UNIVERSIDAD POLITÉCNICA DE MADRID
Escuela Técnica Superior de Ingenieros de Minas y Energía

Harnessing Agricultural Waste for Energy: Improving Decentralised Anaerobic Digestion for Energy Self-Sufficiency

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Cornelis Bumharter
Master in Mechanical Engineering

Madrid, 2024

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Doctoral Degree in Research, Modelling and Analysis of Environmental Risk

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Under the supervision of:
Dr. Marcelo F. Ortega Romero
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Abstract

In light of climate change, sustainability concerns, rising energy and fuel costs, the energetic landscape of Europe stands at a brink of great change. Current waste management techniques in agriculture and livestock industries are extremely harmful to ecosystems and cause issues such as groundwater pollution, eutrophication and climate change acceleration. A solution to these issues can be found in anaerobic digestion technologies, where bacterial microbes are used to degrade either harmful organic waste structures or unutilised residues. A specific business case has been built for low-cost pilot-scale reactors, which are to be installed on a decentralised level to improve energy self-sufficiency of rural communities, large families, and small farms which currently have no access to proper waste infrastructure of an industrial-scale biogas plant.

In order to assess the potentials and viability of biogas generation in pilot-scale reactors, a research methodology was developed in which first, the theoretical potential is identified, followed by a series of experiments to support the business case. The current biogas landscape in Europe was discussed, identifying that compared to its potential, the EU-27 are merely producing 2.65% biogas with 2020 data. After this, a statistical analysis has been performed, where it was found that the carbon-nitrogen-ratio is the most critical design parameter for efficient and stable anaerobic digestion. Various models could be proposed linking pH and retention times to subsequent biomethane proportions.

Following this, the experimental part assessed biochemical methane potential, semi-continuous variable load, and ultimately pilot-scale anaerobic digestion testing. Biochemical methane potential (batch) testing concluded that mixtures of meadow grass and swine manure provided the highest quality biogas, and per mass of substrate added, showed the best conversion factor. Glycerol addition remained below expectations. Semi-continuous testing analysed different feeding rates with the intention to determine the most efficient loading rate to maximise the biomethane production. It was concluded that a system operating at 9 g VS/L.day has the highest conversion rate and produces most biomethane but is also susceptible to reactor acidification. Pilot scale plants should not surpass an organic loading rate of 8 g VS/L.day. Lastly, the pilot scale reactor was successfully operated in an ambient farm environment and could produce high-quality biogas at external temperatures beginning at 15

°C. Despite the successes registered, shortcomings on the heating and agitation strategies reduced reactor efficiency. Due to experimental scaling and resultant inefficiencies, the pilot plant produced 83% and 57% less biomethane than the batch and semi-continuous experiments respectively.

Resumen

A la luz del cambio climático, la preocupación por la sostenibilidad y el aumento de los costes de la energía y los combustibles, el panorama energético de Europa se encuentra al borde de un gran cambio. Las técnicas actuales de gestión de residuos en la agricultura y la ganadería son extremadamente perjudiciales para los ecosistemas y causan problemas como la contaminación de las aguas subterráneas, la eutrofización y la aceleración del cambio climático. Una solución a estos problemas puede encontrarse en las tecnologías de digestión anaerobia, en las que se utilizan microbios bacterianos para degradar las estructuras de desecho nocivas o los residuos no utilizados. Se ha creado un caso de negocio específico para reactores a escala piloto de bajo coste, que se instalarán a nivel descentralizado para mejorar la autosuficiencia energética de comunidades rurales, familias numerosas y pequeñas explotaciones agrícolas que actualmente no tienen acceso a la infraestructura de residuos adecuada de una planta de biogás a escala industrial.

Con el fin de evaluar el potencial y la viabilidad de la generación de biogás en reactores a escala piloto, se desarrolló una metodología de investigación en la que, en primer lugar, se identifica el potencial teórico, seguido de una serie de experimentos para apoyar el caso de negocio. Se ha analizado el panorama actual del biogás en Europa, identificando que, en comparación con su potencial, la UE-27 sólo produce un 2,65% de biogás con datos de 2020. Después de esto, se ha realizado un análisis estadístico, donde se ha encontrado que la relación carbono-nitrógeno es el parámetro de diseño más crítico para una digestión anaerobia eficiente y estable. Se han podido proponer varios modelos que relacionan el pH y los tiempos de retención con la posterior proporción de biometano.

A continuación, la parte experimental evaluó el potencial bioquímico de metano, la carga variable semicontinua y, por último, las pruebas de digestión anaerobia a escala piloto. Las pruebas de potencial de metano bioquímico (batch) concluyeron que las mezclas de césped y estiércol porcino proporcionaban el biogás de mayor calidad y, por masa de sustrato añadida, mostraban el mejor factor de conversión. La adición de glicerol quedó por debajo de las expectativas. Las pruebas semicontinuas analizaron diferentes flujos de alimentación con la intención de determinar el flujo de carga más eficiente para maximizar la producción de biometano. Se llegó a la conclusión de que un sistema que funciona a 9 g VS/L.día tiene el

mayor factor de conversión y produce más biometano, pero también es susceptible a la acidificación del reactor. Las plantas a escala piloto no deberían superar un flujo de carga orgánica de 8 g VS/L.día. Por último, el reactor a escala piloto ha mostrado su eficaz en producir biogás de alta calidad en un entorno ambiental (granja) ton temperaturas ambientales a partir de 15 °C. Sin embargo, se ha identificado problemas de calentamiento y agitación. Debido al escalado experimental y a las ineficiencias resultantes, la planta piloto produjo un 83% y un 57% menos de biometano que los experimentos batch y semicontinuos, respectivamente.

Summary of Publications

JCR Research Articles

A. Bumharter C, Bolonio D, Amez I, García Martínez MJ, Ortega MF. *New opportunities for the European Biogas industry: A review on current installation development, production potentials and yield improvements for manure and agricultural waste mixtures*. J Clean Prod. 2023 Feb 15;388:135867. DOI: https://doi.org/10.1016/j.jclepro.2023.135867
B. Canoira L, Donoso D, Bolonio D, Bumharter C, Lapuerta M. *Desulfurized and Hydrogenated Crude Sulfate Turpentine (HCST): A Biofuel Derived from a Waste of the Pulp and Paper Industries*. Energy & Fuels. 37 (20), 15843-15854. DOI: https://doi.org/10.1021/acs.energyfuels.3c02616
C. Bumharter C, Amez I, Castells-Somoza B, Bolonio D, García Martínez MJ, Ortega MF. *Unlocking Efficiency: Investigating Optimal Co-Digestion Mixtures for Enhanced Biogas Production in Small-Scale Rural Settings*. [To be published in: Waste Management]
D. Bumharter C, Amez I, Ortega MF, Velasquez P, Chacón LM, Acevedo P, Cabeza I. *Determining Optimal Organic Loading Rates for Biomethane Production in Rural Biogas Plants: Balancing Yield and Stability*. [Currently under Review. To be published in: Journal of Environmental Management]

Conferences

A. Bumharter C, Amez I, Castells-Somoza B, Bolonio D, García Martínez MJ, Ortega MF. *Growth of a Novel Inoculum Directed at Anaerobic Digestion of Farm Waste and Swine Manure: Biochemical Methane Potential Tests and Degradation Potential Analysis Through Feedstock Monitoring*. The 18th SDEWES Conference, Dubrovnik, Croatia (Hybrid).

List of Abbreviations

Abbreviation	Description
AcD	Anaerobic Co-Digestion
AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
BOD	Biochemical Oxygen Demand
BOP	Balance of Plant
C/N	Carbon-Nitrogen-Ratio
$CaCO_3$	Calcium Carbonate
CAPEX	Capital Expenditure
CCS	Carbon Capture and Storage
CH_4	Methane
CHP	Combined Heat and Power
CNG	Compressed Natural Gas
CO_2	Carbon dioxide
COP	Conference of the Parties
CPI	Codigestion Performance Index
CSTR	Continuous Stirred-Tank Reactor
EBA	European Biogas Association
EC	European Commission
EGSB	Expanded Granular Sludge Blanket (Reactor)
EU	European Union
GDP	Gross Domestic Product
GHG	Greenhouse Gas
GTP	Global Temperature Change Potential
GWP	Global Warming Potential
H_2	Hydrogen (gas)
HRT	Hydraulic Retention Time
IEA	International Energy Agency
KPI	Key Performance Index
LAD	Liquid Anaerobic Digestion
LCA	Life Cycle Analysis
LCOE	Levelised Cost of Energy
LNG	Liquefied Natural Gas
MBR	Membrane Bio-Reactor
MSW	Municipal Solid Waste

N_2O	.....	Nitrous Oxide
NH_3	.....	Ammonia
NH_4^+	.....	Ammonium
NO_x	.....	Nitrogen Oxides
O_2	.....	Oxygen
OFMSW	.....	Organic Fraction of Municipal Solid Waste
OLR	.....	Organic Loading Rate
pH	.....	Power of Hydrogen, acidity measure
ppm	.....	Parts per million
PV	.....	Photo-Voltaic
R&D	.....	Research and Development
RED	.....	Renewable Energy Directive
RNG	.....	Renewable Natural Gas
RSM	.....	Response Surface Methodology
SCADA	.....	Supervisory Control and Data Acquisition
SO_2	.....	Sulfur Dioxide
SRT	.....	Solids Retention Time
SS-AD	.....	Solid-State Anaerobic Digestion
STP	.....	Standard Temperature and Pressure
TAN	.....	Total Ammonia Nitrogen
TCD	.....	Thermal Conductivity Detector
TKN	.....	Total Kjeldahl Nitrogen
TS	.....	Total Solid
TSS	.....	Total Suspended Solids
UASB	.....	Upflow Anaerobic Sludge Blanket (Reactor)
VFA	.....	Volatile Fatty Acid
VS	.....	Volatile Solids
VS_{red}	.....	Reduced Volatile Solids

1. Introduction

1.1 Context and background

The Intergovernmental Panel on Climate Change reports an increase in average temperatures of 1.09 °C within the 2010-2020 decade, brought upon mainly by "*annual averages of 410 parts per million (ppm) […] of Carbon Dioxide CO_2*" within their 2021 climate change statement (1). As society faces alarming consequences by climate change of human origin, an unequivocal change in policies is required to prevent further damage to ecosystems. The European Union (EU) provides a set of regulations to energy neutrality within their *European Green Deal,* striving towards the challenging objective of reaching full sustainability by 2050 (2). Selected elements of their agenda include "*No net emissions of greenhouse gases by 2050*" and "*economic growth decoupled from resource use*" (2). These two statements are colloquially interpreted as the necessity to fully convert the conventional energy landscape in Europe to an energy system with fully-integrated and flexible renewable energy technologies, independent from fossil fuels.

Renewable energy technologies, mainly focussing on the development of wind power and solar photovoltaics, are often criticised due to their weakness in dealing with power intermittency issues. Additionally, conventional renewable energy sources are not capable of supplying the necessary heat required by industries, so there must be a form of renewable fuel to bridge this gap in the demand profile of energetic sources. Conventional fossil-fuel-based power plants can comparatively increase and decrease power supply based on consumer demand, whilst renewable energy technologies depend on environmental conditions to produce power. The further development of sustainable power generation sources that can accommodate variable demand behaviour is necessary, and a solution has been found in the form of bio- and E-fuels, such as hydrogen, biodiesel, bio-methanol, bio-ethanol and biogas.

The European Commission has defined a roadmap to energy independence, where carbon budgets were introduced to guide member countries via distinct annual milestones towards the 2050 neutrality goal (2). Whilst the use of renewable energy is pivotal for abiding by the Union's regulations, further environmental studies have concluded that various sectors, traditionally independent of fossil fuels, are specifically damaging to ecosystems and indirectly produce greenhouse gases (3). In fact, emissions caused by waste management and unsustainable agriculture play a major role in the carbon budget and are highlighted as primary

contributors to climate change due to their emissions (4). Because of waste mismanagement in these sectors, gases such as methane, carbon dioxide and nitrous oxides amongst others are inadvertently emitted, causing additional strain on both the carbon budget and the environment which the carbon budget intends to regulate (5). The lack of an ideal waste management strategy is thus exacerbating a country's endeavour in following the net-zero guidelines imposed by the EU.

December 2021 marked a period of widespread concern due to the climate change impacts brought upon by large, unsustainable livestock farms. Spanish and other European farming practices prove unsustainable due to large cultivation of monocultures, soil toxification by livestock manure, augmented water consumption and onset of desertification to propel Iberian climate change. Spain's solution proposal is in line with the EU green deal's action statement called the *Farm-to-Fork Strategy*, whose objectives include sustainable food production, preservation of biodiversity, environmental protection and prevention of climate change (2,6). As a result, the Spanish government and the EU independently called for a transformation of the agricultural and livestock sector to become more environmentally friendly, and a carbon neutral industry adapted to the standards of a post-2050 Europe. Currently, the agricultural and livestock sector is responsible for nearly a third of the European Union's gas emissions, bolstering the necessity of emissions reduction in the future (3). A solution applying the principles of circular economy within the agricultural and livestock sectors is proposed as follows: The crop output is distributed to the population and livestock farms for consumption, as previously. Any residue generated through agriculture (i.e. lignocellulosic biomass) and livestock keeping (i.e. manure) is to be collected and transformed into carbon-neutral regenerative fuels to prevent natural decomposition and exacerbation of adverse environmental impacts. The residue after energy recovery and material digestion may then be reintroduced into the primary crop system in the form of organic fertiliser to restart the cycle and maintain energy and alimentary circularity (7).

1.2 Biogas production

Anaerobic digestion (AD) is a set of distinct biological degradation processes to fully oxidise or reduce the organic waste products into biogas and digestate, a liquid waste product containing fatty acids, ammonia and all other products that are not readily degradable. A

community of bacteria synergistically dissect the feedstock inputs to produce biogas, a mixture of carbon dioxide, hydrogen, and methane, simulating the decomposition of waste in nature, such as in wetlands, animal digestive systems, and landfill sites of human origin (8). By using AD, the chemical oxygen demand of a waste product is significantly reduced, effectively lowering the potential for the waste product to use atmospheric oxygen to degrade into greenhouse gases. The chemical oxygen demand can also be interpreted as a measure of calorific value present in the sample. Not only does controlled AD produce a renewable fuel with various applications, but also provides a clean ecological fertilizer to reduce soil toxicity by providing the essential nutrients without risking humus or soil erosion as conventional techniques (4,8).

Biogas production through AD circumvents poor waste-management techniques by using residue materials to produce a renewable, combustible and carbon-neutral fuel (4). To build on this, the EU has set a minimum standard for all organic waste management, which requires producers and consumers to actively seek reutilisation (such as AD) under the Waste Framework Directive (*Directive 2008/98/EC*). This framework provides added benefits of circularity and sustainability (fertilisation and energy recovery respectively) (9). Through a decentralised and developed biogas landscape, energy security and production can be decoupled from major power plants and specific design choices can be adopted to the needs and requirements of end consumers. Risks associated with improper treatment of waste like manure and lignocellulosic biomass include eutrophication, water pollution and ecotoxicity, which can be effectively abated using AD (4). As a result, biogas is quoted to contribute towards the three sustainable development goals of "*2, Zero Hunger; 7, Affordable and Clean Energy; [and] 13, Climate Action*" (10,11).

Anaerobic co-digestion (AcD) is described as the simultaneous digestion of several waste products through substrate mixing prior to reactor feeding. AcD has received widespread attention in the biogas research world due to its improved efficiency and synergy opportunities in substantially augmenting the resultant fuel yield, as described by Scarlat et al. (2018) and through the study of Kougias and Agelidaki (2018) (4,8). Depending on the chemical and protein composition of the substrate, the resultant biogas can have a) a different quality and b) a different quantity of biomethane. During AD, the inert gas CO_2 is to be minimised as it inhibits combustion. By optimising the digestion feedstock one can predict the composition of

gas produced, especially focussing on the ratio of methane to carbon dioxide within the biogas. Further addition of hydrogen to the biogas enhances the combustion capability of a fuel, which has been evaluated in complementary studies of the research group in form of combustion potentials for biogas-hydrogen mixtures (12). Especially the mixture of biogas and hydrogen will play an important part in decarbonising various sectors of the EU economy (13). Through hydrogen addition, the calorific value and fuel enthalpy are improved such that combustion stability is provided by conventional gas burners (12). By initially predicting the biogas yield and richness in the form of the substrates that have been added to a reactor and the performance parameters that allow for adjustment, the ratio of hydrogen addition can be defined at a project beginning, allowing for precise combustion modelling. Due to this, the understanding of feed mixtures and their performance parameters provides great potential to both transform the industry requiring heat and electricity through burning biogas, and for finding a mixture/business case where extremely rich biogas is extracted from reactors, which has a predominant biomethane proportion such that no further addition of hydrogen is required.

The assessment of these mixture profiles to prevent the need for hydrogen additions is covered in this thesis.

1.3 Project summary and proposition

The global installation of bioenergy reached 127 GW in 2021, making it the 4th greatest source of renewable energy worldwide (14). Whilst most industrial units have been built in North America and Europe, Africa and Asia quote large quantities of micro-digesters and prove expertise in a bottom-up implementation approach (15,16). Traditionally, only industrial-scale biogas installation has occurred in Europe due to the benefits of economies of scale and the reduced organisation required for centralised waste management schemes. Because of this, Germany has various biogas plants built across the whole country with a minimal nominal capacity of 750 kW, as this was specifically subsidised by the government in recent years (17).

Pilot scale plants, i.e. plants with a capacity of holding up to 10 m^3 of digestate, have not been installed comprehensively, mainly due to their lower efficiencies and higher associated Levelized Cost of Energy (LCOE) that the resultant biomethane is subject to (18). Due to new

climate directives and issues with waste treatment, low-cost reactors for self-consumption may be an interesting business case which is to be explored in this thesis. No major study or analysis has been performed to date that assesses the economic viability and technical feasibility of pilot-scale anaerobic digesters. This research gap is to be filled by this thesis through evaluating highly potential feedstocks and providing a proposition for improved reactor operations.

Especially when acknowledging previous European security-of-supply issues and the rising energy prices during 2021-2023, there has been recurring interest in further exploring sustainability initiatives that can produce a domestic fuel, especially when the infrastructure has already been developed and the technology of gas distribution and combustion is considered as matured and ready for further application. An additional benefit is that low-cost pilot plants generally require little expert intervention and are very simple to operate, contrary to the industrial counterparts.

With the issues of current agricultural waste in mind and also seeing the potential that further biogas implementation can bring, especially due to high energy prices and the requirement for decoupling the energy industry from foreign imports, a business case was defined in which low-cost pilot-scale reactors would provide additional support through decentralised power generation and consumption. In order to determine the viability of pilot-plant AD reactors, this PhD project was defined in which the technical feasibility and efficiency optimisation of bioreactors on a smaller scale (rather than industrial) are analysed and discussed in depth. Figure 1 shows the breakdown of the overall project plan.

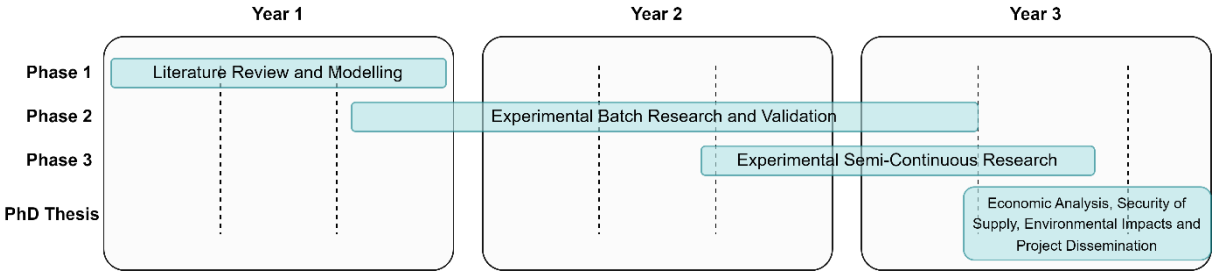


Figure 1: The PhD project timeline

As identified in Figure 1, the project has been divided into four major phases. In each phase, a research paper has been written to summarise and publish the results produced. In the initial phase, a literature review is conducted in which both the installation potentials and statistical

modelling of performance parameters of other biogas generation experiments has been studied and summarised. This literature review has analysed the waste materials and mixture proportions used in AD and identifies the optimal conditions for methanogenesis to occur. This is conducted through a statistical analysis after having compiled data from various studies in recent years.

Following from this, various experiments were planned and conducted in which mixture validation and yield assessments were performed. These were planned on the smallest possible laboratory scale, the batch scale, such that several samples can be tested simultaneously at low project cost. Next, an experimental analysis of material flow rates and microbial kinetics was planned to assess the different quantities of feed that can be added to the biological system. It was important to understand the possible material quantity to feed the biological system to obtain an understanding of the energy generation output based on different feeding rates. Lastly, an experimental assessment of pilot-scale reactors and the verification of their functionality was performed. Different issues and shortcomings were to be identified and discussed with the intentions of mitigating them through improved mixture and feeding profiles (experience gained in the previous experiments).

To conclude all analysis conducted, a further study was performed on the efficiencies registered and quoted in literature to the different experiments conducted. In total, the three experiments planned fall into different experimental scales as batch, laboratory semi-continuous, and pilot-scale plants. To wrap up the fully conducted research project, a further outlook on environmental impacts, security of supply, economic viability and digestate management are given to provide a holistic picture of the analysed technology.

Based on this planning, the primary objectives and research goals could be defined, which are outlined in Section 1.4.

1.4 Project objectives, scope and research question

The objectives and activities of this thesis revolve around the critical evaluation of biogas production characteristics described through different case studies to provide a technologically viable and environmentally friendly solution to assist in the transition to a carbon-free

economy. Through the focus on rural sites and farms, a way to combat waste problems and maximise biogas yields from agricultural and livestock waste will be combined with modern mixing and combustion technologies, such as the production and optimised treatment of agricultural waste. The thesis will contribute to work on simultaneous waste management efforts, such as finding climate-friendly approaches, wastewater treatment and municipal solid waste processing. The work of this project will support farm owners to achieve energy self-sufficiency and decentralised fuel production, which can be supplied to industrial users or used for self-consumption.

Based on this context, a holistic study on the feasibility of implementing pilot scale biogas plants in rural areas is proposed and the underlying objectives of the thesis are defined as:

- To study the European production of waste in agricultural and livestock farms: quantities and types of waste. Propose the optimal parameters of chemical and physical performance of anaerobic digestion based on the knowledge of the raw materials in Europe to produce the highest amount of biogas with the best possible quality. Calculate the potential for biogas production in Europe and the corresponding reductions in GHG emissions and environmental impact.
- Propose a system with ideal anaerobic digestion conditions based on parametric chemical, physical and performance constraints. Through a literature review of previous studies and statistical analysis (with RSM, Response Surface Methodology), optimal mixing ratios of feedstocks can be determined to obtain an efficient symbiosis of microbial cultures within the anaerobic digestion reactor. Chemical analysis of carbon, nitrogen and phosphorus ratios and macronutrient analysis of proteins, lipids and carbohydrates are essential to ensure efficient biogas production. Through the balancing of pH, volatile fatty acids and ammonia, an environment for biomass hydrolysis will be created (at the scale of all small farms in Europe) to increase biogas production. Specific quantification of performance parameters, such as organic loading rate and hydraulic retention time, should also be defined for ideal biogas production.
- Test and analyse such mixtures of raw materials and conditions to optimise anaerobic digestion on a laboratory scale. Investigate the optimal mixtures that have been found according to the literature review.

- Analyse the conversion efficiency of a biological system under different feed rates with the intention of determining the optimum flow rate for a biogas reactor to maximise biomethane production per volatile solid added.
- To set up a pilot scale trial (with the low cost biodigester) and investigate the potential for continuous operation to produce biogas. Define optimal parameters and improve biogas production through changes in daily substrate loading and digestate temperature.
- Analyse the change in biogas and biomethane production resulting from changing the design and scale of the experiment. Quantify the losses and inefficiency produced through scaling up from small scale to medium scale, and finally to pilot scale.
- Quantify the potential of the technology to reduce GHG emissions and environmental impact through a subsequent discussion and produce a financial outlook that verifies the economic viability of implementing a pilot scale (i.e. 1 m^3 biogas reactors) on rural farms to assist in the production of energy for self-consumption. Simulation of use applications detailing the energy savings that occur due to lower emissions from operation and digestate management.

The scope of this project is limited to the determination of these objectives and involves the optimisation and operational improvement of low-cost AD systems, with a specific focus on pilot-scale applications. In this regard, the targets that have been defined are closely linked to the Biogas Roadmap 2022, published by the Spanish Ministry for Industry and Tourism (19). As the biogas map indicates, the direct use of biogas at its place of production and the possibility of displacing the use of natural gas over time makes biogas and hydrogen the most important energy vectors in reducing Europe's energy dependence. Decentralised fuel generation helps to avoid rural depopulation, providing economic value and employment in rural areas, supporting Europe's energy and demographic transition. This policy is also explicitly set out within Spain's '*National Programme for Rural Development (PNDR)*', under Law 13/2013 of August the 2nd, which is funded by the '*European Agricultural Fund for Rural Development (EAFRD)*' (20).

Under the objectives defined by the '*Spanish Strategy for Science and Technology and Innovation*' and under the European Commission's Horizon 2020 research project programmes, the development of biofuels and novel synthesis techniques is encouraged (21).

Furthermore, the 2021 report of the European Biogas Association highlights that, with the accelerated development of advanced biogas plants, 30-40 % of the EU's current gas requirement could be generated from renewable sources by 2050 (22).

The research question of this PhD thesis has been defined as: *How can microbial processes of anaerobic digestion bacteria be improved from a physical, chemical, balance-of-plant and operational perspective to improve the functionality and economic viability of a pilot-scale low-cost bioreactor*.

1.5 Thesis structure

Theoretical analysis is conducted in Chapters 2-4, with accompanying experimental investigation and discussions in chapters 5-7. Chapter 2 provides an overview of the European biogas landscape, including important laws and regulations that paved the way for the current installation amounts. Chapter 3 then explains the different development status of biogas installations in various EU countries, analysing the waste generation of each EU country to analyse the future potential for increased biogas implementation, as compared to their current renewable energy and energy usage share. Lastly, Chapter 4 concludes the theoretical analysis by discussing different biomethane yield modelling studies performed, whilst also summarising a statistical analysis performed by the research group which discusses several conclusions on performance parameters and their impact on total biomethane yield and proportion within the produced biogas.

Following from the theoretical part, Chapter 5 begins the experimental analysis by using the conclusions of the statistical review to validate and determine first approaches of AcD in the smallest (batch) laboratory scale with the intention to receive more insight into the behaviour of different feedstock and their biodegradability by the microbial sample. The experimental scale is increased in Chapter 6, where the ideal amount of nutrition for a bacterial system is analysed with the goal of determining the optimal biomethane yield per amount of feedstock added. After having analysed the different material flow rates, Chapter 7 concludes the experimental analysis by analysing the operational characteristics of a low-cost AD reactor, discussing the operational challenges and shortcomings that should be improved in coming design iterations.

The thesis is finalised with a discussions chapter, where biogas economics, security of supply, environmental impact, digestate managements, benefits and drawbacks of the current technological state are reviewed. Lastly, the conclusions provide a set of main results and summarise the key points of each chapter.

2. European biogas landscape

It is important to discuss the outlook of biogas integration in the European community, and the clearest indications for this outlook can be found through summarising laws and regulations that have recently passed, the political framework and public sentiment on the matter, along with the current annual installation speeds. This chapter will assess these aspects to provide a holistic overview on the biogas landscape in Europe and mainly takes analysis from (13).

AD, along with other forms of bacterial manipulation (fermentation, composting) has been developed before modernisation of society. Main scientific interest in the technology, along with determination of the process pathways, began much later in the 1970s. Through the onset of the first oil crisis and the beginnings of climate change concerns, research activity first increased and has seen exponential growth from the 1990s to date, as seen by databases such as Scopus and Web-of-Science (4,8). Scientific discovery was always correlated with concerns and interests in climate change and sustainability, and as such, biogas and self-sufficiency technologies are hot topics again following the 2021-2023 European energy crisis (8). To date, research article publication is in line with the exponential behaviour of $(year)^{0.135}$ (13).

Figure 2 elucidates the current installation and operation of biogas plants in EU-countries over a 10-year period, along with the global distribution in total installed capacity. Based on the data provided, a 20% average annual installation increase is seen between the years of 2009 to 2019. Europe remains the forerunner in industrial capacity potential, as proven by Figure 2 with a more than 50% capacity share in biogas production (23). Based on installation capacity, Asia then follows with 35%, leaving the Americas as third with 14% (23). Based on energy, the IEA reports a production of 1621 PJ of biogas for the year 2022, with current projections estimating 1789 PJ of calorific biogas content for the year 2024 (24). Of these statistics, Europe takes the largest production share and therefore claims itself as a market leader for industrial scale biogas exploitation (23,25). In comparison to mainly industrial-scale biogas plants present in Europe, other regions, such as Asia, have traditionally focused more on smaller, domestic units. These have only recently received attention in the European markets, with sustainability and energy-independence being the driving factors for their market exploration (23,25).

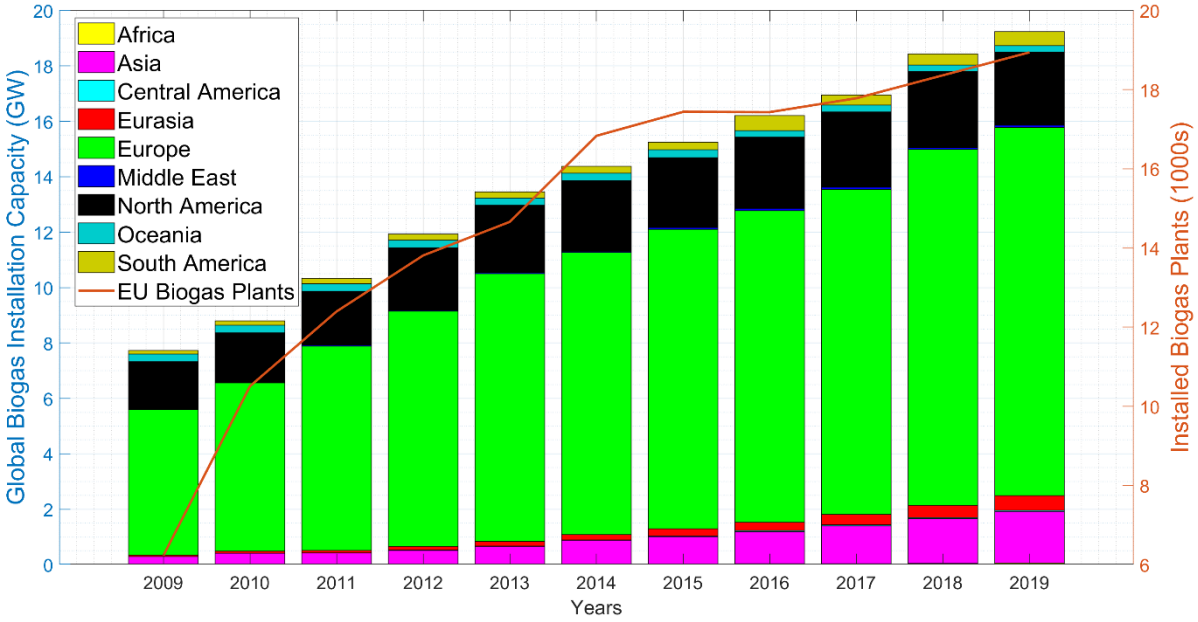


Figure 2: Breakdown of global differences in biogas capacity installation by regions (left, bar chart) and total EU industrial-scale biogas plants operational (right, line plot). Data taken from (14,26–29).

2.1 Biogas production in the EU: Market analysis and industry approach

Biogas production in the EU has doubled from 371 to 765 PJ between 2010 and 2023 (30,31). Even though the development shown in Figure 2 appears to decelerate in more recent years, there is evident movement reported by Kampman et al. (2017) which indicates that the capacity installation will begin accelerating again to 2030 to reach energy independence and sustainability milestones (32). Figure 3 provides a summary of main production players within the EU, the arable land available for agricultural activity, fractions of GDP that correspond to agricultural output, renewable energy shares, the main crop produced in the respective country and their relative abundance compared to the whole county's agricultural output (33–39). Raw data of this summary is available in Table 17 in the appendix. Based on the data provided, Germany is the largest European producer of biogas, with Italy as second and France as third in terms of production quantities (33).

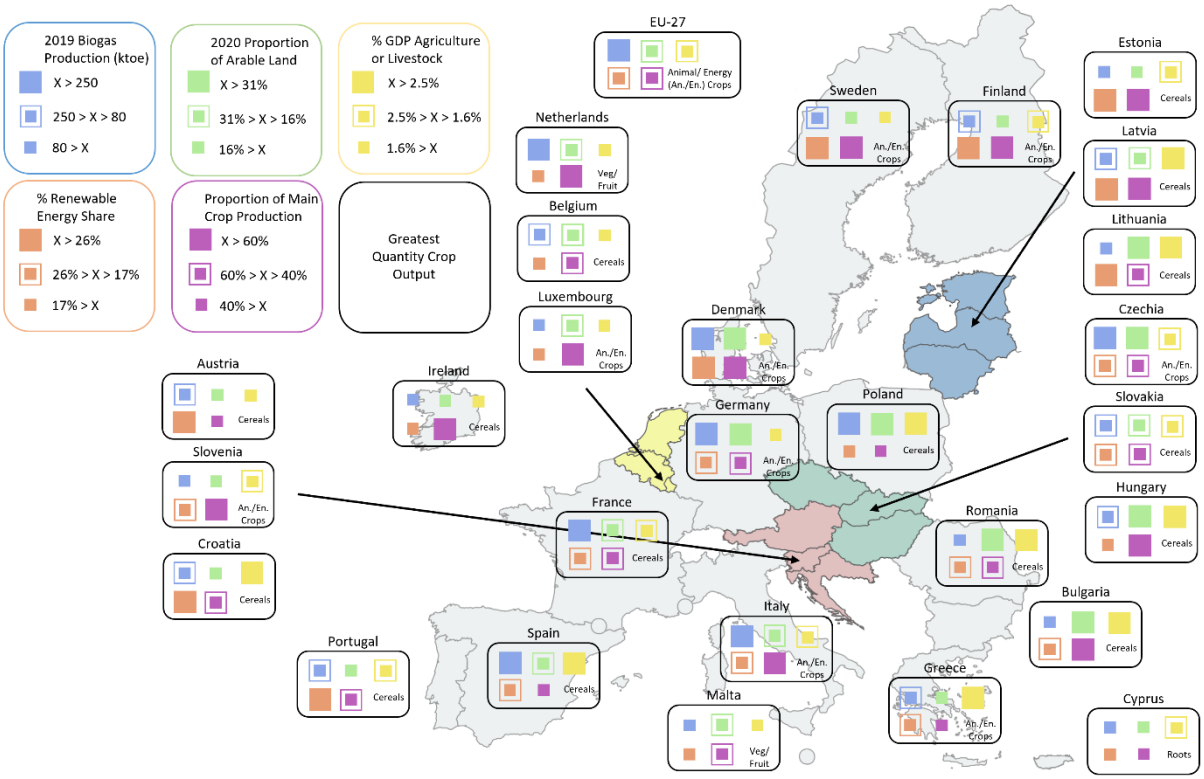


Figure 3: Breakdown of key biogas production statistics. Data mainly taken from Eurostat databases. Data taken from (33–39).

2.2 Regulations and guidelines posed by the EU

The first advancements in setting a legal framework for biogas development in the EU took place in 1997, when the *White Paper for a Community Strategy and Action Plan* first defined milestones and visions for a circular economy and energy self-sufficiency (4,40). A complete overview has been created by published article (A – page xix) that summarises all key regulations that paved the way for the biogas industry and energy policies seen in the EU to date. The whole breakdown of these regulations is reproduced in Table 18 of Appendix 11.2.

The three most notable regulations of the past have been selected and are discussed in more detail in Table 1. These provide the basis for renewable energy implementation, set milestones, and define specific directives to reach emissions-savings as per the European climate agenda (4).

Table 1: The three most influential European regulations passed to provide guidelines for AD.

Year	Directive/Regulation	Summary	Reference
2015	COP21 Paris United Nations Framework Convention	• All member countries agree to limit global warming through anthropogenic sources to a maximum of 2 °C, ideally maintaining warming to 1.5 °C. • Development *of Intended Nationally Determined Contribution (INDC)*, giving each country a pathway of goals and milestones of emissions reductions. • Predecessor of the EU *Clean Mobility Package* (2018) to reduce GHG emissions through transport.	(4,41,42)
2018	Renewable Energy Directive (REDII)	• Revised targets and roadmaps for climate neutrality by 2050. • EU-wide minimum renewable energy consumption target of 32% by 2030, with sector-specific goals. • Revised sustainability thresholds for biofuels to minimise ecosystem damage and indirect land use change (ILUC)	(42,43)
2021	EU Taxomomy Climate Delegated Act	• AD, upgrading, and gas grid injection all recognised under energetic circularity and low-carbon energy generation activities. Allowing for further investments into the sector.	(44,45)

2.3 Outlook and future growth opportunities

The European Biogas Association (EBA) predicts positive and sustainable growth linked to higher capacity installation and development of the sector for the coming years (22,42). This is related to the political agenda of improving waste management and reutilising calorific values of waste for energy generation. On their roadmap to 2030/2050, the EBA expects the European biogas sector to grow in the following way (44,45):

- Renewable natural gas, RNG, which may be derived from microbial processes such as AD, will **surpass the 10% threshold** of total EU natural gas consumption by 2030 (22). Development varies within the EU, with some countries such as Germany having already passed the 10% RNG market share as early has 2015, when it was reported that 12.1% originated from renewable sources (4,22). Pre-treatment strategies increase methane yield in AD by making previously assumed indigestible waste products available for utilization.

- **Biogas energy generation** is projected to double by 2030 as per 2020 (22). Quantifying these claims, this would yield an increase of up to 400 TWh of biogas energy generation compared to 191 TWh in 2020 (22). By 2050, sustainable biomethane projects are expected to cover 30-40% of the gas consumption share in the EU, which corresponds to approximately 1,000 TWh acknowledging industry and population growths (22). The bandwidth of uncertainty claims that any development up to 1,700 TWh of biomethane production is likely by 2050 (22).
- Not only AD-reactors and industrial plants are being built to accommodate sustainability and energy requirements of the next generation. Additionally, **biogas-upgrading infrastructure** is installed at an exponential rate and is seen to become a prevalent future technology (4,22,46). As such, 2021 saw a 40% installation increase of upgrading units as compared to all previous years combined, being a testament to the dynamic and fast-growing industry present in the energy sector (22).
- **Job prospects** are expected to improve and grow in the biogas industry as industrial development accelerates. By 2030, an additional 420,000 employment opportunities are expected to occur because of the additional capacity installation of biogas reactors (22). Following these growth trends to 2050 would imply an increase of up to 500% (compared to the present day) of employment in biogas and bioenergy-related industries as an immediate consequence of industry growth, which would equate an approximated 1 million jobs within the European biogas industry (22).
- The **energy consumption share** will shift further towards renewable energy, and biogas/bioenergy will play a significant role in this transition as well. As such, studies performed by the CE Delft led by the European Commission estimate that by 2030, the potential biogas and biomethane production will account for 2.7% and 3.7% of the EU's total energy consumption respectively (32). These estimations take development tender volumes and future installation projections into account.
- Based on calculations performed by the Intergovernmental Panel on Climate change, the **long-term market penetration** of biomass and biofuels will provide society with 50,000, 75,000 and 89,000 TWh of energetic value by the respective years 2050, 2075 and 2100 (47).

Upgrading technologies allow biogas cleaning such that most of the leftover gas is biomethane, chemically equivalent to natural gas. Through upgrading, distribution is vastly simplified as storage and selling can occur through existing natural gas infrastructure present (4). To properly certify a fuel as "sustainable", the producer must register under the respective energy networks and provide guarantees-of-origin such that, at a different point in the natural gas grid, the energy equivalent can be extracted and sold as RNG. This approach helps businesses buy RNG and reduce carbon emission credits necessary for their operations, even though an upgrading plant may be located far away. Agencies maintaining the grid infrastructure also benefit, as it is easier to track amounts of RNG being fed into the grid, and the share of RNG present in the fuel mix. Especially in Europe, where most countries have very well-developed natural gas grids, this provides an attractive solution to distributing and selling the renewable natural gas.

Further transport fuels often used in industry logistics and heavy-duty vehicles are compressed natural gas (CNG) and liquefied natural gas (LNG). Replacing these energy sources with biomethane as an energy vector is possible through providing decentralised fuelling technologies at gas grid network nodes. In this way, carbon emissions brought through fossil fuels can be heavily reduced and substituted through a sustainable equivalent for road transport fuel. Scarlat et al. (2018) reports that this trend is already occurring, with the biomethane used in transport having augmented by 74% in 2019 to 14 PJ in Europe (4).

Biogas combustion also allows for heat and energy supply in small households in a decentralised manner through combined heat and power plants (CHP). Research is currently being conducted to improve the combustion efficiency of biogas, where hydrogen is mixed into the biogas to improve combustion stability and provide more calorific value to the fuel (12). Further discussions are occurring on incorporating district heat networks from CHPs and other energy sources such that different applications of biogas and its combustion appeal to investors within the energy sector and prove the versatility of the fuel, as per Gustafsson and Anderberg (2021) (48).

When assessing biogas as a carbon-neutral fuel, it is worth mentioning that potentials exist to make biogas generation and combustion carbon-negative, effectively removing CO_2 from the

atmosphere, through coupling the standard CHP-process with carbon capture and storage (CCS) or oxyfuel carbon-sink technologies (49,50).

From a regulatory perspective, the most important factor for industry success has been defined in surveys with industry experts by Gustafsson and Anderberg (2021) (48). They state that continuity and stable legislature are primary drivers for industry growth and reliable investment into the biogas sector (48). What is to be avoided include unpredictable regulatory changes and price instability as barriers to industry growth (48). Economic incentives that governments can take to accelerate biogas industry penetration involve the use of feed-in tariffs or other stability-enforcing mechanisms (48). These economic supports schemes guaranteed by governments are often considered a "double-edged sword": Whilst secured revenues spurs investments and growth in countries such as Germany and Italy, risks associated with "lock-in" effects must be acknowledged. In these risks, specific guarantees will require the taxpayer to continue support a technology even if it may be outdated in the near future, and can thus prevent innovation and technology improvement (48). Lastly, environmental and pollution directives, especially fluctuating restrictions, are seen as a great threat to growth and the industry in general, as they cause uncertainty for investors, developers and operators.

3. European Management of Agro-industrial Waste

More sustainable sector practices are required, especially in the agricultural and livestock sector, to reach the climate objectives defined by the EU. Through accounting for these changes in reduction of environmental impact, up to 33% of the total GHG emissions can be reduced through more effective agricultural and livestock waste management (3). This proves that whilst agriculture and livestock rearing sectors don't have a considerable share on the EU GDP, the global warming potential produced through the sectors are severe and research and/or public funding is required to further mitigate these polluting industries (3).

Figure 3 provides an overview of the EU's main agriculture-heavy countries and the energy share currently seen in each member state (33–39). The raw data that was used for the creation of Figure 3 has been reported in Appendix 11.1 for the interested reader. As to be expected, the larger EU member states featuring Germany, France, Spain, Poland and Romania claim the largest land area available ("utilised area") for agriculture, with each of them providing more than 10 million hectares of their surface area to agricultural activities. The term "utilised area" refers to the space that is provided to any form of cultivation, which encompasses further land-use terms specific to agriculture such as arable land, permanent grasslands, kitchen gardens and most importantly (based on the usage share), permanent crops (38). In contrast, the term "arable land" is used to term land that sees regular agricultural activity, such as trilling and ploughing, where common crop rotations are employed to maintain a healthy soil (38). Further information provided by Figure 3 is the country's primary agricultural output. Evidently, the majority of states produce cereals or animal/energy crops ("plants harvested green") as their main produce (13,33–39). For further information on reference nutritional values of different EU crop outputs, Appendix 11.3 may be consulted. Considering how low of an impact agriculture and livestock rearing have on the national GDP of EU countries, it is surprising how disproportionate these activities contribute to the total GHG emissions reported (33% as per Crippa et al. 2021) (3). Assessing the share in renewable energy implementation in EU member states, disparity is evident, and most member states are far from approaching the 2050 neutrality objective. As such, further potential incentives are necessary to accelerate renewable energy installation and provide adequate infrastructure for distribution and re-dispatching to attain neutrality within the next 25 years.

The optimal conditions for anaerobic digestion will be discussed in detail in Chapter 4. Nevertheless, it is worth mentioning that a high fibre and moisture content is ill-advised, as these waste products either do not degrade, or deteriorate at a very slow rate, thus limiting the immediate biogas yield. After having discussed the main agricultural outputs in different countries, it becomes clear that especially cereals, animal/energy crops and roots are ideal for anaerobic co-digestion, where further support for optimised treatment is found from other sources, such as pig manure. To build on this, detrimental climate impacts of these waste structures can be inhibited by employing post-processing techniques, as these reduce the overall strain on the environment through previous, bacterial degradation and cleaning.

3.1 Agricultural and livestock waste characterization and its impacts

3.1.1 Lignocellulosic biomass

The main advantage linked to using lignocellulosic biomass for AD lies within the fact that its calorific content can be converted into a renewable fuel without the requirement of substantial energy investments and without endangering human and animal food supply (25). The only issue of treating lignocellulosic biomass under AD is that the recalcitrant structure of the feedstock inhibits the hydrolysis process, reducing the efficiency of microbial attack to deconstruct the chemical structure of the cells to simpler monomers, acids and sugars (25). This leads to delayed reaction kinetics, whereby hydrolysis becomes the limitation rate function in the AD process diagram, as seen in Figure 4. Figure 4 shows the process flow diagram of the AD process including the four key processes into which microbial activity can be summarised into.

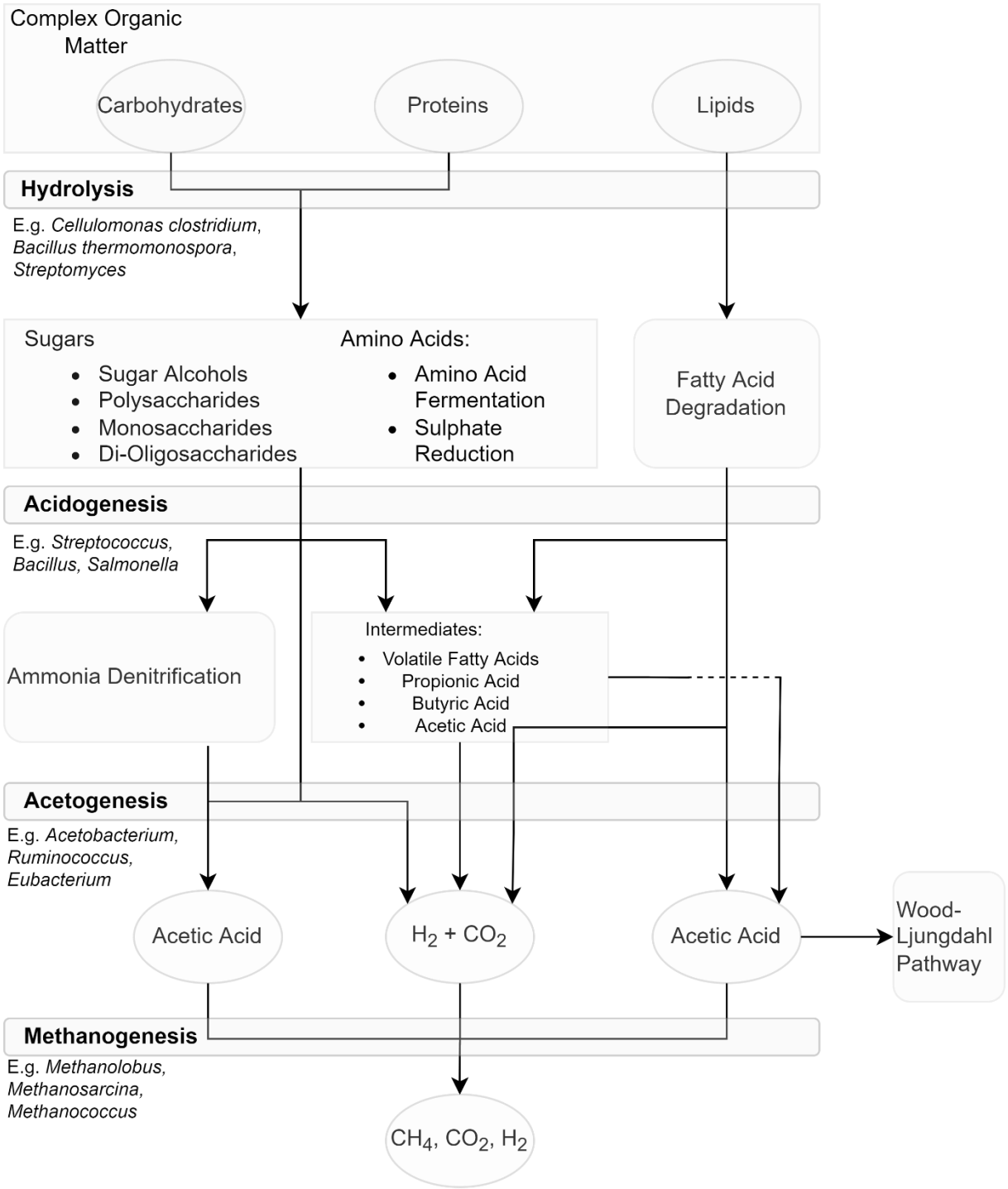


Figure 4: The biogas process mechanisms. Figure recreated based on (11,50–52)*. Information on bacteria taken by* (53)*. The list of bacteria is incomplete as the examples are only included for informational purposes. Chemical compounds and products are indicated as ovals. Specific processes are shown as rounded rectangles. Biogas genesis steps are summarised in bold writing. Intermediate products are visualised using rectangles. Note: both acetic acid ovals are equivalent. For diagram clarity the acetic acid is portrayed twice.*

The production process biologically transforms organic material into potent fuel using the following procedure (8):

a) **Hydrolysis**; Fermentative bacteria treat complex chemical primary compounds such as carbohydrates, lipids and proteins. The primary compounds are composed of long chain molecules and complex chemical substances. A soluble product consisting of oligomers and monomers is formed during the extracellular enzymatic disintegration of the initial feedstock (8,54).
b) **Acidogenesis**; The Acidogenesis stage involves the conversion of primary biochemical products (oligomers and monomers) to produce a series of fatty acids, for instance acetic acid, butyric acid and propionic acid (8). Kougias et al. (8) states that the produced fatty acids contain less than six carbon atoms, along with a series of sugar alcohols.
c) **Acetogenesis**; Acetogenic bacteria convert all previous acids, alcohols and some volatile fatty acids into acetate, hydrogen, formate and carbon dioxide (8,51).
d) **Methanogenesis**; Methanogenesis concludes the production process, converting the residue acetic acid to methane. Hydrogenotrophic and acetoclastic methanogens ultimately produce the desired end-product methane by organic matter mineralisation (54). Trace amounts of hydrogen remain after the conversion process, with the gas majority being composed of methane and carbon dioxide.

