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Roberto Eloy Hernández Regalado

Optimization of the efficiency and flexibility of agricultural biogas plants by integrating an expanded granular sludge bed reactor

PROFESSUR **Wasserwirtschaft**

Universität Rostock Traditio et Innovatio

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Foreword

Biogas production is a key pillar of renewable energy supply, as it can be used to compensate for fluctuating feed-ins from wind and solar energy. In recent years, it became more and more evident that the production of biogas from specifically grown energy plants is neither economically nor ecologically sustainable. Biogas production from biogenic residues is therefore becoming increasingly important. In the agricultural sector, there is great potential, especially in the energetic utilisation of cattle and pig manure. In addition, numerous waste streams in the bioeconomy sector appear suitable in principle.

The main challenges here are often very thin substrates with a low volume-specific energy content or very one-sided compositions that are suboptimal for biological conversion. Starting points for processing such materials include the targeted mixing of material flows and, in the case of very thin substrates, the use of anaerobic technologies with biomass retention.

The aim of this thesis is to combine conventional fermenters with EGSB reactors for the most flexible possible processing of different substrates. Besides, the targeted mixing of different residues for synergistic improve of degradation and methane yield is investigated. In this way, the above-mentioned challenges could be technically mastered largely and a wide spectrum of previously unused biogenic residues could be utilised. The work goes beyond purely technological issues and carries out a well-founded economic comparison with classic fermenters. Even if the concept is not yet economically viable, this is a valuable contribution towards a more comprehensive energy utilisation of biogenic residues.

The work was supervised in close co-operation with Prof. Wetter at Münster University of Applied Sciences. All experimental work was carried out there. We would like to thank him and his team for their active support and fruitful collaboration.

Prof. Dr.-Ing. habil. Jens Tränckner

Universität Rostock

Traditio et Innovatio

Optimization of the efficiency and flexibility of agricultural biogas plants by integrating an expanded granular sludge bed reactor

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Abstract

Biogas produced from anaerobic digestion is a renewable energy source with several advantages, such as the use of organic waste as a substrate, production of local power and heat, rural job creation, reduction of greenhouse gas emissions, and substitution of inorganic fertilizer. However, the development of the biogas sector is highly dependent on the costs of gas, electricity, and heat, while production costs are higher than for other energy sources. Furthermore, the biogas development in Germany was closely related to the bonus payments from the German government. With the disappearance of the bonuses and the cuts in crop cultivation for biogas production, the efficiency of biogas production needs to increase, and new technologies need to be developed or introduced in the biogas sector to increase the palette of wastes that are profitable to digest.

Large unexploited potential rests in liquid manures, industrial wastewater, and other agro-industrial substrates. Nevertheless, with the employment of current reactor technology, slurry digestion is not competitive with other substrates. Therefore, reactors with biomass retention mechanisms, such as the fixed bed (FB), upflow anaerobic sludge blanket (UASB), or expanded granular sludge bed (EGSB), can provide a cost-effective solution by reducing the required reactor size to digest these substrates, thus decreasing investment costs.

This study compared three different treatment concepts (TC) for the mono-digestion of cow manure (CWM) and pig manure (PM): a completely stirred tank reactor (CSTR) as the reference, an EGSB reactor with upstream solid-liquid separation, and a combination of both. Data was collected *via* batch tests and continuous laboratory tests in an EGSB reactor following the German guideline. The results indicated that the integration of the EGSB in the typical biogas plant was better than substituting the typical continuous stirred-tank reactor. Nevertheless, the typical plant was still more profitable than the treatment concept with the EGSB included. Moreover, CWM mono-digestion was more profitable than PM mono-digestion for most of the treatment concepts.

To improve the profitability of the integration of the EGSB, the former procedure was duplicated using anaerobic co-digestion (AcoD). First, batch tests were conducted within a mixture design optimization procedure, and mixtures composition that produced optimal methane yield (MY) and methane production rate (MPR) were obtained by modeling the synergistic and anti-synergistic effects of the substrates within the mixtures.

These mixtures were fed to EGSB reactors in continuous laboratory experiments to obtain the optimal hydraulic retention time (HRT) at which the reactor performed the best in terms of MY and MPR. Once the whole data for co-digestion was collected, the economic analysis of the concept with co-digestion was repeated. The AcoD was found to significantly improve the profitability of the integration of the EGSB if the conditions were adequate.

Abstract

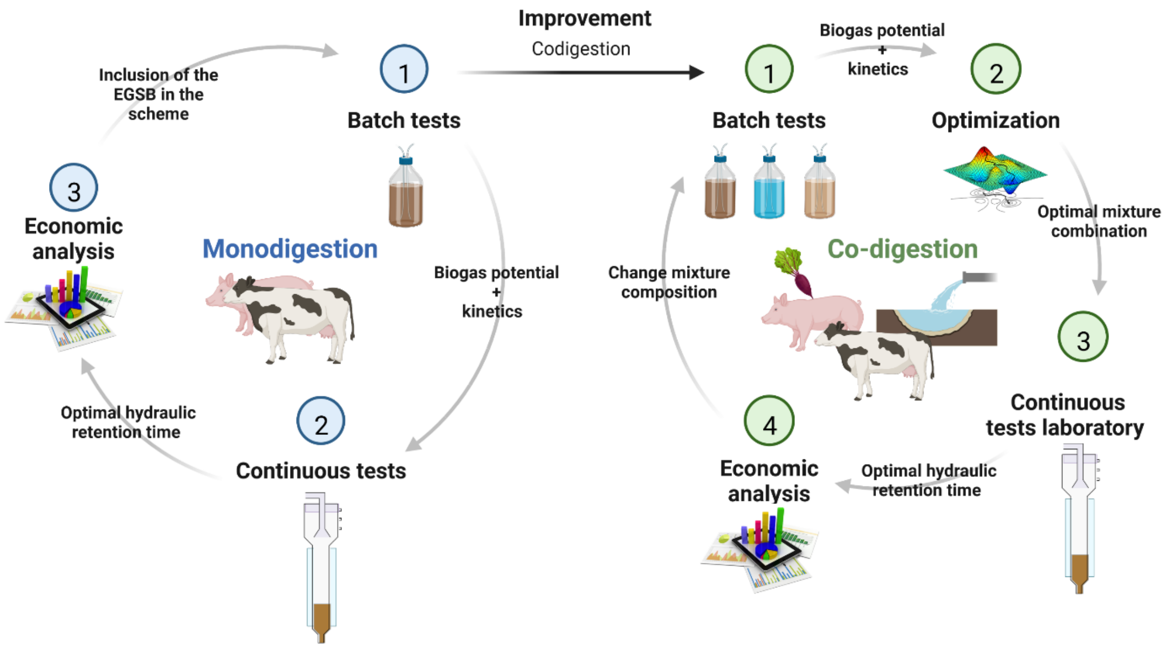
Durch anaerobe Vergärung erzeugtes Biogas aus organischen Reststoffen ist eine erneuerbare Energiequelle, Strom und Wärme vor Ort erzeugt, Treibhausgasemissionen reduziert und helfen kann, Nährstoffkreisläufe durch fachgerechte Nutzung der Gärreste zu schließen. Allerdings sind die Produktionskosten höher als bei anderen Energiequellen, und die Effizienz muss gesteigert werden, damit die Branche ohne staatliche Zuschüsse florieren kann. Um die Rentabilität zu steigern, müssen neue Technologien entwickelt werden. Der Festbett-, der UASB- oder der EGSB-Reaktor (Expanded Granular Sludge Bed) könnten die Investitionskosten senken, indem sie die erforderliche Reaktorgröße verringern, um Substrate wie Gülle und Industrieabwässer abzubauen.

In dieser Studie wurden drei Behandlungskonzepte für die Monovergärung von Rinder- und Schweinegülle verglichen: ein vollständig gerührter Tankreaktor (CSTR) als Referenz, ein EGSB-Reaktor mit vorgeschalteter Fest-Flüssig-Trennung und eine Kombination aus beiden. Die Daten wurden in Batch- und kontinuierlichen Labortests erhoben. Die Ergebnisse zeigten, dass die Behandlungskonzepte mit EGSB-Reaktor und vorgeschalteter Trennung hinsichtlich Substratabbau und Biogasproduktion besser abschnitten als der CSTR. Dennoch war die typische landwirtschaftliche Biogasanlage immer noch rentabler., insbesondere aufgrund der erhöhten Betriebskosten des EGSB Die CWM-Monovergärung war bei den meisten Behandlungskonzepten rentabler als die PM-Monovergärung.

Um die Rentabilität zu verbessern, wurde das Mischungsdesign in Batch-Versuchen für die anaerobe Co-Vergärung (AcoD) verwendet, um eine optimale Methanausbeute und Produktionsrate zu erzielen. Kontinuierliche Laborversuche mit EGSB-Reaktoren wurden durchgeführt, um die optimale hydraulische Verweilzeit zu ermitteln. Die wirtschaftliche Analyse ergab, dass AcoD die Rentabilität der EGSB-Integration deutlich verbessert, wenn die Bedingungen angemessen sind.

Zusammenfassend bietet die anaerobe Vergärung biogener Reststoffe zahlreiche Vorteile. Um mit anderen Energiequellen konkurrieren zu können, muss aber die Effizienz vor allem bei geringer konzentrierten Substraten gesteigert werden. Technologien wie EGSB-Reaktoren und Co-Vergärung können die Investitionskosten senken und die Rentabilität erhöhen.

Graphical abstract



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List of Abbreviations and Symbols

Abbreviation	Parameter
ABR	Anaerobic Baffled Reactor
AC	Anaerobic Contact
AcoD	Anaerobic Co-Digestion
AD	Anaerobic Digestion
ADM1	AD Model No. 1
AF	Anaerobic Filter
AMBR	Anaerobic Migrating Blanket Reactor
ANOVA	Analysis of Variance
BECCS	Bioenergy with Carbon Capture and Storage
BMP	Biomethane Potential
BOD	Biological Oxygen Demand
BPR	Biogas Production Rate
C/N	Carbon to Nitrogen Ratio
CAPEX	Capital Expenses
CFD	Computational Fluid Dynamics
CI	Co-digestion Index
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred-Tank Reactor
CV	Coefficient of Variation
CWM	Cow Manure
DFFR	Downflow Fixed-Film Reactor
DM	Dry Matter
EGSB reactor	Expanded Granular Sludge Bed Reactor
FB	Fixed bed reactor
FLB/EB	Fluidized Bed or Expanded Bed
GBABR	Granular Bed Anaerobic Baffled Reactor
HAc	Acetic Acid
HBu	Butyric Acid
HPr	Propionic Acid
HRT	Hydraulic Retention Time
IC	Internal Circulation
IRR	Internal Return Rate
ISR	Inoculum-to-Substrate Ratio
KI	Kinetic Index
LCFAs	Long-Chain Fatty Acids
MD	Mixture Design
MPR	Methane Production Rate
MY	Methane Yield
NPV	Net Present Value
ŋBOD5	Biological Oxygen Demand on the fifth Day Removal Efficiency
ŋCOD	Chemical Oxygen Demand Removal Efficiency
ODM	Organic Dry Matter
OLR	Organic Loading Rate
OPEX	Operational Expenditures
PABR	Periodic Anaerobic Baffled
PBP	Payback Period

PCA	Principal Component Analysis
Pe_{axial}	Axial Peclet Number
PFR	Plug Flow Reactor
PM	Piglet Manure
r_{CH4}	Specific Methane Production Rate
Re	Reynolds Number
R_{max}	Maximum Biomethane Production Rate
RSM	Response Surface Methodology
RTD	Distribution of The HRT In a Reactor
SBT	Sugar Beets
SMP	Specific Methane Production
SRT	Solid Retention Time
SWW	Starch Wastewater
TA	Total Alkalinity
TC	Treatment Concept
TE	Trace Element
TIC	Total Inorganic Carbon
TPPFR	Two-Phase Plug Flow Reactor
TS	Total Solids
UASB	Anaerobic Sludge Blanket
UASFF	Upflow Anaerobic Sludge Bed Fixed Film
USSB	Upflow Staged Sludge Bed Reactor
VFAs	Volatile Fatty Acids
VOA	Volatile Organic Acids
VS	Volatile Solids
V_{up}	Upflow Velocity

1. INTRODUCTION

1.1 Problem definition

Anaerobic digestion (AD) is an efficient and suitable method for the sustainable management of biowastes and biogas production. The attention paid to AD technology has increased owing to its environmental and economic benefits (Pagés-Díaz et al., 2018). Due to the current high-energy prices in Germany (*TttStrompreise*, 2021), the need to substitute gas imports has risen rapidly. In Germany, the share of the total electrical generation from renewable sources for the public power supply was 45.90%, with biomass representing approximately 16.77% of it. Furthermore, biogas plants play a significant role in the energy mix because they can bridge short-term variations in the wind or solar power supply; they supply up to 7% of the total energy demand in Germany (Cramer et al., 2019) Due to the current amendment to the Renewable Energy Act, the biogas sector is once again facing a challenge (EEG, 2021). The cutdown in the remuneration for plants that do not process agriculture residues, especially manure, has a profound impact on investments in the sector, as Germany has the second-largest amount of biogas plants in the world (German Biogas Association, 2021; *A Perspective on the State of the Biogas Industry from Selected Member Countries of IEA Bioenergy Task 37 | Bioenergy*, 2022). Hence, to be able to operate the biogas process in an economically viable manner despite the cuts in remuneration, alternatives for the flexibilization of the biogas production concerning feedstocks, digester operation, microbial communities, and biogas output must be made available (Schiemenz & Eichler-Löbermann, 2010; Theuerl et al., 2019).

The agriculture sector accounted for 9% of total German emissions in 2020. In comparison to other industries, CO_2 has a smaller role in greenhouse gas emissions in agriculture (13% in 2020). The bulk of emissions is methane and nitrous oxide (46 and 42 percent respectively in 2020). Agriculture alone was responsible for around 63% of all methane emissions and 81% of nitrous oxide emissions in Germany. Regarding emissions, methane is approximately 25 times more detrimental to the environment than CO_2, whereas for nitrous oxide the damaging effect is around 300 times stronger. Methane is largely emitted during the digestive processes of ruminants, particularly beef and dairy cattle, as well as during the storage and dispersion of animal fertilizers such as liquid manure and solid dung. Many of the emissions cited are related to animal husbandry, which accounts for more than 60% of agricultural emissions and around 5% of total emissions in Germany (Guidehouse et al., 2021).

The main feedstock for biogas production for several years was energy crops. However, to reach the climate goals within the agricultural sector, Germany has shifted toward the use of alternative substrates, such as crop residues, livestock waste, and catch crops (Iglesias et al., 2021; Kougias & Angelidaki, 2018). Hence, agro-industrial wastes have gained importance due to their potential as raw materials for obtaining energy (Cremonez et al., 2021). Their use could eventually reduce environmental liabilities and add value to already developed production chains because biogas accounts for AD allowing the establishment of a circular economy (Ghosh & Mukherjee, 2019). The case is especially important with animal manure since on average a German farm has 86 cattle heads, 1 175 pigs, 160 sheep, 14 goats, 1 160 laying hen, and 28 166 broilers, with cattle and pigs generating the largest total manure quantities (Liebetrau et al., 2021).

AD is widely used for waste treatment, but even at an industrial scale, risks of inhibition exist due to the accumulation of ammonia, volatile fatty acids (VFAs), or long-chain fatty acids (LCFAs). One possible solution to overcome these inhibitions is co-digestion, which provides a better supply of macro- and micronutrients, a balanced carbon-to-nitrogen ratio (C/N), and superior buffer capacity, dilutes inhibitors, and enhances biogas production (Di Maria et al., 2015; Salehiyoun et al., 2020).

AD plants can co-digest a variety of waste substrates to increase biogas production and avoid inhibition (Xie et al., 2016). Moreover, anaerobic digesters often work under nominal capacity varying from 15% to 30%. This unexploited potential can be exploited to co-digest other substrates overcoming economic, operating, and environmental challenges by increasing the organic loading rate (OLR) at which they operate (Salehiyoun et al., 2020).

AD technologies can be divided into two broad categories, based on the total solids (TS) content of the substrate: wet (TS: 0-15%) and dry (TS>15%). The limits between the two types of AD technologies are often unclear because they also depend on the employed reactor technology. Moreover, these two categories can be divided into subcategories by the reactor technology or the operation mode of the reactor. Therefore, most of the authors established wet high-rate AD between 2% and 8%, wet with suspended biomass 5%–15%, and solid-state depending on the operation mode (batch or continuous), with the latter being able to process up to 45% in plug flows reactors (Kougias & Angelidaki, 2018; Rabii et al., 2019b; Van et al., 2020). Conventional biogas technology includes a continuous stirred-tank reactor (CSTR), which partially flushes microorganisms out of the system, and usually operates around 10%–13% TS (Weiland, 2010).

High-rate reactor systems minimize biomass washout through different mechanisms (Eberl et al., 2006, pp. 148–150; Khanal, 2008, p. 11). This technological advantage over conventional fermenters allows efficient digestion of substrates with low solid contents (<5% TS) (Kougias & Angelidaki, 2018). A two-stage system combining a wet high-rate system with a wet or solid CSTR can take advantage of the strength of both systems in a fully combined treatment concept (Van et al., 2020). Hence, a combined system could unlock the digestion of a larger diversity of substrates, granting plant flexibility across feedstocks, digester operation, and biogas output. To assure a low solid content, an upstream solid-liquid phase separation step should be included (Wetter et al., 2017). This enables a higher OLR in the

CSTR by using solid manure, whereas the energy-rich, low TS substrate can be utilized in the high-rate reactor, resulting in a higher combined biogas output as the raw substrate (Hernández Regalado, Häner, Brügging, & Tränckner, 2022; Zhang et al., 2013).

Implementing high-rate digestion as a single or multi-stage AD treatment concept can make the digestion of low TS content manures profitable. Therefore, it can play a key role in helping to minimize methane and nitrous oxide emissions and aiding the recycling of critical minerals present in the manures. Hence, the establishment of sustainable circular economy operations within the agricultural sector.

1.2 Goal setting

The current biogas technology does not allow an efficient treatment of liquid agro-industrial substrates, i.e., animal slurry, and wastewater. Thus, implementing anaerobic co-digestion in high-rate reactors provides a more efficient alternative for digesting low total TS substrates. This research aimed to increase the overall economic efficiency and the environmental sustainability of the AD agricultural biogas plant for manure treatment through an increase in reactor efficiency by using high-rate reactors in different treatment concepts.

In the present work, a methodology comprehending three different scales and two operation modes is applied to analyze and optimize the digestion of liquid agro-industrial substrates or mixtures of them from an economic and environmental perspective. The methodology consists of the following logical steps:

1. Performing batch and continuous experiments to determine the profitability of pig manure and cow manure as mono-substrates across three different treatment concepts.
2. To conduct batch experiments to determine the optimal composition of manure-based mixtures to co-digest in the previously analyzed treatment concepts.
3. To employ the optimal mixtures in continuous experiments to select optimal operating conditions for their treatment in an EGSB reactor.
4. To reassess the economic and environmental performance of the treatment concepts using the optimal co-digestion mixtures and to compare them with the use of mono-digestion of cow manure and pig manure.

This work was developed as a cumulative dissertation, with three publications, and supplementary discussion. The specific parts developed in each part of this work are presented in Figure 1-1.

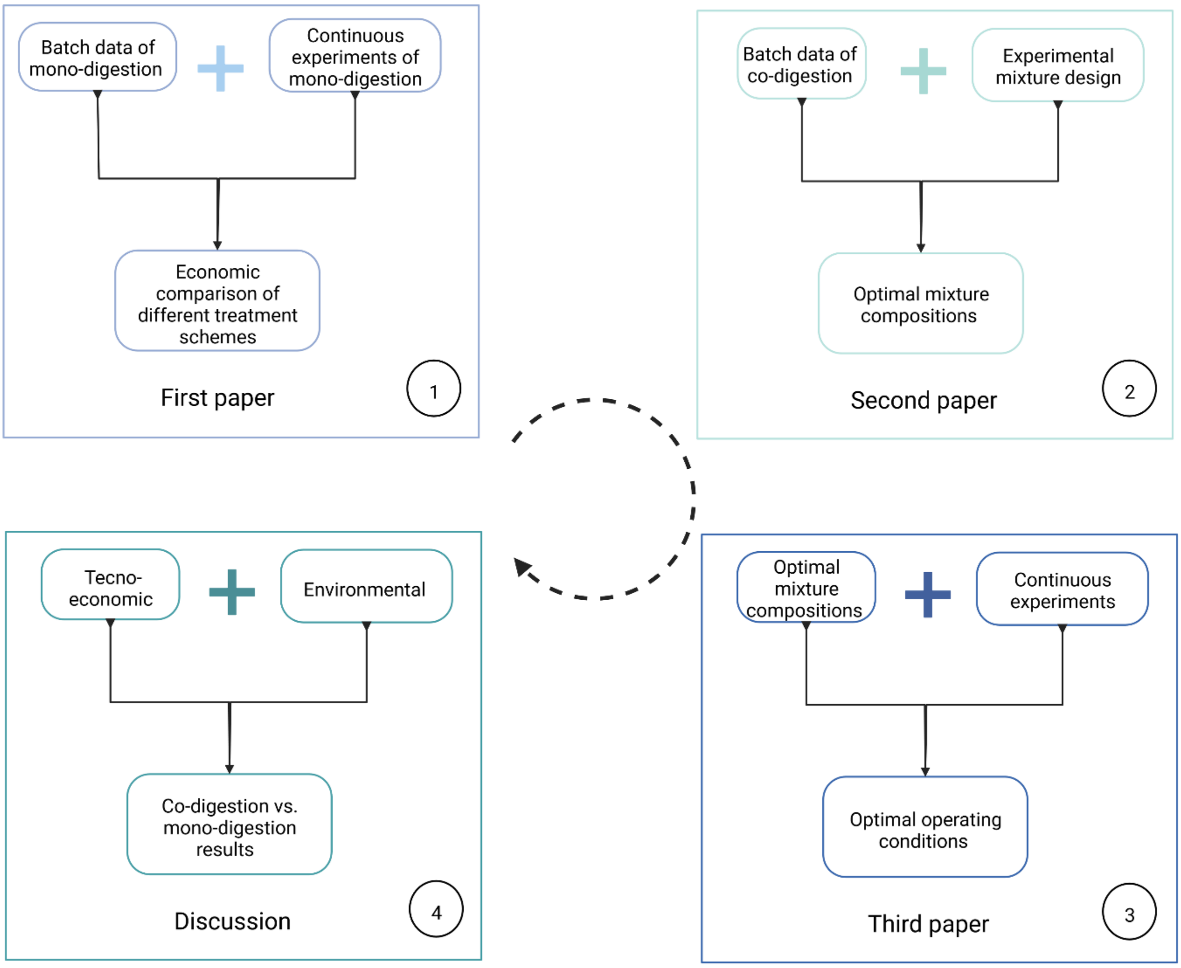


Figure 1-1: Structure of the cumulative dissertation

Figure 1-1 summarizes the four aforementioned logical steps. This work aims to compare reactor technologies within different treatment concepts and substrates by using mono- or co-digestion. The main objectives of the logical steps can be seen at the bottom of the individual squares, while the two main components combined for achieving them are at the top. Hence, the economic assessment of the first paper (mono-digestion), will be economically compared with the combination of the results of the second and third papers (co-digestion) in the discussion section.

2. THE AD PROCESS

AD is a suitable and efficient method for the sustainable management of biowastes and the production of biogas from them (Treichel & Fongaro, 2019, p. 1). AD is a biological degradation process where biomass is converted by the action of a microorganism consortium in the absence of oxygen into a mixture of gases called biogas (mainly methane and carbon dioxide) (Korres et al., 2013, p. 196). The benefits of AD across four categories, namely, energy system, environmental/climate benefits, economic benefits, and other benefits, were summarized by Lauer et al. (2020). The summary is presented in Table 2-1.

Table 2-1: A selection of benefits of biogas plants (Lauer et al., 2020).

Energy system	Environmental/ climate benefits	Economic benefits	Other benefits
• Lower demand for power grid extension (Trommler et al., 2017)	• Reduction of agricultural GHG emissions through the use of manure and other organic waste products (Cuéllar & Webber, 2008; Oehmichen & Thrän, 2017)	• Additional income for farmers (Lauer et al., 2018)	• Source of carbon dioxide for BECCS (bioenergy with carbon capture and storage) (Li et al., 2017)
• Source of carbon for the methanation of hydrogen (Dotzauer et al.)	• Substitution of inorganic fertilizer through the use of biogas digestate (Arthurson, 2009)	• Additional jobs in rural areas (Guenther-Lübbers et al., 2016)	• Reduction in odor and fewer pathogens when manure is used (Yiridoe et al., 2009)
• Cost savings from conventional power plants (e.g., a lower amount of start/stop operations) (Holzhammer, 2015)	• Reduction of GHG emissions and air pollution in the heating sector (Bettina Kampman et al., 2017)	• Positive effect on the added value in rural areas (Guenther-Lübbers et al., 2016)	
• (Decentralized) heat supply and substitution of fossil fuels (Holm-Nielsen et al., 2009)			

AD as a biological process can be divided into four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Rajendran, 2015b). The breakdown of the metabolic pathways is presented in Figure 2-1.

The individual degradation steps are carried out by different consortia of microorganisms; in turn, the microbial consortia consist of several groups of microorganisms, and each performs a specific function. As a whole, they partly stand in syntrophic interrelation and place different requirements on the environment (Abdelgadir et al., 2014; I. Angelidaki et al., 1993; Weiland, 2010).

Hydrolysis is the initial stage of the digestive process. Because polymers cannot be directly metabolized by fermentative microbes, this phase is critical for the AD process (Abdelgadir et al., 2014). As a result, complex components, such as lipids, polysaccharides, proteins, and nucleic acids, are transformed into soluble chemicals, such as amino acids, fatty acids, and short-chain sugar, during the hydrolysis process. Extracellular hydrolytic exoenzymes released by the bacteria present in the medium, such as cellulase, cellobiase, xylanase, amylase, lipase, and protease, are responsible for the catabolism of the more complex molecules (Li et al., 2019; Thiruselvi et al., 2021; Van et al., 2020).

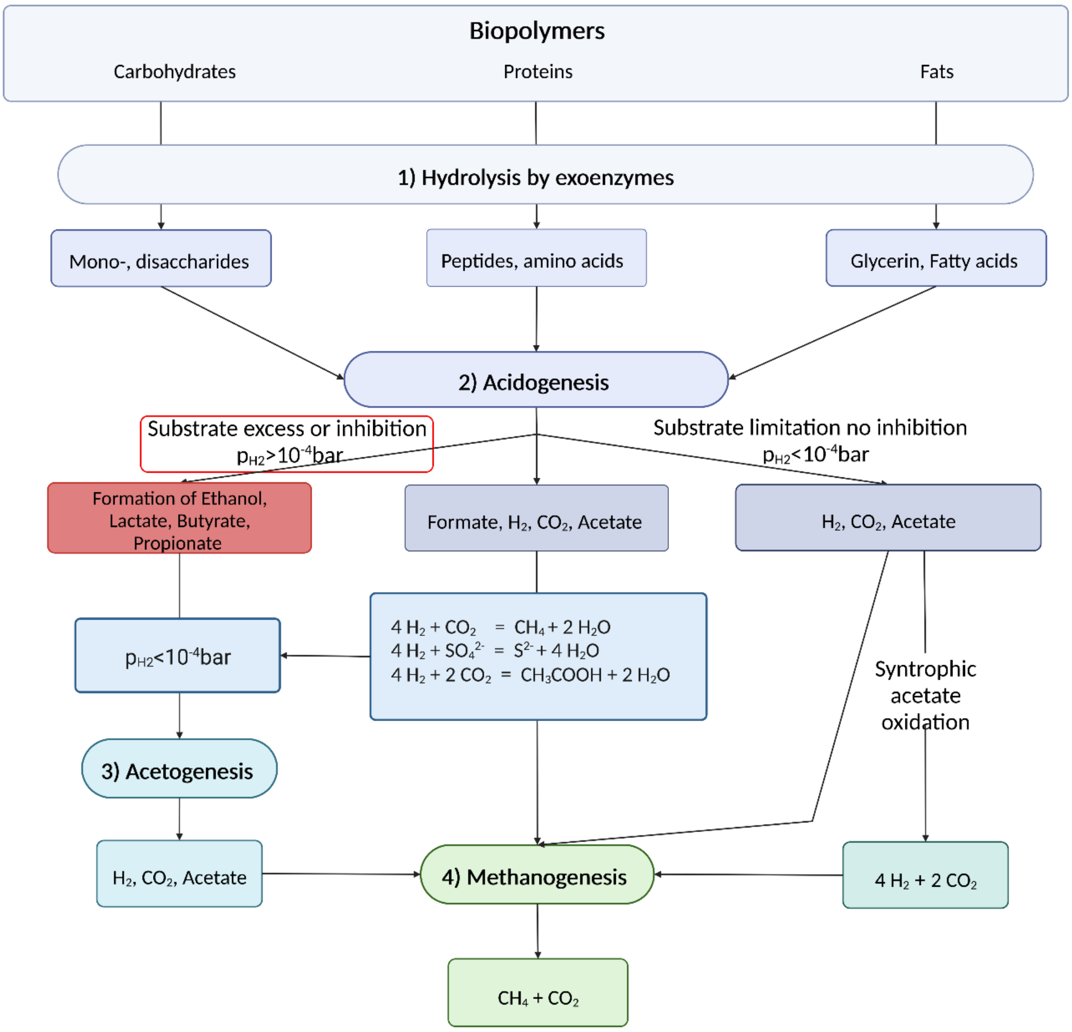


Figure 2-1: Schematics of the anaerobic digestion process based on Rosenwinkel et al. (2015, p. 30).

Hydrolysis often corresponds with the rate-limiting stage of AD because the absorption of these compounds by the bacteria depends on the speed at which the complex substrates are broken down by the enzyme (Cremonez et al., 2021). According to L. Yu and Wensel (2013), the hydrolytic process is described by two mechanisms:

1. Microbes produce enzymes into bulk liquids, which adsorb onto a particle or react with a soluble substrate (Jain et al., 1992).

2. The microorganisms adhere to a particle and degrade soluble chemicals generated by processes catalyzed by enzymes produced locally (Vavilin et al., 1996).

Nonetheless, a first-order kinetic model in terms of the degradable organic matter can usually model the AD properly when hydrolysis is the rate-limiting step (Vavilin et al., 2008).

The second stage is called acidogenesis or fermentation. During acidogenesis, the fermentative bacteria consortia degrade the available soluble hydrolysis products to produce short-chain organic acids, hydrogen, carbon dioxide, ethanol, ammonia, and hydrogen sulfide (Thiruselvi et al., 2021; Weinrich & Nelles, 2021a). Although around 20% acetate and 4% H_2 are directly produced by the acidogenic fermentation of sugars and amino acids, both products are primarily derived from the acetogenesis and dehydrogenation of higher VFAs (Korres et al., 2013, p. 33).

Acidogenesis is carried out by a large and diverse group of fermentative bacteria. Usual species belong to the clostridia group, which comprises anaerobic species that form spores, able to survive in very adverse environments (Chernicharo, 2007, p. 7). Microorganism degradation occurs *via* several metabolic pathways and is greatly controlled by environmental factors such as hydrogen partial pressure and temperature (Rosenwinkel et al., 2015, pp. 39–40).

Acetogenesis is the subsequent step of acidogenesis. Acetogenic bacteria are responsible for converting the products of the acidogenic step into suitable substrates for methanogens. Acetic acid, hydrogen, and carbon dioxide are the byproducts of acetogenic bacteria (Chernicharo, 2007, pp. 7–8; Khanal, 2008, pp. 31–32).

Acetogenesis has a slower growth kinetic than acidogenesis, with a minimum doubling time of 1.5–4 d. Acetogens are strict anaerobes; the presence of oxidants such as oxygen or nitrate is harmful, and they thrive in weak acidic environments (pH 6.0–6.2) (Kondusamy & Kalamdhad, 2014; Ramos-Suárez et al., 2015).

Unless the hydrogen partial pressure is kept below 10^{-4} atm, acetogenesis is considered thermodynamically unfavorable. For energy considerations, high hydrogen content hinders the conversion of acidogenesis intermediate products. Consequently, organic acids including propionic, isobutyric, isovaleric, and hexanoic acids accumulate and inhibit methane production. As a result, acetogenic bacteria (hydrogen-forming bacteria) must coexist in a close biotic community (biocoenosis) with hydrogen-consuming methanogenic archaea, which intake hydrogen along with carbon dioxide during methane formation (interspecies hydrogen transfer), ensuring a suitable environment for the acetogenic bacteria (Friehe et al., 2012, pp. 21–22; Khanal, 2008, pp. 30–31).

The fourth step (methanogenesis) is the most important in the production of methane gas by methanogens. Acetoclastic and hydrogenotrophic methanogenesis are the two basic mechanisms for methane production. Acetotrophic bacteria ferment acetic acid to CH_4 and CO_2. The second type, hydrogenotrophic methanogens, feeds on CO_2 and H_2 (Van et al., 2020). The ratio between both metabolic pathways is around 70% acetotrophic methanogenesis and 30% hydrotrophic methanogenesis (Friehe et al., 2012; Madsen et al., 2011; Rajendran, 2015a; Weinrich & Nelles, 2021a).

Methanogenesis is frequently viewed as the rate-limiting step in modeling efforts as methanogenic bacteria have higher sensitivity and slower growth rates than nonmethanogenic bacteria (L. Yu & Wensel, 2013). Demirel et al. (2008) proposed that interspecies hydrogen transfer is plainly what determines the rate of methane formation.

Methanogens should be kept in a stable environment owing to their poor adaptation to pH variations, and the optimum pH for them is 6.5–7.5. Using oxidation–reduction potential as the basis of judgment, an anaerobic environment is one of the basic conditions for the growth of strictly anaerobic methanogens (Li et al., 2019; Van et al., 2020).

Methane production has been linked to the composition of the AD microbiome, and it is controlled by microbial metabolism, which is thermodynamically dependent on reactor environmental parameters (Campanaro et al., 2020). A summary of the major taxonomies identified in the AD is presented in Table 2-2.

Table 2-2: Examples of different microorganisms shown to be involved in different stages of AD (Korres et al., 2013, p. 264).

Stage of AD	**Major taxonomic entities identified**
• Hydrolysis and acidogenesis	• Fungi *Trichoderma* (e.g., *T. reesei*), *Thermomonospora*, *Ralstonia, Shewanella*, *Penicillium*, *Aspergillus*, and *Humicola* • Bacteria, e.g., *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Cellulomonas*, *Fusobacterium*, *Selenomonas*, *Streptococcus*, *Peptococcus*, and *Campylobacter*. Actinomycetes such as *Streptomyces* • *Pseudomonas mendocina*, *Bacillus halodurans*, *Clostridium hastiforme*, *Gracilibacter thermotolerans*, and *Thermomonas haemolytica*. Synergistetes
• Acetogenesis	• Most acetogens are in the phylum Firmicutes, e.g., *Moorella thermoacetica*. • Spirochaetes • δ-Proteobacteria, e.g., *Desulfotignum phosphitoxidans* • Acidobacteria, e.g., *Holophaga foetida* • Exclusively acetogenic bacteria, e.g., *Acetobacterium* and *Sporomusa* • Genera with acetogenic and non-acetogenic species, e.g., *Clostridium*, *Ruminococcus*, *Eubacterium*, *Thermoanaerobacter*, *Treponema*
• Methanogenesis	• Exclusively anaerobic, methane-producing Archaea from the phylum Euryarchaeota, with • 6 orders: Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, Methanopyrales, Methanocellales, and • 31 genera, e.g., *Methanosarcina*, *Methanobrevibacter/Methanobacterium*, *Methanosaeta*

Sources: (Cirne et al., 2007; Ellis et al., 2012; Franke-Whittle et al., 2009; W. Kim et al., 2010; Nations, Food and Agriculture Organization of the United, 1997; Pobeheim et al., 2010; Rastogi et al., 2008; Sang et al., 2009; Schlüter et al., 2008; Singhania, 2009; Song et al., 2010).

2.1 Influencing process parameters

AD is known to have environmental and economic benefits, such as energy recovery from its organic components and a significant biomass reduction (M. Kim et al., 2017). The AD process can endure a wide range of pH, temperature, and mixing conditions while operating in different reactors, and reactor configurations at different operation modes, using mixed inoculum or isolated cultures. In general, several factors may be considered in the context of a system used in the AD process (Mosquera et al., 2020). Knowing the main parameters that affect the process is critical for decision-making (Ji & Sun, 2022). Hence, an analysis of the main biotic and abiotic variables of the process as well as its interaction is fundamental for the in-depth comprehension of the process.

2.1.1 Nutrient supply

The proper development of anaerobic microorganisms depends on sufficient nutrients with suitable macro- and trace element (TE) composition, along with other factors, such as appropriate water content and a sufficiently large retention time, to allow the proliferation of even the most slowly growing process-relevant microbes (Friehe et al., 2012, pp. 24–26; Lebuhn et al., 2014). The different nutrients are divided into macro- and micronutrients based on their required concentration with a concentration boundary of normally 10^{-4} mol/L (Liu, 2020, p. 58). Nutrients needed in larger quantities are referred to as macronutrients, whereas elements that are only required in small concentrations are known as micronutrients or TEs (Debabrata Dastreiche & Debayan Das, 2019, p. 5).

Various macronutrients and ions are crucially important for the growth and maintenance of microorganisms. They are involved in several key cell metabolic activities such as the synthesis of ATP/NADP or significant enzymes. Different macro- and micronutrients, as well as important cations and their functions, are described in Table 2-3. Due to low growth rates and small biomass yields during AD, only a small amount of macronutrients is needed and is often already sufficiently supplied by the added substrate (Weinrich & Nelles, 2021a, pp. 29–30). However, the long-term mono-digestion operation usually leads to nutrient imbalance (Ganesh Saratale et al., 2018; Kougias & Angelidaki, 2018). Nevertheless, a macronutrient balance normally leads to a micronutrient balance; hence, most studies are focused on the optimization of at least one of the following ratios: C/N, C/N/P, and C/N/P/S (Cremonez et al., 2021; Mao et al., 2015; Neshat et al., 2017; Rabii et al., 2019b).

However, the optimal ratios significantly differ from study to study. Khanal (2008) suggested that the needs of C, N, and P can be roughly calculated using the empirical formula for an anaerobic bacteria cell as $C_5H_7O_2N$ under the following assumptions: 1) 10% of the removed organic matter is used for biomass synthesis 2) N represents 12% of the cell mass, and 3) P requests range [1/7-1/5] of N needs. The information on different optimal ratios by their respective sources is summarized in Table 2-3. Meanwhile, in Table 2-4, the essential nutrients and their functions are presented.

Table 2- 3: Optimal mass ratios of C, N, P, and S by different sources

Source	**C/N**	**C/P**	**C/S**
(Weiland, 2010)	20–40	60.1–120.1	200–298.5
(Van et al., 2020)	15–30	75–150	-
(Cremonez et al., 2021)	20–35	-	-
(Neshat et al., 2017)	15–30	-	-
(Lissens et al., 2001)	10	-	-

Table 2-4: Required essential nutrients for microbial growth (Debabrata Das & Debayan Das, 2019, pp. 6–7).

Nutrients		**Functions**
Macronutrients	Carbon (C)	Required for biomass, product, and energy provision
	Oxygen (O)	An adequate amount of oxygen is needed for the growth of the organism if it is an aerobe
	Nitrogen (N)	Essential for protein synthesis
	Hydrogen (H) Sulfur (S) Phosphorus (P)	Contribute to the components of carbo-hydrates, lipids, proteins, and nucleic acids. Also, they participate in the syn-thesis of energy carriers ATP and NADP
Cations	Calcium (Ca^{2+})	Participates in cell activation, by sup-porting the heat resistance of endospores, for the activity of several enzymes
	Potassium (K^+) Magnesium (Mg^{2+})	Serve as cofactors for many enzymes and stabilize membranes and ribosomes
	Iron (Fe^{2+}, Fe^{3+})	Part of cytochromes, cofactors for en-zymes, and electron-carrying proteins
Trace elements	Manganese (Mn) Zinc (Zn) Copper (Cu) Molybdenum (Mo) Cobalt (Co) Nickel (Ni)	Part of enzymes and cofactors, help in the catalysis of reactions, maintenance of protein structure, etc.

Many micronutrients are involved in the synthesis and activation of important cofactors and enzymes of microorganisms (Weinrich & Nelles, 2021a, p. 31). In general, the need for micronutrients is satisfied in most agricultural biogas plants, particularly when the plant is fed with animal excrement, yet a deficiency in trace elements is very common in the mono-fermentation of energy (Friehe et al., 2012, p. 24). Thus, a reasonable and sustainable alternative to the addition of TE supplements is the balanced addition of manure or grass silage if these resources are easily available (Lebuhn et al., 2014). The recommended interval or optimal concentrations of TE are summarized in Table 2-5.

Table 2-5: Favorable concentrations of trace elements according to various reference sources (Friehe et al., 2012; Rosenwinkel et al., 2015).

Trace element	**Concentration [mg/L]**				
	As in (Seyfried, 1990)	**As in (Preißler, 2009)**	**As in (Bischoff, 2009)[a]**	**As in (M. Bischoff, personal communication, 2009)[b]**	**As in (Sahm, 1981)**
Cobalt (Co)	0.003–0.06	0.003–10	0.06	0.12	0.06
Nickel (Ni)	0.005–0.5	0.005–15	0.006	0.015	0.006
Selenium (Se)	0.08	0.08–0.2	0.008	0.018	0.008
Molybdenum (Mo)	0.005–0.05	0.005–0.2	0.05	0.15	0.05
Manganese (Mn)	n.s.	0.005–50	0.005–50	n.s.	0.005–50
Iron (Fe)	1–10	0.1–10	1–10	n.s.	n. s
Nickel (Ni)	n. s	n. s	n. s	n. s	0.006
Chromium (Cr)	n. s	n. s	n. s	n. s	0.005–50

[a]: Absolute minimum concentration in biogas plants; [b]: recommended optimum concentration.

The values presented in Table 2-5 have very high variance and no information about the process operating conditions to which they are applicable. Hence, Friehe et al. (2012) mentioned that these values are only partly applicable to agricultural biogas plants as in some cases, the studies described in these sources were conducted in the wastewater sector under different initial conditions and using different investigation methods.

2.1.2 Temperature

Chemical reactions, and thus also biochemical reactions, are strongly temperature dependent (Rosenwinkel et al., 2015, pp. 82–83). Temperature is one of the most influential factors in AD as it regulates the growth and activity of the microorganisms involved in AD (Neshat et al., 2017). As the temperature rises, the chemical and enzymatic reactions within the cell occur at a high speed. Thus, the growth and metabolic processes of the species constantly increase until the maximum growth rate is reached and they start to decrease, and a parabolic profile of temperature is therefore found in the praxis (Doran, 2013, p. 468). Hence, temperature influences AD by mainly affecting the thermodynamics of acetogenic and methanogenic reactions (Xie et al., 2016).

The AcoD process is usually applied under either mesophilic (30 °C–40 °C) or thermophilic (50 °C–60 °C) conditions, although psychrophilic conditions (10 °C–20 °C) have also been reported (Xie et al., 2016). Rosenwinkel et al. (2015, pp. 83–84) reported that the growth rate can be almost double at the optimal temperature going from psychrophilic to mesophilic and further to thermophilic. Zoetemeyer et al. (1982) studied the influence of temperature on the maximum growth rate in the first reactor of a two-stage concept to treat glucose. They found that the maximum growth rate in the acidification stage (first reactor) was approximately 40% higher at a temperature of 51 °C than at 37 °C.

Nonetheless, an operation at a higher temperature is not intrinsically better. Table 2-6 presents the comparison of mesophilic and thermophilic AD.

Table 2-6: Comparison of the performance of mesophilic and thermophilic anaerobic digestion (Korres et al., 2013, p. 209).

Performance characteristics	Mesophilic digestion	Thermophilic digestion
Gas production rate	Contradictory reports	Contradictory reports
Pathogen reduction	Lower	Higher
Effluent VFAs	Lower	Higher (contradictory)
Process stability	Higher	Lower (contradictory)
Methane content	Higher	Lower
Energy requirement	Lower	Higher
Odor	Lower	Higher
Product/substrate inhibition	Lower	Higher

Furthermore, Mao et al. (2015) pointed out that other disadvantages of the thermophilic process may be low-quality effluent, increased toxicity, amplified susceptibility to environmental conditions, and large investment cost. On the other hand, it has a rate advantage over mesophilic digestion as a result of its faster reaction rates and higher load-bearing capacity and, consequently, exhibits higher productivity than mesophilic AD (Mao et al., 2015; Neshat et al., 2017).

2.1.3 pH, buffer capacity, and organic acids

pH value is defined as the negative logarithm of the hydrogen ion activity. It is used to indicate the hydrogen (H^+)– or hydroxide (OH^-) ion concentration in an aqueous solution and thus its basic or acidic behavior (Rosenwinkel et al., 2015, p. 85). pH is one of the most important control parameters of AD owing to its influence on the different steps of the process (Cremonez et al., 2021; Korres et al., 2013). pH relates to different enzymatic reactions. In addition, enzymes have their optimum pH; thus, depending on the pH value, the metabolic activity of one or the other microorganism consortia may be favored (Neshat et al., 2017; Weinrich & Nelles, 2021a).

The two main bacterial groups from the taxonomies presented in Table 2.2 in terms of pH distribution are acid-producing bacteria (acidogens) and methane-producing bacteria (methanogens. Khanal (2008) affirmed that acidogens prefer a pH of 5.5–6.5, whereas methanogens prefer 7.8–8.2. Weinrich and Nelles (2021a) reported that the pH values for acidogens range from 4.5 to 6.5, whereas those for methanogens range from 6 to 8.2. Nevertheless, the optimal intervals for single-stage operation are usually contained in an interval that serves all microorganism groups. The different values from the literature are presented in Table 2-7.

