBP during a 30-day period of stable feeding at an SRT of 32 d (**Table 3.3**). After 30th day, stable feeding led to reactor failure, characterized by reduced biogas production and a dwindling methane content in biogas, down to 31.9%.

During the 30-day stable feeding period of MR, the reactor was in overpressure 5 times (from severe foaming). In 3 out of the 5, the digestate sprayed out from the reactor and in all cases >5 L of digestate were lost in the overflow. During the recovery period, after feeding a reduced waste load for 9 days, foaming was so severe, all the digestate adopted a foamy texture, with low density. Subsequent reactor starvation led to an increase in methane content (up to 66%). Nevertheless, severe foaming blocking the gas line persisted for 24 days, and methane content in biogas decreased from 66.8% to 47.8%. Thus, the experiment was terminated. Throughout the first month and a half of Experiment 2, there were often power failures and issues with mixer control, with lack of mixing exacerbating foaming.

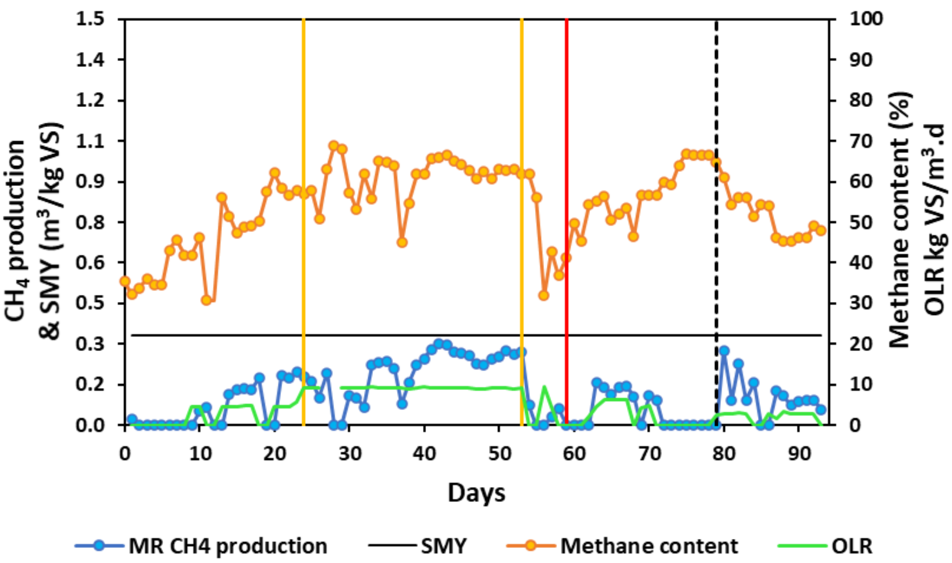

Figure 3. 5. Performance of MR (mesophilic reactor in Experiment 2, inoculated with wet mesophilic digestate from an AD plant treating manure and food and agricultural waste) during its operation time (93 days), in terms of methane content and production. The end of the acclimation period is marked by the yellow bars, which delineate the period of stable feeding at 9.17 ± 0.13 kg VS/m^3.d. The dashed, black bar indicates the beginning of feed dilution and hydrolysis. The red bar indicates when reactor failure took place. SMY: specific methane potential.

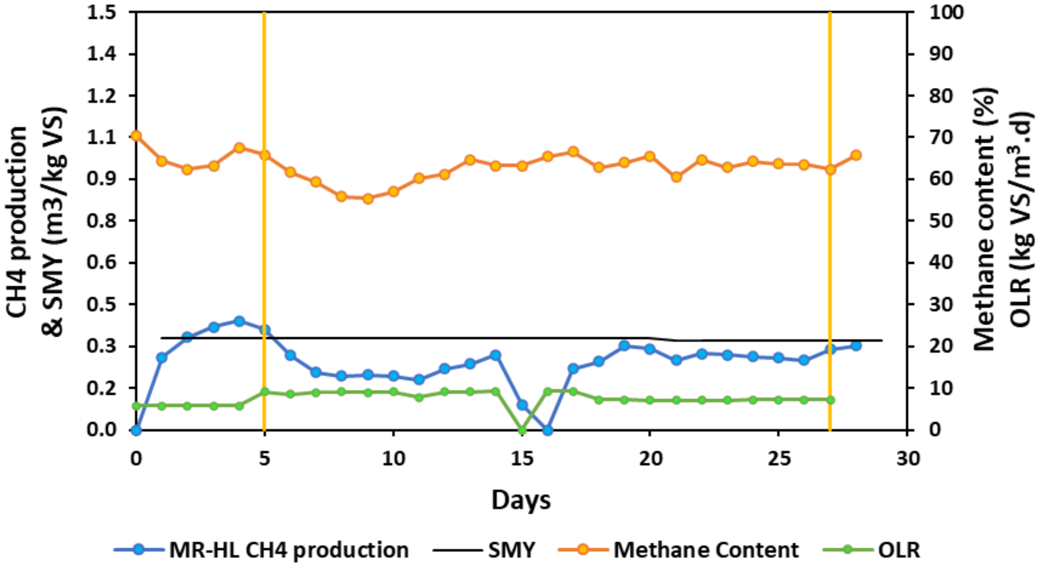

Figure 3. 6. Performance of MR-HL (mesophilic reactor, inoculated with good-quality wet mesophilic digestate from an AD plant treating manure and food and agricultural waste and fed with hydrolysed OFMSW) during its operation time (28 days), in terms of methane content and production. The end of the acclimation period is marked by the yellow bars, which delineate the period of stable feeding at 7.76 ± 1.90 kg VS/m^3.d. SMY: specific CH_4 potential.

In Experiment 3, the MR-HL reactor quickly acclimated to OFMSW. During the stable feeding period of 24 days at an OLR of 7.76 ± 0.13 kg VS/m^3 (SRT 34d), MR-HL had an average biogas production of 71.9 % of TBP (see **Table 3.3**). There was one severe foaming event (reactor in overpressure) 10 days after stable feeding at OLR 9.0-9.2 kg VS/m^3.d was initiated, with around 10 kg of digestate lost in overflow. Nevertheless, there was no reactor crash observed during the 28 days MR-HL was run. Methane content remained > 55 % throughout the experimental run, and > 60% after OLR was reduced from around 9 kg VS/m^3.d to around 7.2 kg VS/m^3.d. Methane production also improved with a decrease in OLR. MR-HL biogas and methane production was higher than that of MR. Average biogas methane productivity was higher in MR than MR-HL due to the differences in applied OLR (see **Table 3.3**). However, more data is necessary to confirm the accuracy of the means and if they are significantly different (at least data throughout 2-3 SRTs). MR also performed worse than MR-HL in terms of severe foaming frequency.

3.3.2.2 Digestate Quality

Changes in digestate quality throughout the operation time of MR and MR-HL are shown in **Figure 3.7**. MR digestate had high initial TAN and HAc concentrations of 4.04 and 2.54 g/kg, respectively. During the reactor acclimation period, HAc accumulated (while HPr and other VFA concentrations remained rather stable), until the reactor was supplemented with 5 kg of fresh digestate. Stable feeding at the target OLR was initiated 3 days after addition of fresh digestate. One week after supplementation, HAc concentration had dropped from 7.03 g/kg to 3.31 g/kg. Despite TAN concentration stabilizing at high levels (>5 g/kg), the FAN fraction remained between 0.26-0.48 g/kg throughout the experiment, due to the mesophilic operating temperature and pH of the digestate (7.61-7.87).

During the period of stable feeding in MR, HAc concentration stabilized between 3.31- and 4.14 g/kg, while propionic and isovaleric acid began accumulating (**Figure 3.7** B). Leading up to reactor failure, HAc accumulated two-fold to 7.610 g/kg, coinciding with a drop in biogas production and methane content. VFA continued accumulating until the end of the experiment, despite the reduced OLR, with HAc and HPr as the main contributors to tVFA content. Feeding with hydrolysed OFMSW was attempted to improve the digestate's physical properties (foamy

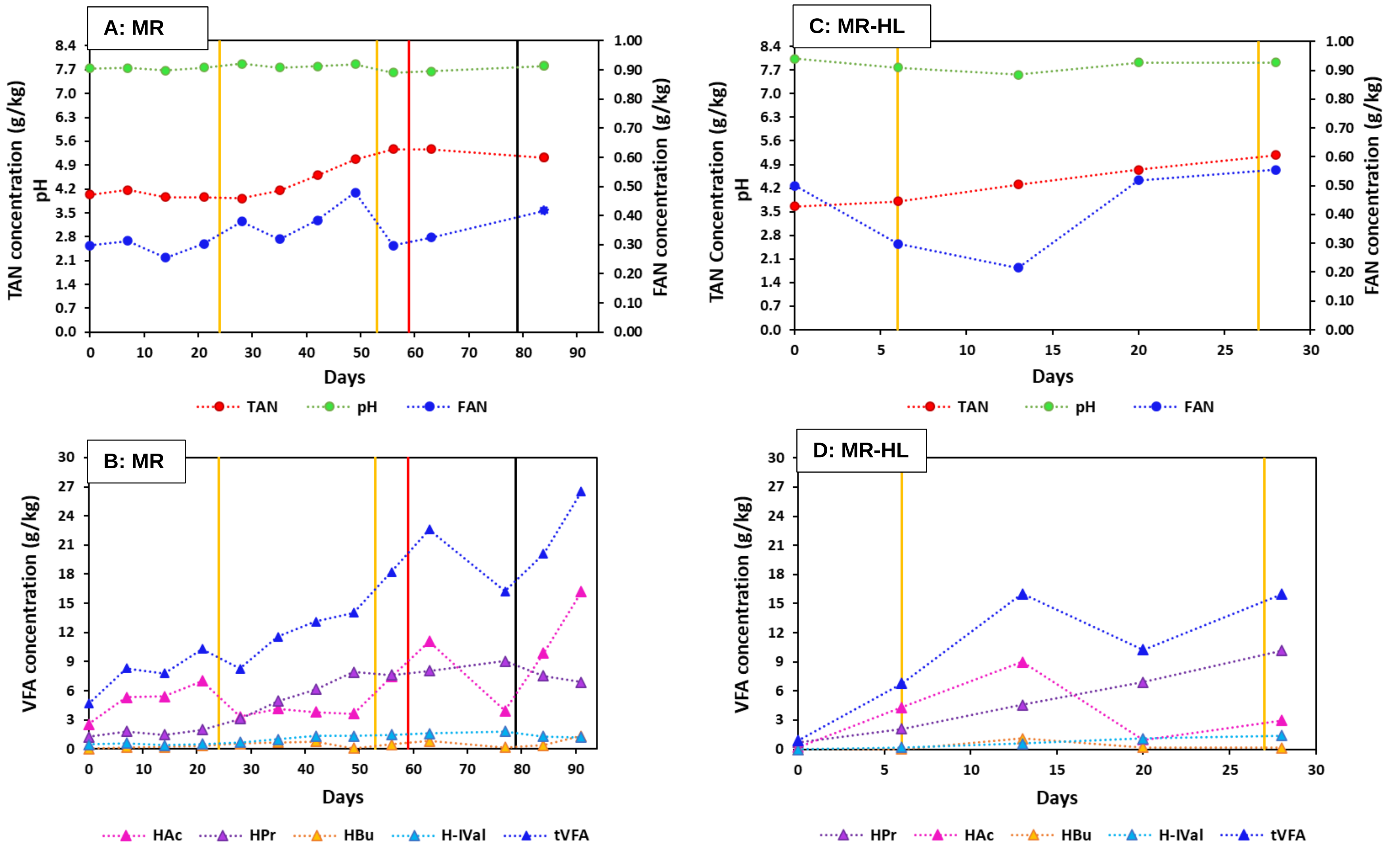

Figure 3. 7. Weekly digestate quality for the mesophilic reactors MR (Panels A and B) and MR-HL (Panels C and D), run to determine if mesophilic conditions mitigated ammonia toxicity. MR and MR-HL were both inoculated with good-quality wet mesophilic digestate derived from the same plant treating manure, food and agricultural waste. The end of the acclimation period is marked by the yellow bars, which delineate the period of stable feeding (9.17 kg VS/m^3.d for MR and 7.76 kg VS/m^3.d for MR.HL). The red bars indicate when reactor failure took place and the black bars the beginning of feed dilution. Panel A and C: Measured values for total ammonia nitrogen (TAN) and pH and calculated values for free ammonia nitrogen (FAN). Panels B and D: acetic acid (Hac), propionic aid (HPr), butyric acid (HBu), isovaleric acid (HI-VAL) and total VFA (tVFA) concentrations.

texture), but extreme foaming persisted. Aluminium (2060 mg/kg), chromium (37,1 mg/kg) and iodine (355 mg/kg) also accumulated in digestate so metal toxicity could be a cause of severe foaming.

Initial inoculum conditions in MR-HL showed low FAN (0.50 g/kg) and VFA content (0.914 g tVFA/kg). Its TAN concentration increased steadily up to 5.18 g/kg and pH remained between 7.56-8.04, with the calculated FAN fraction remaining between 0.21-0.55 g/kg. After 1 week of feeding at OLR 9.0-9.2 kg VS/m^3.d, there was a large accumulation of HAc and HPr. When average OLR was decreased to 7.29 ± 3.11 kg VS/m^3.d (days 15 to 19 of operation), following a severe foaming event, HAc decreased by a factor of 9, while HPr kept increasing (4.59 – 6.94 g/kg) (**Figure 3.7** D). From Day 20 (operation time) onwards, an average OLR of 7.12 ± 0.05 kg VS/m^3.d was applied (target OLR was reduced from 9.2 to 7.33 kg VS/m^3.d) and HAc accumulated once again, but to a lesser extent and HPr continued accumulating at the same rate.

The quality of DMFA was better when it was used to inoculate MR-HL than when it was used to inoculate MR. Initial VFA content in MR-HL was 4 to 5 times less than in MR (HAc concentration was 11 times lower); and initial FOS/TAC in the MR reactor was larger than that in MR-HL, indicating a higher risk for acidification in MR. After more inoculum was added to MR, its digestate quality was still inferior to that of MR-HL. When stable feeding was applied, propionate began accumulating from the first week of stable feeding in both reactors. In MR-HL, HAc also began accumulating linearly, along with HPr; while in MR, HAc only began accumulating after stable feeding had been stopped due to a decline in biogas production and methane content. FOS/TAC in MR-HL remained between 0.6 and 0.8 until the end of the experiment; while in MR, FOS/TAC reached 0.9 during stable feeding and values above 1 after reactor failure. Digestate in both experiments remained within a similar pH range, and TS and TAN content increased at similar rates (**Figure 7** [A & C], **Annex B.9**). FAN remained between 0.21 and 0.55 g/kg for both reactors. TS content did not stabilize during the reactors' operation time, so VS removal at stable conditions was not calculated.

3.3.2.3 Comparison between mesophilic and thermophilic conditions

Average biogas and methane production and productivity was higher in TR-MF (from Exp.1) than in both MR (Exp. 2) and MR-HL (Exp. 3). Foaming events in MR were more common than in TR-MF and MR-HL throughout the reactors' operation period. Both TR-MF and MR failed after a stable feeding period of more or less the same length. MR-HL was operated for a shorter time period, and no reactor failure took place.

Initial TAN concentration was higher in the mesophilic reactors than in TR-MF, stabilizing between 5.2-5.5 g/kg for the three reactors. Before the first failure of TR-MF, its pH and the calculated FAN fraction was higher than the pH and FAN in both mesophilic reactors. TS content in MR stabilized more quickly than in TR-MF due to its higher initial TS content. The adaptation of inoculum to OFMSW in TR-MF and both mesophilic reactors generated an increase in FOS/TAC values. The TS content in MR-HL did not stabilize during the experiment due to a shorter stable feeding period and a lower average OLR.

Acetic acid accumulated in both TR-MF and MR after 3 to 4 weeks of stable feeding; while in MR-HL, VFA accumulated from the first week of stable feeding. HPr accumulated in all 3 reactors from the first week that stable feeding was initiated. HPr accumulation preceded HAc accumulation in MR and TR-MF. In MR-HL, HPr accumulation continued even after HAc concentration decreased (after a reduction in OLR by around 20%). After reactor failure, changes in VFA concentration and FOS/TAC were similar for both MR and TR-MF. VFA decreased slightly when feeding was stopped, and steeply increased when feeding was resumed with diluted OFMSW. FOS/TAC increased after reactor failure, reaching values larger than 1 (**Annexes B.8** and **B.9**). Only MR developed a foamy digestate texture after failure (11 days after its first crash).

Like TR-MF, both mesophilic reactors performed better than TR-OF during stable feeding in terms of reactor performance and digestate quality. Even though the mesophilic reactors did not reach the TBP (156 Nm^3/t) during stable feeding, they consistently had a biogas production >100 Nm^3/t with methane content over 60%, unlike TR-OF. After reactor failure in MR, reactor performance and digestate quality deteriorated similarly to TR-MF (see **Sections 2.1-2.2**), resembling TR-OF, and then worsening.

3.3.3 Thermophilic co-digestion of diluted OFMSW and green waste

In Experiment 4, the CoTR-OF reactor was inoculated with DOF, the same inoculum used for TR-OF in Experiment 1 (sourced from the AD plant under study) and fed with a mixture of OFMSW and GW (3:1 ratio). Co-digestion was tested to assess whether it could mitigate NH_3 toxicity and improve reactor performance and digestate quality when compared to mono-digestion of OFMSW.

3.3.3.1 Reactor performance

CoTR-OF acclimated poorly to the applied feed loads, despite already being adapted to the organic waste. During the stable feeding period of 9 days at an OLR of 5.21 ± 2.73 kg $VS/m^3.d$ (1.3 dilution factor, SRT 40 d), CoTR-OF had an average biogas production of 65.0 ± 12.9 Nm^3/ton (41.8% of TBP). The reactor did not acclimate to the target OLR of 7 kg $VS/m^3.d$ On the third day of stable feeding (22nd day of reactor operation), there was a severe foaming event with the reactor in overpressure. The mixing cycle was then changed to provide continuous mixing and there was no reactor crash observed the days following the foaming event. Methane content remained > 57.3 % during the 9-day, stable feeding period. The daily performance of CoTR-OF throughout its operation is shown in **Figure 3.8** A.