The hydrolysis process path, as explained in Figure 4, is defined through the decomposition of cell structures, lipids, proteins and complex carbohydrates into basic, shorter chemical compounds generally identified as monogomers and oligomers (13,25). To improve the efficiency of hydrolysis on recalcitrant cell structures such as cellulose and hemicellulose, pre-processing techniques of feedstocks is a common tool employed to support the microbes (13,25). Without focussing on the various pre-treatment strategies, as the objective of this thesis is to improve low-cost, medium-sized biogas reactors, Table 18 gives an overview of the key strategies used and their feedstock applications. Table 18 has been reproduced based on existing literature of (13,25). For further information, a summary and literature overview of various pre-treatment strategies that were investigated to date has been provided in Appendix 11.4.

Table 2: An overview of common pre-treatments used for AD of lignocellulosic biomass.

Type	Biomass used	Pre-treatment
Lignocellulosic mass	Wheat Straw. Leaves	Mechanical: milling, shredding, steel-rolling (55,56)
Wood	Birch	Alkaline (NaOH) Pre-treatment (57–59)
	Spruce and Birch	N-methylmorpholine-N-oxide (NMMO) (57–59)
Pine Tree Wastes	Needle leaves, branches, cones and bark	NMMO (60)
Straw	Rice, Barley, Triticale, Winter Rye, Oilseed Rape, Faba Bean, Wheat	NH_3OH, NMMO, Microwave, Mechanical Extrusion, Wet oxidation, Na_2CO_2 and elevated pressure (57–59,61–64)
Water Hyacinth (*Eichhornia crassipes*)	N/A	(1-N-butyl-3-me-thylimidazolium chloride and dimethyl sulfoxide) (65)

An overview of the major agricultural output in the EU is provided by Figure 3. Through inspection, it becomes clear that the common wheat and spelt, alongside maize and corn mixes (commonly grouped as animal and energy crops) are the most utilised crops of EU agricultural area, with 66.07% in 2022 (66,67). Based on definitions provided by Eurostat, grains, industrial plants and similar vegetation used for dietary needs or animal fodder is summarised in the term "animal and energy crops" (68). Additionally, these crops can also be used for the production of biofuels or the biomass can be used for energy production, though less common due to its competition with dietary requirements (68,69). Especially through the cultivation of cereal, large amounts of lignocellulosic biomass are created that are not in competition with dietary requirements (13,69). As such, the straw of cereals and their roots can be readily used for AD, as they are rarely used as fodder and currently do not find any other application. Due to the low economic outlooks of processing these waste products (as there is no lucrative by-product or other revenue stream that can be generated through this waste), roots and recalcitrant parts of the plants are often wasted and left abandoned on fields for natural digestion (70). Specific examples of this phenomenon include maize silage, alfafa, clover and other grain or cereal plants (70). In the world of agriculture, a waste product is defined as any structure that does not produce economic value or immediate benefit for transportation or processing (69). Based on this, many waste products can be identified in the agricultural sector (69).

The status quo of waste management in agriculture is rather simple. Residues of crops are generally dispersed on the open field or land plot with the intention of slow, long-term nutrient release for future growth generations to benefit from old, recycled, nutrients (13,69). Through this technique of ploughing old crop waste into the soil, benefits such as soil health, irrigation efficiency, compost and erosion control are reported (69). With the benefits, certain risks are described as well. Risks associated to incorrect management of biomass cause exacerbated climate effects and are a strain on the environment include leaching (through nitrate, sulphate and phosphate) and water pollution (69). It is important to note that natural fertilisers (i.e. agricultural waste) have a lower environmental impact than inorganic fertilisers, which may push the ecosystem out of its equilibrium and has been considered an unsustainable practice (13,69). Further risks involving biomass mismanagement include denitrification of soil and immobilisation of nutrients, causing the soil to be an ineffective vehicle for future plant growth (69). Through pesticide involvement, further chemicals are introduced to the plants and/or soil that can have detrimental effects on the soil's health (71).

3.1.2 Manure

AD lends itself to the digestion of animal waste products, as these feedstocks traditionally are rich in nutrients and highly efficient in digestion regimes for renewable fuel and organic fertiliser production (28). A clear relationship becomes apparent in developed countries who treat their waste with AD: as the total amount of farms in a country decrease, the number of animals under management becomes substantially larger (28). This principle is known as intensive animal farming (collectivisation) and is a common phenomenon especially for livestock rearing farms (28). Following a similar logic, developing countries exploit economies of scale to build large-scale livestock farms, such that low-cost animal products can be provided to consumers (28).

When estimating the amount of animal droppings available in Europe as a function of total animals present, several variables such as feeding regimes, management strategies, types of production systems and type of shelter systems used must be accounted for (72). All parameters ultimately determine the amount of feed provided to the animals, which stands in direct correlation to their droppings and, thus, feedstock for AD (13,72). Livestock farms, through their high-level emissions potential, cause detrimental global warming potentials due to their dire environmental footprints, unless optimised manure treatment strategies are

employed (73–76). To circumvent the disadvantages described, manure can be used as an agent of the circular economy to promote the energy transition to renewable energies and fuels (77). After having produced the renewable fuel in form of biogas, a further benefit lies in the fertilisation potential: industrial fertilisers may be avoided, and instead a lower chemical-oxygen-demand (COD, term defined in section 4.2.1) manure with equivalent nutrients can be re-introduced to the environment (77). As such, the effect that chemical/industrial fertilisers have on the environment, is substantially reduced (77).

Animal slurry in theory is difficult to be digested by itself in industrial biogas reactors. The reason for this is that its physio-chemical properties do not lend itself for monodigestion. For instance, high nitrogen concentrations in manure, leading to a low carbon-to-nitrogen-ratio (C/N) reduce its digestion efficiency in isolation (13,28). Volatile solids (VS) content in manure is also comparatively low to other feedstocks, and given that the majority content of manure is water, which cannot be degraded, only a small fraction (the VS) of the feedstock is readily available for biological decomposition. Because of this, animal manure is generally digested together with other feedstocks under a co-digestion regimen. The most common and efficient mixtures include co-digestion with lignocellulosic biomass, which helps to improve the waste degradation efficiency and overall aids in the AD process stability (78–80).

To further boost the methane content in the produced biogas and increase the biogas yield overall, various pre-treatment strategies may be employed. An added benefit of these thermal, chemical, biological and physical pre-treatments is that sanitary and environmental regulations can often be combined during the pre-treatment stages, which allows for little added costs. As such, methane increases have been reported in literature. As per Orlando and Borja (2020), pretreatment processes have led to increases of 32%, 45% and 46%, to 238, 271 and 328 Nm^3/ton VS of cow, swine and poultry manure respectively (13,81).

3.1.3 Problems of waste accumulation in the environmental context

Figure 5 provides an overview of the approximated livestock waste accumulated by all swine and bovine animals in the EU annually (36–39,82–84). The raw data, out of which the condensed Figure 5 was generated, is available to the reader in Appendix 11.5. Given the high magnitude of livestock waste generated in the EU, efficient strategies are needed to combat mismanagement and incorrect disposal to reduce environmental damage.

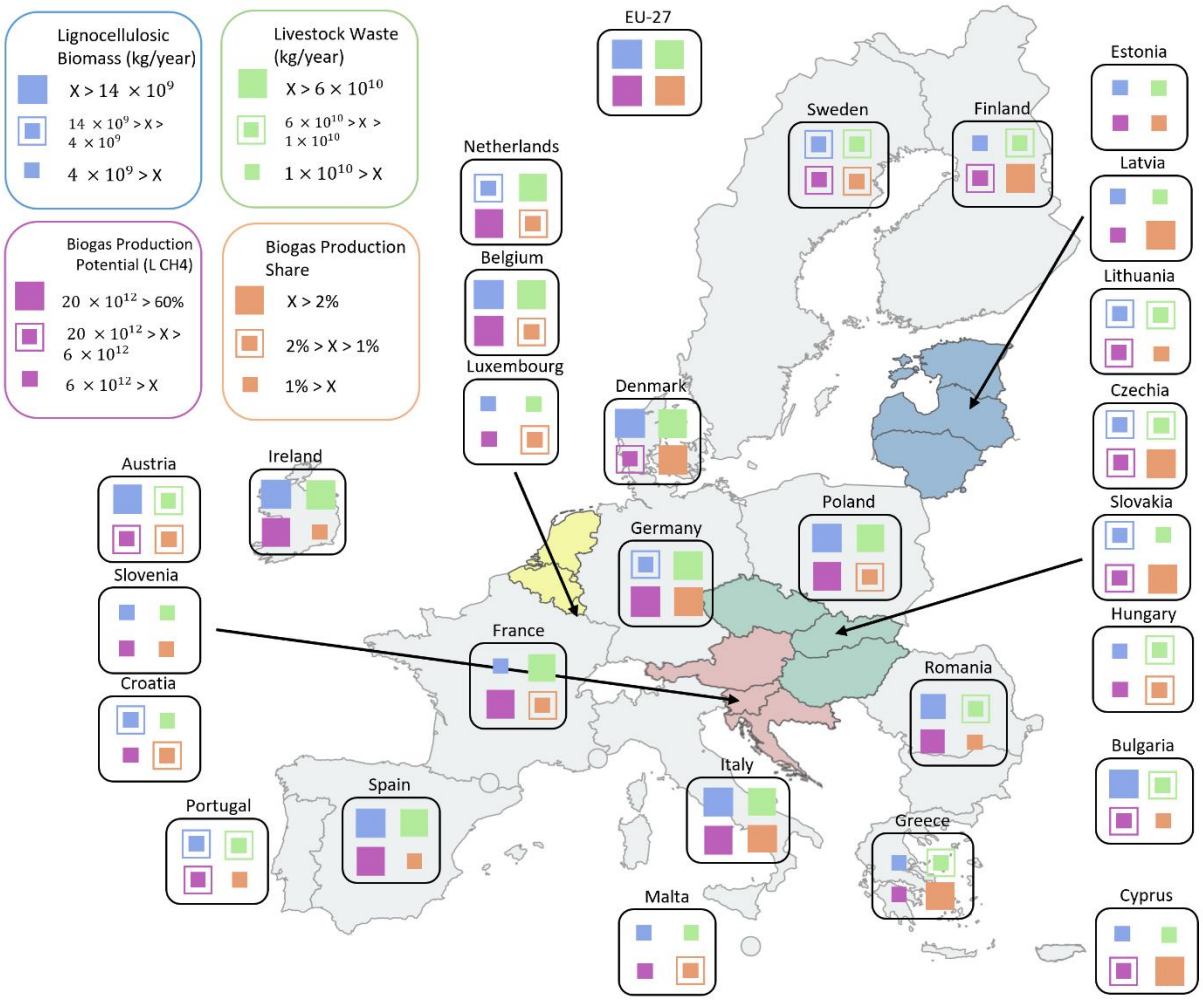


Figure 5: An overview of the EU country's availability of biomass annually, the estimated potential of production capability, and its current production share compared to actual potential (36–39,82–84).

Scheftelowitz and Thrän (2016) report that it is beneficial to conduct post-processing on liquid manure to reduce its chemical oxygen demand, such that the energetic value is reduced once it is used as a fertilizer (70). Simultaneously, all beneficial nutrients of the manure are retained and can be used at a later stage in the process chain (70). Because the nutrients are retained and not used during AD, no competition exists between fertilising and energy recovery practices, allowing manure to be used for both applications (70).

The largest localised occurrence of ammonia (NH_3) occurs in manure storage facilities and stables (85). Volatilisation of ammonia occurs freely once exposed to ambient air, due to the high vapour pressure present in NH_3 (85). Ground water is also at risk of manure, as manure under contact with soil can seep through different layers and cause toxic effects on the ecosystem (85). This has the effect of chemically reducing aquatic biota cultures, leading to toxic ground water (85). Because of this effect, a denitrification process must be added to

domestic wastewaters (85). Water toxicity and extensive eutrophication of surface water are caused by nitrate production through aeration (85). This results in abundant algae growth and low oxygen concentrations in the water, which suffocates fish ecosystems because they lack vital nutrients (85). Through introduction of untreated manure-based wastewater, excessive amounts of nitrogen, potassium, phosphorous, heavy metals, organo-chlorides and salts can enter soil layers and groundwaters, causing high levels of pollution and toxicity (85).

In contrast, several literature sources also quote the advantages of introducing manure waste into soil (86). For example, Segui and Voorburg (1993) suggest that soil fertility and structure are improved, given that the heterogeneous content of manure provides a range of decay velocity, which aids in long-term nutrient release for the soil (86). This provides a good humus and stable organic matter foundation for the soil structure (86). Despite this, Segui and Voorburg also warn of the high soil salt content that prevails in the soil after manure dumping (86). The content of KCl and NaCl in soil has reportedly increased heavily after these practices (86).

The high concentrations of ammonia present in the manure cause nitrous oxide (N_2O) emissions, which reduce air quality and are extremely dangerous to the ozone layer as they are a depleting agent (85). Additionally, strong and unpleasant odours are caused by manure dumping due to the decomposition in air (85). Hydrogen sulphide and ammonia are primarily responsible for these odours (85).

3.2 Waste stream utilisation: agricultural and livestock waste into biogas

Even though the strong correlation between intensive agriculture and livestock farming on climate change and pollution has already penetrated mainstream knowledge, no changes in consumer behaviour have been registered in this sector as further dietary resources must be provided to a growing population. Statistics of live animals and livestock farms prove these trends seen in farming activities (82–84). Even though the livestock and agriculture status quo has been accepted as unsustainable and harmful to the climate, it is important to acknowledge the opportunity of utilising the hazardous waste structures for purposes of circularity, i.e. generating biogas from AD through usage of these waste products. Based on this theory, the work conducted simulates the biomethane production potential in various EU countries based

on data of livestock activities in their respective countries. Data that has been used in this study includes types of agricultural waste, types of livestock present and amounts of heads managed in each country, alongside the current production outputs of biogas (36–39,82–84). The methodology used for this work was taken from the theory presented by Scheftelowitz and Thrän (2016), who also estimated the biomethane yield based on livestock numbers (70). Figure 5 summarises the biomass accumulation, biogas production potential and biogas production share of European countries. The raw data for the creation of this image is available in Table 22 in Appendix 11.5.

The results illustrated in Figure 5 convey how much biogas is currently used as compared to its potential for future development. Solely the EU-27 can provide 2.46 x 10^7 TJ of equivalent energy in methane based on the statistics available in Europe for livestock farming and agriculture. When assessing the current biomethane generation statistics as per the countries' official sources, the stark juxtaposition becomes evident: On an EU-level, a mere 6.51 x 10^5 TJ of biomethane (within the biogas) are currently being generated (13). This difference highlights the untapped opportunity available in utilising European waste streams further by making use of AD practices. With this strategy, the biogas energy landscape can be further expanded to provide more sustainable energy security, waste management and climate change prevention measures.

Within the EU, a 2.65% biogas production share is currently realised (2020 data) as compared to its calculated potential (13). Even after assessing uncertainties and applying correction factors to the data provided, it is clear that generous magnitudes of waste material are left unutilised and can receive a more sustainable waste management strategy than the current status quo. For example, free-range livestock is often left to graze fields and roam freely, making manure collection and storage significantly more difficult. Despite that, a vast share of livestock rearing takes place under controlled conditions in larger facilities, where manure collection can be efficiently coordinated, and waste management is simpler (70). Based on this analysis, it is expected that a generous amount of biogas production potential is available, and it is recommended that these untapped waste magnitudes are utilised for further renewable energy production and GHG emissions reduction.

Of all EU countries presented, Germany has the most developed biogas production statistics at a share of 11.50% compared to its total potential. Experts in the industry support these conclusions, as the German regulative environment has specifically favoured the expansion and private investment of the biogas industry through favourable laws and attractive feed-in tariffs for decades to provide project security and profitability (70). This is further supported by the fact that Germany holds the most biogas plants in any EU country, and in fact has more biogas plants than all other EU countries combined (28,87). 2017 data provides information that 53% of all biogas plants within EU territory are in Germany (28,87).

In Europe, the installation of thousands of pilot-scale AD reactors with a capacity of 1 m^3 can be promoted on farms that are currently too small for industrial biogas production, especially in rural settings. Through this, their dependence on centralised gas production is heavily reduced and a scaling effect is possible. If thousands of farms follow this self-sufficiency doctrine, major fossil-fuel based natural gas purchases can be avoided and a large step towards self-sufficiency can be taken. It is expected that all their gas requirements can be supplied through the recycling of biomass that is generated on-site, thus producing sufficient for decoupling their household use from the natural gas grid. In order to practically implement this vision, Gustafsson and Anderberg (2021) suggest that a governmental support in form of financial incentives and regulations must be enforced to support the increase of new installation capacities and to augment the deployment in future years (48). Just through regulation and financial viability the security of gas supply can be guaranteed.

4. Analytical and Experimental Biogas Yield Production

Chapter 4 begins by discussing various strategies to determine the methane yield based on chemical analyses of macronutrients and physical properties of the feedstock. A model based on Biochemical Methane Potentials (BMP) will also be explained, which serves as a further experimental approach to predict methane yields based on experiments disseminated in literature. Lastly, an in-house literature study is reported, which includes 65 different AcD studies to assimilate various biogas production parameters to mixtures of feedstock added to a biogas reactor. Summary expressions conclude each analytical section, which may be used to model the biomethane yield brought upon by AD, based on differently employed models. Conclusions of this chapter include the statistical analysis, where an expression relating methane, pH, C/N ratio and Hydraulic Retention Time (HRT) could be found. The majority of the analysis has been published in the Journal of Cleaner Production, visible under reference (13).

4.1 Theory and previous analysis by literature

4.1.1 Macronutrient analysis

Macronutrients include substances such as proteins, fats and carbohydrates, which define the chemical composition of a feedstock in its broadest terms. Every feedstock can be characterised and sampled depending on the macronutrient ratio present. Key elements that dictate the macronutrient analysis in AD include carbon, nitrogen, phosphorus and sulphur (in descending order of propensity) (25). Depending on the ratio of nutrients that are prevalent, the metabolic activity of the bacterial consortium can be interpreted up to the decisive factor for biochemical interest: biogas yield and quality (25). Ideal nutrient ratios of 600:15:5:1 for C:N:P:S respectively have been reported by Weiland (2010), even though various ratios have been discussed and debated in literature (88). In addition to macronutrients, micronutrients support bacterial growth and provide the ideal physiological environment for bacterial metabolism to aid in the AD process (25). Several types of micronutrients are necessary to help in degradation of feedstock in AD reactors and to keep a suitable inoculum nutrient balance (25). Studies by Busic et al. (2018) and Holm-Nielsen et al. (2009) report that approximately a concentration of 0.05-

0.06 mg/L is adequate for various types of micronutrients prevalent in the inoculum, with iron being required at a higher concentration of 1-10 mg/L (25,89).

4.1.2 Liquid AD (LAD) compared to solid-state-AD (SS-AD)

Industry standards for AD include both liquid AD (LAD) and Solid-State AD (SS-AD) (25). Liquid and solid AD are differentiated by the amount of TS present in the inoculum during digestion. For instance, SS-AD generally occurs with up to 35% TS (25). In contrast, LAD treats feedstock at a lower TS content, with less than 10% TS. In industrial biogas units that are under continuous digestion regimes, LAD is more common as it lends itself to improved biomass reduction by providing short HRTs and faster reaction rates, given that bacterial distribution into the substrate is easier if this occurs during a liquid state (25).

In contrast, SS-AD generally finds its application in smaller reactor volumes and makes use of a lower energy demand during the AD process, whilst also simplifying the effluent management procedure. This is because insulation of solid matter is improved and because normally, SS-AD does not utilise mixing strategies. Based on various studies performed and as concluded by Brown et al. (2012) (90), no major differences in production efficiencies are evident when utilising SS-AD or LAD, given that the ratio of biomethane yield to energy investments balances in both cases.

4.1.3 Biochemical methane potential (BMP)

The most repeated and protocolled methodology to experimentally determine a substrate's biodegradability factor and its effectiveness for degradation/AD is the BMP test. The BMP is a cost-effective and simple method to estimate the yield of methane generated as per pre-specified conditions relating to inoculant, metabolic activity, reaction kinetics, macro- and micronutrient chemistry and physical inoculum compositions (91). Several experiments have further delved into BMP testing and its effectiveness in devising precise methane yield modelling. Most of these studies have been published in literature and discuss a wide range of organic materials that readily biodegrade. BMP tests provide key data into AD efficiencies and give information on how efficient digestion ratios may be designed. Additionally, BMP tests can support in deducing different substrate synergies in case where AcD strategies are employed (91).

The BMP test is a defined protocol that anaerobically digests a sample for up to 90 days. For adequate scientific validation, various batches of analysis must occur to rule out anomalies, provide statistical and investigative foundations and finally to provide value to the results obtained, which is imperative for reporting. In the process defined by Siddique and Wahid (2018) (91), the management and storage techniques are to be economically assessed, including the cultivation of a stable and effective inoculum mixture. Additionally, the BMP procedure postulates clear methodologies to ensure that performance parameters are maintained, and errors are reduced through repeated result sampling (91). Because the BMP test procedure can be time-consuming and cumbersome, other strategies exist to reduce data collection and yield modelling of organic feedstocks.

A laboratory-scale (or small-scale) experiment utilising batch reactors is required for BMP testing. Any biomass to be treated requires two samples: a "control" mixture (where merely the inoculum is placed into), along with a "control + biomass" mixture (which includes the identical amount of inoculum, and an additional, predefined amount of biomass that is being tested) (91). Both samples are subject to identical conditions and parameters, where the total methane yield of the samples is collected and recorded. Given that the higher-mass sample ("control + biomass") is expected to produce more methane, as the bacteria in the inoculum have received organic material they can degrade and feed on, a subtraction between both samples gives indication of the net methane production induced through addition of the extra biomass in the "control + biomass" sample. This net methane production is known as the theoretical biomethane yield of a specific biomass, hence BMP. Through performing various samples to prove experiment repeatability and allow for statistical analysis, the resultant methane yield can be quoted as the BMP of an organic material. Examples of typical BMP values have been reproduced in Table 3.

Table 3: Selected BMP values of common biomass products

Category	Substrate/Origin	Methane yield according to BMP (mL CH_4 /g VS)	Reference
Lignocellulosic biomass / Agricultural Waste	Wheat	245 – 319	(92,93)
	Rice Straw	279 – 280	(93,94)
	Corn Silage	270 – 298	(95,96)
	Barley	322 – 335	(92)
	Meadow Grass	282 – 388	(92,97,98)
Manure	Cattle	242 – 399	(92,96,97,99)
	Pig	313 – 322	(93,98,100)
	Poultry	107 – 438	(93,97,101,102)

Various sources report that more complex mixtures of biomass have less accurate BMP values, and generally lead to an over-estimation, because inhibition circumstances are disregarded within the characterisation of the feedstock.

4.1.4 Predicting methane yields

Volatile fatty acids (VFA) are a key concept in AD and are considered by many as a primary parameter that dictates inoculum health. If high amounts of sugars are added to the inoculum, the nutrient balance may become unstable as the production of VFA is favoured, thus lowering the pH of the reactor (91). Anaerobic reactor acidification, as previously explored by Llamas Borrajo (1982) (103), is caused by an accumulation of VFA. A primary component found in biomass are carbohydrates, which are classified as complex sugars. Based on this, addition of carbohydrates is to be taken with care to prevent nutrient imbalances and pH deviations from defined optima. Likewise, protein addition leads to the introduction of a nitrogen source into the inoculum. Care must be taken to limit the addition of proteins, as the AD process path favours the production of ammonium ion through the addition of nitrogen-rich proteins. Both high concentrations of VFA and of ammonium can inhibit the efficient production of biogas, because methanogenic microbes identified in Figure 4 cease to survive under pH fluctuations and work at lower chemical kinetics at low and high pHs, which are induced by either high VFA (without balancing with ammonium) and high ammonium (without balancing with VFA) concentrations respectively (104).

The stability of pH-values in the inoculum is maintained through pre-selection and testing of feedstocks that are known to have buffering effects for the AD system. In this regard, the selection of a moderate C/N ratio is crucial to prevent over- or underloading the bioreactor

with feedstock, leading to the system either acidifying or becoming too alkaline. Fatty substances are also degraded by hydrolytic microorganisms to ultimately produce biogas, but care is to be taken for physical difficulties brought upon by the untreatable fats introduced into the reactor (91). For instance, microbial inhibition, physical blockages, foaming and biomass adsorption are risks associated with high fat content of these feedstocks. Long-chain fatty acids are produced through lipid degradation; these acids then may inhibit biogas production through acidifying the inoculum and thus destabilising the production process (91).

Dahunsi (2019) (55) and Tsapekos et al. (2018) (56) have each concluded various models to predict the methane production through different lignocellulosic biomass contents. Depending on the composition of each feed and through employing a mechanical pre-treatment of pressure rolling, Dahunsi (2019) determined a mathematical model that gives insight into the biomethane generated through the macronutrients present, as shown in Equation 1 (55). For this model, six types of feedstock were chemically analysed, pre-treated, and then subject to BMP testing to identify the production yield (55).

$$CH_{4,yield,} = 281.66 + 15.72 \times Arabinan - 3.50 \times Lignin + 28.46 \times Proteins \qquad \textit{Equation 1}$$

Equation 1 gives a CH_4 yield with units (mL/g VS) of digestible material, and the chemical compounds arabinan, lignin and proteins must be indicated in units (g/100g VS) (55). Dahunsi concluded a scientific correlation between the feedstock macronutrients in their study, but also discusses the limitations of their analysis. In this regard, the model precision is the main source for future improvement and research, given that the statistical accuracy R^2 of 0.63 lacks precision in the scientific community (55).

In a similar manner, Tsapekos et al. (2018) (56) analysed the effectiveness of different pre-treatment strategies on meadow grass, yielding a structurally comparable equation as Equation 1. Comparison of the two mechanical pre-treatment strategies of shear brushing and coarse steel rolling were examined to determine the subsequent methane yield of the samples (56). The chemical analysis performed by Tsapekos et al. also focussed on the macronutrients arabinan, lignin and proteins, and assessed the degradation efficiency of the chemical products

through performing multiple linear regressors (56). Equation 2 showcases their modelling summary, where the equivalent unit convention has been used as in Equation 1. It is worth highlighting that likewise for their modelling attempts, a limited modelling precision could be concluded, with a coefficient of determination (R^2) of 0.61 (13,56).

$$CH_{4,yield} = 270.74 + 16.61 \times Arabinan - 3.35 \times Lignin + 27.88 \times Proteins \quad \textit{Equation 2}$$

Furthermore, Tsapekos's research group has been involved in the modelling of methane yields through various chemical substrates and their respective concentrations. A summary of their research has been published in the work of Kougias and Angelidaki (2018) (8) and further describes their prediction statistics and methodology for different macronutrient balances in bioreactors.

4.2 Monodigestion: biogas yield

Other organic substances that may be used for AD and are reported in literature include food waste, kitchen waste, or the organic fraction of municipal solid waste (OFMSW). According to the conclusions of Iglesias et al. (2021) (28), all societies produce an annual sum of two billion tons of MSW, of which a substantial fraction is organic and thus biodegradable. In fact, they state that up to a third of the two billion tons can be managed, recycled or treated more effectively, such as producing biofuels from anthropogenic waste. A further potent waste product is glycerol, derived from the chemical processing industry through the transesterification to produce biodiesel. Given that most biodiesel is derived from agricultural products, glycerol can also be seen as an agricultural waste product and has shown great experimental results in AD through substantially augmenting the methane yield of the treated sample. Through adding small quantities, or "doping" glycerol to other common feedstock mixtures in AcD, glycerol has the ability to improve both the methane yield and proportion in the biogas, as per independent studies of Sillaparassamee (2017) and Astals et al. (2012) (2013) (105–107).

A summary of different experimental mono-digestion biomethane yields through AD has been provided by standalone studies of Scarlat et al. (2018) (4,8)and Kougias et al. (2018) for a wide variety of waste products found in agriculture and MSW. A collated summary of both their analyses of feedstock type and the respective methane yields through experimental discoveries has been reproduced in Table 4, after having been originally published in (A – page xix). From inspection of the methane yields quoted in the table, it becomes clear that there is a large degree of variability in the obtained results. The reason for this skew is traced back to different experimental conditions, measurement equipment and performance parameters. In this regard, different chemical prerequisites and compositions have been utilised to determine the methane yields, and different inocula have been used, which contain different types of consortia to a different degree of concentration.

Table 4: Reproduction of biomethane yields of common organic feedstock subject to AD

Feedstock Type	Category	Methane Yield L CH_4/kg $VS_{reduced}$	References
Manure	Pig Manure	250-350	(4)
	Dairy Manure	200-399	(4,96,97,99,108),
	Chicken Manure	107-438	(93,97,101,102)
Agricultural Waste	Barley	322-335	(108)
	Sugar Beet	230-380	(4)
	Maize silage	250-450	(4,96,109)
	Fruit and vegetable waste	153-342	(4,93,110)
	Meadow Grass	282-450	(4,97,98,108)
	Palm Oil Mill Effluents	282-388	(97,98,108)
	Rice Straw	2200-280	(4,93,94)
	Ryegrass	140-450	(4,95,108)
	Switchgrass	122-450	(4,93,111)
	Wheat	245-319	(93,108)
OFMSW/Kitchen Waste	Kitchen Waste	200-683	(4,93,112)
	OFMSW	300-570	(113,114)
	Slaughterhouse waste	550-657	(4,113)

4.2.1 Chemical oxygen demand (COD)

A further crucial performance parameter necessary to mention within the research world of AD is the COD and biochemical oxygen demand (BOD). Both are characteristics of a substrate or digestate and define the pollution potential of a substance, when exposed to the environment (115). The COD is characterised by the total amount of oxygen taken from the atmosphere to fully oxidise a set solution of material and is a measure of organic, degradable material within

an aqueous solution (116). The BOD, similarly, defines the total oxygen absorption utilised by aerobic biological organisms to fully degrade an organic material whilst dissolved in an aqueous solution (117). COD and BOD performance indicators were purposefully omitted from the statistical analysis in section 4.3.2 as the standardised degradation (or reduction) factor of organic material is the percentage of volatile solids that were reduced. The advantage of VS_{red} is that this value is more uniform and allows for easier comparison.

Different research examined whether there is a relation between the COD and the methane production by conducting AD on organic feedstock. No scientific certainty can be proven to date that would relate COD to methane yield, but studies such as Park et al. (2019) and Aguilar-Aguilar et al. (2021) conclude in their independent analyses that a higher reduction of COD (from substrate to digestate) is inherently linked to a higher rate of methane production (118,119). A different study argues that a scientific model should be defined for methane yield and COD reduction percentages based on experimental data and through the use of the Buswell equation (120). From a chemical perspective this claim is compelling given that mass conservation principles must be adhered to, implying that a higher reduction efficiency should translate to more gas production. A further interesting trend can be seen when examining the addition of glycerol into manure substrates. As reported by Aguilar-Aguilar et al. (2021), glycerol doping produces a higher quality biogas with up to 71.92% CH_4 within the CO_2-CH_4 mixture (118). Because glycerol is known to be a very high-COD compound, this leads to the conclusion that high-COD products tend to support the anaerobic microbes in producing a biogas of high quality.

4.3 Co-digestion: biogas yield

To begin the modelling of methane yield brought by AD of different feedstock, it is important to isolate samples and study their degradation potential and kinetics without other contributions or influences. However, improved biomethane generation yields are generally seen through combination of various feedstocks, as the diverse chemical characteristics of different samples complement each other, and the degradation bacteria can benefit from various advantages in micronutrients that a diverse sample brings to the digestion system. Because of this, AcD is considered the industry standard and has higher yields. As already mentioned in section 4.1.4, chemical inhibition of microbes, either by accumulation of VFA,

ammonia or phenols, can lead to process instability and failure. Through the use of a diverse feedstock sample under AcD, the likelihood of process failure is substantially reduced and the methane generation augmented tremendously due to pH and nutrient stability (8).

A common advantage of AcD is that through the changing of seasons, agriculturally-based feedstocks have varying availabilities for AD. In this regard, certain lignocellulosic material of a specific plant species is only available for a limited time each year, but throughout the seasons, different plants produce organic biomass and can contribute to feed an AD bioreactor on a continuous basis (8). This allows for more flexible feeding regimes and smoothens the production curve of agricultural biomass by incorporating a wider variety of different lignocellulosic biomass substrates for AcD over the span of the whole year with differing seasons. To summarise, the main advantages of AcD include:

- Higher OLRs can be implemented on systems under AcD, because process stability is easier by utilising AcD and the synergy effects contribute to an accelerated degradation rate. Given that more organic matter passes through the reactor at the same time, a larger amount of biogas is produced through the degradation processes (110,121).
- Through AcD, the pH fluctuation is a lot more stable, and through adding various feedstocks, the buffer capacity of the digestate is improved. This has the advantage of keeping the pH within a range that is optimal for methanogenic archae to produce biomethane (122,123).
- Through combining manure-based feedstock and lignocellulosic biomass, the C/N ratio of the substrate mixture is generally improved to optimise the amount of biomethane produced. Additionally, the nutrients present in the digestate are more balanced and different minerals present in the inoculum support the chemical balance of bacteria (98,124,125).
- The presence of additional substrates allows for a balance of different compounds within the inoculant, reducing the concentration of inhibitory chemicals (that would be more likely to cause system failure under monodigestion), that limit the production of biogas (97,126).
- A higher biomethane production is evident as per experimentation performed (108,127).

- Several types of feedstock support a heterogeneous substrate sample, which helps the physical performance of a bioreactor by lowering the probability of pipe blockages or issues with pumping or stirring (128).
- Supports the overall economic viability of a biogas business, as a wider variety of substrates can be used to feed the reactor without the fear of inhibition of destabilisation as with monodigestion (129,130).

A further advantage of co-digestion in the agricultural sector is that on farms and in the rural landscape, where biomass in produced, farm animals are generally also readily available. As such, the supply of both manure and lignocellulosic biomass complements each other, as both tend to require low transport distances as they are located close to each other. Because of that, rural biodigesters are associated with lower costs given that chemical and macronutrient balances are easier to achieve. By mixing agricultural biomass with manure, a suitable nutrient basis is provided for the anaerobic bacteria to optimally produce biogas, as compared to mono-digestion. Through this synergy, there are major benefits of producing in a rural environment and making use of the characteristics present in both feedstock.

4.3.1 Methodology of statistical analysis

A literature analysis on various types of AD experiments was performed with the intention of summarising the performance parameters and feedstocks utilised for various types of manure and lignocellulosic biomass. The objective of this review was to showcase the advantages of employing AcD, especially through the combination of liquid manure and a less readily degradable lignocellulosic biomass. With this in mind, a summary of 65 independent studies has been performed and statistically analysed. The original data has been reproduced for this thesis and is available for viewing in Appendix 11.6. Figure 6 portrays the flowchart of selection used to determine studies that were of interest for this literature review. Key studies that were incorporated include experiments that evaluated co-digestion of various feedstocks, with a special emphasis on manure-lignocellulosic biomass combinations. Studies that have been incorporated include (52,77,105–108,113,118,122–124,126,131–181).

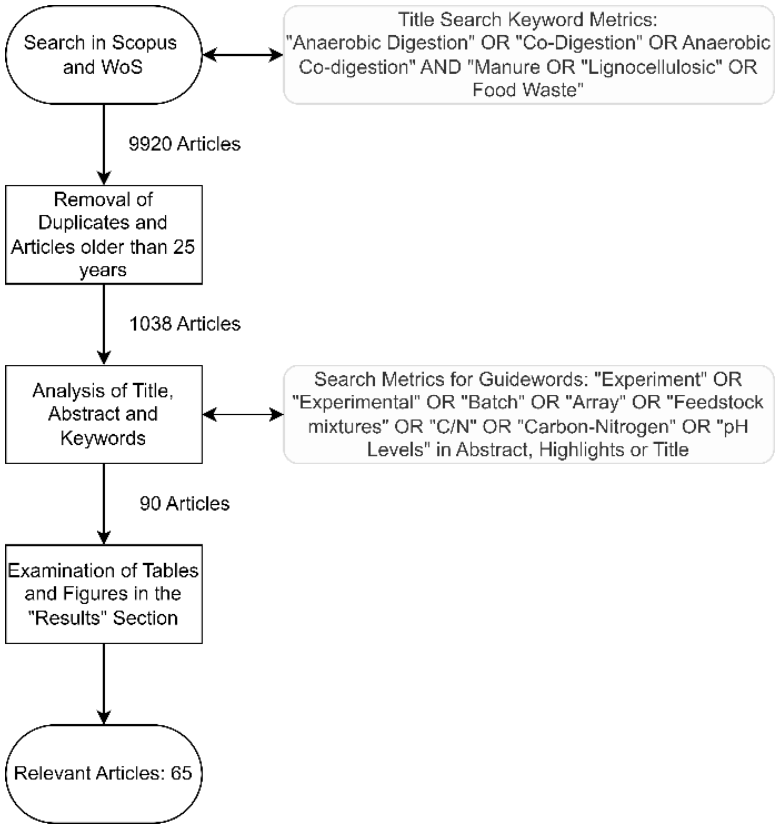


Figure 6: Selection criteria and search strategy employed for the literature review.

4.3.2 Results and discussion

The statistical analysis performed is graphically visualised in Figure 7 - Figure 10. All four figures have been reproduced from the analysis conducted by (13). In total, 65 independent experiments and results have been analysed in terms of methane yield and biogas quality (w.r.t methane proportion) as dependent variables, with different performance parameters of AD systems being the independent variables. The most common independent variables that were discussed in literature, from which the statistical analysis could subsequently fetch results from, were: materials used and their mixture ratio in co-digestion, OLR, HRT, C/N ratio, pH, operational temperature and experimental size. The following analysis mainly spotlights studies in the mesophilic temperature regime, as 85% of the total studies and their published data operated at this temperature. Given that there are three temperature regimes (psychrophilic, mesophilic and thermophilic), the mesophilic regimen, operating at approximately 37 °C, is a common industrial standard and a temperature at which high bacterial efficiency is possible, it was chosen to assess mainly the mesophilic studies for comparison and modelling. Most biological species live at this temperature, including the relevant bacteria. The only downside of working within the mesophilic range is that

temperatures are not high enough to break complex molecules that may release inhibitors. Through performing multivariate analysis of all performance parameters included in the study provides several insights into the behaviour of AD systems and the respective biomethane yield and proportion generated.

The performance parameter with the greatest impact on the biomethane production yield, as per the statistical analysis performed, was found to be the C/N ratio. As the C/N ratio increased, the methane proportion within the biogas decreased. Given that both the C/N ratio and the pH are very related from a chemical perspective, as the proportion of carbon and nitrogen later dictates how many VFA (from carbon) and ammonium (from nitrogen) will be present in the inoculum, it is evident that skewing the C/N ratio to high values leads to system instability. Based on the analysis performed by Siddique and Wahid (2018), an ideal C/N ratio should be within the range of 20-35 (91). From the conclusions of the statistical analysis, it was found that adjusting the C/N ratio to the lower bound of the advised range, at a C/N ratio of approximately 20, leads to ideal results for both methane proportions in the biogas and total biomethane production. This is because a slightly higher C/N ratio favours total biogas production, but for biogas calorific value optimisation (i.e. maximising biomethane), a lower C/N ratio is advisable. All studies that subjected their experimentation to equal parts of VS of lignocellulosic biomass and VS of manure have performed above average. It comes as no surprise that through this mixing ratio, a C/N ratio of approximately 20 is achieved in the AD system. After having reviewed all different parameters on their impact on methane proportions and total biogas production, it became evident that the C/N ratio of all parameters was the best tool to assimilate methane yield. The correlation between C/N ratio and biomethane production is the most evident factor and should be prioritized when designing am AD system. The C/N ratio had a higher impact on the effectiveness of the AD than the temperature regimen, which was not expected based on previous analyses and literature conclusions.

Because the C/N ratio has a proven strong correlation to the pH balance and the macronutrients present in the inoculum, special care must be taken to avoid system inhibition through either acidic (VFA accumulation) or alkaline (NH_3 accumulation) conditions. The higher the C/N ratio, the more carbon is present in the inoculum, which may readily be digested into volatile fatty acids, lowering the pH levels to a level where methanogenic archae cease to degrade material to produce methane. Similarly, the lower the C/N ratio, the more nitrogen is

present in the inoculum, which instead is degraded to ammonia, and causes the reverse effect: an increase in pH. Both fluctuations can be dangerous for the AD system and a nutrient balance is important to have an optimal C/N ratio. To complete the discussion on C/N ratios, it is worth highlighting the positive correlation present between the C/N ratio and the percentage of reduced VS of substrate/digestate material. This is the most evident sign of degradation and leads to the conclusion that organic material is best degraded at higher C/N ratio values.

Figure 7 summarises statistics of C/N ratio to the fraction of reduced VS over different ratios of manure that were present within the samples. Studies included in this analysis were experiments occurring at mesophilic temperatures and at batch experimental scale. As can be seen in the figure, a statistical significance could be identified in the AD systems where the overall manure concentration was inferior to 50% of the total feedstock added (from a VS perspective). However, systems exceeding 50% manure in the total feedstock mixture did not yield statistically significant results.

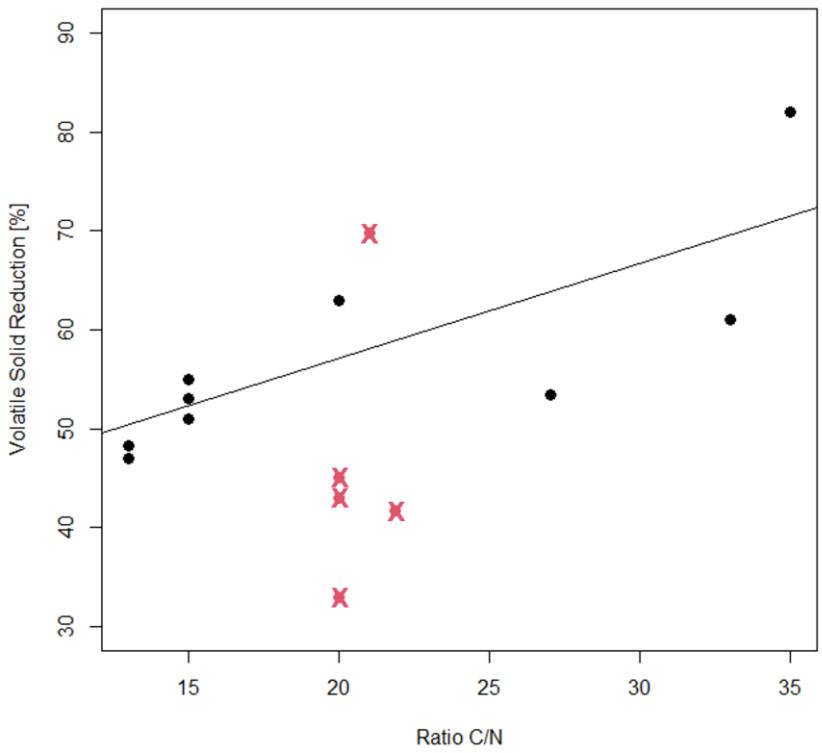


Figure 7: C/N ratios with varying reduction percentages of VS. Data split into two groups: AD systems with less than 50% manure (black, points) and more than 50% manure (red, crosses).

Figure 7 has a specific colour coding present for both systems that were analysed separately. For instance, the red crosses denote digestion experiments where more than 50% manure was present in the feedstock, and no statistical correlation could be identified. In contrast, the black

points, containing less than 50% manure content per VS added, did show statistical significance and have been copied onto a new plot in isolation together with the 95% confidence interval in Figure 8. Figure 8 demonstrates that reducing the manure to a maximum of 50% of the total feedstock is an important design criterion for AD substrates to allow for modelling tasks and methane yield estimations, given that the C/N ratio can be estimated based on the percentage reduction of VS, or analogously, the degree of waste matter degradation potential. In contrast, high-manure feedstock mixtures do not allow for such modelling tasks (but can still be highly effective feedstocks if all performance parameters remain balanced). Even though various strategies to find a suitable model for systems with more than 50% manure were evaluated, none yielded palpable results between methane yield or proportion with different performance parameters that would be worth communicating and discussing.

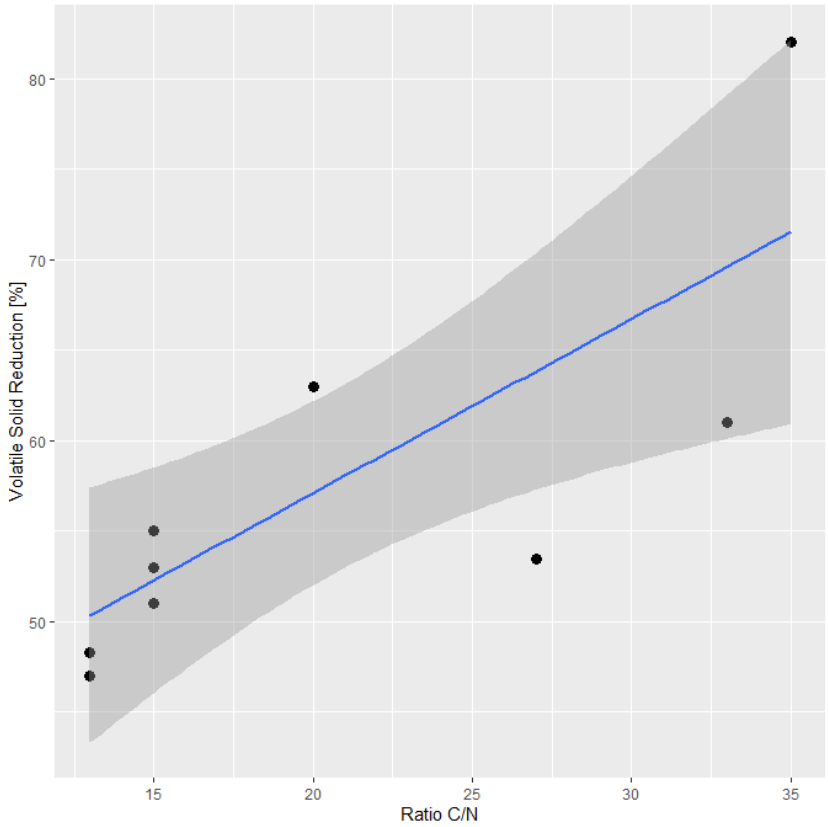


Figure 8: Visualisation of the C/N ratio and VS reduction percentages of low manure (<50% in mixture) content in AD systems.