Table 2-7: Optimal pH intervals for single-stage anaerobic digestion.

Optimal pH interval	Reference
7–7.5	(Weinrich & Nelles, 2021a)
6.8–7.4	(Khanal, 2008; Mao et al., 2015)
6.8–7.2	(Neshat et al., 2017)
7.0–7.2	(Ağdağ & Sponza, 2007)
70.–7.2	(Ganesh Saratale et al., 2018)
6.8–7.4	(Mao et al., 2015)
6.8–7.5	(Rosenwinkel et al., 2015)

The pH value during AD is established within the system by the alkaline and acid metabolic products formed during anaerobic decomposition (Friehe et al., 2012, p. 24). Hence, the pH value changes depending on the strength (dissociation constant) and concentration of individual acids and bases as well as the existing buffer system, concentration, and activity of free hydrogen ions (Weinrich & Nelles, 2021a, pp. 36–37). Consequently, keeping the pH within the narrow optimal interval sometimes poses significant problems in the practical operation of a plant because the value can significantly deviate from the theoretical one based on the substrate and sludge properties (Rosenwinkel et al., 2015, pp. 86–87). Changes in the pH values are often related to changes in the operational parameters. Thus, an accumulation of organic acids (acidification) will typically lower the pH, whereas increased ammonia concentrations or CO_2 removal will lead to an increment in pH values (Kougias & Angelidaki, 2018).

Buffer capacity

A buffered solution resists a change in its pH even when a strong acid or base is added to it. Buffered solutions are important to living organisms whose cells can survive only in a very narrow pH range (Zumdahl et al., 2007, pp. 585–586). A buffer solution is composed of a weak base or weak acid and its corresponding salt (Chang, 2002, pp. 655–656).

The buffer capacity of the medium in AD is usually mainly hydrogen carbonate and ammonium, with ammonium being the most important when treating substrates with high nitrogen content. The buffer capacity normally guarantees a stable pH value. If major changes occur and the pH value shifts out of its optimum range, this is usually a sign of serious disturbances, and action should be immediately taken. With high and diverse nutrient concentrations, such as liquid manure or kitchen waste, the buffering capacity of anaerobic fermentation processes can be strengthened (Friehe et al., 2012; Rosenwinkel et al., 2015; Weinrich & Nelles, 2021a). Hence, when such substrates are present, the accumulation of VFA will not always result in a pH drop due to a surplus of alkalinity which stabilizes the pH value at such an accumulation (Hopfner-Sixt & Amon, 2007; Sommer et al., 2013; Weiland, 2010).

The main buffering species in an anaerobic digester are the VFAs and bicarbonate. Total alkalinity (TA) measured *via* titration to a pH endpoint of 4.3 includes both these species (Association of German

Engineers [Verein Deutscher Ingenieure], 2016; Martín-González et al., 2013). The main buffer equilibria of the AD process are presented in Table 2-8.

Table 2- 8: Dissociation equilibrium of effective buffer systems during anaerobic digestion (Weinrich & Nelles, 2021a, p. 37).

Buffer	Dissociation equilibrium	pKa
Carbonate buffer	$[CO_2 + H_2O \rightleftharpoons H_2CO_3] \rightleftharpoons H^+ + HCO_3^-$	6.35
	$HCO_3^- \rightleftharpoons H^+ + CO_2$	10.33
Ammonium buffer	$NH_4^{+-} \rightleftharpoons NH_3 + H^+$	9.25
Sulfate buffer	$H_2S \rightleftharpoons H^+ + HS^-$	6.99
	$HS \rightleftharpoons {}^- H^+ + S^{2-}$	12.89

Organic acids

VFAs are the intermediate products of the methane production pathway. The main byproducts of AD are acetic, propionic, and butyric acids; other byproducts include formic, valeric, and caproic acids. Acetic acid is the most concentrated carboxylic acid, but it hinders methanogens less than propionic and butyric acids (Marchaim & Krause, 1993), whereas high temperature and alkaline pH conditions facilitate propionic acid production (Li et al., 2019). The presence of formic and valeric acids at above certain concentrations indicates microbial ecosystem imbalances or potential problems in the AD process as the said acids should not form under normal organic matter degradation conditions. These acids are indicators of how the organic matter is decomposed and can be used to assess the performance of AD (Neshat et al., 2017; Rosato, 2018, p. 175).

In general, the most sensitive parameter is the change in VFA concentration because the primary cause of digester failure is an imbalance between acidogenic, acetogenic, and methanogenic organisms (Sakar et al., 2009). Changes in pH values are often related to changes in operational parameters. Thus, an accumulation of organic acids (acidification) will typically lower the pH, whereas increased ammonia concentrations or CO_2 removal will lead to an increment in pH values (Kougias & Angelidaki, 2018).

Many authors have suggested VFAs as control parameters as they are indicative of the activity of the methanogen's consortia. VFA accumulation can be interpreted as either organic overload or inhibition of the methanogens due to the influence of process variables. In either case, action should be taken to avoid reactor failure. The relevance of specific VFAs is still unclear. However, simple short-chained VFAs have been the favorites of many researchers (Madsen et al., 2011).

The complexity and analytical performance of VFA quantification methods vary. Several authors have proposed various titration methods for efficient and low-cost AD process monitoring. This methodology is certainly worthwhile for AD plants seeking a measure of total acidity rather than detailed information about the relative abundance of individual VFAs. However, due to the proximity of the pKa values of the individual VFAs, titration methods cannot distinguish between them (Feitkenhauer et al., 2002; Lahav & Morgan, 2004; Méndez-Acosta et al., 2010).

The chromatographic methods used in industry and research laboratories can separate individual VFAs and provide quantitative measurements of their concentrations. Gas chromatography and high-performance liquid chromatography are the two most commonly used methods (Brondz, 2002).

The individual acids can be used as process indicators, and the extent to which they are produced is evidence of the thermodynamics and kinetics of the processes occurring within the reactor (Mortezaei et al., 2018). Korres et al. (2013, pp. 202–203) conducted a hierarchical cluster analysis to identify the similarity of various physicochemical characteristics of grass silages. They concluded that VFA, for example, will most likely reveal the same information as acetic acid measurements, whereas any of these characteristics can be approximately estimated *via* propionic acid concentration measurements. Furthermore, the propionic acid to acetic acid ratio can be used as a reliable indicator of digester imbalance, and a ratio greater than 1.4 may indicate immediate digester failure (Xie et al., 2016)

2.1.4 Inhibitors

Due to the large number of variables involved in AD, various intermediates and compounds can act as inhibitors. Researchers have demonstrated the inhibitory effect of some agents, and many others are suspected of being AD inhibitors (Friehe et al., 2012, p. 25). Weinrich and Nelles (2021a, p. 31) defined inhibition as primarily a concentration-dependent effect. Hence, even essential components can inhibit AD (Figure 2-2).

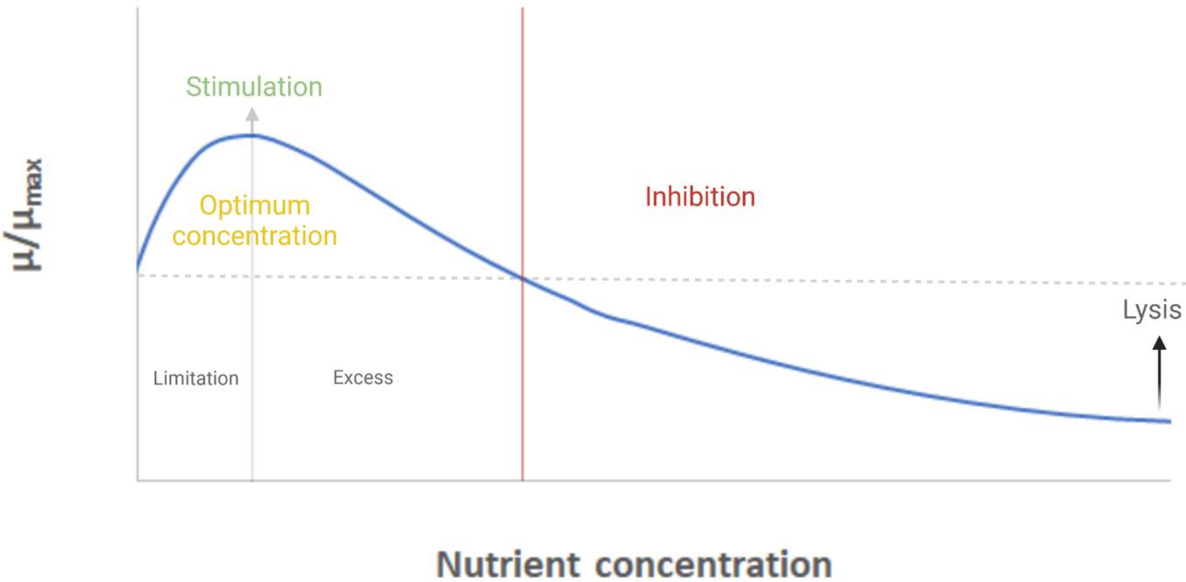


Figure 2-2: Influence of the nutrient concentration on microbial growth, modified from (MacCarty P.L., 1964; Oleszkiewicz & Sharma, 1990; Weinrich & Nelles, 2021a).

Friehe et al. (2012, p. 26) summarized the limit concentration (red line Figure 2-2) for several inhibitors. Nevertheless, many of the concentrations are dependent on the operating conditions and reactor types or configurations (Oleszkiewicz & Sharma, 1990). Therefore, large variations in the inhibition/toxicity levels have been reported due to the complexity of the AD process where mechanisms such as antagonism, synergism, acclimation, and complexing could significantly affect the phenomenon of inhibition (Y. Chen et al., 2008). The summary of the inhibitors in AD is presented in Table 2-9.

AD inhibitors are generally classified into two groups: substances introduced into the system *via* the feed stream and intermediate components that are inhibitory at high concentrations (Neshat et al., 2017). Methanogens are commonly considered the most sensitive to the toxicity of microorganisms in AD. However, the process can acclimatize, and higher concentrations of the toxicant can be tolerated after a period of adaptation (Irini Angelidaki et al.).

Table 2-9: Inhibitors in anaerobic decomposition processes and the concentrations at which they become damaging (Friehe et al., 2012, p. 26).

Inhibitor	Inhibitory concentration	Comments
Oxygen	>0.1 mg/l	Inhibition of obligate anaerobic methanogenic archaea
Hydrogen sulfide	>50 mg/l H_2S	The inhibitory effect rises with a falling pH value
Volatile fatty acids	2,000 mg/l Hac (pH = 7.0)	The inhibitory effect rises with a falling pH value. High adaptability of the microorganisms
Ammoniacal nitrogen	>3,500 mg/l NH_4^+ (pH = 7.0)	The inhibitory effect rises with rising pH value and rising temperature. High adaptability of bacteria
Heavy metals	Cu > 50 mg/l. Zn > 150 mg/l, Cr > 100 mg/l	Only dissolved metals have an inhibitory effect. Detoxification by sulfide precipitation
Disinfectants, antibiotics	n.s.	Product-specific inhibitory effect

Ammonia

Ammonia toxicity increases with increasing temperature, and washout of the microbial population can occur (Irini Angelidaki et al.). Especially the undissociated form of ammonia is considered responsible for process inhibition at concentrations above 80 mg/L (Weiland, 2010). Abdelgadir et al. (2014) reported that the ammonia concentration must be maintained over 40–70 mg $N \cdot L^{-1}$ to prevent biomass activity reduction. Contrarily, Khanal (2008) reported different intervals with their respective effects (Table 2-10).

Table 2- 10: Ammonia nitrogen concentration and its effect on anaerobic treatment (Khanal, 2008, pp. 56–58).

Ammonia–N (mg/L)	Effects
50–100	Beneficial
200–1,000	No adverse effect
1,500–3,000	Inhibitory effect at higher pH values
Above 3,000	Toxic

The results for ammonia–N inhibitory levels are contradictory as they vary in parameters such as pH, temperature, and inoculum adaptation. It is widely accepted that the non-ionized form of ammonia is responsible for AD inhibition; however, pH has a significant impact on the level of ammonia inhibition as the pH value determines the degree of ionization. The equilibrium relation can be used to calculate the free ammonia ratio to the total ammonia/ammonium ratio (Irini Angelidaki et al.; Fotidis et al., 2013).

A decrease in pH reduces ammonia inhibition. Free ammonia inhibition causes VFA accumulation, which lowers pH and decreases the ratio of free ammonia, resulting in the relief of free ammonia inhibition. Owing to this self-stabilizing mechanism, processes can be kept in a stable ammonia-inhibited state with a balance of VFA concentration and ammonia loading (Irini Angelidaki et al.; Y. Chen et al., 2008; Yenigün & Demirel, 2013).

I. Angelidaki and Ahring (1993) conducted experiments on the thermophilic digestion of cattle manure in different ammonia concentrations. The AD of cattle manure was inhibited by ammonia concentrations of 4 g N/L or higher. After 6 months of operation, stable digestion of cattle manure with ammonia concentrations of up to 6 g N/L could be maintained. However, when compared with controls with an ammonia concentration of 2.5 g N/L, the methane yield (MY) was reduced, and the concentration of VFAs increased from 1 to 3 g/L as acetate. Hence, a gradual increase in ammonia concentration reduced the temporary strong inhibition caused by a one-step increase. Furthermore, ammonia toxicity tests on acetate- and hydrogen-using populations revealed that acetoclastic methanogens were more sensitive to ammonia than hydrogenotrophic methanogens.

I. Angelidaki and Ahring (1994) investigated the combined effect of temperature and ammonia concentration in the intervals of 40 °C–64 °C and 2.5–6.0 g-N/L in continuously fed reactors. At the applied retention time of 15 days, poor process performance was observed when the combination of temperature and ammonia loading resulted in a calculated concentration of unionized ammonia (NH_3) exceeding 0.7 g-N/l. When the ammonia load was high, maintaining the temperature below 55 °C increased the biogas yield and improved process stability, as asserted by the depletion of VFAs in the effluent.

One of the most effective ways to avoid ammonia inhibition is the adequate control of the C/N ratio (Irini Angelidaki et al.). A high C/N ratio causes low protein solubilization. On the other hand, a high C/N ratio provides insufficient nitrogen to maintain cell growth and results in rapid nitrogen degradation by microbes resulting in lower biogas production. Excessively low C/N ratio substrates increase the risk of ammonia inhibition, which is toxic to methanogens and results in insufficient utilization of carbon sources. The optimal C/N ratio for AD has been demonstrated to be between 20 and 30 or 20 and 35, with a ratio of 25 being the most commonly used (Karki et al., 2021; Mao et al., 2015; J. Mata-Alvarez et al., 2014).

Organic acids

VFAs act as a double edge blade; they are one of the indicators of the correct balance between hydrolysis, acidogenesis, and methanogenesis. In an anaerobic reactor, instability usually results in VFA accumulation, which can cause a drop in pH (acidification). However, in some substrates with an excess of alkalinity, the VFA accumulation must exceed a certain threshold before it can be detected as a significant change in pH (Weiland, 2010). This means that when a drop in pH is eventually observed in the reactor, the concentration of VFAs is most likely very high, and the process may already be affected (Irini Angelidaki et al.; Verein Deutscher Ingenieure, 2016). Therefore, the VFA accumulation can be seen as a result of an already inhibited process and is not considered the actual reason (Kougias & Angelidaki, 2018).

VFA accumulation can be interpreted as either organic overload or inhibition of the methanogenic microbial communities due to the influence of other factors (Madsen et al., 2011). In a stable anaerobic digester, the concentration of VFAs is about 50–250 mg/L (Khanal, 2008; Neshat et al., 2017). Acetic acid is normally the most concentrated among the carboxylic acids but is less inhibitory to methanogens than propionic and butyric acids (L. Yu & Wensel, 2013). A maximum concentration of 3000 mg/L is considered the stability limit for acetic acid and 1000 mg/L for propionic acid. A very rapid rise in acid concentration should be expected when the loading limit is reached (Verein Deutscher Ingenieure, 2016).

LCFAs are compounds often linked to toxicity in AD. High concentrations of LCFAs are frequently observed in a variety of agro-industrial residues, including slaughterhouse wastes, food wastes, and olive mill wastewater. The inhibition caused by LCFA is due to the accumulation of compounds formed during β-oxidation that cannot be further oxidized because the required reactions are thermodynamically unfavorable. As a result, LCFA inhibits the activity of hydrolytic, acidogenic, acetogenic, and methanogenic bacteria (Kougias & Angelidaki, 2018; Lalman & Bagley, 2002; Pereira et al., 2005).

Sulfur

Another byproduct of the digestion process is hydrogen sulfide (H_2S), which can act as a cytotoxin in the undissociated, dissolved form at concentrations as low as 50 mg/L (Friehe et al., 2012, p. 26). Sulfide is produced by sulfate-reducing bacteria during the anaerobic treatment of sulfur-rich waste streams. The sulfide levels in industrial waste streams from tanneries, petrochemical refineries, coal gasification, and other sources are high. Sulfide is also produced during the breakdown of sulfur-containing organic matter (proteins) in waste, such as swine manure. Unionized sulfide (H_2S) is thought to be more toxic to methanogens than ionized sulfide (HS^-) (Khanal, 2008, pp. 138–139).

The concentration of sulfur species is a strong function of pH, as presented in Fig. 2-3 Lewis. As the pH value falls, the proportion of free H_2S rises, increasing the risk of inhibition. One possible way to reduce the H_2S concentration is *via* precipitation as sulfides with the aid of iron ions. H_2S also reacts with other heavy metals and is bonded and precipitated out, along with the formation of sulfide ions (S^{2-}) (J. L. Chen et al., 2014). However, sulfur is also an important macronutrient. Thus, an adequate concentration of sulfur is necessary for the formation of enzymes, but excessive precipitation in the form of sulfides can also be responsible for the inhibition of methanogenesis (Friehe et al., 2012, p. 106). Figure 2-3 presents the behavior of the activity of the different species of sulfide.

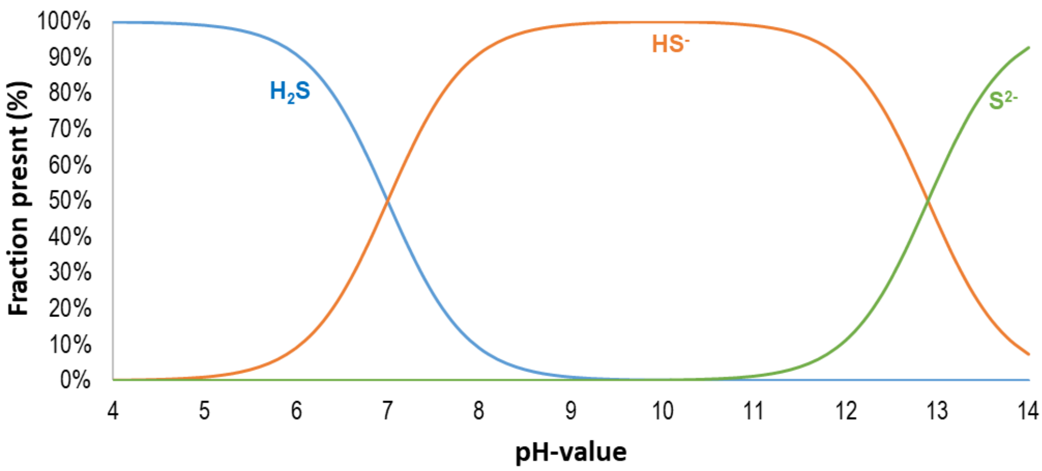


Figure 2-3: pH dependence of sulfide speciation.

McCartney and Oleszkiewicz (1991) observed that sulfide toxicity increased with increasing pH. Other studies on sulfide inhibition indicated that more than one inhibition threshold might be present under different conditions (Y. Chen et al., 2008). Hence, Chernicharo (2007, pp. 37–38) reported that from a practical point of view, it is important to determine the sensitivity of biomass to sulfide. The quantity of sulfides produced in the anaerobic treatment depends on the following main factors:

- Chemical oxygen demand to sulfide ratio (COD/SO_4^{2-}) in the influent (a low ratio results in a high sulfide production).
- Composition of the organic substrate.
- pH and temperature of the medium.
- Competition between sulfate-reducing and methanogenic microorganisms

Additionally, Chernicharo (2007, p. 38) proposed the following measures to counterattack a possible inhibition due to sulfide:

- Increase pH in the reactor, so that the dissociation of H_2S in the liquid phase favors the formation of HS^-.
- Dilute the influent, aiming at reducing the concentration of sulfides in the reactor.
- Precipitate sulfides by using iron salts.

Furthermore, the composition of H_2S in biogas normally ranges from 50 to 10,000 ppm depending on the feed material composition. At high concentrations, H_2S can cause corrosion to the engine and metal parts *via* SO_2 emissions from combustion, especially when the engine is not running continuously. Users have shown little interest in using biogas for power generation because hydrogen sulfide is harmful to the cast iron and steel used in the equipment. Furthermore, many biogas applications have been hampered by the inability to eliminate the corrosive and toxic H_2S in raw biogas. Thus, H_2S needs to be removed before further use (Mamun & Torii, 2015; Nallamothu et al., 2013).

2.1.5 Hydraulic retention time and OLR

The hydraulic retention time (HRT) can be defined as the average time that a fluid element spends in the system, and it is the retention time for a fluid (Chernicharo, 2007, p. 39; Sperling, 2007, p. 100; Winterbottom & King, 1999, p. 268). A more precise definition is provided by Henze et al. (2008, pp. 56–57), in which the volume of the process per unit per volume of influent flow is defined as the nominal hydraulic retention time. To know the actual hydraulic retention time, the sludge underflow and the mixed liquor recycle ratios must be considered.

The mathematical definition of HRT is shown in Equation 2.1.

$$\mathrm{HRT} = \frac{\mathrm{V}_R}{\mathrm{Q}} \tag{2-1}$$

where HRT corresponds to HRT (d); V_R, the volume of the bioreactor (m^3); and Q, the influent flow rate (m^3/d).

When analyzing the performance of bioreactors without biomass retention mechanisms, one of the main criteria is that HRT should be larger than the cell doubling time to avoid the washout of the microorganisms (Environmental Energy Company: Olympia, 2001, pp. 21–22; Kroiss, 1985, pp. 91–92). Hence, the HRT has a large influence on the operation stability of the reactor (Shen, Tian, et al., 2013; Singh et al., 2019).

HRT controls the time the waste remains inside the reactor in contact with the biomass, but the solid retention time (SRT) controls the residence time of the biomass (Khanal, 2008, pp. 173–175). SRT can be expressed in terms of the average time length of microorganisms active in the system (Bui et al., 2019, pp. 107–110). One of the main strategies for increasing the reactor's performance is the decoupling of the HRT and SRT, which can be achieved *via* different retention mechanisms giving birth to different high-rate technologies, such as biofilms and bio-granules (Saravanan & Sreekrishnan, 2006). Hence, in reactors without biomass retention mechanisms, HRT equals SRT.

The influence of HRT is mostly described in non-high-rate reactors, where the settling of an insufficiently large HRT may eventually lead to the acidification of the digester through the accumulation of VFAs (Nkuna et al., 2021). Hence, the establishment of HRT according to the substrate composition is an important part of the reactor's performance optimization (Cremonez et al., 2021). The relationship between biodegradation rate, substrate macromolecule composition, and retention time was semi-quantitatively described by Akunna (2018, p. 7) (Figure 2-4). As can be seen from the figure, the more complex the substrate, the longer it requires to achieve a high degradation rate. Therefore, a higher feed rate as a biodegradation rate will eventually lead to the acidification of the failure of the reactor; therefore, the control of HRT and/or SRT plays a major role in the reactor's performance.

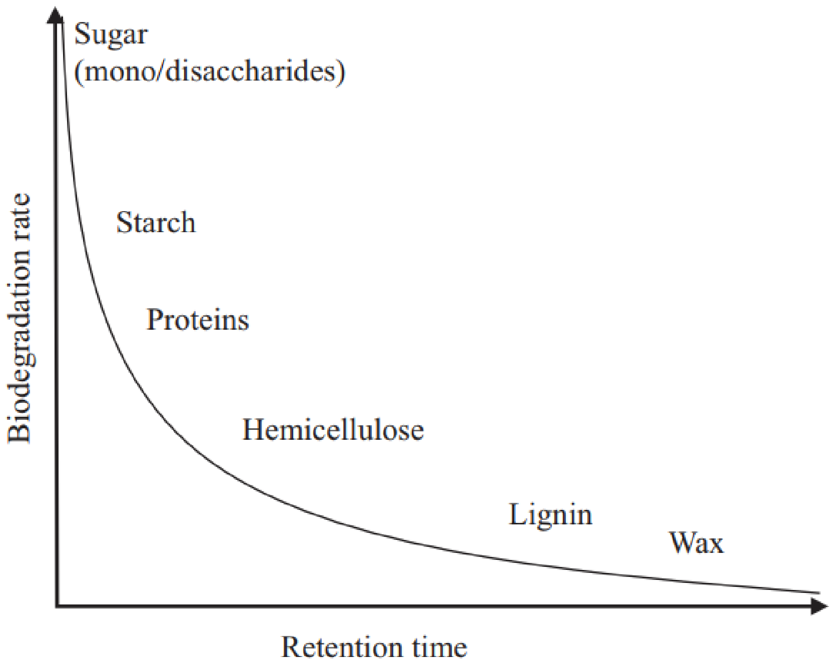


Figure 2-4: Relationship between the rate of degradation and retention time for various types of organic compounds (Akunna, 2018, p. 7).

The organic feed rate per digester volume is known as the OLR (Mahmoud et al., 2003). OLR is also defined as a measure of the AD system's biological conversion capacity (Rabii et al., 2019b). Therefore, it is often one of the main criteria employed in the design of bioreactors (Khanal, 2008, pp. 8–9).

The mathematical definition of OLR is presented in Equation 2.2.

$$\mathrm{OLR} = \frac{Q \cdot COD}{V_R} = \frac{COD}{HRT} \qquad (2\text{-}2)$$

where OLR corresponds to the OLR ($kg_{COD}/m^3 \cdot d$); Q, the flow rate (m^3/d); COD, chemical oxygen demand (kg_{COD}/m^3); V, the volume of the bioreactor (m^3); and HRT, hydraulic retention time (d).

OLR can have a profound impact on microbial community diversity and structure. Microbial community diversity often decreases under high OLR (Bui et al., 2019, pp. 506–509). Low biogas yield is obtained when feeding the system above its sustainable OLR (Rabii et al., 2019b). The optimal OLR is determined by the complexity of each waste component rather than the proportion of different fractions (carbohydrates, proteins, lipids) in it. As a result, the OLR affects all of the microbial communities in the reactor and, as a result, the VFA composition distribution (Vázquez-Fernández et al., 2022). The effect of OLR on AD performance is summarized in Figure 2-5.

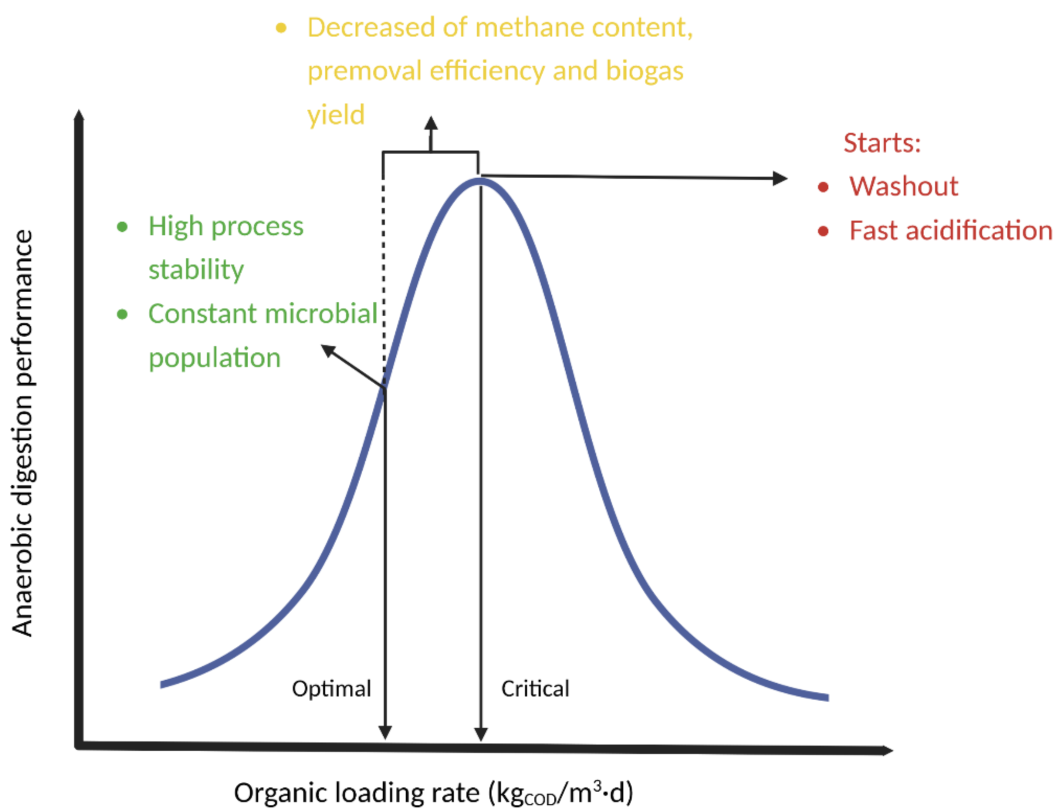


Figure 2- 5: Semiquantitative representation of anaerobic digestion performance based on the organic loading rate, modified from Nkuna et al. (2021).

The critical OLR point varies due to differences in substrate composition and the rate at which these components degrade. Therefore, it is important to identify the optimal OLR for individual substrates or a mixture of them at the targeted operating conditions through exploratory experiments by previously establishing the desired process efficiency and stability.

2.1.6 Flow pattern in the expanded granular sludge reactor

Continuously operated chemical reactors with idealized flow patterns have played a central conceptual role in the development of reactor engineering (Winterbottom & King, 1999, p. 252). There are two ideal flow patterns: plug flow (tubular) and complete mix (Gujer, 2008, pp. 129–130; Sperling, 2007, pp. 100–103). In plug flow, all fluid elements crossing a given plane move at the same speed and in the same direction, resulting in equal velocities. Detailed measurements of velocities in long straight pipes during turbulent flow have revealed a very close approximation to plug flow. Contrarily, in complete mix intensive properties within the vessel, i.e., temperature, composition, and other properties are spatially uniform. When applied to a continuously operated stirred tank, the tank's contents are homogeneous, and the feed quickly mixes with these contents, thus losing its identity (Gujer, 2008, pp. 129–132; Levenspiel, 1999, pp. 258–261; Winterbottom & King, 1999, pp. 268–270). A schema of these two ideal flow patterns is presented in Figure 2-6.

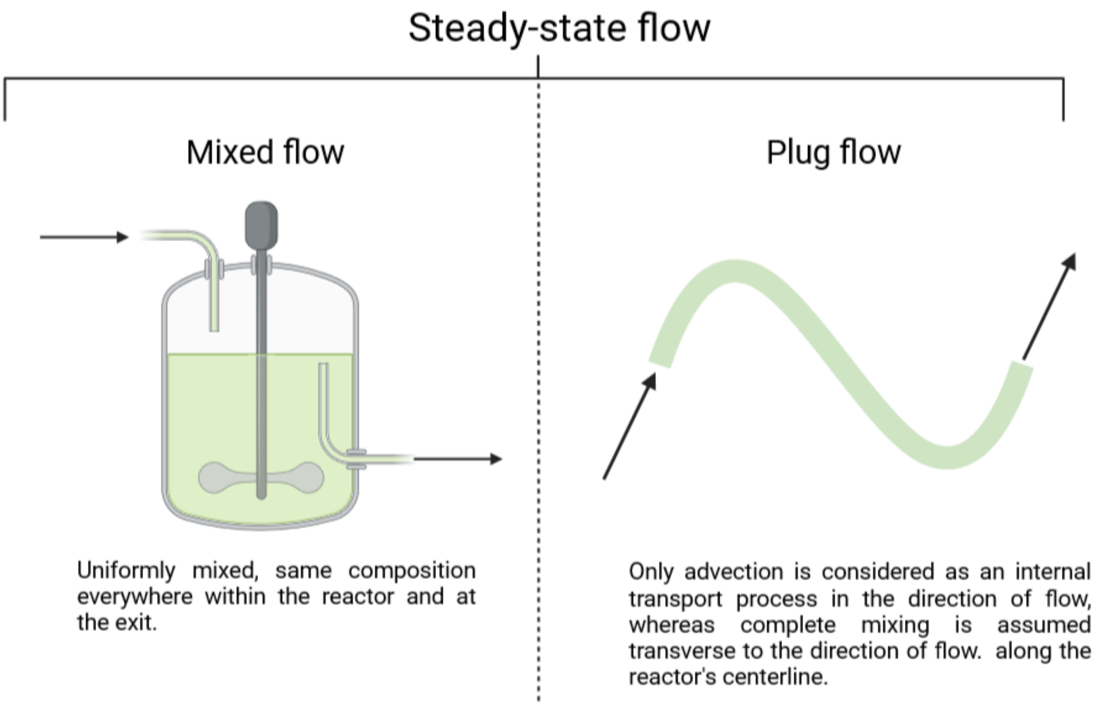


Figure 2-6: Ideal reactor types. Modified from (Gujer, 2008; Levenspiel, 1999).

In a reactor, the design is often beneficial to reach one of these ideal patterns because they are simple to treat; therefore, it is easier to obtain a mathematical description of the optimal reaction path. But a piece of real equipment to a greater or lesser extent always deviates from these ideal flow patterns;

hence, a simplification in their modeling is not possible (Bhattacharyya & Singh, 2010; Monteith & Stephenson, 1978). Factors like the shape of the system, scale, hydraulic characteristics of the input and output structures, presence of biomass, and environmental conditions can produce unfavorable hydraulic circumstances, such as dead zones, inertial currents, and hydraulic short circuits, which reduce the HRT and decrease the efficiency of the system (Londoño et al., 2019). According to Levenspiel (1999), overall, three interrelated factors make up the contacting or flow pattern:

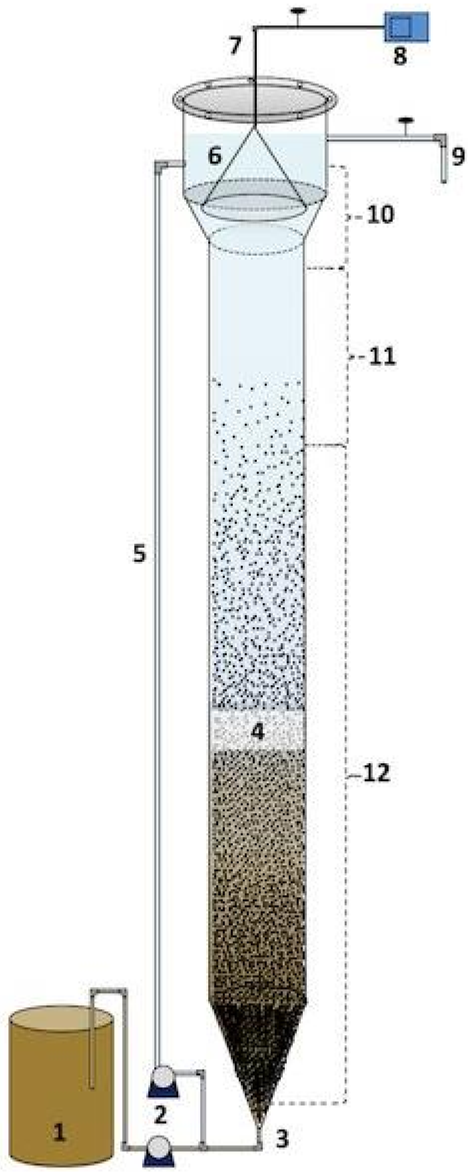
1. The residence time distribution (RTD) of the material that is flowing through the vessel.
2. The state of aggregation of the flowing material, its tendency to clump, and for a group of molecules to move about together.
3. The earliness and lateness of mixing of material in the vessel.

The mixing and internal transport processes in a reactor are characterized by using the distribution of the HRT in a reactor (RTD). The comparison of theoretically computed and experimentally determined RTDs facilitates the development of mathematical models of real-world reactors (Gujer, 2008, p. 129). The study of anaerobic reactor hydrodynamics plays an important role as it can influence the rates of biological reactions through changes in the rate of mass transfer and the distribution of reactions along the reactor affecting its performance (Brito & Melo, 1997; Gleyce et al., 2014).

The EGSB reactors appeared as an improvement on the upflow anaerobic sludge blanket (UASB) reactors where a high height–diameter relation allowed them to work at a higher superficial velocity (>4 m/h EGSB per 1,5 m/h UASB). The main change consisted in the addition of an external recirculation that allows regulation of the mixing independently of the feeding rate, consequently, internal mixing problems of the UASB such as the occurrence of dead zones, preferential flow, and short circuits, among others are solved. In addition, due to the higher superficial velocity, an expansion of the sludge bed occurs, intensifying the hydraulic mixing and giving the EGSB the ability to generate a better substrate–biomass contact within the treatment system (Gleyce et al., 2014; Treichel & Fongaro, 2019, pp. 76–77). Lou et al. (2006) conducted a tracer analysis in an UASB with and without recycling, which resembled a comparison between an EGSB and a UASB. As a result, the UASB with recycling exhibited a significant improvement in overall mixing within the reactor over the no-recycling option.

A schematic operation of the EGSB reactor is presented in Figure 2-7.

Because of the complex nature of the EGSB reactor, its local hydrodynamic behavior is not well documented, even though most of the time, it is described in the literature as a completely mixed reactor. This description has no high deviation from reality when a relatively simple kinetic model can describe the reactor behavior, even when its hydrodynamics present minor flaws (Pérez-Pérez et al., 2017).



(1) Feed tank, (2) peristaltic pump, (3) influent, (4) EGSB bioreactor, (5) recirculation, (6) bell separation, (7) biogas outlet, (8) gas flow meter, (9) effluent, (10) three-phase separator zone or settling zone, (11) transition zone, (12) digestion zone.

Figure 2-7: Schematic diagram of an EGSB bioreactor (A. Cruz-Salomón et al., 2019).

Usually, the consideration of a completely mixed tank comes together with neglecting the liquid mass transfer resistance. However, the exact mixing pattern cannot be generalized. Thus, it should be assessed in each reactor (Fuentes et al., 2011). The hydrodynamic characteristics may be determined by using a tracer material to obtain an RTD curve or exit age distribution curve (E-curve for pulse input or F-curve for step input) (Bhattacharyya & Singh, 2010). Alternatively, computational fluid dynamics (CFD) models can be applied for this purpose.

The amount of mixing in a reactor determines its performance; thus, for a description of a real reactor, the influence of mixing on the mass balance equation must be specified as accurately as possible (Gujer, 2008, p. 129). Despite the complex hydrodynamics of the EGSB, there are some examples in the

literature on the integration of hydrodynamics and mass and balance equations. Fuentes et al. (2011) employed a bioparticle model under the assumption that the EGSB behaved as a CSTR due to the effects of a high recirculation rate. Nevertheless, the expansion of the bed was taken into account. Brito and Melo (1997) conducted tracer studies and found that the EGSB behaved as CSTR and as a consequence of the hydrodynamics liquid film mass transfer resistance seemed negligible in the EGSB. The CSTR model was integrated with zero-order kinetics in the degradation of low-acetate conditions by the granular biomass.
A. Cruz-Salomón et al. (2019) stated that to fully characterize the hydrodynamics of the EGSB bioreactor the fluid dispersion, turbulence in the bioreactor, settling velocity of the sludge, expansion of the sludge bed, and shear rate on the granules should be taken into account in the hydraulic model of the reactor. Nonetheless, a model of a series of five CSTRs each one representing one section of the bioreactor was the most accurate model for Gleyce et al. (2014).

An emerging alternative to tracer studies is CFD. The mixing effect in a digester can be numerically simulated using CFD simulation software. Based on the specified digester geometry, feed location, physical properties, and operating conditions, CFD can also be used to predict anaerobic digester velocity profiles, rates of energy dissipation, concentrations, and flow streamlines. Over the last decade, CFD has been used to predict digester flow patterns in wastewater treatment units such as ponds, lagoons, and tanks (Xie et al., 2016; L. Yu & Wensel, 2013). Nevertheless, the integration of CFD in the mass and energy balance analysis of the reactors has been rather limited mainly due to the complexity of numerical simulation and model stability when biological rate equations are coupled (Xie et al., 2016; L. Yu & Wensel, 2013).

2.2 Choice of the appropriate reactor technology with a focus on the German agricultural biogas sector

Reactor technology is one of the most influential factors in the AD outcome (Treichel & Fongaro, 2019, p. 75). Choosing the right bioreactor type and configuration is critical for maximizing metabolic, and nonoxidative bioenergy production. A reactor designed for bioenergy production may not be suitable for waste treatment (Chernicharo, 2007, pp. 70–71; Khanal, 2008, p. 93).

Some other general considerations when choosing the right anaerobic technology are single-stage vs multistage, wet vs dry AD, batch vs continuous/semicontinuous, and low-rate vs high rate. The selection of one category over its counterpart is ultimately based on the situation by balancing the technical and economic criteria (Korres et al., 2013; Nielsen et al., 2004).

The type of reactor chosen for each application can be influenced by many factors, mainly HRT or organic loading; other specific criteria like hydraulic loading rate or sludge loading rate are not uncommon (Chernicharo, 2007; Henze et al., 2008; Nkuna et al., 2021).

Because anaerobes slowly grow during the metabolic generation of methane, biomass retention capacity is an important consideration when selecting a suitable bioreactor. High-rate reactors are those that can successfully separate HRT from the SRT *via* some retention mechanisms (Halalsheh et al., 2005; Huang et al., 2013). Some of the most common retention mechanisms are biomass immobilization in attached growth systems, granulation, and floc formation (Damien J. Batstone, 2006; Saravanan & Sreekrishnan, 2006).

At present, high-rate technology is widely used in waste treatment owing to its capacity to decouple HRT and SRT. UASB and EGSB are two of the most widely used high-rate reactors due to their ability to form dense aggregates *via* auto-immobilization, which allows them to work at high OLRs, robustness, and market price (Abumalé Cruz-Salomón et al., 2018; Fuentes et al., 2011; López & Borzacconi, 2011; Ratanatamskul & Siritiewsri, 2015).

The treatment of low TS substrates from agriculture like liquid poultry and swine manure is becoming increasingly popular nowadays in high-rate systems because they can be treated to high OLR, which is not profitable for conventional biogas plants due to the coupling of HRT and SRT in the reactor technology used (Häner et al., 2022; C. Rico et al., 2017; C. Rico et al., 2015; C. Rico et al., 2012).

In Germany, agricultural biogas production technology was developed based on the technology used in anaerobic municipal wastewater and sewage sludge treatment plants (Lebuhn et al., 2014). Biogas production technologies are typically simple and robust. Wet fermentation using CSTR is the most common technology. In total, approximately 90% of biogas plants use wet fermentation, with the remaining 10% using solid-material fermentation. Approximately 1% of the plants are discontinuously operated (batch operation/box or garage fermenter). In Germany, 80–100 discontinuously operated dry fermentation plants (garage or box fermenter) were in operation at the end of 2016 (Daniel-Gromke et al., 2018; Thrän, 2015, p. 76).

Although the technological standard of biogas plants has significantly improved over the last decade, most agricultural biogas systems still lag behind industry standards in other branches, particularly in plant safety, automation, and quality control. One reason for this is that at least for farm-scale installations using animal manure as the primary input, construction costs are the most important economic factor for the investor, i.e., the farmer. As a result, except for “large” installations (equivalent electrical output of more than 1 MW) linked to a biogas upgrading and feed-in facility, the level of process control and automation of biogas systems is at best intermediate but frequently low (Lebuhn et al., 2014; Theuerl et al., 2019).

Hence, future agricultural biogas plants should be oriented to increase flexibility concerning feedstocks, digester operation, microbial communities, and biogas output while maintaining stability and reducing susceptibility to disturbances in the reactor operation (Theuerl et al., 2019). For substrates with low dry matter (DM) content, large fermenter volumes are required to guarantee a high degree of biodegradation. This can be avoided by using reactors with biomass retention, where SRT/HRT allows decreasing many times the required reactor volume. However, these reactors do not tolerate high solid loads. Therefore, integration in a multistage treatment schema, with an upstream solid-liquid separation, where the advantages of both reactor types are exploited, may be the best solution. Thus, integrating high-rate technology in conventional biogas plants may increase flexibility across several of the aforementioned categories. A possible multistage process including an EGSB reactor was proposed by Regalado et al. (2021). The schema is presented in Figure 2-8.

The different generations of digesters, their main features, and their relative OLR handling capacities were summarized by Nkuna et al. (2021) (Table 2-11). Other classifications based on their criteria like the amount of TS that the reactor is able to handle or based on the growth rate have been published by Damien J. Batstone et al. (2015) and Khanal (2008), respectively.