3.3.3.2 Digestate Quality

The digestate used to inoculate the CoTR-OF reactor (DOF sourced, from the studied AD plant), contained very high HAc and HPr concentrations (4.08 and 4.96 g/kg, respectively). Given the inoculum was adapted to the OFMSW (and that the digestate was diluted with 2 L of water on day 4 of operation), TS content and TAN concentration in the digestate remained stable during the feeding of diluted OFMSW, between 19-21 % and 5.3-5.07 g/kg, respectively. VS removal averaged between 57.2 ± 2.3 % (see **Annex B.10**). Despite a 10-day period without feeding, total VFA content increased (from 10 to 16 g/kg). Therefore, when stable feeding of the diluted OFMSW was initiated, tVFA content kept increasing, and HAc concentration surpassed that of HPr (P/A ratio from 1.2 to 0.7). HPr concentration seemed to stabilize around 6 g/kg, while HAc kept accumulating until the end of the experiment (up to 9.11 g/kg). There was also a high initial NH_3 content of 1.14 g/kg in the digestate, which peaked at 1.46 g/kg after the first day of stable feeding. Changes in CoTR-OF digestate quality are illustrated in **Figure 3.8** [B & C].

3.3.3.3 Comparing thermophilic co- and mono-digestion of OFMSW

CoTR-OF was fed at an OLR 1.8 times smaller than TR-OF. Under these feeding conditions, CoTR-OF showed better reactor performance than TR-OF in terms of methane production (Nm^3/kg VS) and CH_4 content in biogas ($p < 0.05$). On the other hand, average methane productivity in TR-OF was larger than that in CoTR-OF (**Table 3.3**). The number of

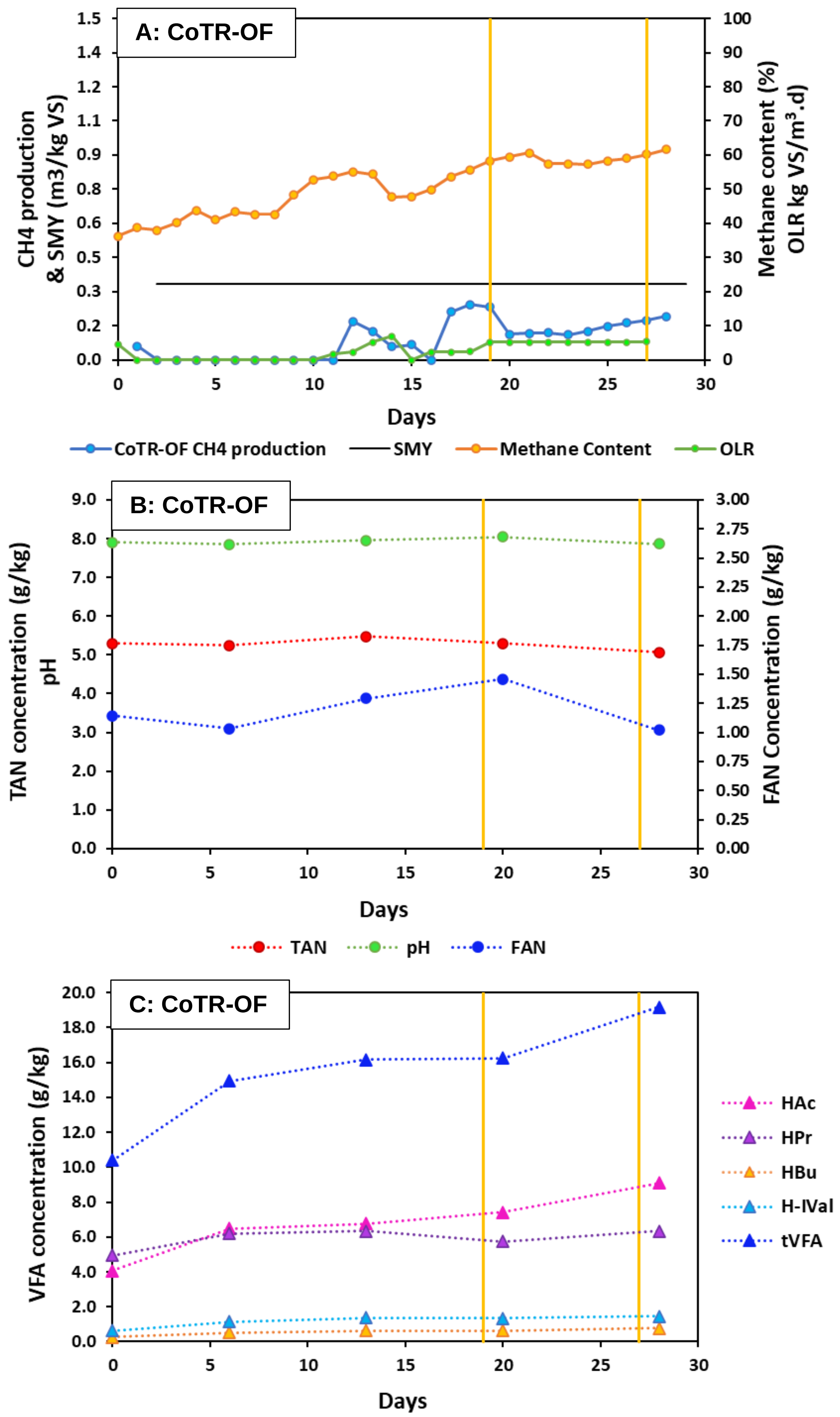

Figure 3. 8. Performance of the reactor CoTR-OF during its operation time (28 days). CoTR-OF is the thermophilic reactor inoculated with digestate sourced from the anaerobic digestion plant under study and run to assess the effectiveness of co-digestion in the mitigation of ammonia toxicity (using green waste and the household organic waste treated by the plant under study at a 1:3 ratio). The yellow bars delineate the period of stable feeding at 5.21 ± 2.39 kg VS/m^3.d. Panel A: Reactor performance in terms of methane content and production. Panel B: Measured values for total ammonia nitrogen (TAN) and pH and calculated values (Equation X) for free ammonia nitrogen (FAN). Panel C: acetic acid (Hac), propionic aid (HPr), butyric acid (HBu), isovaleric acid (HI-VAL) and total VFA (tVFA) concentrations. SMY: specific CH_4 potential.

data points from these reactors is small, so more tests would be needed to confirm normal distribution of the data and better compare group means. Both reactors inoculated with Uvélia's digestate had high initial tVFA, TAN and FOS/TAC levels. FOS/TAC increased from between 0.6-0.8 to values larger than 1 for both reactors. Total VFA also kept increasing until the end of the experiments. TAN concentration in Co-TR-OF had a decreasing trend, contrary to that in TR-OF, due to feed dilution in the former.

3.4. Comparison of full-scale and pilot-scale AD performance

It was not possible to carry out pilot simulations under similar conditions to the full-scale plant given the important fluctuations in the daily feed loads employed and the lack of information concerning feedstock dilution in the full-scale plant. Additionally, it was not possible to stably feed any of the pilot reactors for more than 30 days, thus stable operation at steady state was not achieved (in terms of both feed load and digestate composition), making it more difficult to precisely compare reactor performance between the 2 scales.

Moreover, average monthly SRT in the full-scale plant, taking into account undiluted OFMSW, was always more than 35 d (OLR < 7.5 kg VS/m^3.d), with an average SRT of around 48 d for the year 2020. Average daily feed load was always below the target load. On the other hand, most pilot simulations were carried out at an SRT of 30 d to understand reactor performance when the target SRT is applied. In the sections below, reactor performance and digestate quality of the full-scale process is compared with the pilot simulations.

3.4.1. Comparison between full-scale digesters and pilot reactors TR-OF and CoTR-OF

TR-OF and CoTR-OF were inoculated with digestate sourced from the full-scale AD plant under study. However, in terms of reactor performance, biogas production was much lower in both reactors during stable feeding than in the full-scale. Uvélia's D1 and D2digesters showed an average biogas production of 121-136 Nm^3/t (CH_4 production of 0.268-0.285 Nm^3/kg VS) for average OLRs between 5.3-4.8 kg VS/m^3.d. On the other hand, TR-OF and CoTR-OF had an average biogas production of 62.5 and 65 Nm^3/t (CH_4 production of 0.122 and 0.140 Nm^3/kg VS) for average OLRs of 9.2 and 5.2, respectively. Average methane content in TR-OF during stable operation was lower than the average methane content in D1 and D2, while CoTR-OF had a similar average to D1 and D2. Uvélia's reactors also showed a higher methane productivity (~2.8 Nm^3/m^3.d) than both pilot reactors (0.7 and 1.1 Nm^3/m^3.d) (**Tables 3.2**-**3.3**). Nevertheless, a longer period of operation under stable feeding in the pilot scale (at a sustainable OLR) is necessary to confirm the accuracy of the means and enhance their comparability to the means of the full-scale reactors.

TR-OF and CoTR-OF initial digestate quality was the same as in the full-scale process. However, while the reactors were acclimating to the feed load, FOS/TAC (measured weekly) increased in both reactors to values higher than those generally observed in Uvélia (**Annexes B.7, B.8** and **B.10**). In Uvélia (in 2020), average monthly FOS/TAC (from daily measurements) falls below 0.9, with values larger than 1.0 observed with more frequency only in the month of October. These results show that buffering capacity not only remains compromised in the pilot-cale process due to high VFA content, but also develops an even more reduced buffering capacity compared to the full-scale process. The pH of pilot scale reactors was close to Uvélia's average pH of 7.95 ± 0.12. The pH in TR-OF fell by 0.2 units only after reactor failure. TS content of TR-OF was between 24 to 25 % before and during stable feeding. This is close to the average TS in D1 in 2020 (24 ± 2%), despite D1 having been fed with OFMSW diluted with process water and waste percolate. The TS content in CoTR-OF was lower than Uvélia's average TS content, given the feed was diluted. Dilution of OFMSW by a factor of 1.5 led to

TS stabilization around 19.22%, and dilution by a factor of 1.3 led to stabilization around 20.8%, due to low biogas production.

3.4.2 Comparison between full-scale digesters and pilot reactors TR-MF, MR & MR-HL

During their stable feeding period, TR-MF, MR and MR-HL (biogas production 70-99% of TBP) had a lower biogas production than D1 and D2 (107-108% of TBP) when the latter were fed at an OLR of ~7 kg VS/m^3.d on the month of February in 2020. An OLR of 7 kg VS/m^3.d was the largest one reached in Uvélia in 2020, which is lower than the OLRs applied for TR-MF, MR and MR-HL. However, the mean CH_4 production of TR-MF was 111 % of SMY, larger than the average for D1 and D2 (100 % of SMY at ~ 7 kg VS/m^3.d). The means for methane content in biogas of TR-MF, MR and MR-HL were also larger than that in D1 and D2 (again at OLR ~7kg VS/m^3.d), with a confirmed significant difference between TR-MF, D1 and D2 ($p < 0.05$). However, TR-MF and MR failed and MR-HL was terminated before pseudo-steady state was reached (i.e., a plateau in TS content and TAN). While MR and TR-OF had one or more instances of reactor failure, characterized by methane content dropping below 50%, in the full-scale, only one potential instance of reactor failure was found in 2020, for D1 (where its biogas had low CH_4 content for 5 days [data not shown]). Severe foaming was also a common event in the pilot simulations, however Uvélia's staff did not report issues with foaming in the ful-scale.

Biogas and methane productivity was also significantly different between TR-MF, D1 and D2 reactors ($p < 0.05$), with TR-MF achieving a much higher biogas productivity (5.1 ± 0.5 Nm^3/m^3.d) than the desired 3.79 Nm^3/m^3.d. MR and MR-HL also had an average biogas productivity higher than the full-scale reactors, despite a lower production. Biogas productivity in MR and MR-HL was 3.6 ± 0.4 and 3.1 ± 0.4 Nm^3/m^3.d, respectively; and in D1 and D2, 2.76 ± 0.93 and 2.82 ± 0.88 Nm^3/m^3.d, respectively. The mesophilic pilot reactors' data set was not analysed for a significant difference with the data from D1 and D2 (February 2020) due to the reduced sample size of MR-HL and the frequent outliers in MR (due to constant technical problems during its operational period).

TR-MF, MR and MR-HL were all inoculated with wet digestate with initial TS < 7.5% and FOS/TAC of 0.24, 0.42 and 0.25, respectively. The initial digestate quality of TR-MF and MR-HL, in terms of TS content and FOS/TAC, is similar to the initial digestate quality in D1 and D2 (TS of 6 %, FOS/TAC 0.2). Uvelia's digesters reached a steady-state TS content after 5 months of feeding at dynamic loading rates. With stable feeding at an average OLR of 9.17 kg VS/m^3.d, TS accumulation took place at a faster rate in the pilot reactors. After only 4 weeks of stable feeding, TS content in TR-MF and MR-HL reached ~19 % and MR reached ~23%.

In the full-scale, average FOS/TAC in D1 surpassed 0.4 after 3 months of feeding and D2 after only 1 month. MR-HL and TR-MF surpassed this FOS/TAC ratio after only 14 and 42 days of operation, respectively; while MR had a ratio above 0.4 from the beginning. MR-HL and the full-scale reactors stabilized at FOS/TAC values between 0.6 and 0.9, while in TR-MF and MR, FOS/TAC showed a marked increase after reactor failure. In TR-MF, FOS/TAC kept increasing steadily up to 2.15 after feeding of diluted OFMSW was initiated (see **Annex B.8**). Despite probable differences in feed load (average feed load during inoculum adaptation in the full-scale is unknown), inoculum adaptation to OFMSW in both scales saw a deterioration in digestate quality, in terms of FOS/TAC.

The initial pH in TR-MF was 0.3 magnitudes higher than the initial pH of D1 and D2. TR-MF pH remained between 8.15-8.26 until reactor failure, while the mean pH of D1 showed an increasing trend in a 4-month period, from 7.69 to 8.02. While the average pH of D1 remained between 7.9 and 8.0 in 2020, the pH in TR-MF began decreasing steadily after the first reactor failure, down to 6.98. In MR and MR-HL, pH was the same or lower (7.56-7.93).

CHAPTER 4. DISCUSSION

4.1. Case Study (Uvélia): AD process performance

4.1.1 Operational performance of the Uvélia AD plant

There were large deficits in treatment capacity by Uvélia's AD plant in 2020. Only 20 271 tons of OFMSW were treated, which is 63 % of the planned capacity. This led to a deficit in biogas generation of 971 435 Nm^3 (528 194 Nm^3 CH_4). Assuming an energy content of 9.67 kWh per 1 Nm^3 CH_4, this translates into an energy production deficit of 5.11 GWh (or 51 % of the installed capacity). The reason for the reduced capacity is technical issues with the digestate dryer, preventing it from operating at full capacity (98 t/d). On the other hand, average biogas and CH_4 production was close to or larger than the OFMSW's TBP (>95 %) (**Table 3.2**). However, given the fluctuating feed loads (dynamic feeding), there is a large standard deviation in the data set for biogas production, meaning that daily biogas production (Nm^3/t.d) is actually <80 % of the TBP over half the time in both D1 and D2 in 2020, with the largest daily production values achieved when feed load is <30 t/d (or <68 % of the target load). To meet productivity and TS content targets (without feed dilution), biogas production must be >96 % of the average TBP.

Considering the data and information gathered in this study, the designed SRT and feed load do not match with each other. The target OFMSW treatment capacity of 32 000 t/year, or 88 t/d, implies an SRT of 30. The current target SRT of 21 days would allow the treatment of 45,886 t/year of waste (126 t/d). Granted, SRT not only depends on the feed load, but also on the water provided for dilution and on the digestate recycled. Assuming a biogas production of 90% of the TBP (~123 Nm^3/ton), TS would stabilize at 26 % if feed is left undiluted. Since the target TS in digestate is 23-25 %, the minimum amount of process water to be added is 1000 t/y, and the maximum 3300 tons. Thus, average SRT would be between 27-29 d with dilution, which is still larger than 21 d. Even if digestate recycling is considered at loads estimated by this study (based on the material flow analysis report from 2017), the estimated amounts of recycled digestate correspond to the expected mass reduction of the feed, having a null impact on SRT. In any case, digestate is currently only recycled seldomly, and applying a daily recycle is not planned in the short or medium-term.

It would be easier to determine the average SRT for the process if stable feeding were applied, rather than a dynamic one (i.e., feed loads that vary by more than 5 tons from day to day, causing a large variance in OLR). In this way, there would be less fluctuations in biogas production and TS content from day to day, potentially making it easier to meet daily targets in reactor productivity and to maintain the target TS content. Additionally, goals pertinent to digestate recycle should also be well-defined so pilot simulations can be better tailored to the desired operational parameters. The SRT applied by Uvélia on 2020, as well as the targeted SRT, were within the range of those applied in literature for full-scale and pilot scale studies (14.5 - 50 d) (**Annex A.3**). OLRs applied in literature for the same scales ranged between 0.2 - 6.2 kg VS/m^3.d. Uvélia's applied feed load in 2020 was within this range, while their targeted OLR is larger, at 8.8 kg VS/m^3.d. This shows that the OFMSW treated by Uvélia has a rather high VS content compared to the wastes previously tested in literature at the same (or shorter) SRTs. This reflects differences in OFMSW sorting practices or in the composition of organic waste in the different regions from where OFMSW was sampled.

Regarding CH_4 production from the waste, the average CH_4 production in Uvélia (0.292-0.295 Nm^3/kg VS.d, 77 Nm^3/t) is difficult to compare to other studies, given operational parameters, such as OLR and temperature, differ from study to study. Average CH_4 production is also be affected by differences in the SMY of the OFMSW used in different studies. For example, T.a.s et al. (2020) achieved a higher CH_4 production than Uvélia under mesophilic conditions (0.37-0.39 m^3/kg). However, the SMY of the OFMSW they used (0.515 m3/kg $VS_{consumed}$) was also higher than the SMY determined for the OFMSW treated by Uvélia (0.306 Nm^3/kg VS). Therefore, in terms of fulfilling the OFMSWs' SMY, Uvélia's AD performed better by achieving 95 %. These variances from study to study show, once again, that carrying out optimization studies for existing plants will yield more applicable results than studies that do not simulate an existing process.