All studies that were incorporated into the statistical analysis had a pH within the spectrum of 5.3 to 8.5 (52,77,105–108,113,118,122–124,126,131–181). On the one hand, feedstocks that contained a higher percentage of manure in their substrate mix generally contained a higher operating pH, which translated to a richer biogas being produced. Richer, in terms of biogas,

implies a higher proportion of CH_4 in the CO_2-CH_4 mixture. On the other hand, feedstocks that did not treat manure as a majority substrate did not yield any behaviour between the operating pH and the AD efficiency. Various samples were assessed, but no clear tendency between pH and the biogas yield/richness could be observed for low-manure systems. A possible explanation of this phenomenon is that pH fluctuations had no impact on the methane percentage of the biogas, which when assessing the chemical perspective is evident given that the substrate has a greater buffering capacity when manure is the main feedstock in the substrate. Due to the high buffering capacity of the substrate, the mixture is designed to be extremely resilient to pH changes, and any exterior perturbations to skew the pH to non-optimal pH values is met with the chemical composition shifting the pH back to an ideal setpoint for the AD bacteria to work efficiently and under ideal process kinetics. Additionally, a high system pH was generally associated with higher SRT and HRT as system rate parameters. This can be explained by the AD system processes that are prevalent in the reactors and have been discussed in Figure 4. The acidic sub-products of the hydrolysed feedstock can be converted into VFA, subsequently biogas. In contrast, the basic substances such as ammonia, are not further treated by the bacteria and remain in the inoculum, thus causing a chemical buildup of material. This accumulation ultimately leads to rises in pH.

Bacteria responsible for sample hydrolysis work at a faster rate that methanogenic archae, so it is likely that an accumulation of VFA is caused by a high substrate addition, with the process kinetics of methanogenesis being the bottleneck as the slowest of the four-step process.

Upon assessment of the mesophilic temperature profile present in the studies, several interesting conclusions became evident in the methane proportion in the produced biogas. When comparing mixtures of different manure-lignocellulosic biomass samples and their efficacy in producing a rich biogas, it was found that high-manure samples (and as such, lower lignocellulosic biomass samples) have on average 30% more methane within their biomass than samples where lignocellulosic biomass is the predominant feedstock. Figure 9 elucidates this trend in terms of box and whisker plots. Despite a clear trend being visible, statistical significance could not be proven. With lower mixture concentrations of lignocellulosic biomass (the right part of the plot), a higher proportion of methane was evident in the various studies analysed. In the overall context of optimising the production of calorific content for further usage, it is important to also assess the impact of overall volume of biogas production. With

this in mind, lignocellulosic biomass further supported the biogas production in AcD and can be considered advantageous as more overall biogas has been produced. As a result of this, a compromise must be found where AcD is optimised such that the most amount of calorific content is extracted from the waste material: through maximising the amount of biogas present with the highest methane concentration possible. A further interesting conclusion taken from Figure 9 is that with a high manure content in the substrate, the variability in methane production is higher, both on the lower end as on the higher. Whilst the methane proportion is on average higher, there have been experiments of high volatility that produced substantially more, but also less methane as compared to systems digesting more lignocellulosic biomass (as seen on the left boxplot). The raw data is provided for the interested reader in tabular format in Appendix 11.6 of this thesis.

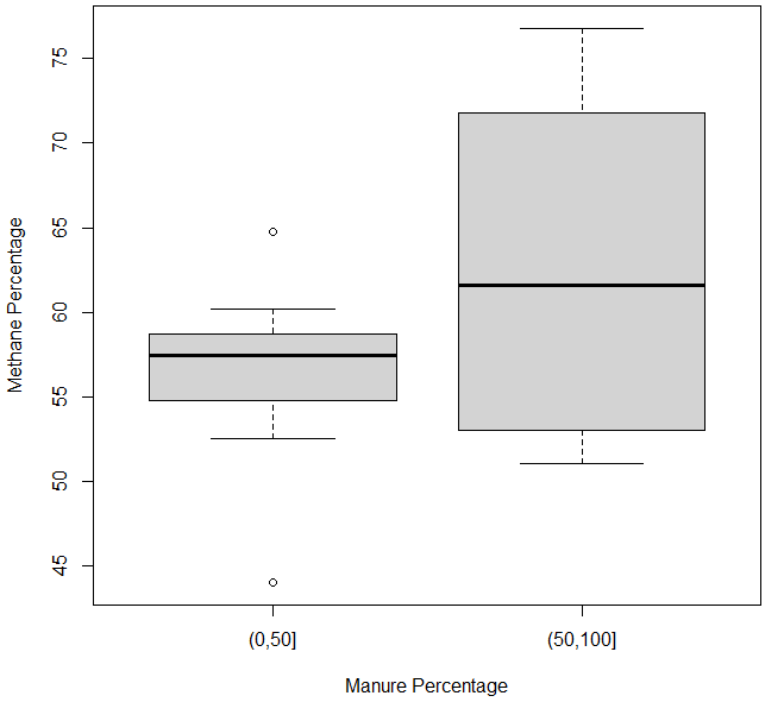


Figure 9: Graphical analysis of methane production/dispersion depending on various manure concentrations prevailing in AcD experiments (53/65 studies incorporated).

A statistically significant model could be determined from multivariate analysis, which has been quoted in Equation 3. These models indicate a relationship between the methane proportion of the resultant biogas to specific performance parameters and feedstock composition criteria. According to Equation 3, the methane proportion increases with a longer retention time of the AD system. This can be explained by chemical kinetics: given that methanogenic archae are the bottleneck of the AD process tree and require the longest time in generating methane, a long

retention time is advantageous to the overall system, giving the bacteria sufficient time in fully converting all available nutrients into methane. In addition, a slightly higher pH proves to be beneficial for the inoculum system as per previous discussions. In this regard, pH ranges discussed between 7.5 and 8.5 would provide an optimal biological system for AD. A further trend in methane proportion behaviour could be identified in Equation 4, but given that it was not statistically significant, the model is not worthy of major discussion and analysis.

$$\%\mathrm{CH_4}\Big|_{\substack{\mathrm{pH}\begin{cases}8.5\\6.5\end{cases}\\ \mathrm{Temperature} = 37\ ℃}} = 2.6797 + 6.3848 \times (\mathrm{pH}) + 0.3632 \times (\mathrm{SRT}) \qquad \textit{Equation 3}$$

$$\%\mathrm{CH_4}\Big|_{\substack{\mathrm{pH}\begin{cases}8.5\\6.5\end{cases}\\ \mathrm{Temperature} = 37\ ℃}} = 9.016 - 0.1003 \times (\%Manure) - 0.6784 \times (pH) + 0.01355 \times (\%Manure \times pH) \qquad \textit{Equation 4}$$

A further conclusion of all studies analysed is that in general, small-scale bioreactors produce biogas with a higher methane proportion than larger-scale reactors. This statement is further supported by Figure 10, where studies of different scales are subject to comparison. Based on these findings, it is suggested to make use of laboratory conditions with technology of customising performance parameters and chemical compositions of feedstock such that these findings and experience can then be translated into larger, low-cost pilot-plant digesters to support a decentralised production landscape in rural areas. In this way, energy needs can be met through self-production initiatives and farm-waste, such as biomass from plants and livestock, receive an immediate additional use. Through the use of an inexpensive pilot-plant reactor discussed in more detail in chapter 7, return-on-investments may be feasible very quickly.

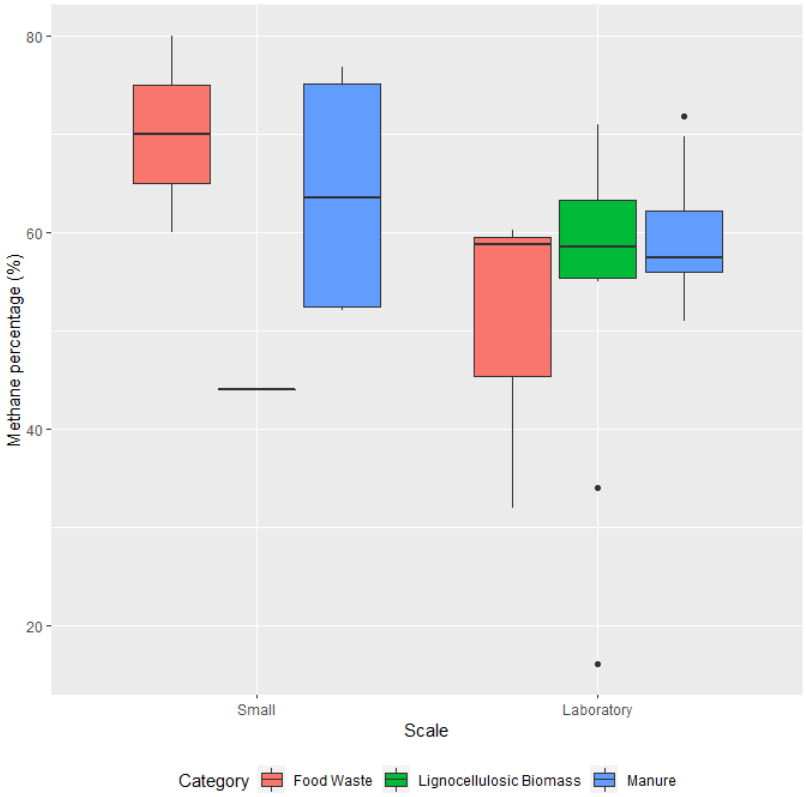


Figure 10: Summary of experimental scaling effects as per different primary (majority) feedstock digested in AD reactors. Methane proportions in biogas examined for mesophilic temperature regimes (53 studies analysed).

5. Biochemical Methane Potential Testing

After having conducted a statistical analysis, it was necessary to experimentally assess the efficacy of statistical findings with in-house validation and further determination of key drivers for improving biogas yield, but now from an experimental viewpoint. The intention was to experimentally analyse well-performing samples that were identified in the statistical assessment, replicate these experiments, and validate their high efficiency through own testing and verification of performance parameters that may be important to the AD system. Through this, the researchers expected to receive further insight into AD process kinetics and overall experimental experience on the lowest-possible experiment scale, before moving to larger biogas systems like pilot-scale reactors. In this sense, the batch testing performed and subsequently described served as an intermediary experimental phase to work towards the optimisation of pilot-scale biogas reactor operation.

The majority of the discussion and conclusion has been evaluated in article (C) in the summary of publications.

5.1 Literature overview of similar analyses

Batch experimentation that was conducted took inspiration from previous studies that followed a similar methodology, where different performance parameters were altered to analyse the effect on the produced biogas yields and biomethane content. A study performed by Xavier et al. (2015) (182) analysed the effect of co-digesting manure with wheat straw through different performance parameters and varying proportions. Their results indicate pretreatment effects on lignocellulosic biomass cause a major impact on the efficiency (final methane yield per mass of VS), with briquetted wheat straw outperforming its shredded equivalent (182). A further addition to their study came in form of their economic analysis, determining that up to 46% of transportation costs are saved by briquetted straw, through its compact storage and transport options (182).

The research group among Wang et al. (2018) (177) investigated performance fluctuations through different co-digestion ratios of pig manure and corn. They concluded that the highest amount of VS added generally improved the efficiency of the sample reactors, achieving a co-

digestion performance index (CPI) of 1.97, implying that a 97% biomethane generation increase was seen through feedstock co-digestion than when either the control sample or the isolated components were digested alone (177). Abouelenien et al. (2013) analysed bacterial inhibition caused by varying pH values (159). They studied the effect on AD efficiency when subjecting samples to pH-values in the range of 4.5 to 8.5, concluding that the range of 7.5 to 8.5 is the optimal environment to maximise biogas yields (159). Further studies analyse the positive effects of adding glycerol as a feedstock to the co-digestion sample, reporting stark increases in biomethane production (105,175,183).

5.2 Experimental aims and objectives, structure of trials, overarching research question

In light of the previous analysis taken and with the intention of gaining own experimental experience in both co-digestion and BMP testing, various experimental objectives can be defined along with an overarching research question that will help direct the investigation into the desired direction. The most relevant objective, under which the different experimental aims will be identified, is the verification and discovery of feedstock mixtures composed of lignocellulosic biomass, manure and glycerol. The intention of this research is to develop ideal mixtures to maximise biomethane yield, for the later use in low-cost biodigesters. Given that performance parameters have already been discussed in detail in Chapter 4, this chapter will assess chemical mixtures in more detail (before assessing continuous operations in Chapter 6). Therefore, various feedstock mixtures will be analysed to determine their efficiency of producing biomethane in a low-cost and low-effort environment.

Further objectives of this experiments were identified alongside the main aim, which mainly encompass the gaining of knowledge and experience of different AD process mechanisms and kinetics in a practical landscape. It was of major interest to the researchers to gain a further understanding of the biomethane-generation potential of glycerol in different feedstock mixtures, as different research groups concluded exciting findings of improved yields from this mixture (105,175,183). Furthermore, the effect and potential improvement of AcD, namely with lignocellulosic biomass and manure, is to be studied and its digestion efficiency analysed in greater detail. Lastly, various types of inoculant are available for practical use. Whilst livestock manure by itself works as a potent starter inoculum that has a well-balanced bacterial

consortium present for efficient AD, other industrial and commercial samples exist that promise cultivation of highly-efficient bacterial consortia for biomethane generation (184). Based on this knowledge, an industrial sample of inoculum was purchased and compared to manure, as a low-cost and high-availability alternative on the market.

The subsequent study will therefore analyse different mixtures in a series of experimental "batch" testing, very similar to the BMP-methodology described in Section 4.1.3. In the first experimental trial, a comparison between the commercial inoculant and swine manure as an AD starter is undertaken. Following from that, the second and third trials assess different mixtures of OFMSW, lignocellulosic biomass, swine manure and glycerol concentrations as mixture feed for AD, evaluating efficiencies and the conversion potentials to biomethane. The aim of the series of "batch experiments" is to find the ideal ratios of inoculant to biomass for AD. The optimal proportions will be defined by those samples producing the highest concentration of methane and overall, the highest amount of biomethane generated.

The research question, of this experimental phase, has been coined as follows: *Through what mechanisms, feedstock mixtures and pre-selected conditions is anaerobic digestion of small-scale samples optimised?*

5.3 Methodology and experimentational setting

5.3.1 Feedstock sourcing

Different feedstock sources were incorporated into the AD experiment conducted. Table 5 shows an overview of the physiochemical characterisation of the various substrates utilised in experimentation. Swine manure was sourced from a biogas plant and farm consortium in Soria, Spain, as the Technical University of Madrid is involved in several research projects within the field of AD and wastewater treatment there. The farm in Soria also provided the meadow grass as a source of lignocellulosic biomass for the batch experimentation. The meadow grass was dried and chopped into a powder prior to substrate mixing.

Table 5: Selected feedstock properties used in batch experimentation. Literature values indicate further details of properties not determined through own investigation.

Property	**Feedstock**				
	Swine Manure (162,185)	Meadow Grass (97,186)	Glycerol (175)	Orange Peel (dried) (123,187)	Orange Peel (fresh) (188)
TS	50.5 (g TS/kg)	46.6 ± 0.7 % (w/w)	376-782 (g/L)	77.66 ± 5.19 % (w/w)	28.88 ± 1.55 (% w/w)
VS	35.4 (g VS/kg)	1.4 ± 0.4 % (w/w)	8.9 ± 0.6 (g/L)	74.84 ± 4.82 % (w/w)	97.48 ± 0.74 (% TS)
Carbon (%)	37.5 % (w/w)	45.1 % (w/w)	39 % (w/w)	46.5 ± 1.5 (w/w)	43.2 % (w/w)
Nitrogen (%)	2.6 % (w/w)	2.2 ± 0.1 % (w/w)	N/A	2.2 ± 0.3 (w/w)	0.42 % (w/w)
C/N ratio	16.4	32.0 ± 3.5	N/A	21.1	-
pH	7.5	4.24	9.00-9.28	-	4.56 ± 0.25
COD	69.7 (g O_2/kg)	1522 ± 18 (mg COD/g VS)	1920-5820 (g/L)	-	380 ± 45(g/L)
NH_4^+ (g/L)	4.4	-	N/A	-	0.95 ± 0.20

The OFMSW used for experimentation, mainly treated through food shredding to decrease its particle size, was kitchen waste which has been provided by the university's cafeteria. In total, 34 kg of kitchen waste were utilised to represent the OFMSW, of which a visual characterisation took place to determine the approximate quantities of each food waste component being present within the mixture prior to processing the waste to liquid consistency. Table 6 provides an overview of the visual inspection and characterisation that the researchers had agreed upon.

Table 6: Approximate proportions of OFMSW used for batch experimentation

Waste	Proportion (%)
Meat and fish	25%
French fries	25%
Cereals: Rice, pasta and bread	10%
Fruits and vegetables: mainly banana, orange and onion peels	10%
Napkins and paper towels	10%
Other	20%

A bacterial inoculum has been supplied by the manufacturer of commercial low-cost biogas plants available for domestic use, by large families or small farms (184). A full set for plant assembly has been procured by this provider alongside the inoculum, to which more description and further analysis is given in Chapter 7 (184). The inoculum is considered a

"starter-set", to be mixed under certain concentrations with lukewarm water as per manufacturer instructions (184).

Lastly, due to specific interest of the researchers, orange peel was also used as a substrate for its BMP analysis and used in co-digestion with other feedstocks. The reason for incorporation relates to the search for additional uses of this waste product outside of its general applications. Orange peel is generally being used in either in the nutraceutical or plastic industry, but other uses as a kerosene-like jet fuel and now biogas has also previously been explored by the research group (189). In this regard, both fresh orange peel and pretreated orange peel, where the D-limonene has been removed from the skin, have been analysed. D-limonene had been removed due to its antibacterial properties, with the concern that this substance would interfere with the bacteria responsible for AD.

5.3.2 Experimental design

Substrates were analysed as described in section 5.2.1, and then mixed such that a constant amount of TS was present in each batch reactor. 4% TS content was chosen for homogeneous mixing, as literature values provide this as a suitable optimum. A constant amount of substrate was added, such that each sample received identical amounts of feedstock to digest.

Duplication of batch experimentation occurred, with a constant amount of both starting inoculum and substrate present in each reactor such that the bacterial activity and the nutrition provided is identical across all trials performed. The batch reactors selected were 250 mL sized bottles with an air-tight lid and rubber septa fixed on the lid for gas extraction and analysis, as seen in Figure 11. Reactors were filled with 200 mL of content, with the remaining 50 mL dedicated for gas accumulation and subsequent biogas generation. All samples were filled with distilled water to the point where the total mixture occupied 200 mL of reactor volume. Distilled water was considered an independent medium not reacting with the other substances present and ensured a constant working volume for all samples, implying the same content of headspace for the biogas to be generated.

Before closing the bottles, a *nitrogen flush* was employed for each reactor at 5 mL/s for the duration of 30 seconds. This consists of bubbling pure nitrogen through the mixture broth prior to closing the reactors with its air-tight lid. The intention of this is to minimise the amount

of oxygen present in the sample, thus recreating anaerobic conditions to the highest degree possible.

Figure 11: Batch reactors used. Image taken from the oven at 37 °C, simulating mesophilic conditions.

Batch reactors were placed in an oven at 37 °C to simulate a mesophilic environment for the bacteria. As soon as the reactors, for the first placement in the oven on day 0 of experimentation, reached the operating temperature of 37 °C, a needle was introduced through the septa to evacuate any pressure build-up that was caused by the temperature increase. Manual agitation of the samples occurred daily. Only two samples were retrieved from the oven at each time for analysis to minimise the temperature fluctuations.

In total, three trial rounds were conducted with different feedstock mixtures and inoculant. The feedstock mixtures of the three trial rounds, as an overview, have been summarised below in Table 7 to Table 9. Round 1 focussed on comparing the commercial bacterial inoculum with swine manure as an inoculum. Rounds 2 and 3 subsequently analysed differing proportions of substrates, whilst keeping the bacterial starter sample identical.

Table 7: Feedstock present in round 1 of batch experimentation.

	Bottle 1	**Bottles 2**	**Bottles 3&4**	**Bottles 5&6**
Manure added (g)	100	0	100	0
Bacterial consortia added (g)	0	15	0	15
Distilled water added (g)	100	185	95	180
Kitchen Waste added (g)	0	0	5	5
Orange peel (dried) added (g)	0	0	0	0

Table 8: Feedstock present in round 2 of batch experimentation.

	Bottles 1&2	**Bottles 3&4**	**Bottles 5&6**	**Bottles 7&8**	**Bottle 9**	**Bottle 10**
Manure added (g)	100	100	100	100	100	100
Meadow grass added (g)	0	0.5	0	0.5	0	0
Distilled water added (g)	100	99.5	93.65	93.15	99	99
Glycerol added (g)	0	0	6.35	6.35	0	0
Orange peel (dried) added (g)	0	0	0	0	1	0
Orange peel (fresh) added (g)	0	0	0	0	0	1
Mixture pH	7.33	7.34	7.33	7.33	7.38	7.40
COD (mg/L)	4643.6	46924.8	47902.4	7576.4	17596.8	N/A
BOD (mg/L)	1315	2990	2995	2600		N/A
TAN (mg/L)	620	360	360	740	700	N/A

Table 9: Feedstock present in round 3 of batch experimentation.

	Bottles 1&2	**Bottles 3&4**	**Bottles 5&6**	**Bottles 7&8**	**Bottles 9&10**
Manure added (g)	100	100	100	100	100
Meadow grass added (g)	0	0.5	0	0.5	0.5
Distilled water added (g)	100	99.5	98	97.5	98.5
Glycerol added (g)	0	0	2	2	0
Orange peel (dried) added (g)	0	0	0	0	1
Orange peel (fresh) added (g)	0	0	0	0	0

5.3.3 Analytical methods

Two measurement strategies were utilised for batch experimentation. Firstly, measurements every other day included the recording of pressure buildup and biogas composition analysis, from which the total volume of biomethane generation at STP conditions could be derived from. Pressure was measured by using the water displacement method in a U-tube to measure the amount of volume displaced by the reactor's internal pressure. A needle connected to one side of the U-tube was passed through the rubber septum of the reactor to identify the pressure difference, with the other end of the U-tube being subject to atmospheric pressure. Figure 12 shows an image of the pressure measurements utilising the U-Tube and gas extraction through the rubber septum.

Figure 12: U-tube pressure measurements (left) and needle system used to pierce the rubber septum (right)

Gas composition analysis included identification of permanent gases such as carbon dioxide, methane, nitrogen and oxygen using gas chromatography. A thermal conductivity detector (TCD) sensor of an Agilent Technologies GC 90 chromatograph was used, with Argon and Helium as transport gases set at a 50 mL/min flowrate. Sulphur content in samples was also analysed using a flame photometric detector via an HP 5890 Series II chromatography unit. Gas losses through both the pressure and composition measurements were accounted for. Figure 13 (left) shows an image of the gas chromatography unit for permanent gas measurement. Figure 13 (right) shows an image of the gas chromatography unit for sulphur content measurement.

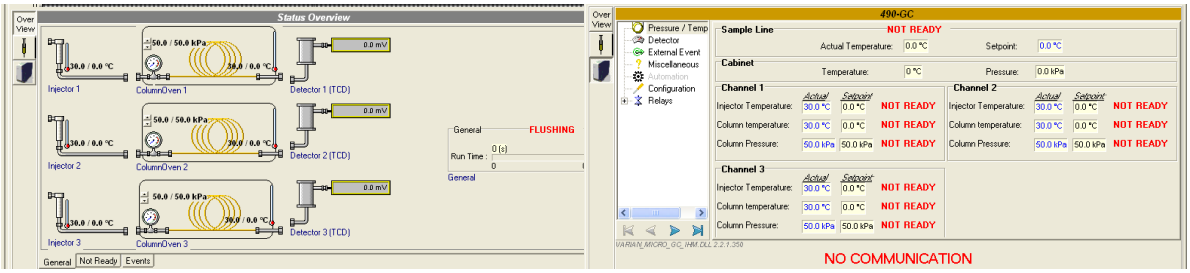
Figure 13: Gas chromatographs used for permanent gas characterisation (left) and sulphur content analysis (right)

Secondly, sample analysis was performed before the experiment began, and after experiment termination. Through this, various assessments regarding the biodegradability of the sample could be concluded in terms of a before vs. after analysis. Sample characterisation of VS, TS and water content took place of all feedstocks described in section 5.2.1 as per standard methods (190). An ORION 250A pH meter was employed for pH measurements. Further chemical properties evaluated as per "*Standard Methods for the Examination of Water and Wastewater*" include COD, BOD, alkalinity and ammonia content (TAN, NH_3 and Kjeldahl) (190). The COD and BOD measurement strategies followed the 5-day test period method incorporating the closed reflux, colorimetric method (190). Alkalinity was determined through joint VFA determination via the Ripley titration method (191). Ammonia compounds were determined by making use of the Nessler method and ammonia-selective electrode method using a known addition (190).

5.3.4 Chromatography

As discussed in section 5.3.3, a TCD-sensor was used for permanent gas characterisation. The TCD-sensor chromatography unit used a 10 metre PPQ heated injection column with injector and column temperature both set at 50 °C. The column pressure was held constant at 200 kPa, using Argon and Helium as carrier gases. All settings could be pre-defined via the graphical user interface of the chromatography software on the auxiliary computer, as seen in Figure 14. The chromatogram measured information such as peak time and area measured under specific peaks.

Every permanent gas will be registered at a different time by the chromatography unit and will cause a "spike" in the chromatogram at a different time interval, as seen in Figure 15.



Used	RT [min]	Name	On/Off	Value
☑	0.00	Set Peak Width		0.1000
☑	0.00	Set Threshold		0.1000
☑	0.00	Turn Integration		
☑	0.00	Baseline Valley-to-Valley		
☑	0.40	Turn Integration		
☐	0.40	Backward Horizontal Baseline		
☑	0.48	Set Threshold		0.1000
☑	0.65	Turn Integration		
☑	1.20	Turn Integration		
☑	1.50	Turn Integration		

Figure 14: Screenshots taken from the graphical user interface method selection menu

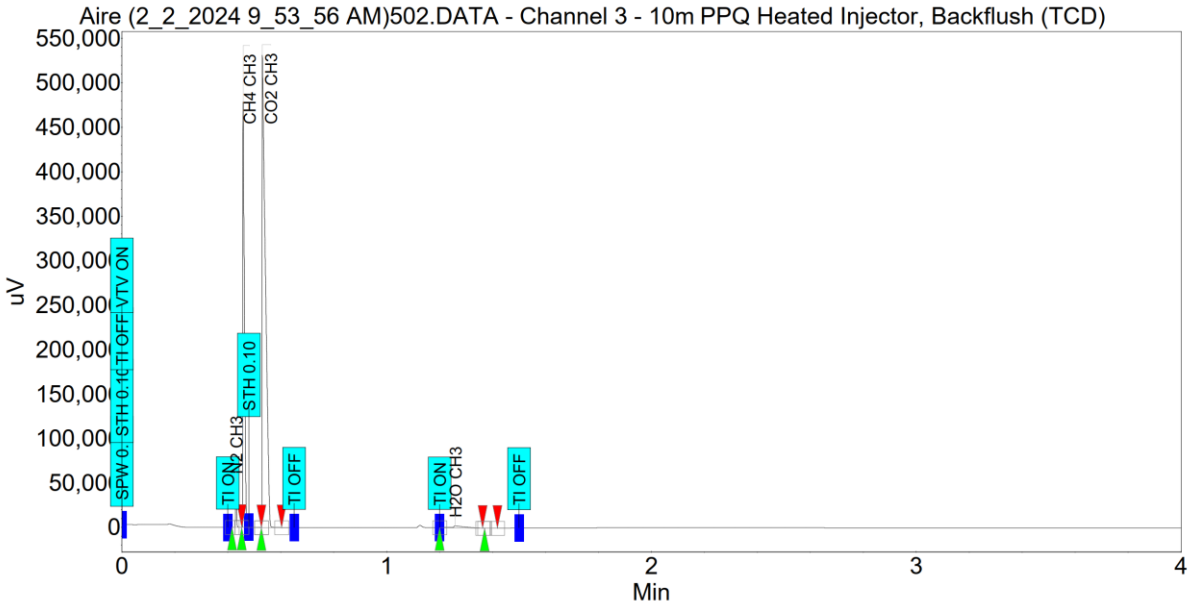


Figure 15: Gas chromatogram extracted from Agilent chromatography software. Peaks correspond to data indicated in Table 10. "CH3" refers to channel 3, based on where the gas column had been installed (three types of measurements can occur simultaneously).

Table 10 provides an overview of the peak times identified for the key gases that were measured throughout the gas chromatography measurement process.

Table 10: Permanent gas chromatography peak times

Permanent gas	Time (min) after injection into column
Nigrogen (N_2)	0.43
Methane (CH_4)	0.45
Carbon dioxide (CO_2)	0.54
Water vapour (H_2O)	1.21

Additionally, the chromatograph measured the area (in µV.min), or intensity, of the peak registered, which stands in direct correlation with the gas content administered from the reactors to the chromatography unit. To accurately determine the correlation between area measured and concentration of permanent gases added, a calibration was conducted in the *Laboratorio Oficial José María de Madariaga (LOM)*, an institute for gas calibration and explosives certification. Specific pre-defined CH_4-CO_2 mixture concentrations could be tested there and measured for peak intensity using the gas chromatograph available. Gas valves called "Brooks 5800S" with pre-defined apertures were used based with diffusivity factors of permanent gases to determine the exact gas intake for the chromatograph, to maintain a controlled calibration.

Data processing of the calibration led to the results summarised in Figure 16. Different mixture concentrations created a calibration curve that could subsequently be used to determine the concentrations of the permanent gases measured through the TCD.

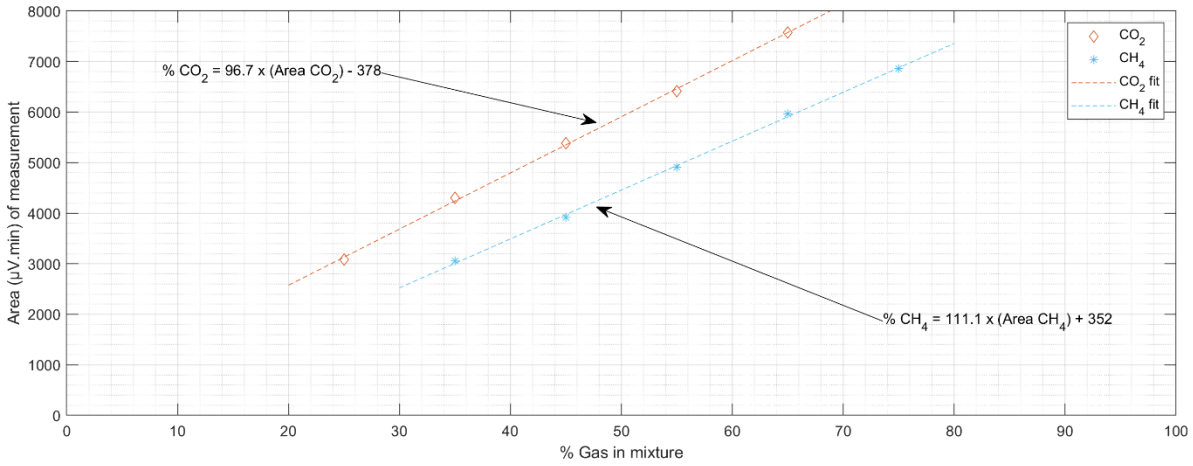


Figure 16: Calibration used to relate chromatography results to gas proportions for carbon dioxide and methane gases

Sulphur composition analysis of the produced biogas was conducted through calibration and measurements of a separate chromatography unit, indicated in Figure 13 (right). This chromatography unit of the HP 5890 Series was fitted with a flame photometric detector, which

can detect the Sulphur content gases and liquids. An HP-1 capillary column (60 m x 0.25 mm x 0.25 µm) with Helium as a carrier gas was employed (192). The column pressure was set at 134 kPa. Injector temperature was tuned to 225 °C, detector temperature at 250 °C, along with a ramp-up heating algorithm: temperature begin 35 °C for 2 minutes; heating increase at 15 °C/min to a final temperature of 250 °C; hold at 250 °C for 0 minutes (192). Figure 17 shows a sample chromatogram extracted during the data acquisition process, as seen in the graphical user interface.

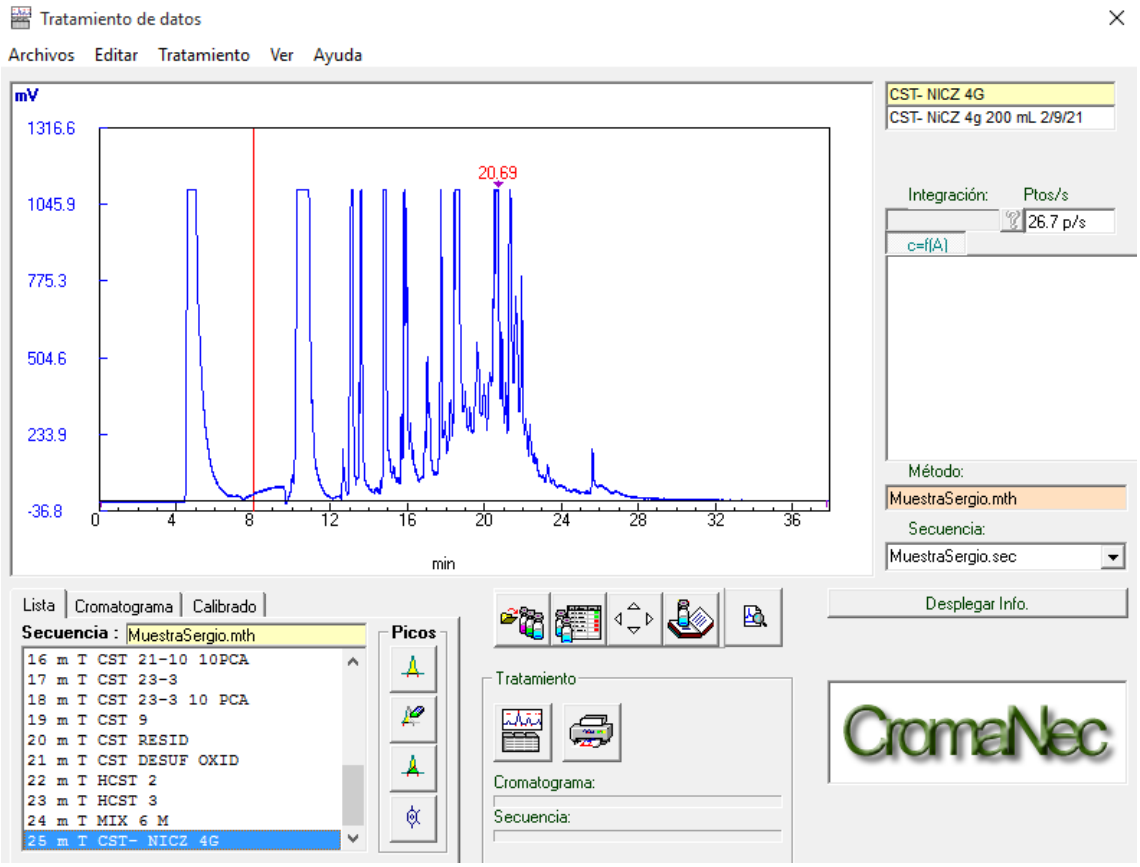


Figure 17: Chromatogram extracted for sulphur analysis and testing

Sulphur content calibration was conducted by subjecting varying Sulphur-containing samples for chromatographic testing, each time analysing the area of activity denoted by the chromatogram. Individual classification of Sulphur compounds did not occur, as merely the total Sulphur content was deemed scientifically relevant for further discussion and interpretation. As such, the full chromatogram area was taken as a standard to derive the Sulphur content present in the samples. Figure 18 provides an overview of the calibration conducted.

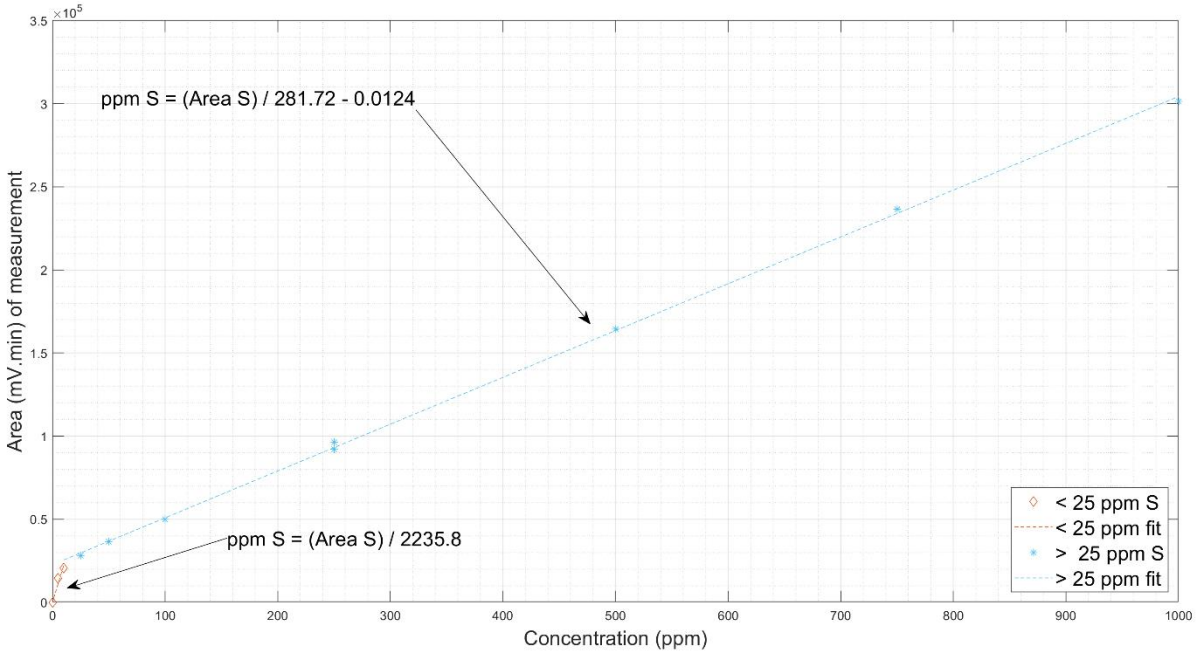


Figure 18: Sulphur calibration curve for flame photometric detection

Overall, Sulphur content was negligible throughout the experimental series, and it was chosen to not report this analysis in the main discussion.

5.3.5 Thermogravimetric analysis and differential scanning calorimetric testing

A Mettler Toledo T-50, located in the *Laboratorio Oficial José María de Madariaga (LOM)* was used for thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) testing. TGA assessment determines the differences in volatilization as a sample is heated with temperature, whereas the DSC analyses the heat exchange experienced by a sample versus its temperature. As a result, the properties of several thermal conversion processes, including pyrolysis, combustion, and oxycombustion can be defined through utilising the TGA-DSC apparatus, which provides interesting insights into the amount of volatile matter present in the discussed samples and its volatilisation regimen under different temperature profiles. TGA was thus used under a continuous heating rate while subject to an air environment with 79% nitrogen and 21% oxygen for subsequent analysis.

The TGA model was set as follows: A 50 mL/min air flow was specified, with 40 ± 5 mg of sample being heated at a rate of 10 °C/min from 30 °C to 800 °C under a 70 µm alumina crucible. Mass loss was noted during the procedure, providing quantification of different volatilisation metrics, including:

- *Induction temperature* (T_i), also known as the temperature at which the reaction accelerates. Given that there are no recorded mass fluctuations, it may be computed as the intersection of the line that the mass loss creates at the start of the reaction (a horizontal line) and the line that creates the slope as the mass drops.
- *Maximum weight loss temperature* (MWLT), which is the temperature at which the mass loss rate reaches is highest. It is determined by calculating the minimum of the TGA curve's first derivative (also known separately as the DTG curve).

Conversely, the sample's heating value was estimated using DSC. In order to do this, a rapid oxidation that results in a fast heat-release is necessary. Due to this heat release, the testing conditions skew from those of TGA analysis. In DSC testing, the heating rate is set to 20 °C/min and volatilisation occurs under a pure oxygen environment with a flowrate of 50 mL/min. Once again, 40 ± 5 mg of sample mass were subject to 70 µL alumina crucibles for heating under the following algorithm: starting temperature 30 °C, final temperature 700 °C. This was conducted because oxidation occurs at temperatures up to 500 °C, therefore allowing for a guaranteed oxidation by heating to up to 700 °C.

Substrate samples and mixtures of round 2 were subject to the TGA and DSC analysis.

5.4 Results and discussion

5.4.1 Biogas production

Experimental findings of each trial round have been portrayed in Figure 19 to Figure 21, as per the substrates seen in Table 7 to Table 9 respectively. Duplication of results are summarised graphically as per the figures, where information on both biogas quality (in blue, with circles) and biogas yield (in orange, as line plots) are visible.

Figure 19 portrays the first set of results, in which a comparison of different inoculants was performed. Certain trends and conclusions are visible very clearly and will dictate the future strategy of experimentation for both future batch and continuous experimentation (as described in this chapter and in Chapter 6), but also for experimental scaling discussed in Chapter 7.

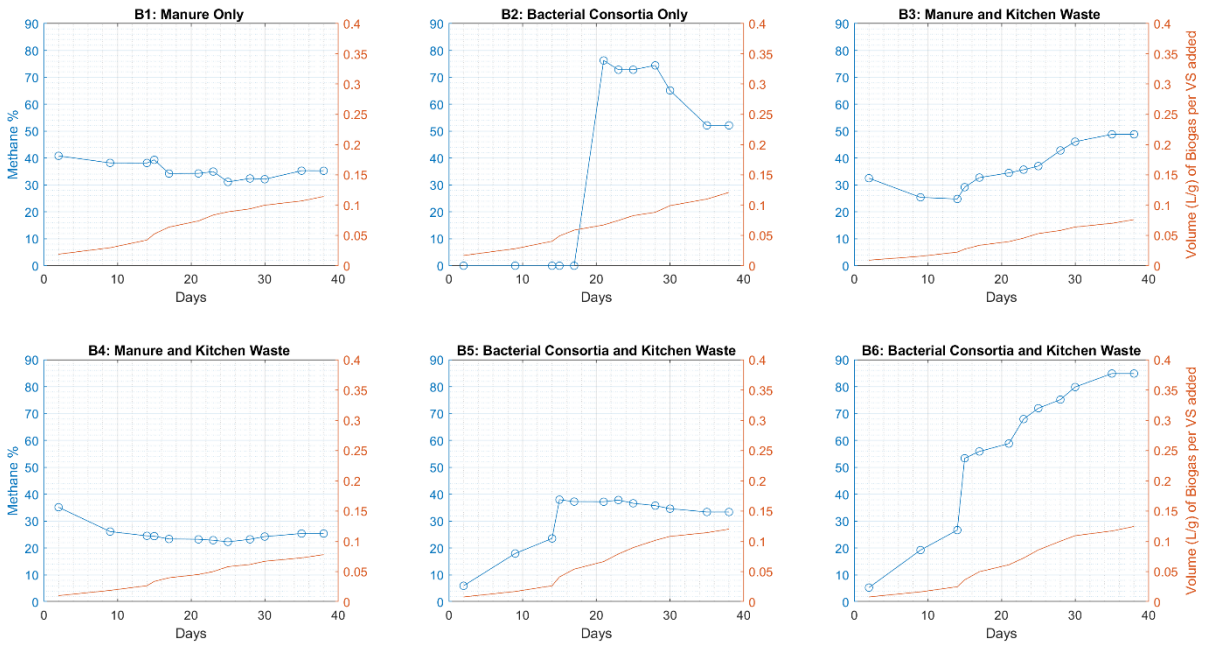


Figure 19: Biogas quality in terms of methane proportion (indicated in blue, circled) and biogas production volume per quantity of VS added (in orange, line plot). Round 1 of experimentation.

As seen in Figure 19, manure and bacterial consortia were assessed based on their methane producing efficiency and their ability of generating biogas. Subplots have been labelled from B1 to B6 to denote the different reactors used in the first round of experimentation, where control samples of manure and the bacterial consortia where compared and co-digested with kitchen waste. The bacteria consortia, as seen in subplots B2, B5 and B6, had a methane production delay of approximately 2 weeks until significant amounts of biomethane were produced. This can be seen in the jump of methane proportion, as per the blue curves in the figure. On the other hand, the manure samples depicted in subplots B1, B3 and B4 did not require an acclimatisation period and produced a similar degree of biomethane throughout the course of the experiment, with the stable production being denoted by the horizontal curves within the range of 30 and 50% methane of the total biogas for all samples tested. Overall, biogas quality was higher in the first two weeks for the manure-based samples, as they started producing methane instantly. After about two weeks of acclimatisation, the bacterial inoculum ramped up its conversion efficiency and substantially improved both the biogas yield and concentration of methane in the biogas. This is especially visible in subplots B5 and B6, where both the biogas volume curves and the quality curves start a steep incline from 15 days onwards. A similar trend is also evident on subplot B2, but here only the methane proportion improved drastically, without any further improvement in biogas generation. The bacterial

inoculant, from a total gas production point-of-view, also outperformed the manure samples, but only after acknowledging the 2-week settling-in period of the commercial inoculant.

Because of these findings, it was determined that the bacterial consortium lends itself better to long-term, continuous biogas generation systems, which can sacrifice two weeks of acclimatisation for subsequent improved and rich biogas generation. In contrast, manure is an efficient inoculant for instantaneous matter degradation and methane production. Because of these intermediate conclusions, it was chosen to base long-term experimentation on the bacterial inoculant, whereas further experimentation with a shorter timescale (as seen in the following discussion and in Chapter 6) would use swine manure as a primary inoculant due to its high efficiency and low acclimatisation time. Because batch experimentation generally occurs within 30 to 40 days, a two-week acclimatisation would yield a lower overall gas production and reduce experiment productivity given the major delay that is to be expected. Pilot plants such as the low-cost biodigester discussed in section 7 however, generally run continuously for at least half a year, in which case it makes sense to use the improved commercial inoculum sample as the overarching objective is to maximise gas production and a high methane quality.

In round 2 of the batch trials, various feedstock mixtures were tested. The intention was to test the efficiency of AcD as compared to treating swine manure in isolation. Figure 20 elucidates the main results obtained. In a similar manner, subplots were created to portray all data of the second experimentational round in one figure.

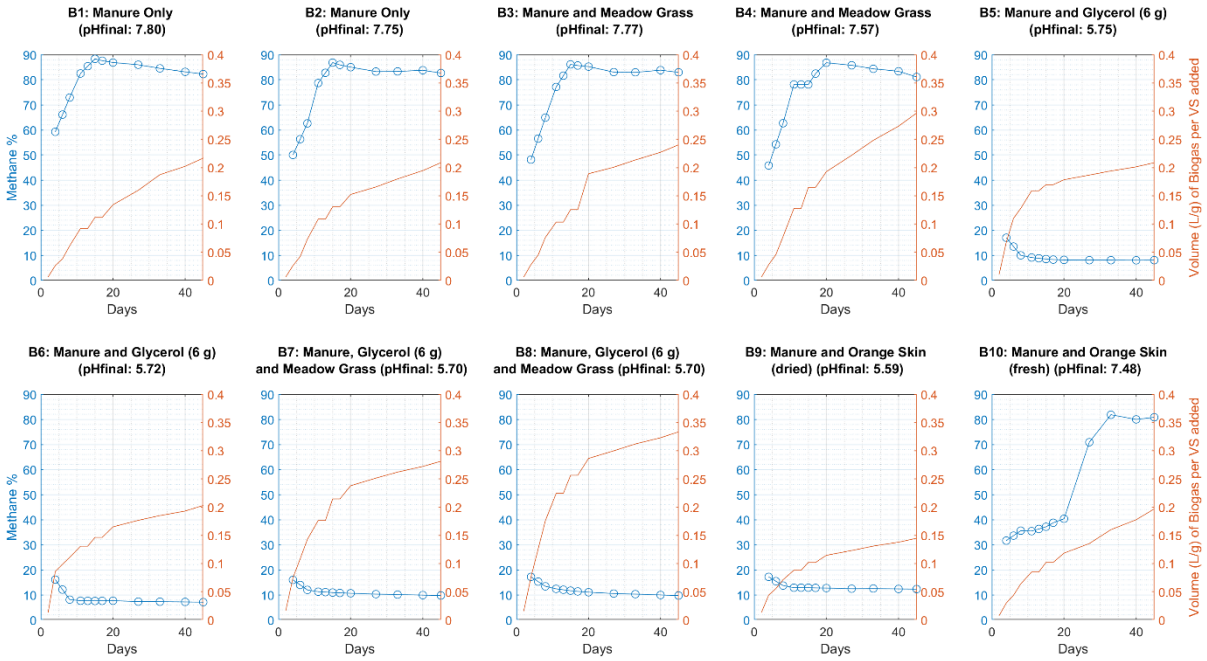


Figure 20: Biogas quality in terms of methane proportion (indicated in blue, circled) and biogas production volume per quantity of VS added (in orange, line plot). Round 2 of experimentation.