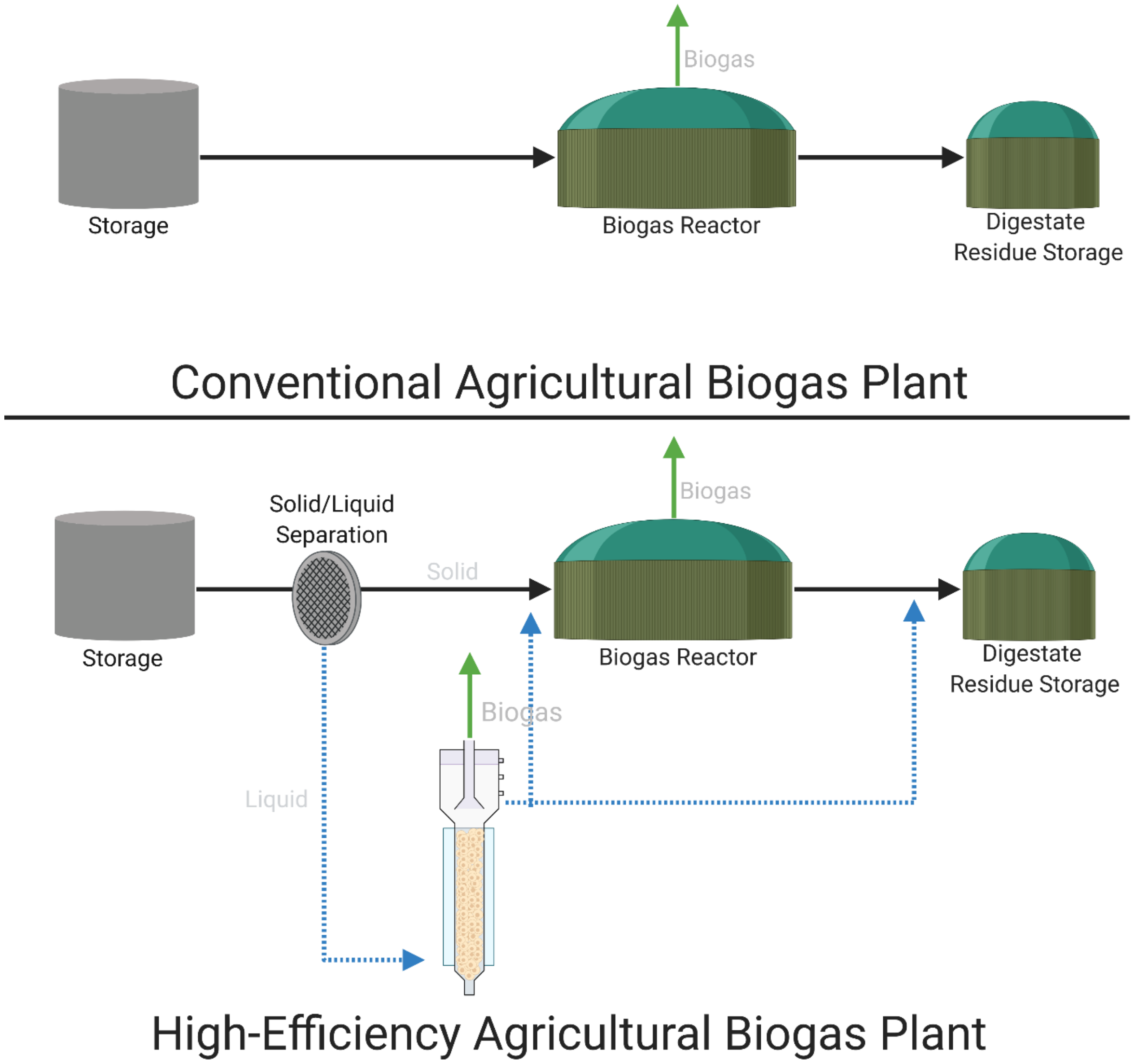


Figure 2-8: Schematic structure of a conventional biogas plant and a high-efficiency agricultural biogas plant (Regalado et al., 2021).

Table 2- 11: Examples of first-, second-, and third-generation digesters and their organic loading rate handling capacities (Nkuna et al., 2021).

Digester class and principle	Examples of reactors	Description	OLR[a] recorded	References
First generation No microbial entrapment which results in high F/M ratios	(PFR) Plug flow reactor. Like a red mud balloon	No agitation in digesters	1.3–5 $kg_{VS}/m^3/d$	(Thorin et al., 2017)
	(CSTR) Continuously stirred tank reactor	Agitation improves the OLR handled	5 $kg_{VS}/m^3/d$ 6 $kg_{COD}/m^3/d$	(Rincón et al., 2008; Sentürk, Ince, & Engin, 2010)
	(AC) Anaerobic contact	Degassed exiting sludge is recycled back into the digester	5 $kg_{COD}/m^3/d$ 0.6–8 $kg_{COD}/m^3/d$	(Sentürk, Ince, & Onkal Engin, 2010; Yousefi et al., 2018)
Second generation Microbial recirculation or retention using support matrices/sludge blanket	Anaerobic baffled (ABR) and periodic anaerobic baffled (PABR)	Compartmentalization assists in biomass retention	10 $kg_{COD}/m^3/d$ 12.5 $kg_{COD}/m^3/d$	(Stamatelatou et al., 2003; Tang et al., 2007)
	(AF) Anaerobic filter	Substrate fed from the bottom through a packed bed	36 g TOC/L/d	(Kennedy & van den Berg, 1982)

	(DFFR) Downflow fixed film reactor	An AF reactor with substrate entering from the top	94 $kg_{COD}/m^3/d$ overload could be successfully reversed	(Rintala & LEPISTO, 1992)
	(UASB) Upflow anaerobic sludge blanket	Naturally forming dense active biomass granules. The granules replace the support material in AF	80 $kg_{COD}/m^3/d$	(Jeris, 1983)
	(FLB/EB) Fluidized bed or expanded bed	Biomass supporting media is kept suspended by the rapid upflow of wastewater	38 $kg_{COD}/m^3/d$ 75.6 $kg_{COD}/m^3/d$ produced severe adverse biodigester response but could be reversed slowly to normal	(Mathiot et al., 1992; Zheng et al., 2012)
Third generation	(EGSB) Expanded granular sludge bed	A UASB expanded by recirculation	45 $kg_{COD}/m^3/d$	(Driessen & Yspeert, 1999)
Additions and modifications to UASB	(IC) Internal circulation	Essentially two-staged UASB with internal circulation and double-phase separation	42 $kg_{COD}/m^3/d$	(Angenent, 2001)

	(AMBR) Anaerobic migrating blanket reactor	Baffles arranged interchangeably vertically downward and upward in a rectangular vessel with intermittent mixing	30 $kg_{COD}/m^3/d$	(Lens et al., 1998)
Phase separation of AD processes	(USSB) Upflow staged sludge bed reactor	Separate reaction zones are created by placing baffles along the UASB reactor length	30 $kg_{COD}/m^3/d$ 43 $kg_{COD}/m^3/d$ 100 $kg_{COD}/m^3/d$	(Angenent, 2001; Onodera et al., 2012)
	(TPPFR) Two-phase plug flow reactor and (GBABR) granular bed anaerobic baffled reactor	These are essentially PFR with baffles (horizontal for TPPFR and vertical ones for GBABR) to facilitate	10.93 $kg_{VS}/m^3/d$ 13.38 $kg_{COD}/m^3/d$	(Liu T., 1998; van Lier et al., 1994)
Hybridization of reactor configurations	AF-UASB	A train of reactors featuring both AF and UASB is implemented	45 $kg_{COD}/m^3/d$ 51 $kg_{COD}/m^3/d$	(Baloch et al., 2007; Borja et al., 1995)
	(UASFF) Upflow anaerobic sludge bed fixed film	Rope matrix (or other packing material) incorporated in a UASB reactor	31 $kg_{COD}/m^3/d$ 35 $kg_{COD}/m^3/d$	(Acharya et al., 2008; Guiot & van den Berg, 1985)

[a] : The organic loading rates (OLR) selected are a few high-end figures recorded in the literature cited.

2.3 AD versus AcoD

2.3.1 The role of AcoD

As a disadvantage, AD is a very complex, sensitive process involving numerous microorganisms with extreme operational and environmental conditions (Treichel & Fongaro, 2019, pp. 2–3). Substrate digestibility and biogas production are influenced by substrate composition, loading rate, mineral and VFA compositions, carbon-to-nitrogen ratio, and pH, as well as reactor temperature and HRT (L. Yu & Wensel, 2013).

High efficiency in energy production is usually achieved under a high OLR; however, stability and effective methane production can be influenced by the system's failure because of the imbalance between acidification and methanation, which may result in severe accumulation of VFAs and a sharp decrease in pH (Rabii et al., 2019b).

Among the most recommended ways of improving AD are pretreatments, enzymes (biocatalysts), anaerobic co-digestion (AcoD), reactor engineering, coupling with dark fermentation, and microbial community (bioaugmentation) (Treichel & Fongaro, 2019).

AcoD involves the simultaneous digestion of two or more substrates. It has been shown as a highly viable option for improving biogas production by alleviating the disadvantages of mono-digestion (Rabii et al., 2019b). It also increases the economic feasibility of the process in existing AD plants by increasing MYs (Rabii et al., 2019b). The advantages of AcoD include the abundant supply of macro- and micronutrients, a balanced C/N, the dilution of reaction inhibitors, a superior buffering capacity, and the enhancement of biogas production (Salehiyoun et al., 2020).

Aside from the advantages, AcoD also presents some drawbacks like feedstock variability, which can affect the stability and performance of the anaerobic digestion process. Increase in cost of transport, handling, and logistic complexity due to the increment in the number of waste streams. Moreover, additional infrastructure, such as storage tanks and feed systems, may be required, which can further add to the overall cost and complexity of the process.

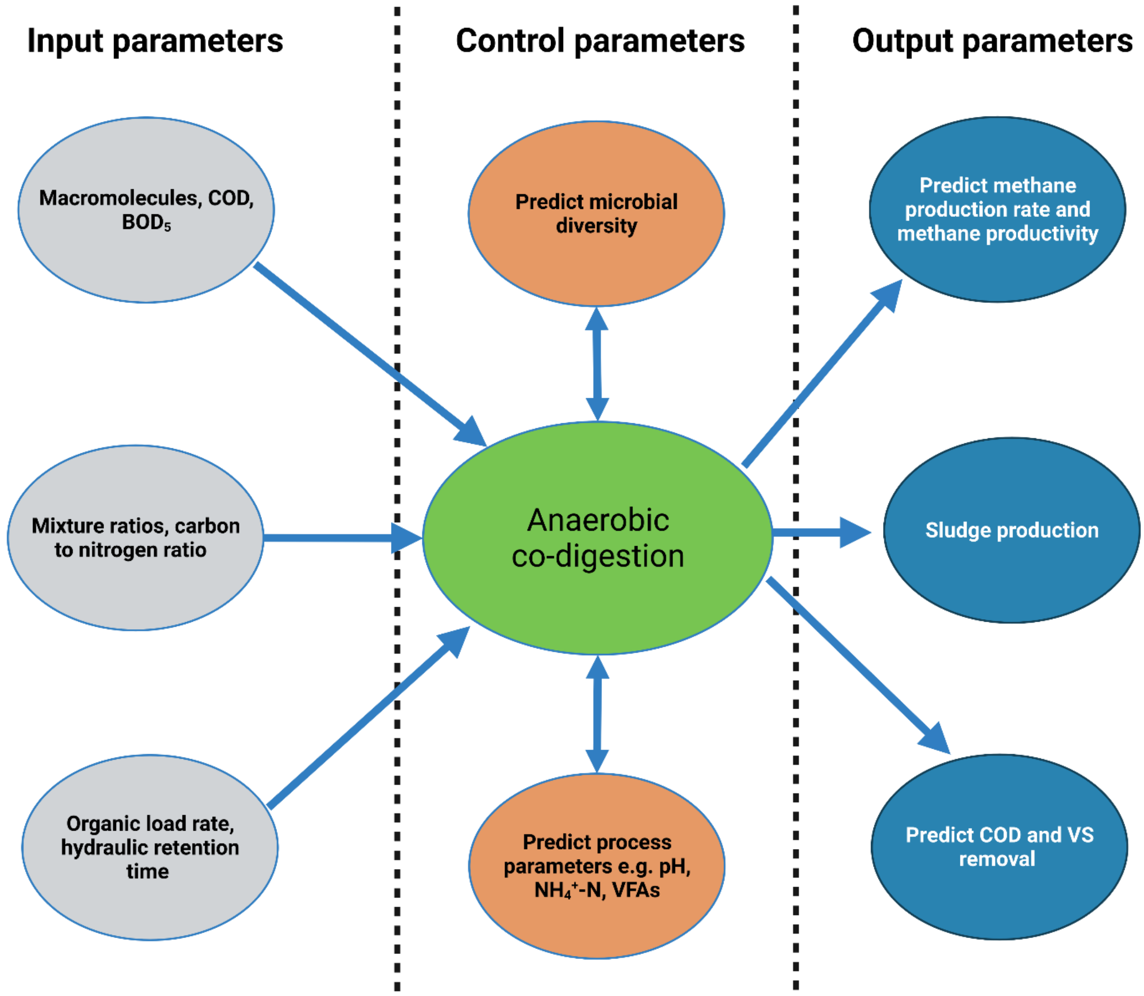
Most of the AcoD's drawbacks can be eluded by the appropriate selection of co-substrates, co-substrate composition within the mixture, and operating conditions of the reactor and selected pre-treatments. Consequently, a poorly researched co-digestion process may result in instability, bringing with it a significant reduction in methane production. Therefore, it is necessary to have a profound comprehension of the co-digestion mixture(s) used at a lab and pilot plant to support full-scale design and operation decisions. All these drawbacks read as avoidable mistakes and not as general drawbacks (Xie et al., 2016; L. Yu & Wensel, 2013).

AcoD in the right proportions yields more biogas than the sum of the biogas produced from the mono-digestion of each substrate (Aichinger et al., 2015). According to Rabii et al. (2019b), co-digestion of different feedstocks with animal manure can increase biogas production from 25% to 400% compared with the mono-digestion of the same substrates. (G. Wang, 2010) observed that wheat straw co-digested with swine manure can increase methane production rate by up to 10% when 46% of wheat straw is added to the digester.

Esposito et al. (2012) concluded that the highest specific MYs reached in the literature were reached by co-digesting organic fraction of municipal solid waste with animal fat (83/17 on dry weight) with a value of 686 L_{CH4}/kg_{VS}. The two highest values observed in the literature by the author were from co-digesting CWM with fruit and vegetable waste (60/40 on wet weight) with values of 490 and 450 L_{CH4}/kg_{VS}, respectively.

The combination of substrates resulting in more biogas is known as the synergistic effect, whereas the opposite is also possible and is known as the anti-synergistic effect (Ebner et al., 2016). The synergistic effect more precisely is an increased specific biogas yield for a mixture of raw sludge and co-substrates over the specific biogas yield for individual substrates (Carucci et al., 2005). I. Angelidaki and Ahring (1997) first reported in lab-scale reactors the synergistic effect of co-digesting swine manure with oil mill waste; they observed more than a doubling of methane production. The synergistic effect was interpreted to occur due to the contribution of additional alkalinity by manure waste and the addition of trace elements, nutrients, and/or enzymes by oil-mill waste (Aichinger et al. 2015). Contrarily, Astals et al. (2014) found that the synergistic effects do not ultimately modify the biomethane potential (BMP). Instead, the synergistic effects were mainly improvement of process kinetics without a significant change in biodegradability. Kinetics improvement was linked to the mitigation of inhibitory compounds, particularly fats dilution.

AcoD operation can be more complex than mono-digestion due to critical factors, such as co-substrate properties and mixture composition. Since co-digestion systems work to a higher OLR system overloading is also more likely if the mixture is not carefully chosen. Substrates proportions within the mixture that lead to antagonistic relationships are a good indication of potential inhibitions (Pagés-Díaz et al., 2014b). Thus, the typical models of AcoD results are more complicated than AD models, and a larger amount of data, as well as more variables, need to be registered (Xie et al., 2016). Nevertheless, adding the substrate ratios can help overcome most of the difficulties (Joan Mata-Alvarez et al., 2011; Rahman et al., 2019). The same is presented in Figure 2-9.



COD: chemical oxygen demand; BOD_5: 5-day biological oxygen demand; VFAs: volatile fatty acids; VS: volatile solids

Figure 2-9: Qualitative model structure for co-digestion.

2.3.2 Batch versus continuous operation implications in the methane yield

The determination of biomethane potential (BMP) is the first stage in the evaluation of the feasibility of a substrate. BMP is frequently defined as the maximum volume of methane produced per gram of volatile solids (VS) of the substrate(s), which is sometimes also defined as specific methane production (SMP). BMP indicates the biodegradability of a substrate and its potential to produce methane *via* AD. The BMP test is a method of establishing a baseline for the performance of AD. The data gathered from these tests are useful for designing AD parameters to optimize methane production (Jingura & Kamusoko, 2017).

This parameter offers valuable information about the operational details as well as the economic evaluation of new biogas plants. Usually, BMP is determined in a batch anaerobic fermentation assay, the so-called BMP test. This method is simple and reliable, avoiding inconsistencies in the collected data (Pagés-Díaz et al., 2018). It consists of adding a known quantity of organic substrate to an active anaerobic inoculum in an air-tight serum bottle (Ohemeng-Ntiamoah & Datta, 2019).

However, there is no standard procedure for BMP tests (Bridgewater, 2017; Koch et al., 2019) despite several guidelines (I. Angelidaki et al., 2009; Verein Deutscher Ingenieure, 2016; Holliger et al., 2016). Furthermore, (Holliger et al., 2016) suggested several criteria to validate BMP data, whereas (Koch et al., 2019) listed most of the critical problems that can be noticed by the use of the SMP curve caused by inoculum storage, inoculum dilution, and inoculum-to-substrate ratio (ISR).

2.3.3 Evaluation of AcoD in batch test

BMP optimization by selecting the optimal substrate ratios is the first step of AcoD optimization according to the road map proposed by (Xie et al., 2016). Discontinuous batch tests are biological test systems that allow for the direct assessment of biogas yield that can be used to estimate the biogas potential and provide additional information on degradation kinetics (Weinrich, 2018).

There are several influential factors mentioned in the literature, such as the test equipment used, blanks and control samples, ISR, inoculum storage, and inoculum dilution (Koch et al., 2019; Weinrich, 2018). While these factors are more related to the correct execution of the test, there are other factors more related to the substrate performance, e.g., raw material composition total and VS, COD and biological oxygen demand (BOD), and C/N or inhibitory substances (Jingura & Kamusoko, 2017).

Because the scope of the test is sometimes unclear, (Koch et al., 2020) pointed out the power and limitations of the batch test, as summarized in Table 2-12.

Table 2- 12: Power and limitations of the batch test (Koch et al., 2020).

Yes	No
✓ Biomethane methane potential (BMP) of a substrate or mixture	x Synergistic or antagonistic effects in the co-digestion of substrate mixtures, by the addition of trace elements, etc.
✓ Anaerobic biodegradability (by dividing the obtained BMP by a theoretical value)	x Long-term effects of nutrients or trace elements due to monotonic feeding
✓ Acute toxicity of a present inhibitor in the substrate or mutually added	x Chronic toxicity of an inhibitor present in the substrate or mutually added
✓ Qualitatively describing the kinetic of the AD process	x Methane yield, process stability, and achievable organic loading rate in a continuously operated system

The influence on the variability of the BMP tests by many influential factors was analyzed (Ohemeng-Ntiamoah & Datta, 2019), and some discrepancies between studies were observed due to the lack of a standard method (Koch et al., 2019, 2019; Ohemeng-Ntiamoah & Datta, 2019, 2019; Weinrich, 2018, 2018). Some validation criteria can be found in (Verein Deutscher Ingenieure, 2016; Holliger et al., 2016), whereas (Ohemeng-Ntiamoah & Datta, 2019) also insisted on the necessity of reporting critical values in the validation of the test.

2.3.4 Determination of substrate optimal mixture

Response surface methodology (RSM) can be defined in several formal ways due to its multiple perspectives; one of the most classical is a set of tools for improving the investigation of a particular experimental region (Oliveira et al., 2019). A more accurate definition is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a dataset to simultaneously optimize the levels of several variables to achieve the best system performance (Bezerra et al., 2008; Oliveira et al., 2019). Furthermore, RSM allows the sensitivity analysis of the optimum values of output variables to variations in operating conditions. In addition, it provides graphical information that allows a visual interpretation of the functional relations between the responses and operational parameters (Mortezaei et al., 2018).

RSM is one of the most popular multivariate statistic techniques and allows the assessment of several parameters on the same duration with fewer experimental sets and offers quantitative and reliable results by using a design experiment; its disadvantage is that its results cannot be generalized (Kainthola et al., 2019). RSM has been broadly applied for optimizing the experimental variables within the AD research field. Mortezaei et al. (2018) used it to simultaneously optimize COD removal and biogas production rate (BPR) in a hybrid reactor composed of an EGSB and a fixed-bed (FB) reactor using yogurt effluent as substrate. The input variables were HRT, COD, and COD/N ratio. The best models for COD removal and BPR were the cubic and quadratic models, respectively. The optimum region of the hybrid EGSB-FB reactor was found at COD of 11,200 mg/L, HRT of 27 h, and COD/N ratio of 51. These variables resulted in a 90% COD removal efficiency and a 180-mL/h BPR. The COD/N ratio and influent COD were the most influential parameters on COD removal and BPR, respectively.

Amani et al. (2015) used a central composite design combined with RSM to quantify the interactive influence of propionic (HPr), butyric (HBu), and acetic (HAc) acids, HRT, and methanogen to acetogen population ratios (M/A) on the response variables VFA removals and BPR. Experiments were conducted in a UASB reactor at thermophilic temperature (55 °C) inoculated with enriched acetogenic and methanogenic cultures. The optimum conditions were found to be HPr = 1.9 g/L, HBu = 2.2 g/L, HAc = 2.5 g/L, HRT = 22 h, and M/A = 2.5. The results of verification experiments and predicted values from fitted correlations were in close agreement at a 95% confidence interval. Analysis of the results of the thermophilic process revealed that the trends and interactive effects of different parameters as well as its optimum conditions were very similar and comparable with the previous study at mesophilic temperature (37 °C).

A special type of RSM is a mixture design (MD) in which the independent factors are proportions of different components of a blend and the proportions of the components must sum to 100% (Buruk Sahin et al., 2016; Kashi et al., 2017). The total amount of material must be held constant to only analyze the dependency on the relative proportions of the components and avoid variabilities in the responses due to the total amount of the mixture (Rahman et al., 2019). The MD analysis provides valuable information about the interactions between independent factors as well as a better understanding of the responses (Cornell, 2002; Kashi et al., 2017; Rahman et al., 2019).

MD has been used by several authors to optimize the BMP and/or the kinetics by determining the optimal proportion of the substrates in the mixture, usually using batch experiments. (Buruk Sahin et al., 2016) investigated the mono and AcoD of two municipal sludge wastes (A and C), grease trap waste (B), and meat processing waste (D) under mesophilic temperature conditions using data from BMP assays and kinetic modeling to perform a simplex-lattice MD. Statistical analyses were also conducted to elucidate the possible synergetic and antagonistic effects of waste interactions on the kinetics and ultimate methane potentials of waste co-digestion. Quadratic models were found to estimate the rate constants of the co-digestion process with good accuracy. Statistical analysis revealed that the interactions among the substrates in co-digestion did not have a significant impact on the ultimate cumulative MYs. Nevertheless, these interactions proved to have synergic and antagonistic effects on the reaction rates, leading to accelerated or hindered methane production rates.

Rahman et al. (2019) researched the AcoD of two sets of mixtures, consisting of poultry droppings, with sugarcane bagasse, and press mud, for set A and poultry droppings with sugar beetroots and tops and press mud for set B. An augmented simplex centroid design was used to design the mixture composition for the AcoD using batch assays. The reactor performances were assessed under identical conditions using cumulative methane yield and percentage VS destruction as the criteria. Synergistic effects were observed by adding a greater proportion of press mud for both sets A and B. Antagonistic effects were obtained by adding a greater proportion of sugarcane bagasse for set A and a higher proportion of sugar beetroots and tops for set B. Response surface methods and modeling were employed to determine the optimum mixture combinations for maximizing methane production. From the optimization plots, the use of PD had antagonistic effects on both sets A and B.

Pagés-Díaz et al. (2014a) investigated the effect of different mixture ratios of solid cattle slaughterhouse wastes, manure, various crops, and municipal solid wastes on MY and specific methane production rate (r_{CH4}). The performance of the process was assessed in thermophilic anaerobic batch co-digestion assays using a four-factor MD, and a modified Gompertz model was employed to quantify both response variables. A MD model was fitted to data to appraise synergistic and antagonistic interactions. Mixing all four substrates resulted in a 31% increase in the expected yield, which was calculated from the methane potential of the individual fractions due to a more balanced nutrient composition enhancing the AD process. However, no significant antagonistic effects were observed.

Although several studies have reported synergistic effects in batch tests (Aichinger et al., 2015; Ebner et al., 2016; Labatut et al., 2011; Pagés-Díaz et al., 2014b), it has been remarked by the Verein Deutscher Ingenieure (2016) that synergistic effects cannot be proven in a batch test as these tests are conducted under optimized conditions concerning buffering capacity, nutrient availability, and substrate-related inhibitory effects. Hence, those results must be necessarily duplicated in continuous operation mode. Furthermore, Astals et al. (2014), by using MD in terms of carbohydrates, lipids, and proteins, concluded that ultimately, the identified synergistic effects in batch tests are an increase in the degradation of the substrates by a better nutrient balance.

3. Techno-Economic Assessment of Solid–Liquid Biogas Treatment Plants for the Agro-Industrial Sector

Abstract: The urgent need to meet climate goals provides unique opportunities to promote small-scale farm anaerobic digesters that valorize on-site wastes for producing renewable electricity and heat, thereby cushioning agribusinesses against energy perturbations. This study explored the economic viability of mono-digestion of cow manure (CWM) and piglet manure (PM) in small manured-based 99 kW_{el} plants using three treatment concepts (TC): (1) typical agricultural biogas plant, (2) a single-stage expanded granular sludge bed (EGSB) reactor, and (3) a multistage EGSB with a continuous stirred tank reactor. The economic evaluation attempted to take advantage of the financial incentives provided by The Renewable Energy Sources Act in Germany. To evaluate these systems, batch tests on raw and solid substrate fractions were conducted. For the liquid fraction, data from continuous tests obtained in a laboratory was employed. The economic evaluation was based on the dynamic indicators of net present value and internal return rate (IRR). Sensitivity analyses of the electricity and heat selling prices and hydraulic retention time were also performed. Furthermore, an incremental analysis of IRR was conducted to determine the most profitable alternative. The most influential variable was electricity selling price, and the most profitable alternatives were TC1 (CWM) > TC1 (PM) > TC3 (CWM). However, further studies on co-digestion using TC3 are recommended because this concept potentially provides the greatest technical flexibility and highest environmental sustainability.

Keywords: cow manure; pig manure; biogas production; anaerobic digestion costs; economic viability

3.1 Introduction

At the end of 2019, global energy demand was projected to grow by 12% by 2030. The share of fossil fuels in the primary energy mix has remained above 80% since the 1950s (Organisation for Economic Co-operation and Development OECD, 2020). COP26 promised to kickstart the urgently needed transition from pledges to real-world actions (Smith et al., 2022). To lessen global warming and the impact of climate change, greenhouse gas (GHG) emissions must be reduced drastically (Arias et al., 2021). "Another important global challenge is the security of energy supply because most of the known conventional oil and gas reserves are concentrated in politically unstable regions" (Weiland, 2010).

In this context, biogas from wastes, residues, and energy crops is expected to play an important role in the future (Glivin et al., 2021; Iglesias et al., 2021; Weiland, 2010). Biogas contributes to the primary targets of the current energy transition by replacing fossil resources and reducing methane emissions related to the disposal of biodegradable waste, thereby reducing GHG emissions. In addition, the resulting digestate can be used to enrich agricultural soils, which contributes to creating carbon sinks.

Methane-rich biogas can also replace natural gas as a feedstock in the production of chemicals and materials (Iglesias et al., 2021; Weiland, 2010).

The Renewable Energy Sources Act (Gesetz für den Ausbau erneuerbarer Energie, EEG) stimulated an increase in biogas plants in Germany from approximately 1000 in 2000 to approximately 9632 operating plants in 2020 (German Biogas Association, 2021; Weinrich & Nelles, 2021a). However, due to changes in the EEG, only small liquid manure plants and waste digestion plants benefit from the original remuneration system outlined in 2012. In addition, economical support for processing biogas that can be put into the natural gas grid (biomethane) has increased. Support for participating in direct marketing (market and flexibility premium) has also increased. Thus, the current funding conditions are directing biogas technology toward decentralized and flexible power generation from biogenic residues and waste materials (Weinrich & Nelles, 2021a, p. 15).

A commonly used criterion to assess the performance of the anaerobic digestion (AD) of a given substrate is the biomethane potential (BMP) (Hernández Regalado et al., 2021). BMP tests are routinely performed in academia and industry to determine the methane potential of a given substrate (Koch et al., 2019). Determining the BMP is the first step in evaluating the digestibility or applicability of a substrate because the BMP parameter provides valuable information about general degradability, expectable energy yield, and the economic evaluation of new biogas plants. Typically, BMP is determined by a BMP test procedure in a batch anaerobic fermentation assay, which is a reliable and straightforward method that avoids inconsistencies in the collected data (Hernández Regalado et al., 2021; Pagés-Díaz et al., 2014a; Raposo et al., 2011).

Profitability is another challenge in the biogas industry (Y. Wang et al., 2019). Broader implementation of the biochemical conversion of biowaste, especially in rural areas, requires an intensive analysis of various technical aspects, e.g., biodigester design and its applications, pre-treatment, and co-digestion processes to enhance the biogas yield. In addition, economics plays a crucial role, and various cost factors, e.g., substrates, substrate collection, transport, biodigester, electricity, and heat selling prices, must be considered. In addition, current and future policies are essential to realizing the practical implementation of new technologies. (Glivin et al., 2021; Y. Wang et al., 2019), and techno-economic models are used to identify the industrialization potential of a project (Rajendran et al., 2014).

An important challenge in the implementation of AD from manure and wastes is selecting the most cost-effective combination of technologies (Vrieze et al., 2018). Two-stage or multistage reactors, in which the hydrolysis/acidogenesis and acetogenesis/methanogenesis steps occur in the same or separate digesters are becoming increasingly popular because they allow targeted control and optimization of the operation, thereby obtaining higher rates and yields of biogas, by separating the steps in which AD occurs (Rabii et al., 2019b). Another two-stage process that has drawn attention is the dual solid-liquid treatment system, as reported by Zhang et al. (2013), who compared the digestion of raw food waste versus the digestion of the liquid and solid phases of the food waste. They found that methane production increased by 13.6% in the dual system compared to raw digestion. In addition, El-Mashad and Zhang (2010) used batch tests to compare the co-digestion of a raw mixture of dairy manure and food waste, as well as its liquid and solid fractions. The mass balance showed that co-digestion of the liquid phase yielded 32% less methane compared to the raw mixture. El-Mashad and Zhang (2010)stated that the yield of the solid phase should also be included in the balance to realize a more complete analysis. Although digestion of the solid phase presents some issues in a continuous stirred tank reactor (CSTR) due to high solid content, it should not be used directly as a fertilizer or as a composting feedstock because this will produce GHG emissions (El-Mashad & Zhang, 2010)[19].

In this study, we attempt to determine the economic profitability of integrating an expanded granular sludge bed (EGSB) reactor and a solid-liquid separation process in a typical agricultural biogas plant to provide flexibility and increase the efficiency of treating raw, solid, and liquid manures (and other agricultural substrates). The assessment was conducted using batch tests to determine the methane yield and methane production rate of the raw, solid, and liquid phases. An economic analysis using the net present value (NPV) as a function of hydraulic retention time (HRT) for the solid and raw phases in a CSTR was conducted to compare the profitability of the dual solid-liquid system compared to raw digestion.

3.2 Materials and Methods

3.2.1 Raw Materials

Two substrates were considered in this study, i.e., piglet manure (PM) and cow manure (CWM). PM and CWM were chosen because the German government has implemented special incentives to address GHG reduction in the agricultural sector and improve the circular use of nitrogen (Guidehouse et al., 2021).

The PM and CWM were previously processed using screw press systems that separated the solid and liquid phases. Here, a separation process with a 100 µm sieve (Klass Wendelfilter, KLASS Filter GmbH, Eresing, Germany) was applied for the PM. The CWM was collected from farmers in a pre-separated form, which was then processed by a second separation using a screw press with a sieve size of 200 µm (Hernández Regalado et al., 2021).

The raw, solid, and liquid fractions of the substrates were characterized by the dry matter (DM) content, volatile solids (VS), macromolecules, and nutrients. The results of these analyses are given in Table 3-1.

Table 3- 1: Characterization of the liquid fraction of substrates.

Variable	Piglet Manure			Cow Manure		
	Solid	**Raw**	**Liquid**	**Solid**	**Raw**	**Liquid**
DM/FM (wt%)	17.50	3.80	1.80	26.70	7.30	5.30
VS/FM (wt%)	90.13	67.90	58.33	89.03	78.50	69.81
VS/DM (wt%)	15.77	2.58	1.05	23.77	5.73	3.70
Crude protein/FM (wt%)	2.40	2.50	0.80	2.50	2.40	2.60
Crude fat/FM (wt%)	0.40	0.30	0.25	0.30	0.30	0.30
Crude fiber/FM (wt%)	5.70	0.00	0.00	9.30	1.40	0.00
Free nitrogen extracts/FM (wt%)	7.50	0.00	0.00	11.70	1.60	0.80
ash/FM (wt%)	1.50	1.00	0.75	2.90	1.60	1.60

DM: dry matter; VS: volatile solids; FM: fresh matter.

3.2.2 Setup, Experimental Validation, and Mathematical Modeling of Batch Tests

The batch assays of the organic substances were conducted according to the Verein Deutscher Ingenieure (2016). The setup is thoroughly described by Regalado et al. [10]. The data used to analyze the methane yield curves (MYCs) were the net daily methane cumulative production (inoculum contribution subtracted). Here, the following criteria based on recommendations from Holliger et al.; Holliger et al. (2016; 2021) were applied to confirm the validity of the experiments.

- Stopping criterion. The test concluded when the relative increase in MY was less than 1% for three consecutive days.
- Plausibility criterion. The existence of abrupt or nonmonotonic trends in the curves requires individual analysis of the affected test.
- Reproducibility/accuracy criterion. After eliminating potential outlier(s) or outlier curve(s), a coefficient of variation (CV) of less than 6% between the curves was required.
- The BMP of the positive control (cellulose) was between 85 and 100% of the theoretical BMP (between 352 and 414 NL_{CH4} kg_{VS}).

Once the MYCs of the raw and solid phases of the substrates satisfied these requirements, a first-order one-step model (Equation (3-1)) was fit using the average of the curves. Note that the liquid phases were characterized using the results from Hernández Regalado et al. (2021), who reported the root-mean-square error s (RMSEs) of the fit.

$$\mathrm{MY}\ (\mathrm{t}) = \mathrm{BMP}_{\infty} \cdot (1 - \mathrm{e}^{-\mathrm{kt}}) \quad (3\text{-}1)$$

Here, MY is the methane yield L_{CH4}/kg_{VS}, BMP_{∞} is the extrapolated MY at infinite retention time in L_{CH4}/kg_{VS}, and k is the first-order reaction constant (1/d).

The degradation fraction (f_d) values were calculated by modifying the equations from Ebner et al.; Raposo et al. (2016; 2011), where rather than using a BMP_∞ value calculated using data from Weender or Van Soest analysis, the BMP_∞ value was taken from the fit from Equation (3-1).

$$f_d = \frac{MY_{tfinal}}{BMP_\infty} \quad (3\text{-}2)$$

Here, MY_{tfinal} is the methane yield L_{CH4}/kg_{VS} at the end of the tests and BMP_∞ is the extrapolated MY at infinite retention time in L_{CH4}/kg_{VS}.

3.2.3 Estimation of HRTs in Continuous Stage

Substrate conversion is time- and process-dependent (Verein Deutscher Ingenieure, 2016; D. J. Batstone et al., 2009). Weinrich (2018) presented a general relation of batch tests and CSTRs with different substrates in Equation (3-3), which assumes that the rate constant k is transferable from a batch to a continuous system. This assumption is based on the correct theoretical determination of biogas potential via model-based extrapolation, as in Equation (3-1).

$$MY\,(t) = BMP_\infty \cdot \left(\frac{k \cdot HRT}{1 + k \cdot HRT}\right) \quad (3\text{-}3)$$

Here, MY is the methane yield L_{CH4}/kg_{VS}, BMP_∞ is the extrapolated MY at infinite retention time in L_{CH4}/kg_{VS} and k is the first-order reaction constant (1/d), and HRT is the hydraulic retention time (d).

Note that an MY(t) must be selected to estimate the operational HRT in a CSTR; thus, 0.8 of the BMP_∞ fit by Equation (3-1) was considered the standard if a quality adjustment was obtained.

The HRTs in the EGSB reactors were assessed based on practical experiments conducted at a laboratory scale published by Häner et al. (2022). Häner et al. (2022)employed filtered pig slurry as a substrate in an EGSB reactor and a fixed-bed (FB) reactor. The author fitted linear regression models for both reactors using the organic loading rate (OLR) measured in g_{COD}/L/d as the independent variable and methane production rate (MPR) as the response variable. The smallest HRT considered efficient was 3 d for the EGSB reactor; however, an inverse relationship between MY and OLR has been described previously (Jafarzadeh et al., 2021; Verma et al., 2014; H. Yu et al., 1998).

Hernández Regalado, Häner, Baumkötter, et al. (2022) performed experiments in the same laboratory using three EGSB reactors, each identical to the reactor used by Häner et al. (2022), to perform co-digestion of the liquid fractions of PM, CWM, starch wastewater (SWW), and sugar beets (SBT) using three 30 L EGSB reactors. The author studied the synergistic effects of two three-substrate mixtures (i.e., PM + CWM + SWW and PM + CWM + SBT) using the PM + CWM mixture as a benchmark. Here, Stover–Kincannon models for the MPR were combined with an inverse function for the MY to find the optimal operational HRT intervals.

In addition, Damien J. Batstone et al. (2015) indicated a linear relationship between the log (HRT) vs. log (% DM) at low DM content values. However, this relationship is only valid up to a DM content of 1%, and both mixtures were greater than this threshold. In the work of Hernández Regalado, Häner, Baumkötter, et al. (2022), the optimal HRTs of both three-substrate mixtures were proportional to the DM ratio between both mixtures. Cremonez et al. (2021) suggested an inverse relationship between the degradation rate and substrate complexity in the order of sugar (mono and disaccharides), starch, proteins, hemicellulose, lignin, waxes, and greases.

Thus, the HRT for the PM liquid was selected in reference to the work of Häner et al. (2022). However, a constraint of 50% ± 5% of the maximal MPR was imposed because optimal intervals were identified by Hernández Regalado, Häner, Baumkötter, et al. (2022) close to this interval. For the CWM liquid, the HRT value of the PM liquid was adjusted by multiplying by **DM (CWM)/DM(PM)** because both substrates have similar complexity.

3.2.4 Economic and Environmental Impact Assessment

Three treatment concepts were analyzed in this study. As shown in Figure 3-1a, the first concept is a typical single-stage agricultural biogas plant using a CSTR. In the second concept, an EGSB replaces the CSTR of the single-stage plant, and a solid-liquid separation stage is included. Here, the liquid fraction is treated by the EGSB reactor, and the solid phase is transported to another plant (with assumed cost-neutral transport), as shown in Figure 3-1b. In the third concept, substrates undergo solid-liquid separation. Then, the liquid phase is treated by an EGSB reactor, and the solid phase is treated using a CSTR (Figure 3-1c).

Economic performance is one of the most important factors affecting a project's viability (Alfonso-Cardero, Pagés-Díaz, Kalogirou, et al., 2021). The economic assessments of the treatment concepts shown in Figure 3-1 are based on special subsidies for biogas plants provided by the EEG, 2021 (2021) (EEG; acronym from untranslated German). Effective as of 2021, the EEG states that biogas plants using predominantly manure (>80 wt%) with an installed electrical capacity of less than 150 kW_{el} are eligible to receive funding corresponding to 22, 23 EUR_{ct}/kWh_{el}. Note that this funding has a yearly degression of 0.5% depending on the year of commissioning, which is also shown in Figure 3-1.

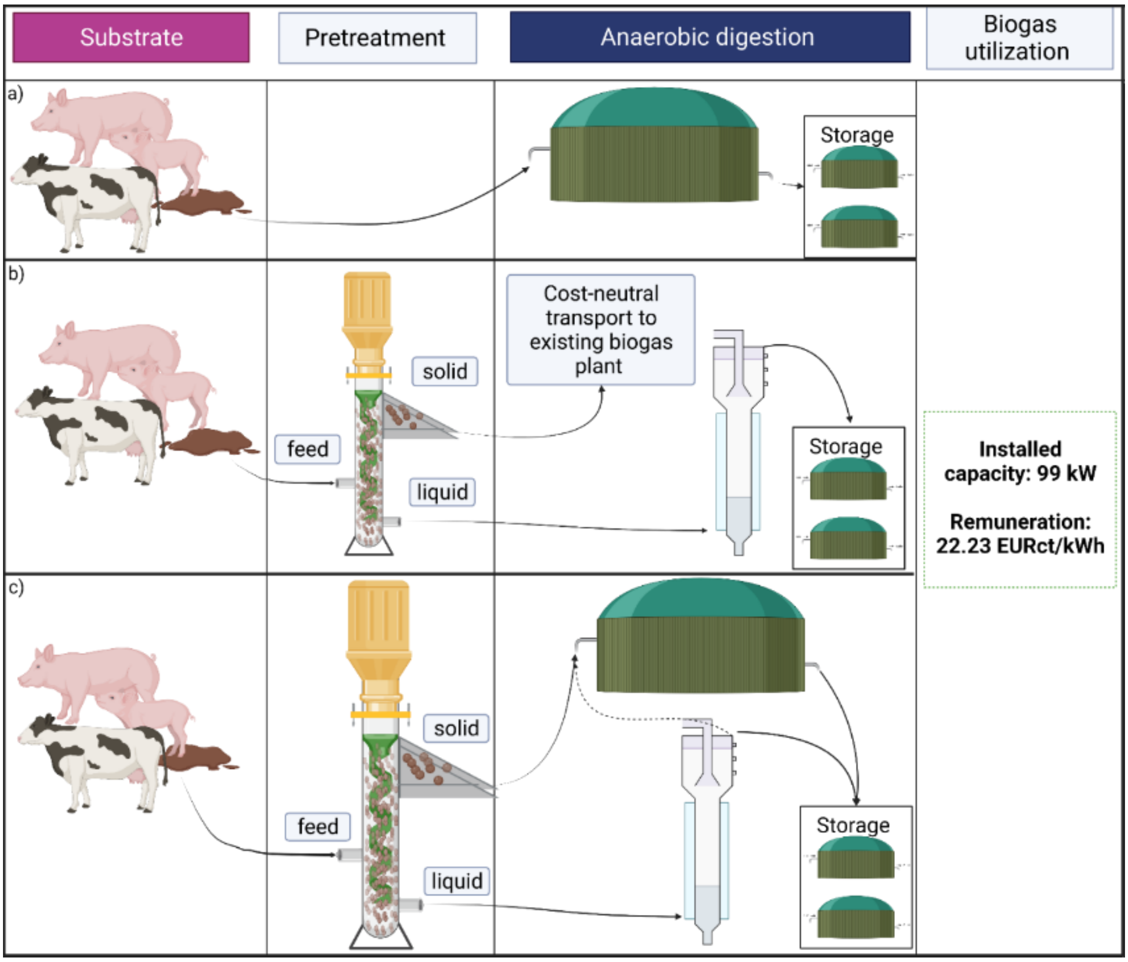


Figure 3- 1: Pig and cow manure anaerobic digestion in (a) treatment concept 1, (b) treatment concept 2, and (c) treatment concept 3.

The economic performance of each concept was estimated based on the material and energy balances. Here, the HRTs were calculated as described in Section 2.3, and the electrical power outputs were fixed to satisfy the requirements for special funding as per the EEG. In addition, a yearly input flow of the respective raw substrate was calculated based on a fixed 99 kW_{el} using the methane yield. The equipment costs were obtained by direct quotations from German companies with respective cost versus scale, and the costs were validated according to the literature.

Capital expenses (CAPEX) were calculated based on the reactor(s) and the combined heat and power (CHP) system costs. For each area, the direct cost and working capital were calculated as a function of the equipment costs (Alfonso-Cardero, Pagés-Díaz, Contino, et al., 2021; Brennan, 2020, pp. 87–90).

In addition, operational expenditures (OPEX) were calculated according to the mass and energy balances. The OPEX calculations for each scenario included the energy costs for pumps, separation, heating, internal electricity, salary, as well as substrate transport costs.

Note that this economic assessment is classified as a preliminary study (budget authorization) with a precision of –20% to +25% according to Don W. Green and Robert H. Perry (2008, pp. 988–989). The general procedure is illustrated in Figure 3-2. The economic profitability of the three treatment concepts was evaluated based on dynamic indicators, e.g., the NPV and internal rate of return (IRR). In addition, an incremental analysis of the IRR was performed to compare profitable alternatives.

3.2.5 Economic Assessment Calculation Flow Diagram

Sensitivity Analysis

A sensitivity analysis was conducted to identify the most influential input parameters on the investment's economic sustainability. Here, three parameters were varied, i.e., HRT, electricity, and heat selling price. These input parameters were varied by ±10%, ±20%, and ±30% compared to the baseline. In addition, the effect of the economy of scale was measured by varying the capacity of the plant for all scenarios because, in many biogas industries, the startup process requires more time, and the plant must occasionally operate at a reduced capacity due to the instability of the process (Rajendran et al., 2014). Note that limiting conditions (NPV = 0) were also investigated for each concept.