4.1.2 Digestate quality

The average TS content in D1 digestate for the year 2020 (24 ± 2 %) was similar to the calculated final TS content based on biogas generation and feed load for the same year (25 %), with an average biogas production of 132 Nm^3/t. This shows that the amounts of water used to dilute feed in 2020 did not have a great effect on TS content nor SRT. Also, if daily biogas production could be maintained at levels equal to or higher than the average TBP (137 Nm^3/t), the level of VS degradation would allow TS to remain below 25% without feed dilution.

The observed increase in pH during acclimation and the stabilization of TAN at large values is consistent with the fact that OFMSW is high in TKN, with a low C/N ratio of 17. Some studies on the mono-digestion of OFMSW have also reported similar or lower C/N values for OFMSW (Nguyen et al., 2017; Yirong et al., 2017), however there have also been cases in which OFMSW has been found to be on the higher end of the optimal CN ratio (Mosquera et al., 2020). The average FOS/TAC values in D1 (**Annex B.7**) were larger than 0.4-0.5, with an average of 0.758 ± 0.138 in 2020, indicating process instability in both digesters (Riau et al., 2010; Scano et al., 2014).

4.2. Pilot simulation of the thermophilic digestion of OFMSW in Uvélia

4.2.1 TR-OF

TR-OF digestate showed inhibited biological activity, with operation at an SRT of 30 d (9.2 ± 0.4 kg VS/m^3.d) leading to VFA accumulation and increased reactor instability. Digestate quality and biogas production were poor from the beginning. The cause of poor performance was probably acetoclastic methanogen (AM) inhibition by ammonia toxicity. Inhibition thresholds of FAN identified in literature are between 0.03-1.45 g/L (Puig-Castellví et al., 2020). Therefore, ammonia toxicity is likely to happen at the high FAN concentration reached (1.5 g/kg). Furthermore, the reactor crash was observed within 8 days after reaching a peak in FAN of 1.6 g/kg (pH 8.1 and TAN 5.5), which might have further inhibited AMs (Capson-Tojo et al., 2017; Puig-Castellví et al., 2020).

The poor biogas production from TR-OF (36 % of TBP) caused by ammonia inhibition made organic overloading and VFA accumulation more likely to occur at an SRT of 30 d, which was indeed the case (**Figure 3.2**). However, the simulation showed that it was still possible to produce biogas with >50 % methane content in the inhibited state, attributable to the activity of hydrogenotrophic methanogens (Puig-Castellví et al., 2020). Stable feeding under the biology's inhibited state might be possible at lower OLRs, given OLR reduction or SRT increase is a common ammonia toxicity mitigation strategy reported in literature (Giménez-Lorang et al., 2021; Jiang et al., 2019).

When comparing TR-OF digestate quality to that of Uvélia's D1, results support that the amount of water mixed with feed in the full-scale plant was not too high and did not greatly affect TS or TAN content. Average TS content in D1 was 24 ± 2 % in 2020 (**Annex B.7**), which is similar to that in TR-OF throughout its operation (24-25 %) (until digestate from TR-MF was fed from Day 34 onwards) (**Annex B.8**). Therefore, the process water and percolate added to the digesters in Uvélia did not have a significant diluting effect on the components of digestate.

From these observations, it can be concluded that TAN concentration in D1 can be estimated to range between 4.7-6.8 g/kg, based on the average TKN in the OFMSW (21.5 ± 4 g/kg).

Taking into account the pH in D1 averaged between 7.9-8.0 (**Annex B.7**), the estimated FAN concentration in D1 is high enough to cause the same inhibitory mechanisms inferred from Experiment 1. However, Uvélia's digesters show less inhibition than TR-OF in terms of reactor productivity and methane production (see **Tables 3.2** and **3.3**), despite similar TAN, FAN and pH. As explained above, this is probably due to the fact that in 2020, D1 and D2 were run at a lower OLR (larger SRT) than TR-OF. Still, the TR-OF reactor behaviour gives insight into the potential outcomes of applying the targeted feed load in the full-scale plant.

4.2.2 TR-MF

From the beginning of stable feeding, TR-MF showed signs of reactor instability. HPr acid accumulation, accompanied by the increase in FOS/TAC above optimal ratios, pointed to the onset of process instability after the first week of stable feeding. However, literature reports that HPr accumulation can be an indicator of various different disturbances, including organic overloading and inhibition by toxic compounds (Capson-Tojo et al., 2017; Gourdon and Vermande, 1987). The data supports that ammonia toxicity, rather than organic overloading, caused reactor failure and subsequent process inhibition. From day 1 to 20 of stable feeding (9.2 ± 0.1 kg VS/m^3), acetic acid concentration and pH remained rather stable (1.2-1.8 g/kg of acetic acid and pH 8.1-8.3). At the same time, an elevated FAN level between 1.3-1.4 g/kg, compared to an initial level of 0.86 g/kg, was sustained for around 3 weeks. As mentioned above, these concentrations are well over ammonia toxicity thresholds (Cavinato et al., 2017; Kang et al., 2021; Puig-Castellví et al., 2020). After this period, HAc began accumulating and pH began declining (**Figure 3.4 D**). Given the high pH of the reactor (>8.0) and stable HAc levels during 21 days, it is more plausible that VFA accumulation in TR-MF was caused by high FAN levels, rather than reactor overloading and VFA toxicity. Therefore, it can be hypothesized that under thermophilic conditions, TR-MF would have been able to maintain stability and good performance at the applied OLR, if the OFMSW had a lower TKN content. This is supported by studies performing the AD of OFMSW under thermophilic conditions, which have found that optimal CH_4 production is be achieved at OLRs of up to 8.6 and 12 kg VS/m^3.d (Fdez.-Güelfo et al., 2011; Nguyen et al., 2017). In these experiments, TKN concentration in the OFMSW used was much lower than its concentration in the OFMSW treated by Uvélia.

The steady increase in HPr preceding the steep increase in HAc is consistent with the effects generated by ammonia toxicity. Additionally, the steady state HAc concentration maintained during 3 weeks was much higher than the initial levels of HAc in the digestate. FAN mainly inhibits AMs, so at the onset of inhibition they might continue degrading HAc, but at a slower rate, making the HAc steady state concentration higher. Moreover, more substrate is available for synthrophic acetate oxidizers (SAO). When SAO degrade acetate, they produce H_2 and increase its partial pressure. The increase in HAc and H_2 shifts HPr equilibrium to the left (i.e., less HPr will be degraded), given they are both products of HPr degradation. Both syntrophic HPr and HAc degradation are thermodynamically unfavorable, so HAc degradation rates by SAO are slower than rates by AM (Capson-Tojo et al., 2017). Acetoclastic bacteria (AB) are also inhibited at high FAN concentrations (Cavinato et al., 2017), so when AM and AB inhibition surpasses a certain threshold, it could be the case that acetic acid quickly accumulates, as it took place in TR-MF.

After reactor failure, TAN levels continued to increase, but FAN decreased given the drop in pH. Digestate quality did not improve substantially after the period of reactor starvation, despite FAN levels falling to generally non-inhibitory levels (< 0.7 g/kg). The reason for this is conceivably due to VFA levels and pH generating conditions that cause VFA toxicity to kick in (days 49 – 55 in **Figure 3.4 D**). As pH decreases, the fraction of undissociated VFAs increases and their inhibitory effect on the digestate's microflora is intensified; and as VFAs accumulate, the undissociated fraction is more likely to reach inhibiting levels (Deublein and Steinhauser, 2008).

4.2.3 Comparison between TR-OF and TR-MF

Experiment 1 showed that the full-scale process will be more prone to instability, failure and a compromised biogas production if feed load is ramped up from the average of ~28 t/d (~5.5 kg VS/m^3.d) per reactor to 44 t/d per reactor (~8.8-9.2 kg VS/m^3.d). Results of TR-OF and TR-MF performance and digestate quality demonstrate that ammonia toxicity was the main inhibitory mechanism in the thermophilic AD process (see **Figures 3.2-3.4** & **Table 3.3**). These initial observations indicate that either a lower OLR should be applied in Uvélia's plant, as is currently the case, or the TKN of the substrate needs to be somehow reduced or balanced to avoid ammonia accumulation.

4.3. Effects of optimization strategies on pilot reactor performance

The optimization strategies applied in the pilot simulations focused on the mitigation of ammonia toxicity in the AD process, following the results obtained in Experiment 1. Applied strategies were: (i) dilution, (ii) mesophilic operation, (iii) substrate hydrolysis, and (iv) co-digestion to improve C/N.

4.3.1 Dilution

Three weeks after TR-MF failure, feeding of diluted OFMSW was initiated. However, VFA accumulation continued and the reactor had poor biogas production. Due to the inhibited biogas production, TS content climbed up to 23 % instead of stabilizing at 16%, when the target dilution factor of 1.3 was applied (reducing OFMSW TS content to 28 %). This indicates that methanogens did not have enough time to recover from ammonia toxicity before feeding was resumed, or that the toxicity event was too severe for recovery to be possible. In fact, (Kayhanian, 1999) warns that, if the methanogen population is severely inhibited, it is difficult to restore reactor by increasing C/N ratio. According to the results observed, this applies to dilution as well.

TAN concentration steadily decreased with dilution; however, it was difficult to maintain it below the target threshold of 4.2 g/kg. This might be due to the heterogeneity of the waste. In any case, reducing TAN concentration did not have a large impact on process stability. This is because when dilution was started, the FAN fraction was already dropping quickly due to VFA accumulation and acidification of the reactor. To properly assess whether dilution could have restored the reactor's activity, it should have been started soon after reactor failure. In **Figure 3.4 C** and **D**, it can be observed that the steep increase in HAc caused pH to drop considerably, along with FAN concentration (well below inhibitory levels). However, the levelling off of HAc concentration allowed for pH to increase once again, causing FAN concentration to shoot up. The renewed ammonia toxicity followed by the steady drop in pH might have contributed to the stunted degradation of HAc and the other VFAs.

4.3.2 Mesophilic Conditions

The mesophilic reactors, MR and MR-HL, had a lower biogas and methane productivity and production than TR-MF during their respective stable feeding period. TR-MF was also able to handle a larger OLR. However, mesophilic reactors performed much better than TR-

OF, which was run at the same temperature as TR-MF. This contradiction is explained by the fact that thermophilic digestion should generally lead to higher biogas production and productivity than mesophilic digestion (as was the case for TR-MF), due to faster reaction kinetics at higher temperature, but that it also has a higher risk of instability (affecting TR-OF) (Rocamora et al., 2020). In this case, instability is due the enhanced inhibitory effects of a high-N substrate, since there is a larger fraction of FAN at higher temperatures (Kang et al., 2021; Yirong et al., 2017). Interestingly, results from **Section 1.2** show that mesophilic experiments yielded a higher optimal methane production when compared to thermophilic ones. Even in optimization experiments where temperature conditions were tested, the mesophilic temperature was chosen as optimal in about half of the cases (see **Annexes A.1** and **A.2**). This might be a recurring trend in the AD of OFMSW due to ammonia toxicity mechanisms. Average methane production for MR and MR-HL was between 1.1 to 2 times lower than values reported in literature (**Annex A.1** and **A.2**).

Under mesophilic conditions, ammonia toxicity was not one of the inhibiting factors in the AD process. Calculated FAN concentration in MR and MR-HL remained between 0.3-0.52 g/kg, which is well below the inhibitory FAN concentration observed for mesophilic AD and acetoclastic bacteria (0.7 g/kg), and for TR-MF and TR-OF (Cavinato et al., 2017; Kang et al., 2021). Yirong et al. (2017) also managed to maintain stable performance of mesophilic reactors at a FAN concentration of 0.45 g/kg. Nevertheless, there have indeed been cases in which the inhibition of the AD process has been observed at lower concentrations than 0.5 g/kg (Puig-Castellví et al., 2020). Nevertheless, FAN concentrations throughout the experiments remained close to the range of the initial FAN concentration in the digestate (0.3-0.5 g/kg), therefore it is unlikely that it induced ammonia toxicity and inhibition.

Additionally, the MR reactor foamed intensively and failed after 1 month of stable feeding, accompanied by VFA accumulation, confirming some type of inhibition was still at play. MR had a steady state HAc concentration 3 times higher than that in TR-MF during stable feeding at the same OLR (SRT 30 d). Moreover, HPr accumulated to concentrations 1-3 units higher in MR during stable feeding (**Table 3.3**). This indicates that the applied OLR led to the organic overloading of MR. Given MR had a lower pH than TR-OF during this period (**Table 3.3**), the higher HAc and HPr concentrations might have led to VFA toxicity and reactor failure (Alavi-Borazjani et al., 2020; Deublein and Steinhauser, 2008).

At one point after failure, the digestate in MR adopted a completely foamy texture. This is a sign of massive microbial die-off, as the proteinaceous compounds released from dead cells generate foam (Moeller and Görsch, 2015). Metal toxicity was unlikely to be the cause for cell die-off, given metal content in MR was generally similar to that in TR-OF and lower than in the full scale digestates (aside from Cr and Ni), and these reactors did not have the same issue (**Annex B.3**). Out of the heavy metals analysed, Cr achieved the largest up-concentration in MR (2.4), but its concentration was 34 g/kg, which is not reported to be toxic (Alkan et al., 1996). Another cause of severe foaming could be the hydrolysis reactions and surface-active intermediates generated during the break-down of OFMSW, such as biosurfactants (produced by microbes for metabolism) (Ganidi et al., 2009). VFA accumulation has also been shown to increase the possibility of foaming in the AD of OFMSW (Kong et al., 2019). TR-MF was fed with the same OFMSW as MR and also reached a similar metal and VFA content (>20 g/kg) after failure, yet it did not adopt a foamy texture. Therefore, differences between TR-MF and MR in other aspects, such as process temperature and TS content, might have played a role in digestate dynamics and exacerbated foaming in MR. Feed hydrolysis was attempted in the next mesophilic experiment (Exp. 3) as a foam mitigation strategy.

4.3.2.1 Feed hydrolysis

In Experiment 3, the stable feeding of hydrolysed, undiluted OFMSW to the mesophilic reactor (MR-HL), at an OLR between 9.0 - 9.2 kg VS/m^3 (SRT 30-31 d), led to VFA accumulation. This confirms that these OLR levels lead to the organic overloading of the mesophilic biology. A sharp decrease in biogas production followed by a severe foaming event pointed to imminent reactor failure. In this case, the reduction of OLR to ~7.3 kg VS/m^3.d prevented reactor failure and restored digestate quality (specifically, a reduction in HAc), process stability and reactor performance (**Figures 3.6** and **3.7 D**). Steady state biogas productivity decreased with the reduction in OLR (while biogas production increased), but methane productivity improved marginally due to an increase in methane content (see **Figure 3.6** and **Annex C.1**). Lower OLRs might allow to maintain the stability in mesophilic AD, without leading to losses in productivity and energy production. Furthermore, the hydrolysis of the feed seemed to mitigate foaming, with MR-HL being in overpressure due to foaming only once, while MR was in overpressure at least 5 times within a similar time frame.

To confirm the latter conclusions, a longer simulation (2-3 SRTs) under the same conditions must be carried out to monitor (i) VFA behaviour and their impacts on reactor performance and (ii) foaming frequency. For instance, HPr concentration continued increasing after OLR was reduced. Studies report that HPr accumulation is an early-warning indicator for a process disturbance that can lead to reactor failure or impaired performance if not addressed (Gourdon and Vermande, 1987), which is supported by results from this study. A longer simulation time would also allow to better understand which mechanisms cause foaming and whether hydrolysis will sufficiently mitigate it. MR-HL was only run for 0.7 SRTs (24 d) in this study due to time constraints, given it was the last experiment run along with Exp. 4.

The significance of future mesophilic, hydrolysis experiments can be further improved by running a control reactor in parallel to the test reactor. In this way, digestate from the same representative sample can be used. This setup would allow to determine to what extent hydrolysis (i) improves process stability and (ii) mitigates foaming when lower OLRs are applied. In this study, the digestate used to inoculate MR and MR-HL were sourced from the same plant. However, the experiments were run in series and the digestate was obtained from two different sampling events on different months. Therefore, the digestate composition differed, given the seasonal variations in wastes. The digestate used to inoculate MR had a worse initial quality than MR-HL's inoculum (**Annex B.2**), which might have affected reactor behaviour during stabilization.

4.3.3 Co-digestion

Co-digestion with GW was tested to increase the substrate's low C/N ratio (C/N 17), given this has been shown to dilute N and mitigate ammonia toxicity (Jiang et al., 2019). GW was chosen because it is a co-substrate readily available to Uvélia and garden waste has been reported to have a high C/N ratio of 50 (Brown and Li, 2013). Co-digestion in Experiment 4 did not mitigate ammonia toxicity in the AD process, nor improve overall performance. Reactor performance and digestate quality in Co-TDOF did not improve compared to TR-OF. It was not possible to acclimate CoTR-OF to the target OLR (~ 7 kg VS/m^3.d) during its operation period, with 5.21 kg VS/m^3.d being the maximum OLR applied. Although CoTR-OF maintained a stable biogas production and high methane content at this OLR, biological activity showed signs of significant inhibition. Biogas was only generated at levels below 50% of the TBP of OFMSW (the TBP of the co-substrate mixture was not determined, but is assumed to be more or equal to that of OFMSW).

Reactor overloading and VFA toxicity was once again dismissed as the main cause for inhibition for various reasons. Firstly, TR-MF showed that, under thermophilic conditions, HAc can be degraded without signs of accumulation (before reactor failure) at almost double the OLR applied in CoTR-OF (**Figure 3.3**). Therefore, it is very unlikely that the reason for high VFA concentrations and VFA accumulation in CoTR-OF is organic overloading. Secondly, VFA concentrations remained rather stable throughout the experiment with only minor accumulation taking place, which can be explained by the NH_3 toxicity mechanisms described in **Section 4.2.1**. Thirdly, HPr does not show an inhibitory effect on the AD process up to a concentration of 6g/l at pH >7.4 and HAc is inhibitory at >6 g/kg at pHs >7.5 (Deublein and Steinhauser, 2008; Gourdon and Vermande, 1987). At pH 7.7, the undissociated fraction of the VFAs is 3 magnitudes lower than their total concentration (Xiao et al., 2013). Given the digestate's high pH (>7.8) and HAc and HPr concentrations in digestate, VFA toxicity was unlikely to happen (see **Figure 3.8 B** and **C**).