Subplots B1 and B2 show the control samples that were tested. As can be seen, both samples generated approximately 0.22 L/g VS of gas at a high comparative methane proportion in the biogas of 86%. Generally, methane content in biogas varies from 50 to 85%, so the biomethane generated is well within the upper limit of what is possible for the sample (13). After an initial peak in methane proportion at about 12 days into the experiment, the methane remained level at 80% over the course of the batch experiment. The total gas content also decreased with time, as shown by the orange line-plots of subplots B1 and B2.

For subplots B3 and B4, the methane proportion is nearly identical as for B1 and B2 (control samples) graphs. The methane proportion of the produced biogas was initially lower for these samples, starting off at below 50% but eventually reaching proportions in line with B1 and B2 as control. An interpretation as to why the methane proportion steadily increased can be developed from the experimental design. As the nitrogen flush occurs, residual oxygen remains in the sample and is prone to disturb the total methane proportion during gas component measurements. Additionally, further oxygen is present in the lignocellulosic biomass that has been added to the sample. When assessing total biogas production, it becomes clear that mixtures with lignocellulosic biomass outperform mixtures where monodigestion occur. It is important to say that additions of feedstock provide further nutrients for the bacterial consortium, so a higher biogas yield will be caused as more VS have been added. Nevertheless,

through normalising the graphs by dividing the amount of VS added helps in understanding the beneficial effects of co-digestion for these sample mixtures. An improved nutrient balance is therefore expected and helps in the process kinetics defined by the digestion process.

This analysis is also in line with the discoveries of both Xie et al. (2011) and Abouelenien et al. (2014), who have independently analysed the behaviour of pH values on AD generation systems, indicating that through an improved nutrient balance, either VFA or ammonia inhibition are very unlikely and the bacteria are under ideal conditions in a stable system, favouring the high production of biogas being rich in biomethane (159,193). This is also supported by previous analysis where an improved C/N ratio through AcD with manure and lignocellulosic material improve the biomethane yield through optimal AD conditions (13).

Subplots B5 and B6 show the addition of glycerol to manure, whilst subplots B7 and B8 perform a co-digestion of glycerol with both manure and meadow grass. By adding trace quantities of glycerol, or "doping" the substrate sample for improved performance, interesting trends could be identified. At the beginning of the digestion cycle, significantly more biogas was produced, as seen by the high slope in plots B5 to B8 for the first days of batch experimentation. Subsequently, the gas production rate reduces as a result of feedstock depletion in the inoculum. Borja et al. (1995) modelled the kinetic behaviour of biogas generation systems and their analysis can be utilised to explain the glycerol degradation process (194). Through the use of their first-order kinetic model employed, they generate an exponential decaying model that shows a similar behaviour as seen through the glycerol absorption process (194). After approximately 14 days, the majority of glycerol has decayed and virtually has no further role in dictating the digestion behaviour. Moreover, through the addition of glycerol other kinetic processes in the inoculum were adversely affected.

Glycerol, as a major cause, is responsible for the poor methane yield at 10-16% of the total generated biogas, with the rest being majority CO_2. Leoneti et al. (2012) explore that glycerol causes increased AD system instability due to its chemical composition (183). They discuss that the high oxygen content of glycerol may cause aerobic conditions for the bacteria, whilst also significantly increasing C/N ratios of the samples (183). This can be seen from the pH-results that have been placed in the title of each subplot. After 45 days, VFA accumulation had occurred in all samples containing glycerol, with a pH range between 5.59 and 5.75, clearly

being too low for efficient biomethane generation (195). There is reason to believe that the other three production phases, as discussed in Figure 4, continued producing as a continuous gas production was registered. However, methanogenesis requires pH-values between 7.5 and 8.5, with any skews severely affecting its productivity. Because of these findings, the conclusion of higher methane yields through glycerol addition as discussed in Siles et al. (2010) were not confirmed (196). However, the study conducted by Rodríguez et al. (2006) was verified, which indicated that glycerol has a high biodegradability factor, which is seen by the significant increase in biogas production curve in the first 15 days of experimentation (197). In the same way as Siles et al., Rodríguez et al. also explore the improved co-digestion of manure with glycerol (196,197). This trend was not visible through the experimental round performed, as glycerol addition resulted in system inhibition. To prevent further inhibition, future analyses should reduce the glycerol content for feed mixtures investigated to allow for further exploration of its use as AD feedstock, assessing the biodegradability and its environmental benefit.

Subplots 7 and 8 of Figure 20 explored the AcD of all three main samples analysed: swine manure, meadow grass and glycerol. These samples produced the highest amount of biogas at 0.34 L/g VS, but methane yield was poor overall at only 10% of the total. Assessing the behaviour of subplots 5 and 6, it makes sense that the addition of glycerol in subplots 7 and 8 also reduced overall efficiency. This is likely also caused by VFA inhibition as per the samples 5 and 6, given that the resultant pH ranges are too low for adequate biomethane generation. Tsapekos et al. (2015) (97) performed a co-dogestion of swine manure and meadow grass, where process stability was achieved despite high ammonia concentration due to the dilation with lignocellulosic feedstock, which generally has a lower C/N ratio and thus balances the pH through VFA creation. In this assessment, through additionally subjecting the co-digestion to glycerol, the samples were skewed to a pH where methanogenesis was impossible. As such, it is advised to reduce the initial glycerol content and allow the inoculum to adjust to the highly potent feed, rather than including high quantities of the biodegradable feedstock in from the very beginning. This is however only possible in continuous or semi-continuous analyses, where daily feeding regimes are possible. Ammonia in itself is not toxic for the bacteria, but rather causes a pH increase. If well balanced with VFA, both ammonia and VFA can work together to provide a stable pH for the bacteria to hydrolyse and generate methane of conventionally

difficult-to-digest lignocellulosic biomass (198). By increasing the amount of lignocellulosic biomass present in a sample that already contains manure substances, a reduced biomass conversion efficiency is seen and biomethanisation occurs at reduced speeds given that reaction kinetics are impeded by the cellulose nature of the biomass, known to be a difficult substrate to hydrolyse (199).

The effectiveness of both fresh and pre-treated orange peel was determined in subplots 9 and 10 of this experiment. In mediterranean countries large amounts of orange peel waste is generated, and it would be interesting to determine whether AD could also be utilised to treat these samples (188). To date, research has been initiated to convert orange peels into biodiesel as a renewable fuel, but research in AD has not been developed yet due to the fact that orange peel contains a substantial amount of limonene, known as a natural antibacterium, which may therefore cause issues in the bacterial kinetics leading to the generation of biomethane. Through comparison of both samples in subplot 9 and 10 it became obvious that the fresh orange peel outperformed the pre-treated sample, both in terms of biogas yield and methane proportion in said biogas. Despite this, both fresh and pre-treated orange peel do not compare well in efficiency to the other samples analysed and fall short of expectations as viable feedstock for AD, given that they produce a mere 35% and 10% methane proportion within the biogas respectively. Gas production was fairly similar across both analysis reactors. The research group among Battista et al. (2020) also analysed the potential of orange peel for AD with a specific focus on limonene, indicating that when limonene is removed from the orange peel through pre-processing, the methane production takes up to twice the time as anticipated (188). This goes against prior belief that limonene acts as an inhibitor and is the reason for low initial methane output (188). In future batch arrays which incorporate orange peel as a feed, it is advised to extend the period of digestion to account for the slower reaction kinetics of pre-treated orange peel.

Summarising the commentary conducted on the second experimentational round, co-digestion mixtures could be successfully tested with the mixture of swine manure and meadow grass producing the most biomethane, with biogas production at 0.3 L/g VS added at a concentration of 86% biomethane. This is an increase of 33.4% compared to the control sample at 0.22 L biogas at a concentration of 86% biomethane. The worst mixture assessed was pre-treated orange peel (even though glycerol additions were not far off) with 0.15 L/g biogas at

12.7% biomethane concentration, 84% less than the control sample. As stated by Abouelenien et al. (2014), major variations in AD efficiencies are caused by different lignocellulosic biomass being added, containing differing properties that can have both positive and negative effects on subsequent digestions (159). The addition of glycerol has been disappointing, which is why experimental round 3 has been dedicated to further explore the improvement of glycerol co-digestion through reducing concentrations and finding an optimum.

The third experimental round focussed on generating biomethane through AcD of the previously unsuccessful samples with glycerol. To prevent experimental failure through VFA inhibition, glycerol concentrations were lowered to 2 and 1 g in all batch reactors, which is a 66% and 83% decrease of previous glycerol additions in round 2 with the intention of effectively reducing the starting C/N ratio of the feedstock to reduce the likelihood of VFA inhibition. Figure 21 summarises the experimental results of all reactors in subplot format, where subplots B7 to B10 portray the results of glycerol co-digestion. Control samples and equivalent co-digestion samples without glycerol were also added in reactors B1 to B6 for experimental validity and statistical accuracy.

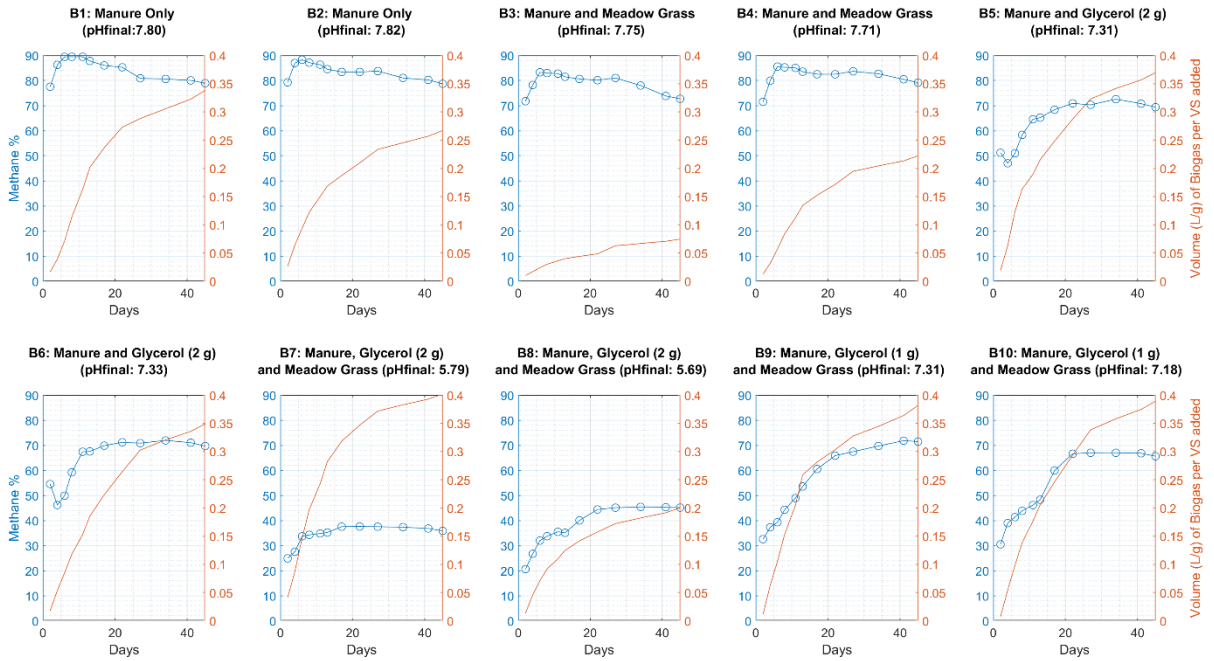


Figure 21: Biogas quality in terms of methane proportion (indicated in blue, circled) and biogas production volume per quantity of VS added (in orange, line plot). Round 3 of experimentation.

Based on the experimental results obtained, it was found that the co-digestion of manure and glycerol was successful but did not yield extraordinary results. This can be seen from a biomethane concentration within the biogas of 70% in plots B5 and B6. The same glycerol content, subject to co-digestion with meadow grass additionally, did not perform well and also caused VFA inhibition at pH-values within the range of 5.69 to 5.79. Similarly, biomethane content in the biogas was comparatively low at 35%. From a total biogas production volume, no difference could be identified between the reactors B5 and B7, indicating that only the methane proportion was majorly affected by the inhibition. A gas leak is likely to have occurred in the batch reactor of B8 given the comparative low production, given that the repeated experimentation on the same septa is not advised and may cause permanent perforations.

Successful AcD can be communicated through reactors B9 and B10, where the three feedstocks swine manure, meadow grass and glycerol were digested together. With a mixture of 1 g glycerol to 100 mL of manure, an optimum ratio could be concluded to achieve similarly high methane yields without the risk of VFA inhibition of the reactor by having added highly-degradable substrate with a high C/N ratio value. The glycerol co-digestion seen in subplots B9 and B10 yet still underperform in comparison to the control mixtures placed into reactors B1 to B4, where pure manure (B1 and B2) or manure and meadow grass mixtures (B3 and B4) were added. This is especially due to the drop in pH-values seen, which have been added in the subplots' titles. Even though this experiment could verify that stability can be obtained through digesting an inoculum with glycerol as a co-feedstock, the feedstock did not meet the expectations of generating significantly higher methane yields as discussed in other literature and was not the best-performing sample tested in the batch experimentations part of this thesis. As a result, it will not be advised to use glycerol for low-cost, pilot-scale reactors when scaling to larger sizes, given that they may cause system instability. Especially when low technology solutions are sought for rural communities, stable systems without the need of excess monitoring are required for continuous biogas production. In this context, glycerol, which does have the ability to produce high amounts of biogas in a short time frame, do not fit the envisaged feedstock profile.

Lastly, the pH values after opening the batch reactors after their specified time lengths, as indicated in the subplot titles, correlate to the final biomethane proportion that was seen in each sample. Low pH-values were a sign of significantly more VFA accumulation present, at

which point the methanogenic archae would not produce methane as efficiently, or not produce biomethane at all. This principle was also further explored by the work of Chen et al. (2023), who analysed the AD performance of manure-based feedstocks with heavily-basic additions to prevent the inhibition through VFA by simulating an alkaline environment, which is favoured by methanogenic archae (200). Given that no experiments conducted in the three rounds of batch experimentation skewed to the high-pH side, it stands to reason that more care should be taken to prevent VFA accumulation rather than ammonia inhibition.

5.4.2 Thermogravimetric analysis and differential scanning calorimetry results

Initially, the untreated substrate mixtures as used directly for AD had been analysed for TGA and DSC testing. However, because the samples were mostly made of water, over 90% of the mass was lost to moisture evaporation before the temperature reached 150 °C. Due to this, the TGA assessments did not produce meaningful data. Instead, a strategy of drying the samples for a whole day at 105 °C prior to treatment was employed, through which an improved analysis was made possible. Table 11 provides a summary of the parameters determined through this analysis, including the MWLT and the induction temperatures.

Table 11: Induction temperature and MWLTs determined through TGA and DTG evaluation

	T_i (ºC)	MWLT (ºC)	Heating value (J/g)
Bottles 1 & 2	239.5	265.2	2315.9
Bottles 3 & 4	170.1	233.5	1282.6
Bottles 5 & 6	219.0	271.8	1332.3
Bottles 7 & 8	252.7	294.6	2485.2
Bottle 10	246.2	303.6	1380.2

Figure 22 (left) provides an overview of the TGA curves generated, including the corresponding derivative thermogravimetry (DTG) profile (right). Based on the results obtained, one can see that samples “Bottles 3&4” and “Bottles 5&6” contain the lowest content of ashes, as the residue after TGA processing was only around 10%. Despite that, “Bottles 1&2” had the highest

residue (almost 50%), indicating a considerable inorganic material content in the sample. No substantial variations exist when comparing samples with moisture content, but samples "Bottles 3&4" and "Bottles 5&6" portray distinct behaviours when the induction temperature is examined, due to their T_i values being lower than those of the other samples evaluated. After analysing DTG curves, distinct differences could also be determined. "Bottles 3&4" and "Bottles 5&6" showed a single peak in the DTG, whilst the other samples contained two peaks. When assessing MWLT, all samples performed as expected with uniform temperatures. This is a clear indication that only one devolatilization step occurs in samples "Bottles 3&4" and "Bottles 5&6", whilst samples "Bottles 1&2", Bottles 7&8", and "Bottle 10" undergo two differentiated processes. Through analysing the control sample, "Bottles 1&2", the resulting curve is similar to the recent research conducted by Wang et al. (2016) and Cuetos et al. (2017) (201,202). The samples with two differentiated peaks indicate that a light volatile degradation occurs prior to a degradation of heavy volatiles. Based on the analysis presented in Figure 22, initial volatilisation of light materials occurs between 200 °C and 350 °C, with the second occurring between 350 °C and 500 °C. The first degradation stage is thereupon involved in a higher mass loss process, which implies that the light volatiles content is greater than the heavy volatiles content.

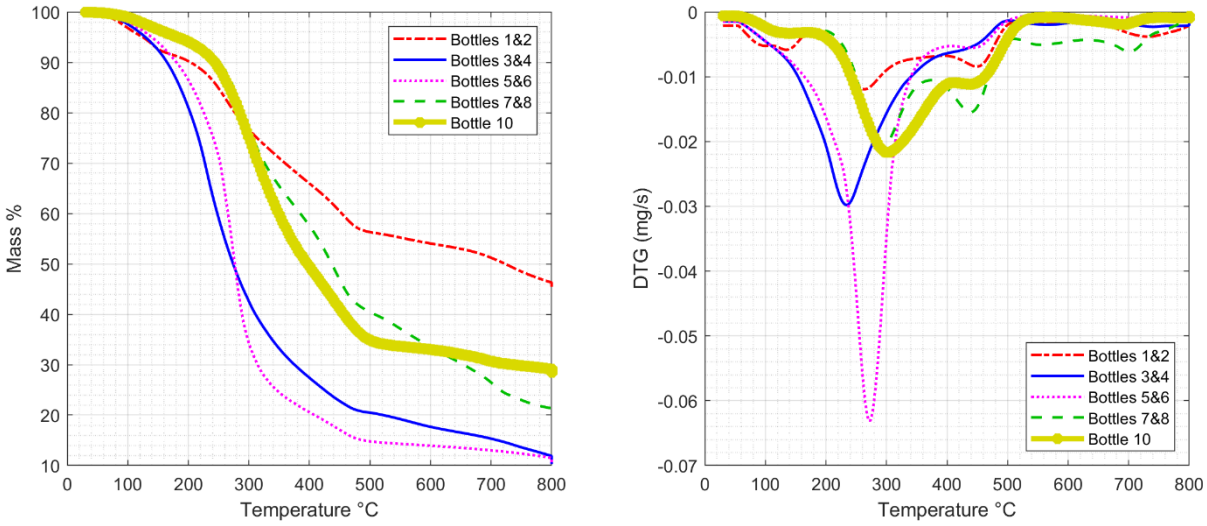


Figure 22: TGA curves for each (dried) sample (left) and its corresponding derivative thermogravimetry (DTG) profile (right)

Figure 23 provides an overview of the DSC analysis (left) and the mass variation in a pure oxygen atmosphere (right). The temperature ranges in which these peaks indicate a fast devolatilization process, which can be assimilated to the high gradients provided in the mass

variation plot. The peaks in each sample are roughly within the same temperature ranges; one peak is found between 200 and 300 °C, but it is not as prominent as the second state, which was determined to be between 400 and 600 °C. Pure swine manure ("Bottles 1&2") generated a notable peak in the first stage, and subsequently, only minor peaks between 400 and 550 °C. This indicates that a highly volatile component, likely to be residue water content present after drying in the manure, which readily dissipates under the heating profile.

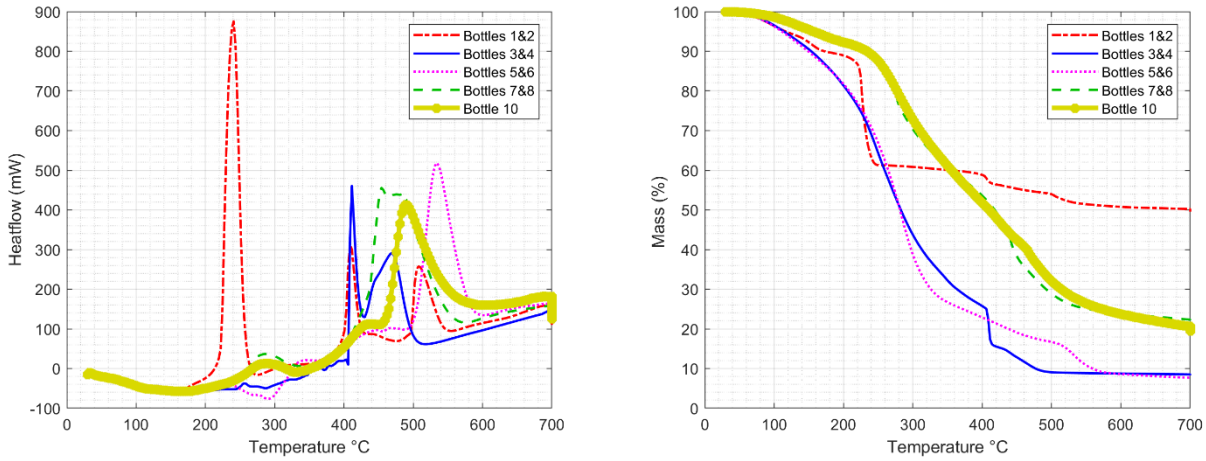


Figure 23: DSC curves (left) and TGA mass variation under oxygen atmosphere (right)

The meadow grass sample ("Bottles 3&4" and "Bottles 5&6") registered a peak at 450 °C, which corresponds with the volatilisation temperature of grass product. Sample "Bottles 5&6", which treated glycerol as a main compound, saw its peak at a temperature of 550 °C. Both of these compounds, however, did not show a significant mass loss, as seen in the corresponding oxygen TGA curves. The results for "Bottles 7&8" exhibit a broader peak. This is because both glycerol and meadow grass were contained within this mixture, which therefore generates a homogeneous, combined peak. "Bottle 10" also has a volatilisation peak between 450 and 500 °C, attaining lower energy than the other samples. After analysing all five substrate mixtures, it becomes evident from the curves that adding a substance to the slurry amplifies the peaks of the second devolatilization phase, given the wider peaks seen from 400 to 550 °C

As was explained in the methodology, the heating value is determined by integrating the peaks from the DSC curves, where results had been published in Table 11. "Bottles 1&2" and "Bottles 7&8" had the highest levels among the samples. "Bottles 1&2" sample's value is the sum of the three peaks, but "Bottles 7&8" sample's high heating value is caused by the width of its peak.

As opposed to “Bottles 1&2” and “Bottles 7&8”, the remaining samples had values that were similar but remarkably lower.

Thermogravimetric analysis is an essential technique for forecasting the behaviour of different biomaterials in AD, providing insightful information for developing and refining small- and medium-sized biogas plants in rural regions. The ability of blends with advantageous decomposition properties, such as low MWLT and high thermal biodegradability, provides the researcher with useful information and insight into biomass combinations that may be worthwhile pursuing. This is based on a material’s potential to generate biogas.

5.5 Experimental conclusions

In the first experimentational round, a comparison between the effectiveness of swine manure and commercial bacterial mixtures is performed. Swine manure provides a continuous biogas production at a stable rate, with high methane yield. In contrast, the commercial inoculant sample requires at least 14 days for acclimatisation, during which barely any methane is generated. After this acclimatisation, very high methane yields are seen at similarly constant biogas ratios. For short-scale experiments, it is advised to use swine manure as inoculant as no activation time is required. For longer experiments, the acclimatisation period may be accounted for given future higher yields of the samples.

In the second experimental round the mixture of meadow grass and manure performed best, outperforming manure only by 34%. Through adding lignocellulosic feedstock, an improved and stable environment was created for the bacterial sample to generate at a high biomethane quality. Glycerol samples all failed due to VFA accumulation, leading to inhibition of the methanogenic archae. The three prior AD-process steps (hydrolysis, acidogenesis, acetogenesis) occurred which can be seen by the high gas production, but methane proportion was very low. Fresh orange skin outperformed pre-treated (limonene-removed) orange skin, despite limonene being known as an antibacterial compound. However, the gas yield overall was not comparable to other lignocellulosic feedstock and the orange skin samples produced less biomethane within the biogas than mixtures of manure and meadow grass. The best performing sample was swine manure mixed with meadow grass, producing 33.5% more

biomethane than the control sample of just swine manure at 0.3 G/g biogas with an average methane content of 86%.

For the third experimental round, glycerol was found to be an effective co-digestion feedstock at 1 g glycerol per 100 g manure added. This is 83% less than the glycerol content added in the second experimental round. Glycerol at 66% less also failed with VFA accumulation. Glycerol only increased biogas production, but biomethane content of this biomethane never reached similar levels as seen with pure manure or manure-meadow grass co-digestion systems. Additionally, glycerol heavily destabilises a system, causing high VFA generation which may lead to pH drops and inhibition due to VFA accumulation. For low-cost digesters without proper monitoring equipment (as the focus of this thesis), it is therefore not advised to further pursue this feedstock for "doping" or improvement purposes, as seen in literature. pH-values are correlated with final biomethane concentration in biogas. No sample in all experiments experienced failure through ammonia inhibition, only VFA inhibition on the low-pH side. This indicates that VFA is a higher risk, and that additional care should be taken to prevent pH reductions due to the feedstock's chemistry. Alkaline additions may be used to properly prevent pH reduction of a sample and maintain efficient process kinetics.

TGA and DCS results concluded that samples "Bottles 3&4" and "Bottles 5&6" showed the lowest ash content, while sample "Bottles 1&2" had the highest, indicating significant the presence of larger quantities of inorganic matter. The TGA and DTG curves highlighted differences in devolatilization processes among the samples, with "Bottles 3&4" and "Bottles 5&6" showing a single peak, suggesting one devolatilization phase, while other samples exhibited two distinct stages, associated with light and heavy volatile degradation. DSC analysis revealed that "Bottles 7&8" and "Bottles 1&2" had the highest heating values, which comes from their broader or multi-peak curves. It could be seen that TGA is a valuable tool in predicting the behaviour of biomass for biogas production, with low MWLT and high thermal biodegradability correlating to higher methane output in anaerobic digestion processes, as seen in the "Bottles 1&2" sample.

6. Experiment semicontinuous

After having conducted various rounds of batch assessments and researched different AcD feedstocks for optimised biological systems, the research group intended to scale batch experiments to larger reactors and change the operation scheme, from batch experimentation (closing the reactors once, and keeping them closed for the course of the experiment) to semi-continuous experimentation (including daily feeding and gas extraction), closely mimicking the operation of actual plants when in operation. Transitioning from 250 mL reactors to larger reactors is necessary to improve understanding of larger biological systems with the intention of gaining experience prior to starting full operations on low-cost pilot-scale plants, with a nominal working volume of up to 600 L. As such, the proximate step included using reactors with a 5 L working volume, representing a 20-fold volume increase. A 5 L working volume was chosen based on the availability of laboratory equipment, as discussed in more detail in chapter 6.2.

This intermediary step, evaluating the efficiency and operations of a 5 L working volume system is crucial to understanding the technical and performance difficulties and intricacies associated with a larger biological system and is the logical stepping stone to subsequently assess a reactor for a pilot-scale. Given that in Chapter 7 a pilot-scale reactor is discussed and successfully operated at a working volume of 100 L, this represents an additional 20-fold increase from the 5-L system. Considering this, it was important to evaluate the performance parameters and operations of larger reactors, still in a laboratory environment, before moving to field-conditions with a pilot-scale reactor.

A further intention of this experiment is to analyse the effects of different feedstock loading with the overarching goal to analyse the behaviour of the biological system when subject to different substrate feeding regimen. An understanding of the optimum loading rates is to be gained, where the bacteria receive their optimum daily feeding in an attempt to maximise the conversion rate of mass of VS to biomethane. With this in mind, an experiment has been devised to assess the efficiency of producing biomethane per grams of VS added at different daily loading rates.

The experimentation summarised here was conducted during the research placement in Bogotá, Colombia, and refers to article (D – page xix) listed in the summary of publications.

6.1 Literature overview of similar analyses

Various studies have been conducted in the field of AD, where different OLRs are examined in an attempt to find either an optimum or an improved operation state for biogas reactors. The following work has taken inspiration by the study of Li et al. (2015), who have investigated the effects of different AcD loading rates in their 40-L reactor under laboratory and mesophilic settings (203). They came to the conclusion that after an OLR of 8 g/L.day, significant compound inhibition along with physical difficulties became apparent and system performance reaches an optimum at an OLR of 8 g/L.day (203). With this in mind, an experiment has been designed to scrutinise their conclusion and validate the optimum OLRs with respect to the methane per mass of waste product that would be generated. Edwidges et al. (2020) have done a similar analysis, but encountered a substantially lower optimum OLR at 3.5 g/L.day, mainly due to the fact that they were not digesting manure-based feedstock but fruit and vegetable wastes, which are more volatile feedstocks given their sugar content and low pH (204).

The research group among Wang et al. (2018) (205) conducted a study on the co-digestion behaviour of fruit and vegetable waste with cow manure, assessing how microorganisms are affected by different loading and mixture profiles under continuous experimentation. It was determined that with an addition proportion of merely 5% of fruit and vegetable waste, the optimum biomethane per mass of VS was generated, whilst also producing the most stable consortium of microbes that had substantial buffering capacities (205). Kaoutar et al. (2020) (206) conducted a mesophilic semi-continuous experiment mixing animal manure and sugar beet waste to improve biomethane production rather than through digesting both samples in isolation. Different OLRs were explored in their research, where OLRs were altered up to the point where inhibition of methanogenic archae began (206). Inhibition started when the HRT was reduced to 5 days, completing their experiment with findings that longer HRTs improve the overall biomethane production as the bacteria is given more opportunity to fully degrade the waste matter present into further substances such as acids, ammonia and ultimately biogas (206). The study conducted by Yu et al. (2023) (207) assimilated the operational characteristics between batch and continuous modes in an AcD of chicken manure and corn stover,

concluding that the highest biomethane yield occurs at a ratio of 2:1 for chicken manure and corn stover respectively. This ratio is associate with up to 28% more methane as the control sample consisting of manure only (207). The continuous experiment performed slightly worse than the batch experimentation under the same conditions and feedstock ratios, producing 4.5% less biomethane due to experimental scaling into continuous operation (207).

6.2 Experimental aims and objectives, structure of trials, overarching research question

Given the previous experimentation (Chapter 5) being performed on batch reactors with the intention of gaining an initial understanding of the operation of biogas reactors and biological systems, the obvious next step included experimental scaling to larger reactors and operating them in a manner closely mimicking the operation on industrial level, by employing semi-continuous feeding and measurement strategies. Various experimental objectives could be defined to support the overall search in operational performance improvements for biogas plants and their application for low-cost, decentralised plants in rural environments. This research focussed primarily on the four semi-automatic reactors located in the Universidad Cooperativa de Colombia, where together with the Universidad de la Sabana and the Universidad EAN, a research placement was held to utilise the state-of-the-art installations present in the laboratories. These state-of-the-art facilities are shown in Figure 24. The primary goal of this research was to improve the efficiency of semi-continuous reactors with the intention of scaling this research to larger, pilot-plant scale reactors. With this in mind, different feeding strategies were tested, being defined as differing OLRs of volatile material entering the reactor as substrate. The biological system present in the bioreactor was analysed and a comparison of the biomethane output per mass of VS added was to be conducted. Through this, efficient feeding modes could subsequently be defined and reactor feeding rates could be assimilated to larger reactors.

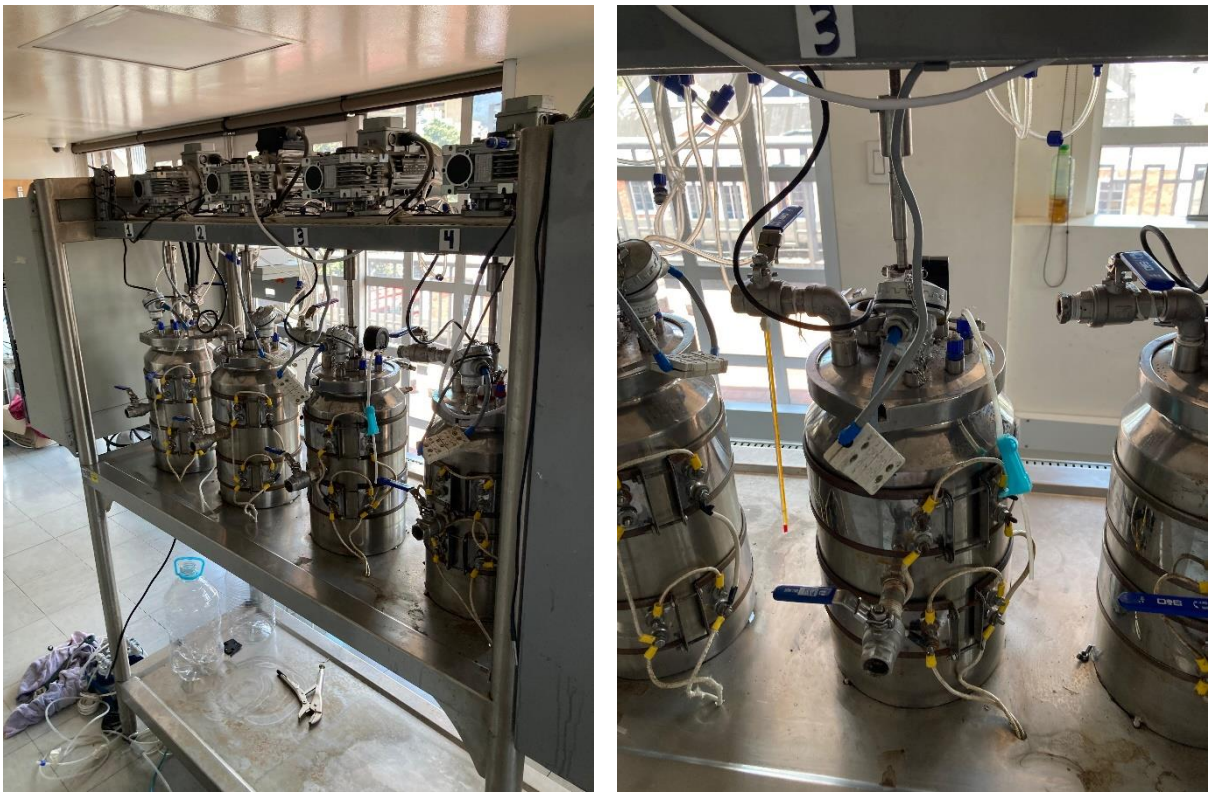


Figure 24: The reactor installation at the Universidad Cooperativa de Colombia, which hold a specialist laboratory dedicated to the research and development of AD and fermentation technologies. Left: whole reactor assembly. Right: detail view of one reactor. Experimentation in Chapter 6 was conducted with these reactors.

Further objectives of this experiment were identified alongside its main aim, which mainly involve the analysis of other operational issues faced with differing loading rates and the analysis of different performance as a result of under- and overloading reactors with varying amounts of feedstock. Considering this, parameters such as Kjeldahl nitrogen content, COD, VS, TS, pH, VFA and alkalinity contents were assessed in line with experimental procedures, including the assessment of biogas in terms of its quantity and richness in calorific output.

The subsequent chapter will explore the experimentation conducted, including its experimental results and conclusions. Following from this, pilot-scale experimentation will commence in Chapter 7.

The research question, of this experimental phase, has been coined as follows: *What is the ideal organic loading rate of substrate for a biological system for it to produce the highest amount of biomethane per mass of volatile matter fed to it?*

6.3 Methodology and experimental setting

6.3.1 Feedstock sourcing

Swine manure was sourced from a nearby farm in Mosquera, Cundinamarca, Colombia, where the university has a campus and conducts research ranging from agricultural techniques to farming yields. Swine manure was chosen as the basis of this experiment due to its stability and previously discussed advantages experienced during the BMP batch tests in Chapter 5. To build on this, swine manure has a known C/N ratio and other physio-chemical characteristics that were previously employed, thus lending itself for the experimental trials conducted subsequently at a larger scale. Additional inoculum was taken as a starter sample to accelerate the biogas generation process and was provided by a wastewater treatment plant from Chía, Cundinamarca, specialised in treating cheese whey and manure mixtures. This bacterial mixture has already been accustomed to treating manure mixtures and was thus chosen to support the fast digestion of feedstock. Table 12 provides an overview of the feedstock used for experimentation, including its physiochemical characterisation.

Table 12: Feedstock characterisation summary

Material	Total Solids (TS)	Volatile Solids (VS)	Ashes	Water Content	Chemical Oxygen Demand (COD) (mg/L)	Volatile Fatty Acids (VFA) (g/L)	Alkalinity (g $CaCO_3$/L)
Inoculum	5.24 %	4.59%	0.65%	89.52%	919	5.2	5.8
Pig Manure	30.33 %	23.58%	6.62%	39.47	13555	4.8	4.8
Distilled Water	0	0	0	100%	0	0	0

The swine manure used for reactor feeding was mixed in specific ratios with distilled water such that a VS-proportion of 4.5% was uniformly present within the mixture. Based on previous literature analysed, a VS-content of 4.5% provides a stable amount of nutrients for bacteria, whilst avoiding any physical difficulties that may be provoked through SS-AD such as tube blockages or excessive foaming. To begin with homogeneous conditions among all reactors, the same mixture content was added to each reactor on day 0 of experimentation. Each reactor was loaded with two litres of wastewater-bacteria mixture, along with an additional two litres of swine manure-distilled water mixture, totalling to a working volume of four litres in each

reactor. Swine manure was added in the first day to give the bacterial sample feedstock to begin the digestion process for the first day of experimentation the following day. The working volume of four litres represents 80% of the capacity of the reactor. This was maintained throughout the experiment, leaving the remaining 20% of the volume for biogas accumulation and storage.

6.3.2 Experimental design

The experimental setup is shown in Figure 25. Figure 24 in Chapter 6.2 showed actual pictures of the experimental setup. The laboratory installations included four semi-automated CSTR-reactors that have been specifically designed for all kinds of biogas and dark fermentation investigation. Different temperature profiles, process kinetics and substrate additions can be examined in this experimental set-up due to its unique design and wide versatility. The set-up, as indicated in Figure 25, includes a heating system that allows for temperature control. In this experiment, the mesophilic temperature range was chosen. Additional stirring motors and blades are attached which allow for uniform mixing of reactor digestate. The reactors are equipped with different valves and openings for feeding, biogas extraction, digestate extraction, pH or temperature monitoring, or acid/base addition for pH regulation. Two control panels are also installed to log temperature and pH profiles and automate heating and pH regulation amongst the reactors. A communication platform acts as a SCADA overlay system providing real-time information on various properties measured inside the reactors, which can be accessed through any internet connection device. A motor control unit has also been installed to implement different stirring profiles.

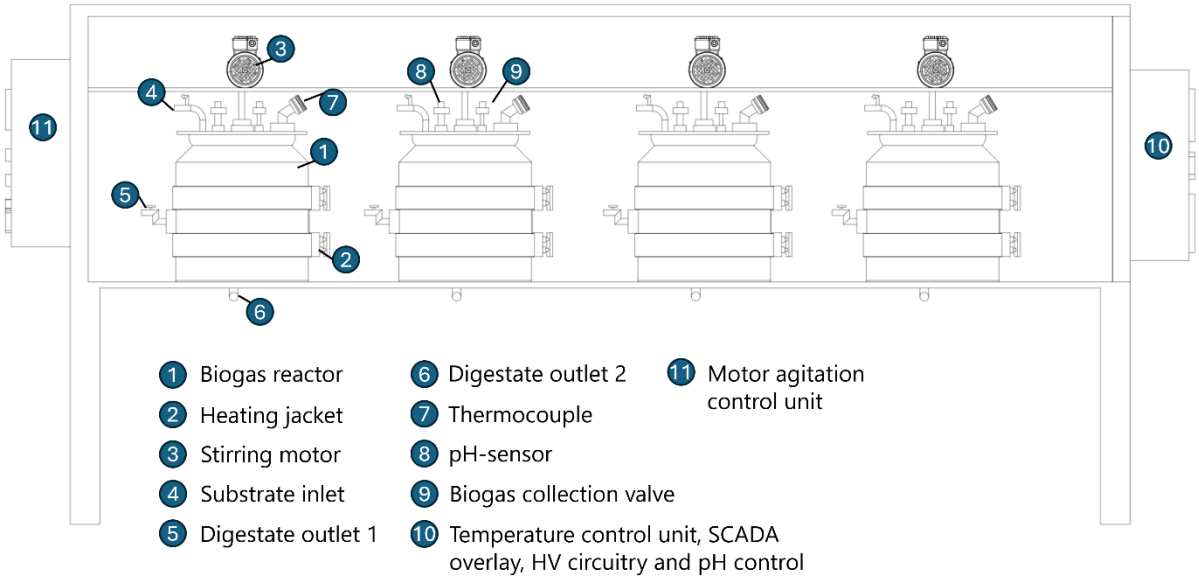


Figure 25: Laboratory overview, with four semi-automatic CSTRs specifically designed for AD

As seen in Figure 25, a biogas collection valve is installed at point #9 of the diagram. This valve remains closed until the daily biogas measurement, where the stored biogas is collected and passed through overpressure (originating from the reactor) into a Tedlar ® bag. The Tedlar bag is then used for daily gas analysis. Digestate samples and substrate feeding was performed through digestate outlet 1, labelled as point #5 in Figure 25. Point #5 was used to minimise introduction of air, and thus oxygen into the reactor.

The experiment was designed to operate for 45 days, given that this is a standard in semi-continuous experiments according to literature. Daily feeding, digestate sampling and biogas characterisation was performed. A different feeding strategy was employed in each reactor with the intention of assessing the effect of OLRs on the biological system. As such, reactors 1, 2, 3, and 4 were loaded daily with an OLR of 5, 7, 9, and 11 g VS/L.day during the 45 days of the experiment.

6.3.3 Analytical methods

Measurements were be divided into two groups: daily and weekly. Daily measurements focussed on characterising the biogas yield, biomethane content in the produced biogas, and pH measurement. Biogas yield was measured through a water displacement, as seen in Figure 26 (left). The water displacement method involved connecting the reactor gas outlet to a manometer, which was filled with water. Through the pressure difference, water was displaced, allowing for volume (in mL) to be read from the manometer. Conversion to STP volume was

accounted for. The produced biogas was then redirected into a Tedlar ® bag for subsequent biogas composition analysis through a special three-way valve, as seen in Figure 26 (right).

Figure 26: Water displacement apparatus (left) and three-way valve (right)

Biogas composition was measured using the Landtec biogas 5000 analysis device, which employs both electrochemical and infrared sensors to determine oxygen, carbon dioxide and methane proportions of the biogas (208). The Landtec biogas 5000 measurement device is shown in Figure 27.

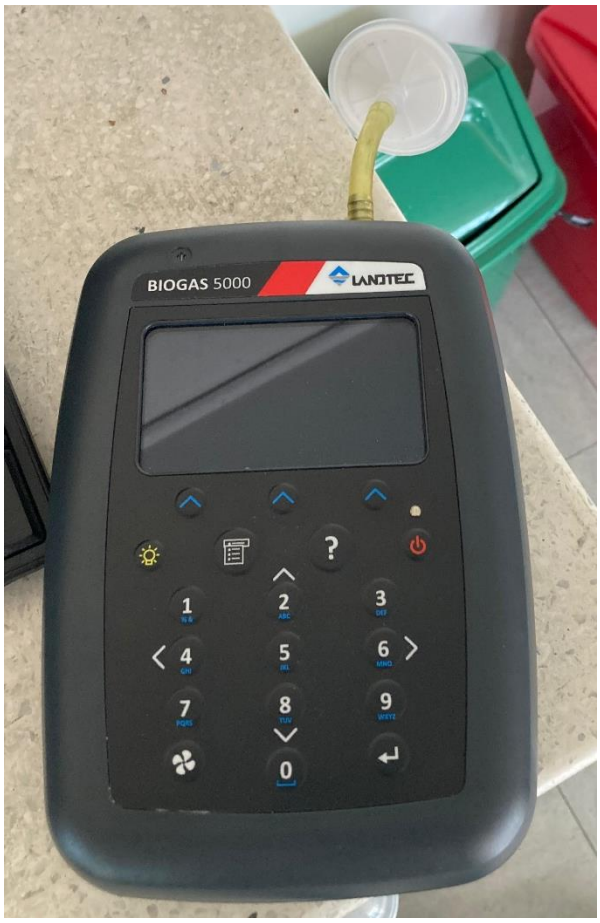


Figure 27: Biogas composition analysis using the Landtec Biogas 5000

Reactor pH was also measured through daily digestate sampling and substrate addition processes. For this, a pH-meter by Hannah Instruments was used, as seen in Figure 28.

Figure 28: A sample pH-meter used for pH sampling of digestate (Image credits: Hannah Instruments)

Digestate physical and chemical characteristics were analysed on a weekly basis to a) demonstrate the consequences of longer-term deterioration, b) analyse the most critical

performance parameters of AD, and c) provide information on the health of the bacterial community contained within the sample. When pH samples were taken, the rest of the digestate at the beginning of each week was stored and later analysed on COD, VFA, TS, VS, ash content, water content, and alkalinity. Standard methods were used to determine COD, VFA, Kjeldahl nitrogen and alkalinity (190). Chemical characterisation on solids and moisture content was conducted under ASTM standards (209). Each analysis was performed in triplicate and separately for each of the four continuously running reactors. Specific protocols following ASTM and APHA standards were devised and adhered to, which can be viewed in Appendix 11.7 for further review and replication.

6.4 Results and Discussion

6.4.1 Data collection analysis

Figure 29 summarises the biomethane percentage and biogas production of all four reactors in a subplot format. As seen in the figure, the concentration of biomethane within the biogas has been mostly constant during the experiment. Biomethane concentration has been recorded at a steady 65-70% for reactors 1 and 2 over the experimental period of 45 days. Whilst reactors 3 and 4 showed a similar profile in biomethane concentrations at the beginning of experimentation, the overall concentration prevalent towards the end of the experiment dropped substantially, especially when comparing reactors 3 and 4 to reactors 1 and 2. This is especially evident when analysing the reduction seen in the blue curves with "+"-symbols of Figure 29. As will be showed later in the experimental discussion, this drop in concentration coincides with a reduction in pH-value, which is attributed to an accumulation of VFA within the reactors. High loading rates, as the ones analysed in this experiment, are prone to occur especially in reactors 3 and 4. In the study by Li et al. (2015) (203), the maximum permissible OLR before VFA accumulation and pH drops was 8 g VS/L.day, which would validate their results with the trends seen within this investigation. The mesophilic, semi-continuous experiments conducted by their research group determined that after an OLR of 8 g VS/L.day considerable chemical and physical problems become apparent that destabilise the AD system,

after having evaluated the effects of various OLRs from 3.0 to 12.0 g VS/L.day on inoculum systems (203).