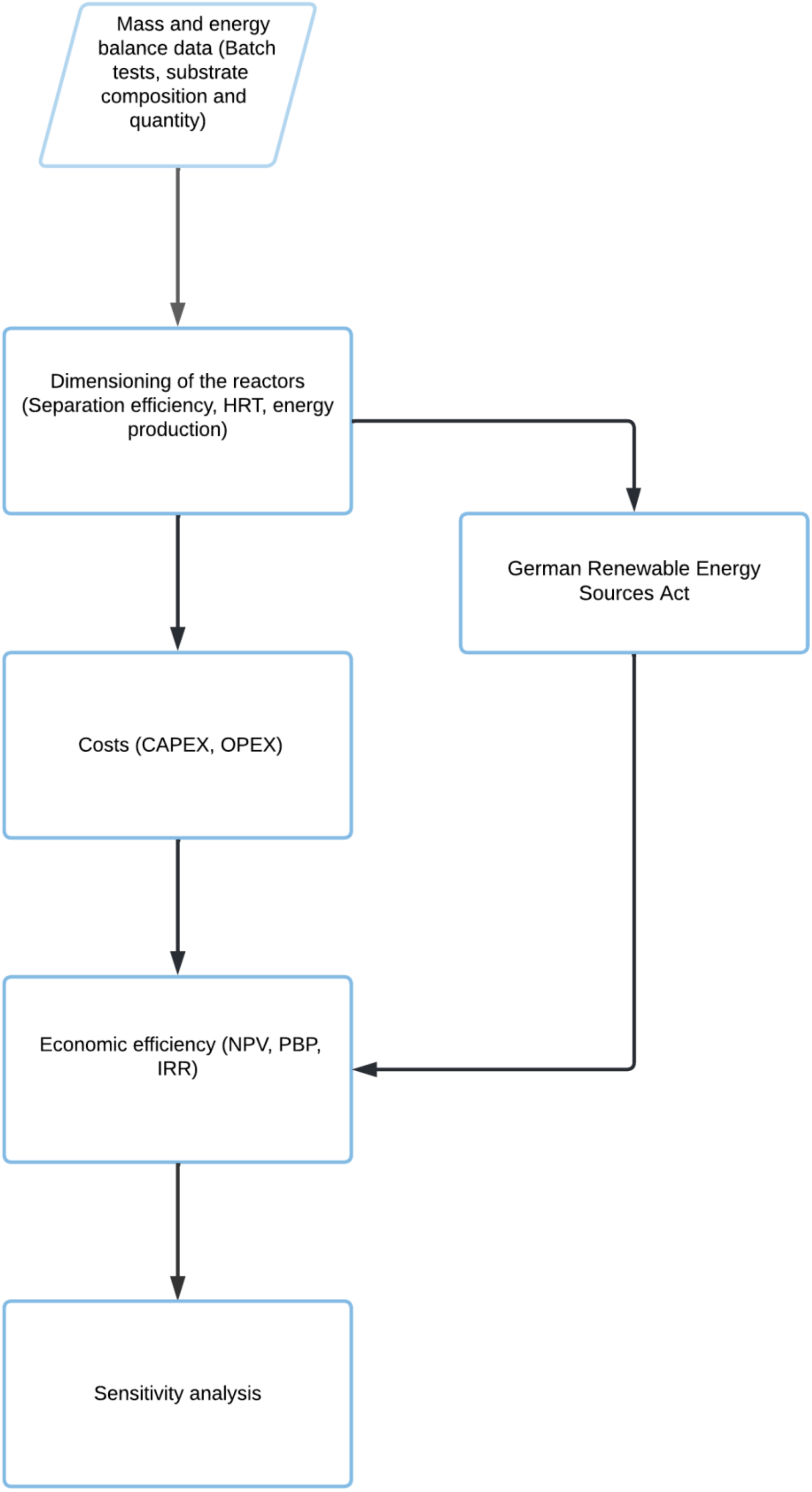


Figure 3- 2: HRT: hydraulic retention time; CAPEX: capital expenditures; OPEX: operational expenditures; NPV: the net present value; IRR: internal rate of return; PBP: payback period.

3.3 Results

3.3.1 Batch Test Results

The dispersion analysis of the MYCs identified the presence of one outlier curve for PM raw, PM liquid, and CWM raw, respectively. These individual MYCs were eliminated to maintain a CV of less than 6%. The curves are shown in Figures 3-3 and 3-4, where the numbers in the legends represent the valid number of replicates for each curve.

The MYCs of the solid and raw phases of the substrates exhibited slow growth on the first days of the tests, which is in agreement with the liquid phase results reported by Hernández Regalado et al. (2021). Hernández Regalado et al. (2021) identified slow methane production at the beginning of the tests characterized by small lag phases. Consequently, the best fit of the three compared models was the modified Gompertz model, as shown in Figures 3-3b and 3-4b. Note that the lag phase was present in the raw substrate; thus, in the solid phase, an overestimation of the biogas production by the first-order model is expected for the first days. Nevertheless, the application of the first-order model was in the interest of the research due to the simplicity and the ability to predict the HRT of a CSTR in the continuous phase using the parameters of the fit model using Equation (3-3) extracted from Weinrich (2018).

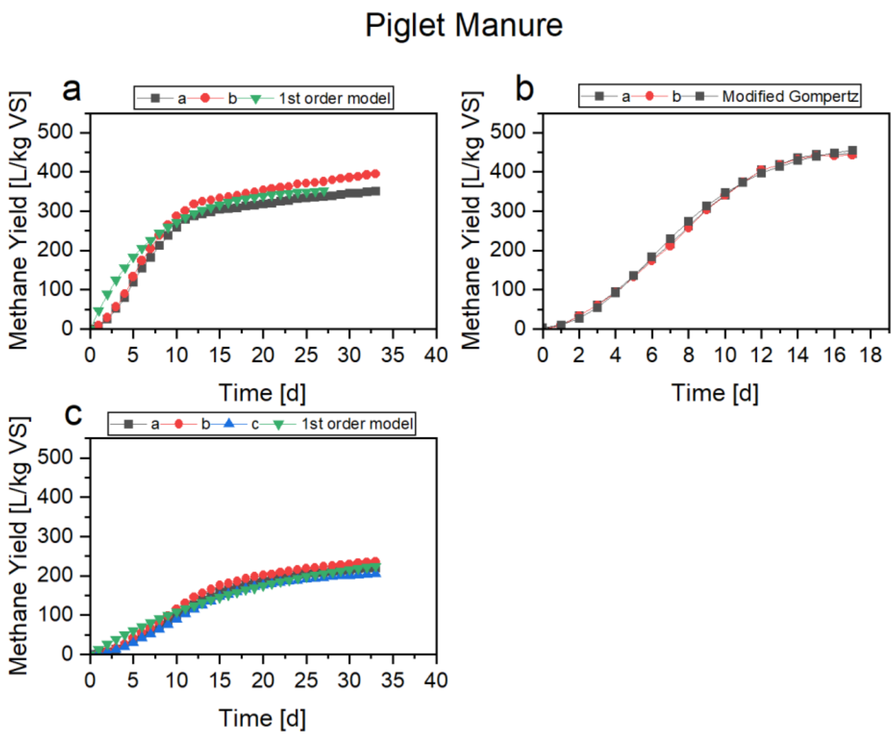


Figure 3- 3: Methane yield curves of piglet manure: (a) raw, (b) liquid, and (c) solid.

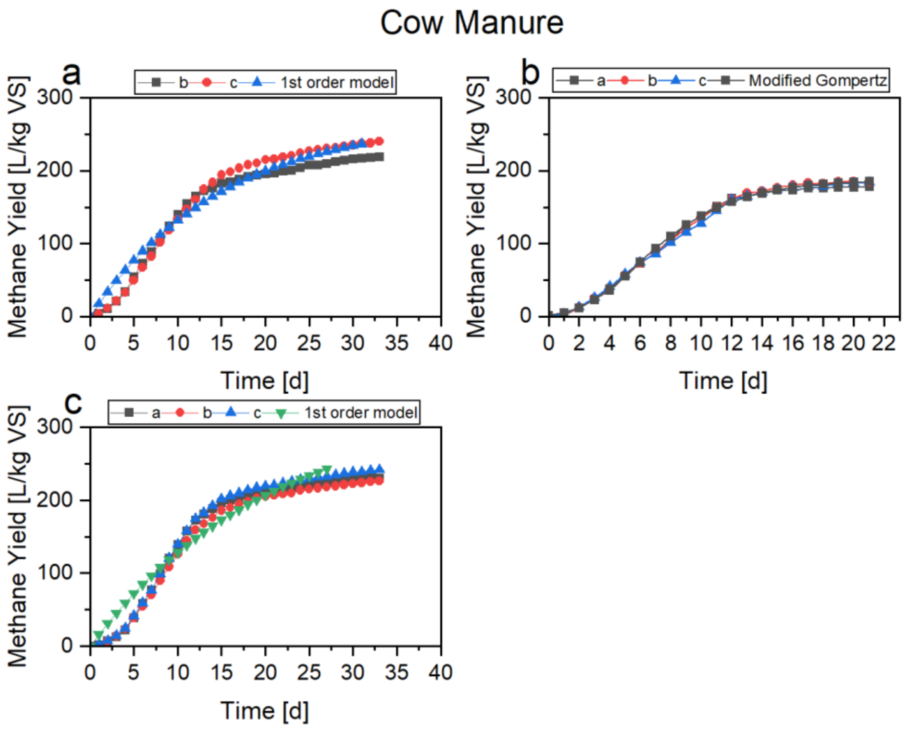


Figure 3- 4: Methane yield curves of cow manure: (a) raw, (b) liquid, and (c) solid.

The fits of the first model for the raw substrate and solid phase of both manure types are summarized in Table 3-2. As can be seen, the difference between the model and the CMW solid was the largest, which is primarily due to the step lag phase. Consequently, CWM solid also had the lowest f_d and relatively short termination of the test by the applied criteria. Despite having an equal k value to the solid PM, the BMP_∞ value was much higher, which indicates overestimation by the first-order model.

The raw PM obtained the best fit, and its k value was approximately three times that of the k value for the solid phase. Nevertheless, a higher value of k is expected in the raw phase than in the solid phase, given that the organic matter is more accessible due to solubilization (J. L. Rico et al., 2007). In addition, the test appeared to reach a degradation value of 0.99 in 27 days. However, the results reported by Hernández Regalado et al. (2021) for the liquid phase were 444.57 L_{CH4}/kg_{VS}, and a test duration of 17 days if the same 1% criterion is followed.

Thus, despite the encouraging results for the raw PM, a comparison between treatment systems using a combination of solid and liquid phases or only the raw substrate was considered necessary to determine the most profitable alternative.

Table 3- 2: Summary of batch test results.

Parameter	Piglet Manure Solid	Piglet Manure Raw	Cow Manure Solid	Cow Manure Raw
BMP_∞ (L_{CH4}/kg_{VS})	280.85	359.77	327.31	270.98
k (1/d)	0.05	0.14	0.05	0.07
RMSE (L_{CH4}/kg_{VS})	12.74	3.17	18.16	13.31
MY_{AVG} (L_{CH4}/kg_{VS})	229.80	356.24	224.49	227.30
f_d	0.82	0.99	0.68	0.84
Time (d)	39	27	27	31

BMP_∞: biomethane potential; k: first-order reaction constant; RMSE: root-mean-square error; MY_{AVG}: average methane yield; f_d: degradation fraction; Time: test duration.

3.3.2 Estimation of Hydraulic Retention Times in Continuous Stage

The operational HRTs of the treatment of the solid and raw substrates in the CSTRs were estimated using Equation (3-3). Here, the target MY value was 0.8 of the BMP_∞ value shown in Table 3-2. For the CWM solid, the target value was the average MY value because the BMP_∞ was likely overestimated by the first-order model, as shown in Figure 3-3c. Nevertheless, the first-order constant of the model was employed in this case. The resulting MY and HRT values are summarized in Table 3-3.

Table 3- 3: Estimation of operational hydraulic retention times in continuous stirred tank reactor.

Parameter	Piglet Manure Solid	Piglet Manure Raw	Cow Manure Solid	Cow Manure Raw
MY	224.68	287.81	224.49	216.78
HRT	81.80	28.06	79.91	59.99

MY: methane yield; HRT: hydraulic retention time.

We found that the raw PM can be processed in less than 30 days, which represents a large time reduction compared to a typical agricultural biogas plant where representative HRT values are between 50 and 150 days (Ruile et al., 2015; Weinrich & Nelles, 2021b). Biogas plants at an industrial scale are rarely operated using mono-digestion of manures. Typically, manures are co-digested with substrates with high DM content to increase OLR to take advantage of the co-digestion positive interactions (J. Mata-Alvarez et al., 2014; Neshat et al., 2017; Tallou et al., 2020). Nevertheless, several studies were conducted at the pilot plant scale or under mono-digestion of manures using CSTR technology. For example, Jurado et al. (2016) operated a CSTR at an HRT of 25 days, and through the AD model no. 1 (ADM1), they found that the operating time was not sufficiently large to assure the disintegration and hydrolysis of the solid manure matter. Consequently, the organic particulate matter did not contribute significantly to methane production. Rodriguez-Verde et al. (2014) operated a CSTR with raw pig manure at an HRT value of 20 days. Damien J. Batstone et al. (2015) suggested a layout recommendation for the treatment by reactor type and DM content, being HRT 10 d the lowest achievable for CSTR (mixed reactors) but at DM content lower than 1%. Thus, the HRT is strongly influenced by substrate complexity and DM content. As a result, to ensure a similar substrate degradation in dry anaerobic to the ones achieved in regular or wet AD, higher operational HRTs should be applied since the organic matter is usually less available (Cremonez et al., 2021; Neshat et al., 2017; Rocamora et al., 2020; Van et al., 2020). Therefore, the model's predictions are consistent with results reported in the literature regarding the industrial use of biogas plants. Nevertheless, studies at lower operating scales suggest that lower HRT values can be realized.

To estimate the HRT of the liquid phase, the model represented by Equation (3-4) was implemented to determine the HRTs interval for which MPR/MPR_{max} equal to 50% ± 5% is encountered. Given that the model is purely empirical, it was only valid for the liquid PM. Then, the HRTs of the liquid CWM were determined by multiplying the limits of the HRTs interval from the liquid PM by DM (CWM)/DM(PM). The determination of the HRT for CWM from PM corresponds to an extrapolation of the relationship between HRT and DM in the feed of the reactor for high-rate AD proposed by Damien J. Batstone et al. (2015). The results are shown in Table 3-4.

$$\mathrm{MPR} = 0.2527 \cdot \mathrm{OLR} + 0.1292 \tag{3-4}$$

Table 3- 4**:** Estimation of operational hydraulic retention time interval in expandable granular sludge bed reactors.

Substrate	**DM/FM (wt%)**	**$COD_{average}$ (g_{COD}/L)**	**OLR Interval (g_{COD}/L/d)**	**HRT Interval (d)**
Piglet manure liquid	1.80	25.48	[4.63–5.77]	[4.41–5.50]
Cow manure liquid	5.30	28.06	[1.73–2.15]	[13.00–16.20]

DM: dry matter; FM: fresh matter; COD: chemical oxygen demand; OLR: organic loading rate; HRT: hydraulic retention time.

A large difference between the HRTs from the liquid PM and CWM was observed as calculated according to the above criteria. Nevertheless, this difference also existed in the treatment of raw substrates using the CSTR reactors despite a critical difference in the underlying assumptions for the HRT estimate. For the CSTR, the calculations were based on batch data, and for the EGSB, the calculations were based on an adjustment by the DM content of the continuous experimental data of the liquid PM obtained in our laboratory. However, large differences were observed between the results of the raw and liquid phases of both PM and CWM. In Table 3-5, the results of different studies using similar substrates and reactor technologies are presented.

Table 3- 5: Comparison of previous studies to the current research on the achievable hydraulic retention times of different manures.

Substrate	Reactor Type	Temperature (°C)	OLR_{max} (g_{COD}/L/d)	HRT_{min} (d)	Reference
PM hydrolysates	EGSB	35	21	1.5	(Rodriguez-Verde et al., 2014)
Separated PM	FB	40	1.82	18	(Wetter et al., 2017)
LF of dairy manure	UASB	25	0.65	34.8	(C. Rico et al., 2012)
PS	UASB	36	16.4	1.5	(C. Rico et al., 2017)
Cattle manure	UASB	55	5.06	7.3	(Castrillón Cano et al., 2019)
Cattle manure	UASB	37	8.63	5.3	(Marañón et al., 2001)
PS	ATAD + EGSB + SBR	35	5.00	6.96	(Lee & Han, 2012)
Filtered PS	FB	40	13.5	1.7	(Häner et al., 2022)
Filtered PS	EGSB	40	8.1	3.0	(Häner et al., 2022)

EGSB: expanded granular sludge bed; FB: fixed bed; UASB: up-flow anaerobic sludge blanket; ATAD: autothermal thermophilic aerobic digestion; SBR: sequencing batch reactor; LF: liquid fraction; PS: pig slurry; PM: pig manure.

The calculated values are within the interval reviewed in the literature (Table 3-4). Lower HRT values and higher OLRs are likely achievable; however, given that MY and COD removal efficiency tends to diminish with lower HRT values (Jafarzadeh et al., 2021; Verma et al., 2014; H. Yu et al., 1998), an HRT that assured a more balanced relationship between MPR and substrate usage efficiency was targeted. However, the values for the mono-digestion of liquid CMW in an EGSB reactor are rather large.

3.3.3 Economic Assessment and Sensitivity Analysis

Capital Expenditures

The economic analysis was performed based on quoted cost curves received for the main equipment. The CAPEX of all treatment concepts at a rated capacity of 99 kWh_{el} is shown in Table 3-6.

Peters and Timmerhaus (1991) recommended Lang factors for the chemical industry with values of 4.13 and 4.83 for plants handling liquids and solids–liquids, respectively. Nevertheless, smaller values are typically used for biogas plants (Amigun & Blottnitz, 2009; Kenneth Ndyabawe & William S. Kisaalita, 2014; Vo et al., 2018). Amigun and Blottnitz (2009) found that a factor of 2.63 can be used to accurately predict the costs of medium or small biogas plants in Africa, and a smaller value of 1.79 is a better predictor for large plants. Vo et al. (2018) employed a factor of 1.79 for a biogas plant with biogas upgrading technology included. Nevertheless, the accuracy of those values was questioned by Kenneth Ndyabawe and William S. Kisaalita (2014), who found that the Lang factor should be 2.40–2.98 depending on the location of the plant. Thus, a factor of 2.69 was selected because it is the average of the values reported by Kenneth Ndyabawe and William S. Kisaalita (2014).

Table 3- 6: Capital expenditures by substrate and treatment concept.

Capital Expenditure (EUR/Year)	Treatment Concept 1		Treatment Concept 2		Treatment Concept 3	
	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure
EGSB reactor and associated costs	-	-	241,153	361,016	213,157	232,913
CSTR and associated costs	181,644	204,927	-	-	74,190	87,583
Separator	-	-	10,378	17,368	8596	11,200
Combined heat and power unit	175,000	175,000	175,000	175,000	175,000	175,000
Lang factor for the fixed plant cost	2.69	2.69	2.69	2.69	2.69	2.69
Total Invest	**959,372**	**1,022,004**	**1,147,368**	**1,488,603**	**1,266,837**	**1,363,012**

The total capital investment of the plants was compared to the values reported by Balussou et al. (2012). The comparison was established between treatment concept 1 and business case A from the work of Balussou et al. (2012), which evaluated a typical biogas plant without a biogas upgrading unit with an output of two 380 kW_{el} CHP units for a total value of 760 kW_{el} from Balussou et al. (2012). All purchase costs were adjusted by capacity in consideration of the inflation factor employing the six-tenths rule and the chemical engineering plant cost index (Sinnott & Towler, 2020, p. 285). The obtained value was 1.11 million euros, which has a relative difference of approximately 13.5% from that estimated for treatment concept 1 in Table 3-6. Thus, the Lang factor values employed were considered valid for the specific case of Germany.

For both substrates, the costs of investment for all treatment concepts were similar, and those from CWM were consistently slightly more expensive than those from the PM. This difference existed due to the substrate properties because CWM requires higher HRT for all treatment concepts. The OPEX values are shown in Table 3-7.

Table 3- 7: Operational expenditures by substrate and treatment concept.

Operational Expenditures (EUR/Year)	Treatment Concept 1		Treatment Concept 2		Treatment Concept 3	
	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure
Electricity costs	24,974	13,279	90,353	65,682	66,002	28,076
Wage rate	5475	5475	5475	5475	5475	5475
Transport costs raw manure	13,875	6221	21,083	17,231	15,401	5524
Internal power consumption	10,193	10,944	10,927	10,927	10,193	10,944
Total operational costs	**54,516**	**35,919**	**127,837**	**99,314**	**97,071**	**50,019**

As can be seen, the largest operating cost was electricity, which increases with the amount of required substrate. Thus, the costs associated with PM are always higher than those for CWM because the DM content of PM is significantly lower than in CWM. The operation costs were significantly higher in treatment concept number 2 because only the liquid fraction of the substrate is employed in this concept.

Introducing the EGSB reactor appreciably increases the electricity consumption of the system due to the high-power consumption of the recirculation pump. Possible alternatives to reduce costs are reducing the upflow velocity (V_{up}) or completely replacing the EGSB with a UASB reactor, which is a very similar high-rate reactor (Castrillón Cano et al., 2019; Abumalé Cruz-Salomón et al., 2018; Gleyce et al., 2014).

The NPV calculations for the base case for each substrate by treatment concept are shown in Figures 3-5a and b.

In both cases, the more profitable concept is the typical agricultural biogas plant, i.e., treatment concept 1. For both substrates, the investment is recovered between years seven and eight. The other profitable alternative under the basic case involves adding an EGSB reactor to treatment concept 1, i.e., treatment concept 3 should be employed to treat CWM. However, the investment is not recovered until after year 13 in this case.

We found that treatment concept 2 is not profitable under the current conditions, being slightly more profitable for PM than for CWM. The main cause for this lack of profitability was the high operational costs, primarily electricity costs.

The NPVs and IRRs for all alternatives are given in Table 3-8.

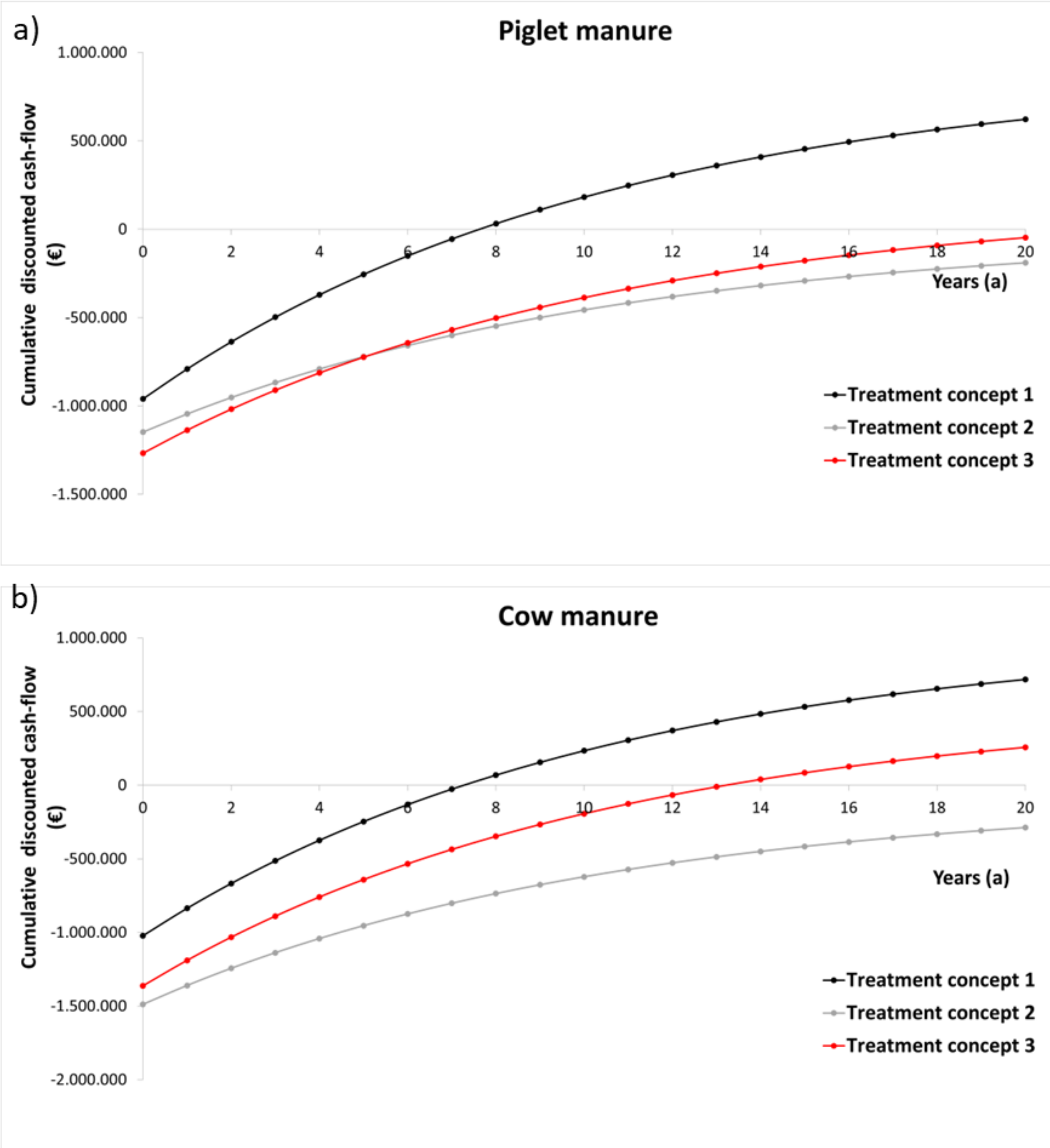


Figure 3- 5: Cumulative discounted cashflow diagrams by treatment concepts for (a) piglet and (b) cow manure.

Table 3- 8: Net present values and internal rate of return of the different treatment concepts.

Dynamic Economic Indicator	Treatment Concept 1		Treatment Concept 2		Treatment Concept 3	
	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure	Piglet Manure	Cow Manure
Net present value (EUR)	622,383	718,084	−189,836	−288,237	−47,373	257,034
Internal return rate (%)	18.70	19.40	7.49	7.00	9.44	12.67

The IRRs of the two alternatives in treatment concept 1 were appreciably better than the others. However, the alternative with the highest IRR is not always the best option; thus, an incremental analysis of IRR was performed. The results demonstrated that the order among profitable alternatives is TC 1 (CWM) > TC 1 (PM) > TC 3 (CWM). Thus, treatment concepts involving high-rate reactors should only be used if special financial support for treating very low DM content is applied.

3.3.3.1 Sensitivity Analysis

To evaluate the profitability of the treatment concepts, a sensitivity analysis of the electricity and heat selling price was conducted for each substrate, where the NPV at year 20 was calculated. The results are summarized in Figures 3-6a and b.

In both cases, the electricity selling price had a larger influence on the profitability of the concepts than the heat selling price, as shown by the slope in Figures 3-6a and b. Treatment concept 1 was profitable under all analyzed conditions, which demonstrates the robustness of the system. In contrast, treatment concept 2 required an increase in the electricity selling price of 12.06% for PM and 18.31% for CWM to reach the break-even point.

Treatment concept 3 reached the break-even point at 3.01% for PM and –16.32% in the electricity selling price. Thus, treatment concept 3 is likely to be profitable based on the recent increments in electricity prices in Europe and specifically in Germany given that the kWh price has risen to 42 cents/kWh for renewable energies (*TttStrompreise*, 2021). This represents a price increment of 200% of the electricity compared to the reference price used in this study. At such a high price, all treatment concepts are profitable. As a reference, treatment concept 2 for CWM was the least profitable system, and its IRR changed from 7.00% to 21.44%.

The most significant change due to the heat selling price is realizing the break-even point at approximately 10.05% for PM. Thus, including the EGSB in the typical treatment concept for both substrates is very close to being profitable.

In addition, sensitivity analysis of the HRT was conducted under the assumption that the MY value remained constant in the interval of ±30% variation in the HRT. The results are shown in Figures 3-7a and b.

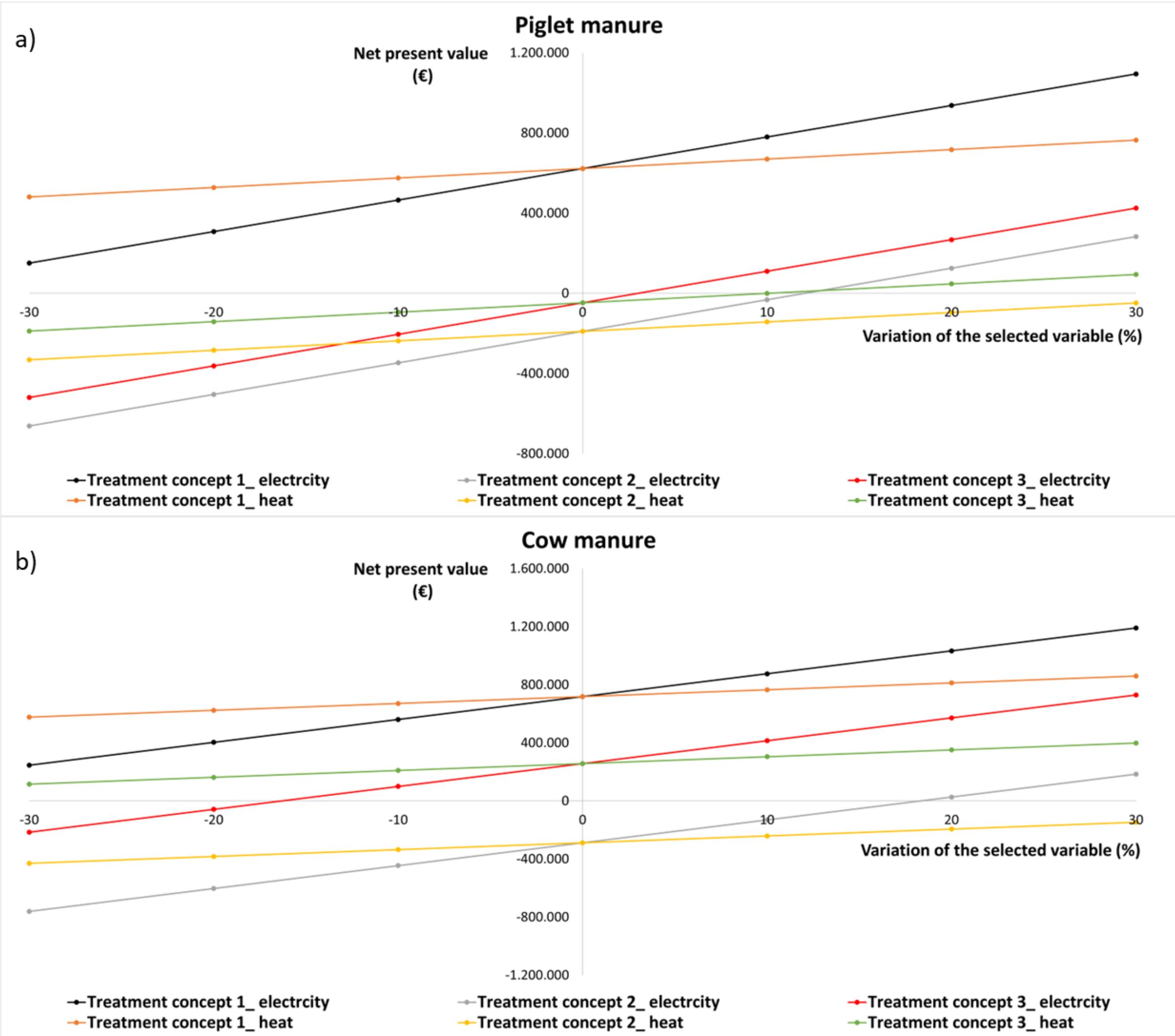


Figure 3- 6: Sensitivity analysis of NPV by electricity and selling price for different treatment concepts for (a) piglet manure and (b) cow manure.

The difference in the slope from the different costs is explained by the difference in electricity costs, which depends on equipment sizing. The high operational costs of the EGSB, particularly electricity costs, are significantly hindered by the applied OLR; hence, the slope of treatment concept 2 diverges between both substrates. In addition, the break-even point for PM is reached when the HRT value of both reactors in treatment concept 3 is reduced by 9.12%.

Of the three variables employed in the sensitivity analysis (electricity selling price, heat selling price, and HRT), the most influential variable was the electricity selling price, which currently has reached a price above the highest one analyzed by the sensitivity analysis.

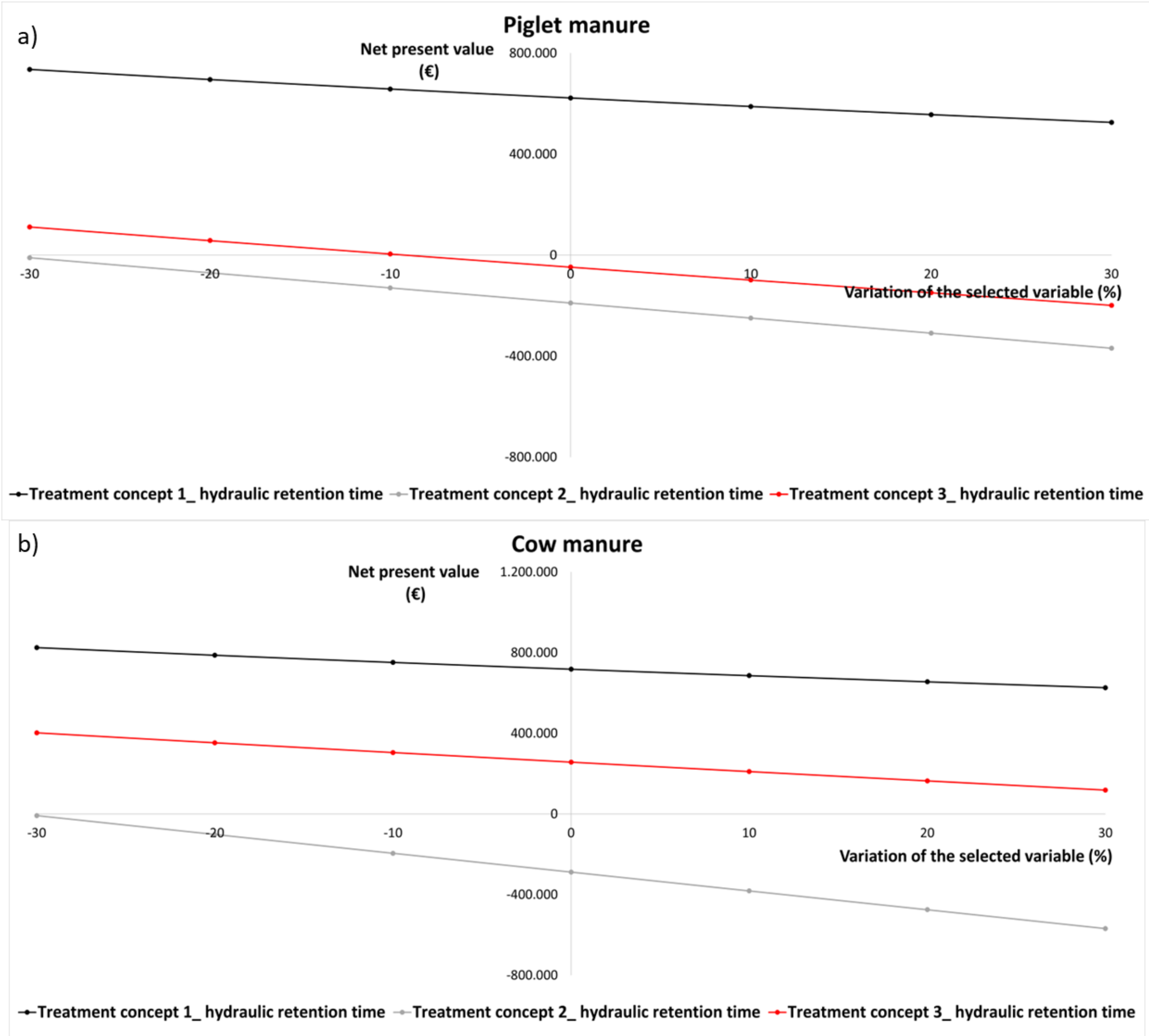


Figure 3- 7: Sensitivity analysis of NPV by hydraulic retention time for different treatment concepts for (a) piglet manure and (b) cow manure.

3.4 Discussion

Biogas plants are much more than energy producers. They are a central piece of sustainable agriculture, especially for livestock farms where optimal use of all resources, including manure, supports increased productivity of grass, forage, and arable land. In addition, the optimal use of livestock manure is a stepping stone on the path to net-zero emissions (Bywater & Kusch-Brandt, 2022; Liebetrau et al., 2021). However, AD involving manure is typically performed using energy crops as a co-substrate, and, in the case of Germany, their mass-specific use is 48% of manures (primarily cattle manure) and 47% of renewable resources (primarily grass silage) (Weinrich & Nelles, 2021a, p. 13). Due to the relatively low fresh mass-specific yields of manures, less than 20% of the energy-related output is attributed to manures (Liebetrau et al., 2021). The German government looks forward to encouraging the use of large quantities of manure in biogas plants by providing incentives to plants that digest at least 80% of manure. However, as of 2019, the total number of plants that take advantage of this incentive was less than 15% of all plants (Majer et al., 2019). Liebetrau et al. (2021) pointed out that the reason for this was that the incentive as written in law for small manure plants only worked for plants with optimal locations with the available substrate to fulfill the installed power of the plant and that the success was hindered by farm size and their distribution in Germany. In addition, less than 20% of the available PM and 33% of available CWM are currently used in German biogas plants. Thus, although economical profitability is an important indicator of project development, it should not be the only factor because the digestion of all available manure could save 3.6 million tons of CO_2 (Liebetrau et al., 2021). Therefore, using an EGSB reactor as an alternative in the mono-digestion of liquid PM and CWM provides an alternative to realize effective digestion (C. Rico et al., 2012; J. L. Rico et al., 2007).

However, the relatively high operational costs of EGSB reactors compared to CSTR reactors represent a barrier to their implementation. Despite the advantages of reducing the operating HRTs the associated costs of the substrate and the energy requirement of the recirculation pump mitigate or even negate the advantages afforded by implementing the EGSB reactors. One possible way to address this issue is to reduce the V_{up} of the reactor; however, this may negatively impact the reactor's flow pattern, which may in turn impact reactor performance (A. Cruz-Salomón et al., 2019; Mahmoud et al., 2003). Thus, an assessment of the most effective V_{up} is required since V_{up} involves a tradeoff relationship, i.e., a higher V_{up} increases the rate of collisions between suspended particles and sludge, which can improve the reactor's efficiency. In contrast, increasing V_{up} can also increase the hydraulic shearing force, which hinders the effectiveness of the removal mechanism by exceeding the settling velocity of a larger number of particles and breaking up or reducing the size of the biomass granules, thereby reducing efficiency (Ratanatamskul & Siritiewsri, 2015). Thus, experiments can be conducted to determine the optimal recirculation rate in the EGSB reactor to enhance reactor effectiveness while potentially reducing OPEX (Fuentes et al., 2011; van der Last & Lettinga, 1992).

Another possible solution could be to replace the EGSB reactor with a UASB reactor, which is the precursor of the EGSB, but without recirculation (Bhattacharyya & Singh, 2010; López & Borzacconi, 2011), which would reduce operational costs. However, the second solution only makes sense if the reduction in power consumption can mitigate the difference in reactor performance. With the UASB, problems occur more frequently in the flow pattern, e.g., short circuits or dead zones (Khanal, 2008; Pérez-Pérez et al., 2017); thus, it is not easy to predict if the overall economic efficiency of the UASB reactor will be better than that of the EGSB. Note that other types of high-rate reactors can be also employed, e.g., anaerobic filters, anaerobic membrane bioreactors (AnMBR), and anaerobic sequential batch reactors or fixed bed reactors (Kougias & Angelidaki, 2018). However, UASB and EGSB reactors have been proven effective in the treatment of liquid manures (Prapaspongsa et al., 2010).

In this study, we found that treatment concept 1 was the most profitable; however, as mentioned previously, only a small fraction of plants in Germany uses large volumes of manures. The flexibility provided by treatment concept 3 can be exploited by taking advantage of incentives afforded by treating substrates (or mixtures of substrates) that are otherwise not profitable due to low OLR, e.g., PM or wastewaters (Liebetrau et al., 2021). Accordingly, Hernández Regalado et al. (2021) selected optimal mixtures with greater than 80% mass-specific content of manure that demonstrated a possible synergistic effect. This was validated by Hernández Regalado, Häner, Baumkötter, et al. (2022) in continuous operation mode, thereby proving synergistic effects between triple mixtures of PM, CWM, and either sugar beets or starch wastewater. Therefore, the treatment of liquid agro-industrial mixtures with 80% manure and an optimal C/N ratio in EGSB reactors represents an opportunity that would typically not be realized in a CSTR (Liebetrau et al., 2021). In addition, the life cycle assessment by Rodriguez-Verde et al. (2014) demonstrated the importance of energy recovery to improve the environmental and economic feasibility of the PM treatment, as well as the improvement in both variables by co-digesting agro-industrial wastes. Prapaspongsa et al. (2010) also stated that AD of manure is the most effective technology for energy recovery while retaining good economic viability.

As a substrate, CWM is known to be difficult to manage in mono-digestion due to its low degradability because it has already been partially degraded by the microorganisms in the cows and its high nitrogen content (Theuerl et al., 2019). Thus, it is typically either mixed for its treatment with carbon-rich substrates or treated in multistage systems (Theuerl et al., 2019; Weinrich & Nelles, 2021a). Thus, the increased flexibility of plants that employ treatment concept 3 would also increase the environmental and economical profitability of the system while avoiding negative effects. Furthermore, it has been found that the use of slurry or manure as a single substrate or co-substrate in AD improves both the performance and environmental sustainability of the process (Bywater & Kusch-Brandt, 2022; Liebetrau et al., 2021; Rodriguez-Verde et al., 2014; Schievano et al., 2012).

Single-stage digesting systems have a wide range of applications due to their low operational complexity and affordability (Cremonez et al., 2021). Nevertheless, AD in multiple stages allows for manipulating the operating variables in each stage in a way that results in optimal conditions for the whole process (Theuerl et al., 2019). Nonetheless, two stages are usually employed for mono- or co-digestion of readily degradable substrates given that the rapidly degradable substrates promote the production of VFAs at a higher rate than those consumed by methanogens, resulting in sudden pH drops and, as a result, process inhibition (Damien J. Batstone et al., 2015; Rabii et al., 2019b; Theuerl et al., 2019). Therefore, multiple-stage systems are arranged with one first stage for the hydrolysis/acidogenesis and the second tank optimizes the acetogenesis/methanogenesis or hydrolysis/acidogenesis/acetogenesis, and the last reactor only with methanogenesis does not seem to be very economically effective, given that manures are relatively complex substrates. Nevertheless, some examples of technologically successful applications of two stages systems for manures can be found in Demirer and Chen; Panichnumsin et al.; Schievano et al. (2005; 2012; 2012).

Furthermore, a different approach for a multistage approach is the treatment of substrate(s) with very different DM content, as pointed out by Van et al. (2020). Van et al. (2020) stated that two-stage systems can be better combined as two wet stages or one dry and wet stage, respectively. Hence, the possibility to combine the output of the EGSB with the input of the CSTR will be investigated to optimize the TC3.

Overall, we believe that breakthroughs in the deployment of AD will depend on various technical aspects and incentives provided by the government (Bywater & Kusch-Brandt, 2022).

3.5 Conclusions

The typical agricultural biogas proved to be more profitable than the alternative of using either a single-stage EGSB or a multistage EGSB-CSTR to treat the liquid and the solid phase, respectively. Nevertheless, a multistage treatment concept involving a high-rate reactor offers a possible solution for establishing a better-rounded circular economy. In addition, a multistage system allows for treating large quantities of manure that otherwise would not be treated, thereby increasing the amount of carbon-neutral energy output, which is beneficial because energy security is currently a major concern.

In addition, reductions in the OPEX of the EGSB reactor through V_{up} will make multistage treatment more competitive from an economical perspective. Furthermore, co-digestion of manure-based mixtures up to 80% FM of manure can potentially make better use of the EEG incentives for small manure-based AD plants. Thus, further research into the life cycle assessment of the co-digestion of agricultural manure-based mixtures is required.

4. Optimization and Analysis of Liquid Anaerobic Co-Digestion of Agro-Industrial Wastes via Mixture Design

Abstract: Anaerobic co-digestion (AcoD) is a widely employed technique to produce biogas from the simultaneous digestion of various biomasses. However, the selection of the optimal proportions of the substrates in the mixtures presents a challenge. This research used a mixture design to investigate the interactions between the liquid fraction of piglet manure (PM), cow manure (CWM), and starch wastewater (SWW). A modified Gompertz model was used to identify the statistically significant parameters of the methane production curves. The optimal compositions of the mixtures were identified based on multi-objective optimization of methane yield (MY) and specific methane production rate (r_{CH4}) parameters. The study was validated using a double mixture of PM and CWM and a triple mixture. The estimated degradation rates for both mixtures were faster than the predicted ones. The absolute relative errors of r_{CH4} were 27.41% for the double mixture and 5.59% for the triple mixture, while the relative errors of MY were 4.64% for the double mixture and 10.05% for the triple mixture. These relative errors are within the normal limits of a process with high variability like AD. Thus, the mixture design supported by the tested models is suitable for the definition of practically advisable mixtures of substrates.

Keywords: anaerobic co-digestion; anaerobic batch-tests; mixture design; statistical optimization

4.1 Introduction

The enhancement of anaerobic digestion (AD) is a commonly researched topic. The improvement can be achieved by several methods. Among the most popular and recommended methods for improving AD, one can find pretreatments or enzymes (i.e., biocatalysts), reactor engineering, coupling AD with dark fermentation, and genetically improving the microbial community (bio-augmentation), and anaerobic co-digestion (AcoD) (Treichel & Fongaro, 2019).

AcoD involves the simultaneous AD of two or more substrates. It has proved to be a viable option for improving biogas production because it alleviates the disadvantages of mono-digestion while increasing the economic feasibility of the process (Rabii et al., 2019b). Distinct advantages of AcoD include the supply of macro and micronutrients, balanced carbon-nitrogen ratio, superior buffer capacity, dilution of inhibitors, and potentially enhanced biogas production (Esposito et al., 2012).