It was expected that ammonia toxicity would not be significantly mitigated by OFMSW co-digestion with GW, since it had a C/N ratio of 21.2, which is also lower than the optimal range of C/N (25-35) (Kayhanian, 1999; Shahbaz et al., 2020). The C/N of the co-digestion mixture was 18, which is only marginally higher than that of the organic waste, 17 (**Annex B.1**). Higher C/N ratios have been reported for GW in literature, but it depends on the woody fraction in the waste. However, these ratios remain around 55, therefore C/N improvements require a high GW:OFMSW ratio (Brown and Li, 2013).

<u>4.3.4 Comparison of the tested strategies</u>

Mesophilic conditions with feed hydrolysis, applied in Experiment 3, proved to be the most successful strategies out of the ones tested. Strategies applied in Experiments 1, 2 and 4 did not improve process stability nor digestate quality. Together, Experiment 2 and 3 showed that under mesophilic conditions, organic overloading takes place at the targeted OLR (9.2 Kg Vs/m^3.d). Experiment 4 showed that co-digestion with low-C/N green waste can improve CH_4 content in biogas during acclimation. Finally, results from Experiment 1, 3 and 4 confirmed that digestate from the full-scale digesters (D1 and D2) was of poor-quality given reactors inoculated with it required a long acclimation period before applying the targeted OLR. On the contrary, TR-MF and MR-HL responded well from the beginning to the initial feed loads applied during the acclimation period (see **Figures 3.3** and **3.6**, respectively). These reactors were inoculated with digestate sourced from different plants, which were known to be of good quality (i.e., low FOS/TAC and low VFA content). The effects of the applied optimization strategies in Experiments 1-4 are discussed in more detail in the sections below.

The NH_3 acclimation strategy was not tested in the pilot scale because it was assumed that the digestate sourced from Uvélia had already gone through this process. (Puig-Castellví et al., 2020) show that the best method for NH_3 acclimation is to apply low N loading rates so final TAN concentration is reached after 75 days of reactor operation. At this rate, the reactor can quickly recover from NH_3 inhibition after the inhibitory concentrations are reached. In Uvélia's case, we can see that feed loading was very low during the reactor's acclimation period in 2019, given steady state TS content was not reached until 5 months after reactor feeding was initiated (compared to 1 month in MR). It can be assumed that TAN increased at the same rate as TS content, given that the TKN in feed is the source of TAN in digestate, and the former is associated to the feed's dry matter content. This means that TAN took about twice as long to reach steady state concentrations than what was recommended by (Puig-Castellví et al., 2020). Therefore, it can be concluded that Uvélia's digestate already went through an NH_3 acclimation process. It is not unusual that even after undergoing a long acclimation, the digestate from Uvélia still shows inhibited activity. The steady state TAN concentration in Uvélia can lead up to 5 times the FAN concentration of 300 mg/L tested by (Puig-Castellví et al., 2020). Moreover, these authors tested acclimation under mesophilic conditions, while it has been shown that thermophilic reactors can be run for long periods of time without showing signs of acclimating to high FAN concentrations (Yirong et al., 2017).

4.3.5 Implications for the full-scale process

Results from the study show that new targeted operational parameters must be defined for the digesters in Uvélia. The new parameters must allow for stable feeding of the reactor without compromising digestate quality and lead to high and stable biogas production, methane content and reactor productivity. The process inhibitors identified for thermophilic conditions (ammonia toxicity, VFA accumulation and severe foaming at OLR > 9 kg VS/m^3.d) should be taken into account when choosing the new operational parameters.
For now, the dynamic feeding regime in Uvélia or the lower OLRs applied (or both) might mitigate the effects of ammonia toxicity and VFA accumulation, including impaired biogas production and foaming. Given that the pilot simulations showed a high risk of foaming, it is important to choose operational parameters very carefully before switching to a stable feeding regime in the full-scale plant. It is highly recommended to test the operational parameters in the pilot-scale before applying them in the full-scale to understand the risk of foaming and devise useful strategies to mitigate it. Aside from feed hydrolysis, this study found that continuous mixing at 30 rpm also mitigate foaming, but longer simulation times are required to confirm this as well.

Dilution is not recommended for use as a mitigation strategy for ammonia toxicity, given this technique did not restore TR-MF activity. Additionally, it would reduce TS content below the target range of 23-25% if optimized process conditions are achieved. Although methane production is higher at a lower TS%, according to reviewed literature (**Annex A.1**), low TS content would make the drying process more complex and energy intensive. At biogas production >80-90 % of TBP, TS would range between 17.95-20.24% with a 1.3 dilution factor. On the other hand, switching to mesophilic conditions could be a promising strategy, given reactor productivity and methane content in the mesophilic simulations were higer than the average productivity of the full-scale digesters in 2020. However, the target productivity would not be reached and the plant's treatment capacity would definitely need to be reduced, since substrate degradation kinetics cannot handle the current targeted OLR. In addition, the observed biogas production would require dilution with up to 1400 t/y of water to maintain TS < 25%, potentially increasing the plant's water footprint.

This study demonstrated the effect of stable feeding at Uvélia's targeted OLR on reactor performance and identified issues affecting biogas quality, production and productivity. These results can inform objectives and the experimental set-up for future optimization studies. Operational parameters can be validated as optimal if reactor stability (in terms of biogas production and productivity, methane content and digestate quality) are successfully maintained for at least 2-3 HRTs. Therefore, more simulations are required to determine the optimal operational parameters for treating Uvélia's OFMSW. When chosen, the optimal operational parameters should be applied in the full-scale process and their effects on reactor performance and stability must be monitored. Comparing the effects of the optimal operational parameters in the pilot and full-scale will allow to understand the scalability of operational parameters between the pilot and full-scale. The recommended simulations, based on this study's results, are discussed below.

4.3.5.1 Mono-digestion of organic waste

The targeted feed load of 44 t/d (SRT 30 d) might cause VFA accumulation and reactor failure in the full-scale. Inhibition of AMs by NH_3 toxicity makes reactor overloading and acidification more likely at higher OLRs and lower SRTs because degradation reactions are slower in this inhibited state. It has been shown that a lower OLR (implying an SRT >30 d) can mitigate the effects of ammonia toxicity (Giménez-Lorang et al., 2021; Jiang et al., 2019). The longer retention time helps because it prevents the active biology from being flushed out too quickly, allowing it enough time to degrade VFAs and stop their accumulation. Short SRTs (<25 d) increase the rate of wash-out of the reactor (Climenhaga and Banks, 2008). Therefore, the targeted SRT should definitely be larger than 21 d. The risk of foaming is also reduced at lower OLRs, due to reduced acidification and lipid and protein loading (Xu et al., 2018).

Therefore, pilot simulations can be designed to assess the effect of reducing the targeted average feed loading rate (44 ton/d) on the performance of the anaerobic digesters. As in Experiment 1, a "control" reactor using good-quality thermophilic digestate should be run at the targeted OLR in parallel to, or before running a simulation with Uvélia's digestate (test reactor). This would give insight into whether or not the new load would lead to reactor inhibition (via the control reactor); and if the test reactor can recover from its inhibited state. The simulations can be run at 2 or 3 OLRs. Based on this study's results and the full-scale data, the suggested OLRs are 7.2 and 8.0 (SRTs ~ 38, 34 d when considering undiluted organic waste). Simulating an OLR of ~7.2 kg VS/m^3.d is interesting because Uvélia's digesters showed the best performance at an average SRT of 38 d (OLR 7.0 ± 1.3 kg VS/m^3.d), with a biogas production and methane productivity of 145 Nm^3/t and 2.3 Nm^3/m^3.d, respectively (which is larger than the defined targets). An OLR of 8 kg VS/m^3.d would give insight into whether it is possible to achieve stable operation at OLRs between 7.2 and the previously applied load of ~9 kg VS/m^3.d. The frequency and intensity of foaming should always be compared between the experiments. If switching to mesophilic digestion becomes of greater interest for Uvélia, simulations at lower OLRs are also recommended for a period of 3 SRTs (6.5-8.0 kg VS/m^3.d). The feasibility of operating the mesophilic process at a TS content > 25% could also be tested.

4.3.5.2 Co-digestion of organic waste

Co-digestion of OFMSW with a high C/N waste remains an interesting option to achieve the targeted loading rates without compromising process stability. A pilot simulation can be designed to assess reactor performance and stability at an SRT of 30 d, when fed with a co-substrate mixture with a C/N between 25-35. Given ammonia toxicity was determined to be the cause of reactor failure and instability, an adjusted C/N ratio within the optimal range might have a diluting effect on TAN/FAN concentration and allow for the application of high OLRs (Capson-Tojo et al., 2017; Kayhanian, 1999). An accessible waste stream with a high C/N ratio must be identified to be used in the simulation. Paper and cardboard waste (PCW) could be a good option, given these wastes have a very high carbon content (C/N >300) and have been shown to have high degradability and to improve the digestion of FW (Shahbaz et al., 2020). Besides, the company that delivers organic waste to Uvélia also collects municipal PCW. Sawdust (C/N >500) is not recommended as co-substrate, given it is not readily degradable thus reducing biogas production from OFMSW and increasing TS content in digestate (Pastor-Poquet et al., 2019).

Due to PCW's high C/N, an estimated load of 8 t/d per reactor (5840 t/year total for an OFMSW/PCW ratio of 1:4.5) could increase feedstock C/N to 25. This load of PCW would increase the feedstock's average TS content to ~47% (from 37 %). TS content can inhibit the AD process after a certain concentration. (Pastor-Poquet et al., 2019) determined that it was possible to run the reactor up to a 30% TS content without reactor acidification taking place. Therefore, it is important to choose different dilution factors that can be tested in the pilot simulation as well, to determine an optimal TS content for the process. These can be chosen based on the TBP of the mixture and the final TS required for digestate drying. The dilution in the pilot scale should be simulated with process water and percolate collected by Uvélia. Depending on the chosen co-substrate and need for dilution, OFMSW treatment capacity would be reduced; but Uvélia staff is open to consider a reduction in treatment capacity if it resulted in enhanced reactor productivity ($Nm^3/m^3.d$).

4.4. Opportunities for collaboration

4.4.1. Improvement of research methodology alongside plant design and start-up

An interesting area of collaboration between AD plants and academia is in the optimization of process parameters during the plant's design and start-up. For AD plants, this would (i) allow the evaluation of the acclimation process with different digestates and (ii) aid them in choosing the inoculum that would lead to the shortest stabilization period and the best digestate quality. Also, pilot simulations carried out with the desired design parameters would help diagnose issues that arise due to substrate characteristics and/or other process parameters. In this way, the desired operating parameters could be validated or changed, if necessary, and strategies to counteract process instability could be identified. The validated process parameters can then be applied when the AD-plant is started-up. For the academic sector, working with AD plants during their start-up and operation could aid in the improvement of research methodology in the field of AD optimization and provide results that are more readily applicable in the field. Proposed improvements of methods and experimental set-up for optimization studies are discussed below.

4.4.1.1 Reporting reactor performance in literature

Both biogas production and reactor productivity are important measures in the industrial AD setting. However, reactor productivity was seldom reported in the literature reviewed in **Section 1.2.** Biogas production was reported more often in full and pilot scale studies than in lab-scale ones. The majority of the studies reported reactor performance in terms of methane production.

Data on CH_4 productivity and biogas production at given OLRs is arguably just as important in full-scale AD as data on methane production. Methane productivity gives more insight than CH_4 production into the energy potential of an AD plant (if average methane content is known to be > 50%). In this study, it was observed that methane production does not significantly increase (or even decreases) with increasing OLR, as was observed by other authors (Nguyen et al., 2017; Ossa-Arias and González-Martínez, 2021; Shahbaz et al., 2020); while reactor productivity had a rather strong positive correlation with OLR (**Annex B.6**). Biogas production is more informative than CH_4 production to assess treatment efficiency and mass reduction. Therefore, it would be useful to report all 3 parameters in AD optimization studies. Depending on an AD plant's digestate post-treatment and end-use, certain plants might need to aim for the most efficient treatment of waste (a measure of biogas production), while others might be more interested in producing as much biogas as possible (a measure of productivity). More optimization studies with a focus on reactor productivity could be useful in the sector.

4.4.1.2 Simulating dynamic feeding

As stated earlier, this study did not simulate the applied operational conditions in the studied AD. If the dynamic feeding conditions employed in Uvélia must be simulated in future experiments, the reactor should first be stabilized. After stabilization, dynamic feeding can be based on the different daily OLRs provided to the full-scale reactor in a given month (at a given and known average SRT). A control reactor should be run in parallel to confirm if dynamic feeding impacts reactor performance and stability differently than stable feeding (for the same organic load).

To stabilize reactors inoculated with digestate sourced from Uvélia, an OLR of 5.2 kg VS/m^3.d can be tested. To stabilize reactors fed with high N feedstock, but inoculated with good-quality digestate adapted to the process temperature, a stabilization method that does not compromise process performance and stability must be designed. Dilution to maintain TAN levels equal to or below initial levels can be tested.

4.4.1.3 Experimental set-up

When working on process optimization with AD plants that are already in operation, it is important to determine whether their digestate is of good quality. If quality indicators monitored by the plant operators show digestate is poor, it is important to firstly determine the mechanism of inhibition. An effective methodology to define this is to perform parallel pilot simulations with (i) one reactor inoculated with digestate sourced from the AD plant under study (R1) and (ii) another reactor inoculated with digestate sourced from an AD plant known to have a stable process and good digestate quality (R2).

Results from R1 would provide the baseline for assessing results from R2. Results from R2 will give insight into the nature of process performance issues (technical or biological) and into which specific parameters cause digestate quality to decay (e.g., organic loading rate, temperature, pH, ammonia concentration, metal content…). After determining the cause of poor reactor performance and quality in R1 and R2, strategies to mitigate reactor instability can be chosen. Such strategies should then be tested in another reactor (R3) inoculated with fresh digestate, from the same source as the one used to inoculate R2. With R3, the effectiveness of the chosen strategies in preventing digestate quality degradation and reactor failure is evaluated. Using fresh, good quality digestate accelerates the strategy selection process, given some might be capable of preventing issues, while unable to restore activity after inhibition.

Successful strategies can then be tested on the poor-quality digestate (R4) to test whether or not (and how efficiently) they can restore good quality and performance. If possible, R3 and R4 should be run in parallel with control reactors inoculated with digestate from the same representative sample. In this way, limitations in repeatability and comparability related to (i) scale and (ii) the heterogeneity and seasonality of solid waste can be partially addressed. For example, reactor performance at the pilot scale might not be sufficiently representative of the performance of a full-scale reactor. Therefore, if the application of a certain strategy leads to a significant improvement in a pilot reactor compared to a control, it is more likely that this strategy would also improve full-scale reactor performance in relation to its performance under previous conditions.

Due to the available time and resources, it was not possible to run parallel control reactors in Experiments 3 and 4. Reactors run in previous experiments (TR-OF and MR) served as controls, but data from reactors run in parallel (i.e., when running TR-OF and TR-MF) was more easily comparable than data from reactors run in series.

4.2 Improvement and deployment of anaerobic digestion technology

Collaboration between the industrial and academic sectors could also accelerate the improvement and deployment of AD for municipal, agricultural and industrial waste treatment. For example, testing strategies that accelerate the establishment of thermophilic AD is of particular interest, given thermophilic conditions improve biogas production (i.e., both waste degradation and biogas generation). At the moment there are much less thermophilic plants in operation than mesophilic ones because it is more difficult to control the thermophilic process due to a higher risk for instability (Franca and Bassin, 2020; Rocamora et al., 2020). AD under different conditions could be better understood and improved more quickly if more scientists publish results from work with existing plants to optimize process design and control.

Partnerships between researchers and small AD installations would be mutually beneficial. For researchers, the needs of different AD plants can inspire the development of new technology. Small plants would benefit from tailored research that can help them improve their performance. The partner plants could also help fund the studies on their process optimization, as this requires less investment than building their own infrastructure for R&D. If both sectors collaborate in R&D, technology improvement and deployment could be accelerated. This could lead to more investment in AD within the waste treatment sector which could open more opportunities in research. As AD technology is rendered more robust, more and more plants might envision to invest in biogas upgrading and other up-and-coming sustainable technologies that can be integrated with AD, such as algal technology (promoted with the EU project, ALG-AD). Specific technology that can be researched for AD optimization by working with plants like Uvélia is discussed below.

4.2.1 Ammonia stripping

Ammonia stripping and recovery from recirculated digestate can be used to mitigate ammonia toxicity under thermophilic conditions and recover mineral N. This technology was recently demonstrated in the plant Aqua & Sole in Italy as part of a European project (SYSTEMIC [systemicproject.eu]). This plant has 4 times the treatment capacity of Uvélia and 5.1 times its active volume and successfully treats high solids, high-N waste under thermophilic conditions (**Annex A.2**). Differently from Uvélia, this plant operates at a minimum SRT of ~ 50 d (OLR 2.0 ± 0.5 kg $VS/m^3.d$) due to its larger size relative to its capacity (Pigoli et al., 2021). Therefore, it would be interesting to test whether this ammonia stripping technology improves the stability of high-N and high-solids thermophilic AD at lower retention times. To test this in pilot simulations, novel methodology could be developed to facilitate testing.

Uvélia already operates with a digestate recycling system and an NH_3 scrubbing unit, so it should be possible to redirect the stripped NH_3 to the scrubbing unit and the stripped digestate towards the recycling system. Considering the average TKN in the OFMSW collected by Uvélia is 21.5 ± 4.2 g/kg TS and the up-concentration factor of TAN in digestate is 1.2, steady state TAN should be ~ 5.76 g/kg under theoretical conditions, without feed dilution. This estimated concentration is consistent with results from TR-OF, where TAN increased from 4.84 to 5.62 g/kg with feeding of undiluted OFMSW. Results from TR-MF show that FAN levels should be kept below 1 g/kg when pH is between 8.0-8.3. Based on the latter, a steady state TAN concentration of 2.5 g/kg could be tested. This implies a digestate recycle ratio of 0.52 times the inflow and an ammonia stripping unit of around 3.0 m^3 (at ~ 2 t/h max recycle flow) capable of a 90 % removal efficiency. Nevertheless, a steady state TAN concentration at which FAN levels do not lead to reactor failure must be defined. The proposed configuration is illustrated in **Annex C.2**.