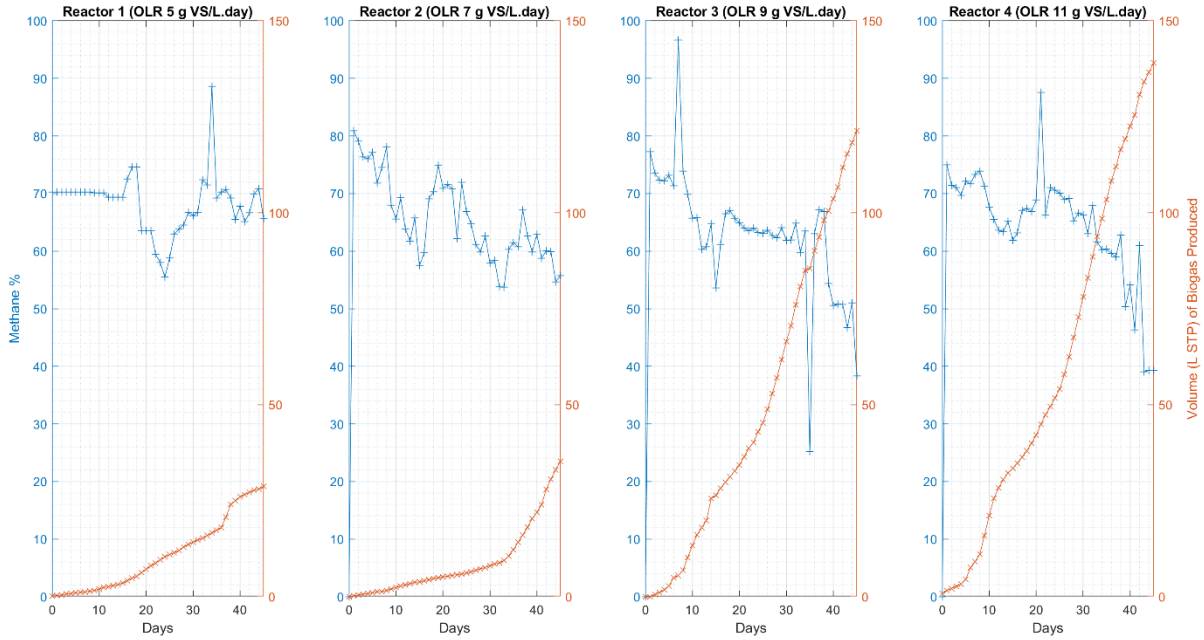


Figure 29: Biomethane proportion (left, blue, "+") and biogas production (right, orange, "x") throughout the experimental length

When analysing the biogas production, visible in orange in Figure 29, one can see that both reactors 1 and 2 have produced a very similar amount of biogas, as they have been loaded below the optimal OLR of 8 g VS/L.day. A large jump occurred to reactors 3 and 4, subject to 9 and 11 g VS/L.day respectively. It is worth discussing and highlighting this stark jump, given that it occurred at a production threshold occurring for AD systems between 7 g VS/L.day and 9 g VS/L.day, as was performed between reactors 2 and 3 respectively. The difference in gas production is notable, with 95 L of gas at STP conditions. Given that both systems deviate from the optimum (based on literature at 8 g VS/L.day for manure mixtures) by only 1 g VS/L.day, it becomes evident that it makes more sense to overload a reactor rather than underloading to allow for the high gas production seen in this analysis (203). Based on Figure 29, reactor 3 stands out as being the most efficient reactor when considering the amount of mass of VS that has been added to the system. Given that reactor 3 has received 9 g of VS/L.day as opposed to 11 g of VS/L.day in reactor 4, and the fact that it has generated biogas at a volume comparable to reactor 4 suggests that the biomethane content per mass of VS is ideal in this system.

The change in pH (magenta, right, "x") compared to the COD (red, left, "+") is summarised in Figure 30 in subplot format for each OLR assessed. As can be seen by the COD curves, the oxygen demand grew in the digestate of reactor 1, as during the experimental procedure more wastewater was replaced by COD-rich manure in the reactor, causing the COD to increase. In reactor 1, the COD is steadily kept within the range of 5000 and 10,000 mg/L, which indicates that process stability will have occurred in this system. In contrast, the COD-values of the other reactors investigated, in reactors 2 to 4, are not a constant value but rather an increasing property as the experiment progresses. This is also attributed to the feeding rate, but rather than no digesting equilibrium can be reached: as the experiment progresses and by conducting visual inspections of the digestates of reactors 2 to 4, it becomes clear than the substrate added to the reactors had not received sufficient time to fully digest and degrade into biofuels. This phenomenon is seen across reactors 2 to 4, where the COD-values are substantially increasing and at very high values. Photometry was used to determine the COD-value of samples. In certain cases, the digestate had a COD-value too high and was too dense for the photometry device to give accurate readings, especially when measuring on samples 3 and 4. The error "*measurement out of range*" was common, after which a value of 15,000 mg/L was plotted for continuity purposes. It is expected that the actual COD of these samples to be within the range of 15,000 to 30,000 for both reactors 3 and 4, as a trend can be seen that increasing the OLR of the reactors also increases the digestate COD. To conclude, it was presumed that from all reactors, especially for reactors 2 and 4, large amounts of feedstock were not ultimately digested and substantial amounts of calorific value were left unused during the experiment. This feedstock, with minimal digestion, then left the reactor in form of digestate and remained with a high COD-value. Whilst in reactor 1 the COD-values also increased, a steady COD-range could be observed after the transient increase, indicating that for this reactor, the feedstock did have sufficient time to degrade in the reactor under the conditions prescribed by the experimental design.

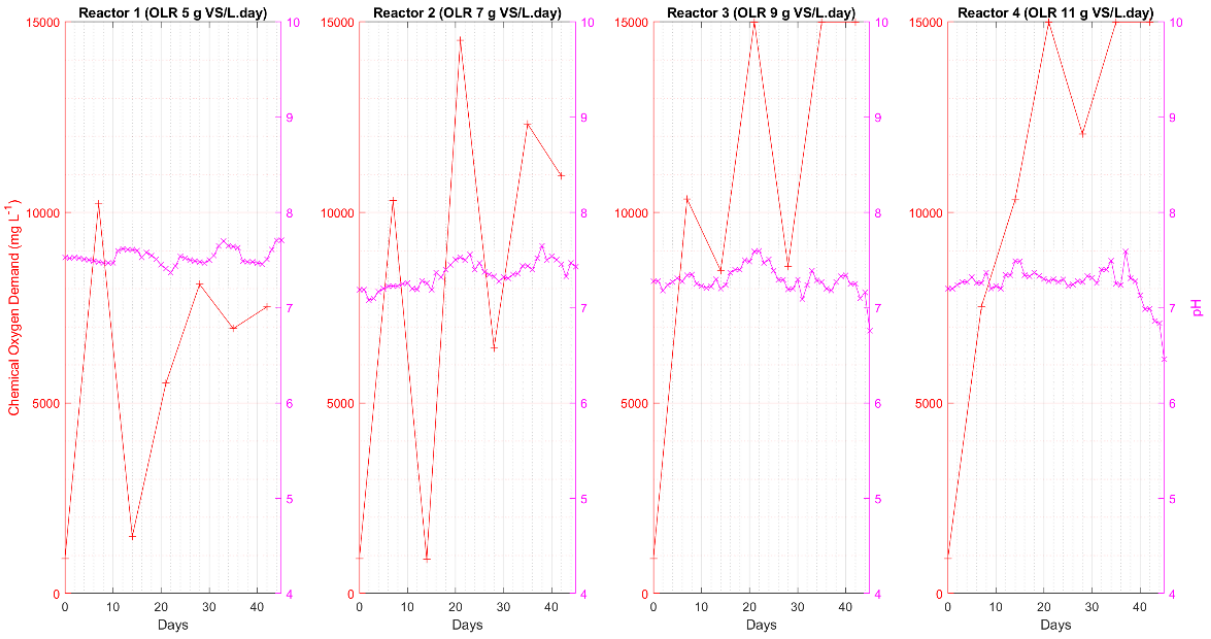


Figure 30: COD (left, red, "+") and pH (right, magenta, "x") throughout the experimental length

Based on Figure 30 and its COD-curves one can see that reactor 1 is most efficient in degrading feedstock. This is mainly because a lower volume of material has been fed into the reactor, effectively increasing the HRT of the substrate in the reactor. It makes sense that a high reduction efficiency is therefore seen in this reactor. When analysing pH-values in Figure 30, interesting trends can also be concluded. Whilst mostly stable variations are present in reactors 1 and 2, there has been a notable downward trend for pH in reactors 3 and 4. These reductions in pH coincide with the reduction of methane proportion in the biogas as well, leading to deduct that the reduced pH has been responsible for suboptimal conditions for the anaerobic archae. The methanogenesis process is known for being very pH-sensible, so slight deviations from ideal pHs in the range of 7-8.5 will cause inefficiencies (159).

Edwiges et al. (2020) (204) discuss the effect of pH variations in anaerobic systems. In their study, they argue that pH deviations are caused from VFA generation, accumulation and degradation in the biological system (204). When VFA accumulated, they reduce the pH of the inoculum, thus impacting methane generation kinetics and inhibiting the sample by preventing these archae of properly degrading the generated acids into biomethane (204). Other studies, as conducted by Jiang et al. (2012) (210)and Alvarez and Lidén (2008) (211) proved that low-pH digestate causes system inhibition due to VFA, which leads to no further biomethane production. Llamas Borrajo (1982) (103) also describes this issue in his work, indicating that a feedstock with a high nitrogen content was used for digestion (103). This meant that the

addition of sewage sludge or other ammonia-heavy add-in was not required to provide reactor stability.

Figure 31 shows the behaviour of VFA and alkalinity in the samples tested. As seen in the graphic, reactors 1 and 2 follow the same trend and have stable VFA contents between 3 and 7 g/L within their digestate. Alkalinity, similarly, is also constant throughout the course of the experiment and varies at a lower concentration between 1 and 4 g $CaCO_3$/L for both reactors

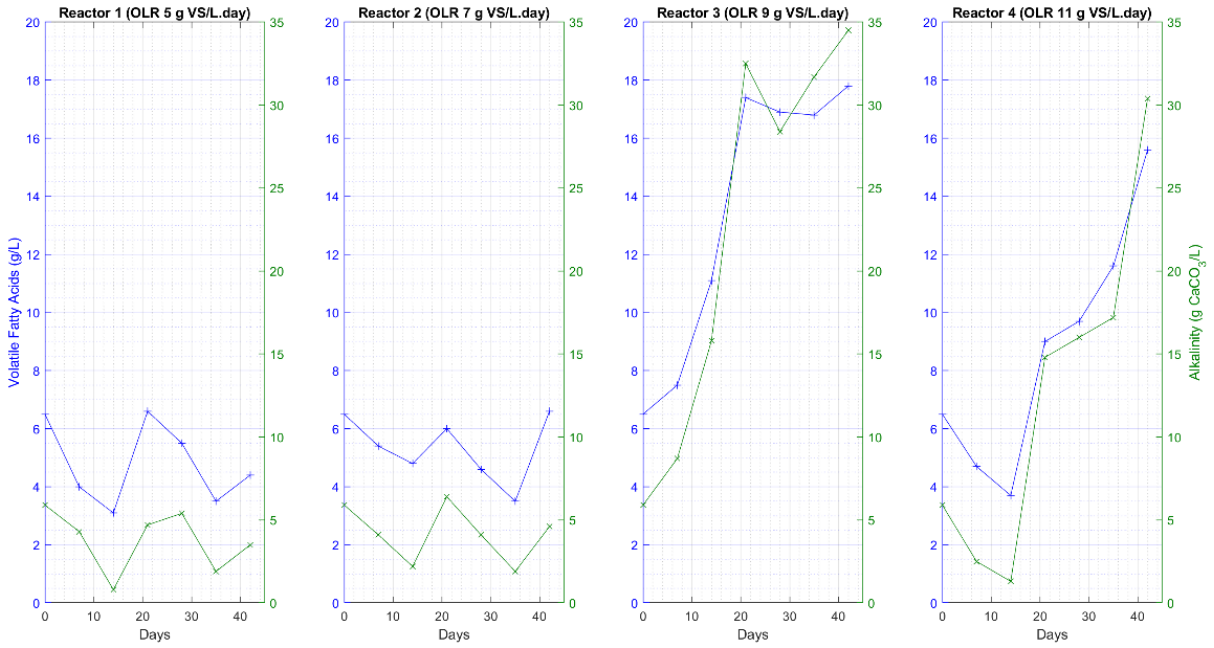


Figure 31: VFA (left, blue, "+") and alkalinity (right, green, "x") throughout the experimental length

Reactors 3 and 4, on the other hand, saw an increase in the concentration of both alkalinity and VFA throughout the course of experimentation. The amount of VFA that has been registered is likely to have caused inhibition due to acidic accumulation, halting further methane production by the archae. Given the trend identified in Figure 30 with the reduction of pH-values in reactors 3 and 4, it is likely that the pH reduction would persist to the point where VFA accumulation is confirmed. This is especially caused by the high OLRs present in both reactors and the inability of bacterial consortia to adapt sufficiently quickly to the high feeding strategy, thus leading to higher alkalinity and VFA values as seen in Figure 31.

During the initial phase of experimentation, the VFA and alkalinity behaviour was uniform across all reactors. As the experiment progressed and the reactors were subject to higher OLRs, an accumulation of VFAs and a stark increase in alkalinity was visible in both reactors 3 and 4, as compared to reactors 1 and 2. This is most likely to have been the reason that system

efficiency had decreased, to the point where less relative methane was produced in reactors 3 and 4 as compared to reactors 1 and 2. Edwiges et al (2020) (204) has studied a similar phenomenon in their experiment, where an inhibition due to VFA increase was recorded at OLRs of 3.5 g VS/L.day. These system instabilities occurred at an OLR far lower than postulated in this experiment and in literature overall, with the main experimental distinction being that a different feedstock was used during their AD trials (204). Edwiges et al. (2020) (204) utilised fruit and vegetable waste as a substrate during their experiment, which causes higher system parameter fluctuations due to the augmented sugar content and low pH of the substrate mixture. With this fact in mind, it is evident that a biogas system is more likely to fail from VFA accumulation at lower OLRs as a result of the initial feedstock properties. In comparison, swine manure has a neutral pH and provides substantially more system stability. Other studies such as Shen et al. (2013) (212) along with Wang et al. (2014) (212,213) have also discovered that a VFA accumulation is likely to occur from substrate chemical compositions blocking acetogenic bacteria to further degrade propionic and acetic acids, a necessary chain-reaction element prior to methanogenesis.

The research conducted by Rico et al. (2015) concluded that high alkalinity values is a prime indicator of higher methane content within the generated biogas (214). This is due to a higher concentration of the compounds H_2CO_3, HCO_3^- and CO_3^{-2} submerged aqueously within the water, which helps bind dissolved CO_2, thus effectively reducing the CO_2 in the generated biogas (214). This trend was observed in their experiment for OLRs between 2 and 4 g VS/L.day, however was not identified in a similar manner within this experiment (214). Despite high alkalinities present in reactors 3 and 4, along with lower pHs, no improved methane concentrations could be registered.

Figure 32 illustrates the VS and TS content of digestate leaving the four reactors during the experiment. From a physical characterisation perspective, values for VS and TS did not fluctuate or increase to the degree that other performance parameters have increased, with both VS and TS remaining within the range of 1 and 3% of the total mass throughout the course of the experiment. Volatile matter within the substrate, which was intended to be converted into gaseous compounds, acted as expected. As to be seen by the initial peaks, a steady optimum could be found after the initial transient period. Given that a high COD-value remained present within the reactors, it became clear that further amounts of volatile matter had not received

the opportunity yet to be fully converted to biogas, and full energetic waste recovery had not occurred. Estimating from the graphs, it can be assumed that only between 50-70% of volatile matter could be removed. Additionally, TS content remained within the digestible broth as well, as seen in Figure 32.

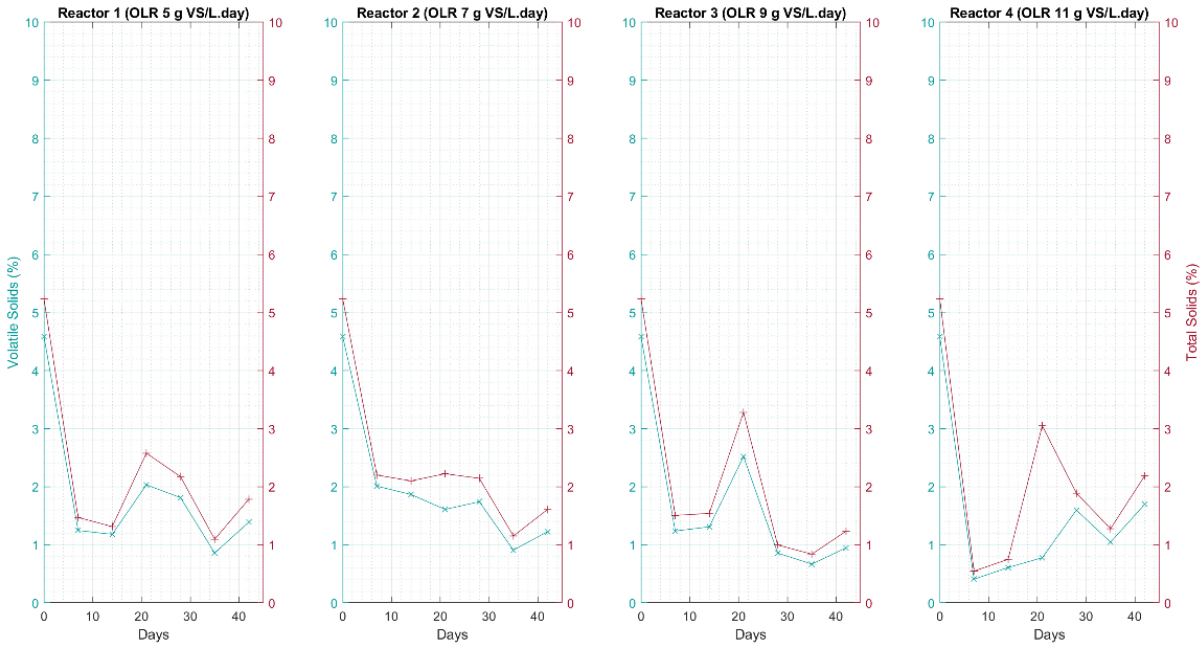


Figure 32: VS (left, light-blue, "+") and TS (right, dark-red, "x") throughout the experimental length

The research group among Theaker et al. (2021) also analysed the effect of different loading rates on biomethane production and performance (215). Their most interesting conclusion included a statement that different feeding rates can be employed depending on the demand of biogas and biomethane, and the fuel supply should be coupled more closely to the demand profile (215). In their continuous experimental setup with two AD reactors, they never experienced overloading and saw stable VS and TS contents throughout the experimental trials between 2.6% and 3.9% at the highest OLD (215). When comparing reactors, it is worth stating that the higher loading profiles generated 14% more specific biomethane than the control system. They concluded that this higher production is linked to a higher VS reduction proportion (215). A likewise behaviour was recorded in the experiment conducted here, especially when analysing the curves visible in Figure 32 and Figure 33, where it can be identified that the mean TS and VS content is reduced to a higher degree in reactors 3 and 4 than in reactors 1 and 2. Despite these conclusions, Theaker et al. (2021) (215) highlight that improper sample digestion and thereupon not fully exploiting the digestate's calorific content can cause further damage to ecosystems by subsequently digesting uncontrollably, with these

emissions contributing to the GHG-effect. This energetic value should, ideally, be utilised and both experiments (Theaker et al. and the one presented here) have not fully utilised the feed to its highest potential.

Figure 33 illustrates calculated (processed) experimental results when assessing the daily biomethane yield and the mass-specific biomethane yield and was mainly derived from Figure 29. Figure 33 is especially helpful to contrast the biomethane generation efficiency, of each OLR system, as the different subplots easily allow for comparison.

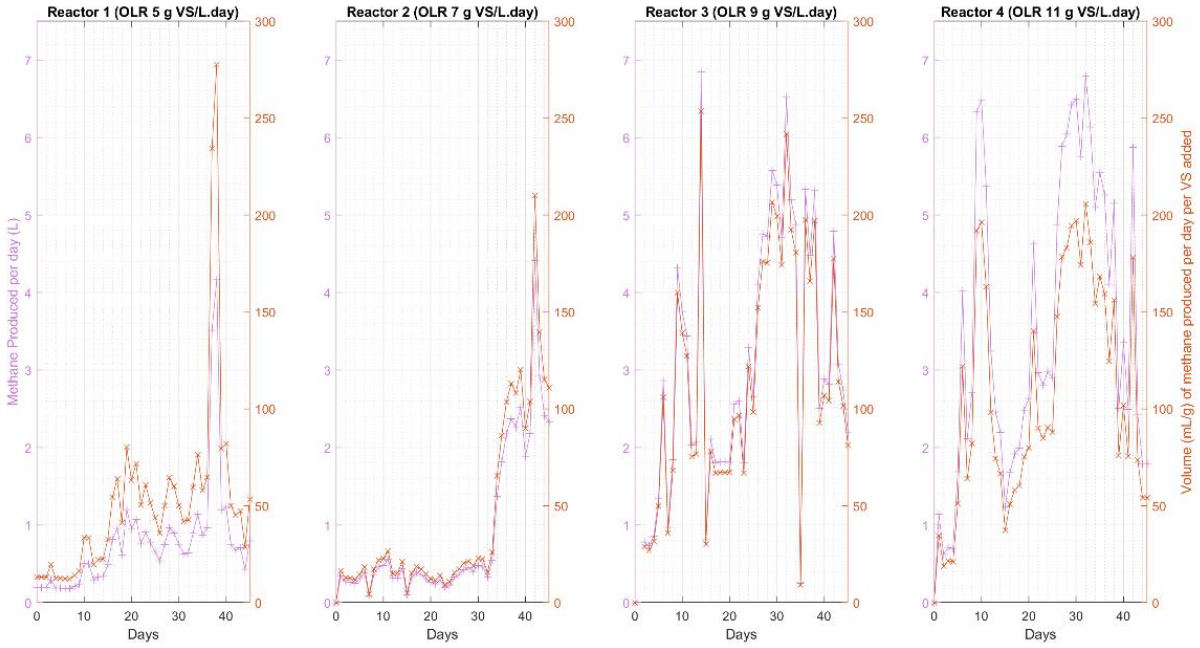


Figure 33: Daily methane output (left, pink, "+") and biomethane generated per VS added (right, orange, "x") throughout the experimental length

The biomethane generation profiles were very similar for the first two reactors and the reactors 3 and 4. Despite reactors 3 and 4 producing a lower proportion of biomethane in their biogas in the last few days of experimentation, likely as a result of acidification, they both outperformed reactors 1 and 2 in total biomethane production. Especially the shift in production between reactors 2 and 3 is worth highlighting, which can be interpreted as a consequence of optimisation-driven digestion protocols.

The area under the curve gives an indication of the total biomethane that has been produced per VS, and is the crucial factor that should be compared across the four subplots. Figure 33 shows that reactors 3 and 4 follow this behaviour by producing substantially larger biomethane amounts than reactors 1 and 2. A review of the literature indicated that for the substrate utilised

at a given TS content added in this system, an OLR of 8 g VS/L.day would be ideal for efficient biomethane production given a limited biomass available. The difference identified between reactor 2 at 7 g VS/L.day and reactor 3 at 9 g VS/L.day is stark, and it can be clearly seen that surpassing the optimal OLR has been advantageous for the biological system under the experimental conditions investigated here (203). Further conclusions of this experiment and especially Figure 33 include that higher methane conversion efficiencies were prevalent past the optimum OLR point, rather than before the OLR. By testing reactor 3 at 9 g VS/L.day at exactly 1 g higher than the perceived optimal, substantially more biomethane could be extracted than when working at 7 g VS/L.day, or exactly 1 g VS/L.day lower than the optimal. This statement also implies that if there are no waste supply issues, then it is recommended to exploit high OLRs and thereupon generate more renewable fuel per mass of added material, as reactors 3 and 4 both significantly outperformed reactors 1 and 2 in biomethane production. Both reactors 1 and 4 were 3 g VS/L.day in OLR away from the optimal, but the production differences are drastic, and do not follow a standard hyperbolic behaviour. The generation of biomethane in the reactors has exceeded the expectations when regarding the optimum, and it is advised to exploit these conclusions by operating at high OLRs.

Despite the advantages discussed thus far with high OLRs, there are risks and challenges evident as a result of acidification. Especially when high OLRs are employed, it is very likely that reactor overloading occurs as a result of hydrolytic microbes having faster process kinetics than methanogenic archae. When operating low-cost anaerobic digesters, with the intention of autonomous operation without any remote surveillance or chemical parameter analysis, subjecting the system to unstable conditions to produce more biomethane may be very risky. Any deviations in pH and other unstable behaviour should be abated as much as possible, and given that instabilities became evident when surpassing an OLR of 8 g VS/L.day, it is advised to maintain this OLR as an upper limit to guarantee safe and stable operations. Beyond this OLR, pH and other chemical property fluctuations are likely, which cannot be worked against in a rural setting with a low-cost anaerobic digester.

6.4.2 Results before versus after

Table 13 provides an overview of how the main parameters perform prior and after AD when subject to the four OLRs examined. It can be determined from this partially raw and processed data that reactor 3 performed best out of all four samples. This is because the highest fraction

in VS reduction was seen in this reactor, which is a good indicator that feedstock volatilisation and conversion to biogas (thus, full anaerobic degradation) has taken place. When comparing reduction efficiencies in terms of VS, it is important to mention that after surpassing the most efficient sample at 9 g VS/L.day, the efficiency reduced substantially and a major decline was recorded in reactor 4, where only 63% of the total VS were reduced. This is the worst factor out of all four reactors tested. Reactors 1 and 2 both outperformed reactor 4, but lagged behind the good degradation performance registered in reactor 3.

Table 13: Pre- and post-analysis of key parameters investigated

Property	Reactor 1	Reactor 2	Reactor 3	Reactor 4
OLR (g/L.d)	5	7	9	11
pH initial	7.53	7.24	7.28	7.2
pH final	7.71	7.65	6.76	6.46
Total Biogas Produced (L)	28.7	31.4	121.4	139.0
% Biomethane	67.80%	66.53%	60.20%	62.94%
COD Initial (mg/L)	13555	13555	13555	13555
COD Final (mg/L)	7524	10959	>15000	>15000
VS Initial (%) (g/L)	4.59%	4.59%	4.59%	4.59%
VS Final (%) (g/L)	1.39%	1.23%	0.95%	1.70%
VFA Initial	5.2	5.2	5.2	5.2
VFA Final	4.4	6.6	17.8	15.6
Alkalinity Initial ($CaCO_3$/L)	5.8	5.8	5.8	5.8
Alkalinity Final ($CaCO_3$/L)	3.5	4.6	34.5	30.4
TS Initial	5.24%	5.24%	5.24%	5.24%
TS Final	1.79%	1.61%	1.23%	2.19%
% Reduction VS (%)	69.7%	73.2%	79.3%	63.0%
% Reduction COD (%)	44%	19%	Out of range	Out of range
Biomethane/OLR	3.89	2.98	8.12	7.96
Biomethane/Total VS added (L/g)	0.0216	0.0166	0.0451	0.0442

When analysing CODs, it is important to highlight that the reactors 3 and 4 performed under expectations and it was confirmed that a high amount of calorific potential was left unutilised by these reactors. The digestate was still very rich in swine manure and full digestion has not occurred based on final COD values being "*out of range*" for the measurement device. The final COD of reactor 1 was the lowest, which is in line with expectations given that the HRT of this reactor was the longest out of all four reactors and OLRs tested. The substrate had more time to fully degrade and be processed by microbes in reactor 1, causing this COD to be the lowest

out of all systems analysed. Based on this behaviour, it was determined that it is probable that the total % reduction in COD is less than 10% for both reactors 3 and 4.

After evaluating the specific biomethane production per OLR of VS added to the reactors, surpassing the optimal OLR at 8 g VS/L.day can yield improved results rather than to underload a reactor. The reactors with OLRs at 9 and 11 g VS/L.day have produced substantially more biomethane per mass of VS than the reactors operating under the defined optimum. This implies that reactors should be fully loaded and charged with high amounts of feedstock to exploit this feature, if possible. An inherent risk brought upon by the high loading is bacterial washout, and reactor acidification. Bacterial washout was not seen, and is unlikely given that manure is added, which itself has a stable consortium of bacteria present capable of performing the AD process chain. Partial acidification was recorded in both reactors 3 and 4, where the pH-values dropped from 7.2 to 6.76 and 6.46 respectively. If this acidification can be circumvented or worked against by active monitoring or addition of basic substances, it is recommended to operate at high OLRs in this specific scenario.

In terms of biomethane yield per VS added at different OLRs, reactor 3 showed optimal conditions at highest overall yield. Conclusions of this experiment are consistent with the findings of a different study performed by Li et al. (2015) (203), where it was found that the optimal OLR for AD was 8 g VS/L.day. After having already discussed partially in section 6.4.1, the pH variation seen in reactors 3 and 4 poses a significant risk to sustained operation. In order to reduce the risk seen by VFA accumulation, which would be seen through a pH reduction, it is advised to control reactor loading to a maximum such that the acidification potential is limited to what the bacterial consortia can actively handle. As such, when effective monitoring and corrective actions are not within the scope of the low-cost pilot scale reactor, either through technological or financial limitations, an OLR of 7 g VS/L.day should not be exceeded.

6.4.3 Operational challenges encountered

Through completing this experiment, different operational drawbacks and challenges were discovered that are worth discussing. Due to high loading rates experienced, reactor 4 (with highest OLR) experienced an explosion of an auxiliary gas measurement tube as a result of the

overpressure experienced. Upon opening and inspecting the reactor, it was found that there was significant bubble and foam buildup within the digestate broth, leading to the overpressure that ultimately caused the explosion.

To build on this, the research group concluded that it was impossible to achieve nutrient and bacterial washout with swine manure being used as a feedstock, given that all necessary nutrients and microbes are prevalent within the substrate. Furthermore, acidification was also difficult because swine manure has buffer characteristics which prevent the skew of pH-values to high or low bounds. This is why, previously, swine manure was used immediately as an inoculant in experimentation, whilst in this experiment it was utilised as a feedstock. Feeding occurred with a highly balanced substrate, having excellent buffer capacities and already containing a stable consortium capable of performing AD by itself, to itself. Although an indication of inhibition was registered in reactors 3 and 4, where a pH drop did occur, inhibition was not fully verified during the 45 days of experimental procedure. It can however be identified that the decreases in methane conversion efficiency are correlated to the pH drops when overlaying pH and methane proportion graphs.

A lot of calorific content within the digestate was left unutilised, which could be seen though daily sample analysis and weekly COD assessments. This is a direct consequence of having loaded the reactor with large daily volumes and volatile masses, totalling more than 0.5 L of feedstock on a daily basis on average. Large amounts of bacteria and microbes were introduced through the swine manure substrate, which were treated prematurely through digestate washout before proper digestion could occur. To improve future digestion and degradation ratios, it is advised to extend the HRT of the experiment, or by adding less volume on a daily basis to reduce the total flow of material.

On the weekend days of Saturday and Sunday, only a single measurement and feeding cycle was performed. Even though no new substrate was added on Sunday, AD continued without any perturbations brought upon through the changed feeding cycle. Measurements could not detect a day where the measurements had not occurred either. In the event that there was no feeding or measurement on Sunday, for instance, the operating pressure on the following Monday was twice as high, indicating to double the biogas production seen on a subsequent day. This yielded in the conclusion that there was still a large amount of digestible biomass

present in the reactors and the high OLRs juxtaposed with the low HRTs did not allow for full energetic amortisation of the substrate. The total calorific output was therefore not utilised, and highly-digestible feedstock exited the reactor prematurely and would have continued to produce further biogas if given the opportunity in the reactors. Several strategies exist to utilise the residue digestate to a higher degree. For instance, multi-stage AD processes can be employed, where the resultant digestate is stored in a larger tank to allow for continued digestion at a higher HRT (or slower digestion rate). Through this, more carbon can be extracted from the digestate, effectively lowering its COD.

6.5 Experimental conclusions

The conducted experiment evaluated the maximum feeding strategies and their effects on the biological system present in the reactors. As such, the four available AD reactors were subject to semi-continuous mesophilic loading regimes with OLRs at 5, 7 9 and 11 g VS/L.day. From a biomethane production perspective, there was no notable difference between reactors 1 and 2, which have been subject to 5 and 7 g VS/L.day respectively. When assessing the biogas and biomethane production proportion, both reactors also acted very similar. In the same way, reactors 3 and 4 also showed similar trends where both reactors produced similarly high quantities of biogas as compared to reactors 1 and 2. The concentration in biomethane in reactors 3 and 4 was overall less than in reactors 1 and 2, which is attributed to the reduction in pH. The pH is likely to have reduced due to an accumulation of VFA, which will slowly cause reactor inhibition due to reactor overloading. Both reactors ended the experimental period with a pH between 6.46 and 6.76, which is lower than the optimum required by methanogenic archae to work efficiently.

The most efficient OLR based on experimental results was concluded to be 9 g VS/L.day, present in reactor 3 of the experimental arrays. This was concluded because this reactor universally performed best when assessing different KPIs, such as the VS highest reduction percentage (at 79.3%), the highest ratio of biomethane per OLR (at 8.12) and the best biomethane yield per accumulative VS substrate fed to the reactor (at 16.77 L/g). Building on this, both reactors 3 and 4 indicate lower averaged VS and TS contents in their digestate, being a clear indication of improved degradability of material. If reactor kinetics allow and

appropriate monitoring is possible, it is advised to exploit high OLRs and subject the bacteria to high feeding regimen. It is more efficient to overload a reactor past its optimum of 8 g VS/L.day than to underload it by the same amount, so it is recommended to comply with this proposal if monitoring of pH, TS/VS and VFA is possible to ensure reactor stability.

Operational challenges must also be highlighted, most notably the tube explosion caused by excessive foaming and subsequent pressure build-up in the reactor treating the highest OLR (11 g VS/L.day). Also worth highlighting is that the digestate samples taken daily from the reactors were still very rich in calorific value, and their high COD-values indicated that further biomethane could have been generated from these samples. In order to do so, the HRT should be adjusted in future experiments.

In low-cost biodigesters which are to be optimised and further studied in this thesis, it is generally not possible to employ precise temperature, pH, or other parameter monitoring or logging due higher R&D costs and associated efforts. As such, if a low-cost pilot-plant reactor is subject to such high OLR, special care must be taken because pH-regulation is not easily feasible. The risk of VFA accumulation should be minimised, therefore it is recommended to not surpass an OLR of 8 g VS/L.day.

7. Experiment scaling to larger reactors

7.1 Hypothesis and experimental setting

This thesis began by explaining the European biogas landscape and current legislation that has brought substantial infrastructure development for renewable fuels and renewable energy technologies in general. The tools and key laws were discussed that paved the way for future installation strategies and political roadmaps. Following from this in Chapter 3, the potential for further biogas installation capacities in the EU were identified. It could be derived that there was a need for smaller, decentralised solutions, like power plants smaller than the current industrial scale. As such, the low-cost biodigester was discussed as a potential solution to improve the carbon economy and reduce GHG emissions brought through intensive agriculture practices.

Given that the low-cost pilot plant was identified as a key technology driver for future emissions improvement and self-sufficiency tool, a roadmap was developed to fully evaluate all possible optimisations and knowledge dissemination responsibilities that this technology may incur. In a primary step, a literature analysis was performed together with a statistical analysis with the intention of defining high-performing co-digestion samples and potential modelling trends seen across all feedstocks examined. From a substrate side, key discoveries could be concluded.

In the subsequent analysis, two experimental phases focussed on assessing the operations of biogas plants. On an initial batch scale, various feedstock mixtures were tested for their validity under laboratory conditions and first experiences of AcD could be gained by testing the effectivity of biomethane production through various high-performing samples identified in the literature review. Following from the batch assessment, various performance parameters further analysed the operational performance of biogas reactors through a set of semi-continuous experiments conducted. Here, an understanding of reactor operations was developed, where the feeding requirements of a biological system were investigated and how AD reactors should be operated in the most efficient manner given their performance parameters.

After having researched and found all these optimisation recommendations and experiences through joint experimental and theoretical review, it was important to merge all results

together and apply them to a final experiment, where a low-cost pilot plant for popular use on farms is used in a decentralised manner. Given that ultimately this technology is envisioned to provide a potential solution to the overlying problem in the field of agriculture, this chapter aims at analysing the technical difficulties and experiences seen with operating current commercially-available AD reactors available on the market.

All knowledge from previous analyses was used for this chapter to focus on the operation of a low-cost, pilot-scale reactor, after which the main results, findings and discussions have all been disseminated. This chapter uses experimental conclusions and discussions that are further explored in article (C).

7.2 Literature overview

Different previous studies have been performed where the operational efficiencies of pilot-scale reactors were investigated and compared to different OLRs, smaller-size reactors, AcD improvements and temperature strategies employed. For instance, Goberna et al. (2010) (216) conducted an analysis on the co-digestion of olive mill waste and cattle manure in a 75-L CSTR. They compared the mesophilic and thermophilic behaviour regarding the efficiency of co-digestion of olive mill waste and cattle manure to monodigestion (216). In the mesophilic temperature regime, a 337% methane production increase could be recorded through co-digestion of the two substrates as compared to monodigestion of cattle manure. Comparing the different temperature operations, thermophilic production generated 17.3% more biomethane than the mesophilic regime (216). One of the reactors, digesting olive mill waste in mono-digestion at mesophilic conditions, failed due to VFA accumulation, likely due to a higher copper concentration that was detected in this sample, which was toxic for the methanogenic archae (216).

Giuliano et al. (2013) (166) assessed various performance parameters and feedstock ratios in four 230-L pilot-plants under semi-continuous feeding conditions and installed an external heating system to simulate mesophilic/thermophilic conditions. Two of their reactors were running on different temperature regimen, while the other two reactors on different OLRs (166). Through this, the four reactors were compared based on the efficiency of generating biomethane (166). In various experimental runs, different feedstock were tested, including cow

manure, energy crops and agro-waste mixtures (166). No apparent differences in production yield could be concluded between low and high OLRs. Process stability was present over both OLRs assessed, which were comparatively low at 2 and 4 g VS/L.day (166).

Research by Sun et al. (2013) (176) was similar to the investigation of Giuliano et al. (2013) (166) by analysing the effect of different OLRs for mixtures of food waste and waste activated sludge. A 1600-L CSTR was used under mesophilic conditions at an initial loading rate of 4 g VS/L.day (176). OLR increases took place from 4 to 6 and ultimately to 8 g VS/L.day, without experiencing VFA or ammonia inhibition, thus not experiencing any operational difficulties as discussed in chapter 6 (176). The best-performing OLR was identified as 4 g VS/L.day, where from a degradability standpoint, 58.5% of the COD could be degraded (176). During low-OLR phases (2 g VS/L.day), hydrolysis was the rate-limiting step. In contrast, during high OLRs (8 g VS/L.day), methanogenesis was the rate-limiting step (176).

Further research by Chuenchart et al. (2020) (163) investigated the mono and co-digestion of food waste with chicken manure under thermophilic conditions in an 87-L CSTR (163). Different OLRs in the range of 1 to 4 g VS /L.day were analysed and it was proven that AcD outperformed the monodigestion of feedstock. The best performance was identified to be the highest OLR at 4 g VS/L.day, where the most biomethane was generated and 89.9% more methane was produced as compared to monodigestion (163). Process stability was maintained without any indication of overloading or acidification of the reactor (163). Co-digestion systems allowed for higher loading rates where stability is still maintained. Loading a reactor in monodigestion is not possible to the same degree as when co-digestion is employed, due to the synergistic benefits discussed in chapter 4.3 (163).

7.3 Research questions and objectives

After having analysed the operational performance in both batch scales at 200 mL and laboratory bench tests at 5 L working volumes, the next step was to analyse the performance and replicate experimental procedures on a larger, pilot scale. Therefore, the primary objective of this section and the associated experimental research has been to operate and assess the biomethane production capacities of a low-cost pilot-scale anaerobic digestor. This system is primarily of research interest because biological systems at this scale behave significantly

different and require closer attention to ensure a successful start-up of the reactor. As such, close monitoring is necessary, and the experience gained through previous experimentation is vital to ensure project success.

Further secondary objectives were defined to support experimental design and project scope. With this in mind, different feedstock and inoculum specimen are to be analysed for their performance on a pilot-scale. When starting experimentation and the operations on a pilot-scale reactor, further measurements of performance parameters are also to be taken with the intention of gaining operational experience and understanding of how the biological system reacts to different perturbations and loading profiles throughout the course of experimentation. Lastly, any optimisations are to be identified and studied in further detail, mainly when assessing operations (heating, agitation), BOP (reactor environment, waste processing) and economics (heating and agitation effort, installation costs).

The following chapter will summarise the experimental conclusions gained and briefly discuss the challenges and opportunities that were encountered to fully conclude the optimisation of biogas plants from a feedstock, performance parameter, and process scaling perspective.

The research question, of this experimental phase, has been defined as: *How can a pilot plant be loaded and operated effectively under non-laboratory (e.g. site) conditions?*

7.4 Methodology

7.4.1 Feedstock sourcing

Inocula used for experimentation came from the same sources as already discussed in chapter 5.3.1. Similarly, the food waste used for experimentation follows the same proportions as indicated in Table 6 and originates from the university's cafeteria. For swine manure utilised, the same farm in Soria, Spain was asked to provide the feedstock such that reliable results can be compared against each other. Table 14 provides a more detailed overview of the macronutrient analysis present within the feedstock, which was characterised as per the study conducted in article (C).

Table 14: Feedstock characterisation for pilot-plant experimentation

Waste	Proteins (%)	Carbohydrates (%)	Lipids (%)
French fries, other fried foods	3.28	39.4	11.9
Fish	25.1	0	10.4
Meat, croquettes and nuggets	15.6	19.4	11.6

7.4.2 Experimental design

A low-cost anaerobic bioreactor was sourced from the industrial manufacturer PUXIN with dimensions 120 cm x 96 cm x 96 cm (height x length x width) and is pictured in Figure 34 (184). The total volume of the reactor was 1.2 m^3, with a maximal working volume capped at 1.0 m^3. The digester parts were assembled based on the construction manual that was provided by the manufacturer. All necessary parts were supplied in three large boxes, which after assembly resulted in a medium-sized digester that could be used by either a large kitchen or family, or also small-scale farms for self-consumption. As seen in Figure 34, the reactor has various parts and subsystems that are necessary for the biological system to function. Fresh substrate is added through a stainless-steel sink with an attached processor that mixes and residue with water and grinds any large particles into a liquid phase. The mechanical processor connects to the digestate bag via a transparent tube, through which the substrate enters the reactor at a height of 50 cm above-ground. The blue digestate, or fermentation bag is manufactured from a strong, elastic, and durable plastic material which is held in place through an aluminium structure. Lastly, the aluminium structure is covered with an additional translucent plastic sheet to prevent sunlight from degrading or excessive heating of the blue plastic fermentation bag. It also protects against any other environmental impacts, such as sharp objects penetrating and heating capture.

The fermentation reactor has three openings: one for the substrate addition, one for the digestate exit, and one opening for gas extraction. The digestate exit opening is located at the opposite side of the substrate addition entry and located at ground level (to ensure that all digestate can exit when the reactor is cleaned. The digestate exit also serves as a convenient location for digestate sampling to conduct subsequent characterisations.

Figure 34: The Biodigester PUXIN

The third, and last outlet of the fermentation bag is intended for gas extraction. This outlet is located on top of the fermentation bag, where a rubber pipe can be inserted and fixed to the reactor for gas extraction, analysis and connection to home appliances and heating equipment. After the pipe has been inserted into the fermentation bag, the gas is directed through an external circuit for cleaning and improvement processes, which can be seen in Figure 35. This external gas circuit consists of a manometer for gas pressure readings, a dehumidification chamber, a desulfurization column, a gas pump, a further manometer for gas readings, along with two valves (at the beginning and the end of the circuity). The intention of this gas circuit is to improve the quality of the biogas by extracting any humidity and by scrubbing the sulphur out of the biogas prior to combustion, given that sulphur can lead to toxic oxides. After the gas circuitry, any appliance such as a gas stove or a heating unit can be connected to the exit tube for gas combustion and consumption. For the experimental purposes and biogas analysis, no further appliance was added as biogas quality was measured at this point. All parts of the auxiliary gas circuit and a starter inoculum, including a stove burner for kitchen use, were also supplied by the manufacturer PUXIN (184).

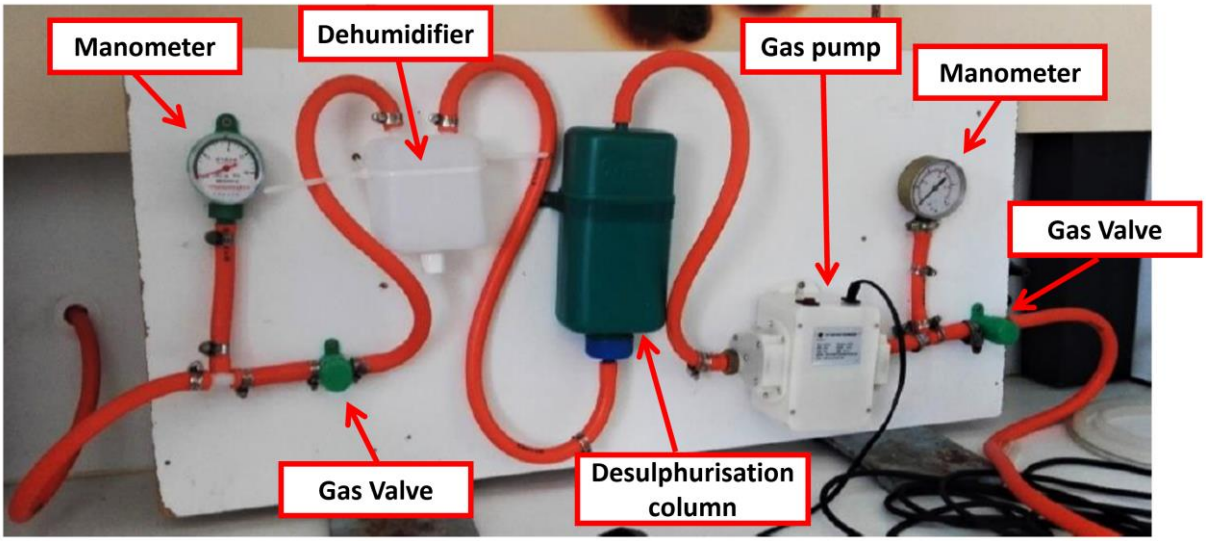


Figure 35: The supplementary gas circuit components.

In total, four experimental trials were performed with the pilot-scale reactor. In the first two experimental trials a mixture of kitchen waste and the commercial starter-inoculum were used given that a conclusion of the batch-experimentation was that for larger systems, the inoculum will produce methane at a higher concentration. The main aim of this experiment was to verify the performance or a low-cost pilot-reactor under larger-scale conditions, so no co-digestion was employed to reduce experimental complexity. Kitchen waste was crushed and mixed with water in a 1:6 ratio. The inoculum was also prepared as per manufacturer's recommendations, with 37 g of bacteria being mixed with a litre of water (184). In total, 500 L of inoculum were mixed with 50 litres of water-feedstock mixture. 10% of the reactor's total volume was utilised given that 200 L of digestate had been introduced into the fermentation bag. This was conducted in order to reduce the cleaning and post-treatment process after the experimental trial had been concluded.

For the subsequent two experimental trials, swine manure was digested in mono-digestion as performed in the continuous-style experimentation discussed in detail in chapter 6. The reactor was transported to the farm in Soria (where swine manure was sourced from) and was subject to environmental conditions instead of in a laboratory setting. 10 L of fresh swine manure were added as substrate at a daily rate, fixing the HRT at 10 days as per recommendations of Llamas Borrajo (1982) (103), who recommended critical HRTs within the range of 10-15 for successful AD. The primary aim of this experimental phase was to produce high-quality biomethane under ambient conditions and validate the effectiveness of the system at conducted AD under non-ideal conditions. With this in mind, trial 3 had no heating strategy was employed, whilst trial 4

did employ a heating method where the digestate was subject to floor-heating for 50% of the time. Experimentation occurred during the Spanish winter months: trial 3 was conducted December to February (with ambient temperatures between 0 °C and 10 °C), and trial 4 started in March (with ambient temperatures between 1 and 13 °C), when temperatures and daylight hours had already increased significantly. Despite the Spanish winter being suboptimal conditions for biomethane production, it was deemed essential that the pilot reactor is tested at the worst conditions. Given that continuous, year-round production is envisaged, the researchers were certain that if biomethane could be generated during the lowest temperatures, the system could withstand higher temperatures as well. Because of this, winter experimentation was preferred.

No chemical pretreatment was performed on either substrate added, as the risk of pH alteration was to be minimised. Physical pretreatment in form of griding and particle size reduction was performed to improve the surface area for bacterial processing.

7.4.3 Analytical methods

Gas composition was determined using the MRU Optima 7 Biogas meter, as seen in Figure 36 (left). This device was specifically designed for field testing of industrial biogas units and employs infrared sensors for CO_2 and CH_4 monitoring, along with electrochemical sensors for O_2 and H_2S recording (217). Digestate pH-values were recorded using the ORION 250A pH meter. The pH meter also contained a built-in thermostat for temperature measurements, as shown in Figure 36 (right).

Figure 36: The MRU Optima 7 biogas characterisation meter (left) and Orion 250A pH measuring device (right).

System pressure was also recorded, both via the installed manometer (left) and through visual inspection of the fermenter inflation volume (right), with sample measuring pictures provided in Figure 37. Daily measurements were performed of all analytical methods discussed in this paragraph. Testing was scheduled for 20 days, given that after this time specific trends in biomethane production become evident.