There exist different criteria to assess the performance of AD processes, however, the most well-accepted and commonly used is the biomethane potential (BMP) procedure. BMP is defined as the capacity of a substrate to be converted into methane and carbon dioxide. Determination of BMP is the first step in evaluating the digestibility or applicability of a substrate. The BMP parameter provides valuable information about general degradability, expectable energy yield, and the economic evaluation of new biogas plants. BMP is usually determined by employing a BMP test procedure in a batch anaerobic fermentation assay. This method is reliable, straightforward, and avoids inconsistencies in collected data (Pagés-Díaz et al., 2018; Raposo et al., 2011). The BMP test consists of adding a known quantity of an organic substrate to an active anaerobic inoculum in an air-tight serum bottle (I. Angelidaki et al., 2009; Ohemeng-Ntiamoah & Datta, 2019).

BMP optimization is the first step in AcoD optimization. BMP optimization for a substrate mixture is usually conducted based on statistical methods and variables. Substrate ratios and the inoculum/substrate ratio are the most important experimental factors. However, additional process variables may be included (Pagés-Díaz et al., 2018; Xie et al., 2016).

A popular statistical optimization method is response surface methodology (RSM). RSM is a group of mathematical and statistical techniques that identify process improvements based on the fit of empirical models to measured experimental data (Bezerra et al., 2008). A special type of RSM is the mixture design. Mixture design is an effective method for determining the optimal proportions of ingredients in a mixture (Buruk Sahin et al., 2016).

In mixture design experiments, the independent variables are the proportions of the investigated components. The total amount of material must be held constant in a mixture design experiment. This allows for analysis of the dependency of the component proportions without confounding variability due to changes in the total amount of the mixture. The mixture design analysis provides valuable information about the interactions between independent factors. It also provides a better understanding of the response variables (Cornell, 2002).

Mixture design has been previously used to understand how substrates interact during AcoD. Pagés-Díaz et al. (2014a) used a four-factor mixture simplex-centroid design, which employed solid cattle slaughterhouse wastes, manure, various crops, and municipal solid wastes. Methane yield (MY) and specific methane production rate (r_{CH4}) were the response variables. Rahman et al. (2019) used two sets of mixtures. The first set consisted of poultry droppings with sugarcane bagasse. The second set consisted of press mud and poultry droppings with roots, tops, and press mud from sugar beets. An augmented simplex-centroid design was applied to describe the interactions.

This paper generated experimental data for the integration of high-rate anaerobic reactors in conventional agricultural biogas plants. The objective was to find the optimal compositions of mixtures of three substrates that are usually found in great quantity in the agro-industrial sector, especially in the region of North Rhine-Westphalia, Germany. AD plants' profitability and flexibility can be improved by using information about the interactions between these substrates. The substrates used in this study were piglet manure (PM), cow manure (CWM), and starch wastewater (SWW). A three-factor mixture design was employed to analyze and describe the interactions between the substrate BMP tests. The

BMP tests were only conducted using the liquid fraction of the selected substrates. This was necessary because the study used expanded granular sludge bed (EGSB) reactors that operated in a continuous mode with a dry matter (DM) content of less than 8 wt%.

4.2 Materials and Methods

4.2.1 Raw Materials

In this study, the three selected substrates were PM, CWM, and SWW. In NRW, animal manure management is a topic of interest due to the large livestock population (Heinrich-Böll-Stiftung, 2020). PM and CWM were selected to address this issue. Both PM and CWM provide a strong buffer capacity that supports pH balance in livestock management operations. SWW was selected due to its low DM content. Low DM content avoids clogging in the reactors of a long-term operation. Also, the high content of readily degradable carbohydrates in SWW may increase the biogas yield and the co-digestion degradation rate of the mixture (L. Yu & Wensel, 2013). In Germany, SWW is highly available because it is obtained from crops that have already been treated by AD, including potatoes, corn, and wheat (Rosenwinkel et al., 2015, pp. 419–423).

Previously, PM and CWM were processed using screw press systems that separated the solid and liquid phases. For PM, a dual screw press (i.e., Vakusep from BETEBE GmbH) with a sieve filter size of 100 µm was applied. CWM was collected from farmers in a pre-separated form which was then processed by a second separation using o a screw press with a sieve size of 200 µm.

The liquid fractions of the substrates were used to characterize the content of DM, volatile solids (VS), macromolecules, and nutrients. The results of these analyses are shown in Table 4-1.

Table 4- 1**:** Characterization of the liquid fraction of the substrates.

Variable	Piglet Manure	Cow Manure	Starch Wastewater
DM/FM (wt%)	1.80	5.30	1.70
VS/DM (wt%)	58.33	69.81	82.35
VS/FM (wt%)	1.05	3.70	1.40
Crude protein/FM (wt%)	0.80	2.60	0.40
Crude fat/FM (wt%)	0.25	0.30	0.20
Crude fiber/FM (wt%)	0.00	0.00	0.00
Free nitrogen extracts/FM (wt%)	0.00	0.80	0.80
ash/FM (wt%)	0.75	1.60	0.30
Total nitrogen/FM (wt%)	0.36	0.36	0.07
Ammonium nitrogen /FM (wt%)	0.29	0.19	0.01

DM: dry matter; VS: volatile solids; FM: fresh matter.

4.2.2 Batch-Test Setup

Batch assays were conducted on fermentation tests of the organic substances, based on the VDI 4630 guidelines. Each test was performed using a 1000 mL glass vessel. Five grams of VS were added to the vessel of each test. A defined amount of substrate and a previously calculated quantity of inoculum were weighed into the reaction vessel. When necessary, the reaction vessel was carefully filled with warm water to reach a reaction volume of 800 mL. To prevent inhibition, the substrate inoculum rate $VS_{substrate}/VS_{inoculum}$ was kept constant at 0.5. Each bottle was vigorously agitated, sealed with a rubber stopper, and clamped down with a plastic screw cap connected to a eudiometer tube (as shown in Figure 4-1). The produced gases were transferred through PVC hoses into 1000 mL eudiometer tubes. This process enabled daily measurements of gas volume and quality. The eudiometers were sealed with a barrier fluid comprised of water and 5 wt% sulfuric acid. The acidification of the barrier liquid prevented carbon dioxide (CO_2) from dissolving in the gas mixture. Additionally, 7.5 wt% sodium sulfate (Na_2SO_4) was added to prevent the entry of CO_2 into the barrier fluid (Verein Deutscher Ingenieure, 2016). The batch assays were developed over 42 days.

A test vessel with cellulose, as the reference substrate, was carried out to compare and ensure adequate biological activity by the inoculum. The biogas potential of cellulose is known. Thus, cellulose can be used as a reference for evaluating the reliability of an experiment. Also, an inoculum-only batch fermentation test or zero-test was conducted. In the zero-test, the gas production value from the inoculum was subtracted from the gas production value from the substrate inoculum. Each fermentation test was performed at least three times, including the cellulose reference sample and the zero-test sample (Verein Deutscher Ingenieure, 2016; Holliger et al., 2016). The pH was measured at the beginning and the end of every batch test because changes in pH are usually correlated with other operational parameters. The accumulation of organic acids (acidification) typically lowers the pH, while increased ammonia concentrations or CO_2 removal increases the pH (Kougias & Angelidaki, 2018; Weide, Baquero, et al., 2020).

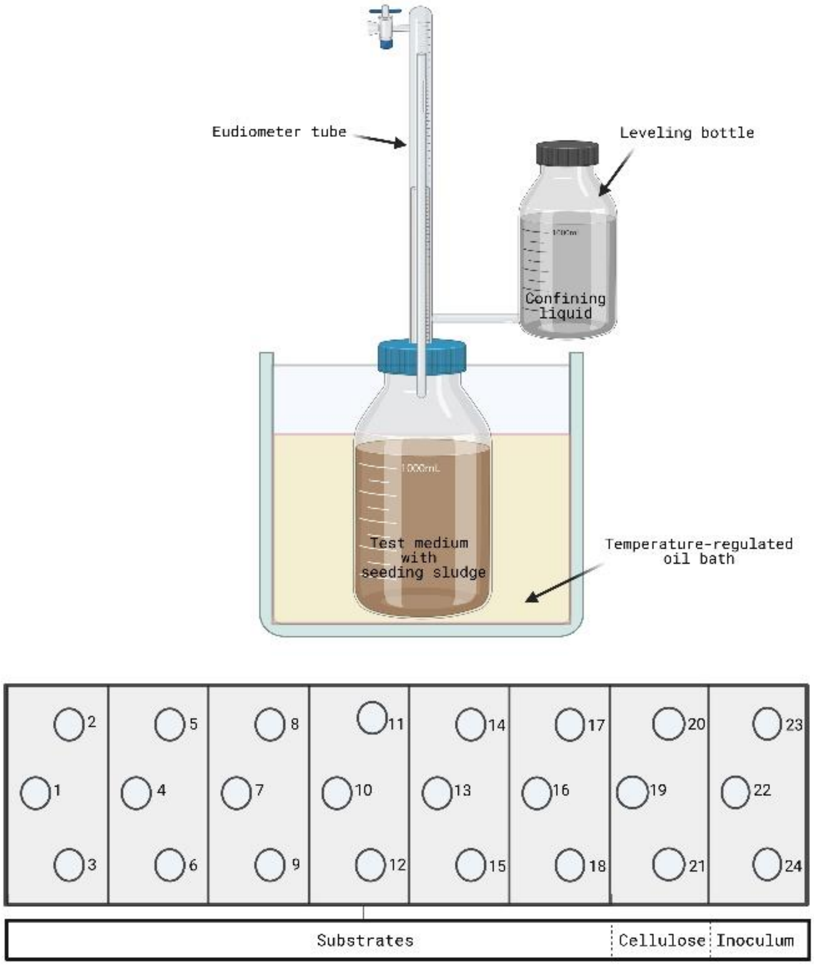


Figure 4- 1**:** The schematic diagram of the test stands.

4.2.3 Mathematical Modeling

Before modeling the methane yield curves (MYC), pre-processing of the measured data was necessary. The pre-processing of the measured data involved the application of three criteria:

1. Stopping criterion: The test was concluded when the relative increase of MY was less than 1% for three consecutive days.
2. Plausibility criterion: The existence of abrupt or non-monotonic trends in the curves requires individual analysis of the affected test.
3. Reproducibility/accuracy criterion: After deleting possible outliers, a coefficient of variation (CV) smaller than 5% between the curves was required.

If any of the above criteria were not met, the sample from all three batch tests was eliminated from the study. The three criteria are based on the recommendations by Holliger et al. (2016).

For validation, BMP results from the experimental tests were compared to reported BMP values from the literature and calculated theoretical BMP values. The theoretical BMP values and the degradation fraction (f_d) values were calculated using the equations from Ebner et al.; Raposo et al. (2016; 2011), shown in Equations (4-1) and (4-2), respectively.

$$BMP_{theo} = 415 \cdot X_{Carbohydrates} + 496 \cdot X_{Proteins} + 1014 \cdot X_{Lipids} \quad (4\text{-}1)$$

$$f_d = \frac{BMP_{measured}}{BMP_{theo}} \quad (4\text{-}2)$$

Where:

Xi: fraction of the macromolecule expressed in g of macromolecule per g of total so-lids

BMP_{theol}: theoretical BMP calculated using Equation (4-1) (L_{CH4}/kg_{VS})

$BMP_{measured}$: measured BMP obtained as a result of the practical tests (L_{CH4}/kg_{VS})

The most appropriate models to simulate MYCs include quantification of important parameters like maximal MY, r_{CH4}, and, if it exists, lag phase time (λ). The most commonly used of these models are the first-order one-step model (I. Angelidaki et al., 2009), the first-order two-step model (Brulé et al., 2014), and the modified Gompertz model (Pagés-Díaz et al., 2014b). All three models were fitted to the measured data. Goodness-of-fit statistics were compared to identify the model with the best fit. The non-linear regression tool of the software Minitab 19 was used to fit the models to the measured data. The root-mean-square error (RMSE) statistic was used to evaluate goodness-of-fit. An RMSE value less than 10 L_{CH4}/kg_{VS} was designated as acceptable goodness of fit. The first-order one-step model, the first-order two-step model, and the modified Gompertz models are shown in Equation (4-3), Equation (4-4), and Equation (4-5), respectively (Table 4-2).

Table 4- 2**:** Models employed to describe the methane yield curves.

Model	**Equation**	**Reference**
First-order one-step	$BMP(t) = BMP_{\infty} \cdot (1 - e^{-k \cdot t})$ (4-3)	(I. Angelidaki et al., 2009)
First-order two-step	$BMP(t) = BMP_{\infty} \cdot (1 + \frac{k_{vfa} \cdot e^{-k_{hyd} \cdot t} - k_{hyd} \cdot e^{-k_{vfa} \cdot t}}{k_{hyd} - k_{vfa}})$ (4-4)	(Brulé et al., 2014)
Modified Gompertz	$BMP(t) = BMP_{\infty} \cdot (e^{-e \cdot (\frac{R_{max}}{BMP_{\infty}} \cdot (\lambda - t) + 1)})$ (4-5)	(Pagés-Díaz et al., 2014b)

BMP_{∞}: Extrapolated BMP at infinite retention time in L_{CH4}/kg_{VS}. k: First-order reaction constant (1/d). k_{hyd}: First-order reaction constant of the first step (hydrolysis/acidification) (1/d). k_{vfa}: First-order reaction constant of the second step (volatile fatty acids degradation) (1/d). R_{max}: Maximum biomethane production rate (L_{CH4}/kg_{VS}/d). λ: Lag time (d). t: Time (d).

4.2.4 Mixtures Characterization

The synergistic and antagonistic effects of the individual mixtures on the BMP and reaction rate were characterized by two indices: (1) the co-digestion index (CI) (Equation (4-7)) and (2) the kinetic index (KI) (Equation (4-8)). In these two equations, the parameter values for the BMP and reaction rates were estimated from the three selected models that describe the MYCs.

$$BMP_{additive} = \sum_{i=1}^{n} (BMP_i \cdot x_i) \quad (4\text{-}6)$$

$$CI(\%) = \left(\frac{BMP_{fitted}}{BMP_{additive}} - 1\right) \cdot 100\% \quad (4\text{-}7)$$

$$KI(\%) = \left(\frac{R_{max_{mixture}}}{R_{max_{fastest}}} - 1\right)\cdot 100\% \quad (4\text{-}8)$$

Where:

$BMP_{additive}$: Calculated BMP based on substrates individuals' BMP (L_{CH4}/kg_{VS})

BMP_{fitted}: The fitted value of BMP during the batch-test by the selected model (L_{CH4}/kg_{VS})

CI (%): Co-digestion index (%)

R_{max}: Fitted maximum biomethane production rate by the selected model ($L_{CH4}/kg_{VS}/d$)

$R_{max_{fastest}}$: Fitted maximum biomethane production rate of the fastest substrates in the mixture by the selected model ($L_{CH4}/kg_{VS}/d$)

KI (%): Kinetic index (%)

The CI (%) expresses the relative increase in MY compared to the sum of the individual substrates in the mixture. Thus, it detects if the interaction between the substrates is positive or negative. The positive interactions are interpreted as synergistic effects. However, this term should be used with caution since batch tests are inoculum-biased. Instead, the term "acute effects" is suggested to describe the positive interactions in batch tests (Verein Deutscher Ingenieure, 2016; Koch et al., 2020).

The KI (%) expresses the relative increase in the degradation rate compared to the fastest substrate in the mixture. A positive KI (%) is likely related to an improvement in continuous operation mode (Brulé et al., 2014).

4.2.5 Experimental Design

A three-factor simplex-centroid mixture design with seven design points was used to evaluate the interaction between the substrates for the response variables. Based on the procedure described in Section 2.2, the seven design points were replicated three times for a total of 21 data points. The response variables were MY and r_{CH4}. The mixture design's experimental points employed in this study are detailed in Table 4-3.

The compositions of the mixtures are expressed in VS ratios (% VS) since the total VS in each batch test was held constant at 5 g to avoid confounding variability from the response variables. Based on Equation (4-9), each component's proportion varied between 0 and 1, and the variable xi represents the proportion of its constituent in the mixture.

Table 4- 3: Summary of the experimental design points.

Mixture	Number	Ratio (% VS)
Pure component	3	100 %
Double mixture	3	50 % + 50 %
Triple mixture	1	33 % + 33 % + 33 %

VS: volatile solids.

$$\sum_{i=1}^{3} x_i = x_1 + x_2 + x_3 = 1 \quad (4\text{-}9)$$

The effect of the mixtures on the response variables was modeled by a special cubic model shown in Equation (10) (Buruk Sahin et al., 2016; Cornell, 2002; Rahman et al., 2019):

$$\hat{Y} = \sum_{i=1}^{n} \beta_i \cdot x_i + \sum_{i=1_{j=1}}^{n} \beta_i \cdot x_i \cdot x_j + \sum_{i=1_{j=1_{k=1}}}^{n} \beta_i \cdot x_i \cdot x_j \cdot x_k \quad (4\text{-}10)$$

The βij and βijk coefficient values indicate the strength of the interaction between the substrates. The sign of the βij and βijk coefficients indicates whether the interaction is positive or negative. An analysis of variance (ANOVA) was used to identify the terms in the model. In an ANOVA, the p-value associated with the statistical confidence level determines if a term should be included in the model (Kashi et al., 2017). The response optimization method was employed to identify the combination of substrate proportions that simultaneously optimize both responses. A desirability function was applied in the optimization procedure. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The desirability function approach is one of the most widely used methods for optimizing multiple response processes. This method identifies the operating conditions that provide the most desirable response values given the specified assumptions. The characteristics of a goal may be altered by adjusting the weight or importance of each variable or the ranges within the optimization performance (Kashi et al., 2017).

4.3 Results

4.3.1 Analysis of the Curves

The MYCs were predominantly smooth with a slight leaning toward the logistic growth and signs of small lag phases. Subsequently, the dispersion analysis of the batches identified an outlier in one of the three PM replicate runs. This run was eliminated, to keep a CV of less than 5%. Furthermore, to make symmetrical the mixture experimental design the outlier curve was replaced during the mixture design analysis by the average of the two remaining curves. The rest of the CVs were approximately ≤5%, which is the validation criteria recommended by Holliger et al. (2016). The curves of the single digestions are presented in Figure 4-2, the numbers in the legend represent the valid number of replicates of each curve.

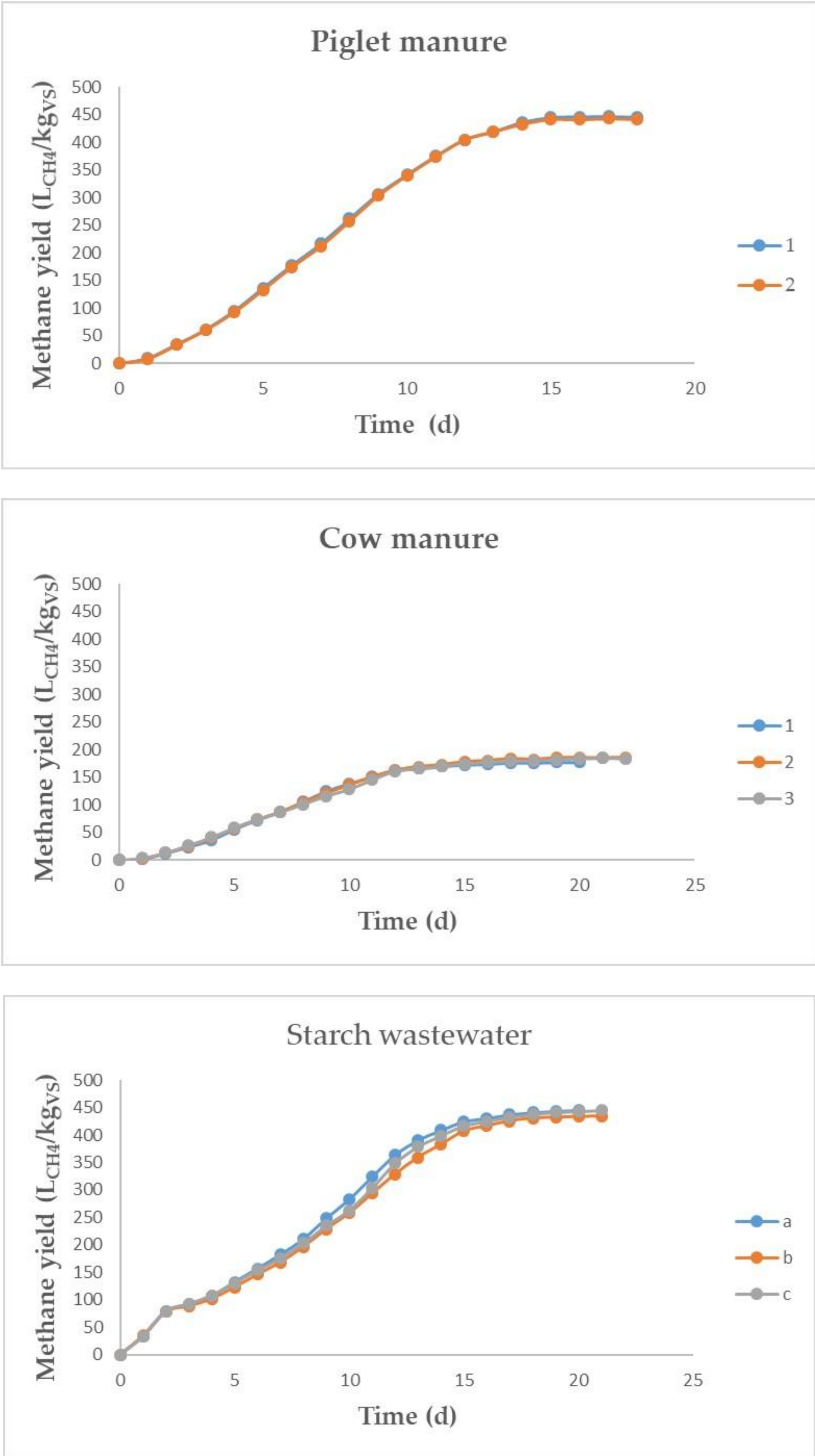


Figure 4- 2**:** Single degradations of piglet manure, cow manure, and starch wastewater.

Both PM and CWM had reasonably small lag phases. This was attributed to the relatively large presence of nitrogen-associated compounds, which are common in these substrates (Kougias & Angelidaki, 2018). Nevertheless, the ammonia concentrations were very different from the inhibition values reported by (Fotidis et al., 2013). Thus, the lag phases were probably related to an adaptation phase rather than inhibition. SWW was the only readily degradable substrate because of the relatively large presence of nitrogen-free extracts. Consequently, no lag phase was detected for SWW. Instead, a sharp change in slope occurred after the second day, indicating slight diauxic behavior. Thus, it was assumed that a second substrate was consumed after the second day. However, no plateau phase was observed, and the MYC was considered monotonic.

Most of the batch tests had small lag phases. However, none of the lag phases were long enough to cause inhibition. Similarly, the MY values were compared to the theoretical and literature values (shown in Table 4-4).

Table 4- 4**:** Validation through literature and theoretical values of the measured methane yield values.

Substrates	**Theoretical Methane Yield** (L_{CH4}/kg_{VS})	**Measured Methane Yield** (L_{CH4}/kg_{VS})	**Degradation Fraction** (%)	**Literature Methane Yield** (L_{CH4}/kg_{VS})
Piglet manure (PM)	577.79	489.90	84.79	[400–443.60] (Cu et al., 2015; M. et al., 2013)
Cow manure (CWM)	520.49	190.12	36.53	[175–212.00] (Garcia et al., 2019; Kouas et al., 2017)
Starch wastewater (SWW)	523.71	456.15	87.1	466.87 (from previous laboratory experiments)

For each substrate, the measured values were smaller than the calculated theoretical values but relatively close to the values reported in the literature. The MY of PM was above the interval reported by the literature. However, it has been reported that different factors like age, sex, type of feeding, and separation processes of the manure can cause significant variations in MY results (Cu et al., 2015; Garcia et al., 2019; Kouas et al., 2017; M. et al., 2013).

The measured MY of CWM was significantly different from the calculated theoretical value, though it was within the interval reported by the literature. Since the three batch tests behaved similarly with a CV of 2.34%, this finding was attributed to cows being ruminants. Thus, the organic matter was partially degraded before AD. Furthermore, the presence of microorganisms provided high VS values in the analysis of the macromolecules. Despite part of those VS not being available for the production of biogas. However, VS are included in the calculation of the BMP_{theo} in Equation (4-1).

The f_d was approximately 85% in PM and SWW. This result was expected because the AD occurred in the liquid phase, and no fiber content was measured in the substrates. Thus, hydrolysis was probably not a rate-limiting step. Next, the batch-test data were processed for compatibility with the model fitting procedures.

4.3.2 Model Fitting

The three models to describe MYCs were fitted to the measured data from the 20 batch tests, which met the three data pre-processing criteria. Table 4-5 shows the average RMSE results of the fit for each substrate and mixture. The modified Gompertz model had the best fit in all cases. According to Koch et al. (2019), the modified Gompertz model is a better fit when a lag phase is necessary to describe a curve. However, the lag phases detected were rather small.

Table 4- 5: The root-mean-square error (RMSE) for each model by substrate and mixture.

RMSE (L_{CH4}/kg_{VS}) / Substrates	Modified Gompertz Model	First-Order One-Step Model	First-Order Two Steps Model
PM	8.29	32.39	15.68
CWM	2.87	13.48	5.98
SWW	16.20	23.38	21.44

PM: piglet manure; CWM: cow manure; SWW: starch wastewater; RMSE: root-mean-square error

The fit to the first-order one-step model was not acceptable, based on the RMSE value of 10 L_{CH4}/kg_{VS}. This poor fit is due to small lag phases in all MYCs and hydrolysis being unlikely the rate-limiting step. A similar explanation applies to the poor fit from the first-order two-step model. However, the first-order two-step model accounts for a second degradation constant of VFAs. Thus, the first-order two-step model fit to the MYC was better than the first-order one-step model fit. However, the first-order two-step model did not meet the RMSE criteria for four of the seven substrate or mixture cases. Moreover, the values of the constants were predominantly the same, indicating that the process had only one rate-limiting step.

None of the three models adequately fit the MYCs of the SWW. Thus, a two-substrate model would provide a better fit, despite lacking a visually observed plateau phase in the SWW curve. However, for consistency in the mixture experimental design, all substrates must be described using the same model. Table 4-6 summarizes the average values from the fitted modified Gompertz model along with the measured pH before and after the AD.

Table 4- 6: Modified Gompertz model fitting summary.

Substrate	Maximum Specific Methane Production (L_{CH4}/kg_{VS})	Specific Methane Production Rate ($L_{CH4}/kg_{VS}/d$)	Lag Time (d)	pH at the Beginning	pH at the End
PM	476.38	47.05	2.19	8.26	7.55
CWM	187.81	18.66	2.09	8.10	7.36
SWW	506.75	32.13	1.06	8.05	7.43
PM + CWM	328.48	34.39	2.27	8.30	7.48
PM + SWW	438.67	43.91	0.95	7.88	7.48
CWM + SWW	311.16	28.80	0.98	8.02	7.45
PM + CWM + SWW	511.07	50.54	1.27	7.15	7.52

PM: piglet manure; CWM: cow manure; SWW: starch wastewater.

The adjusted lag times were relatively short. The lag times from the samples containing SWW were approximately one day slower than those without it. This was due to better C/N in the mixtures from the supply of carbohydrates readily degraded by the SWW. It was also due to high nitrogen content in substrates, like manures. Also, substrates with an initial pH of ≥ 8.10 corresponded to a lag phase of at least two days. Substrates with initial pH values < 8.10 had a maximum lag phase of 1.27 days. This drop in performance was associated with a shift in the NH_4+-NH_3 equilibria. This was because a higher pH moves the equilibria to NH3 production. It was reported that ammonia inhibition is commonly found in protein-rich substrates like the digestions of the manures and their double mixtures (Kougias & Angelidaki, 2018). The pH was always in the optimal recommended interval of 7.4 to 7.6 (Khanal, 2008, pp. 14–15) at the end of the AD. This indicated that the manures provided adequate buffer capacity.

Continuous surveying of the operational parameters during the batch tests was not feasible. However, the measured initial and final pH values combined with the continuous gas production indicate that the process was kept in stable operating conditions.

The adjusted BMP_∞ of PM and CWM were smaller than the measured BMP. This was assumed to be due to the cancellation of noise from the measured data by the model, associated with the 10% measurement uncertainty of the eudiometers.

4.3.3 Mixture Characterization

The mixtures were further analyzed based on the CI and KI parameters; the results are shown in Table 4-7. No positive interactions were found for either the MY or r_{CH4} of the double mixtures. However, Ebner et al. (2016) used CI to characterize nine double mixtures of manure and a second substrate. The CIs ranged from −32% to 21% for a mixture proportion of 70:30% *w*/*w*. The mixtures of manures and carbon-rich substrates showed statistically significant positive effects. This was attributed to the buffering of the VFAs by the manure when it is digested together with carbon-rich substrates, as explained in Joan Mata-Alvarez et al. (2011). The mixture from CWM + SWW carries this assertion since despite having both negative CI and KI, an increase in both the methane yield and the rate was observed when compared to the individual digestion of CWM. This suggests that the augmentation of the design could find optimal double mixtures PM + SWW and CWM + SWW by providing new data and improving the interpolating capacity of the model.

Table 4- 7: Comparison of the constructive/destructive effects of the mixtures.

Type of Mixture	Mixture	CI (%)	KI (%)	Prot/VS (%)	Fat/VS (%)	FNE/VS (%)
Double mixture	PM + SWW	−10.76	−6.68	0.49	0.18	0.33
Double mixture	CWM + SWW	−10.40	−10.32	0.47	0.28	0.25
Double mixture	PM + CWM	−1.09	−26.89	0.73	0.10	0.17
Triple mixture	PM + CWM + SWW	32.26	7.42	0.62	0.12	0.26
Double mixture	PM + SWW	−10.76	−6.68	0.49	0.18	0.33
Double mixture	CWM + SWW	−10.40	−10.32	0.47	0.28	0.25
Double mixture	PM + CWM	−1.09	−26.89	0.73	0.10	0.17

PM: Piglet Manure; CWM: Cow manure; SWW: Starch Wastewater; VS: Volatile Solids; Prot: crude Protein; Fat: crude Fat; FNE: Free Nitrogen Extracts; CI: Co-digestion Index; KI Kinetic Index.

For the triple mixture, both parameters showed a positive effect with a CI value of 32.26% and a KI value of 7.42%. The triple mixture was the only mixture with a pH close to neutral at the beginning and a constructive effect for both CI and KI, which in batch processes benefits the acidogenic microorganisms (Khanal, 2008). Also, Astals et al. (2014) found that ‘‘mixing a carbohydrate and/or protein source to lipids is a feasible option to reduce long-chain fatty acids (LCFA) inhibition, mainly due to the dilution’’. Furthermore, he concluded that AcoD leads to an enhancement of AD kinetics, but rarely to a methane yield increase. However, in the triple mixture, both are observed. Thus, the superior performance of the triple mixture was attributed to better macro and micronutrient balance.

4.3.4 Mixture Design

An extra batch test was manually added to the twenty measured batch tests. The added test resulted from the average between the two fitted PM curves. Consequently, all substrates had three tests in the mixture design. The response variables were MY and r_{CH4} and the input data were the parameters resulting from the fitted curves by the modified Gompertz model.

Special cubic models were fitted for each variable. Model selection was made using a stepwise procedure with a 90% confidence interval for the parameters. The goodness-of-fit statistics for the models are detailed in Table 4-8.

Table 4- 8: Goodness-of-fit statistics for the special cubic models by response variable.

Variable	R^2	Adjusted R^2	Predicted R^2
Methane yield (L_{CH4}/kg_{VS})	0.99	0.99	0.99
Specific methane production rate ($L_{CH4}/kg_{VS}/d$)	0.97	0.96	0.93

The statistics indicated very good goodness-of-fit. Thus, the model was deemed acceptable for prediction purposes. The predicted model equations for the response variables were as follows:

$$\widehat{MY} = 476.27 \cdot PM + 187.70 \cdot CWM + 506.64 \cdot SWW - 14.47 \cdot PM \cdot CWM - 2\ 10.72 \cdot SWW - 143.61 \cdot CWM \cdot SWW + 4372.78 \cdot PM \cdot CWM \cdot SWW \quad (4\text{-}11)$$

$$\widehat{r_{CH_4}} = 47.03 \cdot PM + 18.63 \cdot CWM + 32.11 \cdot SWW + 6.16 \cdot PM \cdot CWM + 17.39 \cdot PM \cdot SWW + 13.74 \cdot CWM \cdot SWW + 373.08 \cdot PM \cdot CWM \cdot SWW \quad (4\text{-}12)$$

Where:
PM: Piglet Manure
CWM: Cow Manure
SWW: Starch Wastewater

The predicted model includes all the mixtures that participated in the design since all terms were statistically significant in the equations. The response equation for MY indicated a very negative interaction between two double mixtures: (1) PM and SWW and (2) CWM and SWW. A slight negative interaction was observed between PM and CWM. Additionally, the strength of the negative effect in the double mixtures negatively correlated with the protein ratio in the mixture. The triple mixture interaction was very positive, as previously detected in the characterization. Furthermore, Pagés-Díaz et al. (2014b) found qualitatively similar results having the highest positive effects among triple, while Kashi et al. (2017) found the best results in a mixture of four substrates, as well that the mixture was very sensitive to changes in their composition. Moreover, the interactions between the variables in the equation were consistent with the characterization of the mixtures by the KI and CI parameters. Therefore, it served as a practical validation of the model.

The equation that described r_{CH4} showed positive interactions for all terms. The positive interactions did not necessarily contradict the characterization by KI. Unlike CI, KI only compares the fastest component of the mixture and not the mixture's predicted rate from the combination of individual substrates. The finding that all rate equation influences are positive is promising for further development in this research area. This finding indicates that it is more likely that the kinetics interaction transfers to the continuous stage rather than to the yield (Verein Deutscher Ingenieure, 2016; Koch et al., 2020). Therefore, the equation for r_{CH4} provided a better description of the interactions than the equation for KI.

Based on the r_{CH4} equation, the weakest positive interaction occurred in the PM + CWM mixture. Also, the magnitude of the positive interaction between double mixtures positively correlated with the percentage of readily degradable carbohydrates. It should be noted that the model was calibrated based solely on the seven mixtures but interpolated for the entire VS fraction range of 0 to 1 for each substrate. Thus, the positive interactions in the double mixtures that were outside the range of measured ratios indicated a need to improve the model by recalibration with additional runs.

The multi-objective optimization was performed after the constructed models were validated by acceptable matching with the practical values obtained from the characterization of the curves.

4.3.5 Optimization

The optimization was conducted with three constraints to treat a significant fraction of the piglet manure and to increase the chance of success in a continuous long-term operation. The three constraints were as follows:

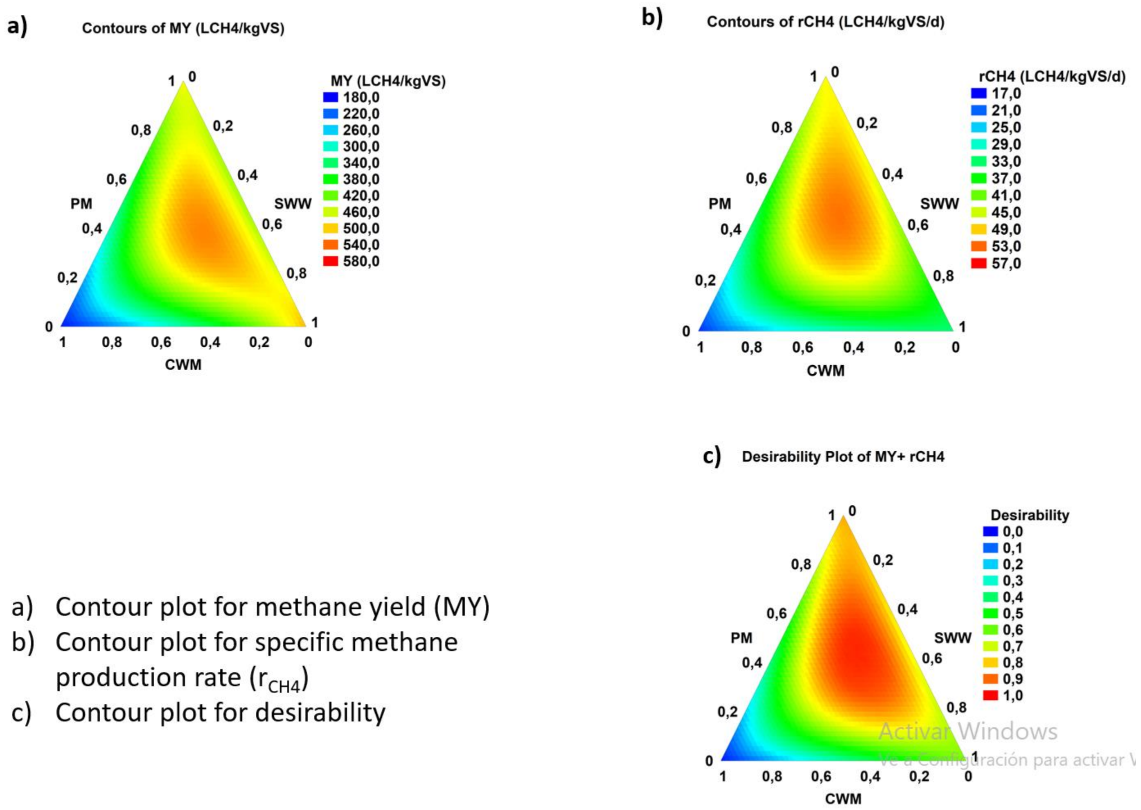
1. Maximize the specific methane production rate.
2. Bound the BMP with a minimum value of 450 L_{CH4}/kg_{VS}.
3. Require a minimal fraction of 0.4 of volatile solids in the piglet manure.

The optimization goal was to detect the optimal region(s) where coupled strong positive kinetics interacted with high MY quantities. The constraint that the minimum VS fraction was at least 0.4 guaranteed that most of the PM was treated due to its low VS content in terms of FM. The CWM content was indirectly restricted by giving a higher weight in the optimization to the r_{CH4} rather than the MY. This was due to the CWM deceleration effect on the degradation. However, it was advantageous to limit the DM content in the subsequent high-rate continuous operation.

A single common optimal region was found. Contour plots display the optimal region in Figure 4-3**.**

The red colored zone indicates the most powerful interaction, while the blue indicates the least powerful interaction. Figure 4-3c resulted from the superposition of Figures 4-3a and b. The rate (Figure 4-3b) had its maximal values in the region close to the center point of the mixture design. The model predicted a zone of higher rates compared to the center point of the mixture design because it was near the exterior border of the optimal rate region.

The optimal region in the contour plot of MY (Figure 4-3a) was bigger than the optimal rate region, but both were similarly located. Consequently, the yield region contained most of the rate region. However, the third imposed constraint bounded the optimal range to the section between the PM vertex and its inferior bound on 0.4 PM. This resulted in an optimal area that did not contain most of the high MY area. Nevertheless, the relatively large size of the optimal zone represents a practical advantage since it can provide some resilience to measurement errors in VS content and still be able to operate inside the optimal region.



a) Contour plot for methane yield (MY)
b) Contour plot for specific methane production rate (r_{CH4})
c) Contour plot for desirability

Figure 4- 3: Contour plots of the optimal region for piglet manure (PM), cow manure (CWM), and starch wastewater (SWW).

The three best solutions found in the optimal desirability region are shown in Table 4-9.

Table 4- 9: Goodness-of-fit statistics for the special cubic models by response variable.

Number	PM	CWM	SWW	Methane Yield (L_{CH4}/kg_{VS})	Specific Methane Production Rate ($L_{CH4}/kg_{VS}/d$)	Desirability
1	0.53	0.20	0.27	513.07	51.93	0.91
2	0.40	0.31	0.29	513.03	51.29	0.89
3	0.40	0.14	0.46	513.05	50.16	0.86

PM: Piglet Manure; CWM: Cow Manure; SWW: Starch Wastewater.

The three optimal solutions were triple mixtures with high predicted values for both CI and KI. In the first two mixtures, at least 71% of the VS in the mixtures were formed by the manures. Consequently, large quantities of manure can be treated by AcoD. Also, due to the buffer capacity of the three mixtures and their low VS values, the mixtures provide an opportunity to reach a stable operation in continuous high-rate reactors. The mixtures are composed of macro and micronutrients that provide high yields. No significant differences were found in the response variables' values between the three mixtures. This provides practical flexibility for continuous operation since the substrate's availability is sometimes a limiting factor. (Kougias & Angelidaki, 2018). Additionally, other constraints can be imposed to find optimal solutions for the double mixture, but alternative approaches were not investigated in this study.

4.3.6 Validation of the Models

Two mixtures were selected to validate the models, namely, PM + CWM and PM + CWM + SWW. This allowed evaluation of the effect of adding SWW to a base mixture of PM + CWM. The constraints were as follows:

1. The mixture had to remain within the optimum zone.
2. The PM composition had to be the same in both mixtures.

The validation used fresh substrates that were collected during a different time of year than the substrates used in the modeling. The mixtures selected after optimization are detailed in Table 4-10.

Table 4- 10: Mixtures selected after Optimization.

%Volatile Solids / Mixture	Piglet Manure	Cow Manure	Starch Wastewater
1	0.53	0.20	0.27
2	0.40	0.31	0.29
3	0.40	0.14	0.46

One batch test from the co-digestion of PM + CWM had to be eliminated to keep the CV value under 5%. Furthermore, the curves showed a smooth trend, and the lag phase was barely noticeable. This is a good sign for the practical application, despite affecting the fit of the modified Gompertz model. Moreover, as predicted by the model, the addition of SWW to the mixture of both manures produced a significant increase in performance. The comparison of the methane yield of the double and the triple mixture is shown in Figure 4-4.

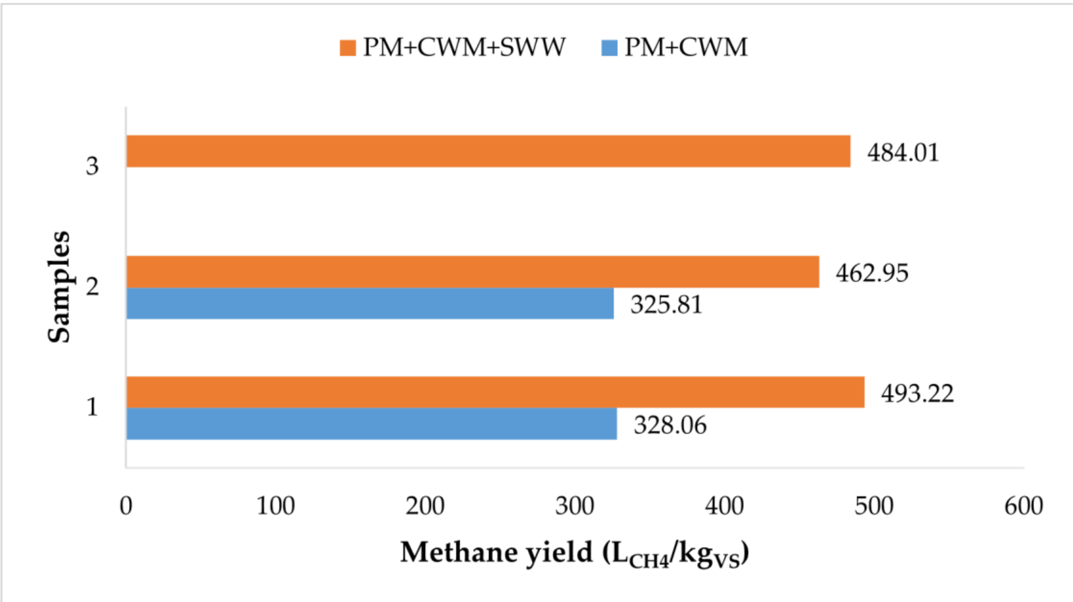


Figure 4- 4: Comparison of the methane yields of the mixtures employed in the validation. PM: piglet manure; CWM: cow manure; SWW: starch wastewater

The average MYCs were fitted by the modified Gompertz model. The fitted rates were superior to the predicted rates by the resulting mixtures' design model due to the lack of a lag phase. Thus, an increase in the rate was observed, and the modified Gompertz model was not the best possible fit. However, the modified Gompertz model was employed to establish a direct comparison between the predicted and the measured results from the validation. Still, this model was a good fit with an RMSE value of less than 10 L_{CH4}/kg_{VS}. The results of the predicted and measured values are detailed in Table 4-11.

Table 4- 11: Mixtures selected after Optimization.

Mixtures	**Obtained MY (L_{CH4}/kg_{VS})**	**Predicted MY (L_{CH4}/kg_{VS})**	**Obtained r_{CH4} ($L_{CH4}/kg_{VS}/d$)**	**Predicted r_{CH4} ($L_{CH4}/kg_{VS}/d$)**	**Relative Error MY (%)**	**Relative Error r_{CH4} (%)**
PM + CWM	326.94	342.83	45.58	35.77	**4.64**	**−27.41**
PM + CWM + SWW	480.54	534.21	54.59	51.7	**10.05**	**−5.59**

PM: piglet manure; CWM: cow manure; SWW: starch wastewater; MY: methane yield (L_{CH4}/kg_{VS}); r_{CH4}: Specific methane production rate.

The predicted values were used as references in the relative error calculations. The relative errors in MY were relatively small considering the multiple sources of error, including the age of the inoculum, differences in the VS content between the substrates used for modeling, and human error in the preparation of the batch tests.

The relative error for r_{CH4} in the PM + CWM mixture was quite large, but it was encouraging because the measured value was superior to the predicted one. This occurred because the deceleration of the CWM decreased compared to the CWM used in the model fitting. The average rate of the single digestion of CWM was 22.40 to a previous value of 18.66 $L_{CH4}/kg_{VS}/d$.

Thus, it was concluded that the models were valid for practical application and can be used for prediction. However, smaller values of MY and larger values of r_{CH4} should be expected. These two mixtures are currently being continuously tested in two separate high-rate reactors.

The developed mixture design model provided a reliable prediction and description of the interaction of the substrates at a macro scale, although it does not present profound insights into the biochemical interactions of the substrates in the mixture. This issue can be overcome by the development of a more complex mechanistic model. However, it usually requires the estimation of several non-measurable parameters, which is time-consuming and often does not assure the same level of precision as the empirical models.