4.2.2 Process configuration

Temperature-phased AD (TPAD) could improve the AD process for the treatment of high N waste, like OFMSW. Recent papers studying TPAD all propose a 2-stage process where hydrolysis and methanogenesis are decoupled (Amodeo et al., 2021; Fernández-Rodríguez et al., 2016, 2015; Nguyen et al., 2021; Qin et al., 2018). Stage 1 is generally thermophilic in order to accelerate hydrolysis reactions (normally the rate-limiting step). In Stage 2, mainly methanogenesis takes place under mesophilic conditions. Stage 1 must have a shorter SRT than Stage 2 for successful decoupling. The application of thermophilic conditions for hydrolysis would not induce ammonia toxicity since acidogens and acetogens are not greatly inhibited by NH_3 (Capson-Tojo et al., 2017). With a second mesophilic reactor for methanogenesis, high TAN concentrations are unlikely to cause ammonia toxicity due to lower temperature leading to a reduced FAN fraction (Kang et al., 2021; Yirong et al., 2017). Lower SRTs can be applied for a 2-stage system compared to the single stage without inducing VFA toxicity (Nguyen et al., 2021).

When we consider our case study, the following research opportunity arises: evaluating TPAD when phasing from mesophilic to thermophilic conditions (MTPAD). Conventional TPAD is not compatible with Uvélia's permit or infrastructure: a 21-day pasteurization at minimum 50 °C is required and both digesters are the same size (not possible to apply different SRTs). These conditions do not allow for the decoupling of hydrolysis and methanogenesis. MTPAD is an interesting alternative given the mesophilic conditions mitigate the NH_3 toxicity problem during biogas generation and a thermophilic post-digester would provide the necessary pasteurization, while degrading residual matter and producing biogas. An extra reactor would have to be introduced for the post-digestion step in order to (i) prolong the SRT of the mesophilic stage to avoid organic overloading and (ii) to meet the minimum pasteurization time. To achieve this, pilot simulations should first be run to determine the optimum OLR for mesophilic conditions and choose an appropriate SRT based on the results. Then, pilot simulations applying MTPAD could be carried out to determine the technical and economic feasibility of this design in Uvélia. If results are positive, this configuration could be considered when further investments are done to modify the process. The proposed configuration is illustrated in **Annex C.2**.

4.2.3 pH control for high N thermophilic digestion

Research in improving pH controllers in full-scale anaerobic plants could help make anaerobic digestion of high-N substrates more robust. Nikulina et al. (2021) showed that pH can have a strong effect on the start-up phase of a digester. There are pH controllers that have been developed for wastewater and fermentation processes (e.g., Fortrans Model 5000B pH control system and Eurotherm Process Automation PID control module with Smith predictor), but there is little information available on the use of pH control for high solids AD. Suitable controlled and measured parameters that allow for a quick and accurate response of pH in AD processes can be investigated, as well as the feasibility for using and installing such a control system in a high solids process.

The control of pH could be especially useful during the start-up phase of reactors treating high-N feedstock. For example, in TR-MF, initial conditions and low feed loading rates allowed it to maintain low VFA concentration in its digestate. However, the increasing TAN concentration caused the reactor's pH to increase above 8.0, which led to high FAN levels and ammonia toxicity. Therefore, controlling pH between 7.6-7.7 g/kg (for FAN < 0.85 g/kg, at TAN of 5.6 g/kg) would not have induced VFA toxicity and could have prevented the high TAN concentration from causing ammonia toxicity or reactor failure. To control the pH levels observed in TR-OF, minimal amounts of concentrated acid would be required. It is would also be possible to recover the CO_2 generated from CHP engines to lower and maintain pH (CO_2 technology applied by Fortrans pH control).

CONCLUSIONS

The studied AD plant consists of two 1320 m^3 digesters that can provide the target waste treatment capacity of 32 000 ton of OFMSW per year when operated at an SRT of 30d. Due to technical factors, the plant operated at 63% of its treatment capacity (SRT 48 d) and met 48% of its target energy production in 2020. In Experiment 1, applying an SRT of 30 d to 2 thermophilic pilot reactors (TR-OF and TR-MF), it was possible to establish that ammonia toxicity inhibited the AD process when Uvélia's targeted operational parameters were applied. Ammonia toxicity stems from the high TKN content and low C/N in the OFMSW treated by the plant. Feeding this OFMSW to the reactors led to a high pH and TAN concentration in the reactors' digestate, which translated into a large FAN fraction (1.0-1.5 g/kg) under thermophilic conditions. Ammonia toxicity caused inhibition in TR-OF performance from the beginning of its acclimation period, even though this reactor had been inoculated with digestate sourced from the AD plant under study, and thus adapted to the OFMSW used. At an SRT of 30 d, its biogas production was only 40% of the OFMSW's TBP. On the other hand, TR-MF, which was inoculated with non-adapted digestate sourced from a different plant, showed good performance during acclimation and during the first 28 days of stable feeding. At the applied SRT, its biogas production was 99% of the TBP and 111% of SMY. However, its digestate quality showed signs of the onset of ammonia toxicity during this period, with high FAN concentration, a high steady state HAc concentration and HPr accumulation, which eventually caused the reactor to fail.

Dilution, co-digestion and mesophilic conditions were tested as strategies to mitigate ammonia toxicity. With dilution and co-digestion (at low C/N), it was not possible to restore the activity nor the quality of digestates that were already inhibited. Under mesophilic conditions, ammonia toxicity was mitigated given FAN content remained low. However, organic overloading took place when the mesophilic reactors were operated at an SRT of 30 days, leading to VFA accumulation and potentially causing severe foaming. Feeding of hydrolysed OFMSW at an OLR of ~7.3 kg VS/m^3.d (SRT of 38 d) under mesophilic conditions showed the best results in terms of reactor stability and digestate quality.

In the full-scale AD, inhibition by ammonia toxicity was not as obvious as it was in the pilot reactors, with biogas production being ~97% of the average TBP in 2020. This might be due to the fact that, in 2020, the full-scale plant was fed dynamically and operated at a much larger SRT than the pilot reactors. Biogas production and methane productivity under mesophilic conditions did not meet the targets defined by Uvélia, nevertheless, the average biogas productivity and methane content (3.1 Nm^3/m^3.d and 62.3 %) were higher than values achieved in the full-scale process in 2020 (2.76 and Nm^3/m^3.d and 58.5 %). Moreover, TR-MF achieved a higher biogas production and methane productivity than the full-scale digesters ($p > 0.05$), before inhibition. This shows that if ammonia toxicity were mitigated, it could be possible to stably operate the full-scales digesters at a lower SRT, with an improvement in both treatment efficiency and energy production.

The case study allowed to identify synergies between the academic sector and the AD industry. The main benefits that can be reaped from collaboration are the improvement of research methodology and plant design and the acceleration of AD implementation under different operational conditions. All in all, this study demonstrated that pilot simulations are useful in order to anticipate potential issues that can arise in the full-scale process when new operational parameters are applied. The next steps would be to further conduct pilot experiments in order to determine the operational parameters that lead to an optimized biogas production and methane productivity and evaluate their effect on the full-scale AD.

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ANNEXES

Annex A. 1: Summarized methods and results reported in the lab and pilot scale optimization experiments (<100 L) reviewed. Numbering indicates process, parameters or additives tested in series, otherwise the parameters were tested in parallel. Pretreatment refers to the pre-treatment of the substrate.

Substrates (source)	Scale	Applied, Tested and Optimal Conditions	Biogas/ CH_4 production	CH_4 content	VS removal[a]	Reference
OFMSW (composting facility) and SS (WWTP) *- Khanjapur, India*	Lab (120 mL)	**Applied:** 35°C | I/S-1:1 & 1:1.5[b] | HRT 21 & 43 d[b] | Batch **Tested: 1.** M-AD vs. Co-AD | **2.** Co-substrate type (PS, WAS, MS) | **3.** Co-substrate ratio-OFMSW:MS (1:1, 3.5:1.5, 4:1, 9:1) | **4.** TS (6, 9, 12, 15%) **Optimal**: Co-digestion | OFMSW+MS (4:1) | TS 9%	0.967 m^3 biogas/kg VS	NS	37%	Ahmed et al., 2020
OFMSW (MSW landfill site) -*Ostrava, Czech Republic*	Lab (13 L)	**Applied:** TS (52-58%) | Semi, M-AD **Tested:** Particle size: 3-15 mm & 15-24 mm (only at 40°C) | T: 40 & 55 °C (only for 15-24 mm particle fraction) **Optimal:** 55°C | Particle size: 15-24 mm	0.176 m^3 CH_4/kg VS	63%	NS	Basinas et al., 2021
OFMSW (waste transfer station) -*Mexico City, Mexico*	Lab (500 mL)	**Applied:** HRT 25 d | Batch, 2-stage, M-AD **Tested:** Pretreatment: Silage | 20, 35, 55 °C (for both silage and AD) | I/S: 1/1.5, 1/1, 2/1 | TS: 10, 20, 28% **Optimal**: Ensiled OFMSW | 35°C (silage and AD) | TS 10% | I/S 2:1	0.431 Nm^3 CH_4/kg VS	NS	NS	Castellón-Zelaya & González-Martínez, 2021
OFMSW (university community garbage bins) -*Bombay, India*	Lab (250 mL)	**Applied:** 37°C | pH (initial)-7.0 | I/S-1:1[c] | HRT 30 d | Batch, M-AD, Dry (20% TS) **Tested:** Pretreatment: alkali hydrolysis with 5M NaOH | pH 8.0,9.0, 10.0, 11.0, 12.0, 13.0 (for hydrolysis) **Optimal**: Pretreatment, at pH 10	0.2805 m^3 CH_4/kg VS	68.9%	70%	Dasgupta & Chandel, 2020
FW (one household) and BW -*Beijing, China*	Lab (800 mL)	**Applied:** OLR-2.7 kg VS/m^3/d | HRT 30 d |Semi, Co-AD (KW+BW [1:1]), Wet (<15% TS) **Tested:** 35, 55 °C | Reactor configuration: single and 2-stage CSTR (35°C) **Optimal**: 2-stage at 35°C, volume ratio of 1:3 (stage 1: stage 2)	0.450 ± 0.5 L biogas/d	NS	97% tCOD	Giwa et al., 2021
Mock FW (rice, chicken, vegetable, mixture of the 3)	Lab (90 mL)	**Applied:** 55°C | I/S-1:1.5 | Batch, M-AD, Wet (<15% TS) **Tested:** Additive (Biochar) | Biochar Source: pinewood, cattle bone, and eggshell-banana-squash **Optimal**: Amended with mixed FW-biochar	0.167 m^3 CH_4/kg VS	NS	NS	Leininger and Ren, 2021

Rural OFMSW (MSW classification & disposal center) *-Xuzhou, China*	Lab (350 mL)	**Applied:** 37 ± 1°C | Batch BMP tests, M-AD **Tested:** Additive-Prussian blue analogue nanoparticles (PNP) | TS: 8, 10, 12, 15% (for S/I of 3.43, 4.49, 5.60, 7.74) **Optimal**: Amended with PNP | TS 8%	0.302 m^3 CH_4/kg VS	70%	NS	Liu et al., 2021
Syn-BDI and CIR, OFMSW (1 household), PM (farm), SS (WWTP) *- Colombia*	Lab (200 mL)	**Applied:** 35°C | HRT 21 | Batch, Co-AD. **Tested:** Co-substrate mixture OFMSW+CIR, OFMSW+BID, BDI+CIR | C/N 25, 35, 40 | N-source-SS, PM | VS concentration 10, 6.25 and 2.5 kg VS/m3 **Optimal**: OFMSW+BID | SS as N source | C/N ratio 40 | 2.5 kg VS/m^3	0.382 (MARS)&0.350 (BB) m^3 CH_4/kg VS	NS	NS	Mosquera et al., 2020
FW from university cafeteria *-P city, South Korea*	Lab (10 L)	**Applied:** Dry (TS 22%), Semi-continuous, m-AD **Tested:** 38, 55 °C **|** HRT 100 – 25 d (OLR 2.16 – 8.62 kg VS/m^3.d) **Optimal**: 55 °C | HRT 25 d (OLR 8.62 kg VS/m^3.d)	0.73 m^3/kg VS fed	60%	80.98%	Nguyen et al., 2017
OFMSW (AD plant)- *Backnang-Neuschöntal, Germany*	Pilot (57 L)	**Applied:** HRT 28 d | Batch, Dry (TS 32-35% [OFMSW] 55% [manure]) **Tested: 1.** I/S: 1/4, 1/1.5, 1.5/1 (at 37.6 °C, m-AD-digestion OFMSW) **| 2.** T and percolation substance: 37.6 (I/S 1:4, LI) and 52.2 °C (I/S 1:4 with LI; I/S 1:1.5 with water) **| 3.** Co-substrate ratio 3:1, 1:1, 1:3 (FW:CM, TS basis; at I/S 1:4 and 37.6°C) **Optimal**: M-AD | 52.2 ±0.8°C | Percolation of LI | I/S 1:4	0.236 m^3 CH_4/kg VS (fed)	54.5%	NS	Nikulina et al., 2021
OFMSW (waste transfer station) *-Mexico City, Mexico*	Lab (1.5 L)	**Applied:** OLR (initial)-3.6 kg VS/m^3/d | Semi, M-AD, Dry (27.8 % TS) **Tested:** 20, 35, 55 °C (quickest Start up and stabilization time) | OLR: 3.6, 4.5, 5.8, 7.4 kg VS/m^3/d (at 35°C) **Optimal**: 35°C | OLR 4.5 kg VS/m^3	0.483 ± 0.003 Nm^3 CH_4/kg VS	69%	NS	Ossa-Arias & González-Martínez, 2021
Industrial FW (deconditioning unit) *-Issé, France*	Lab (5L)	**Applied:** 35 °C| OLR-0.5 kg COD/m^3/d | HRT 25 d| Semi, M-AD, Wet (<15% TS) **Tested:** NH_4^+ loading rate: specified in terms of days of operation after which final concentration of 183 mg NH_3/L is reached (75, 50, 25, 14 and 2 days) **Optimal**: Low NH4 loading rate (75 d)	NS	85%	NS	Puig-Castellví et al., 2020
OFMSW (OFMSW-AD plant) *-South Germany*	Lab (2L)	**Applied:** I/S 1:0.3 (VS basis) | HRT 56 d | Batch BMP Tests, Wet (<15% TS) **Tested:** 23,25, 35, 40°C (using digested sewage sludge for inoculum) **Optimal**: T 25°C	0.364 ± 0.025 m^3 CH_4/kg VS	58.37 ±1.41 %	NS	Sailer et al., 2021

FW (restaurant, boneless) and ms-PW, CW, TW (MSW treatment plant) *-Beijing, China*	Lab (800 mL)	**Applied:** 35 ± 1 °C | I/S-1:1[d] (15 g VS/L) | C/N 25 | HRT 30 d | Batch & Semi[e], Co-AD, Dry (36 %TS) **Tested: 1.** Co-substrate type: PW, CW, TW (1:6.51, 1:6.07 and 1:7.15 [Co-S: FW] ratios, respectively, for a C/N of 25, batch) | **2.** OLR: 2, 3, 5 kg VS/m^3/d (for FW+PW, semi) **Optimal**: FW+PW | OLR 2 kg VS/m^3/d	13 m^3 CH_4/kg VS	70-75%	96%	Shahbaz et al., 2020
OFMSW-High N (collection service); Low N (synthetic) *-Hampshire, UK*	Lab (4L)	**Applied:** M-AD, Dry (TS 23.91% [high-N] and 22.48 % [low N]) **Tested:** 37, 55°C | OLR: 2-4 kg VS/m^3/d (HRT 74-95.5 d, at 37 °C) and 0.5-2 kg VS/m^3/d (HRT 127.7 d, at 55°C) **Optimal**: T 37°C | OLR 4 kg VS/m^3	0.460 m^3 CH_4/kg VS	55-60%	81.20%	Yirong et al., 2017
Ammonium-rich synthetic medium	Lab (0.2-1 L)	**Applied:** 55 °C | M-AD, Wet (<15% TS) **Tested: 1.** TAN concentration: 3, 4, 5, 8 mg/L (at 1.25E+4 µmol/L/d and 3 working volumes [0.2, 0.5, 1 L] in batch) | **2.** Photon number and HRT: 1.25.10^4 and 0.42.10^4 µmol/L/d at HRTs 20, 15, 10 (OLR 0.09, 0.18, 0.72 g OC/L/d) **Optimal**: Photon number- 1.25 µmol/L.d | HRT 20 d (0.72 g organic carbon/d)	0.541 m^3 CH_4/kg DOC removed	NS	NS	Zhu et al., 2021
Dried OFMSW (waste management site) *-Tehran, Iran*	Lab (2 L)	**Applied:** 37°C | HRT 12.5 d | Batch, Dry (15-30% TS) **Tested: 1.** Co-substrate ratio in terms of C/N: 20 (m-AD), 25, 30, 35 (at I/S of 2 and TS of 20%) | **2.** I/S ratio: 1:4, 1:2, 1:1, 2:1 (at 1:2 Co-S ratio and TS 20%) | **3.** TS: 15, 20, 25, 30% (at 1:2 Co-S ratio and I/S ratio 2:1) **Optimal**: Co-AD, C/N 25 (Co-substrate ratio 1:2 [Sawdust:OFMSW]) | I/S 2:1 | TS 15%	0.37 m^3 CH_4/kg VS	NS	NS	Ziaee et al., 2021

TS in % fresh waste (FM). AD: anaerobic digestion, BDI: bottled fruit drinks industry wastewater, BW: blackwater, CIR: cocoa industry residue, CSTR: continuous stirred tank reactor, Co-AD: Co-digestion process, COD: chemical oxygen demand, Demo: demonstration, DOC: dissoved organic carbon, FW: food waste, m-AD: m-AD-digestion process, HRT: hydraulic retention time, I/S: inoculum-substrate ratio, MS: mixed sludge (equal fractions of PS and WAS), OLR: organic loading rate, LI: liquid inoculum, NS: Not specified, OFMSW: Organic fraction of municipal solid waste, ms-OFMSW: mechanically sorted OFMSW, PM: pig manure, PS: primary sludge, Semi: semi-continuous, SS: sewage sludge, Syn: synthetic, sCOD: soluble chemical oxygen demand, tCOD: total COD, TS: total solids, T: temperature, WAS: waste activated sludge, VS: volatile solids.

a. Removal efficiency of organic matter in substrate is reported in VS basis (unless indicated otherwise)
b. I/S of 1:1 and HRT 21 for tested parameters 1-3; I/S of 1:1.5 and HRT 43 days for tested parameter 4.
c. VS basis (Dasgupta)
d. I/S used for batch experiments when choosing optimal co-substrate
e. Batch experiments to choose co-substrate type. Semi-continuous experiments applied to choose optimal OLR

Annex A. 2: Methods and results from pilot (>100 L working volume) and full-scale optimization experiments and operational parameters found for full-scale plants in the literature reviewed in **Chapter I**.