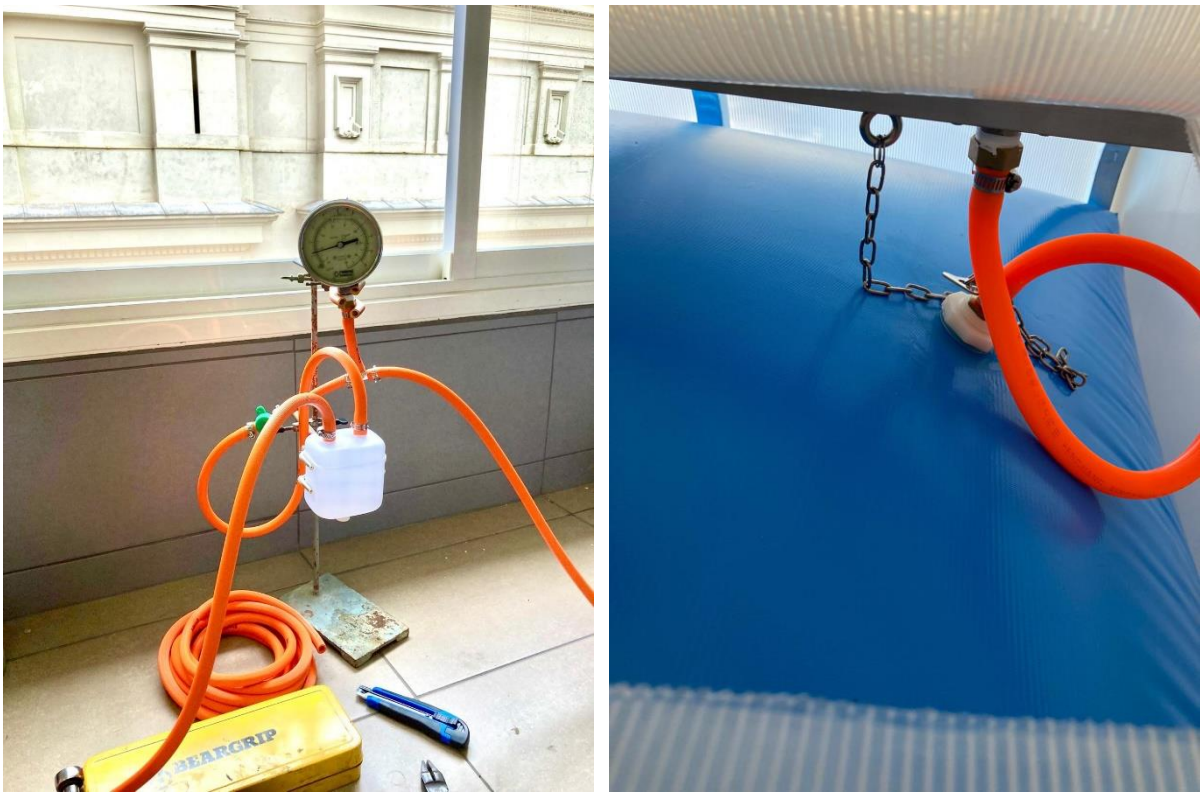


Figure 37: Pressure measurements performed through manometer (left) and via visual inspection of volume generated (right)

Sulphur chromatography measurements for the produced gas, as described in section 5.3.4, were also conducted. This was done to determine the absence of large amounts of hydrogen sulphide in the resultant biogas that was produced and will be explored in more detail in section 7.5.1.

7.5 Results and discussion

7.5.1 Data collection and analysis

The variation of pH and digestate temperature has been plotted in Figure 38. As can be seen in the diagram, the pH remains significantly below the ideal operational pH for methanogenesis to occur. In fact, a similar process to AD called dark fermentation has been registered. Dark fermentation is characterised through similar process steps of anaerobic conversion, but mainly leads to the production of biohydrogen under acidic conditions without the final process step, methanogenesis (218).

From the very beginning, VFA has occurred as can be seen by the very low pH-values. Between days 6 and 12 of both trials there is an increase in pH, but it never approaches the neutrality that is necessary for the growth of methanogenic bacteria and for them to be in a suitable environment to perform methanogenesis.

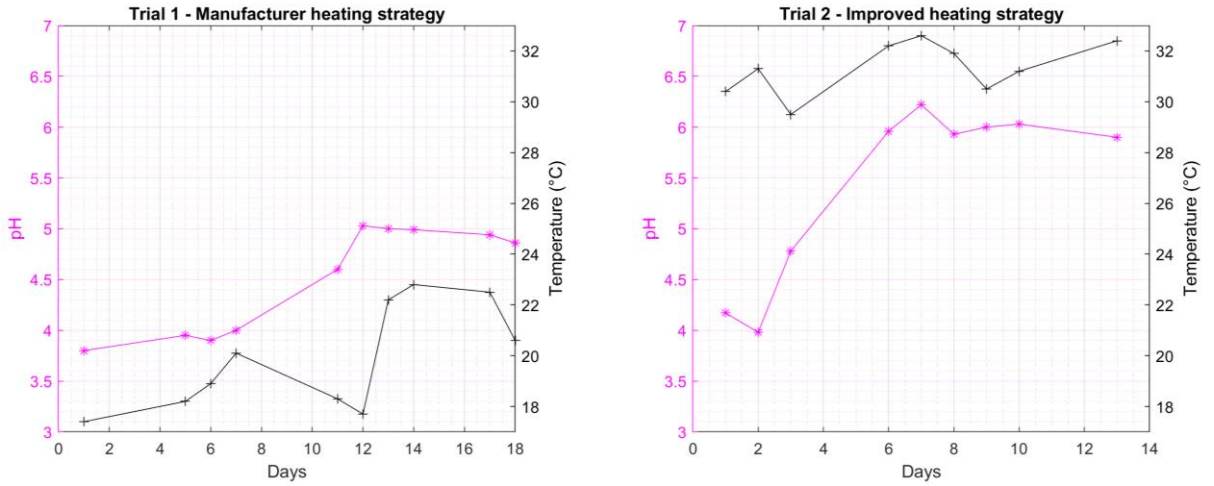


Figure 38: pH (left, magenta, "") and temperature (right, black, "+") profiles over the first days of experimentation for trials 1 and 2 (kitchen waste and commercially available inoculant).*

In both trials, a constant temperature was kept by employing a heating mat at a nominal power of 200 W to heat the digestate from the floor. In trial 1, it was identified that the heating strategy was not sufficient to heat the digestate to the mesophilic range. Therefore, in trial 2 a second heating mat was procured and both mats were utilised for heating the digestate. As can be seen by the parameter behaviour in Figure 38, the experiment was not successful and no major biomethane production was registered. This was also because the substrate particles entering the digestate bag were big at about 25-35 mm in diameter, compared to the optimal substrate size being lower than 20 mm in diameter (219). Therefore, rapid sedimentation took place where the main amount of calorific value of the digestate was unused and was deposited at the bottom of the reactor. Furthermore, the absence of an agitation or stirring device meant that homogeneous mixing was not possible.

Biogas readings indicated than no biomethane had been produced in neither the first, nor the second experimental trial. Measurements of the first trial occurred on day 6, where 53.3% and CO_2 and 2223 ppm of H_2S were recorded. A further reading occurred on day 11, where 62.2% CO_2 and 2100 ppm of H_2S were detected by the biogas sensors. A third reading on day 17 of experimentation yielded identical biogas proportions as on day 11, confirming that

experimental failure had occurred. Given that no methane was registered through the measurements, it was concluded that VFA accumulation and inhibition had occurred, as seen by the low pH. The electrochemical sensor of the MRU optima biogas reader cannot distinguish between hydrogen and hydrogen sulphide measurements, according to the technical support specialists (217). As a result, and with the intention to rule out the high production of hydrogen sulphide, flame photometric detection chromatography was used to verify that only trace amounts of sulphur are contained within the produced biogas. Therefore, the produced hydrogen sulphide was indeed hydrogen, and dark fermentation had ocurred.

Even though the second trial saw an improvement of the pH-values as a result of the augmented heating, no biomethane was registered throughout the experimental procedure either. Results were found to be similar with the first set of proportions indicated for trial 1. In both trials, the fermentation bag did also not reach its maximum volume capacity, indicating that gas production was low overall. The reason why no biomethane was generated is inevitably linked with the low pH present in the digestate, which in turn originates from the feedstock that has been added to the system. Table 14 shows an overview of the feed that has been added, of which the majority is very fatty and rich in lipids. Lipids are generally responsible for VFA accumulation, whilst proteins augment the likelihood of ammonia creation. In this experiment and the mixtures investigated, it was deemed that reactor overloading with fatty material occurred, which in turn resulted in inhibition through VFA and dark fermentation occurring rather than AD (218).

As a reaction to the poor performance of trial 1, trial 2 employed a different feeding strategy. The proportion of fruit and vegetable waste was increased in this trial from 10 to 25% in an attempt to reduce the likelihood of VFA accumulation. As can be seen on the right subplot of Figure 38, the addition of fruits and vegetable waste improved the system pH to a value of approximately 6, which was however still too low for effective methane production. From a temperature perspective, the mesophilic regimen was reached, with the digestate temperature varying between 29.5 and 32.5 °C. The reason for this is that a second heating pad was used after the experiences made in the first experimental trial, and that the digestate could not be heated sufficiently with only the heating pad provided by the manufacturer. The heating algorithm was programmed such that for every two hours, the digestate would be heated,

following two hours of no heating. This was done to reduce the energetic requirements of the system.

Specific difficulties could be identified with the pilot-scale AD system by PUXIN even though, in general, an easy operation was promised and also experienced. When analysing the digester capacity as per the operation manual, the biodigester is expected to produce a daily energetic value of 0.8 kWh worth of biomethane when subject to ideal conditions, such as an optimal pH and efficient loading of 35 kg of organic matter per day (184). Acknowledging that food waste as examined in this study has an average density between 200 and 400 g/L and approximately 75-85% of organic matter is present in the substrate that has been fed at a daily rate, calculations performed expected 0.8 kWh of biomethane are generated on a daily basis. If the whole fermentation bag were to be filled with biomethane, the total calorific potential available would be 11.7 kWh, given that one cubic metre of methane provides 11.7 kWh of energy. As such, when incorporating the theoretical biomethane generation and the fermentation capacity, it would take approximately two weeks for the digester to be fully inflated with gas.

When assuming that of the total biogas produced, 65% is methane (as a conservative average, knowing that methane in biogas ranges between 50 and 75%), only a mere 0.8 kWh/d of biomethane is expected from the biodigester given its current configuration and performance parameters employed. This is juxtaposed with the energy demand necessary for heating purposes, which is attributed to 1 kWh/d (220). No operational budget can therefore justify such high heating requirements to the theoretical yield experienced by the pilot-scale reactors. The strategy of heating in trials 1 and 2 was not sufficiently efficient, and future research and development efforts should focus on improving insulation and reactor design to circumvent the experienced issues related to digestate heating.

A further cost reduction that has been employed was that no stirring mechanism or external agitation has been designed in the low-cost digester purchased (184). Even though the assembly process is made significantly simple through the absence of agitation, the operational drawbacks became evident: high amount of sedimentation and reduced homogeneity of the digestate reduced the reactor's efficiency and overall biogas output.

When assessing the results obtained in the initial two phases, along with the theoretical output expected through Puxin's operation manual, it became obvious that starting operations on this

system were more difficult than initially anticipated. Various life cycle analyses have been performed where the benefits of biogas reactors and AD systems are discussed from a self-sufficiency, but also environmental perspective, reducing the onset of climate change, water eutrophication and soil toxicity. Different research groups have all concluded that effective waste management can produce sufficient biomethane that, at current levels, 64% of the current anthropogenic natural gas use could be displaced with biological technologies like AD (221). This provides the incentive to further research the state-of-the-art technologies and provide recommendations for efficiency improvements in the next generation of low-cost AD that the biogas market will see.

Swine manure was utilised as an inoculant in trials 3 and 4 due to their higher buffer capacity, thus reducing the likelihood of experiencing VFA accumulation and subsequent inhibition again. For both trials 3 and 4, the low-cost bioreactor was placed on site in Soria, Spain for experimentation under ambient conditions. 10 L of swine manure were added daily to the reactor for semi-continuous loading, whilst monitoring gas production and compositions through the aforementioned methods. As already mentioned in the methodology section, trial 3 was conducted during the winter months with environmental conditions between 0 and 5 °C, whereas trial 4 occurred during spring, where temperatures increased to 10 to 15 °C during the days and 5 °C at nighttime. Biomethane composition in the generated biogas has been summarised in Figure 39.

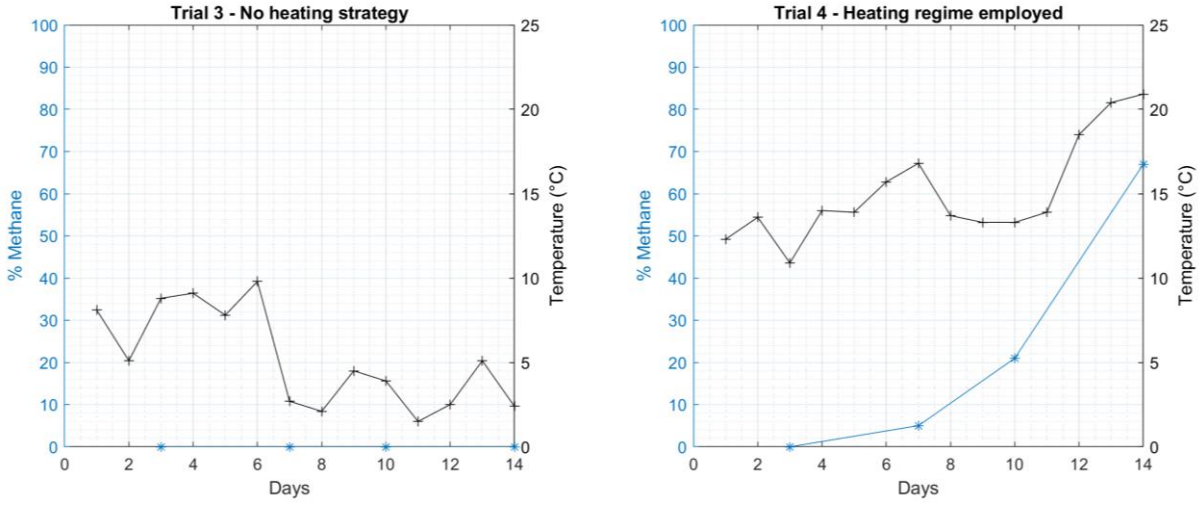


Figure 39: Methane proportion (left, blue, "") and temperature (right, black, "+") profiles over the first days of experimentation for trials 3 and 4 (swine manure).*

Biomethane production in trial 3, as per Figure 39, did not occur. The reason for this is mainly attributed to the low ambient temperatures, where the bacterial kinetics are so slow that no notable gas production could be seen in the fermentation bag. Because no heating mat employed, the bacteria present in the swine-manure based digestate suffered from below 0 °C temperatures at night, rendering effective degradation. Due to this shortcoming, the heating system was added in the fourth trial when temperatures had already begun to increase with the onset of spring, as seen by the black "+" marks in Figure 39. The ambient temperatures were now higher, and the heating system steadily produced heat for the digestate at 200 W.

Heating occurred for two hours, after which the system was turned off for another two hours. Through doing this, the consortia could be effectively animated to degrade the substrate provided and produce biomethane at higher quantities for every day at which the experiment progressed. Trial 4 managed to operate under the psychrophilic temperature regimen, which promises improved bacterial system efficiencies than operating at temperatures around the freezing point. It could therefore be verified that through the support of an auxiliary heating system, the pilot-scale biodigester was capable of producing biomethane in non-laboratory conditions as intended under psychrophilic temperature control. A total biogas yield of 0.559 m^3 was recorded, at an average biomethane concentration of 67%.

It is worth noting that from an energy generation and consumption balance, the AD reactor did not produce sufficient biomethane in its current configuration to justify the thermal investment in form of the heating system that had been installed afterwards. It was determined that the pilot-scale reactor that had been purchased lacked in thermal and operational design to guarantee a positive energy balance in this system, which is why low amounts of biomethane could be generated throughout the experimental trials. It was important to determine the reactor's performance in worst conditions, as this would be the break-scenario of the system, and it can be said with confidence that during spring, autumn and summer months the system will have an improved energetic turnover than what was analysed in these experimental trials. It must be noted that a different pilot-scale system has been subject to identical environmental conditions in the farm in Soria, Spain, and did not experience the same operational difficulties as the domestic bioreactor investigated in this thesis. It is therefore concluded that the design specifics (and not the experimental conditions of scale) of the purchased bioreactor where not

ideal, and further optimisations are required to reach full amortisation of substrate added to the fermenter.

7.5.2 Experimental scaling

It is ultimately interesting to discuss the yields and proportions of methane that were registered in each experimental trial as the scale was increased. As already discussed, batch experimentations assessed working volumes of 200 mL. Semi-continuous experiments then scaled the working volume by a factor of approximately 20, conducting the experiment with a digestate volume of 4 L. Following from this, a further step and 20-fold increase in working volume is conducted to treating 100 L of working volume in the pilot-scale reactor. When analysing these experimental scales, it is vital to discuss how different inefficiencies or challenges that were encountered have reduced the overall biomethane generation that was registered. For this, Table 15 has been generated, which provides an overview of the yields and proportions seen in the biogas throughout experimental scaling.

Table 15: Best results summarised for all experimental scales investigated: batch, semi-continuous, and pilot-scale

	Batch	Semi-continuous	Pilot
% Methane	86.0%	60.2%	67.0%
Total biogas (L STP / VS added)	0.135	0.0749	0.0291
Biomethane generation (L STP/ g VS added)	0.116	0.0451	0.0195

As seen in Table 15, the batch experiments have produced the highest proportion of biomethane in their experimentation and have also been the most efficient from a biomethane generation perspective. This is because the batch reactors, in a purely laboratory setting, were always subject to 37 °C and were agitated twice daily. On semi-continuous level, only 60.2% biomethane could be extracted from the biomethane. On average, the total biogas per mass of VS added has also decreased substantially, resulting in a total biomethane reduction of 61% when moving from batch to semi-continuous experimentation. Lastly, pilot-scale experimentation was even less effective. This is not only attributed to the increase in scale, but also the fact that only a psychrophilic temperature regimen was followed and that no agitation was possible for the pilot-scale reactor. Comparing to the batch and semi-continuous

experiments, the pilot-scale experiment has seen a reduction in biomethane generation per mass of VS of 83% and 57% respectively.

7.6 Experimental conclusions

Concluding from the pilot-scale experimentation and after having investigated the effect of scaling experimental sizes, various conclusions could be devised that will be summarised as follows. In the pilot-scale experiments, trials 1 and 2 had failed. The substrate pH was too low and immediately caused VFA inhibition due to pH drops and VFA accumulation. In fact, dark fermentation occurred rather than AD, characterised by the gas (carbon dioxide and hydrogen gas) production under acidic conditions (218). As a result, trials 3 and 4 utilised swine manure due to its improved buffer capacity.

Further operational difficulties were experienced regarding both heating and agitation. The supplier had provided one heating mat to keep the digestate at mesophilic conditions, but in laboratory settings, no temperature higher than 25 °C could be reached. Out of experimental curiosity, a second heating mat was employed, where digestate temperatures then reached up to 32 °C. When considering agitation and stirring mechanisms, it is important to state that given the low-cost nature of the system, not adding agitation into its design and more complex assembly is a design decision taken to simplify both the manufacture and assembly stages. However, this comes at the cost that the digestate is not homogeneous and operational inefficiencies are more likely, as sedimentation occurs and the bacteria are not as mobile as in stirred or agitated reactors.

Through assessing the heating requirements of the BOP, the energy consumption of heating was higher than the calorific value of the produced biomethane, highlighting the inefficiency of the bioreactor and the requirement for improved insulation and/or process efficiency. Biogas production was proven to be impossible at temperatures under 10 °C, but the pilot plant could successfully be employed at ambient temperatures of 15 °C with the heating system engaged.

An analysis was also conducted where the scaling of the three conducted experiments is assessed from a biomethane proportion and yield viewpoint. Given that no agitation nor the same heating regimen could be followed, the pilot-scale reactor saw a biomethane generation ratio per mass of VS which was 83% and 57% lower as compared to the batch and semi-

continuous experiments respectively. The batch experiments performed the most efficient per amount of added material, at 86% biomethane concentration and a total biomethane generation per mass of VS added of 0.116 L/g VS.

As of current technologies, low-cost AD can provide a solution to many self-sufficiency and environmental issues in the agro-livestock industry. Materials installed in the reactors are functional and meet quality standards, however, specific design flaws such as the lack of insulation, poor heating qualities and the absence of agitation heavily reduce the efficiencies of this system. A higher electrical consumption could be proven than the calorific value of the biomethane created. It must be noted that other systems installed in the farm, under the same scale and environmental conditions, did successfully produce biogas at a high quality with minimal heating requirements. It is thus concluded that low-cost pilot plants of these specifications must be further optimised in future design iterations to become commercially competitive with industrial-scale reactors, especially when incorporating improved heating and agitation systems into the reactors whilst only marginally increasing the CAPEX costs.

8. Big picture: biogas and the future

8.1 Economics

Economics and profitability of biogas reactors are arguably the most important aspect to discuss in order to secure the future of the AD industry and improve climate impacts for future generations. Different characteristics of a business plan must be acknowledged when designing an AD system such that an efficient, fine-tuned and independent enterprise can be constructed around the technology. For instance, characteristics such as feedstock types (and sourcing), treatment techniques, methane yield assessments, supply contracts of energy/fuel, distances/logistics/transportation, power plant design and digestate management should be agreed upon and tested for profitability prior to starting a feasible project (28,54).

Two conventional business models have proved their efficacy under current politic-economic conditions: large-scale centralised power plants that are profitable due to high efficiencies, economies of scale and industrialised technologies, and small-scale "pilot-plant" reactors, that have a comparative low efficiency but operational costs are minimal (222). The industrial-scale biogas plant is often state-owned or under a governmental feed-in-tariff that uses local contractors of the municipality to treat their waste and thus generates an energy carrier that is utilised directly by the local community (through passive heating, energy generation, or methane cleaning and selling). Current infrastructure and appliances are suitable for biomethane and therefore for injection into the gas grid. On the other hand, the small-scale "pilot-plant" is a fraction of the size, significantly more versatile in application, and operates in a decentralised manner (222). Little support schemes such as feed-in-tariffs can be identified for this technology, mainly due to its reduced efficiency. The main objective of this technology is to promote energy independence and self-sufficiency, by using the waste accumulated in large families or in small agricultural enterprises to reduce the transport distances to the larger industrial installations. In this manner, the technology mainly supports individual, smaller scale farms and is a low-technology approach for less-developed communities in treating their waste without additional costs, providing energy, and reducing their carbon footprint. The major benefit of this practice is energy independence and the fuel storage possibilities.

The most common types of reactors currently used for the large-scale industrial applications include Continuous Stirred Tank Reactors (CSTR). CSTRs have been specifically designed to treat

liquid manure and other liquified feedstocks within the range of Total Suspended Solids (TSS) of between 30 and 80 g/L (8). The reason that CSTRs are most popular in industry is because through the operation principles of a CSTR, containing large panels/vanes that are rotating within the reactor, homogeneous mixing can occur and bacteria for degradation can easily spread to all regions of the reactor, thus improving efficiency (8). Additionally, through mixing different feedstock synergies can be exploited. The most well-known sludge retention bioreactor is commonly referred to as the Upflow Anaerobic Sludge Blanket (UASB) reactor, which is mostly used in applications where feedstock wastewater and other light mixtures with less than 3 g/L TSS are digested (8). In UASB reactors, a granular sludge blanket, suspended in the tank, is used to interact with wastewater introduced at the bottom of the tank (223). Through a pressure difference, the wastewater is pushed upward, passing through the granular sludge blanket (223). The blanket remains stationary within the reactor due to a force equilibrium: gravitational pull versus upward flow (drag induced by the flocculants) (223). Further types of common reactor models include membrane reactors, most commonly referred to as Membrane Bio-Reactor (MBR) or Expanded Granular Sludge Blanket (EGSB) reactors (224). These reactors are mainly used in the treatment of wastewater and water upcycling in decontamination plants to produce sanitary, potable water (224). However, these processes generally incur significant costs (224).

In contrast to the well-established industrial-scale AD reactors, small-scale AD reactors are gaining popularity due to their low cost and multiple installation benefits (see chapter 8.5). These types of systems have the primary benefit of providing energy independence from the gas infrastructure and can be used by small-scale farms and other waste sources (e.g. restaurants, large families, etc.). What separates industrial from small-scale units is not only the size: operation and maintenance efforts required for commercially available small-scale reactors are a fraction of the large industrial AD units, and this design decision was taken on purpose. Whilst trained personnel must operate the power plant, any unspecialised person can operate the small-scale reactor without difficulty. These systems, often standing out with their low cost and size between 1-5 m^3, are suitable for small farms or other domestic applications to provide energy for self-consumption in the form of heating and cooking fuel. To build on this, the resulting combustion gases are carbon neutral, and the environmental footprint is considerably reduced by giving the waste products a second use, rather than heating/cooking

with fossil fuels (thus reducing net GHG emissions). To date, it is reported that German livestock farms treat less than 30% of the accumulated manure in AD facilities (225). Upon analysing this major incongruency, it was determined that there is a mismatch between the quantities of manure available and the biogas plant specifications, leading to capacity bottlenecks and thus manure surpluses that cannot be treated effectively or economically (225). This is also an economic issue, as viable feed is not readily degraded due to discrepancies in reactor sizes, feedstock volume, and overall efficiency losses of industrial-scale reactors.

Comparing these issues with low-cost, pilot-scale biodigesters provides an interesting business case for the existing market segment of manure treatment and renewable energy carriers (225). By making use of smaller, decentralised plants, further flexibility is provided to the farmer in treating the manure, as these reactors must not run on full-capacity to be commercially and technologically competitive. To build on this, the produced biomethane boosts self-sufficiency of energy purchases and landowners/communities benefit from these decentralised production methods. One must also report the disadvantages of small-scale systems. For instance, pilot-plant bioreactors have a lower energy-efficiency as compared to industrial units. Additionally, there is a lower energy-efficiency from a balance-of-plant perspective, as the energy investments into the system as a form of heating and agitation are not negligible. Despite this, major cost-savings are to be realised through smaller-scale digestion schemes, as major CAPEX costs and expensive monitoring can be avoided. Moreover, there is a high degree of feedstock availability and the amount of waste material available in smaller quantities, ideal for a smaller reactor, are readily available in the rural setting. This provides a unique selling point for low-cost reactors, which have to date not experienced a market penetration as the industrial units, and thus provide major potential for the further saving of GHG emissions and reduction of energy costs.

When assessing costs for reactor assembly and machinery, various items and engineering stock must be procured to design a functional system. The most basic components generally include pumps, mixing apparatus, fermentation reactors, gas storage units, along with Balance-of-Plant tools and connecting pipes (70). A further major cost of biogas units must be acknowledged if the biogas is desired to be cleaned to biomethane quality, often necessary for commercial selling or applications in cars, industry or storage facilities. If biogas cleaning is desired/required, further cleaning steps must be incorporated into the existing design,

elevating CAPEX-costs significantly. Further items responsible for CO_2 removal, sulphur cleaning and dehumidification must be incorporated into the existing design to adhere to industrial standards and regulations to be considered an analogous fuel to natural gas, or by conforming to standards provided by the combustion industry. Biogas cleaning and refinement to the state of natural gas is generally not feasibly from a financial perspective, given the high competition with natural gas suppliers and the further burden of investments and maintenance required by more complex machinery within reactor plant design (226). Further analysis on the budgeting of low-cost reactors was performed by Amigun and von Blottnitz (2010) (227). They concluded costs of 1427 USD for a 6 m^3 bioreactor including all auxiliary systems and BoP (227). A full breakdown of these budgeting costs have been visualised in Figure 40 (13,227).

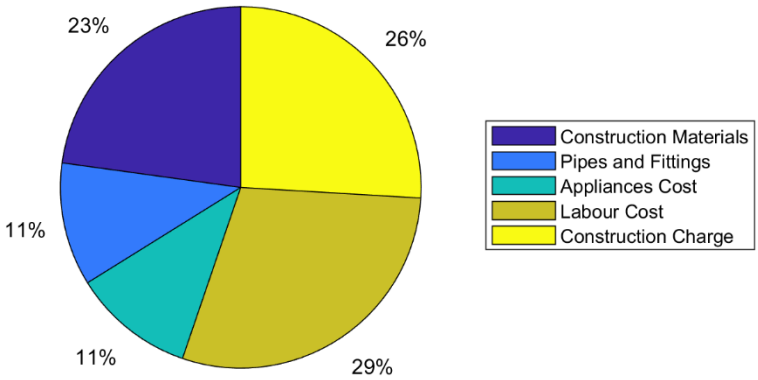


Figure 40: The Proportions of CAPEX-costs, as analysed by Amigun and Blottnitz (2010) (227).

Major improvements in economic viability are proposed by the conclusions of the work of Scheftelowitz and Thrän (2016) (70), who state that the technical effort (such as operation stability) negatively correlates with plant size. They state that investment costs can be estimated at € 3500/kWh_{el} for industrial reactors above the rated power capacity of 1 MW_{el}, whilst smaller units at 50-75 kW_{el} rated power suffer from a higher levelized cost of energy (LCOE) at up to € 9000/kWh_{el} (17,70,228). The trend exemplifies that the smaller the biogas reactor, the higher the specific LCOE of installation. Because of this conclusion on the cost development, their research suggests that reactors starting at 50 kWh_{el} would be economically viable for operation, but smaller reactors would prove difficult to maintain from an economic perspective (70). Reactors of smaller size would to date require the support of external funding sources, such as government subsidies and other initiatives to raise awareness for the promotion of self-consumption of waste to produce biogas on farms or in decentralised infrastructures (70).

The mean size of European agricultural entities was reported by Eurostat to be 16.1 Ha, producing a total biomass that is insufficient to feed an industrial biogas plant at capacity (229). Because of this landscape, O'Connor et al. (2021) argues that smaller AD reactors can fill this market gap and be implemented on a wider scale of farms and communities where the minimum capacity limitations of industrial units are not easily met (226). As most farms produce animal manure, containing low energy density but very polluting liquid-waste, biogas plants must be built in immediate proximity to reduce transportation costs to the minimum (ideally, the plant is located on-site) (70). As discussed in section 4, co-digestion can significantly augment the process efficiency and support the production of more methane per added biomass. This conclusion suggests that by using manure, the generation efficiency of lignocellulosic biomass and manure mixtures provide an economically viable alternative to current generation practices (70). In this regard, smaller quantities of pilot-plant AD reactors provide an outlook for smaller farms to produce biogas effectively (70). To build on this, the specific methane yield (per mass of TS) introduced by the manure is low at 0.9 to 23%, mainly due to the low dry matter content of manure, which implies that transport distances are a crucial factor to be minimised as low methane yield can be expected from a high mass and volume of manure. As such, manure-based bioreactors should be placed in the vicinity of the farms such that the impact of the low dry-matter content, and the associated costs, are minimised (70,230,231). Additionally, biogas plant sizes can be easily adapted and designed to fit the specific needs of the farm, such that efficient operations are guaranteed, and all waste of the livestock population can be managed through one ongoing digestion cycle (13,70).

The main challenge and barrier to smaller biogas reactors to date, as discussed, is the lower volumetric turnover of agricultural waste and the high cost associated with transport of the waste products. Because of this, there was low economic incentive for self-consumption of the waste products in order to provide a stable production of domestic fuel. After the 2021-2022 energy crisis in Europe, the political and market circumstances have changed to a situation where domestic, independent production sources are actively sought and the importance of decoupling energy and fuel necessity from foreign countries was proven. Because of the volatile energy prices experienced, self-consumption became very popular and a great push in research output was sought. Furthermore, in light of future carbon taxation and emissions reduction protocols led by the European Union to achieve energy neutrality by 2050, alternative

energy sources must be actively pursued and all sectors must reduce their emissions, most notably the agricultural sector.

The most successful support scheme for biogas capacity increase is commonly quoted to be the Feed-in-tariff scheme, as they guarantee specific output revenues and can easily be applied to various biogas reactor technologies. German legislation has paved the way for a high degree in installation capacity within their renewable energy source act, through which reactors with up to 75 kW rated capacity receive benefits and give the operators security of a successful project, even on a smaller scale (226).

8.2 Security of supply

Energy security is a pivotal factor for political success and stability. Countries with large reserves of fossil fuels, or with oil/gas fields under their territory, do not voice the same urgency for energy independence as countries who are dependent on energy imports (48). Because of energy independence requirements, many countries are actively pursuing alternative energy generation strategies (such as AD) to lower energy costs and improve their sustainability (232). This analysis of comparing a countrys' renewable energy policies depending on their import/export behaviour was conducted by Wilkinson et al. (2011) (232). They outline that Germany, known as an energy importer, provides clear roadmaps to energy independence by 2045 and further plans to becoming fully self-sufficient from fossil fuel sources on a later timeline (232). To displace the fossil fuels, a strict agenda of pushing renewable energy sources is promoted and subsidised, such as the generation of biomethane via AD (233). In contrast, countries with large fossil fuel reserves and geographical energy abundance do not share the same necessity for energy independence, and therefore do not implement the same degree of long-term strategies for energy independence and sustainability (234).

A bottom-up infrastructure plan was suggested by Lindfors et al (2020) to bring the most effective energy transition to renewable fuels with biogas plants (235). This approach plans to actively involve communities on the lowest political levels to provide sufficient resources and collaborate for the installation of biogas plants. Political entities should focus on providing incentives for biogas plants on the lowest level, through which other communities become aware of the potential to self-sustain their energy needs (235). Through a joint collaboration of

various communities, a decentralised biogas/biomethane infrastructure is developed that works independently from large gas pipelines and does not require major energy purchases from external sources. In this manner, through the joint effort of various communities, an efficient and economical alternative can be created to the current fossil fuel dependence that rural communities face to date. Through these measures, centralised resources of governments can be decoupled from rural settings, as planning, logistics and waste management strategies occur on the lowest political structure, which allows individual communities to improve their standard of living in terms of energy independence, environmental sustainability, and waste management.

Chapter 3 has shown that extensive resources are present in Europe for waste conversion to biomethane. Sufficient resources are available for the current fossil fuel share to be displaced substantially through the incorporation of biogas or biomethane, as currently an average of 2.65% of the total biogas is produced despite vastly higher resources being produced in all member states. Given that the fuel cost of biomethane is comparatively low (in absence of transportation, logistics and prime material costs), a positive outlook for future profitability is expected due to concerns for energy imports and dependence of foreign countries and growing sustainability concerns. 15 million TJ worth of natural gas are consumed annually by all EU countries according to a 2020 report (37). By using the waste products available and knowing that this waste will continuously be produced, a steady supply of resource is available for biomethane production, whilst simultaneously promoting sustainability and energy independence in the EU and other countries with similar sustainability agendas.

A challenge that may be faced in the rural environment, where only certain resources are available, is that optimal waste mixtures may not be at disposal to the same degree as in laboratory settings. Due to this, deviations from optimal biogas compositions are likely because ideal biochemical mixtures are not possible on a larger, farm-based scale. This further highlights the importance of having collated information of various studies in chapter 4 to support knowledge on how various differing mixtures of waste interact with each other, and what biomethane quality and yield they produce. Most farms will currently sacrifice optimal biogas quality (i.e. high biomethane proportions) in order to maximise on yield and treat the maximum material available on the farm. This has, from a sustainability perspective, an added benefit as more waste material is degraded and more overall energy is generally available

(according to LCAs conducted) for local heating and other energetic purposes. A further challenge experienced is that biogas, because of its chemical composition, cannot be transported and distributed as easily as natural gas. If self-consumption is not fully feasibly, distribution may be difficult as biogas (with its high carbon dioxide share) cannot be injected into natural gas grids and cannot be compressed to adequate pressures for efficient transportation without rising costs and the requirement of additional equipment and energy. Cleaning to biomethane quality (i.e. extracting the CO_2, H_2O and H_2S) would be the minimum requirement for the distribution of the gas in a decentralised environment, but this strategy was exactly what is to be avoided given the exacerbated costs posed by this procedure. Further infrastructure, burners, generators and logistics would be required to either convert the chemical energy into electrical storage, or to clean the gas until it is apt for transport and selling on the gas market. Both of which increase the costs to the point where the business case collapses.

8.3 Environmental impact

8.3.1 Main GHG emissions

The primary gases responsible for contributing to the GHG effect and are associated with being produced during the AD process or in subsequent biogas combustion are nitrous oxides, carbon dioxide and methane (5). Poeschl et al. (2012) (236) have conducted an LCA study to determine the emissions associated with biogas installations and its subgroups in terms of CO_2-equivalent emissions: feedstock sourcing, plant operation and maintenance, digestate release and postprocessing and infrastructure development. A major finding of their study included that AD technologies have a negative CO_2-balance, implying that net CO_2 is extracted from the environment and captured through photosynthesis, which is then sequestered in form of digestate management in soils and other applications. This, as a result, substantially improves the air quality and reduces the GHG concentrations currently seen in the atmosphere (5,236).

Compared to CO_2, methane emissions are drastically more damaging for the environment and for the GHG-effect, leading to 28-32 times the global warming potential than CO_2 (5,237). The only positive note is that the total amount of methane emitted into the environment is generally substantially less than CO_2, such that on a global scale, CO_2 is the main driver of

climate change. In terms of contribution to the GH-effect, methane is therefore seen as the second greatest anthropogenic gas, based on the quantities emitted and the global warming potential it exhibits (237). Poeschl et al. (2012) (236) also launched a study assessing the emission rates of methane through cattle manure management under incorrect feedstock and digestate management (allowing for AD). They concluded that with a methane emission rate of 5 g/kg of feed from incorrect management techniques proves fatal for the environment and disastrous levels of methane emissions would be simulated through incorrect feed and digestate management (236). This again highlights the importance of maintaining a closed AD system and removing the toxic substances from natural degradation processes to circumvent these amounts of methane to be emitted naturally.

In third place in terms of GHG emissions in their potential to cause damage are nitrous oxides (N_2O). Nitrous oxide is not an immediate product of AD but is created through biogas combustion in air. Life cycle analyses have been performed to compare the impact of nitrous oxide with other GHG emissions, concluding values between 0.10 – 0.40 kg CO_{2eq}/kWh_{el} for the impact of nitrous oxide in terms of CO_2-equivalent emissions on a specific energy level (238). To compare the figures of GHG-impact however, it is worth noting that the emissions associated with N_2O are 22-75% lower than the total GHG emissions of Germany in their 2018 energy mix (238), indicating that nitrous oxide have a limited overall impact compared to carbon dioxide and methane. It may be argued that nitrous oxide is the most significant GHG over the course of 100 years, even outweighing CO_2 and methane, depending on the climate metric and decomposition behaviour seen by the molecules in the atmosphere (238). When assessing the Global Temperature Change Potential (GTP-100) metric, nitrous oxide is quoted as the main driver for climate change (238).

8.3.2 Emissions savings and climate improvement strategies

There is a clear relation between the environmental impact and the emissions produced through bioreactor technologies and the AD process stream. On top of that, the use of biogas also has environmental benefits, because use of fossil fuels is abated, which can be substantially more harmful to the environment as proposed by LCA studies. Through biogas production, various gases are generated that can be attributed to greenhouse gases and the global warming effect, such as methane, carbon dioxide and nitrous oxide. These mainly originate

from the production of biogas in the reactor, and from subsequent combustion for the generation of heat and/or electrical energy, as in CHP plants (5).

Bachmaier et al. (2010) (239) analysed the global warming potential and environmental impact via LCA analyses for ten different bioreactors. They summarised their findings in global warming potentials and GHG saving potentials, indicating that -85 to 251 g CO_{2eq}/kWh_{el} and 2.31 to 3.16 kWh_{fossil}/kWh_{el} are respectively emitted and saved as a result of utilising the benefits of biogas reactor technology (239). The wide ranges in savings and emissions are often associated with different data sets and boundary conditions that are being used in the LCA models, as discussed within their analysis. For example, all ten different plants that were incorporated into the LCA model have portrayed different strategies to deal with key operational topics, and stipulate different environmental impacts due to varying degrees and strategies that were implemented in categories such as: heat recycling, transport necessities, digestate processing and feedstock processing (5).

A different case study by the research team among Battini et al. (2014) (240) has been prepared where the GHG emissions of dairy farms were compared with, and without a biogas plant to treat the resulting livestock waste. The case study portrayed their findings with a clear benefit of employing AD in Italy: emissions are reduced by a notable fraction between 23.7-36.5%, indicating that livestock emissions are reduced substantially through this practice, thus reducing the climate change impact seen from intensive livestock farms (240). Further assessments were conducted by Kaparaju and Rintala (2011) (241) in the Finnish livestock sector, where sow, pig and dairy farms equipped with an AD reactor for subsequent manure processing led to GHG emissions savings of 87.7, 125.6 and 177 $CO_{2,eq}$/yr respectively.

Different strategies exist to further reduce the environmental impact and GHG emissions of biogas plants, which have been outlined by Hijazi et al. (2016) (242). They could identify that the main driver for a worsened global warming potential originates in form of methane production and subsequent leakage from the AD production system (242). Given that methane boasts 28-36 times the global warming potential (GWP) as carbon dioxide, any leakage is recommended to be either sealed immediately or burned to (less harmful) carbon dioxide (5). In order to reduce the overall effect of having to flare off methane leakages, Hijazi et al. make the following recommendations for plant design: exhaust flares to reduce any flue methane to

enter the environment, additional or reinforced tank coverage for leakage abatement, improve CHP efficiency, reutilise and recirculate electric and thermal power where possible, and reduce pipes and linkages as these are the main sources of leakages (242,243). In a similar study conducted in Umbria, Italy, Buratti et al. (2013) (243) concluded similar results, especially emphasizing the importance of digestate storage as an additional measure necessary to reduce the potential of GHG emissions from the overall biogas system. By successfully limiting the leakage potential of these digestate emissions, for example by a closed covering of digestate, a GHG saving factor of 68.9% is estimated by their study given the decrease in methane leakage into the environment (243).

8.3.3 Combustion pollution

Combustion of biomethane and biogas leads to inevitable by-products such as CO_2 and water, as per chemical combustion definitions. Given that combustion mechanisms are not perfect and biogas impurities exist, different combustion products are formed alongside the main products, which are polluting substances such as carbon monoxide (CO), nitrogen oxides (NO_x) and sulphur dioxide (SO_2) (5). The quantity of combustion emissions varies between the three substances, but experimental studies by different research groups quote the emissions in the range of 310, 25 and 540 g CO_2/GJ_{el} respectively (240,244,245). The amount of sulphur dioxide emitted is heavily dependent on the amount of sulphur contained in the feedstock and resultant biogas samples produced by the AD reactor, and whether desulphurisation equipment has been added or not (245). Carbon monoxide is mainly a gas generated through incomplete and inefficient combustion mechanisms of methane, where the specific turbines and combustion technologies must be improved to reduce the pollution via this gas. The most detrimental environmental impact from AD is caused by NO_x, which are up to 300% the expected ranges seen when combusting in natural gas engines (244). The reason for these comparatively high nitrogen oxide emissions stem from suboptimal combustion mechanisms and the absence of using chemical strategies like selective catalytic reduction and/or scrubbing to clean the effluent gases post-combustion (246,247). High-level research is currently focussed on improving the environmental impact on industrial-scale biogas plants, such that on a global level, the technology becomes more sustainable (246,247). NO_x is extremely dangerous for the environment due to its versatile nature of causing adverse impacts. NO_x causes acidification of rainwater and soils (based on LCAs, by 5.5% to 6.1%), particulate matter

emissions (0.7% to 1.4%), photochemical ozone formation (41.6% to 42.3%) and eutrophication effects (approximately 0.8%) (240).

In addition to the aforementioned pollutants, further NO_x and ammonia emissions are caused through the storage and fertilisation of digestate, causing adverse environmental effects (5). Based on the research of Boulamanti et al. (2013) (248), the majority of particulate matter circulating in the atmosphere was caused by NO_x emissions, with a significant share originating from agricultural practices. Emissions of this kind can be further controlled and reduced by employing closed storage techniques, where the overall amount of particulate matter originating from NO_x and ammonia could be diminished effectively (5).

8.4 Digestate sustainability

Digestate, as per its definition, is any tangible substance (other than the resultant gas) leaving a bioreactor after the digestion has taken place. The intention of digestate is to be low in calorific value and less polluting to the environment than other waste substances such as substrates, which have not been treated via AD yet. Therefore, Europe, with its major biogas infrastructure, generates large amounts of clean, affordable and readily available digestate that can be used for further applications. For instance, digestate re-introduction as fertiliser into the agricultural sector is often discussed (8). To date, the EU has defined specific directives to regulate AD feedstocks that are apt for use as fertiliser under the EC communication 1774/2002 (249). The intention of this regulation is to prohibit dangerous practices such as waste dumping and prevent other risks such as soil contamination, pesticide poisoning, disease spread or groundwater contamination (249). This regulation also calls for the use of AcD feedstocks made from lignocellulosic biomass treated with pesticides and livestock waste to increase digestate hygienic standards (249).

8.4.1 Digestate Emissions

The digestate material can emit various gases as part of its natural degradation process, including methane, ammonia, nitrous oxides and volatile hydrocarbons (5). These emissions must be analysed from an ecological perspective to determine the risks these substances pose to the environment when digestate is distributed as fertiliser or left to degrade in the atmosphere (5).

An analysis of the nitrogen offtake and soil emissions from manure-based digestate distribution has been conducted by Möller et al. (2015) (250). They concluded that soil remains fertile and healthy, along with quoting the low environmental consequences of fertilising in this manner, leading them to conclude that fertilising with manure-digestate is a sustainable practice (250). Comparing both fresh organic matter and COD-depleted digestate when distributed as fertiliser, it became evident that the digestate degrades significantly faster and is more readily available for the subsequent generation of crops (251). In contrast, fresh organic matter has more carbohydrates available (given it was not previously digested by bacteria), which are absorbed easily by new plants to help in the growth of lignin and other non-hydrolysable lipids that plants use in their cell structures (251). These substances, as a result of the fresh organic matter distribution, are not degraded easily, neither during natural degradation nor via AD, causing exacerbation of feed availability for future AD applications (252). In this regard, Tambone et al. (2015) (252) argue that pig slurry digestate provides a very stable fertiliser for soils given their balanced nutrient properties and their degradation kinetics.

If manure-based digestate is used as a fertiliser, without being further processed or treated, it can raise concerns due to environmental risks regarding soil contamination and pollution of various ecosystems (253). The emissions of untreated digestate have been discussed in Eickenscheid et al. (2014) (253), where it was concluded that both methane and ammonia emissions did not change whether post-processing occurred or not. However, digestates that showed high amounts of carbon loadings did lead to exceeding N_2O emissions that can adversely affect the environmental conditions and have a negative environmental impact (13,253). In general, and as discussed by Oshita et al. (2014) (254) untreated biomass (including biomass not treated prior by AD) shows significantly more methane and nitrous oxide emissions and pollution than treated biomass, leading to the conclusion that AD digestate as well is less environmentally harmful than fresh organic matter to be distributed on fields and other waste deposits.

AD accelerates the rate of degradation of manure as compared to other feedstocks that may be used for fuel processing. As a result, the manure digestate has reduced N_2O emissions, including lower anoxic microsites and slower microbial degradation kinetics than fresh feedstock, which has not been treated via the four AD pathways yet (253–255). Building on this, the lower methanogenic potential of manure-treated digestate as compared to lignocellulosic-

based digestate leads to evidence that further emissions, such as CO_2 and CH_4 after AD has occurred, is substantially lower than the original biomass samples (236). A notable highlight is identified for manure, where ammonia emissions are quoted to be higher after AD has been performed than for when the sample is left untreated (250,256). Matsunaka et al. (2006) (257) supports this claim by concluding a 13% ammonia volatilisation rate of cattle manure digestate as it is being subsequently used as fertiliser on grassland.

The greatest environmental threat is perceived by the nitrogen release from digestate use as a fertiliser to support soil fertility. According to the work performed by Paolini et al. (2018) (5), a specific annual dosage of nitrogen is recommended to maintain a sustainable, healthy and rich soil apt for future crop development. Lastly, as the emissions associated with digestate storage are comparatively larger than those linked with fertilisation, effort and investments should be primarily placed on sealing digestates and feedstocks from the environment in order to reduce emissions on a lifecycle basis (5,243,255).

8.4.2 Digestate storage and associated risks

Storage of digestate can be a major source of gas emission and pollution if the chemical compounds are not contained in a controlled environment that is closed-off and regulated sufficiently (5). By using a closed-to-air facility and preventing any gas leakage occurring from the digestate, harmful GHG emissions are reduced to a minimum as aerobic digestion is no longer feasible for the digestate specimen. A digestate sample which is manure-rich and is contained in a controlled facility therefore has no further possibility of producing and leaking N_2O into the environment, preventing any source of ecological or global warming damage that can be related to the emissions of this GHG (248).