4.4 Conclusions

The simultaneous anaerobic digestion of two or more substrates presents the challenge of selecting the correct proportions of substrates in the mixture. Mixture design describes a solid approach to finding the optimal proportions and understanding the interaction between the substrates in a mixture. The statistical models obtained in this experimental design presented physical meaning, also they seemed to describe accurately the constructive and destructive interactions between the substrates observed in the experimental data. The same models predicted the existence of an optimal zone where several triple mixtures presented many advantages for future continuous operation, and this existence was properly validated.

Thus, mixture design is advisable as the first step of a substrate-specific methodology for optimizing and understanding the co-digestion of a specific group of substrates.

5. Continuous co-digestion of agro-industrial mixtures in laboratory scale expanded granular sludge bed reactors

The results of this study will be used in the construction and operation of a state-of-the-art biogas pilot plant in the Bioenergy Park of Saerbeck in Germany. This study was relevant to the product development activities of PlanET GmbH.

Abstract: Anaerobic co-digestion often improves the yields and stability of single anaerobic digestion. However, finding the right substrate proportions within mixtures and corresponding optimal operating conditions using a particular reactor technology often presents a challenge. This research investigated the anaerobic digestion of three mixtures from the liquid fractions of piglet manure (PM), cow manure (CWM), starch wastewater (SWW), and sugar beets (SBT) using three 30 L expanded granular sludge bed (EGSB) reactors. The synergistic effects of 2 three-substrate mixtures (*i.e.*, PM+CWM+SWW and PM+CWM+SBT) using the PM+CWM mixture as a benchmark were studied. These were used to detect the predicted synergistic interactions found in previous batch tests. The methane productivity of both three-substrate mixtures (~1.20 $L_{CH4}/L_{react}/d$) was 2x the productivity of the benchmark mixture (0.64 $L_{CH4}/L_{react}/d$). Furthermore, strong indications of the predicted synergistic effects were found in the three-substrate mixtures, which were also stable due to their appropriate carbon-to-nitrogen ratio values. Moreover, the lowest averaged solid-to-hydraulic retention times ratio calculated for samples obtained from the top of the reactors was > 1. This confirmed the superior biomass retention capacity of the studied EGSB reactors over typical reactors that have been used in agricultural biogas plants with a continuously stirred tank reactor.

Keywords: Anaerobic co-digestion; Synergistic effects; Expanded granular sludge bed reactor; Agro-industrial substrates

5.1 Introduction

Anaerobic digestion (AD) is an efficient and suitable method for the sustainable management of bio-wastes as well as the production of biofuel (Treichel & Fongaro, 2019). It is a biological degradation process where biomass is converted into a mixture of gases called biogas, which consists mainly of methane and carbon dioxide, by the action of a microorganism consortium in the absence of oxygen. It is typically divided into four main stages hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Rajendran, 2015b).

However, AD is a very complex and sensitive process, which involves diverse microorganism groups that require different environmental and operational conditions (Treichel & Fongaro, 2019, pp. 2–3). For example, biomass substrate digestibility and biogas production are significantly affected by substrate composition and chemistry such as carbon-to-nitrogen ratio (C/N), mineral and volatile fatty acid composition, and pH (L. Yu & Wensel, 2013). These are also affected by the operational conditions including hydraulic retention time (HRT), substrate loading rate, reactor temperature, and so on.

High-efficiency energy production by AD has been commonly achieved through the use of a high organic substrate loading rate. However, the high loading rate affects the stability and efficiency of methane production due to the imbalance between acidification and methanation, which typically results in a significant accumulation of volatile fatty acids and a sharp decrease in pH leading to system failure (Rabii et al., 2019a).

The most popular approaches to improve AD for biogas production include anaerobic co-digestion (AcoD), coupling with dark fermentation, microbial community bioaugmentation, reactor engineering, substrate pretreatments, and use of enzymes as biocatalysts (Treichel & Fongaro, 2019).

AcoD in particular involves the simultaneous digestion of two or more substrates. It has been shown to be a highly viable option to improve biogas production by alleviating the disadvantages of mono-digestion (Rabii et al., 2019a). It also increases the economic feasibility of the process in existing AD plants by increasing the methane yields (Rabii et al., 2019b). The advantages of AcoD include the ample supply of macro and micronutrients, balanced C/N, dilution of reaction inhibitors, superior buffering capacity, and enhancement of biogas production (Salehiyoun et al., 2020). Although AcoD has its benefits, it also has some downsides that can arise from poor choices regarding co-substrates, their composition, and operating conditions. Consequently, a poorly researched co-digestion process may end up in process instability bringing along a significant reduction in methane production. Therefore, it is first necessary for a profound comprehension of co-digestion mixture(s) employed at lab and pilot plant to support full-scale design and operation decisions (Xie et al., 2016; L. Yu & Wensel, 2013).

Germany with 9,632 operating and 9,692 forecasted biogas plants in 2020 and 2021, respectively is the largest producer of biogas in Europe (German Biogas Association, 2021). Its main feedstock for biogas production was initially energy crops. However, due to the current policy framework, Germany has shifted toward the use of alternative substrates such as crop residues, livestock waste, and catch crops (Iglesias et al., 2021; Kougias & Angelidaki, 2018). Hence, agro-industrial wastes have gained importance due to their potential as raw materials for obtaining energy (Cremonez et al., 2021). Their use could eventually reduce environmental liabilities and add value to already-developed production chains. In 2018, 95% of Germany's mass-specific substrates came from animal excrements (48%) and renewable resources (47%) such as maize or grass silage (Weinrich & Nelles, 2021a, p. 13). AD has been carried out either as a single or co-digestion system. Production plants have been equipped with a gas-tight storage tank and a minimum of two digesters that are connected in series (Ruile et al., 2015).

However, optimization of HRTs is still required to reach high degradation values (Ruile et al., 2015; Weinrich & Nelles, 2021a).

A large share of the studies on anaerobic co-digestion are concerned with the enhancement of biogas production while increasing methane content and shortening the retention time. Nevertheless, the typical anaerobic digestion systems are not sufficiently efficient for today's demand (Neshat et al., 2017). The alternative may be a combination of modern reactors with enhanced biomass retention capacity and optimized digestion conditions (pH, Temperature, HRT, among others) to obtain higher methane yields and productivities (Kougias & Angelidaki, 2018; J. Mata-Alvarez et al., 2014; Neshat et al., 2017).

In the selection of a suitable bioreactor, the biomass retention capacity is an important consideration, because anaerobes grow slowly during the metabolic generation of butanol, ethanol, hydrogen, and methane (Khanal, 2008, pp. 14–15). This is particularly important in a bioreactor configuration that decouples HRT from solids retention times (SRT). These reactors which are usually named high-rate reactors were initially developed in the late 1970s, with the introduction of the up-flow anaerobic sludge blanket (UASB) reactor (Abdelgadir et al., 2014).

The decoupling of HRT and SRT enables the maintenance of a significantly higher SRT/HRT ratio than in a continuous stirred tank reactor (CSTR) and prevents the washout of slow-growing anaerobes. Therefore, high-rate anaerobic systems are maintained at a sufficiently high biomass level inside the bioreactor (Environmental Energy Company: Olympia, 2001). In addition, environmental conditions are well preserved under optimal bioreactor performance parameters. The organic loading rates in these systems typically vary from 5 to 30 $kg_{COD}/(m^3 \cdot d)$ although higher rates have been reported (Fuentes et al., 2011; Khanal, 2008).

The two main types of high-rate systems include suspended and attached growth. The expanded granular sludge bed (EGSB) reactor is a suspended high-rate system, which has been used in industrial wastewater treatment. The implementation of EGSB reactors for biogas production has grown very fast in the last two decades (A. Cruz-Salomón et al., 2019). Interestingly, EGSB reactors appeared as an improvement of UASB reactors, which allow high height-diameter relations for achieving high superficial velocities of >4 m/h EGSB per 1.5 m/h UASB (Gleyce et al., 2014; Seghezzo et al., 1998). The EGSB reactor technology was developed to optimize internal mixing and solve problems, which are typically found in the practical operation of UASB reactors such as the occurrence of dead zones, preferential flows, and short circuits, among others. Consequently, EGSB reactors provide better substrate–biomass contacts within the treatment system by expanding and intensifying the sludge bed and hydraulic mixing, respectively (Gleyce et al., 2014; Treichel & Fongaro, 2019, pp. 77–78).

This study aimed to assess the performance of the AcoD of three manure-based agro-industrial mixtures in three different EGSB reactors in continuous operation mode. The AcoD of manure-based mixtures has acquired more relevance due to changes in the German Renewable Energy Sources Act (EEG), provoking that only small liquid manure plants and waste digestion plants continue to benefit from the original remuneration system outlined in 2012 (Weinrich & Nelles, 2021a, p. 14). Furthermore, the German government encourages the use of natural fertilizers to reduce greenhouse gas emissions by recycling nitrogen (Guidehouse et al., 2021). The substrates were collected in the federal state of Nordrhein-Westfalen, Germany. In particular, the optimal compositions of two of the considered agro-industrial mixtures were determined using an approach initially designed by our group. The optimal composition of the third mixture was determined using another approach by Hernández Regalado et al. (2021), which was an extension of our previous study. The observed performance characteristics of the laboratory-scale reactors will be the basis for operation optimization and scale-up to pilot plant scale.

5.2 Materials and Methods

5.2.1 Mixtures and inoculum characterization

Mixture 1 was a combination of piglet manure (PM) and cow manure (CWM). It served as the benchmark to measure the change in the performance by the addition of a third substrate, which was found in mixtures 2 and 3. The information on the mixtures and their inoculums is summarized in Table 5-1.

Table 5- 1: Characterization of the mixtures and their inoculums.

Reactor	Substrates	Dry matter (wt.%)	Organic dry matter (wt.%)	Carbon-to-nitrogen ratio (%)
1	Pellets 1	7.79 ∓ 0.16	87.59 ∓ 0.06	
	PM+CWM	2.92∓ 1.40	60.08∓9.32	13.70
2	Pellets 2	8.84 ∓ 1.75	89.47 ∓ 1.45	
	PM+CWM+SWW	1.76 ∓ 0.94	58.33∓9.07	16.32
3	Pellets 3	7.93 ∓ 0.29	87.56 ∓ 0.10	
	PM+CWM+SBT	3.14 ∓ 0.99	64.55 ∓ 8.52	18.87

piglet manure (PM); cow manure (CWM); sugar beets (SBT); starch wastewater (SWW)

5.2.2 Bioreactor setup and operation

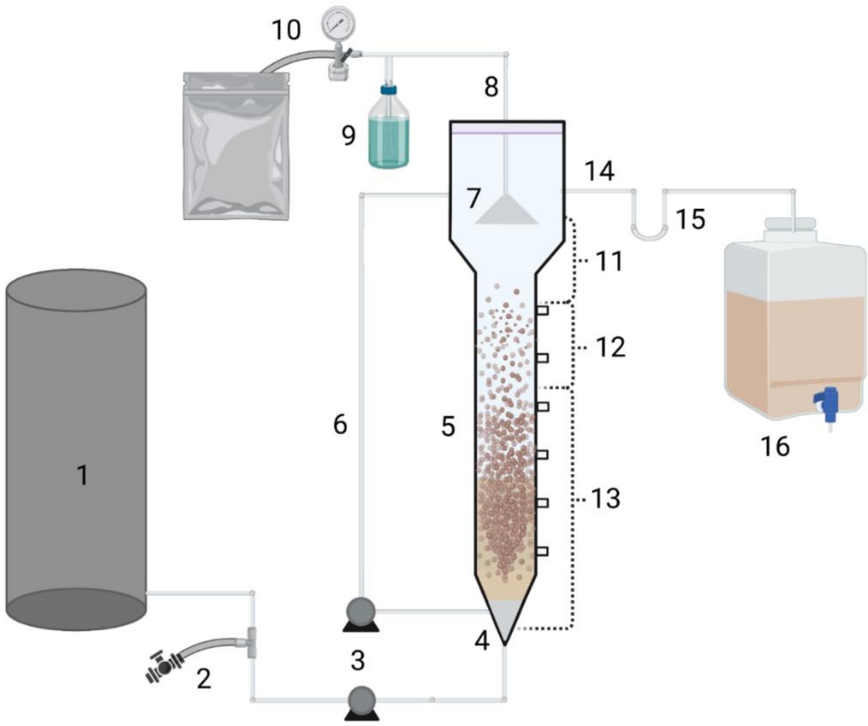
Three EGSB reactors with a height-diameter ratio of 3 units were employed in a continuous operation mode. The reactors were inoculated with 20 L of mesophilic inoculum with a spherical shape and dark green color. The EGSB reactors were operated at 6 different HRTs for 15, 10, 7, 5, 3, and 1 day(s). The HRTs were automatically altered by changing the feeding time of the pump to a constant flow. The HRT was calculated by equation (5-1) (A. Cruz-Salomón et al., 2019; Abumalé Cruz-Salomón et al., 2018).

$$HRT = \frac{V_R}{Q} \tag{5-1}$$

Where, the HRT, the volume of the reactor (V_R), and influent volumetric flow are in day(s), m^3, and m^3/day units, respectively.

The recirculation pump was continuously working at an upflow velocity of 5 m/h. Each reactor was connected to a 100 L tank that was kept under a nitrogen atmosphere and temperature of 4 °C to prevent premature aerobic degradation. The concept for a single reactor is shown in Figure 5-1.

All three reactors were operated under mesophilic conditions with temperatures between 37 and 40°C and pH values close to 8 by regulating the feed of the reactor which is mainly possible due to the buffer capacity of the manures (Joan Mata-Alvarez et al., 2011; Weide, Hernández Regalado, et al., 2020). The procedure for the setting and monitoring of the continuous operation was as described in the reference (Verein Deutscher Ingenieure, 2016). The measured and set variables are summarized in Table 5-2. Other relevant values were calculated from registered variables such as organic loading rate (OLR) ($kg_{COD}/m^3/d$), methane production rate (MPR) ($L_{CH4}/L_{reactor}/d$)), methane yield (MY) (L_{CH4}/kg_{VS}), removal efficiencies of chemical oxygen demand (η_{COD}) (%), and biological oxygen demand on the fifth day (η_{BOD5}) (%).



The parts are (1) feed tank, (2), three-way sampling valve, (3) eccentric screw pumps, (4) mixer for influent and recirculation, (5) bioreactor, (6) recirculation, (7) bell separator, (8) biogas outlet, (9) foam trap, (10) gas flow-meter, (11) three-phase separator or settling zone, (12) transition zone, and (13) digestion zone, (14) effluent, (15) siphon, (16) digestate storage.

Figure 5- 1: Schematic diagram of the reactor.

Table 5- 2: Registered variables in the monitoring of reactor operations.

Variable	Input	Inside the reactor	Output
temperature (°C)		x	
dry matter (%)	x	x	x
organic dry matter (%)	x	x	x
C/N (%)	x		
chemical oxygen demand (mg_{O2}/L)	x		x
biochemical oxygen demand on the 5th day (mg_{O2}/L)	x		x
loading rate per unit volume ($kg_{COD}/m^3 \cdot d$)	x		
hydraulic residence time (d)	x		
pH value (-)	x		
gas composition in volume fractions (%, ppm)			x
the ratio of volatile organic acids to total inorganic carbon (-)		x	

5.2.3 Data cleaning and analysis

Data cleaning was aimed to obtain a data set, which will not contain obvious failures, start-up periods, or clear mistakes in an operation. The data cleaning was performed using the three main criteria as described below.

1. The HRT is ≤ 30 d.
2. The MY < biomethane potential of the mixture at HRT_∞ (BMP_∞). BMP_∞ was taken from Hernández Regalado et al. (2021).
3. The chemical oxygen demand removal is ≥ 0.

5.2.3.1 Overview of each reactor's operation

A comparison of the different operation points of a reactor was made using the variables of MY, MPR, η_{COD}, and biological oxygen demand removal (η_{BOD5}). An analysis of the practical operation of each reactor was completed using these pieces of information and the complementary information on the mixtures involved. Box and scatter plots were employed to visualize a reactor's operation.

5.2.3.2 Principal component analysis

PCA has been an adaptive exploratory method, which can be used on numerical data of various types. From a mathematical point of view, principal components are linear combinations of original variables so that they are orthogonal to each other (Jolliffe & Cadima, 2016; L.E. López de la Maza et al., 2019). This method increases the interpretability of the data and at the same time minimizes information loss. For each reactor, a new data set was created using the average values of all the operation points for the above for all four response variables. The new datasets were used in a principal component analysis (PCA) to compare the reactors by operation points as a part of a multivariate analysis. Up to five components were acceptable and three components were desirable. The goal of the PCA was to rotate the data into an axis system where the greatest amount of variance was captured in a small number of dimensions (Aggarwal, 2015).

The PCA involved the calculation of eigenvectors and eigenvalues of a sample covariance or correlation matrix. Furthermore, the calculation of the principal components was carried out using a singular value decomposition (SVD) (Møller et al., 2005). As a plus, the PCA was employed for outlier detection due to its robustness (Dempster, 1971; Møller et al., 2005)

5.2.4 Solids retention time to hydraulic retention time ratio (SRT/HRT)

A high-rate reactor such as an ESGB reactor can decouple HRT and SRT, thereby increasing the residence time of a biomass element within the reactor (Chernicharo, 2007, pp. 65–66; Eberl et al., 2006, p. 148; Khanal, 2008, pp. 93–95). One of the main selection criteria for a reactor is a high SRT/HRT ratio, which prevents the washout of slow-growing methanogens (Khanal, 2008, pp. 92–97).

The sludge age (SRT) in d is given by equation (5-2).

$$SRT = \frac{Mass\ of\ sludge\ in\ reactor}{Mass\ of\ sludge\ wasted\ per\ day}\ (d) \tag{5-2}$$

If a steady-state was assumed, equation 2 can be written as equation (5-3).

$$SRT = \frac{x_i \cdot V_R}{Q_{eff} \cdot X_{eff}}\ (d) \tag{5-3}$$

Where, x_i, V_R, Q_{eff}, and X_{eff} are the viable biomass concentration inside the reactor (kg_{VS}/kg_{FM}), the volume of the bioreactor (m^3), the effluent flows out (L/d) of the reactor, and viable biomass concentration in the effluent (kg_{VS}/kg_{FM}), respectively. Since the input and output flows were equal (steady-state condition), equation (5-3) was transformed to (5-4).

$$\frac{SRT}{HRT} = \frac{x_i}{X_{eff}}\ (d) \tag{5-4}$$

The ratio SRT/HRT values were calculated in all three reactors using biomass, which was sampled from the top of each reactor where biomass concentration was lowest. The ideal SRT/HRT ratio should be > 3 (A. Cruz-Salomón et al., 2019; Environmental Energy Company: Olympia, 2001; Khanal, 2008)

5.2.5 Characterization of synergistic effects

The synergistic effects of the three-substrate mixtures (PM+CWM+SWW and PM+CWM+SBT) were compared using the two-substrate mixture (PM + CWM) as a benchmark. Since it was not possible to operate a single digestion of each substrate, the hypothesis employed used equation (5) to validate the synergistic effects.

$$\left(\frac{MY_{MAX\_TM}}{MY_{MAX\_DM}}\right)_{continuous} \cong \left(\frac{MY_{MAX\_TM}}{MY_{MAX\_DM}}\right)_{batch} \tag{5-5}$$

Where, MY_{MAX_TM} and MY_{MAX_DM} are the maximum MY of the three- and two-substrate mixtures, respectively.

Since calculated ratios were based on the yield of the two-substrate benchmark mixture, the ratio for the PM + CWM mixture was equal to 1. As for the batch data results from Hernández Regalado et al. (2021) and some complementary unpublished data, the MY_{MAX} value corresponding to the maximum value of each operating point was used. In addition, a comparison between the MY_{MAX} in the continuous and batch operation processes was made for each mixture.

5.2.6 Characterization of hydraulic behaviors

Anaerobic reactor hydrodynamics was studied because they significantly influence the rates of biological reactions. They particularly affect the rates of mass transfer and distribution of reactions along a reactor, which determine a reactor's overall performance (Brito & Melo, 1997; Gleyce et al., 2014). The amount of mixing in a reactor also determines the performance of a reactor; therefore, to describe the real behavior of a reactor, the influence of mixing on the mass balance equation must be specified correctly (Gujer, 2008, pp. 129–130). In this study, hydrodynamics were characterized by the non-dimensional numbers given by Peclet and Reynolds.

The mixing intensity of the fluid within a reactor is well described by the axial Peclet number (Pe_{axial}) (see equation (5-6)).

$$Pe_{axial} = \frac{V_{up} \cdot H}{D_A} \tag{5-6}$$

Where V_{up}, H, and D_A are the upflow velocity (m/h), bioreactor height (m), and axial dispersion coefficient (m^2/h), respectively. When $D_A \rightarrow \infty$, the value of Pe_{axial} becomes 0 since Pe_{axial} is an inverse function of D_A. Consequently, the system will operate as a plug-flow reactor since there is no mixing in the axial direction. On the other hand, when $D_A \rightarrow 0$, the system will behave as a complete mixture reactor (A. Cruz-Salomón et al., 2019). Various transfer functions have been proposed to estimate the dispersion from either the Reynolds number or a flow velocity (Inglezakis & Poulopoulos, 2006; Levenspiel, 1999; Rapp, 2017; Rodríguez-Reinoso, 2002; Šolcová & Schneider, 2002). Here, we used an approach described by equations (5-7) and (5-8), which assessed D_A as a function of flow distance.

$$D_A = 1.03 \cdot {V_{up}}^{1.11} \cdot 0.009^{n_j} \tag{5-7}$$

$$n_j = \frac{z}{H} \tag{5-8}$$

Where, n_j, z, and H are the values of the normalized height, axial position (m), and height (m), respectively of the bioreactor.

The amount of turbulence is characterized by the Reynolds number (Re) and is given by equation (9) (A. Cruz-Salomón et al., 2019; Raju, 2010, pp. 21–26). Where, V_{up}, d, μ_w, ρ_w, and V_{up} are the upflow velocity (m/h), bioreactor diameter (m), dynamic viscosity (Pa·s), density (kg/m^3), and kinematic viscosity (m^2/s), respectively Reynolds describes a relationship between inertial to viscous forces (Rapp, 2017). It should be noted that despite the prevalence of Equation 5-9, there exist alternative forms of the Reynolds number that are specifically applicable to noncircular conduits, packed beds, and mixing impellers.

$$Re = \frac{V_{up} \cdot d}{\vartheta_w} = \frac{\rho_w \cdot V_{up} \cdot d}{\mu_w} \tag{5-9}$$

Meanwhile, turbulence is the rotational and three-dimensional chaotic movement in all directions of flowing elements, where the resulting net flow is unidirectional. The rapid mixing associated with turbulence enhances the momentum, heat, and mass transfer processes. The intervals of Reynolds include Re < 2300, 2300 < Re < 4000, and Re > 4000, which correspond to laminar, transient, and turbulent regimens, respectively. However, a typical turbulent regimen truly manifests itself from values of Re > 10000.

5.2.7 Modeling of reactors

5.2.7.1 Stover–Kincannon model

The MPRs of the reactors were modeled using a variation of the Stover–Kincannon model for an anaerobic filter reactor (equation (5-10)), which was proposed and implemented by Jafarzadeh et al.; Verma et al.; H. Yu et al. (2021; 2014; 1998).

$$MPR = \frac{MPR_{max} \cdot OLR}{M_B + OLR} \tag{5-10}$$

Where, MPR, MPR_{max}, and M_B are the methane production rate (L_{CH4}/L_{react}/d), maximum MPR (L_{CH4}/L_{react}/d), and constant ($kg_{COD}/m^3 \cdot d$), respectively. OLR is the organic loading rate ($kg_{COD}/m^3 \cdot d$). A non-linear regression procedure was employed using the calculated cleaned average data of all reactors. To identify similarities and differences in the kinetic behavior of all possible combinations, the averaged data of reactors 1, 2, and 3 were arranged to have a total of 7 datasets. The goodness of fit was measured by a root-mean-square error (RMSE). For the most meaningful dataset(s), a simple regression analysis was performed for MY and η_{COD} removal using OLR as the independent variable. The goodness of the fit was compared by the R^2 value, simplicity, and Durbin-Watson coefficients to determine the most significant dependency. R^2 values of < 0.7 were automatically dismissed and those >0.8 were identified as desirable.

5.2.8 Reactor's optimization

Once the significant models were identified, their dependencies with OLR were plotted to perform a graphical optimization. In a graph, the ordinates represented the values of the individual variable divided by their maximum measured value (V_i/V_{Max}), which was expressed in %. Thus, the ordinates represented values between 0 and 100 % for each plotted variable.

5.3 Results

5.3.1 Reactors operation' overview

The data cleaning was performed according to the set criteria in section 2.3. The two-substrate mixture of PM+CWM (pellets 1) had a BMP_∞ of 342.83 L_{CH4}/kg_{VS}. Meanwhile, the BMP_∞ values for the three-substrate mixtures of pellets 2 and 3 were 534.21 and 530.28 L_{CH4}/kg_{VS}, respectively (Hernández Regalado et al., 2021; Regalado et al., 2021). After the data cleaning, the resulting data sets have sizes of 162, 181, and 203 instances for reactors 1, 2, and 3 respectively. To accurately characterize the performance of a reactor, four employed output variables including MPR ($L_{CH4}/L_{reactor}$/d), MY (L_{CH4}/kg_{VS}), η_{COD}, and η_{BOD5} (%) were used (Verein Deutscher Ingenieure, 2016; Friehe et al., 2012). The overview of reactor 1 is shown in Figure 5-2. The MPR suffered a sudden drop at the operating point of 5 d. This interrupted the upward trend that was observed from HRT of 15 to 7 d. From 3 to 1 d, a sustained increase in the MPR was observed, with a value that was almost 2x the second-highest average value observed at 7 d. This suggested that a punctual failure occurred at 5 d, which was not due to reaching an operational HRT limit. MY had the highest mean value of 272 L_{CH4}/kg_{VS} at 15 d. After 15 d, the values significantly decreased and a similar sudden drop in MPR was observed at HRT of 5 d. However, MY did not experience significant recovery after the inhibition, unlike MPR. Thus, considering simultaneously both variables of MPR and MY, the operation can be divided into two main stages which are before and after inhibition. There is a noteworthy difference between these two stages. The former reached considerably higher yields than the latter; however, similar values of MPR were found in both stages.

The removal efficiencies in these two stages were not as evident. BOD_5 removal efficiency values noticeably dropped to 10 and 1 d. The low BOD_5 removal values may explain the drop in MY at 10 and 1 d in the previous operation point. This was most likely due to low reaction completions (Sperling, 2007). A minimum average value for the chemical oxygen demand (COD) removal efficiency was observed at 5 d. However, the trend followed by the average η_{COD} values had a smaller variation compared to both MPR and MY. Furthermore, both BOD_5 and removal efficiencies behaved differently since the calculated BOD_5/COD ratios were fluctuating.

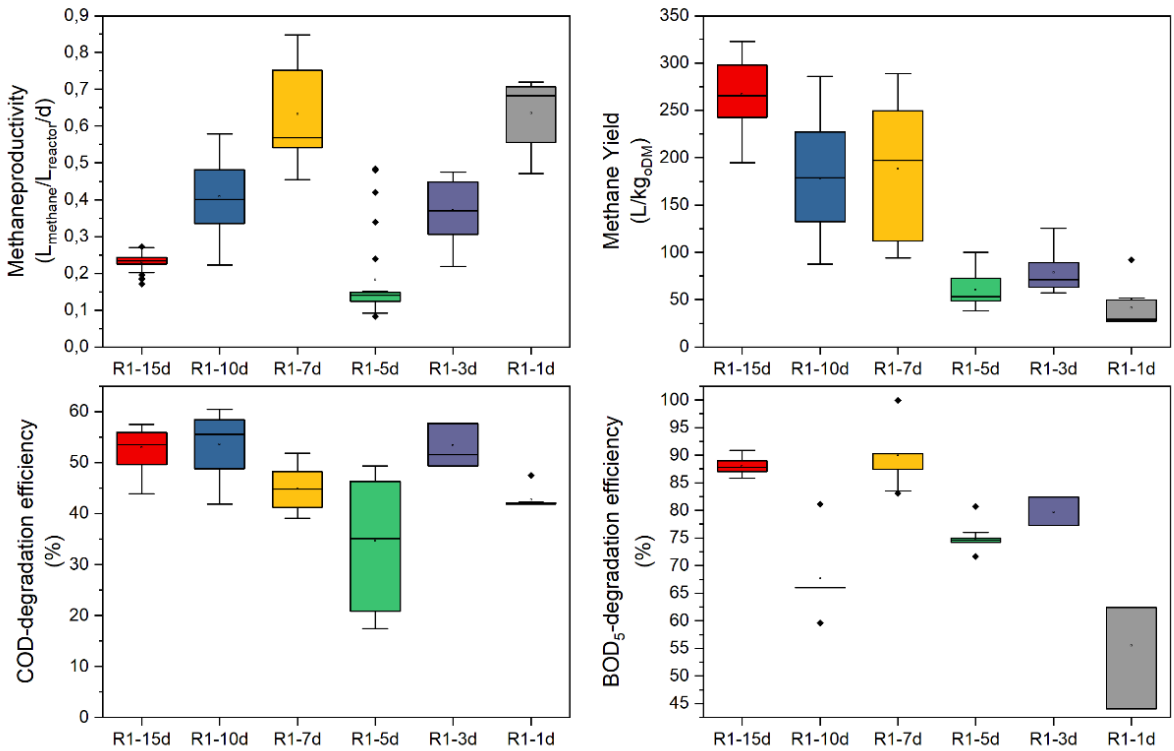


Figure 5- 2: Summary of the main response variables for reactor 1.

To identify the potential causes of inhibition, the control variables of OLR and volatile organic acids to total inorganic carbon ratio (VOA/TIC) were employed. The results are summarized in Figure 5-3. The high OLR values observed were not an indicative cause of inhibition. In particular, the OLR value was not very high at 5 d. Moreover, the system did not run at very high OLR values despite a constant decrease in the HRT values. Therefore, the COD values in the inflow suffered sizable fluctuations as shown in Figure 5-4.

The fluctuation of the OLR was not strongly correlated with the VOA/TIC, suggesting that a failure was not caused by a system overload caused by overfeeding. Instead, they seemed to be more connected to the quality of the feed (Figure 5-4). However, the VOA/TIC results showed that an acid accumulation occurred at 10 d. The VOA values decreased sharply and approached zero at the operating point of 5 d. Hence, two possibilities were weighted, such as the failure of the system due to VOA accumulation and the system's delayed response without failure, both due to VOA accumulation at 10 d. The VOA concentration was not high enough to trigger inhibition or system failure and there were no signs of strong inhibition before 5 d. Additionally, the latter close to zero VOA/TIC values indicated that if the system failed, it was not because of VOA accumulation.

Regarding the latter possibility, the system had a delayed response to VOA accumulation at 10 d, which seemed unlikely, given how large the delay had to be. In addition, the MPR values increased from 10 to 7 d, while the MY values were practically the same. Therefore, both possibilities were rejected and acetogenesis as the limiting-rate step or very low quality of the feed were instead considered.

COD_{in} and $VOAs\_{in}$ values were determined on the feed to investigate whether the quality of the feed was responsible for the inhibition. The results are summarized in Figure 5-4. All targeted acids were found except for valeric and caproic acids. A rapid decline in the total acetic acid concentration and equivalent occurred after 10 d. Likewise, the concentration of the acids was almost zero at 5 d. This behavior was consistent with the inhibition observed at the operating point of 5 d. Thus, the occurrence of failure due to the lack of VOA in the feed (low quality of the feed) was accepted, which accordingly, caused meager VOA/TIC values. However, the phenomena seemed somewhat unrelated to process inhibitions in previous operational points and rather a punctual problem at 5 d.

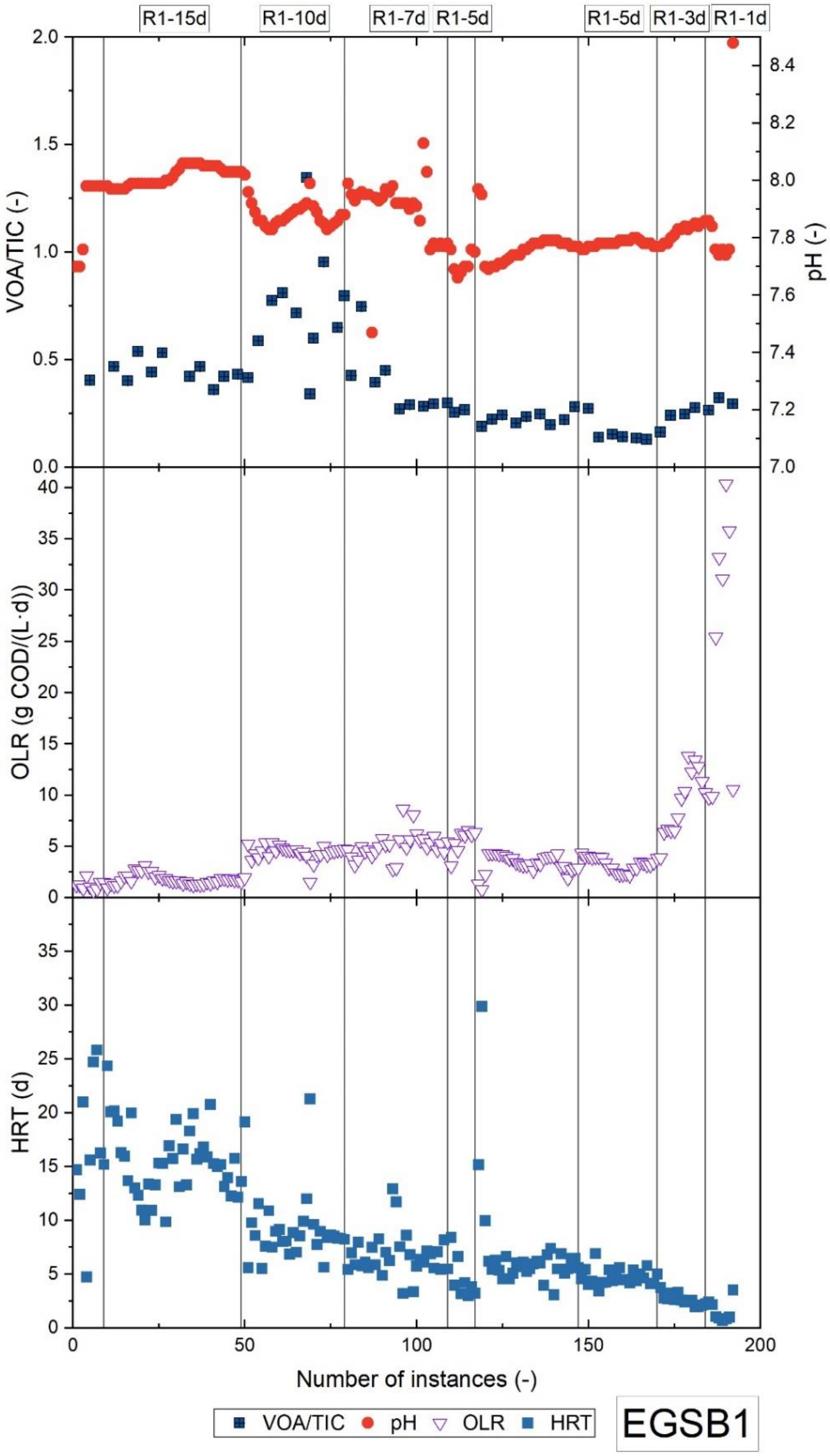


Figure 5- 3**:** Volatile organic acids to total inorganic carbon ratio (VOA/TIC), organic loading rate (OLR), and hydraulic retention time (HRT) in reactor 1.

The observed COD_{in} fluctuations explained the behavior of the OLR with the gradual reduction of HRTs. It was expected that the intensive variables such as COD_{in}, VOA concentrations, and BOD_5/COD ratio will show stable behavior. However, these variables fluctuated due to the lack of proper mixing in the feeding tank. Continuous mixing was not ensured during operations even though the mixtures were vigorously mixed in the tank during preparation. This induced the settling of particulates and instability of the feed, which caused the first excess of VOAs observed at 10 d. The concentration of VOAs was approximately zero at 5 d, which suggested the existence of a substrate limitation on the system. This limitation substantially influenced the operation since the tank had to be refilled several times, which caused variations in the preparation. Therefore, heterogeneities in the composition of substrate mixtures during a year of operation are expected due to seasonal behavior (Weinrich, 2018).

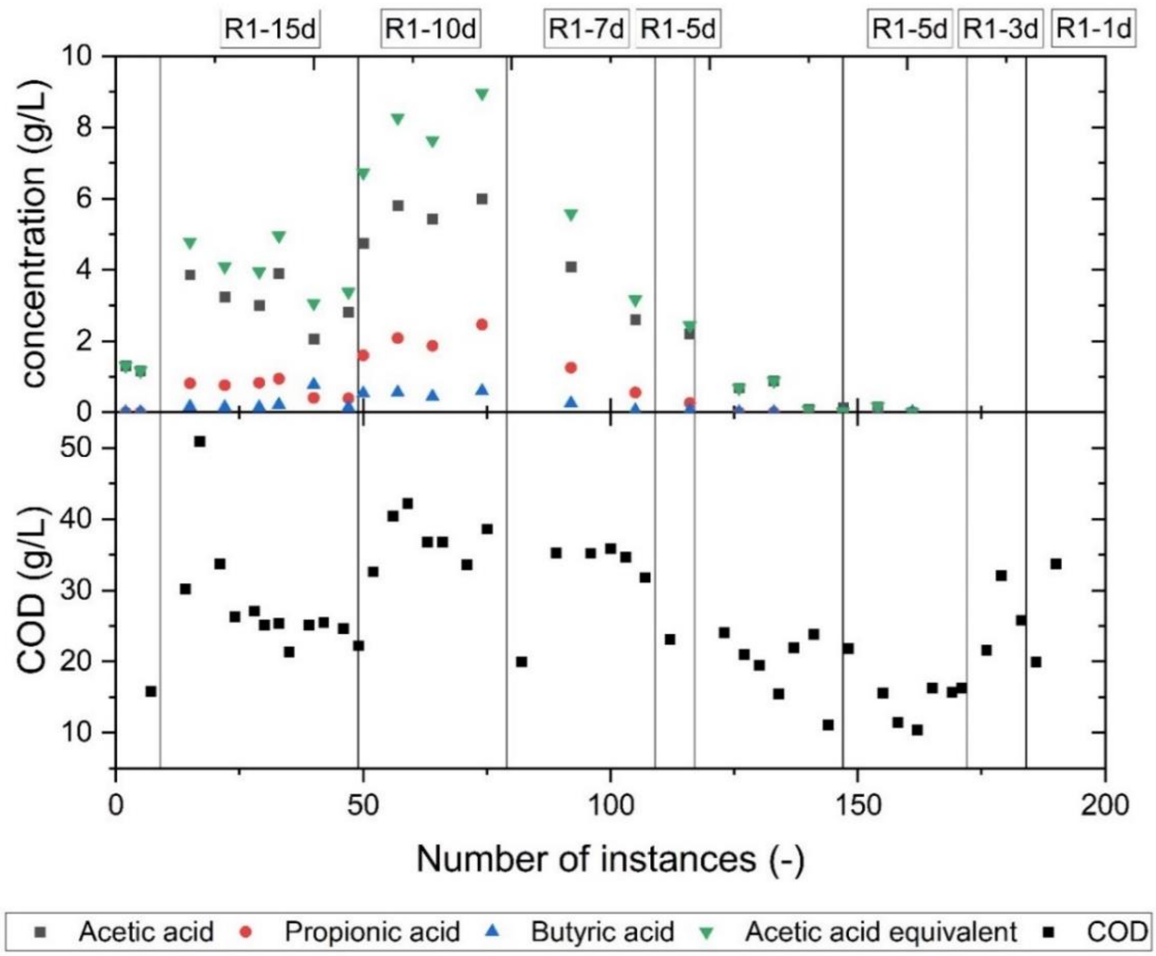


Figure 5- 4: Volatile fatty acids and chemical oxygen demand (COD) in the feed of reactor 1.

Similar trends to reactor 1 were observed for reactors 2 and 3. For reactor 2 in particular, the inhibition was the least abrupt due to a lower dry matter (DM) content (*i.e.*, almost 80% of SWW on a fresh matter basis), which reduced the effect of the seasonal behavior observed in the manures (Table 5-1). A summary of the averaged behavior by an operation point for each reactor is found in Table 5-3, which uses a three-color scale by column. The colors were ordered red, yellow, and green to show the increase from lower to higher values. The intensity of each color determined its proximity to the lowest, middle, or highest value.

Table 5- 3: The averaged values by operation point for each reactor.

Operating points	*Mean values COD removal efficiency (%)*			*Mean values of methane yield* (L_{CH4}/kg_{VS})			*Mean values of methane production rate* ($L_{CH4}/L_{react}/d$)			*Mean values* BOD_5 *removal efficiency (%)*		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
Operating Point 1 (15d)	52.7	49	59.5	265.7	295.6	217.6	0.23	0.15	0.29	88.13	84.98	76.84
Operating Point 2 (10d)	53.6	58.6	76.2	180.2	261.6	382.5	0.41	0.21	0.83	68.77	83.64	85.34
Operating Point 3 (7d)	45.3	82.5	63.7	189.2	395.8	317	0.64	0.46	0.96	90.16	95.22	97.69
Operating Point 4 (5d)	34.2	73.9	53.8	61.1	158	158.8	0.19	0.42	0.6	73.03	90.93	81.23
Operating Point 5 (3d)	53.2	77.3	55.7	78.1	200.3	193.9	0.34	0.91	0.79	78.21	91.09	77.47
Operating Point 6 (1d)	42.6	64.1	43.7	41.4	77.6	89.4	0.64	1.2	1.21	84.56	92.77	84.56

The colors are ordered red, yellow, and green from lower to higher values. R is the reactor.

The maximum value of η_{COD} removal efficiency observed in reactor 1 was much smaller than in reactors 2 and 3. The same trend was observed with MY. The COD removal efficiency has been interpreted as a degree of reaction completion (A. Cruz-Salomón et al., 2019; Levenspiel, 1999; Sperling, 2007). Thus, a strong relationship between COD removal and reaction completion is expected. Meanwhile, a similar relationship should be found between MY and η_{BOD5}; however, due to fluctuations in the BOD_5/COD, no clear visual correlation can be established. Furthermore, the drop in the MYs of all three reactors at HRT 5 d, was linked with the lack of organic dry matter (ODM) or VOA in the feed as previously shown in Figure 5-4.

The MYs in reactors 2 and 3 were also significantly higher than those in reactor 1. The maximum MY of reactors 2 and 3 were similar at 7 and 10 d, respectively. The improvement observed from the addition of a third substrate to the PM+CWM mixture was probably related to a higher C/N ratio. The C/N ratio balance in feedstocks was significant for the stable operation of an AD. Substrates with high

C/N ratios have poor buffering capacity; therefore, nitrogen will be consumed rapidly by methanogens to meet their protein requirements. This results in low methane production and produces excess VOA during fermentation. In typical feedstocks with a low C/N ratio, nitrogen has been found to accumulate in the form of ammonia, which inhibited the methanogens and prevented methane production (Li et al., 2019; Rabii et al., 2019b).

The lowest recommended limit for C/N is 20 (Weinrich & Nelles, 2021a, p. 30); thus, a value of 15 may be sufficient for our purpose (Neshat et al., 2017). The values of C/N in Table 5-1 are between 15 and 20 for the three-substrate mixtures. Meanwhile, the value was < 15 for the two-substrate mixture. This supported our finding that three-substrate mixtures were more stable and produced more methane. Nonetheless, Lissens et al. (2001) have affirmed that substrates with a C/N ratio < 10 can support a stable process; however, they require a multistage system to avoid reactor overloading.

The maximum values observed for COD and BOD_5 removal efficiencies, as well as MY for reactors 2 and 3, were at HRTs of either 10 or 7 d. The same operation interval was observed by A. Cruz-Salomón et al. (2019) and regarded as the optimal operation interval for EGSB reactors.

All three reactors reached their maximum MPR at 1 d. The observed stable operation of EGSB reactors at HRTs 3 and 1 d presented some novelty in our operation with the agricultural substrates. Castrillón Cano et al. (2019) were able to operate reactors at HRTs of as low as 8 hours; however, they only used a 3.4 L effective volume to perform their residence time distribution (RTD) experiments with water in the presence and absence of biomass. In another study, Dereli (2019) effectively operated a full-scale EGSB of 1200 m^3 at an average HRT of 7 d for the treatment and digestion of confectionery industry wastewater. Meanwhile, Abumalé Cruz-Salomón et al. (2018) performed continuous tests with a 3.3 L EGSB reactor with HRT of between 3 and 9 d for the treatment of coffee processing wastewater. In addition, C. Rico et al. (2015) operated a UASB reactor with an external settler and effluent recycling for alkalinity supplementation for the co-digestion of cheese whey and liquid fraction of dairy manure. Under a constant HRT of 2.2 d, their system demonstrated a stable operation with up to 75% cheese whey fraction in the feed. This was with an applied OLR of 19 kg_{COD} m^{-3} d^{-1}, obtaining a η_{COD} and MPR of 94.7% and 6.4 m^3_{CH4} m^{-3} d^{-1}, respectively. They observed critical biomass washout when the cheese whey fraction in the feed was 85% with an HRT of 2.2 d. An operation at a constant 60% cheese whey fraction in the feed mixture enabled a stable operation under an OLR and HRT of 28.7 kg_{COD} m^{-3} $d^{-1}$and 1.3 d, respectively. In addition, the η_{COD} and MPR values were 95.1% and 9.5 m^3_{CH4} m^{-3} d^{-1}, respectively. Therefore, we conclude that there is a novelty in our successfully operated EGSB reactors for the AD from the agricultural substrates in mixtures 1 and 3 at small HRTs. Notably, C. Rico et al. (2015) suggested that a manipulation of the mixture proportion at constant HRT can also lead to improvements in both stability and MPR.