Substrates (source)	Capacity	Parameters	Biogas/CH_4 production	CH_4 content*	VS removal*	Potential Electricity	Reference
ss-OFMSW (composting plant) *-Lombardy, Italy*	160 L	**Applied:** 38°C | **OLR** 6.2 kg VS/m^3/d (**HRT** 26 d) Dry (21.4 % TS) | Continuous, Plug flow, m-AD	0.41 m^3 CH_4/kg VS	61.2%	83% COD	101 kW (885.125 MWh/year)	Chinellato et al., 2021
OFMSW (university restaurant) *-Recife, Brazil*	9.6 m^3	**Applied:** Mesophilic | **OLR** 0.2 kg VS/m3/d (**HRT** 45 d) Semi-continuous, covered lagoon	0.584 m^3 biogas/kg VS	50%	NS	44 MWh/year	De Sousa et al., 2021
LFD (OFMSW AD plant) *-Barcelona, Spain*	43.8 m^3	**Applied:** 33 to 37°C | Wet | Continuous, M-AD **Tested**: **OLR** 1.5-5.4 kg COD/m^3/d (**SRT** 7.3-24.3 d) **Optimal:** Positive co-relation between SRT and COD removal & between SRT and NH_3 toxicity mitigation	BMP: 0.112 ± 0.021 Nm^3 CH_4/kg tCOD	73 ± 2%	36.8% tCOD 70% sCOD	NS	Gimenez-Lorang et al., 2021
FW (commercial, grease separator, industrial) *-Germany*	2000 m^3 (2 reactors) 16 000 t/y	**Applied:** Mesophilic | **OLR** 2.8 kg VS/m^3/d | **HRT** 29 d | m-AD	-	-	-	860 kW (installed)	Moeller & Görsch et al., 2015
FW (kitchen, grease separator, old food)|Paper sludge| Poultry manure *-Saxony, Germany*	40 000 t/year	**Applied:** Mesophilic | 2-stage, Co-AD	-	-	-	-	Moeller & Görsch et al., 2015
Leftovers *-Thuringia, Germany*	72 000 t/year	**Applied:** Mesophilic | M-AD	-	-	-	-	Moeller & Görsch et al., 2015
ms-OFMSW (household and restaurants) *-Ho Chi Minh, Vietnam*	5.2 m^3	**Applied**: Pretreatment[a] at 55 °C, 2.5h | 32°C (Stage 1) | 35°C (Stage 2) | Semi-continuous, 2-stage, m-AD **Tested**: **OLR** -1.6, 2.5, 3.8 kg VS/m^3/d **Optimal:** 2.5 kg VS.m^3/d (**HRT** 54 d)	0.578 ± 0.280 m^3 CH_4/kg VS	NS	83-87%	23 MW (201.48 GWh/year)	Nguyen et al., 2021

Dewatered SS, OFMSW percolate and OFMSW digestate -*Lombardy Region, Italy*	4500 m^3 (n=3, in series)	**Applied:** 55 °C | **OLR** 0.7-3 kg VS/m^3.d (**HRT** 50 d) | Dry, co-AD, Continuous	0.200 ± 0.029 m^3 CH_4/kg VS	NS	NS	1.6 MW	Pigoli et al., 2021
ms-OFMSW and ss-OFMSW (MBT plant) -*Oława, Poland*	1 100 m^3 (15 000 t/year) per digester [n = 2]	**Applied:** 54°C | **FLR** 35 t/d (ss) 40 t/d (ms) | **HRT** 31 d (ss), 28 d (ms) | Dry [TS 46.2 ± 0.3% (ss), 49.8 ± 0.3% (ms)] | Kompogas reactor, Continuous, M-AD **Tested**: ms-OFMSW and ss-OFMSW **Optimal:** ss-OFMSW AD	111.1 m^3 biogas/ton	58-60%	NS	3 933 MWh/year	Seruga et al., 2020
ms-OFMSW (MBT plant) Corn Stillage (distillery plant)|FW (restaurants and kitchens)|Cleaning effluent (chocolate factory) - *Oława, Poland*	1135 m^3 (4000 t/year) per digester [n =2]	**Applied:** 54°C | **FLR** 32 ton/d | **HRT** 31 d | Kompogas reactor, Continuous **Tested**: m-AD vs. co-AD | Co-S type (CS, FW, CE) | Co-S ratio: 5, 10, 15% (Co-S:OFMSW) **Optimal:** co-AD**,** Cleaning effluent, 5.7:1 (OFMSW:CE)	134.6 m^3 biogas/ton	53%	NS	273.90 MWh increment/y	Seruga et al., 2018
OFMSW and landfill leachate (MSW processing facility) -*Hyderabad, India*	42 m^3 (n=3)	**Applied:** Ambient T (24-39°) | **OLR** 6.2 kg VS/m^3/d | **HRT** 14.5| Co-S ratio 1:1 | Semi-continuous, gas lift **Tested**: m-AD vs co-AD | **C/N** 24 (OFMSW), 15.3 (LL), 19.7 (OFMSW+LL) **Optimal Parameter:** OFMSW mono-digestion (C/N 24)	0.61 m^3 biogas/kg VS consumed	60-64%	46%	27 MWh/year (1 reactor)	T.A.S. et al., 2020

AD: anaerobic digestion, Co-S: co-substrate, CE: cleaning effluent, tCOD: total chemical oxygen demand, co-AD: co-digestion, CS: corn stillage, FW: food waste, HRT: hydraulic retention time, LFD: liquid fraction of digestate, OFMSW: organic fraction of MSW, OLR: organic loading rate, ms-OFMSW: mechanically sorted OFMSW, MBT: mechanical biological treatment, MSW: municipal solid waste, FLR: feed loading rate, SRT: solids retention time, ss-OFMSW: source-sorted OFMSW, T: temperature.

a. Pretreatment refers to the pretreatment of the substrate

Annex A. 3: Comparison between optimal and applied values for different parameters, disaggregated by scale, reported in the literature reviewed in **Chapter I** (see **Annex A.1** and **A.2**). The count "n" refers to the number of studies considered for each parameter per category (each column being a separate category). n_T is the total number of studies considered per category. Numbers in parenthesis represent the number of studies reporting the same specific value for the given parameter.

		Applied Values [a]			
				Similar to Optimal values [b]	
Parameters	Optimal Values (all scales) [c] [n_T =22]	Lab Scale [n_T=17]	Full/Pilot Scale (n_T =11)	Lab Scale [n_T=17]	Full/Pilot Scale (n_T =11)
T (°C)	25, 35(3), 37, 52, 55(2) [n=8]	35(4), 37(3), 55(2) [n=9]	Ambient (24-39), Mesophilic (4), 35, 33-37, 38, 54 [n=9]	35, 37, 55 [n=9]	33-38, 54 [n=3]
OLR (kg VS/m³/d)	2, 2.5, 4, 4.5, 8.62 [n=5]	2.7, 3.6 (initial) [n=2]	0.2, 2.8, 6.2 [n=3] Ton_{waste}/d : 32-35, 40 [n=2]	2.7 [n=1]	2.8 [n=1]
I/S	2:1(3), 1:4 [n=4]	1:0.3 (VS basis), 1:1(3), 1:1.5, 1:1.5 (VS basis) [n=7]	-	None	None
HRT (days)	20, 24.3, 25, 54, 74 [n=5]	12.5, 21(2), 25(2), 28, 30(3), 34, 43, 56, 62, 122, 138, 163 [n=10]	14.5, 26, 28, 29, 31, 45, 50 [n=7]	25-28 [n=3]	26-28 [n=2]
Co-substrate type	MS, BW, BDI+SS, PW, Sawdust, CCE [n=6]	-	-	None	None
Co-substrate ratio [d]	5.7:1 (CCE), 4:1 (MS), 1:2 (Sawdust), [n=3]	1:1(BW) [n=1]	1:1 (LL) [n=1]	None	None
C/N ratio	24, 40 [n=2]	25 [n=1]	-	None	None
TS, feed (% FM)	8, 9, 10, 15 [n=4]	<15(5), 20, 22.5, 23.9, 27.8, 15-30, >32, 36, 52-58 [n=12]	<15, 21.4, 46.2 ± 0.3, 49.8 ± 0.3 [n=3]	<15-49.8 [n=3]	<15 [n=1]
Mode	Co-AD (3), M-AD (2), 2-stage [n=6]	1-stage (2), 2-stage, M-AD (9), Co-AD (3), Continuous, Semi (5), Batch (9), BMP test (2) [n=16]	1-stage(7), 2-stage (2), M-AD(5), Co(1), Continuous (4), Semi(3) [n=10]	m-AD, Co, 2-stage [n=2]	m-AD, Co, 2-stage [n=6]
Pretreatment	Sieving (15-24 mm), silage (35°C), alkali hydrolysis (pH 10) [n=3]	-	Sieving/shredding (60 mm), heating (55°C) [n=5]	None	Hydrolysis [n=2]
Other strategies	Mixed-FW biochar, PNPs, LI-percolation, illumination, ss-OFMSW [n=5]	-	-	None	None

CH_4 production [e]	Working V <100 L[f]: 0.167-0.483 [n=9] m^3/kg VS Working V >100 L[f]: 0.37-0.39 and 0.578 ± 0.280 m^3/kg VS; 64.4-71 m^3/ton_{waste} [n=4]	NA	NA	NA	NA

AD: anaerobic digestion, BDI: bottled fruit drinks industry wastewater, BW: blackwater, CCE: chocolate tank cleaning effluent, FM: fresh matter, I/S: inoculum-substrate ratio, LI: liquid inoculum, MS: mixed primary and secondary sludge, NA: Not applicable, PNP: Prussian blue analogue nanoparticles, PW: paper waste, SS: sewage sludge, ss-OFMSW: source sorted OFMSW, VS: volatile solids

a. Optimal values for the operational parameters are those yielding the best methane production in AD optimization experiments.
b. The applied values are the operational parameters applied (i.e., not tested) to carry out anaerobic digestion in the studies or in full-scale plants.
c. Applied values similar to the values reported to be optimal in optimization studies (seen in the “optimal values” column).
a. The main substrate is always OFMSW or FW. Ratios are reported as OFMSW(FW):Co-substrate
b. CH_4 production under the optimal conditions identified in optimization studies.
c. Lab scale experiments are defined as those having a working V < 100 L, and pilot and full-scale experiments as those having a working V >100 L

Annex B. 1: Characterization of the OFMSW treated by Uvélia and the resulting digestate (DOF) and the green waste used for co-digestion in this study. Values are expressed as the averages with standard deviations. Concentrations and gas production units are expressed per kg fresh weight of OFMSW, unless otherwise stated.

	Uvélia [a]		This study [b]		
Parameters	**OFMSW**	**DOF**	**OFMSW**	**GW**	**DOF**
TS (% FW)	37.0 ± 3.2	24.3 ± 1.92	36.98 ± 1.26	42.03	22.87 ± 1.14
VS (% TS)	71.2 ± 5.8	-	74.32 ± 3.05	66.96	55.62
Biodegradable VS	52.1 ± 13.6	-	-	-	-
TKN (% TS)	2.15 ± 0.42	-	2.480 ± 0.041	1.76	2.72
TAN (g/kg)	-	-	-	-	4.96
TC (% TS)	-	-	45.490 ± 2.733	-	34.16
TOC [c] (% TS)	-	-	41.3 ± 1.7	37.2	
C/N	16.6 ± 3.4	-	16.6 ± 0.7	21.2	11.8
Protein (kg/t)	-	-	48.5 ± 4.6	-	-
Carbohydrate (kg/t)	-	-	112.4 ± 12.3	-	-
Lipids (kg/t)	-	-	32.8 ± 3.7	-	-
pH	-	7.95 ± 0.12	4.80 ± 0.10	-	8.21 ± 0.28
FOS/TAC	-	0.758 ± 0.138	-	-	0.66
EC (mS/cm)	-	-	-	-	24.15
Biogas potential (m^3/ton)	136.8 ± 15.1	-	156 ± 8	-	
Biogas potential (m^3/kg TS)	370 ± 35.1	-	-	-	-
CH_4 potential (m^3/ton)	-	-	91 ± 4	-	-
SMY (m^3/kg VS)	-	-	0.332 ± 0.015	-	-
Theoretical methane content (%)	-	-	0.59 ± 0.01	-	-

EC: electrical conductivity, FOS/TAC: VFA to alkaline buffer capacity ratio, GW: green waste, OFMSW: SMY: specific methane yield, TAN: total ammonia nitrogen, TC: total carbon, TOC: total organic carbon, TKN: total Kjeldahl nitrogen, TS: total solids, VS: volatile solids

a. Average OFMSW values were obtained from a report from an external laboratory based on the characterization of samples obtained monthly for a 10-month period in 2020. Average digestate values were calculated from digestate quality data provided by Uvélia for the year 2020.
b. Average OFMSW values were calculated from 9 OFMSW samples collected on 7 separate days in January and April 2021. Digestate quality values were obtained from analysis of one sample collected on April 2021.
c. Calculated from assumption that around 55 % of VS is organic carbon (Iglesias Jiménez and Pérez García, 1992)

Annex B. 2: Initial conditions of the inoculum used for the different pilot experiments (Experiments 1-4) and the initial quality of the wet, mesophilic digestate used to inoculate the two full-scale digesters in Uvélia. Concentrations are expressed per kg fresh weight.

Parameters	**DOF (E 1)**	**DMF (E 1)**	**DMFA (E 2)**	**DMFA (E 3)**	**DOF (E 4)**	**Full-scale AD Inoculum [a]**
TS (%)	23.48	4.87	7.32	6.69	22.46	5.58
VS (% TS)	52.09	72.9	59.19	55.45	58.04	-
TAN (g/kg)	4.84	2.63	4.04	3.66	5.3	-
FAN [b] (g/kg)	1.49	0.86	0.30	0.50	1.14	-
pH	8.11	8.15	7.74	8.04	7.9	7.63
EC (mS/cm)	30.43	23.2	35.6	32.32	27.56	-
FOS/TAC	0.69	0.24	0.42	0.25	0.81	0.175
Acetic Acid (g/kg)	1.869	0.273	2.545	0.211	4.076	-
Propionic Acid (g/kg)	5.841	0.027	1.265	0.703	4.961	-
Isobutyric Acid (g/kg)	0.412	0.009	0.296	<0.008	0.26	-
Butyric Acid (g/kg)	0.044	0.007	0.061	<0.006	0.464	-
Isovaleric Acid (g/kg)	0.543	0.012	0.487	<0.010	0.648	-
Valeric Acid (g/kg)	0.025	0.013	0.02	<0.011	<0.029	-
Caproic Acid (g/kg)	0.028	0.025	<0.022	<0.022	<0.057	-
P/A	3.1	0.1	0.5	3.3	1.2	-

DOF: dry thermophilic digestate sourced from the studied AD plant (treating the organic fraction of municipal solid waste), DMF: wet thermophilic digestate from manure, maize and food waste treatment, DMFA: wet mesophilic digestate from the treatment of manure and food and agricultural waste, EC: electrical conductivity, FAN: free ammonia nitrogen, FOS/TAC: VFA to alkaline buffer capacity ratio , TAN: total ammonia nitrogen, TS: total solids, VS: volatile solids.

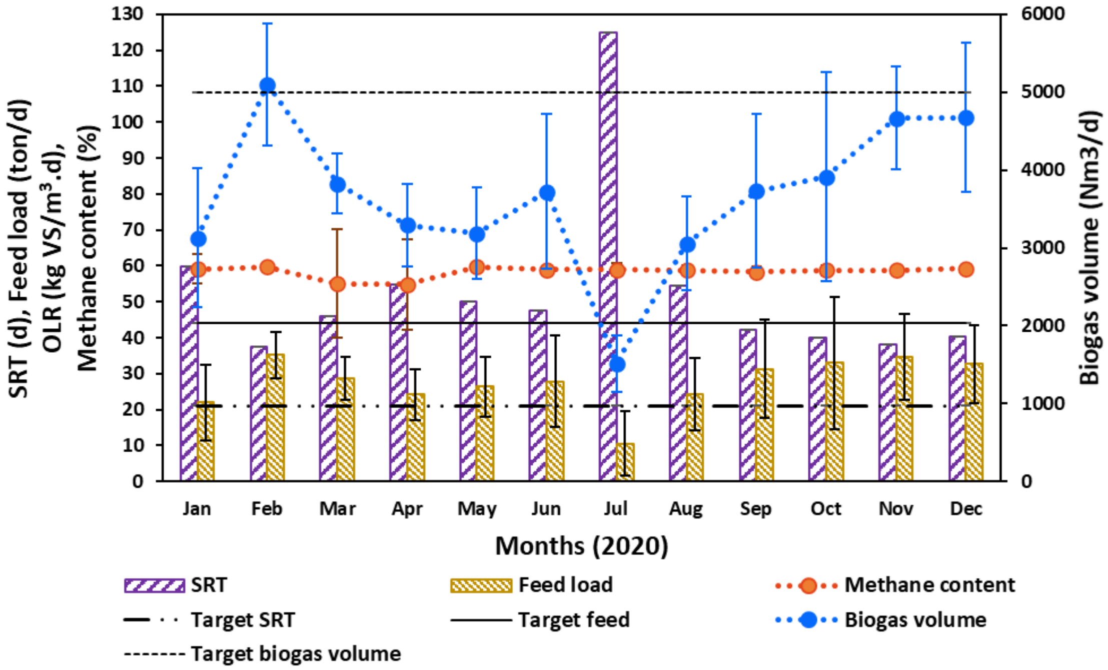
a. Initial digestate quality data from Digester 1. These values are very similar to initial values in Digester 2
b. FAN is calculated from TAN, pH and temperature (Puig-Castellví et al., 2020).