Most ammonia emissions originating from the livestock or agricultural sector take their origin in incorrect waste management or storage practices. In simulations, bad waste management damages the overall AD production chain. In practice, this leads to the production of a toxic chemical mostly associated with nitrate leaching, groundwater contamination and environmental pollution (248). Studies have shown that through effective mitigation measures of digestate pollution, GHG emissions can be reduced by up to -36.5% (with respect to -23.7% of general AD) by setting up appropriate infrastructure for digestate control, management and storage units (240). Lastly, effective storage in air-tight tanks also prevents pollution through

odour and reduces the production of other chemicals associated to ammonia, which are also proven to be harmful to the environment and originate from the AD process of a bioreactor (5).

Nevertheless, it must always be stated that with the current practices of livestock rearing and agriculture and no planning for manure or waste control are currently taken, that the consequences for the environment are significantly worse without conducting AD on the waste structures, than when incorporating AD into the waste treatment process. In this regard, even the sole AD process and subsequent mis-treatment of digestate products is still better for the environment than no AD at all. Adding a planned digestate treatment as well further improves the environmental footprint of the waste processing pathway.

8.5 Advantages and Disadvantages

To summarise at the end of this thesis, both the benefits and drawbacks have been reviewed in condensed format in Table 16.

Table 16: Anaerobic Digestion: Advantages and Disadvantages

Advantages	
Biogas Generation	Organic waste products receive a treatment procedure within a controlled environment in which hygiene standards are kept and ecological risks are reduced to waste dumping into the environment (258). Calorific content is fully exploited, and resources produced by/for humankind are used to their full value, improving efficiency and reducing the total need of material for anthropogenic energy needs (8). Reduction in the GHG emitted through agricultural processes and livestock rearing/management. Implementation of an effective treatment strategy that improved the carbon footprint of the agricultural sector and prevents waste dumping on landfills or water basins (5). Energy independence is boosted through generating decentralised, domestic fuel with no security-of-supply issues. Creation of an easily stored energy vector with existing infrastructure in place (232).
Biogas and biomethane applications	Renewable fuel is created with versatile applications: heat, power and storage capabilities. This fuel can be used with modern-day technology to support the electricity grid by acting as an intermittent source of power, or be used as storage at a later date (54).

	Environmental pollution is reduced, as substances such as methane, carbon dioxide, particulate matter and nitrogen oxides are now generated within a controlled environment and not left to decay or produce other harm within the environment (5). The technology is an active player in the guarantee-of-origin trading and can be used as a carbon-offsetting technology. The technology also allows for carbon-negative energy accounting if CCS (Carbon Capture and Storage) is employed either during biomethane refining or after biogas combustion (236).
Digestate reutilisation	The European "farm to fork" policy of circular economy is supported, and nutrients previously used in fertilisation are still readily available to be re-introduced to the agricultural sector (259). Digestate distribution on agricultural fields supports the foundations of humus and adds structural integrity of the soil (69). Higher yields expected from fertilisation efforts, thus increasing the economic outlook for agriculture in Europe (260). Reduce the dependency of chemical-based fertilisers that were manufactured artificially through fossil-fuels (69). Reduction of bad-odour complaints through inadequate manure dumping (85). Boost of organic farming practices (259).
Socioeconomics	Auto-production and consumption reduces overall energy purchases, thus lowering gas and electricity bills for producers (260). Self-sufficiency from third-party suppliers of gas/energy (232). Due to the fact that no net carbon emissions are produced through the technology, no efforts for carbon budgeting are required as with the exploitation of fossil fuels (260).
Environmental	Emissions reduction and protection of the ozone layer through lower emissions of gases damaging the atmosphere (85). Lower environmental damage: decrease in pollution, water contamination, water eutrophication and toxification of soils (85). Lower GHG impact as the previously emitted methane is displaced by less harmful CO_2 through combustion (5).
Disadvantages	Comparatively slow process kinetics due to its biological nature. Other biofuels, treated through chemical engineering, are produced at a faster rate (8,25). Initial investment efforts must be made to provide the local infrastructure, BOP and pilot-plant reactors for farmowners and entrepreneurs. This may be a deterrent to self-sufficiency due to the limited financing options (261). Many uncertainties given by legislative organs give little planning prospects for major investment and security for the biogas sector. As such, technological innovation has been decelerated in recent years (48). Inefficient use of land if the crops grown are primarily used for biogas production. Specific yield (kJ/m^2 of crop) is with the lowest of all technologies, compared with solar PV or wind power (248). High research potential for different technologies implies that the technology has not matured yet, and is still relatively expensive. As such, technologies such as multi-stage AD, BOP optimisation, pre-treatment strategies, prediction/simulation of yields/concentrations must still be researched in higher detail (54,248).

Different discussions have been held to criticise biogas production as a sustainable practice, especially in regard to high land area requirements, competition with dietary needs, and alternate emissions caused by combustion (5,85,248).

Biogas/biomethane combustion produces other gases and substances such as nitrous oxides and particulates that are harmful to the environment and must be acknowledged for in environmental impact assessments (5).

Additional difficulty brought upon by co-digestion manure-rich feedstock mixtures, given the necessity to balance several performance parameters (C/N ratio, pH, TSS and TKN).

9. Conclusions

The following chapter will summarise the key findings and improvements to be sought for future studies intending to optimise the waste utilisation and conversion to renewable fuels via anaerobic digestion (AD) technologies.

9.1 Industry analysis

The industry analysis concluded that bioreactor implementation on European level was previously uneconomical, mainly due to the transport distances that were required for waste products to be distributed to centralised processing plants. Pilot-scale reactors, given their smaller size, can effectively minimise these transport distances to the point of origin of these waste products, thus providing a good business case for increased biowaste processing and biofuel generation infrastructure. Distributed and decentralised production is thus promoted especially in rural European communities by a more diverse biomethane production landscape. The EU farm-to-fork policy is thus supported and the barriers to ecological and environmental protection are lowered through intensive agricultural activities.

Rising natural gas and energy prices have reopened the topic of sustainability and energy self-sufficiency in recent years, with countries promoting their agendas to become energy self-sufficient through renewable energy technologies. Especially in line with the future development of carbon taxation, low-cost options for the generation of renewable fuels become interesting to continue the usage of existing technologies and infrastructure in a world less dependent on fossil fuels. Germany, given its highest scale of biogas infrastructure development, was taken as a case study. To date, 70% of German manure is wasted, with other countries in the EU using even less of their waste for renewable practices. This contributes to climate change and has dire ecological effects. Based on the calculations conducted, only 2.65% of the biomethane is currently produced compared to its potential, as has been calculated in the statistical analysis. More development is needed to decarbonise the agricultural and livestock sectors to improve the sector outlook with its new sustainability directives in mind.

9.2 Literature review

The literature review conducted provided great insights into modelling biological systems undergoing AD process kinetics. Different performance parameters could be discussed, such as carbon-nitrogen ratios, organic loading rates, hydraulic retention times, pH-values, biomethane yields and proportions, volatile solids reduction rates and operation temperatures. The carbon-nitrogen ratio was found to be the most influential design parameter for determining the methane content in the biogas produced. A high carbon-nitrogen ratio generally results in a lower methane concentration in the biogas, given that the production of volatile fatty acids is favoured to a higher degree than ammonia generation. Small changes in carbon-nitrogen-ratio of a substrate can cause the most impact on the yield and calorific content of the biogas. The carbon-nitrogen ratio is therefore the most decisive design parameter of all variables assessed.

A high pH-value is also advantageous for manure-rich feedstocks that are treated in co-digestion with lignocellulosic biomass. This is because higher pH-values provide sufficient chemical stability of the fatty acid production during hydrolysis, acetogenesis and acidogenesis stages in the digestion process cycle, where the high pH can sufficiently act as a buffer to the onset and accumulation of acids generated. Various models and relations between carbon-nitrogen ratio, pH, solids retention time and manure content were found which can help optimise the biogas yield and the aforementioned methane proportion within that biogas yield. Lastly, several generalised behaviours could be identified between carbon-nitrogen-ratios and percentages of volatile solids reduction, with the conclusion that for samples containing more than 50% manure, further optimisation of carbon-nitrogen-ratios yields no tangible results in improved biogas yield or calorific value. Only systems with less than 50% manure allow for improvement of biomethane yields through carbon-nitrogen-ratio adjustment.

9.3 BMP/Batch experimentation

Biochemical methane potential experiments were performed to verify the previous literature analysis conclusions through in-house testing. Through the literature review, mixtures of manure and lignocellulosic biomass could be identified as high yield and high-potential feedstock for digestion and biomethane production.

Based on experimental results where swine manure and commercial inoculants were compared, it was found that swine manure digested foreign matter (and its own) instantaneously, whilst commercial bacteria consortia required a 14-day acclimatisation time. Thereon, the commercial consortium outperforms swine manure disregarding the 14-day acclimatisation period. Swine manure co-digested with meadow grass (as a sources of lignocellulosic biomass) outperformed all other samples at a methane percentage of 86% within the biogas. Glycerol, often concluded to be a highly-efficient substrate in literature, is only effective in miniscule additions, and poses the risk of destabilising the digestion system due to a perturbation in carbon-nitrogen ratio. Glycerol addition only improved gas production, but methane proportions fell short of expectations. It is advised to keep glycerol addition to under 1 g glycerol for each 100 mL of digestant inoculum. The digestion of fresh orange skin also outperformed dried and pre-treated orange skin, proving that the antibacterial contents of limonene have little effect on the overall digestion efficiency.

9.4 OLR/Semi-continuous experimentation

Following from the batch experimental scale, the size of reactors was increased to 5 L reactors and an assessment of different feedstock loading profiles was conducted with the intention to determine the ideal feeding amount to maximise the biomethane production per mass of substrate added.

Different feeding strategies at organic loading rates of 5, 7, 9 and 11 g VS/L.day were assessed. Reactors 1 and 2 (5 and 7 g VS/L.day) produced a similar amount of biomethane, as did reactors 3 and 4 (9 and 11 g VS/L.day). Biomethane concentration in the biogas was mostly stable at between 60 and 65% content in the biogas. Reactors 3 and 4 experienced a drop in pH towards the end of experimentation, which is attributed to an accumulation of VFA and therefore less biomethane was produced at the end of experimentation. Both reactors ended the experimental period with a pH below 7, with 7 being defined as the lower range for optimal methanogenesis.

An OLR of 9 g VS/L.day could be defined as the optimum, because when incorporating different KPIs, it saw the best reduction efficiencies and biomethane production per mass of VS fed to it. The highest VS reduction with 79.3% and highest specific biomethane yield with 0.0451 L/g

of volatile solid was attributed to this reactor system. High organic loading rates are advised if appropriate reactor monitoring of performance parameters (chemical oxygen demand, pH, volatile solids, total Kjeldahl nitrogen) is possible, as overloading was shown to be more efficient. If reactor monitoring is not possible, as with pilot-scale plants, it is advised to not surpass an OLR of 8 g VS/L.day. To minimise the risk of volatile fatty acid overload, and to maintain a low-cost reactor design, it is ill-advised to approach maximum reactor capacities.

Operational challenges were also seen, such as tube explosions and excessive foam formation at 11 g VS/L.day. Digestate samples also indicated that lots of digestate left the reactor which was still very rich in calorific value, signifying that complete degradation had not occurred and more biomethane could have been extracted from the digestate when it was extracted on a daily basis. The hydraulic retention time should be increased in future experiments.

9.5 Low-cost/Pilot plant experimentation

After having gained experience with two prior experimentational phases, further scaling occurred, moving from 5-L semi-continuous reactors to 100-L low-cost pilot-scale reactors. The intention of this experiment was to verify the successful operation of the pilot-scale plant through using experiences gained and methods employed in the previous experiments.

The experimentation was split into four trial runs. Trials 1 and 2 were conducted under laboratory settings and used the commercially supplied inoculum and kitchen waste as a substrate. Both experiments have yielded no success in producing biomethane, due to VFA accumulation and subsequent pH reductions. Dark fermentation of the digestate had occurred and caused a substantial production of hydrogen and hydrogen sulphides rather than biomethane.

Trials 3 and 4 took place in site conditions under ambient temperatures. These trials utilised swine manure because this inoculum has proven to be extremely stable due to its buffer capacities. Biomethane could be generated under ambient conditions in Spanish spring weather and a high methane proportion of 67% could be proven. Biogas production started at an ambient temperature of about 15 °C, anything lower than 10 °C caused the bacteria to be dormant and no gas production could be registered.

Operational difficulties could also be identified. Issues with the heating system and the lack of agitation lowered overall methane output. The heating mat provided by the supplier was ineffective to heat the digestate to mesophilic conditions, especially when working on-site instead of in the laboratory. A second heating mat was employed to reach a digestate temperature of 32 °C. However, from an energy balance viewpoint, the electricity requirement of heating did not cover the additional biomethane yield of the heated digestate, so further efficiency improvements must be sought to make this system viable. It is also with highlighting that a different low-cost bioreactor has been installed under the same environmental conditions in the farm in Soria, Spain. This system did provide a beneficial business case and produced a net-positive energy balance when incorporating biomethane output and BOP energy investments. This leads to the conclusion that specifically the tested system has underlying design flaws, but not the domestic biogas plant-operation in itself. It is recommended to continue improving the design of the low-cost biogas systems such that lower heating requirements of the evaluated system are required, especially in the winter months when experimentation took place.

Experimental losses due to plant scaling were also assessed. Due to the inefficiencies discussed, the pilot-plant had a reduced biomethane generation compared to the batch and semi-continuous experiments, where 83% and 57% lower biomethane yields were registered respectively. Batch experiments, given their small scale and ideal laboratory setting, performed best at 86% biomethane concentration and a biomethane yield of 0.116 L biomethane per g VS added.

9.6 Future work

A set of future improvement and objectives to continue the research field of low-cost pilot-scale anaerobic digestion bioreactors could be identified, which will help provide insights into the operation and design of the future generation of reactors. Through this, a key contribution to European sustainability objectives will be provided on a decentralised level.

The current work has not incorporated any analysis on wastewaters and sewage sludge as possible feedstock or inoculants for anaerobic co-digestion. The specific focus of this study was to improve the co-digestion of manure and lignocellulosic biomass, given that the scope

was defined within agro-industrial waste management. The statistical analysis performed can be complemented by a more diverse mixture of feedstock types and organic waste structures.

The industry analysis conducted has been confined to the European biogas market and agricultural activities based in Europe. This analysis could be extended from a European scale to a global scale to assess further potentials and the opportunity for major biogas production centres. Through this, specific regions and countries can be defined where waste management can be improved and directed governmental funding can provide large impacts.

As part of this study, various performance parameters were assessed in their effectivity in predicting both biomethane yield and richness in the produced biogas. However, a single modelling equation that defines the biological behaviour could not be identified. This equation, or a model describing the behaviour, must be further refined and shall engulf all major performance parameters, such that more accurate yield and quality predictions can be conducted. This model should include various experimental scales: laboratory, pilot, and industrial scales. Also, chemical analysis of the feedstock compositions and their physical attributes shall be fed into this model.

Lastly, from a design perspective further effort shall be placed on improving pilot-plant reactors. Especially, improvements in insulation, heating systems and stirring mechanisms are required to improve reaction kinetics to yield a higher biomethane output. Currently sedimentation of VS occurs at the bottom of the fermentation bag, where the bacteria have no access to effectively degrade the volatile matter. These design improvements shall not result in a major increase in either operational or capital price increases. Further research of pilot-scale systems is to occur in moderate climates where temperatures do not drop below 15 °C, like barns or sheds, or more temperate climates (tropics, or along the coast).

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11. Appendices

11.1 Appendix 1: Raw data taken from Eurostat to create Figure 3

Please note: data from this table was collected from the following resources/references: (33–39).

Table 17: 2020 Data of agricultural activity of various EU countries (selected and collected data)

GEO	Utilised Agricultural Area (1000 Ha)	Proportion of Land for Agriculture	Arable Land (1000 Ha)	Proportion of Land Arable	Main Agricultural Output by Percentage	% Agricultural and Livestock rearing activities of GDP	% Share of energy from renewable sources
EU-27	1.62E+05	38.49%	9.88E+04	23.44%	Animal/Energy Crops (40.40%)	1.65%	22.09%
Belgium	1.37E+03	44.78%	8.69E+02	28.48%	Cereals (52.72%)	0.64%	13.00%
Bulgaria	5.05E+03	45.73%	3.48E+03	31.51%	Cereals (71.16%)	3.51%	23.32%
Czechia	3.52E+03	44.68%	2.49E+03	31.57%	Animal/Energy Crops (49.08%)	1.92%	17.30%
Denmark	2.62E+03	61.04%	2.37E+03	55.20%	Animal/Energy Crops (65.50%)	1.31%	31.65%
Germany	1.66E+04	46.44%	1.17E+04	32.64%	Animal/Energy Crops (55.87%)	0.74%	19.31%
Estonia	9.85E+02	21.79%	6.94E+02	15.34%	Cereals (82.08%)	2.17%	30.07%
Ireland	4.51E+03	64.64%	4.43E+02	6.35%	Cereals (88.20%)	0.93%	16.16%
Greece	5.27E+03	39.89%	1.76E+03	13.37%	Animal/Energy Crops (35.37%)	4.23%	21.75%
Spain	2.44E+04	48.30%	1.19E+04	23.46%	Cereals (39.35%)	3.15%	21.22%
France	2.89E+04	45.64%	1.80E+04	28.50%	Cereals (55.79%)	1.60%	19.11%
Croatia	1.51E+03	26.61%	8.89E+02	15.71%	Cereals (49.44%)	3.22%	31.02%
Italy	1.31E+04	43.44%	6.91E+03	22.87%	Animal/Energy Crops (71.41%)	1.98%	20.36%
Cyprus	1.36E+02	14.65%	1.07E+02	11.51%	Roots (32.31%)	1.93%	16.88%
Latvia	1.97E+03	30.49%	1.33E+03	20.65%	Cereals (85.04%)	4.03%	42.13%
Lithuania	2.94E+03	45.08%	2.25E+03	34.42%	Cereals (45.11%)	3.24%	26.77%
Luxembourg	1.32E+02	51.10%	6.23E+01	24.10%	Animal/Energy Crops (84.70%)	0.20%	11.70%
Hungary	5.00E+03	53.73%	4.10E+03	44.10%	Cereals (62.13%)	3.36%	13.85%
Malta	1.07E+01	33.93%	7.77E+00	24.64%	Vegetables and Fruits (53.18%)	0.44%	10.71%
Netherlands	1.81E+03	43.68%	1.00E+03	24.19%	Vegetables and Fruits (79.39%)	1.58%	14.00%
Austria	2.65E+03	31.56%	1.32E+03	15.76%	Cereals (34.58%)	1.10%	36.55%
Poland	1.45E+04	46.32%	1.09E+04	34.93%	Cereals (35.40%)	2.50%	16.10%
Portugal	3.97E+03	43.04%	9.73E+02	10.55%	Vegetables and Fruits (59.53%)	2.11%	33.98%
Romania	1.36E+04	57.01%	8.92E+03	37.40%	Cereals (59.74%)	3.97%	24.48%
Slovenia	4.84E+02	23.87%	1.76E+02	8.69%	Animal/Energy Crops (67.85%)	2.12%	25.00%

Slovakia	1.91E+03	38.95%	1.35E+03	27.45%	Cereals (47.89%)	1.76%	17.35%
Finland	2.27E+03	6.71%	2.24E+03	6.63%	Animal/Energy Crops (67.62%)	2.45%	43.80%
Sweden	3.01E+03	6.85%	2.54E+03	5.79%	Animal/Energy Crops (62.49%)	1.37%	60.12%

11.2 Appendix 2: A complete breakdown of the European regulations and policies relating to the biogas industry.

Table 18: A complete breakdown of the European regulations and policies relating to the biogas industry.

Year	Name/Communication	Description	Reference
1991	*EU Nitrates Directive*	• Hinders and limits the use of nitrogen-based manure digestate after AD post-processing	(42,262)
2007	*Energy and Climate Change* Package	• Providing an initial European energy policy • Quoting the goal to limit climate change temperature to 2 °C • 20% GHG emission reduction by 2020 as compared to 1990 levels	(4,263,264)
2009	*Renewable Energy Directive (RED)*	• Guide transition to more RE (20% total renewable energy share (RES) in energy, 10% in transport) • Distinct national objectives and mandatory targets were defined • Roadmaps for RE implementation targets	(4,265,266)
2009	*Fuel Quality Directive (FQD)*	• Supplement to the RED • 6% GHG emission reduction in transport sector by 2020 • Promotion of biofuels and alternatives to fossil fuel resources in transport	(4,267)
2010	*Common Agricultural Policy* (CAP)	• Incentivises biogas production in rural settings to reduce emissions of methane from animal waste and manure	(268)
2011	*COM(2011) 112 final* statement and *COM(2011) 885 final* statement	• 80-95% carbon emission reduction in each EU member country by 2050 • RES of 55-75% in consumption • Decarbonisation and Energy Roadmap for 2050 published	(4,266,269,270)
2012	The bioeconomy strategy: *COM(2012) 60*	• Fossil fuel substitution to natural resources in chemical and material industries. • Revision in 2018 to define recyclable and bio-based fertilisers within a circular economy context: AD digestate is recognised as a fertiliser, but must still be registered under the REACH programme as a hazardous substance.	(4,42,266,271,272)
2015	*Directive 2015/1513*	• Limitation of biofuel share in transport to 7% by 2020 • (only applicable to biofuels derived from food or energy crops) • Biofuels from waste products must be included with at least 0.5% share	(4,273)
2015	*COP21 Paris United Nations Framework Convention*	• General agreement to limit global temperature rise to 1.5 °C • Production of the *Intended Nationally Determined Contribution (INDC)* for each country	(4,41,42)

		to define goals in preventing exceeding of climate change by 2 °C • Production of the third EU *Clean Mobility Package* (2018)	
2016	*COM(2016) 767 final/2* statement	• Limitation of biofuels derived from food/energy crops of 3.8% of the fuel mix	(4,274)
2018	*Renewable Energy Directive (REDII)*	• Revision of targets and roadmaps to reach climate neutrality by 2050 • EU-wide minimum renewable energy consumption target of 32% by 2030 • Heating sector: 1.3% annual increase in renewable energy share (RES) • Transport sector: 14% RES by 2030, including 3.5% mixture share of biologically synthesised fuels • Support for renewable natural gas transportation in existing gas networks and trade through origin certificates • Revised sustainability thresholds for biofuels: for public funding eligibility, biofuels must provide at least 65-80% GHG savings depending on the application sector. this was implemented to reduce the incentive for damaging ecosystems (wetlands, forests and peatlands, i.e. natural carbon sinks) to produce more energy crops for biofuel production (Indirect land use change, ILUC)	(42,43)
2021	*EU Taxonomy Climate Delegated Act*	• AD (and upgrading with gas grid injection) recognised a sustainable and low-carbon activity, promoting investments in the biogas sector	(44,45)
2021	*Fit-for-55 Package*	• 55% Carbon emission reductions by 2030, net-zero pollution by 2050 • Proposal of further biofuel deployment to abide by EU climate law	(22,275)

11.3 Appendix 3: Reference nutrient breakdown of agricultural waste

Table 19 gives a breakdown of the main nutritional proportions seen in the various waste streams. Based on these, it is possible to use discussed equations (reviewed in chapter 4) to assess the feasibility of the waste to produce biogas.

Table 19: A reference nutrient breakdown of key agriculture/livestock products

Waste	Water content	Proteins	Carbohydrates	Fats	Fibre
Cereals (276)	10%	12%	69%	3%	6%
Animal/Energy Crops (277)	10%	13%	71%	5%	1%
Vegetables/Fruits (278)	95%	1%	1%	1%	1%
Roots (279)	22%	9%	50%	8%	12%
Manure (106)	87%	4%	6%	1%	2%

11.4 Appendix 4: A literature review on pre-processing techniques and methods

The objective of pre-treatment is to augment the biodegradability of feedstock for more efficient AD and increased reduction of volatile solids (54). Siddique et al. (2018) state that pre-treatment is used to make the nutrients within lignocellulos structures more available and to increase the chemical oxygen demand of the substrate (91,280). As a result of this, the quantity of specific biogas is increased, leading to a more efficient and economical process (281). Especially for lignocellulosic biomass, pre-treatment may be essential for the economic viability of a biogas project, as without a treatment strategy, the cellulose and hemicellulose walls of biomass reduce the biodegradability within AD to an extent where feedstock is not reacting in the hydrolysis stage and HRTs are extended substantially. The use of a pre-treatment strategy must therefore be meticulously designed and chosen, as the feedstock compound must be degraded in an advantageous manner for AD, without formation of inhibitory compounds that may prevent straightforward biogas production (282).

The most common pre-treatment methods are chemical pre-treatment, mechanical pre-treatment, and the biological methods of fungal pre-treatment, addition of microbial consortia (MC), and enzyme addition (54). Biological pre-treatment is a popular strategy due to its simplicity and relatively cheap incorporations into the production chain (54,283). To build on this, biological pre-treatment sources are environmentally friendly and require low energy investments for pre-treatment, proving their economic feasibility and ease of implementation (283). Biological pre-treatments come with the disadvantage of time constraints, which lead to high HRTs and, inadvertently, large reactor volumes to accommodate the long HRTs (284). The

common disadvantage of long HRTs and large digestions vessels is the necessity for augmented heat investment for limited biogas production. Iglesias et al. (2021) summarises the types of pre-treatment strategies into 4 distinct groups, as shown in Table 20 (28):

Table 20: A Summary of Pre-treatment Techniques

Type of Pre-treatment form	Example of Method
Physical	Grinding Milling Irradiation/Electromagnetic
Physio-chemical	Steam Explosion Liquid hot water
Chemical	Alkali Acid Oxidising Agents Organic Solvent Enzymatic
Biological	Ammonia Fibre Explosion Ionic liquid Fungal Microbial Consortia Ensiling Micro-aeration Composting Genetic and Metabolic Engineering

11.4.1 Conventional pre-treatment

Thermo-alkaline strategies (chemical pre-treatment)

Thermo-alkaline strategies generally involve the use of a strong basic solution as storage medium (under a set temperature) for the agricultural waste to break down the lignocellulosic bonds prior to AD. In that regard, several different treatment trials have been performed in literature and are generally accepted, together with mechanical pre-treatment, as the most conventional technique to improve biogas yield in AD.

A study by Solé-Bundó et al. (2017) improved the methane yield by 15% by mixing wheat straw in a solution of 10% CaO before oven-drying the specimen for one day at 72 °C (285). Further very common strategies include the use of NaOH as additive for improve biodegradability. For instance, the thermo-alkaline pre-treatment of peanut hulls enhanced biogas yield by 66.15% through pre-treatment of 3 g NaOH per 100 g TS and oven storage at 55 °C for one day (286). A further common base used for chemical pre-treatment is KOH (91). The work of Avicenna et

al. (2015) investigated pre-treatment strategies in co-digestion, where corn husks were soaked in NaOH for five days at room temperature, and then co-digested with cattle manure (287). They demonstrated an improved methane yield from 60-80%, along with reduced heating energy investments because no oven for heating has been used (287). A further efficient chemical pre-treatment technique is using an ammonia solution to reduce the lignin content in lignocellulosic biomass (288).

Mechanical Pre-treatment

Not only is mechanical pre-treatment important to break recalcitrant material within lignocellulosic biomass, but it may also help with improving digester operation by preventing clogging, foaming, material floating and heterogeneity of substrates (55,56). Mechanical pre-treatment examples, as seen in Table 2 of section 3.1.1, include the processing of feedstock with machines to grind and press substrates into smaller and more easily degradable constituents (55). The theory behind mechanical pre-treatment is linked to increasing the effective reactive area of the substrate, allowing for more enzymatic attack and bacterial degradation of the material (55).

Common pre-treatment methods include subjecting the lignocellulosic biomass to shear forces with a rotated plastic sweeping brush against a steel roller assembly, as studied by Tsapekos et al. (2018) (56). Further types of physical processing include coarse steel rolling, which subjects the material to compressive forces and intends to break further cellulose layers. Finally, milling and structural destruction by machines is often employed in an attempt to increase the effective reactive area of biomass. Mechanical comminution machines hold great potential in enhancing the feedstock biodegradability by reducing particle sizes, altering surfaces, and partially drying the biomass (289).

Often it is essential to analyse the energy balances within mechanical pre-processing. For instance, large processing machines require vast amounts of energy to run. The improvement in biogas yield must amortise and surpass the energy investment of running pre-processing machines. Therefore, it is advised to closely follow research advances and adhere to pilot scale practice runs before changing feedstock and pre-processing strategies at industrial scale. For the study conducted by Dahunsi (2019), a large net positive energy improvement was observed with electrical energy production from biomass and pre-treatments within the range of 399-

523 kWh/t TS, confirming the economic viability of mechanical pre-treatments in their use case (55).

Commercial applications generally employ chemical or mechanical pre-treatment forms to reduce HRT and reactor size, resulting in improved economic viability of the business plan (54).

Electromagnetic Pre-treatment

A few studies have been performed assessing ultrasonic and microwave irradiation on lignocellulosic structures to reduce their recalcitrant structures (91). Ultrasonic pre-treatment allows for the degradation of polymer layers at high temperatures, leading to improved biogas yields. Siddique et al. (2017) improved methane yields of wastewater by 25% using ultrasonic pre-treatment methods (290). Furthermore, food waste and waste agricultural sources were pre-treated in a study by Naran et al. (2016) to improve biogas yields by 56.2% using an energy intensity of 360 kJ/L (291). Microwave irradiation also proves to promote feedstock degradability to improve biogas yields, as shown by Siddique et al. in their 2017 study, which enhanced biogas production of agricultural wastes by 53% (290).

Fungal Pre-treatment

The high amount of lignin contained within lignocellulosic biomass acts as a barrier for the biodegradable carbohydrate polymers contained within the cell structures (292). Within literature, it is commonly accepted that the most successful pre-treatment tool is the introduction of fungi for lignocellulosic degradation (293,294). For instance, a widely used fungi is the wood-rotting fungi called *white rot fungi* or Anaerobic fungi (AF), which delignify the impermeable materials contained within agricultural waste substances at low temperatures (293,294). This class of fungi, containing several fungi species, has the advantage of simultaneously degrading cellulose, hemicellulose and lignin at similar rates, allowing for the minimisation of time as no major bottlenecks are created (295,296). The process is terminated with residue simple sugars and feedstock that is now susceptible to improved enzymatic degradation (according to enzymatic pre-treatment in section 6.5) (54).

Performance of fungal pre-treatments is dependent on their ability to colonise on the host material and the propensity of lignolytic enzyme production like manganese peroxidase, lignin peroxidase and lacasse (54,297,298). The fungus is also a well-known species due to its ability

to cultivate on many biomass feedstocks and allows for improved gas production of up to 500% and methane yield increases because of augmented nutrient availability (297,299). The research group among Zhao et al. (2013) discovered that fungal pre-processing of yard trimmings resulted in a methane yield improvement by 154% at a moisture content of 60% with natural aeration, proving the importance of correct ambient conditions to maintain optimal fungal performance (300).

Fungal pre-treatment is expensive (54), and coupled with plant upgrading (in the case of the *white rot fungi*) to accommodate these changes renders its economic viability. The greatest benefit of AF is their ability to be used during the AD process, thus allowing for simultaneous degradation and fungal reproduction within the biogas digester without the need of a more-complicated two-stage system or a rigorous prior pre-treatment regime (301).

Tabatabaei et al. (2020) quote an improvement in methane production in between 15-500% (54). This method is commonly accepted as the best strategy to delignify lignocellulosic biomass. The eco-friendly approach has a fast growth rate, and the fungi are generally very easy to cultivate. The feedstock is further processed so that is it more susceptible to feedstock decomposition in the enzyme liquefaction step (54,284,293,297,302). Disadvantages of fungal pre-treatments include the high retention time required, as the actual process is generally slow. Investment and planning costs may be extensive, and careful installation of fungal pre-treatments is essential. The fungi may also consume a part of the generated sugars, which will reduce biogas yields and reduce the sugar efficiency. Finally, a sterilisation step is required before fungal pre-treatment may occur, further raising the energy investments (54,284,293,297,302).

Microbial consortium (MC) pre-treatment

It has been suggested in various research streams that the use of mixing independent microorganisms into specialised MC can lead to significant advantages in biogas yield improvement (303). In contrast to fungal pre-treatment strategies that focus primarily on the degradation of lignin, MC, which generally are developed to contain between 2-4 independent strains, treat cellulose and hemicellulose material within the agricultural waste (299). The additional benefit of using multiple strains of microbes comes in the form of the diversity they bring and the subsequent improved adaptability within the biogas digester (304). With

improved adaptability, it becomes easier for microbes to grow, consume biodegradable material faster, stabilise pH easier and perform enzymatic saccharification at a faster rate than if solely one strain of microbiomes is used (305).

As an example, Zhong et al. (2011) performed AD of corn straw with a MC which resulted in specific methane yield increases within the range of 25-96.6% (302). Their MC consisted of various microbial systems such as yeast species, cellulytic/hemicellulytic/lignolytic bacterial species, and lactic acid bacteria (302). The synergies of the MC became obvious at cellular level: yeast bacteria reduced the crystallinity index of lignocellulosic biomass, allowing the cellulytic and hemicellulolytic bacteria easier access to the now exposed cellulose/hemicellulose/lignin compounds which were contained within the agricultural waste. Lignolytic bacteria supported the AD process by stabilising pH levels within the preferred range, which not only improved yield, but also reaction kinetics (302).

The strategy of MC is independent of sterilisation steps and is easily incorporated into the established AD process through simultaneous pre-treatment (54). A meticulous physiological examination is necessary to develop a MC which is capable of decomposing lignocellulosic biomass efficiently (54). This extremely scientific barrier is currently inaccessible to industrial biogas plants, as analysis of microorganisms is seldom conducted in laboratories rather than industrial reactors (54). Furthermore, specific MC may only work for limited types of lignocellulosic biomass, and special fine-tuning may be required if the operator wishes to change feedstock. This further complicates application as co-digestion regimes are practically ubiquitous in the current industry biogas-landscape.

Biological pre-treatment strategies like fungal and MC might be slower than chemical and mechanical counterparts but save energy in the process and are generally more environmentally friendly as no release or contact with toxic chemicals occurs (91). MC prove a high ability to enhance methane yields, with literature concluding improvements of 16-97% (54,284,299,302,304,306–308). The eco-friendly solution provides an improved metabolic diversity, high adaptability and rapid growth potential. More feedstock may be digested simultaneously, as various microbes work with synergies to digest nutrients more quickly (54). This leads to a higher OLR to be used, thus savings in reactor size and costs. MC also do not

require a sterilisation step and the pre-treatment method may occur in parallel with the actual AD process ((54,284,299,302,304,306–308).

Enzymatic pre-treatments

Cellulases, cellobiases, endoglucanase, ligninolytic, pectinases and xylanases, along with amylases and proteases are used in different studies to transform lignocellulosic material into readily biodegradable sugars to improve biogas yield (309,310). Given the enzymatic processes, it is advised to perform a different pre-treatment (for example fungal or MC) before employing enzymatic aftertreatment to fully maximise on the yield production potential (54). The reason for this lies within the fact that enzymes readily degrade materials within lignocellulosic biomass, whilst fungal treatments and MC further support the degradation of cellulosic cell walls.

Hydrolytic enzymes can be used in conjunction with fungi to improve degradation of lingnocellulosic biomass (311). The methane yield is doubled by the AD study on chicken feathers by Patinvoh et al. (2016) (312), whilst a 28% biogas increase of sugar beet pulp pre-treatment with hydrolytic enzymes is performed by Zieminski and Kowalska-Wentel (2015) (313).

The metabolic cost associated with the addition of microbial proteins/enzymes and their synthesis using peptides and amino acids is cheaper than using nitrogen sources such as ammonium ions, leading to high availabilities and high biogas yields within the product (54). Furthermore, due to the high availability and uptake of amino acids and peptides, there is no compound build-up within the digester, minimising the threat of inhibiting materials during the AD process (314).

Generally, it is required to sterilise the feedstock after enzymatic processing to prevent sugar consumption prior to AD by the endogenous microorganisms (54,315). Moreover, economic feasible enzyme pre-treatments are only viable if the enzymes and biosurfactants can be created on a low-cost and low-effort basis. Enzymatic pre-treatment guarantees a high sugar yield for further digestion (299,309,310,315). The strategy comes with a few disadvantages as well. For instance, a further pre-treatment strategy is often needed to improve enzymatic liquefaction and to attain high efficiencies. Methane yields are generally improved by 0-34%,

(54). Finally, enzyme development and procurement may make installation and maintenance costs soar, rendering the effectiveness of the project (299,309,310,315).

11.4.2 Other pre-treatment techniques

Ensiling

Ensiling is a successful aerobic strategy used to deplete oxygen from the feedstock prior to AD (316). Following the exhaustion of oxygen in the first step of ensilage, lactic acid fermentation is initiated, which converts water-soluble carbohydrates into organic acids (such as lactic acid) under anaerobic conditions (317). The pH of the feedstock is reduced to below 4.5 due to the presence of the acids, obstructing the development of unwanted microorganisms (318). Ensiling is said to improve hydrolytic breakdown of lignin layers along with creating pores within the lignocellulose matrix (54), allowing for improved colonisation of fungi and penetration into cell walls for improved mass utilisation of the feedstock, contributing to the degradation of material during AD (319).

The benefit of ensilaging is that unfavourable microorganisms are not formed with the same propensity as in other processes due to lactic acid formation (54,295,319–321). There is an improved hydrophilicity of lignin, augmenting the biodegradability of the substrate. Ensiling provides methane yield enhancements of 7-15%, but requires a high degree of accuracy and experience for successful implementation (54,295,319–321).

Microaeration

Microaeration is an aerobic biological pre-processing tool used to treat complex substances by using slight amounts of oxygen in an inexpensive process, as compared to the previous main types of pre-treatments examined (54). Microaeration improves the mixture of the microbial community within the substrate, allowing for enhanced performance during the hydrolytic phase of AD (54,295,322,323). The strategy is a very cost-effective, but it may be difficult to regulate the oxygen necessary for optimal microaeration. The process also incurs a loss of substrate, so careful planning of oxygen control is decisive to maximise biogas yield (54,295,322,323).

Composting

The objective of using composting is rooted in the aim to increase the feedstock temperature prior to usage within AD, so as to reduce heating and maintenance costs of the biodigester (324). However, the process of composting inevitably leads to degradation of organic material in aerobic conditions, which causes a reduction in biogas production due to losses experienced within the composting stages. It is therefore advised to plan composting strategies carefully to minimise the degradation and minimise organic matter losses (54). The advantages of composting include that there is no necessity of employing highly technical equipment for the composting process, along with the relative ease and speed at which composting can occur (54).

Genetically- and metabolically-engineered microorganisms

Various metabolic engineering strategies have been used to refine strain performance in AD, such as screening, metagenomic sequencing, gene synthesis and enzyme/pathway engineering (325,326). Further advancements in reporting technology and tools for bioinformatics support the development of genetically modified microbial consortia for boosted biogas production (327).

A metabolic engineering project recently investigated the performance enhancement of hydrolytic microorganisms through genetic modification (328). A different strategy was to use non-hydrolytic bacteria that grow at a fast rate as host bacteria, then genetically changing the bacteria to encode the production of hydrolytic enzymes in the fast-growing host microbes (328). As a result, enzymes like cellulases and hemicelluloses are created and produced quickly within the feedstock material (328). Research efforts are also focussed on genetic engineering of enzyme production to develop enzyme clones for efficient hydrolysis (329,330).

Regulations are stringent and care must be taken when researching and implementing genetically modified substances on biomass. On an industrial scale, the use of engineered strains is vastly restricted based on feedstock regulations and unknown scientific consequences due to the novelty of the research area (328).

11.5 Appendix 5: Livestock headcounts in EU countries and derivation of manure estimations for Figure 5

Table 21: Headcounts of livestock populations in the EU for the year 2020, including a derived annual manure production.

GEO	Bovine Total Heads	Swine Total Heads	Manure Estimation (kg/Year)
EU-27	7.94E+07	1.39E+08	1.80E+12
Belgium	2.50E+06	6.18E+06	6.42E+10
Bulgaria	5.98E+05	6.38E+05	1.50E+10
Czechia	1.41E+06	1.54E+06	2.87E+10
Denmark	1.57E+06	1.24E+07	6.24E+10
Germany	1.24E+07	2.87E+07	2.93E+11
Estonia	2.58E+05	2.80E+05	5.22E+09
Ireland	7.22E+06	1.60E+06	1.15E+11
Greece	6.20E+05	7.69E+05	1.38E+10
Spain	6.09E+06	2.39E+07	1.92E+11
France	1.90E+07	1.36E+07	3.84E+11
Croatia	4.18E+05	9.45E+05	9.95E+09
Italy	6.11E+06	8.38E+06	1.33E+11
Cyprus	5.37E+04	2.65E+05	1.77E+09
Latvia	4.35E+05	3.61E+05	9.17E+09
Lithuania	7.40E+05	6.27E+05	1.53E+10
Luxembourg	2.01E+05	9.23E+04	3.81E+09
Hungary	8.48E+05	2.98E+06	2.53E+10
Malta	1.47E+04	4.16E+04	3.96E+08
Netherlands	4.25E+06	1.25E+07	9.95E+10
Austria	1.93E+06	2.88E+06	4.09E+10
Poland	5.95E+06	1.10E+07	1.32E+11
Portugal	1.57E+06	1.88E+06	3.47E+10
Romania	1.85E+06	4.14E+06	5.58E+10
Slovenia	4.86E+05	2.73E+05	8.39E+09
Slovakia	4.52E+05	4.84E+05	9.43E+09
Finland	9.09E+05	1.23E+06	1.69E+10
Sweden	1.49E+06	1.35E+06	2.73E+10

The data portrayed in Table 21 has been reduced from further data available in the Eurostat databases. Original tables also include data relating to animal age ranges, animal applications and detailed types/races of animals reared. Through the support of supplementary literature and by using these additional information sources, an estimation of the annual manure accumulation through livestock rearing can be taken for all EU countries.

The work conducted by Scheftelowitz and Thrän (2016) has been used to simulate manure accumulation, along with the resulting biomethane production potential. The raw data for Figure 5 can be seen in Table 22. All available biomass that can be readily digested through AD

is derived from the crop productions and percentage shares of lignocellulosic biomass that are publicly available in waste databases on biomass in the Eurostat portal for each EU country (36,38). Through knowledge of the waste fractions accumulated in each type of lignocellulosic waste, a sum of crop residues, fodder crops and grazed biomass can be taken to obtain an aggregated reference of each country's unutilised waste products in the agricultural sector. When taking reference values of VS content reduction and conversion rates to biomethane (described in further detail in Chapter 4), a multiplication factor incorporating the conversion of VS content to biomethane will yield an estimated value of the biomethane that would be generated if all existing registered waste sources are fully utilised in each country.

In a similar manner, the Eurostat database information was used to determine various types and propensities of bovine and swine animals in the EU (37,39,82–84). By taking animal weight ranges and their application in the livestock sector, it was possible to make estimations on their respective manure production on a daily basis (331–335). Though utilising the same reference values of biomethane production through VS reduction that has been conducted in the literature review in Chapter 4, the conversion of all available manure to biomethane can be estimated for each EU country (70). Furthermore, an additional scenario was built where both feedstocks are digested under a co-digestion regimen. Given that co-digestion generally produces a higher efficiency, substantially elevated methane production potentials were obtained by the use of improved degradation of the VS present in the mixture and by improved degradation conditions present. As such, a factor of 350 L CH_4 / $VS_{reduced}$ in comparison to 150 L CH_4 / $VS_{reduced}$ was taken, which is in line with other research conclusions on the efficiency of VS reduction to biomethane production, and is discussed in further detail in Chapter 4.

Table 22: Overview of biomethane generation potentials and current production shares based on existing waste accumulation

GEO	Available Biomass from Lignocellulosic Biomass (kg/annum)	Available Biomass from Livestock Waste (kg/annum)	Biomethane Potential through Lignocellulosic Biomass (L CH4 STP / annum)	Biomethane Potential through Livestock Waste (L CH4 STP/ annum)	Biomethane potential of combined waste streams with improved co-digestion efficiency factor (L CH4 STP/ annum)	Current Biomethane production (est.) (L CH4 STP / annum)	Production share
EU-27	4.04E+11	1.80E+12	6.06E+13	2.03E+11	6.17E+14	1.63E+13	2.65%
Belgium	2.66E+10	6.42E+10	3.99E+12	7.76E+09	2.43E+13	2.70E+11	1.11%
Bulgaria	1.82E+10	1.50E+10	2.73E+12	1.59E+09	1.08E+13	5.91E+10	0.55%
Czechia	1.30E+10	2.87E+10	1.95E+12	3.06E+09	1.29E+13	6.59E+11	5.10%
Denmark	2.03E+10	6.24E+10	3.04E+12	8.98E+09	1.60E+13	5.66E+11	3.54%
Germany	1.12E+10	2.93E+11	1.68E+12	3.48E+10	7.47E+13	8.59E+12	11.50%
Estonia	2.85E+09	5.22E+09	4.27E+11	5.45E+08	2.56E+12	2.20E+10	0.86%
Ireland	3.17E+10	1.15E+11	4.76E+12	1.10E+10	4.95E+13	5.80E+10	0.12%
Greece	9.70E+08	1.38E+10	1.45E+11	1.45E+09	4.44E+12	1.50E+11	3.38%
Spain	5.26E+10	1.92E+11	7.89E+12	2.43E+10	5.86E+13	3.59E+11	0.61%
France	3.78E+09	3.84E+11	5.67E+11	3.89E+10	1.21E+14	1.26E+12	1.04%
Croatia	8.26E+09	9.95E+09	1.24E+12	1.18E+09	5.34E+12	9.22E+10	1.73%
Italy	5.26E+10	1.33E+11	7.89E+12	1.47E+10	5.48E+13	2.24E+12	4.08%
Cyprus	5.93E+07	1.77E+09	8.90E+09	2.34E+08	3.54E+11	1.47E+10	4.16%
Latvia	3.15E+09	9.17E+09	4.73E+11	9.54E+08	3.86E+12	8.90E+10	2.30%
Lithuania	1.35E+10	1.53E+10	2.03E+12	1.57E+09	9.46E+12	4.28E+10	0.45%
Luxembourg	9.66E+08	3.81E+09	1.45E+11	3.76E+08	1.57E+12	1.99E+10	1.27%
Hungary	8.35E+08	2.53E+10	1.25E+11	3.23E+09	5.50E+12	9.92E+10	1.80%
Malta	1.18E+08	3.96E+08	1.77E+10	4.90E+07	1.30E+11	1.49E+09	1.15%
Netherlands	8.90E+09	9.95E+10	1.33E+12	1.20E+10	2.66E+13	4.61E+11	1.73%
Austria	1.50E+10	4.09E+10	2.25E+12	4.60E+09	1.62E+13	2.34E+11	1.44%
Poland	7.09E+10	1.32E+11	1.06E+13	1.54E+10	5.83E+13	3.58E+11	0.61%
Portugal	8.00E+09	3.47E+10	1.20E+12	3.69E+09	1.29E+13	9.18E+10	0.71%
Romania	2.92E+10	5.58E+10	4.37E+12	6.28E+09	2.51E+13	2.05E+10	0.08%
Slovenia	3.38E+09	8.39E+09	5.07E+11	8.52E+08	3.79E+12	2.99E+10	0.79%
Slovakia	9.95E+09	9.43E+09	1.49E+12	1.00E+09	6.23E+12	1.45E+11	2.33%
Finland	3.70E+09	1.69E+10	5.55E+11	1.84E+09	6.09E+12	1.87E+11	3.08%
Sweden	1.08E+10	2.73E+10	1.61E+12	2.90E+09	1.18E+13	2.06E+11	1.75%

11.6 Appendix 6: Master table of all summarised experiments and studies

The following summary shows the master table of all studies incorporated into the statistical analysis in Chapter 4 (52,77,105–108,113,118,122–124,126,131–181).