5.3.2 Principal components analysis PCA

PCA was conducted using the data shown in Table 5-3. The results are shown in Figure 5-5. The score of 5 (a) shows the distribution of the data in the reactor number combining the shapes and colors shown in the figure legend. The HRT is shown above each point. The axes of the graph were created by a linear combination of the variables. This is represented in 5 (c) with MY and COD removal being the most influential variables on the x and y-axis. respectively. Meanwhile, 5 (b) shows the combination of 5 (a) and 5 (c). The red and blue points are the variable and data points, respectively. The x-axis was the most significant since it explained most of the variability of the data (e.g., up to 99%). PCA has been used for reducing the dimensionality of large datasets (Adam et al., 2015; Jolliffe & Cadima, 2016; L.E. López de la Maza et al., 2019). However, since the dataset employed was small, PCA was used for descriptive purposes only.

The MPR was not able to describe well the variability of the data since the most influential variables were found between the two external ellipses in Figure 5-5 (c). MY was the most important and efficient according to the PCA results within the low-right quarter of the ellipse in Figure 5-5 (a). The green diagonal line in both 5-5 (a) and 5-5 (b) represents the difference between efficient and non-efficient operations. Hence, the best points were 7, 10, and 7 d for reactors 1, 2, and 3, respectively. These points reached their high values simultaneously in all four variables (Table 5-3). The operation of reactor 1 reached a comparable efficiency to reactors 2 and 3 at HRT 15 d only. Nonetheless, more efficient operation at lower HRTs was possible with the three-substrate mixtures.

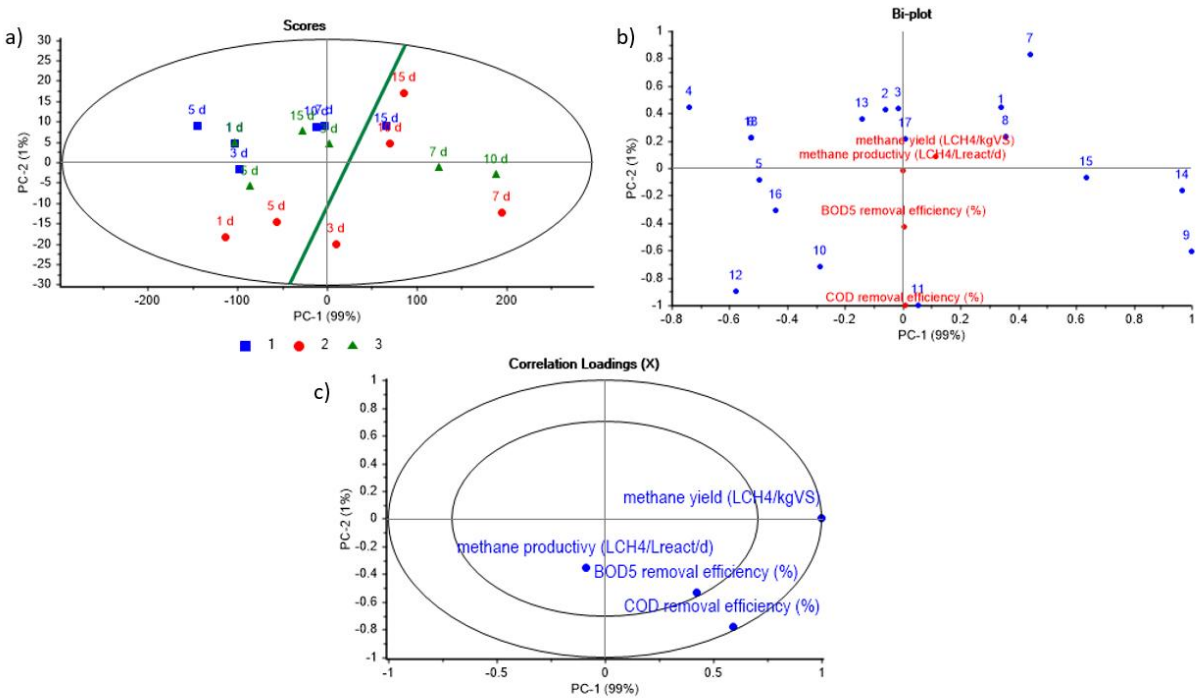


Figure 5- 5**:** Principal components analysis overview of the averaged data. (**a**) Score plot, (**b**) bi-plot, and (**b**) correlation loading.

Most of the operating points for HRTs at < 7 d were considered inefficient, except for reactor 2 with HRT at 3 d. Therefore, the HRTs at < 7 d were generally feasible; however, they are not recommended due to their low efficiency.

5.3.3 Solids retention time to hydraulic retention time ratio (SRT/HRT)

EGSB can potentially reach much lower HRTs than CSTR reactors, which are typically used in agricultural biogas plants. In these reactors, the HRTs are equal to SRTs. However, an ESGB reactor can decouple the retention times by increasing the residence time of the biomass within the reactor (Chernicharo, 2007, pp. 65–66; Sperling, 2007, pp. 100–102). The SRT/HRT values were calculated by equation 5-4 for each reactor using a biomass sample from the top of a reactor, where biomass concentration was lowest. The steady-state condition was assumed in the calculations and the results are shown in Table 5-4.

The ideal SRT/HRT ratio should be > 3 (Environmental Energy Company: Olympia, 2001); however, this was far from being accomplished by sampling at the top of a reactor. In all cases, the averaged SRT/HRT was > 1, nevertheless, in three instances (one from each reactor) SRT/HTR ratios smaller than 1 were calculated. This demonstrated that even by sampling at the least biomass concentration, an average EGSB reactor can retain biomass better than a typical CSTR. Minimal washout was observed in all three reactors. Unfortunately, data were not collected between HRTs of 10 and 7 d.

Table 5- 4: SRT/HRT results for the 3 reactors (R1, R2, and R3)

Operating points	Solid retention time to hydraulic retention time ratio		
	R1	R2	R3
Start-up (15d)	1.10	1.29	1.24
Operating Point 1 (15d)	1.72	1.41	1.59
Operating Point 1 (15d)	0.82	0.83	1.16
Operating Point 4 (5d)	1.22	1.33	1.15
Reactor recovery (5d)	1.30	1.68	1.18
Operating Point 4 (5d)	1.20	1.42	0.99
Operating Point 5 (3d)	1.22	1.28	1.11
Average value	1.23	1.32	1.20

The red and green highlighted numbers were the lowest and highest values in the columns, respectively.

5.3.4 Characterization of synergistic effects

To study the possible synergistic effects suggested by the interactions identified as acute effects in Hernández Regalado et al. (2021), the ratios between the maximum MYs in the continuous operation and batch validation tests were compared using equation 5-5. The results are shown in Table 5-5.

Table 5- 5: Methane yields and ratios based on piglet and cow manure yield in the batch and continuous tests.

Mixture	MY_{MAX} in continuous tests (L_{CH4}/kg_{VS})	MY ratios in continuous tests	$MY_{predicted}$ by the model in batch tests (L_{CH4}/kg_{VS})	MY ratios in batch tests	continuous to batch MY ratio
PM+CWM	265.70	1.00	342.83	1.00	0.78
PM+CWM+SWW	395.80	1.49	513.07	1.50	0.77
PM+CWM+SBT	382.50	1.44	530.76	1.55	0.72

MY: methane yield; MY_{MAX}: maximum methane yield; $MY_{predicted}$: predicted methane yield

The MY ratio of the mixture with SWW was almost the same on both scales. The relative difference in the ratios obtained for the mixture with SBT was 7.01%. This confirmed the acute effects of adding a third substrate to the two-substrate mixture. The third substrate provided the same boost in the continuous stage and batch tests.

Also, the methane yield ratio obtained during the transfer from batch tests to the continuous stage was between 0.72 and 0.78. It was expected that obtained MY from the continuous stage would be smaller than in the ultimate biomethane potential from the batch test, which was described by Weinrich (2018). Similar intervals in the continuous stage to batch tests methane yield ratios have been identified in the literature. For example, Mahnert et al. (2005) obtained ratios from 0.73 to 0.8 from the use of different grass species. Obiukwu and Nwafor (2016) reached a ratio of 0.81 from the use of grape pomace. Meanwhile, Chowdhury and Fulford (1992) used mesophilic digestion of cattle dung in both batch and semi-continuous digestion with four reactors and six semi-continuous reactors, respectively. Their results showed higher rates in the semi-continuous operation; however, biogas yields were lower compared to the batch test. Their batch tests reached 67% COD efficiencies, which was lower than the results in this study. In addition, Holliger et al. (2017) suggested that BMPs can be used to estimate biogas production at full scale; however, the BMP value should be multiplied by a factor of 0.8–0.9 to avoid overestimation.

5.3.5 Hydraulic analysis

The results for both hydraulic parameters of the reactors are shown in Table 5-6.

Table 5- 6**:** Hydraulic parameters of the reactors

Parameter	Influent	Reactor tube	Separation zone
Re	295.42	295.42	295.42
Peaxial	1.10	9.33	38.87

The Pe_{axial} results showed values that were very close to 0, even in the separation zone. This indicated a flow pattern that was close to a completely mixed system (Londoño et al., 2019). The value of the Reynolds was also very similar to the one obtained by Brito and Melo (1997). These authors fitted an EGSB reactor to a CSTR with a characteristic coefficient of determination of 0.92. The inclusion of a short circuit increased the coefficient of determination to 0.95. Therefore, a CSTR model for simplicity was accepted and successfully used in their mass balance equations. Similarly, López and Borzacconi (2011) assumed a CSTR behavior based on a high recirculation ratio and expansion of a bed. The combination of these two effects resulted in significant mixing of the liquid and solid phases, as well as uniform gas production. However, their mass balance equation for the biomass included a washout effect, which was attributed to the high V_{up} at which the reactor was operated.

Nevertheless, the relative increase of the Peclet's value from one zone to another was considered significant. Consequently, due to the different behavior of the zones in the reactor, the zones can be modeled as different reactors in series as described by Gleyce et al. (2014). Gleyce et al. (2014) divided a reactor into two major zones, i.e., the separator and reactor tubes. The reactor could be modeled either as two plug-flow reactors in series or five CSTRs with three separators and two tubes with coefficients of determination of 0.94 and 0.95, respectively.

5.3.6 Modeling of reactors

The Stover–Kincannon model was used to fit the five different combinations of data from the reactors. The average values were employed, and the size of a combination was up to 18 points. The results are summarized in Table 5-7. The fit for R2 was very good and the best among all datasets. The datasets for R1 and R3 have the worst fit, which suggested that the largest difference in the kinetic behavior among all possible combinations existed in these reactors. The RMSE values for R1 and R2 were also rather large, which meant that there were significant differences in the behavior of these reactors. The goodness of fit in R1 and R3 were equal; however, R3 can produce a maximum amount of methane that was more than double the daily amount of methane from R1. R3 was also able to handle a larger OLR, which was related to the intrinsic properties of the substrate mixtures. In addition, the model predicts that R2 was far from reaching its maximum production that can be handled by its largest OLR.

Table 5- 7**:** Fit analysis using the Stover–Kincannon model.

Data set	**RMSE (-)**	**M_{max}** ($L_{CH4}/L_{react}/d$)	**M_B ($g_{COD}/L/d$)**
(R1, R2, R3)	0.214	1.29	6.08
(R1, R2)	0.188	1.25	8.21
(R1, R3)	0.245	1.07	4.20
(R2, R3)	0.118	1.48	5.07
(R1)	0.132	0.68	3.59
(R2)	0.031	1.76	9.99
(R3)	0.132	1.52	5.12

R: reactor; RMSE: root-mean-square error.

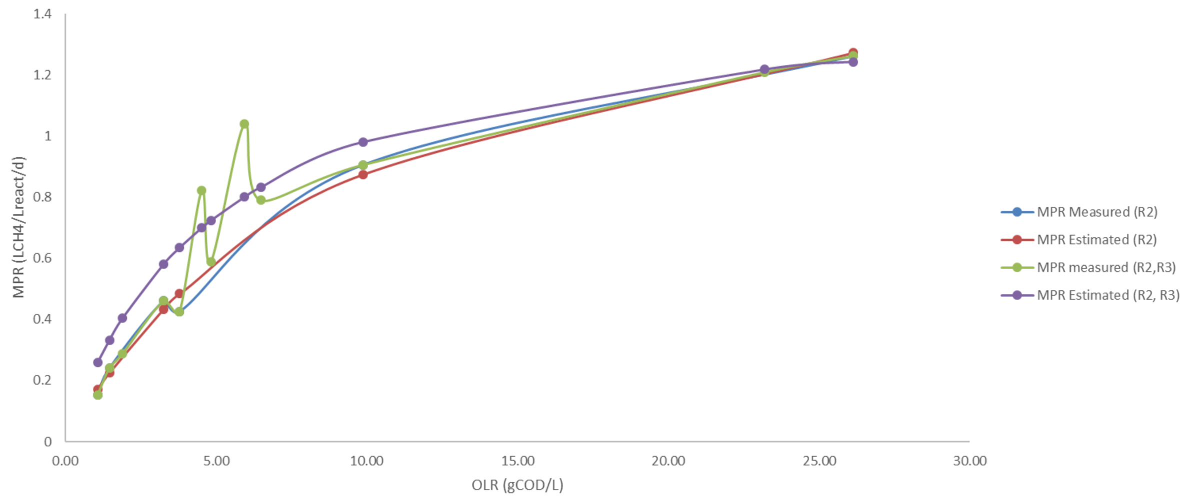


Figure 5- 6**:** Stover–Kincannon model fitting for the datasets of R1, R2, and R3.

The datasets for R2 and R3 were of special interest. Since the mixtures in these reactors showed synergistic effects and they seemed to behave similarly, we examined if the mixtures followed similar kinetics. We found that the difference among them was moderate (R2, R3); however, the differences between R1 and R2 (R1, R2) or R1 and R3 (R1, R3) were larger. The fits and measured data are shown in Figure 5-7. By following both estimated models (red and violet lines), it was noticed that significant differences existed at the low OLRs. These were much smaller at higher OLRs. The fluctuations in R3 between 4.5 and 6.5 gCOD/L were most likely the main cause of the misfit, which was observed in the green but not in the red line. The performance of R3 (green line) was closely related to mixture preparation and degree of mixing in the feeding tank since mixture 3 had the highest DM content, contrary to the smooth behavior of R2 (light blue line).

Therefore, we decided to work on both mixtures individually, given that neither model was able to converge in most of the working intervals. Since working at low HRTs usually reduce the MY (H. Yu et al., 1998), we took into account the other response variables to establish an optimal operation OLR. Hence, empirical models of MY and η_{COD} versus OLR were also fitted.

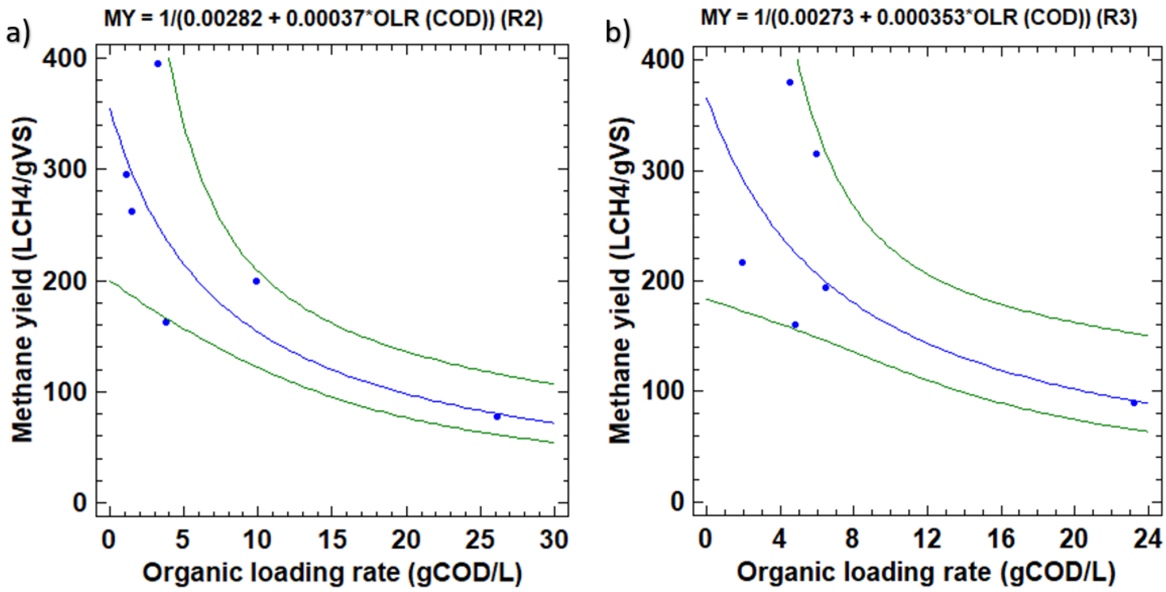


Figure 5- 7**:** Comparison of the Stover–Kincannon model against measured data for EGSB reactors (R2 and R3). Blue and green lines corresponded to the model and confidence limits of the model at 95 %, respectively.

No significant fit was found for η_{COD}, as per the pre-established criterion set for R^2. The R^2 values for R2 and R3 were 0.649 and 0.635, respectively. Therefore, no strong dependency on OLR existed. The fits should be described by more complex models that consider mass transfer relationships. MY was satisfactorily described by its inverse relationship with OLR for R2. The R^2 was 0.881 with a D-W of 3.6 (p-value = 0.990). While the fit for R3 was smaller, the R^2 of 0.782 with a D-W of 2.22 (p-value = 0.4709) was still significant. The inverse relationship between MY and OLR has been described by several authors (Jafarzadeh et al., 2021; Verma et al., 2014; H. Yu et al., 1998). Since both p-values above were greater than 0.05, there was no indication of serial autocorrelation in the residuals at the 95.0% confidence level. The fitting for both reactors is shown in Figure 5-7. All the points were contained or at least very close to the confidence limits of the prediction lines (green lines). Therefore, with all the above information combined, the models were considered acceptable.

5.3.7 Optimization of a reactor

The two equation systems developed for R2 and R3 combined the Stover–Kincannon model and the reciprocal model for MY. Hence, the optimal OLR to simultaneously optimize MPR and MY for R2 and R3 are described by equations [(5-11) and (5-12)] and [(5-13) and (5-14)], respectively.

$$MPR = \frac{1.76 \cdot OLR}{9.99 + OLR} \tag{5-11}$$

$$MY = \frac{1}{0.00282 + 0.00037 * \mathrm{OLR}} \tag{5-12}$$

$$\mathrm{MPR} = \frac{1.52 \cdot \mathrm{OLR}}{5.12 + \mathrm{OLR}} \tag{5-13}$$

$$MY = \frac{1}{0.00273 + 0.000353 * \mathrm{OLR}} \tag{5-14}$$

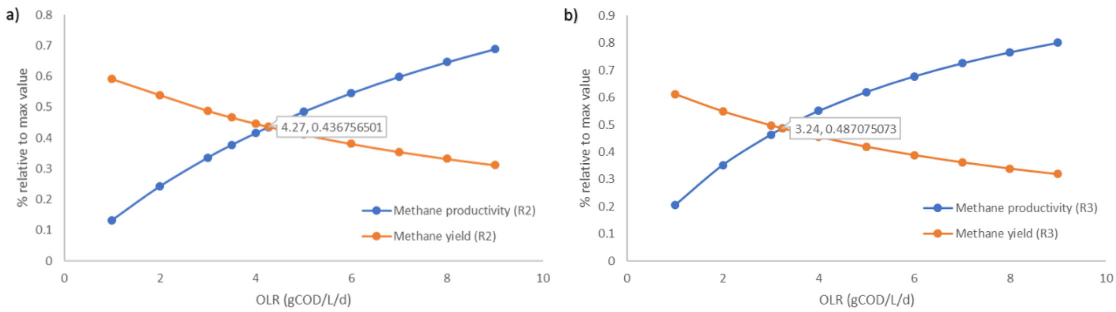


Figure 5- 8: Graphical optimizations of the optimum MPR and MY of R2 and R3 using the OLR.

The graphical optimizations are shown in Figure 5-8. The ordinates represent the % from the MPR_{max} or the MY_{max} measured. The call-out represents the point where both functions meet each other. R2 can handle a higher OLR; however, the yields were less from both functions than R3. Nevertheless, both reactors have similar working intervals, which provided reasonable yields from 3 to 5 gCOD/L for both response variables.

Using the averaged value of the COD in the feed, the working intervals were between 4 and 7 d and 6.5 and 11 d for mixtures 2 and 3, respectively. The upper value for mixture 3 was slightly above the suggested interval of 10 d (HRT) for agro-industrial wastewaters by Abumalé Cruz-Salomón et al. (2018). Meanwhile, mixture 2 had a working interval that was slightly lower than the selected interval. This was attributed to the lower COD content in the mixture.

5.4 Discussion

The AD of PM+CWM, PM+CWM+SWW, and PM+CWM+SBT substrate mixtures in a continuous operation mode using the three different EGSBs reactors yielded three main conclusions regarding the mixtures (see below).

1. The synergistic effects described by the batch model in (Hernández Regalado et al., 2021) were also found in the continuous operation.
2. The maximum methane yields in the continuous operation of any mixture of these four substrates were predicted using the batch model and multiplying the BMP_{∞} by a coefficient between 0.7 and 0.8.
3. The employment of the Stover–Kincannon model showed that all three mixtures had a different kinetical behavior, which can even be noticed among the two triple mixtures.

The synergistic effects due to the addition of a third substrate were most likely related to the C/N values. The high C/N values in the three-substrate mixtures explained the good performance observed; however, a higher ratio was no indication of a better performance of mixture 3 versus 2. Hence, the performance of the co-digestion of these mixtures should not be oversimplified by the C/N values, without having considered other influential factors. Nevertheless, the most recommended C/N values in the literature were from 0 to 30 (Neshat et al., 2017; Paulose & Kaparaju, 2021; Rabii et al., 2019b). We note that all our mixtures have a C/N value < 20 (Table 5-1), which strongly suggested the increased proportion of the carbon-rich-substrates within the mixtures.

The concept of integrating an EGSB reactor in a typical agricultural biogas plant is also of relevance. Compared to a typical agricultural biogas plant where the representative HRT values are between 50 and 150 d (Ruile et al., 2015; Weinrich & Nelles, 2021b), high-rate reactors provide an alternative system for the treatment of liquid substrates or their liquid fractions at much smaller HRTs. Substrate mixtures that have influenced HRTs should be applied as suggested by Paulose and Kaparaju (2021). They stated that a degradation rate follows an inverse function with HRT depending on substrate complexity. Consequently, higher HRTs needed to be applied and lower degradation rates were expected for lignin-rich substrates than for protein- or sugar-rich substrates. Agricultural biogas plants in Germany typically co-digest animal manure with either maize or grass silage (Akunna, 2018, p. 7; Weinrich & Nelles, 2021a, pp. 13–14); therefore, higher HRTs are expected for the three-substrate mixtures digested in this paper due to their complexity. However, the differences in the HRTs were always noticeably large. Ruile et al. (2015) studied 21 full-scale plants in the region of Baden-Württemberg (southern Germany), which performed either single digestion or co-digestion of cattle manure, maize silage, and grass silage at different solid contents. They found that high values of degradability were reached at HRT of $\geq$ 100 d. Thus, a more sophisticated concept of treatment that involved multistage processes has been suggested for more efficient energy production (Paulose & Kaparaju, 2021; Rabii et al., 2019b). Thus, the integration of a high-rate reactor in a typical treatment plant could lead to an increment in energy production as found by Shen, Yuan, et al. (2013) in their co-digestion of fruit/vegetable and food wastes in two stages (UASB+CSTR). This approach allowed them to work at higher OLRs and increase MPR values up to 15 % over single-stage digestion (UASB).

We were able to operate all three reactors for up to 1 d, where the tanks were refilled daily. Consequently, the daily preparation of the mixtures was a logistical and practical challenge. Also, the MYs obtained at HRT of 1 d were the lowest among the three reactors. It was probably not ideal to run the reactors at such a low HRT; however, this was possible and can be useful especially useful when the demand for biogas is peaking or excess amounts of substrates need to be processed.

The results of the continuous operation were significantly influenced by the lack of proper mixing in the storage tanks and the seasonal behavior of the substrates. The latter was more obvious in the manure substrates and buffered by the addition of a third substrate Mixture 2 was the least affected since it had the lowest ODM content in the feeds. Consequently, this mixture had the highest substrate homogeneity inside the reactor due to the mixing by recirculation and increased biomass-substrate contact, which facilitated the operation (A. Cruz-Salomón et al., 2019; Gleyce et al., 2014).

The obtained results support the technical feasibility of the AcoD of liquid manure-based mixtures using EGSB reactors. Thus, it opens the possibility of designing new treatment concepts employing EGSB reactors for the AD of liquid agro-industrial mixtures while significantly reducing the required operating time.

The calculation of the hydraulic dimensionless numbers strongly suggested CSRT behavior. The assumption of a single reactor with a CSTR behavior simplified the modeling of an EGSB reactor. This was strongly considered when applicable. Due to the lack of biomass sampling along the reactor, and without an adequate computer flow dynamics (CFD) model or RTD study, the consideration of one CSTR seemed the better option. However, the measurements of biomass concentration together with CFD modeling or an RTD study were highly recommended to thoroughly model a reactor (Xie et al., 2016; L. Yu & Wensel, 2013).

Likewise, the operation intervals for an operation in a pilot plant scale were laid down for the two three-substrate mixtures, since both mixtures were likely more profitable than the two-substrate mixture considered. This was in the context of EEG. Furthermore, we recommend the development of more complex models, which will allow the simultaneous control of several process variables as well as describe the potential interactions involved within these variables.

5.5 Conclusions

The AcoD of the liquid fraction of PM+CWM and a third carbon-rich substrate such as SWW or SBT was successfully carried out in EGSB reactors, which were operated continuously. This work provided an alternative to typical CSTR systems used for manure and liquid manure treatments. The flow pattern of the studied reactors behaved similarly to a complete mixture reactor. Notably, the hydraulic behavior of our reactors was similar to those found in the literature. Moreover, the results from the batch test were successfully transferred to a continuous scale through the development of empirical and statistical modeling and optimization of operating OLR intervals. We will consider more complex mechanistic models in the future. Further experiments are going to be carried out on the pilot plant scale in the Saerbeck bioenergy park using one automatically controlled 500 L EGSB reactor.

6. FINAL DISCUSSION

The AD of manures, especially the ones with high-water content, is not very economically competent with the currently used technologies despite the substantial remunerations provided by the German government through EEG. In the following final discussion, the results of the individual contributions are discussed in the sum of the impact of performing efficient AD of manures. The results are structured and interpreted thematically based on three categories: (i) technical-economic, (ii) ecological, and (iii) energy security.

6.1 Manure digestion: Germany, a case study

Manure digestion has increasingly gained importance as the abundance of manures keeps increasing in Germany. Germany became an exporter of meat in 2005 (Thrän et al., 2020), and as the animal farming industry grew, a noticeable increase in waste streams, i.e., bones, blood, and manure, was observed. AD involving manure is normally performed using energy crops as co-substrate with mass-specific use of 48% of manures, mostly cattle manure, and 47% of renewable resources mostly grass silage. Nevertheless, due to the relatively low fresh mass-specific yields of manures, less than 20% of the energy-related output belongs to manures. The German government looks forward to encouraging the use of large quantities of manure within the biogas plants by giving incentives to the plants that digest at least 80% of manure. However, the number of plants that took advantage of this incentive by 2019 was less than 15% of the total number (Liebetrau et al., 2021; Majer et al., 2019). Moreover, less than 20% of the PM available and 33% of CWM are currently used in Germany in biogas plants because, with the use of CSTR as the main technology, liquid manures have one of the lowest profitability among the most commonly available substrates.

As a result, at the beginning of the 2000s, barely any manure was used in AD in Germany, whereas catch crops were mostly employed by a large margin. As the manure premium was established by law, the recommended amount of manure started to increase, whereas that of the catch crop was limited, resulting in the use of co-digestion in almost 50% manure to 50% catch crop mixture in FM basis (Daniel-Gromke et al., 2018; Oehmichen & Thrän, 2017; Thrän et al., 2020). Nevertheless, manures used in AD remain the main co-substrate, and the percentage of their use in the AcoD mixture was mostly a byproduct of the imposed limitation of the use of energy crops in agricultural biogas plants.

The aforementioned premium for plants that digest at least 80% manure was used to break the tendency of using manure mostly as a secondary co-substrate (till 50% FM basis in the mixture) and instead use it as the main co-substrate (at least 80% FM basis in the mixture). Nevertheless, the premium by itself according to several authors is not sufficient to change the extended use of catch crops in the

German biogas industry. The main reasons are the farm size and technology employed (Liebetrau et al., 2021; Theuerl et al., 2019; Weinrich & Nelles, 2021a).

The term cost of lost opportunity refers to the foregone benefits or potential gains that could have been realized. Don W. Green and Robert H. Perry (2008, p. 1017) defined it as the cost or value sacrificed when an investment is pursued, often used as a benchmark. Essentially, the cost of lost opportunity represents the cost of not choosing an alternative path. In the context of anaerobic digestion, if manure is digested instead of a more energy-intensive substrate, there is a financial loss. However, when the market for these energy-intensive substrates becomes saturated or enters a stagnation phase, not digesting manure becomes a missed opportunity as potential earnings remain unrealized. This is particularly relevant in Germany, where the maize cap and environmental concerns related to energy crop production have pushed the energy production market close to stagnation or, at the very least, market maturity.

Edel et al. (2017) estimated a potential unexploited power production of manure in the interval of 32–40 TWh/a. Therefore, the AD of manure needs a transforming element to make it a more profitable market. To add to this idea, Thrän et al. (2020) concluded that to use this unexploited potential, the AD of manure needs to be monetized in other forms, such as GHG reduction, apart from the existing incentives, which are mainly focused on energy production to develop new business cases for biogas producers. Theuerl et al. (2019) argued that market regulations are not sufficient to have a well-developed biogas market. For this purpose, a significant technological change must be carried out in the form of control, optimization, digitalization, and new reactor technologies for the biogas industry to reach parity with other German industries.

6.2 Inclusion of new technologies: EGSB, a solution for slurry treatment

The most common technology is wet fermentation based on a CSTR. In total, about 90% of the biogas plants use wet fermentation, and 10% use solid-material fermentation. Approximately 1% of the plants are operated discontinuously (batch operation/box or garage fermenter). At the end of 2016, 80–100 discontinuously operated dry fermentation plants (garage or box fermenter) were in operation in Germany. Around 5%–9% of the biogas plants use the principle of a horizontal plug flow for the fermentation of mainly energy crops. The fermentation systems ring-in-ring and the specific Pfefferkorn technology are only common in a few biogas plants (Daniel-Gromke et al., 2018; Majer et al., 2019).

In the industry, there has been a need for the digestion of low DM content substrates since the energy crisis of the 1970s when AD was first introduced (Wilkinson, 2011). Steps toward the right direction were accomplished by Lettinga et al. (1980), with the design and feasibility tests of the UASB, hence making available the first of the second-generation AD reactors. The UASB has been so far the most popular among the high-rate reactors (Saravanan & Sreekrishnan, 2006). High-rate reactors enable the decoupling of the SRT of the HRT. Hence, smaller reactor sizes are needed, allowing for a reduction in CAPEX and increasing the profitability of the AD of the substrates with low DM content.

The UASB has been extensively used in the digestion of industrial wastewater. However, the introduction of the UASB in the agricultural sector has been rather slow due to the need for very low DM content with very small particle size and flow pattern problems that decreased the performance of the reactor, especially as the scale increased (Leitão et al., 2011; C. Rico et al., 2017).

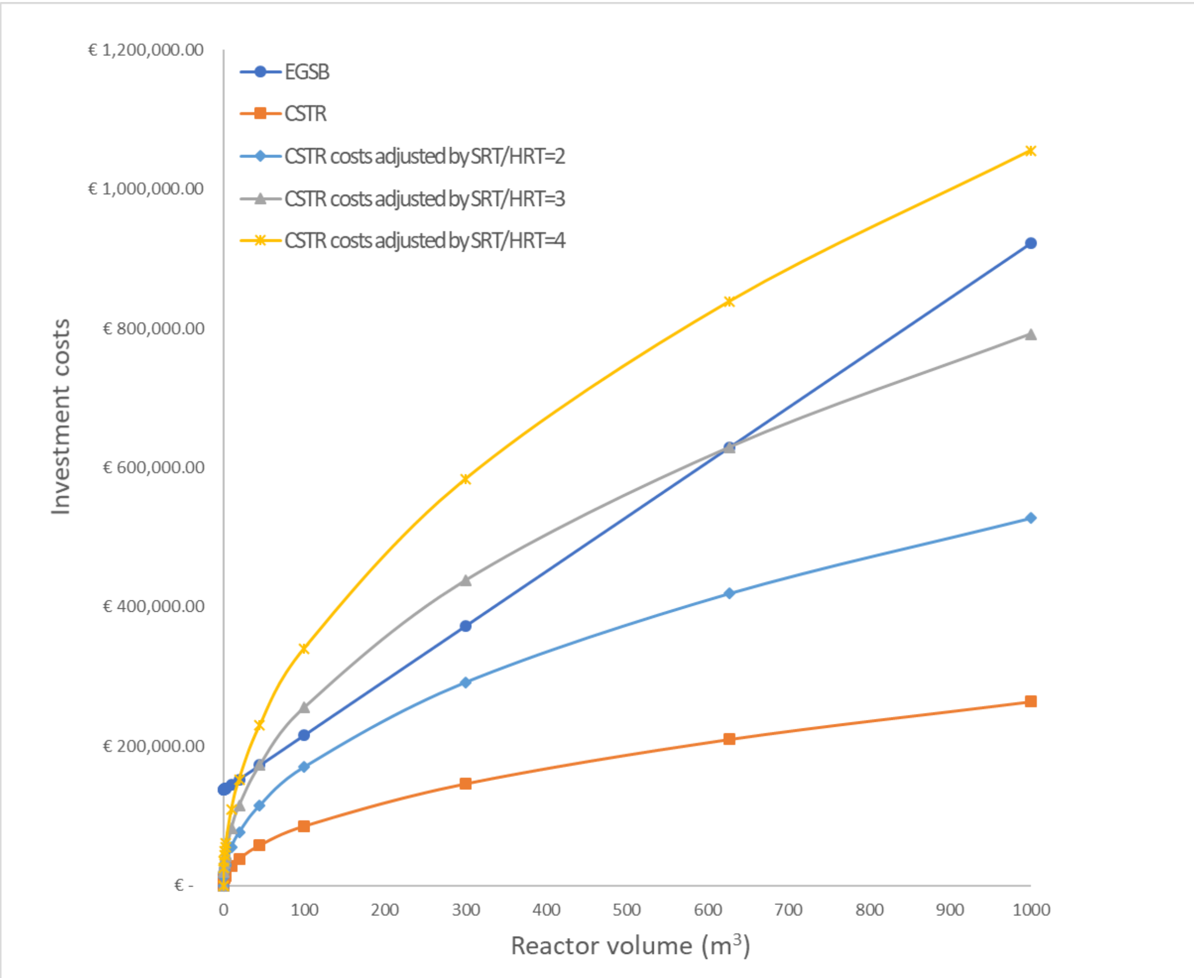
The EGSB as a third-generation reactor eliminated the flow pattern problems of the UASB by adding recirculation of the liquids, and hence, a better long-term performance can be reached (Gleyce et al., 2014). Nevertheless, its profitability in comparison to a CSTR is hindered by the need for previous solid–liquid separation and operation costs of the recirculation pump. Therefore, the comparison is not as straightforward as it may seem. Häner et al. (2022) compared two high-rate reactors Fixed bed vs. EGSB treating pig slurry. The comparison revealed that the technical efficiency was higher for the FB at a lower OLR, but when the OLR started increasing the EGSB's MPR was superior. Nevertheless, superior technical efficiency does not automatically mean higher profitability. Hence, a further comparison is needed to determine at which OLR one reactor is superior to the other. However, one conclusion can be drawn: for a fixed COD and reactor size, the EGSB can treat a larger amount of substrate at a higher efficiency than the CSTR and FB. Therefore, the EGSB reactor has a big potential for application where large quantities of manure need to be treated with shorter HRTs. Moreover, the ability to digest large loads of substrates may be pivotal to optimizing earnings by flexibly producing electricity and heat during peaks in electricity prices and decreasing the load during low-demand hours.

Another techno-economic advantage of the EGSB over the CSTR is the lesser use of land. The CSTR has a higher requirement for land than the EGSB because of the EGSB's larger H/D ratio. Hence, the use of land is one of the competitive advantages of the EGSB for the likely upcoming plant expansion of a large part of the biogas plants in Germany due to the expiration of the 20-year EEG compensation.

Typically, the inclusion of a new reactor in a plant implies significant investment costs. Nonetheless, multistage biogas plants normally separate the different stages of AD because methanogenesis has significantly different operating conditions than the rest of the stages. Hence, the separation of one substrate in its solid and liquid phases has not proven to be as popular as the separation of AD by stages since some advantages are not immediately realized in calculated capital gains. Instead, the advantages appear as technical benefits which may be later translated to capital gains, as discussed in Chapter 3.4. Yet, according to the results of Chapter 3.3.3, the economic efficiency of a typical biogas plant and one typical plant with an included EGSB are very similar. However, the gains in flexibility are very large in terms of biogas output and substrate digestion. Moreover, two stages of liquid manure separating the stages of AD is likely unnecessary because liquid manures do not contain large quantities of complicated chemical components, i.e., lignin or hemicellulose.

Therefore, the inclusion of an EGSB in a plant expansion will allow the processing of substrates with very low DM content at very short HRTs, whereas the more energy-dense substrates are processed at higher HRTs in a CSTR. Therefore, biogas production can be manipulated to time better the energy market hourly peaks, while accessing different premiums by processing large quantities of different substrates that are not normally processed in a biogas plant. Thus, possibly improving the earning potential of the plant. An additional technical-economic advantage of the EGSB is that a part of the output can be used as process water decreasing the water needs of the plant.

Figure 6-1 presents the comparison of the CAPEX curves of the EGSB and the CSTR used by Hernández Regalado, Häner, Brügging, and Tränckner (2022). The curves compare the CAPEX by reactor volume.



SRT: solid retention time; HRT: hydraulic retention time.

Figure 6- 1**:** Comparison of the employed cost curves of an expanded sludge bed granular reactor and a continuous stirred-tank reactor.

To make an equivalent comparison between the two reactors, a series of three curves representing the costs of a CSTR with an enhanced biomass retention capacity are represented. The curves at which SRT/HRT is larger than 1 indicate the equivalent cost of building a CSTR with the retention capacity of an EGSB. These curves at different ratios of SRT/HRT (2, 3, and 4) allow for a better comparison of the operating effectiveness of the reactors. Hence, the curves allow the determination of the combination of biomass retention and volume(s) at which the EGSB is more likely to be a more cost-effective technological solution. The EGSB is significantly more expensive at very small reactor volumes independently of the effectiveness of the biomass retention. An SRT/HRT ratio of 2 is not sufficient to substitute a CSTR at any reactor volume. The sampled SRT/HRT ratios of the three analyzed EGSB reactors presented in Chapter 5.3.3 ranged from 1.20 to 1.32, with data proceeding from the top of the EGSB reactors. Given that EGSBs develop a gradient of biomass concentration, where the bulk of the biomass is found at the bottom of the reactor, these ratios by themselves do not allow concluding to what extent the biomass retention mechanism functions. Consequently, sampling across the reactor column is recommended to determine whether the reactor may reach profitability or not.

At a ratio of 3, in the approximate interval of 45–627 m^3, the EGSB is at least as cost-effective as the CSTR, whereas from 627 m^3 on the CSTR, it has a lower investment cost. Conversely, for a ratio of 4 starting at approximately 19.5 m^3, the EGSB becomes a more profitable option. Therefore, the profitability of the EGSB is largely dependent on the settling properties of the biomass.

One very influential factor in the analysis of Figure 6-1 is the shape of the curves of the EGSB and the CSTR(s). Usually, the scale-up of industrial equipment follows a power function as in the six-tenths rule. The six-tenths rule is a commonly used rule of thumb in engineering to estimate the scaling cost of industrial equipment. The rule states that as the size of a piece of equipment increases, the cost to manufacture it will increase by a factor of 0.6 raised to the power of the scaling ratio. The six-tenths rule allows engineers to estimate the cost of manufacturing larger pieces of equipment based on the cost of manufacturing small pieces of equipment. However, the rule is only an estimate and actual manufacturing costs may vary depending on factors such as material costs, labor costs, and manufacturing processes. Therefore, the six-tenths rule should be used as a starting point for estimating scaling costs and should be refined based on actual manufacturing data and experience.

Nevertheless, the EGSB presents a straight line; this linear dependency can be partially explained by technology diffusion in the biogas market. Despite the EGSB being often used in industrial wastewater treatment plants, its use in biogas plants is so far almost exclusively experimental. With a larger technology diffusion of the EGSB in the biogas market, the CAPEX can be driven down as the know-how, and the different components of the manufacturing and supply chain of its production become more efficient. Another driver of the EGSB cost is the associated electricity cost for pumping, for which experiments of efficiency vs V_{up} are needed to find the optimal techno-economic efficiency curve for the treatment of manure and manure mixtures.

Furthermore, an economic assessment following the methodology described in Chapter 3.2 including the mixtures employed in Chapter 5 was carried out. Figure 6-2 presents the results of the calculated net present values by treatment concept and mixture. The results validated the literature-based assumption presented in Chapter 3.4 that the AcoD can improve the profitability of the concepts.

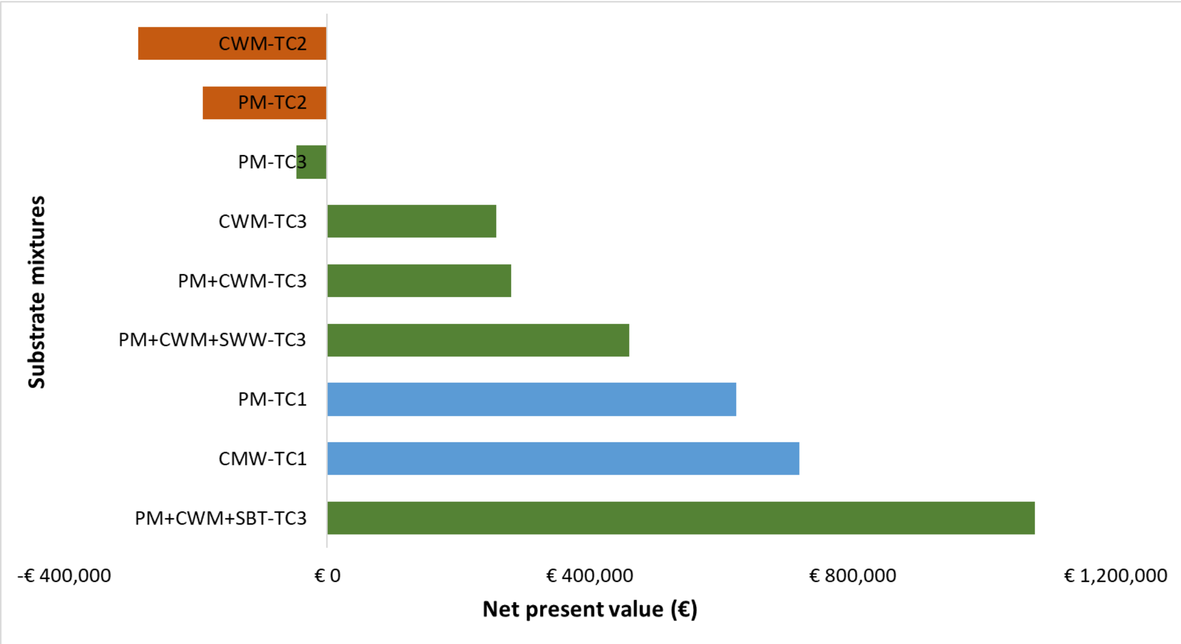


Figure 6-2**:** Comparison of mixtures by treatment concept. TC: treatment concept, PM: pig manure, CWM: cow manure, SWW: starch wastewater, SBT: sugar beets.

The most profitable option was the triple mixture of manure and sugar beets using the two-stage EGSB-CSTR concept. Afterward, the mono-digestion of manures using only the CSTR yielded the best economic performance. Afterward, appeared three business cases with an advantageous economic balance: the triple mixture containing starch wastewater, the double mixture of the manures, and the mono-digestion of cow manure, all of them using the two-stage concept. Later, three business cases with a negative economic balance appear: mono-digestion of pig manure using the two-stage treatment concept, the mono-digestion of pig manure, and later cow manure using only an EGSB reactor.

From the concepts containing one EGSB reactor, the option combined were the triple ones, which can be attributed to the synergistic effects predicted in Chapter 4 and discussed in Chapter 5. The difference between the profitability of both mixtures was explained by having a higher DM content in the mixture containing SBT, given that the MPR prediction experimental results were very similar.

A limitation of this analysis is that the costs of the substrates are not included. According to IEA, 2018, the specific price of production on 1 m^3 of biogas from manure is more profitable than the one produced from crop or crop wastes, and food waste. Therefore, the cost of AD using manure might be somewhat more favorable. Nevertheless, the fraction of other substrates employed within the mixtures is small. Therefore, it is possible to disregard the impact of substrate prices without introducing significant errors.

Therefore, the EGSB has a place in the near future in the German agricultural biogas market if the conditions are previously optimized. In addition, there is further room for optimization given that the C/N never reached 20, and C/N is considered one of the most influential and simple parameters to optimize co-digestion. Therefore, within the optimal zones identified in Chapter 4.3, there are likely some mixtures with better performance than the ones chosen for further testing. Moreover, the limits of profitability and stability of the reactor regarding the DM content of the feed should be further explored.