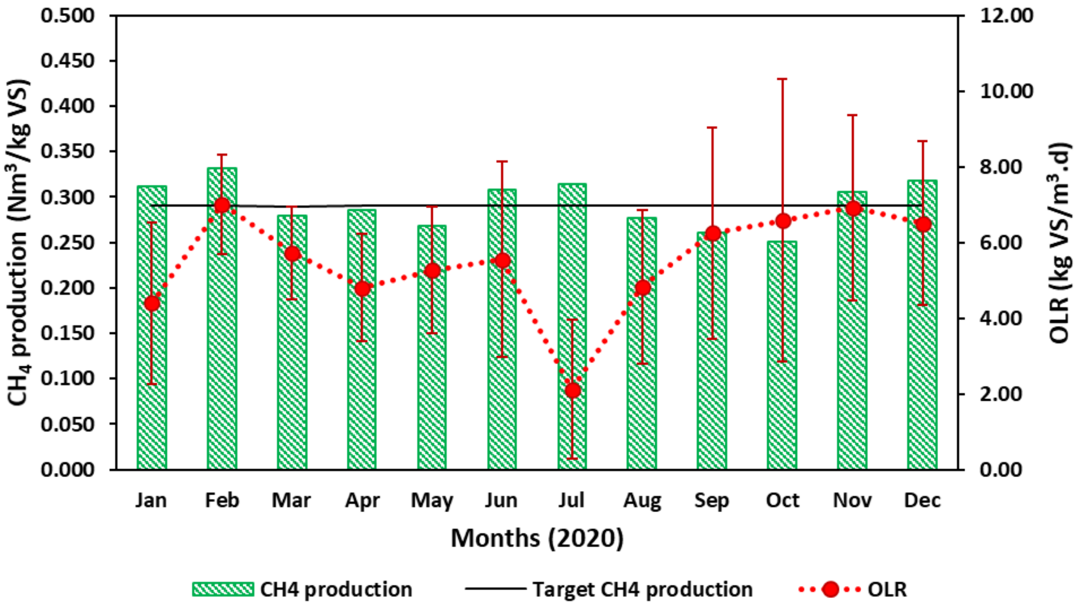
Annex B. 3. Metal content in the organic fraction of municipal solid waste (OFMSW) treated by the anaerobic digestion plant under study and resulting concentrations in the digestate of the full-scale digesters and the pilot reactors run in Experiment 1 (TR-MF: thermophilic reactor inoculated with wet thermophilic digestate from treatment of manure, maize and food waste) and Experiment 2 (MR: mesophilic reactor inoculated with wet mesophilic digestate from treatment of manure, food waste and agricultural waste). Accumulation factors for the metals in the reactors are shown in parenthesis.

Parameters	**OFMSW**	**Full scale reactors**	**TR-MF (E 1)**	**MR (E 2)**
TS content (%)	37 ± 1	23 ± 1	16	23
Micronutrients (g/kg TS)				
Ca	87.4 ± 5.4	224 ± 15 (2.6)	55.1 (0.63)	50.4 (0.58)
K	24.0 ± 0.9	72.8 ± 0.2 (3.0)	17.6 (0.73)	16.4 (0.69)
Mg	11.9 ± 1.2	29.9 ± 0.9 (2.5)	8.00 (0.67)	6.61 (0.56)
Na	15.4 ± 0.7	39.7 ± 0.1 (2.6)	10.25 (0.67)	10.2 (0.66)
P	10.1 ± 1.1	28.4 ± 0.7 (2.8)	8.38 (0.83)	6.96 (0.69)
S	7.66 ± 0.54	19.7 ± 0.5 (2.6)	5.27 (0.69)	5.78 (0.79)
Heavy Metals (mg/kg TS)				
Cd	1.98 ± 0.70	5.76 ± 1.44 (2.9)	<0.481 (-)	<0.470 (-)
Cr	65.8 ± 40.7	44.8 ± 4.2 (0.7)	213 (3.2)	161 (2.4)
Cu	63.3 ± 8.8	208 ± 12 (3.3)	385 (6.1)	145 (2.3)
Zn	558 ± 240	1141 ± 27 (2.0)	523 (0.9)	443 (0.8)
Pb	35.5 ± 11.0	114 ± 4 (3.2)	44.6 (1.3)	31.6 (0.9)
Al	7220 ± 2649	17147 ± 572 (2.4)	8563 (1.2)	8957 (1.2)
Ni	28.0 ± 18.4	18.0 ± 1.2 (0.6)	99.4 (3.6)	70.9 (2.5)
Co	5.01 ± 1.02	11.5 ± 1.5 (2.3)	3.66 (0.7)	2.97 (0.6)
Fe	8812 ± 1607	26036 ± 748 (2.9)	6813 (0.8)	6913 (0.8)
Mn	830 ± 90	2025 ± 76 (2.4)	448 (0.5)	474 (0.6)

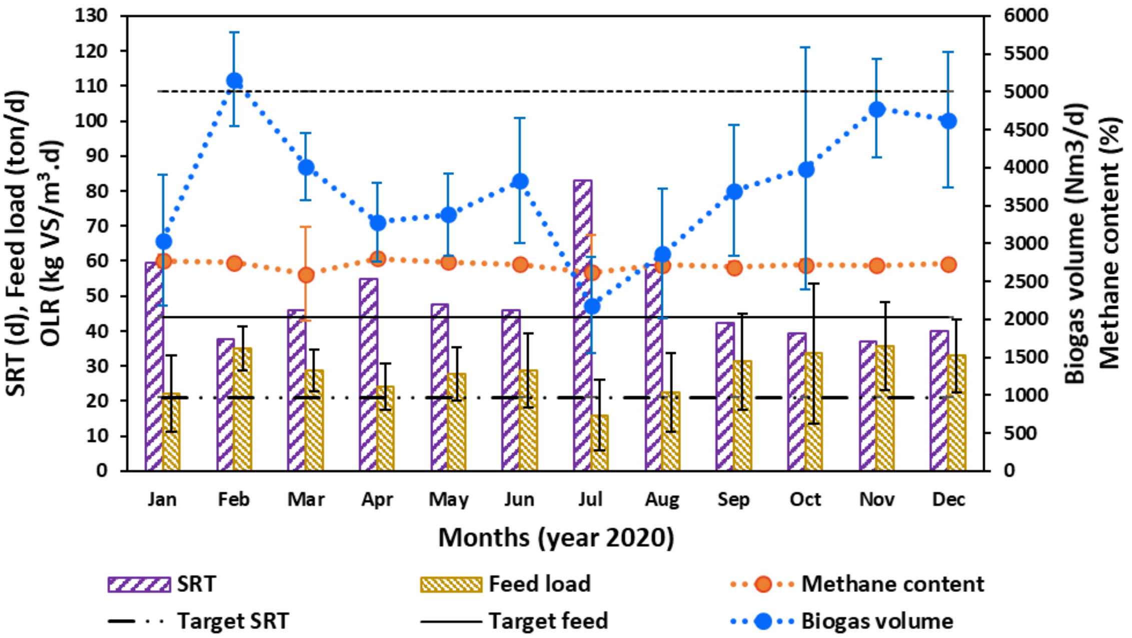
TS: total solids

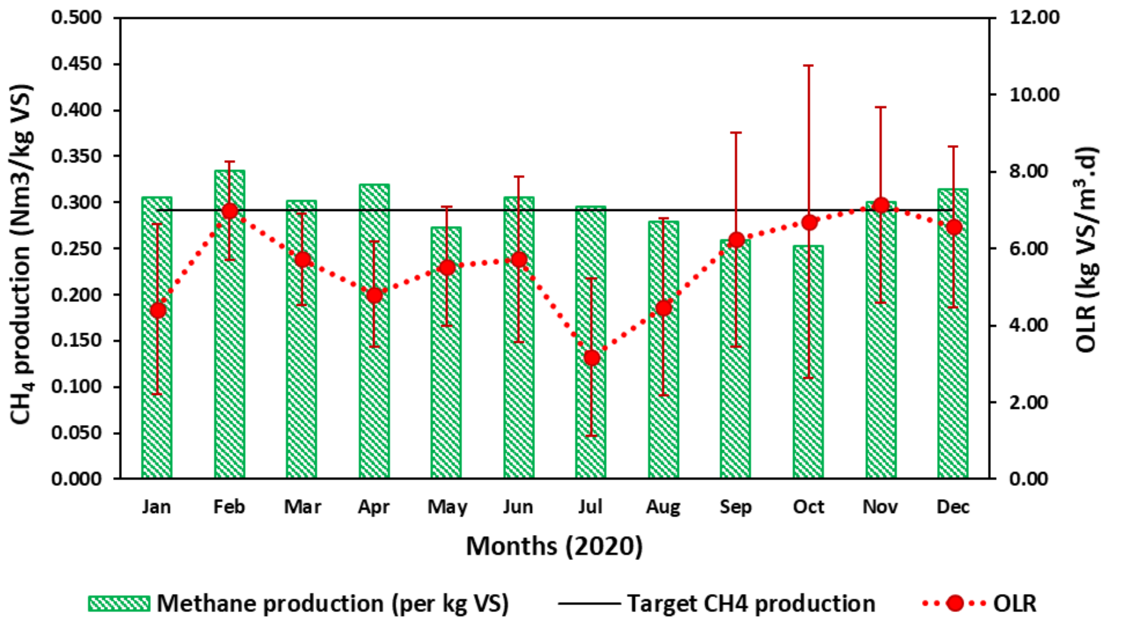
Annex B. 4. Monthly averages in the full-scale Digester 1 (D1) in 2020 for reactor performance indicators (top graph: methane content and biogas volume; bottom graph: methane production) and operational parameters (top graph: feed load, solids retention time [SRT]; bottom graph: organic loading rate [OLR]). The error bars represent the standard deviation of the means.



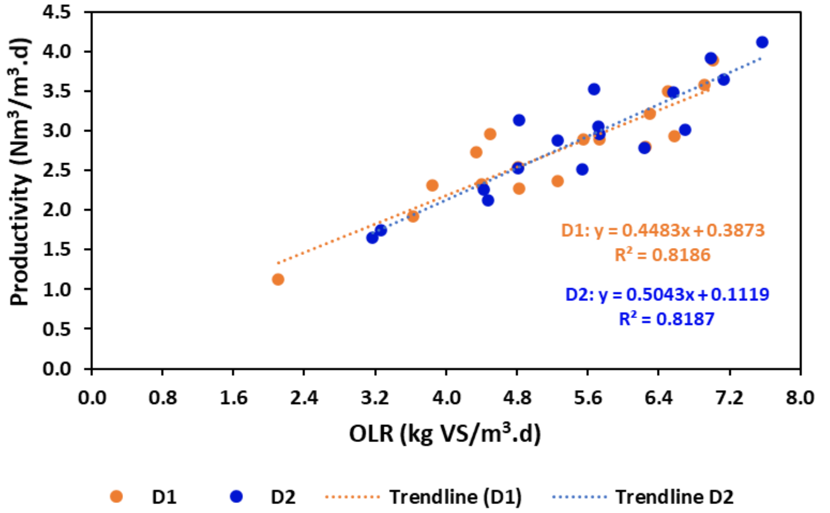


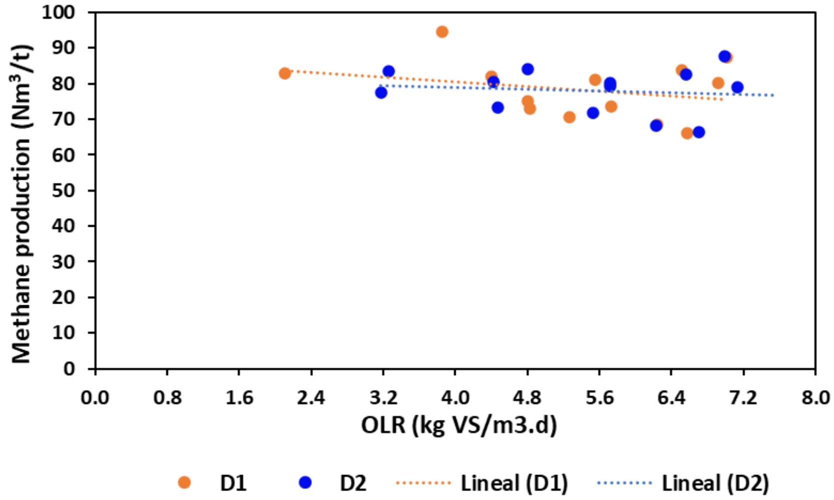
Annex B. 5: Monthly averages in the full-scale Digester 2 (D2) in 2020 for reactor performance indicators (top graph: methane content and biogas volume; bottom graph: methane production) and operational parameters (top graph: feed load, solids retention time [SRT]; bottom graph: organic loading rate [OLR]). The error bars represent the standard deviation of the means.



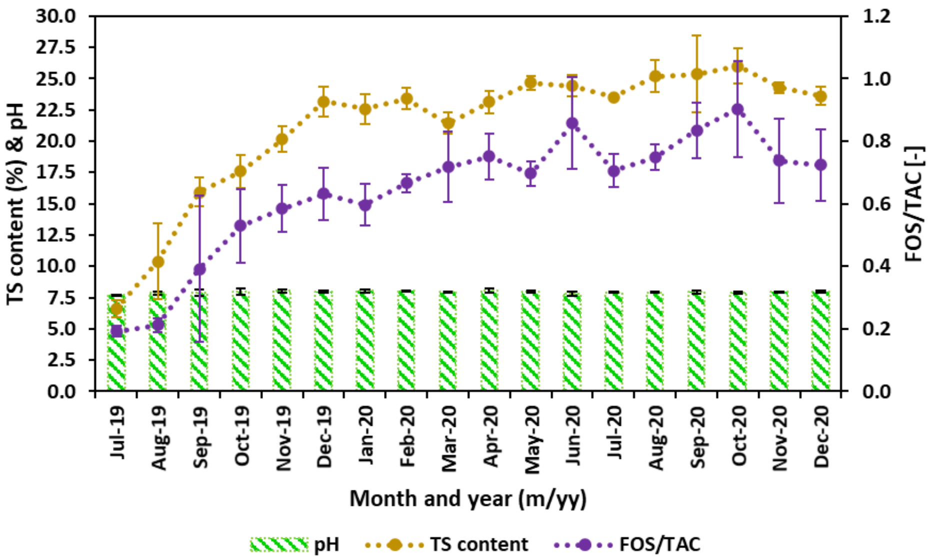


Annex B. 6: Correlation between OLR and productivity for D1 and D2 (the 2 digesters studied and operated by Uvélia).

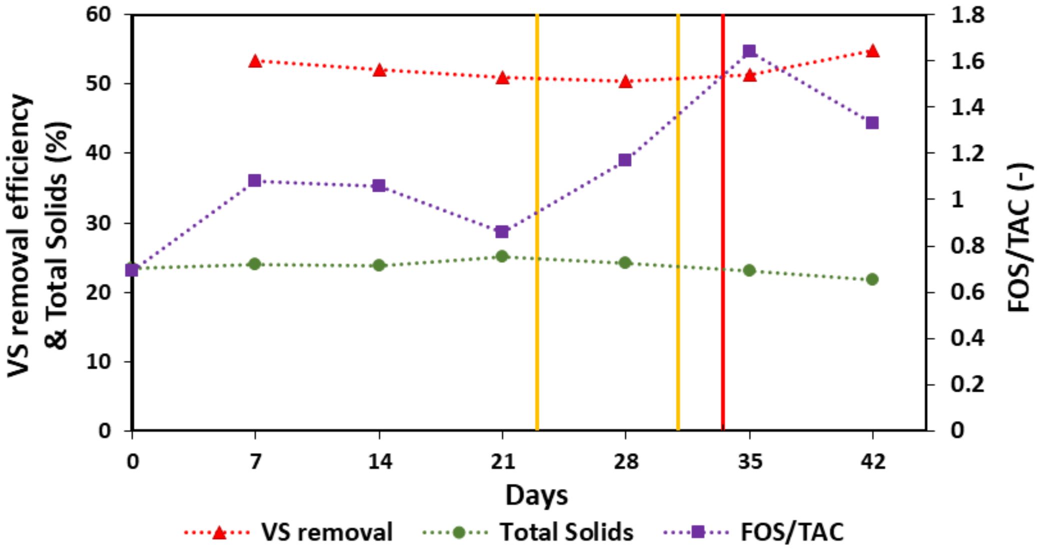


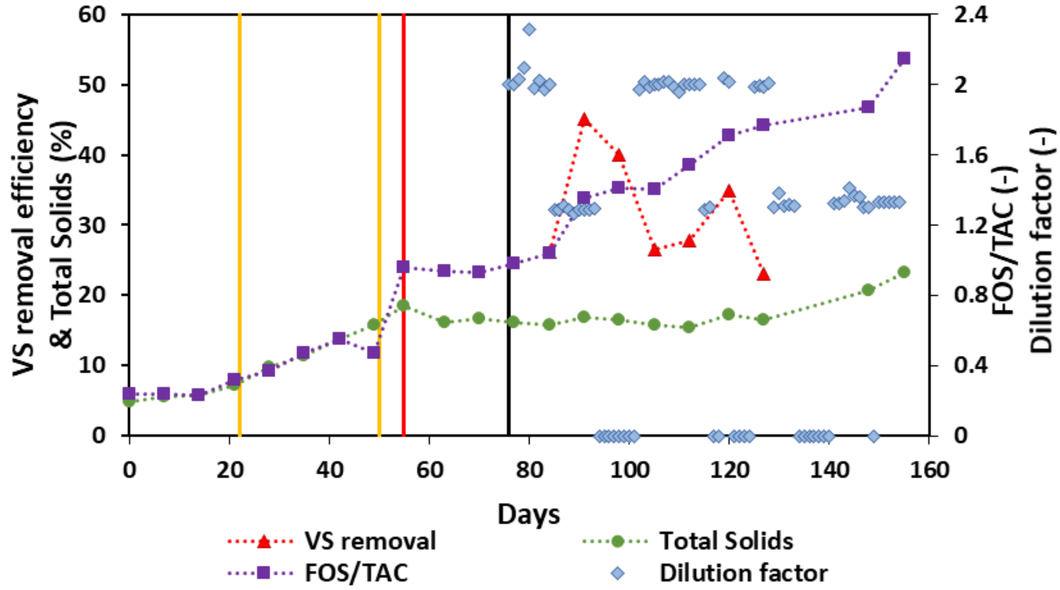


Annex B. 7. Monthly averages of digestate quality parameters monitored by Uvélia for D1 in 2020. Error bars represent the standard deviation of the data sets.