Table 23: The master table of all data summarised in the statistical analysis of Chapter 4

Table X: The Master Table of 65 Studies summarised

Category	Substrates	Feedstock Ratio	C:N Ratio Numerical	C:N Ratio	OLR Numerical	OLR (kg VS / m^3 . Day)	SRT/HRT Numerical	SRT/HRT (days)	Experiment Scale?	Temperature Regime?	Experiment scale (Pilot, laboratory, bench?)	Reactor Type?	Pretreatment? Preprocessing? Pre-processing?	pH Numerical	pH level	VS Reduction N	VS reduction	Methane Yield N	Methane yield (L/kg VS)	Methane % percentage increase	Methane composition (in bi	Methane composition (in biog	Further Remarks/Comments/Pretreatment?	Reference
	Straw : Cattle Manure	78:22 % VS	30	30	2.8	2.8	25	25 days	Laboratory	mesophilic	Laboratory	CSTR reactor (5L)	Steam Explosion	7.65	7,5-7,8	N/A	N/A	170	130 - 210 L/kg VS	N/A	55.5	53-58%	the impact of a pretreatment (steam explosion) on gas yield and process stability during co-digestion of the straw with cow manure was investigated.	Risberg (2013)
	Jatropha Seed Cake : Bagasse	2:1 ratio	25.05	Between 26.5:1 and 23.6:1	N/A	N/A	15	15 days	Laboratory	mesophilic	Laboratory	batch	Addiction of Fe2+ to JSC and bagasse mixture	N/A	N/A	N/A	N/A	203	203 L/kg VS	N/A	66	66%		Sen (2013)
	Liquid Hydrolysate of Wheat Straw : Seaweed Hydrolysate	01:01	N/A	N/A	3.6	3.6 ± 0.5 kg COD/m3.d)	2.7	2.7 ± 0.5	Pilot	mesophilic	Pilot	UASB	Pretreatment of the straw hydrolysate with nutrient supplementation in a UASB reactor	6.91	6.91	N/A	COD reduction: 95%	220	220 ± 0.07 L/kg VS	N/A	56	56 %± 2%		Nkemka (2013)
	Algal Biomass : Glycerol	5,5:20,02 g VS/L and 9,76 g VS/L of inoculum concentraction	N/A	N/A	N/A	N/A	N/A	N/A	Laboratory	Thermophilic	Laboratory	N/A	N/A	N/A	N/A	N/A	N/A	231	2.31 mL/g VS	N/A	N/A	N/A		Sittijunda (2020)
	Blue Algae : Corn Straw	65:35 %	20	20:1 ratio	6	6.00 kg VS / m3 . Day		10 days	Laboratory	mesophilic	Laboratory	Continuous feed digesters	N/A	7.42	7.42 ±0.11	63	63%	234	234 L/KG VS	46% Compared with the algae alone	62.35	62.35%± 6.14%		Zhong (2013)
	Wheat Straw : Microalgal Biomass	50:50 % VS	13	13:1	0.97	0.97 ± 0.02 kgVS/m3 d	20	20 days	Laboratory	mesophilic	Laboratory	mesophilic lab-scale reactor	Thermoalkaline pretreatment (10% CaO at 75ºC for 24 h)	7.49	7.49 ± 0.16	48.3	48.3% ± 2.9%	240	240 ± 2 L/kg VS	77%	67	67.0% ± 0.7%	The pretreatment increased the methane yield by 15% compared to the untreted mixture All data is about the production with thermo-alkaline pretreatment	Solé-Bundó (2017)
	Crop Residues : Sugarcane scum	75:25 VS	24.7	24.70	N/A	N/A	N/A	N/A	Batch	mesophilic	biochemical methane potential tests	batch	ACR (agricultural crop residues) through particle size reduction, and SCS (sugarcane scum) with dilution	7.37	7.37 ± 0.16	77	77%	276	276 L/kg VS	N/A	N/A	N/A		Mendieta (2020)
	Cassava Pulp : Swine Manure	CP:PM = 60:40	33	33:1	3.5	15	15	15 days	Laboratory	mesophilic	CSTR experiment	a semi-continuously fed stirred tank reactor (CSTR)	N/A	7.11	7.11 ± 0,05	61	61% ± 1%	306	306 ± 13 L/kg VS	60%	57	57%		Panichnumsin (2010)
	Sugarcane Filter Cake : Bagasse	70:30 %	41	41:1	3	3 kg VS / m3 . Day	35.8	35.8 days	Laboratory	mesophilic	Laboratory	semi-continuous stirred-tank reactor mesophilic conditions (38 ± 1 C)	N/A	6.45	6.45	N/A	N/A	320	320 L/kg VS	N/A	55	55% v/v	semi-continuous feeding	Janke (2016)
	Vinasse : Sugarcane Press Mud Cake	75:25 and 50:50 % VS	N/A	N/A	2.2	2.2 kg VS / m3 . Day	24.1	24.1 days	Laboratory	mesophilic	Laboratory	CSTR reactors	N/A	7.4	7.4	N/A	N/A	365	365 L/kg VS	N/A	62.4	62.40%	Digestion process was stopped after 30 days when no more than 1% of daily methane volume was produced	López (2017)
Lignocell ulosic Biomass	Cassava Pulp : Swine Manure	77:23 (wet w/w)	35	35:1	5	4-6 kgVS/m3 d	N/A	N/A	Laboratory	Mesophilic	Laboratory scale mesophilic digester	Single stage **semicontinuously** stirred reactor with total volume of 10 L and working volume of 7 L	N/A	7.68	7.68 ± 0.08	82	82%	380	380 L/kg VS	N/A	60	60%		Glanpracha (2016)
	Microalgae : Barley Straw/Beet Silage/Brown Seaweed	N/A	25	25	4	4.0 kg VS / m3 . Day	N/A	N/A	Batch	mesophilic	N/A	batch	N/A	N/A	N/A	N/A	N/A	404	404 L/kg VS co-digestion with beet silage	62%	N/A	N/A		Herrmann (2016)
	Agro-Residues : Slaughterhouse Wastes	50:50 ww or 62:38 VS	N/A	N/A	N/A	N/A	26	26 days	Laboratory	Thermophilic	laboratory	batch	N/A	8.39	digestate: 8,39	N/A	N/A	647	647 L/kg VS	31%	N/A	N/A	this study concludes that a mixture of four substrates (slaughterhouse wastes (SB), manure (M), various crops (VC), and municipal solid wastes (MSW) have better results	Pagés-Dias (2014)
	Palm Oil Decanter Cake : Crude Glycerol	2:0,75% TS	N/A	N/A	N/A	N/A	17	17 days	Laboratory	mesophilic	N/A	CSRT	N/A	5.3	highter than 5.3	59	Waste reduction based on TS (59%)	736	736 L/kg VS	N/A	71	71%	The reactor with working volume of 4 L	Kanchanasuta (2017)
	Microalgae Residues : Poultry Manure	8.7:91.3 (VS)	4.29	Aprox 4.29	N/A	N/A	N/A	N/A	Laboratory	mesophilic	Laboratory	Batch	In this study, we can find several pretreatments such as: Pretreatment with dilute acid (5% H2SO4) for 1.2 h (Chlorella sp.) Thermal pretreatment at 75 °C for 10 h. (Chlorella sp. and Microalgae-bacteria consortium) Autohydrolysis co-pretreatment at 55 °C. and Thermal pretreatment at 120 °C for 60 min.	7.59	7.59	N/A	N/A	124.62	124.62 L/kg VS	N/A	16.14	16.15 %		Torres (2021)
	Vinasse : Glycerol	50:50 % COD	N/A	N/A	10.1	10.1 kg COD/m3.d	N/A	N/A	Laboratory	Thermophilic	Laboratory	batch	N/A	7.4	7.4 ± 0.1	N/A	N/A	139.32	139.32 mol CH4 m−3 day−1	N/A	83.1	83.1 %± 0.1		Lovato (2019)
	Oat Straw : Cow Manure	2:1 ratio (based on TS)	27	27:1	N/A	N/A	N/A	N/A	Batch	mesophilic	batch experiments	batch	N/A	8.1	7.80–8.40	53.46	53.46%	841.77	841.77 L/kg VS	N/A	44	The methane content was stable between 35% and 53%	higher CM concentrations were found to be inhibitory.	Zhao (2018)
	Palm Pressed Fiber : Cattle Manure	3:1 VS	N/A	N/A	N/A	N/A	N/A	N/A	Laboratory	mesophilic	laboratory	batch	N/A	7.9	7-8.8	91.1	91.1%	93.6	93.6 L/kg VS	N/A	34	34.0-77.0 %	3:1 recommended for practice	Bah (2014)
	Pulp and Paper Sludge : Dairy Manure	50:50 ratio	N/A	N/A	14.91	14,91±0,42 kg VS / m3 . Day	10	10 days	Laboratory	mesophilic	Laboratoy	semi-continuous (2L)	N/A	7.24	7,24±0,03	55.74	55,74±0,27	N/A	N/A	N/A	56.54	56,54±4,21%,	In comparison with PPS and PM alone, obviously co-digestion improved the methane production efficiency of 35.66% and 9.39%, respectively.	Chen (2013)
	Food Waste : Chicken Manure	the cycle was of three days, exactly, two days fed with FW and the third day fed with CM	N/A	N/A	2.5	2,50 kg VS / m3 . Day	35	35 days	Laboratory	mesophilic	semi-continuous anaerobic digestion	CSTR with a total volume of 5 L and a working volume of 3.5 L	N/A	7.59	7.59	N/A	N/A	507.58	507.58 L/kg VS	N/A	58.78	58.78%	The raw CM was pretreated via ammonia removal for semi-dry or dry anaerobic digestion	Wang (2014)
	Food Waste : Chicken Manure	70:30 g VS	20	20.0	4	4 kg VS / m3 . Day	N/A	N/A	Laboratory	mesophilic	N/A	CSTR with the working volume of 87 L	N/A	7.2	7.2	63.01	63.01%	655	655 L/kg VS	89.9%	N/A	N/A		Chuenchart (2020)
	Food Waste : Dairy Manure	8:4 g-VS/L (batch) 10:5 g-VS/L (CSRT)	15.8	15,8 (batch)	10	10 kg VS / m3 . Day (CSRT)	N/A	N/A	Laboratory	mesophilic	Laboratory	batch and CSTR	N/A	7.5	7,5 (batch) 7.1 (CSRT)	N/A	N/A	388	388 L/kg VS in batch test 317 L/kg VS in CSRT test	55,2 % in CSRT	60.2	60.2% (CSRT)	Addition of cattle manure enhanced the buffer capacity (created by NH+4 and VFAs), allowing high organic load without pH control. The C/N ratio and the higher biodegradation of lipids might be the main reasons for the biogas production improvement.	Zhang (2013)
	Food Waste : Fruit and Vegetable Waste : Sewage Sludge	2:1:1 ratio	N/A	N/A	4	4.0 kg VS / m3 . Day	16	16 days (Pilot experiment and ORL=8)	Pilot	mesophilic	bench-scale tests and pilot-scale experiments	Bench-scale tests were carried out in a 6-L semicontinuous stirred tank reactor (S-CSTR) with a working volume of 4 L Pilot-scale experiments were conducted in a 2-m3 S-CSTR with a working volume of 1.6 m3	N/A	6.79	pH of 6.79	N/A	N/A	420.6	417.5 L/Kg VS for the bench-scale tests and 420.6 L/Kg VS for the pilot-scale	58.50% of total COD was converted to methane	N/A	N/A		Sun (2013)
Food Waste	Food Waste : Dairy Manure	30:70 %w/w	N/A	N/A	N/A	N/A	N/A	N/A	Laboratory	mesophilic	biochemical methane potential (BMP) assays.	N/A	N/A	7.55	7.2 to 7.9	N/A	N/A	456	180 to 732 L/kg VS (it depends on what the food waste is made of)	N/A	N/A	N/A		Ebner (2016)
	Food Waste : Sophora Flavescens Residue	7:3 Co-difestion ratio 4:1 Inoculum-to-substrate ratio	25.8	25.80,	N/A	N/A	N/A	N/A	Batch	mesophilic	batch anaerobic experiments	batch	N/A	N/A	N/A	N/A	N/A	640	640 L/kg VS	N/A	60	60%		Ma (2019)
	Food Waste : Spent Coffee Grounds	67:33 % VS	16	16	N/A	N/A	N/A	N/A	Laboratory	mesophilic	Laboratory (BMP tests were performed in 120-mL serum bottles with a 100-mL working volume, which were filled with equal volumes of the inoculum and a feedstock mixture)	BMP	N/A	N/A	N/A	N/A	N/A	535	535 L/kg VS	N/A	N/A	N/A		Kim (2019b)
	Food Waste : Cow Manure	50:50 %	N/A	N/A	N/A	N/A	N/A	N/A	Laboratory	mesophilic	laboratory	N/A	N/A	N/A	N/A	72.1	72,1±6,4%	410	410 L/kg VS	N/A	32	32%	Potato wastewater Brewery wastewater is used as a seed inoculum	Monou 2008
	Agri-Food Waste : Glycerol	straberry extrudate:fish waste: glycerol 54:5:41 VS	N/A	N/A	4.26	4.26 kg VS / m3 . Day	N/A	N/A	Laboratory	mesophilic	laboratory	N/A	N/A	7.2	7.2	97	97%	308	308 L/kg VS	N/A	N/A	N/A		Serrano (2014)
	Kitchen Waste : Cow Manure	1:1 (wet weight basis)	N/A	N/A	N/A	N/A	N/A	N/A	Laboratory	mesophilic	laboratory	Lab-scale anaerobic digesters with working volume of 1 L contained 700 mL total mixture with 200 mL inoculant.	Concentrated NaOH was used to adjust the initial pH to the values previously selected	7.8	between 7,7 and 7,9	N/A	N/A	179.8	179,8 L/Kg VS	N/A	N/A	N/A		Zhai (2015)
	Food Waste : Sewage Sludge : Glycerol	2:1:3%(v/v)	N/A	N/A	N/A	N/A	N/A	N/A	Batch	mesophilic	two-stage acidogenesis-methanogenesis anaerobic system under mesophilic conditions (35 C).	batch	the anaerobic sludge used as inoculum in the BMP tests was not subjected to heat-shock pretreatment, but only acclimated to the operating conditions (temperature of 35 C and rotation at 150 rpm) for 5 days for establishment of the methanogenic biomass	7.25	7–7.5	N/A	N/A	342	342 L CH4/kg VS	N/A	80	above 50% with maximum values close to 80%.		Silva (2018)
	Pig Manure : Corn Stover : Cucumber Residues	5 : 2 : 3 (wet basis)	14.5	14.5	N/A	0	40	40	Batch	Mesophilic	Batch testing Mesophilic operation at 35°C	1 L glass reactors	N/A	N/A	N/A	N/A	N/A	305.4	305.4	27 - 246 % Compared to Monodigestion	N/A	N/A	A reduced amount of VFA/TA and hemicellulose content was measured Studies proved that cucumber, pig manure and corn stover mixtures improved methane yields by 1.27 - 3.46 times as compared to mono-digestion The best performing mixture also proved to contain the largest amount of soluble substance (%TS) A CPI (Co-digestion Performance Index) of 1.97 was determined) Cucumber addition changed the diersity of microbes within the mixture, optimising methane yield. Compared to monodigestion, double the total nitrogen content and double the NH4+-N concentration is observed by co-digestion	Wang (2018)
	Dairy Manure : Meat and Bone Meal : Crude Glycerol	1 : 0.7 : 0.3 (VS)	13	13	2.65	2.65	30	30	Laboratory	Mesophilic	Laboratory Scale. Bench Experiment Semi-continuous operation Mesophilic (38°C)	12 L Semi-CSTR	N/A	7.22	6.77 influent 7.67 digestate	47	47%	420	420	~40% higher than monodigestion and 7.5% higher than co-digestion without glycerol	64.75	64.75%	Addition of crude glycerol lowered the pH The most successful trial had the highest C:N	Andriamanohiarisoamanana (2018)
	Chicken Manure : Food Waste : Wheat Straw	60 : 20 : 20	21.87	21.87	2.0	**2.0 kg TS /m3.d (best)** 2.5 kg TS /m3.d 3.0 kg TS /m3.d	20	20	Laboratory	Mesophilic	Semi-continuous operation Mesophilic at 37 ±1 °C	500 mL Glass Reactos with incubated shaker at 100 rpm	N/A	N/A	N/A	41.7	**OLR2.0: 41.7 ± 2.8** OLR2.5: 36.4 ± 9.4 OLR3.0: 35.3 ± 6.7	350.5	Biogas yields: OLR2.0: 350.5 ± 10.1 OLR2.5: 279.9 ± 9.8 OLR3.0: 220.1 ± 11.4	cw monodigestion chicken manure OLR2.0: 93.4 % OLR2.5: 117 % OLR3.0: 167.2 %	69.7	OLR2.0: 69.7 ± 5.1 OLR2.5: 68.6 ± 2.6 OLR3.0: 68.2 ± 3.2	For all AD reactor trials an OLR of 2 g TS/ L.d performed best An overloading in the reactors caused a drop in pH As not all substrates were degradable, they brought upon a subsequent pH increaseFor OLR 3.0, the VA content increased substantially, causing an inhibitory effect More undigested cellulose was found at high OLRs	Zahan (2018)
	Cattle Manure : Cheese Whey	50 : 50 (v/v)	N/A	N/A	1.7	1.7 kg COD/m3.d	20	20	Laboratory	Mesophilic	Bench-scale Continuous System Mesophilic at 35 ± 1°C	Two-stage Bench-scale reactor 500 mL working volume	N/A	6.3	Acidogenesis reactor: 5.1 ± 0.3 Methanation reactor: 7.5 ± 0.3	83	83 ± 6 % (sCOD)	258	258	N/A	60	60 ± 6	Two-phase led to doubling methane production as compared to the one-phase trial Maximum VFA concentration and H2 accumulation (68 ± 4%) ocurred after 5 days, fixing the HRT for acidogenic to 5 days Productivity: 031 ± 0.04 L/L.d CH4; 0.02 L/L.d H2 and 0.51 L/L.d Biogas	Bertin (2013)
	Swine Manure : Olive Mill Wastewater	60 : 40 (N/A)	N/A	N/A	4.4	4.4	30	30	Laboratory	Mesophilic	Countinuous testing at different OLRs Mesophilic conditions at 35 ± 1°C Magnetic stirring	CSTR with magnetic stirring device Two reactors with 3.9 L and 3.0 L working volumes	N/A	7.37	7.37 ± 0.06	71.22	71.22 ± 0.25%	373.00	373 ± 0.6	N/A	N/A	N/A	Various OLRs investigated: an increase in OLR did not negatively affect miomethanation, despite a higher concentration of inhibition compounds due to higher OLRs There was no change in methane yield despite compound inhibition by phenols The highest OLR also showed the highet degradation efficiency, at 77.78 ± 0.12 Also: 277 mL CH4/g COD	Kougias (2014)
	Chicken Manure : Apple Pulp	2 : 1	18.50	18.5	4.8	4.8	25	25	Batch	Mesophilic	Semi-continuous operation Batch Testing Mesophilic (37°C)	250 mL conical flasks for batch testing; 2 L working volume flaskwith 20 rpm stirring for semi-continuos testing	N/A	8.3	8.01 - 8.59	N/A	N/A	340	340	N/A	74.5	74.50%	Despite VFA and TAN accumulation, no process inhibition was observed as compared with chicken manure monodigestion Paper's objective is to determine adequate feedstocks to reduce likelihood of ammonia inhibition for different OLRs	Li (2018a)
	Pig Manure : Corn Stover	4 : 1	24.50	24.5	2.4	2.4	50	50	Batch	Mesophilic	Semi-continuous operation Batch Testing Mesophilic (37°C)	251 mL conical flasks for batch testing; 2 L working volume flaskwith 20 rpm stirring for semi-continuos testing	N/A	8.3	8.01 - 8.59	N/A	N/A	301	301	N/A	76.8	76.80%	Pig Manure and Corn Stover were the optimum ratio without containing Apple Waste and chicken manure	Li (2018a)
	Swine Manure : Corn Straw	1.22 : 1 (N/A)	25.00	25	2	2	20.73	20.73	Laboratory	Mesophilic	Mesophilic (37 ± 1°C) operaton, Laboratory scale, semi-continuous	8 L working volume CSTR 130 rpm	Corn Staw: 5% NaOH with 1 : 10 solid : liquid (g : ml) ratio for 5 days, manual mixing twice a day	N/A	N/A	N/A	N/A	514.75	Biogas: 514.75 Methane: 320.02	Biogas increase: 80.63%	62.17	62.17%	Study assessing C/N, OLR and TS contents on production performance A higher methane yield was produced compared to swine manure monodigestion Biogas slurry performance ocurred at C/N of 35	Ning (2019)

Manure	Chicken Manure : Agricultural Waste (coconut waste, cassava waste, coffee p	21 : 1.3 : 3.0 : 0.8 (w/w)		19.05	17.1 - 21	N/A	N/A	N	N/A	Batch	Thermophilic	Thermophilic (55 ± 2°C) and mesophilic (35 ± 2°C) conditions Semi-continuous batch testing	500 mL working capacity vials	Ammonia stripping of chicken manure	8	7.4-8.6	N/A	N/A	502	Thermophilic: 502 Mesophilic: 506	compared with manure only: Thermophilic: 93% Mesophilic: 50%	N/A	N/A		Pretreated chicken manure (ammonia stripping) further increased methane yield by 42% to 695 mL/g VS under mesophilic conditions Extremes in pH involved low bounds of 4.5 and high bounds of up to 8.5 100% ammonia inhibition occurs from concentrations of 8-13 g/L Ammonia accumulation is reduced by 39% due to pretreatment Improvement was brought upon by doubling the OLR, high biodegradability of crude glycerol, optimised C/N, and reduction in free ammonia concentrations Microorganisms obtained large amounts of nutrients from the glycerol	Abouelenien (2013)	
	Pig Manure : Crude Glycerol	97 : 3 (w/w)		27.00	27	2.6	2.6 ± 0.1		15	15	Laboratory	Thermophilic	Thermophilic (55°C) Semi-continuous Semi-continuous operation	4 L working volum semi-CSTR stirred at 60 rpm	N/A	7.85	Influent: 7.7 ± 0.1 Effluent: 8.0 ± 0.1	52.5	52.5 ± 2.4 %	470	Biogas: 470 ± 20	Biogas: 180% increase	N/A	N/A		The digestate cannot be used immediately as soil fertiliser, as still many degradeable products were measured within it -> a long OLR or post treatment is advised for optimum digestate management (in comparison, pig manure may immediately be used) Whilst ratio 1:9 initially produced most, all ratios produced a lot of biogas from early on in the experiment	Astals (20	*gives loads of insight into Analytical methods
	Dairy Manure : Rice Straw	3 : 5 (w/w)		15.00	15	1.6		1.6	47	47	Laboratory	Mesophilic	Laboratory scale Mesophilic (35°C)	0.8 L working volume cups	Mechanical grinding of Agricultural waste	6.125	pHinital: 5.25, pHstable: 7.00	51	51 ± 5 %	N/A	>17.000 L	N/A		57.45	50.4-64.5%		Ratio 7 : 3 had the highest initial biogas production rate	Li (2014)	
	Dairy Manure : Corn Stalks	4 : 5 (w/w)		15.00	15	1.6		1.6	47	47	Laboratory	Mesophilic	Semi-continuos operation Laboratory scale Mesophilic (35°C)	0.8 L working volume cups	Mechanical grinding of Agricultural waste	6.125	pHinital: 5.25, pHstable: 7.00	53	53 ± 5 %	19.428	19.428 L	N/A		57.45	50.4-64.5%		Possible analysis for bad performance of straw residue : Dairy Manure (9:1): C/N imbalance; Lack of trace nutrients; too high OLR for AD; Excessive carbohydrate level, leading to accumulation of VFA	Li (2014)	
	Dairy Manure : Wheat Straw	5 : 5 (w/w)		15.00	15	1.6		1.6	47	47	Laboratory	Mesophilic	Semi-continuos operation Laboratory scale Mesophilic (35°C)	0.8 L working volume cups	Mechanical grinding of Agricultural waste	6.125	pHinital: 5.25, pHstable: 7.00	55	55 ± 5 %	19.127	19.127 L	N/A		57.45	50.4-64.5%		There was no notable difference in biogas production speeds for different feedstock ratios tested (1:9, 3:7, 5:5, 7:3, 9:1)	Li (2014)	
	Manure (2 : 1 ; Cow : Pig) : Raw Meadow Grass	53 : 47 VS	N/A	N/A		3.8		3.8	15	HRT: 15 days	Laboratory	Thermophilic	**Continuous** laboratory scale Thermophilic	CSTR 3.5 L working volume	Simple Mechanical Pretreatment (Milling)	7.95	7.6 - 8.3	N/A	N/A	270	270 ± 20	114% (cf manure only)	N/A	N/A		Co-digestion of various agricultural products () resulted in a 62 - 221% higher methane production compared to AD of agricultural product only According to BMP testing, 72.78% of the expected methane yield was obtained Study on different OLRs and agricultural fat addition	Sondergaard (2015)	
	Dairy Manure : Agricultural Waste (fats)	75 : 25 (w/w)		13.40	13.4	3.7		3.7	19	19	Laboratory	Mesophilic	Laboratory scale, continuous mesophilic operatio	12.5 L CSTR	N/A	N/A	N/A	N/A	N/A	450	450	N/A		61	61%		Past OLRs of 3 kg VS/m3/day, the linear relationship of biogas production to OLR breaks down into decreased organic matter degradation. Conclusions show that lower than 25%w/w fat inclusion has no effect on methane productivity, whereas fat ratios exceeding 60% w/w destabilise the	Guillaume and Lendormi (2015)	
	Dairy Manure : Olive Mill Waste	3 : 1 (N/A)		12.60	Mesophilic: 11.86 Thermophilic: 12.6	5.5	Mesophilic: 5.5 g COD/L/d Thermopilic: 5.5 g COD/d		21.4	21.4	Laboratory	Thermophilic	Two-phase Continuous operation Mesophilic (37°C) and thermophilic (55°C)	75 L CSTR	N/A	7.6	Stable aboe 7.6	54	Mesophilic: 53.4 ± 1.7% Thermopilic: 54.0 ± 1.8%	210	Mesophilic: 179 ± 18 Thermopilic: 210 ± 30	Mesophilic: 73.3% (cw mono) Thermopilic: 17.3% (cw Meso)			62	Mesophilic: 62.8 ± 1.2% Thermopilic: 62.0 ± 1.5%		Comparison between thermophilic and mesophilic operations Thermophilic operation produced 17.3% more methane An alkalinity exceeding 0.8 renders effectivity of a digester The manure buffered the low alkalinity and pH level of the Wastewater, enabling efficient biodegradation in the absence of pretreatments or additives No dilution of olive mill wastewater was required, nor the addition of chemicals for efficient biogas production	Goberna (2010)	
	Poultry Manure : Olive Mill Wastewater	75 : 25 (v/v)		21.00	21	1.7	1.7 gl/reactor.day		20	20	Laboratory	Mesophilic	Laboratory Scale Mesophilic Operation (35°C) Continuous operation	25 L Barrel with recirculating mechanism	N/A	8.05	8.05	69.8	69.8%	9.33	Biogas: 0.52 L/Reactorv		17.2%		71.8	71.80%		Foam was manually removed, and not measured on VS basis Olive Mill Wastewater proves to be an effective influent due to ist high lipid content The pH of both laboratory and pilot reactors remained unchanged within 7.8-8.0	Gelegenis (2007)	
	Cattle Slurry : Vegetable Waste (9.2% TS) : Digester Inoculum	70 : 20 : 10 (% w/w)	N/A	N/A		N/A		0	N/A	N/A	Laboratory	Mesophilic	batch experiments - Laboratory scale Mesophilic	CSTR	N/A	7.8	7.7 - 7.9	52.1	52.10%	230.00	230 ± 10	N/A	N/A	N/A		Total loading was 72.8 kg VS/m3 Vegetable waste produced a decrease in methane yield as compared to the control system with cattle slurry alone Vegetable waste produced the majority of its gas within five days, far quicker than the other trials which had only produced half the relative methane	Callaghan (1999)	
	Cattle Slurry : Fish Offal (8.7% TS) : Digester Inoculum	70 : 20 : 10 (% w/w)	N/A	N/A		N/A		0	N/A	N/A	Laboratory	Mesophilic	batch experiments - Laboratory scale Mesophilic	CSTR	N/A	7.85	7.7 - 8.0	47.3	47.3	375	375 ± 10	N/A	N/A	N/A		Total loading was 79.2 kg VS/m3 Fish offal produced an increase in methane yield as compared to control system with cattle slurry alone Biogas increases in production were attributed to an increase of OLR and synergistic effects of N:N ratios.	Callaghan (1999)	
	Pig Manure : Crude Glycerol	96 : 4 (wet basis) VS: 25 : 75		48	48	6.8	1.7 ± 0.1 (g VS/Vreactor.day) 6.8 ± 0.4 (g VS/L.d)		20	20	Laboratory	Mesophilic	Mesophilic Operation Semi-continuous	4 L working volume CSTR 60 rpm	N/A	7.7	Influent: 7.6 ± 0.1 Effluent: 7.8 ± 0.1	77.7	77.7 ± 1.5 % COD: 84.9 ± 2.0 %	N/A	Biogas: 780 ± 20	~380% increase compared to mor	N/A	N/A		Analysis of macro-compounds, proteins, lipids, carbohydrates and fibres revealed that removal efficiencies decrease in co-digestion as a result of the nutrients provided by the glycerol Digestate quality remained high as a result of co-digestion Addition of 21 : 79 (VS) glycerine led to organic overloading	Astals (2012)	
	Cow Manure : Grass Silage	70 : 30		20.00	15 - 25	2		2	20	20	Laboratory	Mesophilic	Laboratory Scale Semi-continuous feeding Mesophilic Operation (35 ± 1°C)	4 L working volume CSTR		7.5	7.2 - 7.8	43	43%	268	268 ± 29		72.90%		53.00%	53 ± 2		Co-digestion of manure and crops with up to 40% VS was verified Increasing the proportion of crop feed further led to a decline in specific methane yield by 4-12% Doubling the OLR resulted in a deline of the specific methane yields by 16-26% Thedigestate post-methanation potential was quoted within the range of 0.9-2.5 m3 CH4/L wet weight digestate (12-31% of total CH4 production)	Lehtomäki (2007)	
	Cow Manure: Sugar beet tops	70 : 30		20.00	15 - 25	2		2	20	20	Laboratory	Mesophilic	Laboratory Scale Semi-continuous feeding Mesophilic Operation (35 ± 1°C)	4 L working volume CSTR	i) Agricultural chopping ii) 24h of prewilting	7.5	7.2 - 7.8	45	45%	229	229 ± 54		47.74%		56	56 ± 1		NH4-N concentrations of 33-48% were measured during all trials, despite feedstock concentration of NH4-N was lower at 30-38%	Lehtomäki (2007)	
	Cow Manure: Oat Straw	70 : 30		20.00	15 - 25	2		2	20	20	Laboratory	Mesophilic	Laboratory Scale Semi-continuous feeding Mesophilic Operation (35 ± 1°C)	4 L working volume CSTR	iii) Silage additive: lactic acid bacteria inoculant iv) Further chopping with garden chopper	7.5	7.2 - 7.8	33	33%	213.00	213 ± 17		37.42%		51	51 ± 1		Increasing the OLR from 2-4 led to an increase of 30-37% of post-methanation potential of the digestate Different OLRs were investigated. At an OLR of 5.4 g COD/L.d, the propionic acid to acetic acid ratio was higher than the critical threshold limit for metabolic imbalance	Lehtomäki (2007)	
	Cow Manure : Glycerol	20 : 80 (COD)		20.00	20	1.6	1.6 g COD/L.d		18	38	Laboratory	Mesophilic	Discontinuous operation Mesophilic	UASB-Type reactors, operational volume 5.3 L	Pretreatment of glycerol with sulfuric acid	7.4	7.1 - 7.7	N/A	>80% by COD	320	320 L/kg COD	N/A		54		54%	The maximum methane yield of 240L/kg COD removal was achieved at an OLR of 1.3 g COD/L.d Satisfactory operation within OLR ranges of 1.3-2.6 g COD/L.d	Nuchdang (2012)	
	Cow Manure : Rice Straw	1 : 1 (VS)		15.20	15.2	6		6	19	19 ± 1	Batch	Mesophilic	Batch testing & continuous bench experiments Mesophilic Operation (37 ± 2°C)	2.5 L (batch), manual mixing twice daily 40 L (bench), mixing 6 times a day at 80 rpm for 30 minutes	Rice straw chopping and grinding into less than 1 m	7.25	7.0 - 7.5	N/A	N/A	201.337	Batch testing: 196.03 Biogas yield: 383.5	Batch tests: Methane increase of 5.8% compared to monodigestion Continuous: N/A			52.5	50-55%		At an OLR of greater than 8 kg VS/m3.d, substantial foaming ocurred Batch testing yielded an anaerobic digestion efficiency (Methane yield/Total methane potential) of 44.2% 20 % Manure trial experienced VFA inhibition due to inadequate C:N; [N] concentration in broth too low This led to accumulation of intermediate products that were not further digested	Li (2015)	
	Pig Manure : Glycerol	80 : 20 (weight basis)		23.40	23.4	0		0	30	30 days	Laboratory	Mesophilic	Laboratory scale, mesophilic (35°C)	250 mL reactors	N/A	7.4	7.4	90.9	90.90%	249.6	249.6 L/kg VS 215 L CH4/kg COD	125% more than pig manure mon	N/A	N/A		20% Manure trial contained large concentrations of acetic, propionic and butyric acid The propionic/acetic acid ratio should not surpass 1.4	Astals (2011)	
	Manure : Wheat Straw	Lab: 95 : 5 (shredded or briquetted) Full: 91 : 9 (shredded and briquetted) Fresh Matter	N/A	N/A		N/A	N/A	Lab: 20 days Full: 25 days	20		Laboratory	Mesophilic	Lab Scale & Full-scale Mesophilic operation at 49 ± 1°C Continuous operation	Lab: CSTR 15 L at 100 rpm, fed daily Full: CSTR 30 m3. fed daily	Briquetting (+ shredding): 100 kWh/t Shredding only: 60 kWh/t	7.19	Lab: 7.19 ± 0.09 Full: N/A	N/A	N/A	213.6	Lab: 213.60 ± 6.46 (shredded) & 217.08 ± 8.77 (briquetted) Full: 351.33 ± 195.97	Lab: 31% improvement to only manure Full: 33% increase to only manure	N/A	N/A		Lab: 387 ± 9 (shredded) and 410 ± 25 (briquetted); Full: 410 ±31 ppm; Control Full-scale with manure only: 2320 ± 350 ppm Energy production: 116 m3 CH4/t for shredded, 120 m3 CH4/t for briquetted 46% of transport costs are saved due to briquetting Less than 10% of energy produced is for mechanical preprocessing	Xavier (2015)	
	Manure : Energy Crops (triticale and maize silage): Lignocellulosic Biomass (Onion and Potato Waste)	50 : 25 : 25 (VS)	N/A	N/A		3	Low: 2 High: 4	N/A	N/A	N/A	Pilot	Thermophilic	Continuous operation (390 days) both mesophilic and thermophilic profiles evaluated Semi-continuous feeding three times a day	Pilot-Scale CSTR 0.23 m3 working volume	N/A	8.5	LowMeso: 8.5 ±0.1 HighThermo: 8.5 ± 0.1 LowThermo: 8.5 ± 0.1	N/A	N/A	530	HighMeso: 480 ± 10 LowMeso: 470 ± 20 HighThermo: 540 ± 10 LowThermo: 520 ± 10	N/A		53.5	All % HighMeso: 52 ± 1 LowMeso: 54 ± 1 HighThermo: 53 ± 1 LowThermo: 54 ± 1		Analysis of Manure only and Manure+energy crops only as wellStable biological process due to low VFA accumulation No noticeable differences between different OLR: one may either increase the OLR or reduce design volume The biogas yield of 674.4 L/kg VS was 71.67% and 10.41% higher than the comparative monodigestion of rice straw and ping manure respectively	Guiliano (2013)	
	Pig Manure : Rice Straw : Kitchen Waste	1.6 :1 : 0.4		21.70	21.7	N/A		0	45	45	Laboratory	Thermophilic	Single-stage batch mesophilic experiments (37 ±	2L working volume filler bottle manual mixing twice daily	N/A	6.19	6.19 initial	55.8	55.80%	383.9	383.9	71.6 % increase compared with monodigestion of Rice straw 17.5 % increase compared to monodigestion of all individual components			56.92	56.92%		Addition of more than 26% kitchen waste brought upon production inhibition due to VFA accumulation Propionate and acetate dominated the VFA composition that have accumulated (50-70% concentration)	Ye (2013)	
	Cattle Manure : Corn Stover	3 : 1 (dry mass) + 15 g inoc		18.55	18.54655634	N/A		0	50	50 days	Laboratory	Mesophilic	Mesophilic Operation (35 ± 1°C)	0.8 L working volume Bottle	Liquid Fraction of Digestate, Ammonia Solution, and NaOH Comparison	7.5	7.5 (after AD)	55.45	55.45% total (ove	228.95	228.95	N/A	N/A	N/A		Pretreatment with Ammonium solution (concentration) yielded the highest cumulative biomethane production of 9911.31 mL CBP resulted in 25.40-32.01% higher yield as compared with untreated sample Digestion time of pretreated samples were accelerated, 20-37.14% faster	Wei (2015)	
	Swine Manure : Corn Stover	60 : 39.2 60 ml inoculum		25.00	25 : 1	N/A		0	30	30 days 12-13 recommended	Batch	Mesophilic	Semicontinuous System at 35°C (Mesophilic) ope	Serum bottles, 150 mL working volume Bath shaker at 120 rpm	NaOH at various concentrations (2%, 4%, 6%) and temperatures (20°C, 35°C, 55°C)	7	7.0 Phosphate buffer used	N/A	N/A	350	350	34.59 %	N/A	N/A		Of all pretreatments, ammonia (28 day) required the longest time to produce 80% of the maximal biogas production, as compared to 22 days for the other samples A lignin removal rate of 54.7% - 79.49% has been measured through pretreatment 6% NaOH at 35°C produced the highest methane yield, 34.59% higher than the untreated control All trials were forced to have a 25:1 C:N ratio, powdered Corn stover was mixed with a solution of The optimum addition of glycerol is expected to be within 5-10% addition	You (2014)	
	Cattle Slurry : Crude Glycerol	90 : 10 (wt weight)	N/A	N/A		2.3	2.3	N/A	N/A		Laboratory	Mesophilic	Laboratory scale, mesophilic (35-37°C)	3 L working volume CSTR	N/A	N/A	N/A	N/A	N/A	825.7	825.7	14.3%	N/A	N/A		15% glycerol addition yielded process failure due to process instability, as the acetogenic and methanogenic bacteria is impeded through the onset of metabolic products such as VFA/acidification	Robra (2010)	
	Swine Manure : Crude Glycerol	22.5 : 15 (grams)		17.47	17.47	N/A		0	20	20 days	Batch	Mesophilic	Laboratory scale 28 °C -> psychophilic?	100 mL reactors with 60% working volume	N/A	7.605	7.95 ± 0.23 initial 7.26 ± 0.03 final	N/A	N/A	344.13	344.13 ± 32.31 ml /g COD	N/A		52.04	52.04		Increasing Crude Glycerol to a certain degree impedes biogas production as a result of impurities, high pH and C/N ratio A gradual increase in methane yield observed from 3-6% addition of glycerol; It is not advised to increase	Aguilar-Aguilar (2021)	
	Pig Manure : Glycerine	94 : 6 (FM)	N/A	N/A		N/A		0	N/A	N/A	Batch	Mesophilic	batch experiments - Laboratory scale Mesophilic	1 L Eudiometer	N/A	N/A	N/A	N/A	N/A	617	617 ± 37.04	N/A		55.38599641	55.38599641		glycerol composition further	Amon (2006)	

Pig Manure : Maize Silage : Maize Corns : Rapeseed Meal : Glycerine	46 : 27 : 13 : 9 : 5 (FM)	N/A	N/A	N/A	0	N/A	N/A	Batch	Mesophilic	batch experiments - Laboratory scale Mesophilic	1 L Eudiometer	N/A	N/A	N/A	N/A	N/A	432	432 ± 5.05	N/A		61.62624822	61.62624822	Addition of Rapeseed Meal worsened performance (in terms of CH4 yield) compared to the Pig Manure : Maize Silage : Maize Corns : Glycerine	Amon (2006)
Cattle Manure : Cardoon Silage	60 : 40 (VS)	N/A	N/A	N/A	0		45 45 days	Batch	Mesophilic	Batch Reactors, Mesophilic	Glass Vessel Reactor, no stirring	N/A	N/A	N/A	N/A	N/A	308	308	N/A	N/A	N/A		NaOH pretreatment improved solubilization of milk thistle stalks by 77.7 % Cardoon Silage and pretreated Milk Thistle are good substitutes for Maize Silage	Kalamaras (2014)

Key:
1) The category is dictated by the substrate with the highest proportion.
2) Reference has the paper linked
3) OLR: Organic Loading Rate
4) SRT: Solids Retention Time
5) FM: Fresh Matter =? TS?

11.7 Appendix 7: Experimental protocols for COD, VS, TS, water content, VFA and alkalinity characterisation.

The following PDFs have been written as a guideline for daily/weekly analysis. They serve as a procedure to be followed to allow for knowledge dissemination and can be replicated/amended for future compound characterisation. They were used primarily for data collection in the semi-continuous experiment detailed in Chapter 6.

Fecha		Investigadores	

Reactor 1	Calibración Inicial	x Veces de sacar gas	Última vez	Suma de volumen
pH	%CH4	%CO2	%O2	Comentario

Reactor 2	Calibración Inicial	x Veces de sacar gas	Última vez	Suma de volumen
pH	%CH4	%CO2	%O2	Comentario

Reactor 3	Calibración Inicial	x Veces de sacar gas	Última vez	Suma de volumen
pH	%CH4	%CO2	%O2	Comentario

Reactor 4	Calibración Inicial	x Veces de sacar gas	Última vez	Suma de volumen
pH	%CH4	%CO2	%O2	Comentario

Instrucciones:

1. Medir volumen de Gas
    - 1.1 Apagar los motores de agitación
    - 1.2 Conexionar la bolsa tedlar (para almacenar el gas)
    - 1.3 Empezar con la calibración. La calibración te dirá una pista para que puedas trabajar más rápido
    - 1.4 Una vez que sepas el volumen de la calibración, puedes anotarlo en el cuadro
    - 1.5 Saca gas hasta que la bolsa tedlar está llena (o el reactor está vacío de gas)
    - 1.6 Cuando no hay más gas en el reactor y no alcancas hasta el marco 50, tienes que hacer la última medicion manualmente (igual a la calibración)
    - 1.7 Anota las veces de sacar gas en el cuadro. Haz el cálculo para saber la suma de todo el volumen
2. Medir compuestos de gas
    - 2.1 Encender el equipo y pulsar "Next" después de que dice "self-test failed"
    - 2.2 "Next ID"
    - 2.3 "Meter la bolsa de gas al equipo"
    - 2.4 "Next", "No ID", "Next"
    - 2.5 Anotar los compuestos de gas en la tabla
3. Sacar cierta cantidad de digestato del reactor (del grifo al lado)
    - 3.1 De R1 a R4: 310 mL, 440 mL, 560 mL, 690 mL (2 L en total)
    - 3.2 Medir el pH de cada muestra de digestato. Anotar
    - 3.3 Botar la muestra (semanalmente, se guarda la muestra, i.e. cada lunes!)
4. Alimentar los reactores
    - 4.1 Abrir la entrada para el pH-imetro
    - 4.2 Meter sustrato
    - 4.3 De R1 a R4: 310 mL, 440 mL, 560 mL, 690 mL (2 L en total)
    - 4.4 Cerrar la entrada
    - 4.5 Poner en marcha la agitación

Fecha		Investigadores	

Reactor 1	V	W	X	Y
Muestra A				
Muestra B				
Muestra C				

Reactor 2	V	W	X	Y
Muestra A				
Muestra B				
Muestra C				

Reactor 3	V	W	X	Y
Muestra A				
Muestra B				
Muestra C				

Reactor 4	V	W	X	Y
Muestra A				
Muestra B				
Muestra C				

Instrucciones:

1. Encender el equipo y preparar las muestras
    - 1.1 Encender el horno a 105°C
    - 1.2 Preparar los crisoles necesarias (necesitas 12 crisoles en total)
    - 1.3 Mete los crisoles al horno, y deja calentar para una hora
    - 1.4 Pon los crisoles en el recipiente de silicona, y dejar enfriar
    - 1.5 Llama el crisol (S1R1A, para reactor 1, primera semana, muestra A), por ejemplo
    - 1.6 Pesar el crisol. Eso será tu valor para **V**
    - 1.7 Añade cierta cantidad de digestato al crisol. Pesar otra vez. Eso será tu valor para **W**.
2. Meter los crisoles al horno para 24-36 horas
3. Comprobar por si todavía queda humedad dentro del crisol
    - 3.1 Sacar uno de los crisoles, anotar el peso. Devuelvelo al horno.
    - 3.2 10 minutos después saca el mismo crisol, y anotar el peso.
    - 3.3 Si el peso ha cambiado mucho, es senal de que todavía hay humedad en la muestra.
      Si el peso no cambió, saca la muestra con las pinzas, y pesala inmediatamente. Eso será tu valor para **X**.
4. Encender la mufla a 550°C
    - 4.1 Pon las muestras en la mufla para 1 h

    !! Cuidado !!

    La mufla es muy caliente. Es necesario usar las guantes de protección thermal y las pinzas

    La marca que anadieron en paso 1.5 (S1R1A) va a desaparecer después de 1h en la mufla. Es importante recordar por donde pusieron las muestras en la mufla. (A mí, me gusta hacer una foto para acordarme.

    La mufla va a producir muchas gases tóxicas durante el tratamiento. Es importante encender el ventilador y salir del laboratorio

    - 4.2 Sacar las muestras y pesarlas inmediatamente. Eso será tu valor para **Y**.
    - 4.3 Dejar enfriar las muestras, y luego limpiarlas.
5. Cálculos
    - 5.1 Solidos volatiles SV = (X - Y)/(W - V)
    - 5.2 Solidos totales ST = (X - V)/(W - V)
    - 5.3 Solidos fijos SF = (Y - V)/(W - V)
    - 5.4 Humedad = 1 - SV - ST - SF
    - 5.5 (se puede cambiar el formato a % si quieren)

Fecha		Investigadores	

Reactor 1	COD A	COD B	COD C

Reactor 2	COD A	COD B	COD C

Reactor 3	COD A	COD B	COD C

Reactor 4	COD A	COD B	COD C

Foto 1

Foto 2

Instrucciones:

1. Sacar muestra de digestato
2. Abrir tubos con reactivo preparado de HANNA "COD A-C" como indicado en la foto 1.
    - 2.1 Cuidado: la muestra preparada lleva ácido sulfirico y otros compuestos muy
        Sólo abrir con guantes, tapabocas y gafas de seguridad
3. Diluición del digestato
    - 3.1 Sacar 5 mL de muestra con una bureta y dejar en un vaso
    - 3.2 Meter 45 mL de agua distilada para diluir la muestra
    - 3.3 Mezclar bien la mezcla
4. Meter 2 mL de la mezcla a los tubos del reactivo
    - 4.1 Cerrar bien los tubos
5. Colocar los tubos con (reactivo + muestra) al reactor
6. Enciende el reactor y deja calentar a 150 °C para 2 horas
7. Después de enfriarlas, sacar los tubos y meter cada uno al photometro
    - 7.1 El método pre-programado se llama "COD HR (O_2)"
    - 7.2 Anotar el valor y seguir con la siguiente mustra

Foto 3

Fecha		Investigadores	

Reactor 1	HCl (A)	NaOH (A)	HCl (B)	NaOH (B)	HCl (C)	NaOH (C)

Reactor 2	HCl (A)	NaOH (A)	HCl (B)	NaOH (B)	HCl (C)	NaOH (C)

Reactor 3	HCl (A)	NaOH (A)	HCl (B)	NaOH (B)	HCl (C)	NaOH (C)

Reactor 4	HCl (A)	NaOH (A)	HCl (B)	NaOH (B)	HCl (C)	NaOH (C)

Procedimiento:

1) Diluir y preparar las muestras: 10 mL de muestra, con 40 mL de agua distillada (en total, necesitarás 150 mL)
   Necesitas 3 muestras para cada reactor. En esta hoja referimos a las tres muestras como A, B y C.
2) Bajar el pH a 4.0 (con HCl). Anotar la cantidad
3) Bajar el pH a 3.0. No hace falta anotar la cantidad
4) Calentar la muestra hasta el punto de embullición
5) Dejar enfriar a aproximadamente 40°
   Sabes que la muestra tiene 40° cuando puedes tocar el vidrio sin que te duele
6) Subir el pH a 6.5 (con NaOH), anotar la cantidad
7) Terminar el experimento con limpieza y los cálculos

Notas:

8) Metes 0.1 M NaOH y HCl en este experimento. Este variable será nuestro **N**
9) Para calcular la alcalinidad y cantidad de AGV:

X = (HCL (A) + HCl (B) + HCl (C)/3

Alcalinidad (g/L)= X * N * 50 * 5/50

Y = (NaOH (A) + NaOH (B) + NaOH (C))/3

AV (Acidos Volatiles) (g/L)= Y * N * 50 * 5/50

Si AV < 180, **AGV = AV**

Si AV > 180, **AGV = 1.5 * AV**