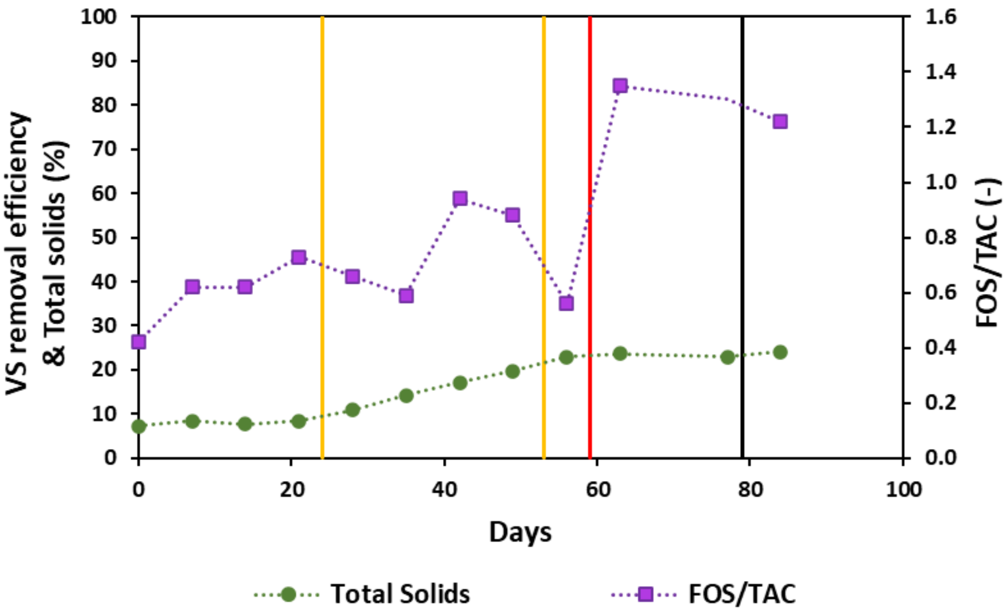


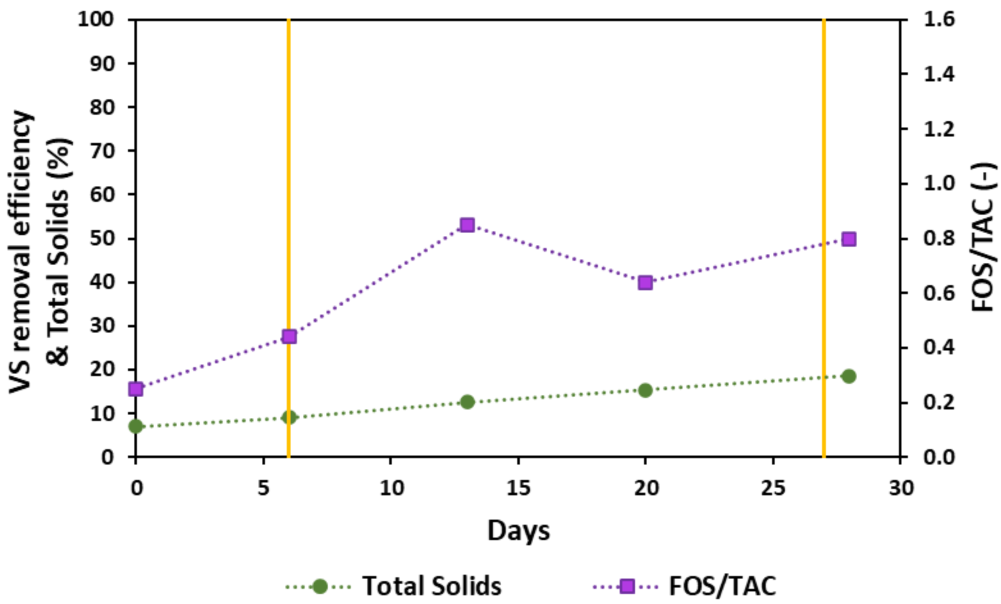
Annex B. 8. Weekly total solids (TS), VFA to alkalinity ratio (FOS/TAC) and volatile solids (VS) removal efficiency for the thermophilic reactors TR-OF (top graph) and TR-MF (bottom graph) throughout their operation. VS removal was calculated only when TS reached a steady concentration. TR-OF was inoculated with digestate from the plant under study (derived from organic household waste). TR-MF was inoculated with digestate sourced from a different plant, treating maize manure and food waste. The end of the acclimation period of the reactors is marked by the yellow bars, which delineate the period of stable feeding simulating an SRT of 30 d (OLR ~9.2 kg VS/m^3.d). The red bars indicate when reactor failure took place and the black bars the beginning of feed dilution (as an ammonia mitigation strategy). For TR-MF, the daily dilution factor applied is shown.



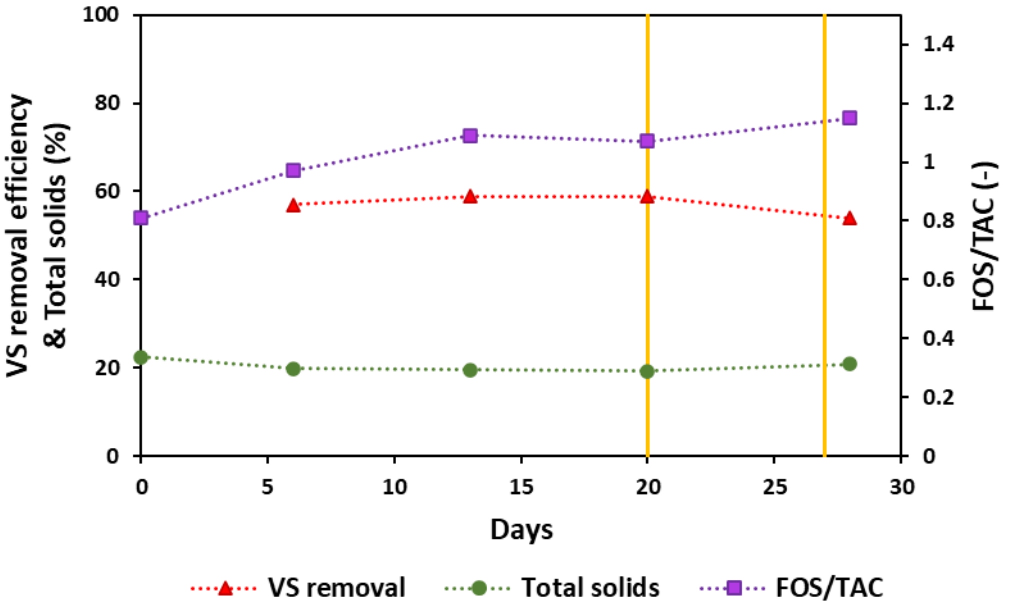


Annex B. 9: Weekly total solids (TS) and VFA to alkalinity ratio (FOS/TAC) for the mesophilic reactors MR (top graph) and MR-HL (bottom graph), run to test if mesophilic conditions mitigated ammonia toxicity. MR and MR-HL were both fed with organic waste collected from the plant under study and inoculated with digestate derived from the same plant (treating manure, food and agricultural waste), but MR-HL was fed with hydrolysed organic waste The end of the acclimation period is marked by the yellow bars, which delineate the period of stable feeding (9.17 kg VS/m^3.d for MR and 7.76 kg VS/m^3.d for MR.HL). The red bars indicate when reactor failure took place and the black bars the beginning of feed dilution.

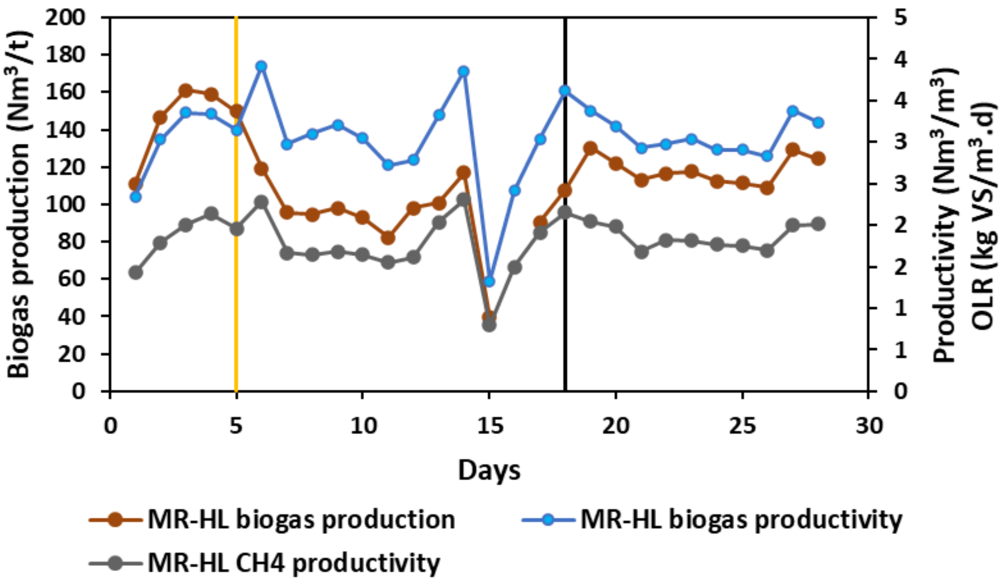




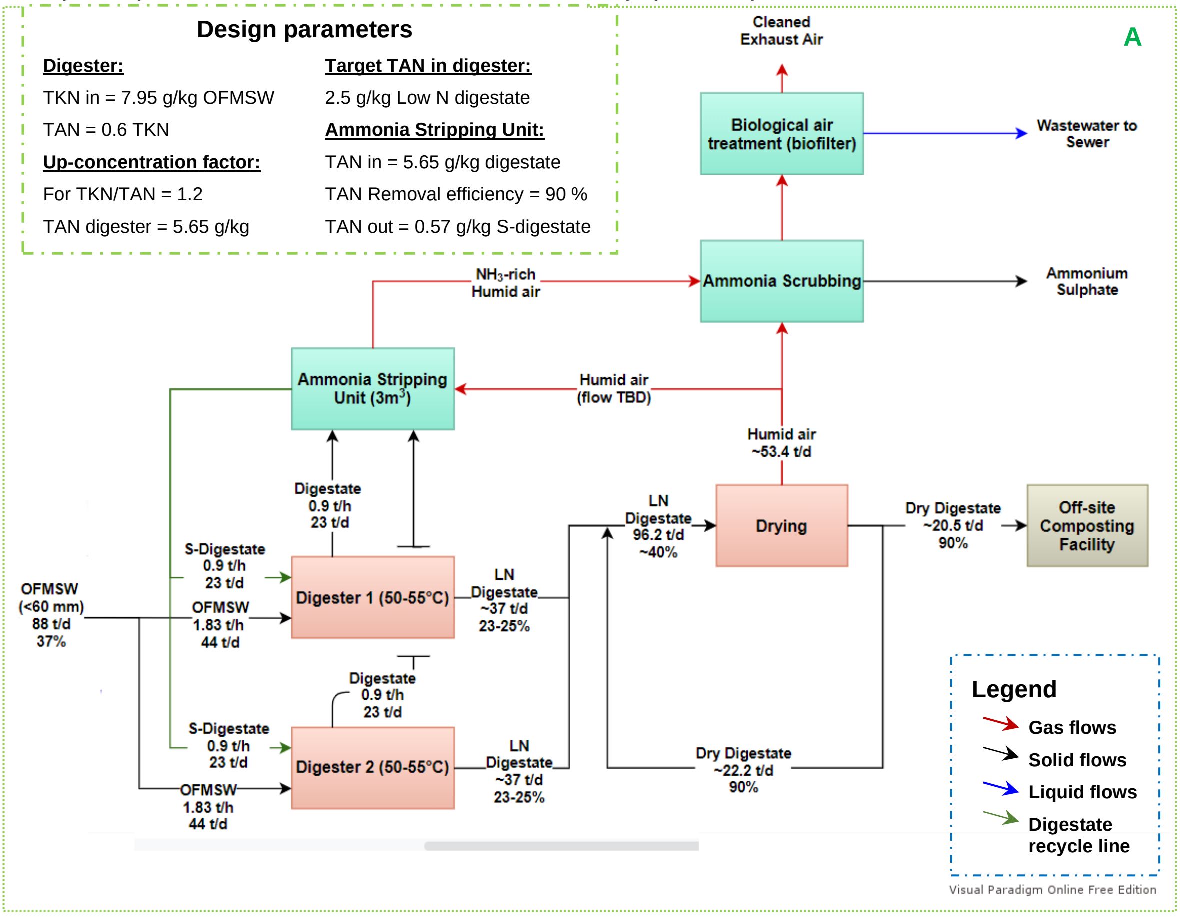
Annex B. 10. Weekly total solids (TS) and VFA to alkalinity ratio (FOS/TAC) and volatile solids (VS) removal efficiency (calculated only when TS reached a steady concentration) for CoTR-OF during its operation time. Co-TROF is the thermophilic reactor inoculated with digestate sourced from the anaerobic digestion plant under study and run to assess the effectiveness of co-digestion in the mitigation of ammonia toxicity (using green waste and the household organic waste treated by the plant under study at a 1:3 ratio) The yellow bars delineate the period of stable feeding at 5.21 ± 2.39 kg VS/m^3.d.



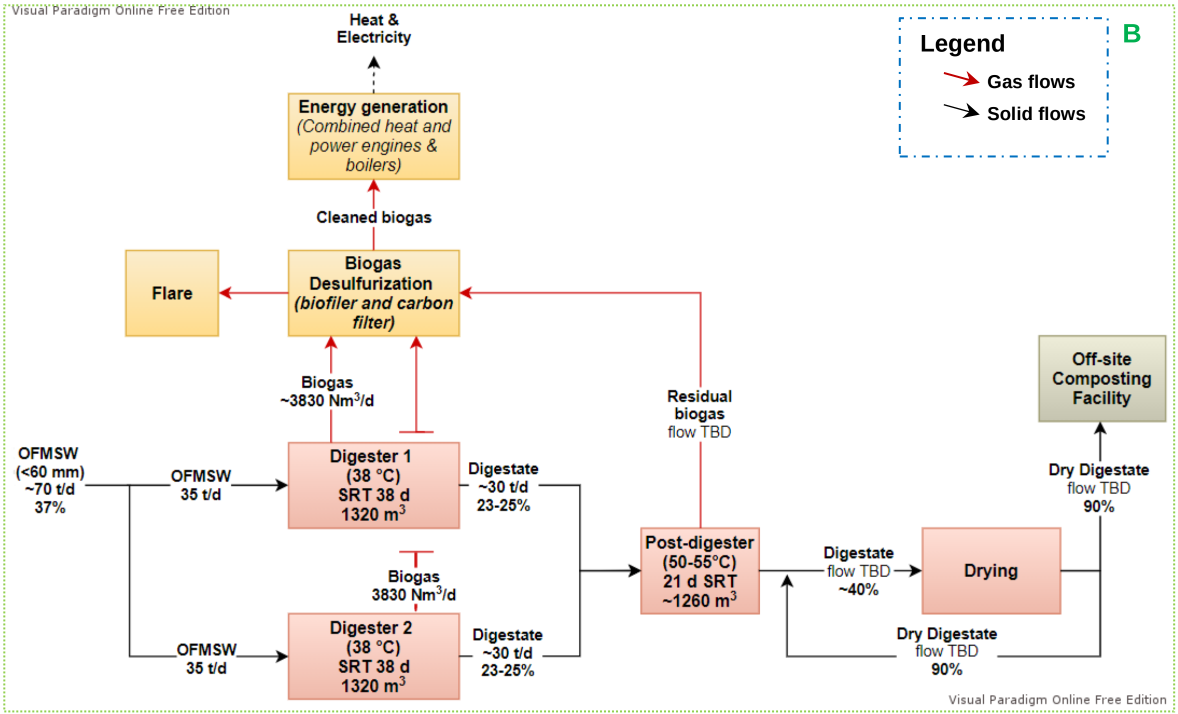
Annex C. 1: Changes in biogas and methane productivity and biogas production with the change in target OLR in the mesophilic reactor run in Experiment 3 (MR-HL, inoculated with digestate from manure, and food and agricultural waste treatment and fed with hydrolysed substrate). The yellow bar indicates the beginning of stable feeding at the initial target OLR (~9.2 kg VS/m^3.d); the black bar indicates the change in target (and applied) OLR to ~ 7.3 kg/m^3.d)



Annex C. 2: Proposed configuration and mass flows for integrating an ammonia stripping unit (Figure A) or a temperature-phased process (Figure B) in Uvélia's anaerobic digestion plant. Mass flows were calculated using the target parameters defined in **Table 3.2** in the text. Figure A: the parameters taken into account to calculate the digestate recycle flow through the NH_3-stripping unit are shown. Figure B: OFMSW load based on OLR (~7.3 kg VS/m^3) tested in the mesophilic pilot simulation carried out in this study (MR-HL).



TBD: to be determined, S-digestate: stripped digestate, LN digestate: low nitrogen digestate



TBD: to be determined