6.3 Manure AD, a key component of the sustainable agricultural and energy sector

In this discussion, sustainability is going to be defined using three pillars: not causing irreversible changes to the environment, being economically viable, and providing social benefits (Cavicchi, 2016; Horschig et al., 2020). The economic viability of the EGSB reactor and under what conditions it can be achieved have already been discussed. Thus, the environmental and social impacts of its technical application need to be investigated.

With the Renewable Energy Directive, a recast for 2030 of the climate change mitigation goals for European and therefore for Germany has been established. Some of the main points of the redirection are as follows:

- To reduce GHG emissions by 65% instead of 55% by 2030.
- To reach net zero emissions by 2045 instead of 2050.
- To reach negative GHG emissions by 2050.

Furthermore, at a national level are present regulations for the risks of diffuse N pollution to ground and surface waters, control ammonia emissions, control the recycling and transportation of fertilizers from animal excrements, and secure plant nutrition and soil fertility, while averting damage to humans, animals, and the environment. Therefore, manure management plays a decisive role in moving toward more sustainable agricultural and energetic sectors, with possible ramifications to the transportation or power and heat market if gas purification or liquefaction are considered.

Oehmichen and Thrän (2017) conducted a life cycle assessment (LCA) of GHG in a 75-kW_{el} biogas plant fed with 8500 t/a of cattle slurry and 2100 t/a of pig slurry. The study aimed to determine the effects associated with the use of manure from livestock breeding regarding two aspects: (i) mitigation of GHG emissions due to improved manure management and (ii) replacement of electricity from the German electricity grid by producing electricity from manure-based biogas. The study determined that 50% of the manure emissions of the plant can be avoided by bringing it quickly into the digester instead of storing conventionally (open silos), and the overall GHG emission reduction potential was estimated for a total of 3.5 Mt CO_2eq through manure utilization. Hence, to mitigate climate change, the ability to rapidly digest continuously produced manure is crucial, especially when there is insufficient storage capacity for undigested manure. Methane emissions from undigested manure significantly hinder the emission reduction capacity of anaerobic digestion. Additionally, the evaporation of organic matter reduces methane yield, ultimately impacting the profitability of the process.

The EGSB reactor is especially well suited to promptly processing slurries and low DM manures due to the low operating HRT because of the biomass retention system. Furthermore, the existence of a premium payment certification by the German government of the equivalent CO_2 saving from biogas production from manure could make the EGSB economically sustainable and help its diffusion in the biogas market.

Another largely unexploited potential in Germany is the agro-industrial wastes with an unexploited potential of 33–41 TWh, according to Edel et al. (2017). Rodriguez-Verde et al. (2014) analyzed the environmental impact of the AcoD with four different agro-industrial co-substrates (molasses, fish, biodiesel, and vinasse residues) using the LCA methodology. The AcoD of all co-substrates with manure exhibited improvements over the mono-digestion of PM in four categories, i.e., acidification, eutrophication, global warming, and photochemical oxidation potentials, with no significant difference in the co-substrates employed. Moreover, Rodriguez-Verde et al. (2014) also linked the OLR with the environmental performance of the different mixtures. The results indicated that the higher the OLR if the COD removal was kept constant, the higher the environmental benefits.

According to the values presented in Table 2-11, the EGSB can process up to nine times the OLR of a CSTR. Therefore, huge environmental benefits are expected from the substitution of the CSTR with the EGSB as the *de facto* reactor for liquid manure digestion. Furthermore, the reason for the positive impact of AcoD over manure mono-digestion was mainly linked to higher MYs. Chapter 4.3.4 presents a methodology to elevate the MY and MPR of manure and agro-industrial co-substrates by quantifying the possible synergistic and anti-synergistic effects of the substrates in the mixtures. Conversely, Chapter 5.3.4 validates these predictions in continuous experiments using EGSB reactors. Therefore, the possible environmental benefits of integrating the EGSB reactor in the agricultural biogas plants with the methodology outlined in Chapters 4 and 5 can conceivably surpass the benefits of simply integrating the EGSB or even implementing it with co-digestion without considering the influence of the mixture composition in MY.

In any case, the EGSB can potentially surpass the CSTR in terms of the potential for GHG reduction, and a complementary LCA analysis to the techno-economic comparison in Chapter 3.3.3 should be conducted to study the possibility of obtaining certifications for CO_2 savings and their impact on the economic efficiency of the TC analyzed, given that a market of GHG savings is being established in the EU.

Despite all the positive possible impacts of AD, biogas is still regarded as the least desirable energy carrier in Germany according to a survey by Herbes et al. (2014). The main issue associated with biogas mentioned by the 1000 interviewed persons in Karlsruhe and Stuttgart, Germany, was the competition with food production for land and the final use of the crops. In addition, some isolated cases mentioned the local impacts, such as odor nuisance. Horschig et al. (2020) pointed out that one of the most influential factors in this perception was the reporting of the press, and both academia and plant operators agreed that both reporting and decision-making should be more scientific-based. Hence, proper reporting regarding the environmental benefits of the AD of manure or agro-industrial residues and its role in the establishment of a circular economy should be intensified.

6.4 German energy security during the energy transition: the role of biogas

Some of the most mentioned negative side effects of the German energy transition by the public scientific debate are increasing CO_2 emissions, electricity prices, dependence on gas imports (as currently visible with the challenging replacement of Russian Gas by LNG), and negative environmental externalities from wind power due to the fast rise of renewable intermittent energy sources (*The European Dimension of Germany's Energy Transition*, 2019)

Kunze and Lehmann (2019) argued that the fact that the German energy transition has not resulted in a significant decline in its energy-related CO_2 emissions was driven by developments in international fuel and carbon markets—not only, if at all, by the phase-out of backup energy sources like nuclear during the ongoing renewable energy ramp up. The authors argued that the coal binge could have occurred even without the German energy transition and that a real transition is not possible without phasing out coal. The critics of this argument stated that a developed nation like Germany can't phase out at once both nuclear and coal power plants.

Whether the coal ramp-up is linked to the German energy transition or not, an increasing proportion of intermittent renewable energies require further technologies for balancing demand and supply in the energy system. The difference between Germany and other industrialized nations is the presence of a large installed capacity of biogas plants. Furthermore, in the current technological stand, biogas plants are the most important dispatchable renewable energy. According to the Federal Statistical Office (2022), the share of biogas in the gross electricity production in 2021 was 19.14%.

Moreover, the projections for the energy security of Europe are not very favorable due to the consequences of the ongoing conflict in Ukraine. Hence, in the public debate, there has been a shift from decarbonization to energy security (Mišík, 2022). The forecasts for the upcoming months are not very encouraging as Germany reopens coal power plants to make up for the shortages in imported Russian biogas and the alarm due to gas shortage rings (Coleman, 2022; Delfs & Dezem, 2022). As a consequence, the price of energy and goods reached 40 years high (Murray, 2022).

Nevertheless, the reforms introduced by the (EEG, 2017; EEG, 2021) limit the annual expansion of biogas plants to a maximum of 150 MW (2017–2019) and 200 MW (2020–2022). Therefore, the actions of the German Government in 2016 toward the reduction of the installed capacity and the electricity generated by biomass (and biogas) plants within the next decades, as many of them start their phase-out after 20 years of remuneration, have probably contributed to the deterioration of the energy security of the country. The former statement becomes more assertive because the decrease in the renewable load-base power of the country was accompanied by the phase-out of nuclear energy.

Furthermore, Lauer and Thrän (2018) analyzed the total system costs of varying biogas extension paths and modes of operation for the period of 2016–2035 by using a nonlinear optimization model. The authors found out that without considering the costs for biogas plants, the increasing extension of biogas plants may be more cost-effective for the system integration of intermittent renewable energies compared with their reduction or phase-out. The phase-out was the worst scenario, and the higher the flexibility of biogas plants, the higher the impact on the residual load curve. However, the findings indicated that the biogas extension path backup may be a more economically feasible way to integrate intermittent renewable energies into the electricity system than the continuous increase in the extension path increase.

One confusion identified by the authors that might have influenced policymaking is that dispatchable biogas plants are associated with a higher LCOE compared with intermittent renewable energies or flexibility options, such as battery storage. Nevertheless, the LCOE does not typically consider the total system costs of system integration of intermittent renewable energies by using flexible conventional power plants.

In another study, Lauer et al. (2020) conducted an economic assessment of the different extension paths and modes of operation of the biogas plants in Germany's future electricity system for the period of 2016–2035. This entailed carrying out a cost–benefit analysis that included the costs incurred for the flexibilization and installation of new biogas plants and the costs saved for onshore wind turbines and additional saved opportunity costs. Here, the most cost-effective path was the low construction rate of biogas plants. Furthermore, the economic feasibility of biogas plants benefits from an early phase-out of lignite- and coal-fired power plants.

Hence, AD should be either expanded or kept as backup power because Germany can use them as base load or for flexible energy production. Also, biogas could replace up to 40% of Russian biogas imports, and biogas represents the main source of renewable heat (Daniela Thrän et al., 2022). The contribution of the EGSB can come as a new investment in plant expansion that can help create flexibility with substrate and biogas output. The capacity to increase biogas production by using manure represents around 35% of the untapped potential. Nevertheless, the flexibility of power generation requires investments in gas storage and combined heat and power units.

7. Final conclusions

The AD of manure plays a multifaceted role in the German energy transition. AD can function as a power load base for energy security, as a low- or zero-emission source, and as circular carbon, with important side roles as a cornerstone for sustainable agricultural, sanitation, and circular economy of soil minerals. Nevertheless, the current stand on reactor technologies and policy framework does not allow the exploitation of the full potential of manure within the biogas industry.

This study aimed to introduce the EGSB as a technological alternative to enhance the techno-economic efficiency of manure digestion. The EGSB reactor can be a flexible investment that allows plants to access possible premiums for CO_2 and biogas output flexibility. This can be achieved by enabling the profitable digestion or co-digestion of some typically unprofitable low-TS substrates, rarely found in the agricultural biogas plant landscape. The EGSB can play a decisive role in extending the life of biogas plants in the post-EGG era, especially in facilitating their redirection toward biomethane production. Consequently, The EGSB can be a small but crucial building block in the European biomethane strategy.

Furthermore, the work puts in place a methodology to find, quantify, and transfer synergistic effects from standard batch tests to continuous tests with the EGSB reactor. This methodology is reproducible with any combination of substrates. Furthermore, the procedure is especially beneficial with manure because of its relatively very low MYs. Moreover, this methodology can be applied with different composition constraints, for example, 80% manure in fresh matter basis to access premium payments access as in the case of Germany. Summarizing, the constraints can offer considerable flexibility and can be adapted to a particular framework to optimize profits, environmental impact, or both at once, depending on the desired output.

Biogas is an essential part of the German renewable energy mix, a country whose energy transition and security have suffered significant drawbacks due to the Ukraine conflict. Hence, efforts toward renewable energy carriers' production, such as biomethane, should be maximized to minimize the risks of a future energy crisis. In the German sustainability strategy, biomethane from agricultural wastes, especially from manure, plays a central role. Furthermore, the current European political climate has revealed the potential and importance of further developing the biogas/biomethane sector. Fortunately, there is room for improvement, given that the German biogas industry is still not on par with the rest of the German industrial landscape regarding standardization of industrial equipment, automation, and process control. Introducing new reactor technologies, applying mathematical modeling, and optimizing feedstock composition to increase profitability are some techniques implemented in this thesis to bring the biogas industry a step closer to the gold standard of the German industrial landscape.

8. RECOMMENDATIONS AND FUTURE WORK PERSPECTIVES

Looking forward to the actual widespread market use of the EGSB in the biogas industry, two main issues need to be resolved. The first is the value of upflow velocity (V_{up}) and its influence on mixture intensity. Therefore, a task for the near future is to mathematically describe the influence of V_{up} on the performance of the reactor. Consequently, a curve of operational costs against biogas sales can be created, and indirectly, the economically optimal V_{up} can be established. For more precision in the experiments, it is recommended that the inferior limit of the V_{up} will be zero, reaching a no-recirculation operating condition, which would be the equivalent of comparing an EGSB with its predecessor, UASB.

Also, experiments with different mixtures and the influence of TS should be described to determine how much both the stability and profitability of the reactor are connected to TS because the literature reports upper limits ranging from 2% to 8%.

To further advance the EGSB in the biogas field, completing the ongoing pilot-plant experiments with the automatically controlled 550 L EGSB reactor in the Saerbeck Bioenergy Park facilities is crucial. Additionally, building a mechanistic model is recommended to accurately predict key variables in AD, such as methane flow, biogas composition, and inhibitory concentrations. The model should also emphasize the influence of the SRT to HRT ratio, as it has been proven to be definitive in the profitability of the EGSB. Moreover, the model would enable tailored control strategies to be developed for the reactor.

Furthermore, examining the phase separation in the two-stage treatment concept could help reduce costs and further optimize the process. This step is crucial in determining the solid content in each reactor. For very liquid manure (TS<6%), it may be more cost-effective to substitute the separation with a filtration step to avoid clogging in the EGSB and reduce operating costs significantly. In addition, analyzing the influence of the TS content in the EGSB on variables such as stability, methane yield, and methane production rate would provide complementary information for this optimization approach.

9. References

Abdelgadir, A., Chen, X., Liu, J [Jianshe], Xie, X., Zhang, J., Zhang, K., Wang, H., & Liu, N. (2014). Characteristics, process parameters, and inner components of anaerobic bioreactors. *BioMed Research International*, *2014*, 841573. https://doi.org/10.1155/2014/841573

Acharya, B. K., Mohana, S., & Madamwar, D. (2008). Anaerobic treatment of distillery spent wash - a study on upflow anaerobic fixed film bioreactor. *Bioresource Technology*, *99*(11), 4621–4626. https://doi.org/10.1016/j.biortech.2007.06.060

Adam, G., Lemaigre, S., Goux, X., Delfosse, P., & Romain, A.-C. (2015). Upscaling of an electronic nose for completely stirred tank reactor stability monitoring from pilot-scale to real-scale agricultural co-digestion biogas plant. *Bioresource Technology*, *178*, 285–296.

Ağdağ, O. N., & Sponza, D. T. (2007). Co-digestion of mixed industrial sludge with municipal solid wastes in anaerobic simulated landfilling bioreactors. *Journal of Hazardous Materials*, *140*(1-2), 75–85. https://doi.org/10.1016/j.jhazmat.2006.06.059

Aggarwal, C. C. (2015). *Data Mining: The Textbook*. *Springer eBook Collection Computer Science*. Springer. https://doi.org/10.1007/978-3-319-14142-8

Aichinger, P., Wadhawan, T., Kuprian, M., Higgins, M., Ebner, C., Fimml, C., Murthy, S., & Wett, B. (2015). Synergistic co-digestion of solid-organic-waste and municipal-sewage-sludge: 1 plus 1 equals more than 2 in terms of biogas production and solids reduction. *Water Research*, *87*, 416–423. https://doi.org/10.1016/j.watres.2015.07.033

Akunna, J. C [Joseph C.]. (2018). *Anaerobic waste-wastewater treatment and biogas plants: A practical handbook* (First edition). CRC Press an imprint of Taylor and Francis. https://www.taylorfrancis.com/books/mono/10.1201/9781351170529/anaerobic-waste-wastewater-treatment-biogas-plants-joseph-akunna https://doi.org/10.1201/9781351170529

Alfonso-Cardero, A., Pagés-Díaz, J., Contino, F., Rajendran, K., & Lorenzo-Llanes, J. (2021). Process simulation and techno-economic assessment of vinasse-to-biogas in Cuba: Deterministic and uncertainty analysis. *Chemical Engineering Research and Design*, *169*, 33–45. https://doi.org/10.1016/j.cherd.2021.02.031

Alfonso-Cardero, A., Pagés-Díaz, J., Kalogirou, E., Psomopoulos, C. S., & Lorenzo-Llanes, J. (2021). To dream or not to dream in Havana: Multi-criteria decision-making for material and energy recovery from municipal solid wastes. *Environmental Science and Pollution Research International.* Advance online publication. https://doi.org/10.1007/s11356-021-17360-2

Amani, T., Nosrati, M., Mousavi, S. M., & Elyasi, S. (2015). Study of microbiological and operational parameters in thermophilic syntrophic degradation of volatile fatty acids in an upflow anaerobic sludge blanket reactor. *Journal of Environmental Chemical Engineering*, *3*(1), 507–514. https://doi.org/10.1016/j.jece.2014.12.016

Amigun, B., & Blottnitz, H. von (2009). Capital cost prediction for biogas installations in Africa: Lang factor approach. *Environmental Progress & Sustainable Energy*, *28*(1), 134–142. https://doi.org/10.1002/ep.10341

Angelidaki, I [I.], & Ahring, B. K [B. K.] (1993). Thermophilic anaerobic digestion of livestock waste: The effect of ammonia. *Applied Microbiology and Biotechnology*, *38*(4), 560–564. https://doi.org/10.1007/BF00242955

Angelidaki, I [I.], & Ahring, B [B.K] (1994). Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Research*, *28*(3), 727–731. https://doi.org/10.1016/0043-1354(94)90153-8

Angelidaki, I [I.], & Ahring, B. K [B. K.] (1997). Codigestion of olive oil mill wastewaters with manure, household waste or sewage sludge. *Biodegradation*, *8*(4), 221–226. https://doi.org/10.1023/A:1008284527096

Angelidaki, I [I.], Alves, M., Bolzonella, D [D.], Borzacconi, L [L.], Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P [P.], & van Lier, J. B [J. B.] (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Science and Technology*, *59*(5), 927–934. https://doi.org/10.2166/wst.2009.040

Angelidaki, I [I.], Ellegaard, L [L.], & Ahring, B. K [B. K.] (1993). A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: Focusing on ammonia inhibition. *Biotechnology and Bioengineering*, *42*(2), 159–166. https://doi.org/10.1002/bit.260420203

Angelidaki, I [Irini], Ellegaard, L [Lars], & Ahring, B. K [Birgitte Kiœr]. Applications of the Anaerobic Digestion Process. In (pp. 1–33). https://doi.org/10.1007/3-540-45838-7_1

Angenent, L. (2001). Development of anaerobic migrating blanket reactor (AMBR), a novel anaerobic treatment system. *Water Research*, *35*(7), 1739–1747. https://doi.org/10.1016/s0043-1354(00)00447-4

Arias, P., Bellouin, N., Coppola, E., Jones, R., Krinner, G., Marotzke, J., Naik, V., Palmer, M., Plattner, G.-K., Rogelj, J., Rojas, M., Sillmann, J., Storelvmo, T., Thorne, P., Trewin, B., Achutarao, K., Adhikary, B., Allan, R., Armour, K., . . . Zickfeld, K. (2021). *The Intergovernmental Panel on Climate Change AR6*. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group14 I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; Technical Summary* (V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Conners, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou, Eds.).

Arthurson, V. (2009). Closing the Global Energy and Nutrient Cycles through Application of Biogas Residue to Agricultural Land – Potential Benefits and Drawback. *Energies*, *2*(2), 226–242. https://doi.org/10.3390/en20200226

Association of German Engineers. (2016). *Fermentation of Organic Materials–Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests: VDI 4630.*

Astals, S [S.], Batstone, D. J [D. J.], Mata-Alvarez, J [J.], & Jensen, P. D. (2014). Identification of synergistic impacts during anaerobic co-digestion of organic wastes. *Bioresource Technology*, *169*, 421–427. https://doi.org/10.1016/j.biortech.2014.07.024

Baloch, M. I., Akunna, J. C [J. C.], & Collier, P. J. (2007). The performance of a phase separated granular bed bioreactor treating brewery wastewater. *Bioresource Technology*, *98*(9), 1849–1855. https://doi.org/10.1016/j.biortech.2006.06.014

Balussou, D., Kleyböcker, A., McKenna, R., Möst, D., & Fichtner, W. (2012). An Economic Analysis of Three Operational Co-digestion Biogas Plants in Germany. *Waste and Biomass Valorization*, *3*(1), 23–41. https://doi.org/10.1007/s12649-011-9094-2

Batstone, D. J [D. J.], Tait, S., & Starrenburg, D. (2009). Estimation of hydrolysis parameters in full-scale anaerobic digesters. *Biotechnology and Bioengineering*, *102*(5), 1513–1520. https://doi.org/10.1002/bit.22163

Batstone, D. J [Damien J.] (2006). Mathematical Modelling of Anaerobic Reactors Treating Domestic Wastewater: Rational Criteria for Model Use. *Reviews in Environmental Science and Bio/Technology*, *5*(1), 57–71. https://doi.org/10.1007/s11157-005-7191-z

Batstone, D. J [Damien J.], Puyol, D., Flores-Alsina, X., & Rodríguez, J. (2015). Mathematical modelling of anaerobic digestion processes: applications and future needs. *Reviews in Environmental Science and Bio/Technology*, *14*(4), 595–613. https://doi.org/10.1007/s11157-015-9376-4

Bettina Kampman, Cor Leguijt, Thijs Scholten, Jurga Tallat-Kelpsaite, Robert Brückmann, Georgios Maroulis, Jan Peter Lesschen, Koen Meesters, Natasa Sikirica, & Berien Elbersen (2017). Optimal use of biogas from waste streams : An assessment of the potential of biogas from digestion in the EU beyond 2020. https://research.wur.nl/en/publications/optimal-use-of-biogas-from-waste-streams-an-assessment-of-the-pot

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965–977. https://doi.org/10.1016/j.talanta.2008.05.019

Bhattacharyya, D., & Singh, K. S. (2010). Understanding the Mixing Pattern in an Anaerobic Expanded Granular Sludge Bed Reactor: Effect of Liquid Recirculation. *Journal of Environmental Engineering*, *136*(6), 576–584. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000187

Bischoff, M. (2009). *Erkenntnisse beim Einsatz von Zusatz-und Hilfsstoffen sowie von Spurenelementen in Biogasanlagen.*

Borja, R., Banks, C. J., & Wang, Z. (1995). Performance of a hybrid anaerobic reactor, combining a sludge blanket and a filter, treating slaughterhouse wastewater. *Applied Microbiology and Biotechnology*, *43*(2), 351–357. https://doi.org/10.1007/BF00172838

Brennan, D. J. (2020). *Process industry economics: Principles, concepts and applications* (Second edition). Elsevier. https://ebookcentral.proquest.com/lib/kxp/detail.action?docID=6202066

Bridgewater, L. L. (Ed.). (2017). *Standard methods for the examination of water and wasterwater* (23rd edition). American Public Health Association.

Brito, A. G., & Melo, L. F. (1997). A Simplified Analysis of Reaction and Mass Transfer in UASB and EGSB Reactors. *Environmental Technology*, *18*(1), 35–44. https://doi.org/10.1080/09593331808616510

Brondz, I. (2002). Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography, and related techniques. *Analytica Chimica Acta*, *465*(1-2), 1–37. https://doi.org/10.1016/s0003-2670(01)01467-2

Brulé, M., Oechsner, H., & Jungbluth, T. (2014). Exponential model describing methane production kinetics in batch anaerobic digestion: A tool for evaluation of biochemical methane potential assays. *Bioprocess and Biosystems Engineering*, *37*(9), 1759–1770. https://doi.org/10.1007/s00449-014-1150-4

Bui, X.-T., Chiemchaisri, C., Fujioka, T., & Varjani, S. (2019). *Water and Wastewater Treatment Technologies*. Springer.

Buruk Sahin, Y., Aktar Demirtaş, E., & Burnak, N. (2016). Mixture design: A review of recent applications in the food industry. *Pamukkale University Journal of Engineering Sciences*, *22*(4), 297–304. https://doi.org/10.5505/pajes.2015.98598

Bywater, A., & Kusch-Brandt, S. (2022). Exploring Farm Anaerobic Digester Economic Viability in a Time of Policy Change in the UK. *Processes*, *10*(2), 212. https://doi.org/10.3390/pr10020212

Campanaro, S., Treu, L., Rodriguez-R, L. M., Kovalovszki, A., Ziels, R. M., Maus, I., Zhu, X., Kougias, P. G., Basile, A., Luo, G., Schlüter, A., Konstantinidis, K. T., & Angelidaki, I [Irini] (2020). New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters. *Biotechnology for Biofuels*, *13*, 25. https://doi.org/10.1186/s13068-020-01679-y

Carucci, G., Carrasco, F., Trifoni, K., Majone, M., & Beccari, M. (2005). Anaerobic Digestion of Food Industry Wastes: Effect of Codigestion on Methane Yield. *Journal of Environmental Engineering*, *131*(7), 1037–1045. https://doi.org/10.1061/(ASCE)0733-9372(2005)131:7(1037)

Castrillón Cano, L., Londoño, Y. A., Pino, N. J., & Peñuela, G. A. (2019). Effect of Benzophenone-3 on performance, structure and microbial metabolism in an EGSB system. *Environmental Technology*, 1–12.

Cavicchi, B. (2016). Sustainability that backfires: the case of biogas in Emilia Romagna. *Environmental Innovation and Societal Transitions*, *21*, 13–27. https://doi.org/10.1016/j.eist.2016.02.001

Chang, R. (2002). *Chemistry* (7th ed.). McGraw-Hill.

Chen, J. L., Ortiz, R., Steele, T. W. J., & Stuckey, D. C. (2014). Toxicants inhibiting anaerobic digestion: A review. *Biotechnology Advances*, *32*(8), 1523–1534. https://doi.org/10.1016/j.biotechadv.2014.10.005

Chen, Y [Ye], Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, *99*(10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Chernicharo, C. d. L. (2007). *Biological wastewater treatment*. IWA publishing London.

Chowdhury, R., & Fulford, D. J. (1992). Batch and semi-continuous anaerobic digestion systems. *Renewable Energy*, *2*(4-5), 391–400. https://doi.org/10.1016/0960-1481(92)90072-b

Cirne, D. G., Lehtomäki, A., Björnsson, L., & Blackall, L. L. (2007). Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *Journal of Applied Microbiology*, *103*(3), 516–527. https://doi.org/10.1111/j.1365-2672.2006.03270.x

Coleman, J. (2022, July 8). Germany Reopens Coal Plants Because Of Reduced Russian Energy. *Forbes*. https://www.forbes.com/sites/juliecoleman/2022/07/08/germany-reopens-coal-plants-because-of-reduced-russian-energy/

Cornell, J. A. (2002). *Experiments with mixtures: Designs, models, and the analysis of mixture data* (3rd ed.). *Wiley series in probability and statistics*. Wiley. https://doi.org/10.1002/9781118204221

Cramer, M., Rinas, M., Kotzbauer, U., & Tränckner, J. (2019). Surface contamination of impervious areas on biogas plants and conclusions for an improved stormwater management. *Journal of Cleaner Production*, *217*, 1–11. https://doi.org/10.1016/j.jclepro.2019.01.087

Cremonez, P. A., Teleken, J. G., Weiser Meier, T. R., & Alves, H. J. (2021). Two-Stage anaerobic digestion in agroindustrial waste treatment: A review. *Journal of Environmental Management*, *281*, 111854. https://doi.org/10.1016/j.jenvman.2020.111854

Cruz-Salomón, A [A.], Ríos-Valdovinos, E [E.], Pola-Albores, F [F.], Lagunas-Rivera, S [S.], Meza-Gordillo, R [R.], Ruíz-Valdiviezo, V. M., & Cruz-Salomón, K. C. (2019). Expanded granular sludge bed bioreactor in wastewater treatment. *Global Journal of Environmental Science and Management*, *5*(1), 119–138. https://doi.org/10.22034/gjesm.2019.01.10

Cruz-Salomón, A [Abumalé], Ríos-Valdovinos, E [Edna], Pola-Albores, F [Francisco], Lagunas-Rivera, S [Selene], Meza-Gordillo, R [Rocío], & Ruíz-Valdiviezo, V. (2018). Evaluation of Hydraulic

Retention Time on Treatment of Coffee Processing Wastewater (CPWW) in EGSB Bioreactor. *Sustainability*, *10*(2), 83. https://doi.org/10.3390/su10010083

Cu, T. T. T., Nguyen, T. X., Triolo, J. M., Pedersen, L [L.], Le, V. D., Le, P. D., & Sommer, S. G [S. G.] (2015). Biogas production from Vietnamese animal manure, plant residues and organic waste: Influence of biomass composition on methane yield. *Asian-Australasian Journal of Animal Sciences*, *28*(2), 280–289. https://doi.org/10.5713/ajas.14.0312

Cuéllar, A. D., & Webber, M. E. (2008). Cow power: The energy and emissions benefits of converting manure to biogas. *Environmental Research Letters*, *3*(3), 34002. https://doi.org/10.1088/1748-9326/3/3/034002

Daniela Thrän, Harry Schindler, Peter Kornatz, Martin Dotzauer, & Michael Nelles. (2022). *Die Rolle von Biogas für eine sichere Gasversorgung in Deutschland*. https://slub.qucosa.de/id/qucosa:80062

Daniel-Gromke, J., Rensberg, N., Denysenko, V., Stinner, W., Schmalfuß, T., Scheftelowitz, M., Nelles, M., & Liebetrau, J. (2018). Current Developments in Production and Utilization of Biogas and Biomethane in Germany. *Chemie Ingenieur Technik*, *90*(1-2), 17–35. https://doi.org/10.1002/cite.201700077

Debabrata Das, & Debayan Das. (2019). *Biochemical engineering: An introductory textbook*. Jenny Stanford Publishing. https://www.taylorfrancis.com/books/mono/10.1201/9780429031243/biochemical-engineering-debabrata-das-debayan-das https://doi.org/10.1201/9780429031243

Delfs, A., & Dezem, V. (2022, June 23). Germany Warns of Lehman-Like Contagion From Russian Gas Cuts. *Bloomberg*. https://www.bloomberg.com/news/articles/2022-06-23/germany-to-trigger-phase-two-of-three-stage-emergency-gas-plan

Demirel, B [B.], Neumann, L., & Scherer, P. (2008). Microbial Community Dynamics of a Continuous Mesophilic Anaerobic Biogas Digester Fed with Sugar Beet Silage. *Engineering in Life Sciences*, *8*(4), 390–398. https://doi.org/10.1002/elsc.200800010

Demirer, G. N., & Chen, S [S.] (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Process Biochemistry*, *40*(11), 3542–3549. https://doi.org/10.1016/j.procbio.2005.03.062

Dempster, A. P. (1971). An overview of multivariate data analysis. *Journal of Multivariate Analysis*, *1*(3), 316–346. https://doi.org/10.1016/0047-259x(71)90006-6

Dereli, R. K. (2019). Modeling long-term performance of full-scale anaerobic expanded granular sludge bed reactor treating confectionery industry wastewater. *Environmental Science and Pollution Research International*, *26*(24), 25037–25045. https://doi.org/10.1007/s11356-019-05739-1

Di Maria, F., Sordi, A., Cirulli, G., & Micale, C. (2015). Amount of energy recoverable from an existing sludge digester with the co-digestion with fruit and vegetable waste at reduced retention time. *Applied Energy*, *150*, 9–14. https://doi.org/10.1016/j.apenergy.2015.01.146

Don W. Green, & Robert H. Perry. (2008). *Perry's chemical engineers' handbook* (8. ed.). McGraw-Hill. https://www.accessengineeringlibrary.com/content/book/9780071422949

Doran, P. M. (2013). *Bioprocess Engineering Principles, Second Edi*. Elsevier Ltd.

Dotzauer, M., Pfeiffer, D., Thrän, D., Lenz, F., & Müller-Langer, F. *1.1 Bioenergie innerhalb des Forschungsprojekts TF_Energiewende*. https://epub.wupperinst.org/files/7082/wr13-1.pdf#page=10

Driessen, W., & Yspeert, P. (1999). Anaerobic Treatment of Low, Medium and High Strength Effluent in the Agro-industry. *Water Science and Technology*, *40*(8), 221–228. https://doi.org/10.2166/wst.1999.0425

Eberl, H., Morgenroth, E., Noguera, D., Picioreanu, C., Rittmann, B., van Loosdrecht, M., & Wanner, O. (2006). *Mathematical modeling of biofilms*. IWA publishing London.

Ebner, J. H., Labatut, R. A., Lodge, J. S., Williamson, A. A., & Trabold, T. A. (2016). Anaerobic co-digestion of commercial food waste and dairy manure: Characterizing biochemical parameters and synergistic effects. *Waste Management (New York, N.Y.)*, *52*, 286–294. https://doi.org/10.1016/j.wasman.2016.03.046

Edel, M., Kühnel, C., & Reinholz, T. (2017). *Rolle und Beitrag von Biomethan im Klimaschutz heute und in 2050*. https://www.dena.de/fileadmin/dena/Dokumente/Pdf/9218_Analyse_Rolle_Beitrag_Biomethan_Klimaschutz_2050.pdf

Ellis, J. T., Tramp, C., Sims, R. C., & Miller, C. D. (2012). Characterization of a Methanogenic Community within an Algal Fed Anaerobic Digester. *ISRN Microbiology*, *2012*, 753892. https://doi.org/10.5402/2012/753892

El-Mashad, H. M., & Zhang, R. (2010). Biogas production from co-digestion of dairy manure and food waste. *Bioresource Technology*, *101*(11), 4021–4028. https://doi.org/10.1016/j.biortech.2010.01.027

Environmental Energy Company: Olympia. (2001). *Dairy Waste Anaerobic Digestion Handbook*. http://www.symbiosisbiosystems.com/linked/research/dairy_waste_handbook_2001.pdf

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., & Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, *11*(4), 325–341. https://doi.org/10.1007/s11157-012-9277-8

The European Dimension of Germany’s Energy Transition. (2019). Springer, Cham.

Federal Statistical Office. (2022, July 18). *Gross electricity production in Germany*. https://www.destatis.de/EN/Themes/Economic-Sectors-Enterprises/Energy/Production/Tables/gross-electricity-production.html

Feitkenhauer, H., Sachs, J. von, & Meyer, U. (2002). On-line titration of volatile fatty acids for the process control of anaerobic digestion plants. *Water Research*, *36*(1), 212–218. https://doi.org/10.1016/s0043-1354(01)00189-0

Fotidis, I. A., Karakashev, D., Kotsopoulos, T. A., Martzopoulos, G. G., & Angelidaki, I [Irini] (2013). Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. *FEMS Microbiology Ecology*, *83*(1), 38–48. https://doi.org/10.1111/j.1574-6941.2012.01456.x

Franke-Whittle, I. H., Goberna, M., Pfister, V., & Insam, H. (2009). Design and development of the ANAEROCHIP microarray for investigation of methanogenic communities. *Journal of Microbiological Methods*, *79*(3), 279–288. https://doi.org/10.1016/j.mimet.2009.09.017

Friehe, J., P. Weiland, & A. Schattauer. (2012). *Guide to Biogas from Production to Use*. Fachagentur Nachwachsende Rohstoffe e. V. (FNR).

Fuentes, M., Scenna, N. J., & Aguirre, P. A. (2011). A coupling model for EGSB bioreactors: Hydrodynamics and anaerobic digestion processes. *Chemical Engineering and Processing: Process Intensification*, *50*(3), 316–324. https://doi.org/10.1016/j.cep.2011.01.005

Ganesh Saratale, R., Kumar, G., Banu, R., Xia, A., Periyasamy, S., & Dattatraya Saratale, G. (2018). A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. *Bioresource Technology*, *262*, 319–332. https://doi.org/10.1016/j.biortech.2018.03.030

Garcia, N. H., Mattioli, A., Gil, A., Frison, N., Battista, F., & Bolzonella, D [David] (2019). Evaluation of the methane potential of different agricultural and food processing substrates for improved biogas production in rural areas. *Renewable and Sustainable Energy Reviews*, *112*, 1–10. https://doi.org/10.1016/j.rser.2019.05.040

German Biogas Association. (2021). *Biogas market data in Germany 2020/2021*. https://www.biogas.org/edcom/webfvb.nsf/id/EN-German-biogas-market-data/$file/21-10-14_Biogasindustryfigures_2020-2021_english.pdf

Renewable Energy Sources Act, 2017. https://www.bmwi.de/Redaktion/EN/Downloads/renewable-energy-sources-act-2017.pdf%3F__blob%3DpublicationFile%26v%3D3

Renewable Energy Sources Act, 2021.

Ghosh, S. K., & Mukherjee, T. (2019). Circular Economy Through Treatment and Management of Industrial Wastewater. In *Waste Water Recycling and Management* (pp. 1–13). Springer.

Gleyce, Teixeira, Corrêia, Tania, Pérez-Pérez, Ileana, Pereda-Reyes, Deny, Oliva, Merencio, Marcelo, Zaiat, Wu, Hong, & Kwong (2014). Mathematical Modeling of the Hydrodynamics of an EGSB Reactor. https://www.semanticscholar.org/paper/Mathematical-Modeling-of-the-Hydrodynamics-of-an-Gleyce-Teixeira/c962e1884a8db02bf12f339ac6618c8226bfca1e

Glivin, G., Kalaiselvan, N., Mariappan, V., Premalatha, M., Murugan, P. C., & Sekhar, J. (2021). Conversion of biowaste to biogas: A review of current status on techno-economic challenges, policies, technologies and mitigation to environmental impacts. *Fuel*, *302*, 121153. https://doi.org/10.1016/j.fuel.2021.121153

Guenther-Lübbers, W., Bergmann, H., & Theuvsen, L. (2016). Potential analysis of the biogas production – as measured by effects of added value and employment. *Journal of Cleaner Production*, *129*, 556–564. https://doi.org/10.1016/j.jclepro.2016.03.157

Guidehouse, T. S., Beyschlag, L., Bruhin, A., Kerres, P., Lotz, B., & Oppermann, L. (June 2021). *Climate Action in Figures (2021) (EN) - BMUV-Publikation: Facts, Trends and Incentives for German Climate Policy 2021 edition.* Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU). https://www.bmuv.de/publikation/climate-action-in-figures-2021-en

Guiot, S. R., & van den Berg, L. (1985). Performance of an upflow anaerobic reactor combining a sludge blanket and a filter treating sugar waste. *Biotechnology and Bioengineering*, *27*(6), 800–806. https://doi.org/10.1002/bit.260270608

Gujer, W. (Ed.). (2008). *Systems Analysis for Water Technology*. Springer-Verlag. https://doi.org/10.1007/978-3-540-77278-1

Halalsheh, M., Koppes, J., Elzen, J. den, Zeeman, G [G.], Fayyad, M., & Lettinga, G [G.] (2005). Effect of SRT and temperature on biological conversions and the related scum-forming potential. *Water Research*, *39*(12), 2475–2482. https://doi.org/10.1016/j.watres.2004.12.012

Häner, J., Weide, T., Naßmacher, A., Hernández Regalado, R. E., Wetter, C., & Brügging, E. (2022). Anaerobic Digestion of Pig Slurry in Fixed-Bed and Expanded Granular Sludge Bed Reactors. *Energies*, *15*(12), 4414. https://doi.org/10.3390/en15124414

Heinrich-Böll-Stiftung. (2020, October 13). *Fleischatlas 2016 - Deutschland Regional | Heinrich-Böll-Stiftung*. https://www.boell.de/de/dossier-zum-fleischatlas-deutschland-regional

Henze, M., van Loosdrecht, M. C., Ekama, G. A., & & Brdjanovic, D. (2008). *Biological wastewater treatment: Principles, modelling and design*. IWA Publishing.

Herbes, C., Pustisek, A., McKenna, R., & Balussou, D. (2014). Überraschende Diskrepanz bei Biogas: lokal akzeptiert, global umstritten. *Energiewirtschaftliche Tagesfragen*, *64*, 53–56.

Hernández Regalado, R. E., Häner, J., Baumkötter, D., Wettwer, L., Brügging, E., & Tränckner, J. (2022). Continuous Co-Digestion of Agro-Industrial Mixtures in Laboratory Scale Expanded

Granular Sludge Bed Reactors. *Applied Sciences*, *12*(5), 2295. https://doi.org/10.3390/app12052295

Hernández Regalado, R. E., Häner, J., Brügging, E., & Tränckner, J. (2022). Techno-Economic Assessment of Solid–Liquid Biogas Treatment Plants for the Agro-Industrial Sector. *Energies*, *15*(12), 4413. https://doi.org/10.3390/en15124413

Hernández Regalado, R. E., Weide, T., Baumkötter, D., Wettwer, L., Häner, J., Brügging, E., & Tränckner, J. (2021). Optimization and Analysis of Liquid Anaerobic Co-Digestion of Agro-Industrial Wastes via Mixture Design. *Processes*, *9*(5), 877. https://doi.org/10.3390/pr9050877

Holliger, C., Alves, M [Madalena], Andrade, D., Angelidaki, I [Irini], Astals, S [Sergi], Baier, U., Bougrier, C., Buffière, P., Carballa, M., Wilde, V. de [Vinnie], Ebertseder, F., Fernández, B [Belén], Ficara, E., Fotidis, I., Frigon, J.-C., Laclos, H. F. de, Ghasimi, D. S. M., Hack, G., Hartel, M., . . . Wierinck, I [Isabella] (2016). Towards a standardization of biomethane potential tests. *Water Science and Technology*, *74*(11), 2515–2522. https://doi.org/10.2166/wst.2016.336

Holliger, C., Astals, S [Sergi], Laclos, H. F. de, Hafner, S. D., Koch, K., & Weinrich, S. (2021). Towards a standardization of biomethane potential tests: A commentary. *Water Science and Technology*, *83*(1), 247–250. https://doi.org/10.2166/wst.2020.569

Holliger, C., Fruteau de Laclos, H., & Hack, G. (2017). Methane Production of Full-Scale Anaerobic Digestion Plants Calculated from Substrate's Biomethane Potentials Compares Well with the One Measured On-Site. *Frontiers in Energy Research*, *5*, Article 12. https://doi.org/10.3389/fenrg.2017.00012

Holm-Nielsen, J. B [J. B.], Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilization. *Bioresource Technology*, *100*(22), 5478–5484. https://doi.org/10.1016/j.biortech.2008.12.046

Holzhammer, U. (2015). *Biogas in einer zukünftigen Energieversorgungsstruktur mit hohen Anteilen fluktuierender Erneuerbarer Energien*. https://scholar.google.com/citations?user=-fnoka8aaaaj&hl=es&oi=sra

Hopfner-Sixt, K., & Amon, T. (2007). Monitoring of agricultural biogas plants in Austria—Mixing technology and specific values of essential process parameters. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.555.8236&rep=rep1&type=pdf

Horschig, T., Schaubach, K., Sutor, C., & Thrän, D. (2020). Stakeholder perceptions about sustainability governance in the German biogas sector. *Energy, Sustainability and Society*, *10*(1). https://doi.org/10.1186/s13705-020-00270-5

Huang, Z., Ong, S. L., & Ng, H. Y. (2013). Performance of submerged anaerobic membrane bioreactor at different SRTs for domestic wastewater treatment. *Journal of Biotechnology*, *164*(1), 82–90. https://doi.org/10.1016/j.jbiotec.2013.01.001

Iglesias, R., Muñoz, R., Polanco, M., Díaz, I., Susmozas, A., Moreno, A. D., Guirado, M., Carreras, N., & Ballesteros, M. (2021). Biogas from Anaerobic Digestion as an Energy Vector: Current Upgrading Development. *Energies*, *14*(10), 2742. https://doi.org/10.3390/en14102742

Inglezakis, V. J., & Poulopoulos, S. G. (2006). Heterogeneous Processes and Reactor Analysis. In V. J. Inglezakis & S. G. Poulopoulos (Eds.), *Adsorption, ion exchange and catalysis: Design of operations and environmental applications* (1st ed., pp. 57–242). Elsevier. https://doi.org/10.1016/B978-044452783-7/50003-3

Jafarzadeh, M. T., Jamshidi, N., Talebiazar, L., & Aslaniavali, R. (2021). Performance evaluation of an anaerobic hybrid reactor treating petrochemical effluent. *International Journal of Geology*, *14*, 51–57. https://doi.org/10.46300/9105.2020.14.10

Jain, S., Lala, A. K., Bhatia, S. K., & Kudchadker, A. P. (1992). Modelling of hydrolysis controlled anaerobic digestion. *Journal of Chemical Technology & Biotechnology*, *53*(4), 337–344. https://doi.org/10.1002/jctb.280530404

Jeris, J. S. (1983). Industrial Wastewater Treatment Using Anaerobic Fluidized Bed Reactors. *Water Science and Technology*, *15*(8-9), 169–176. https://doi.org/10.2166/wst.1983.0165

Ji, C., & Sun, W. (2022). A Review on Data-Driven Process Monitoring Methods: Characterization and Mining of Industrial Data. *Processes*, *10*(2), 335. https://doi.org/10.3390/pr10020335

Jingura, R. M., & Kamusoko, R. (2017). Methods for determination of biomethane potential of feedstocks: a review. *Biofuel Research Journal*, *4*(2), 573–586. https://doi.org/10.18331/BRJ2017.4.2.3

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences*, *374*(2065), 20150202. https://doi.org/10.1098/rsta.2015.0202

Jurado, E., Antonopoulou, G., Lyberatos, G., Gavala, H. N., & Skiadas, I. V. (2016). Continuous anaerobic digestion of swine manure: ADM1-based modelling and effect of addition of swine manure fibers pretreated with aqueous ammonia soaking. *Applied Energy*, *172*, 190–198. https://doi.org/10.1016/j.apenergy.2016.03.072

Kainthola, J., Kalamdhad, A. S., & Goud, V. V. (2019). Optimization of methane production during anaerobic co-digestion of rice straw and hydrilla verticillata using response surface methodology. *Fuel*, *235*, 92–99. https://doi.org/10.1016/j.fuel.2018.07.094

Karki, R., Chuenchart, W., Surendra, K. C., Shrestha, S., Raskin, L., Sung, S., Hashimoto, A., & Kumar Khanal, S. (2021). Anaerobic co-digestion: Current status and perspectives. *Bioresource Technology*, *330*, 125001. https://doi.org/10.1016/j.biortech.2021.125001

Kashi, S., Satari, B., Lundin, M., Horváth, I. S., & Othman, M. (2017). Application of a mixture design to identify the effects of substrates ratios and interactions on anaerobic co-digestion of municipal sludge, grease trap waste, and meat processing waste. *Journal of Environmental Chemical Engineering*, *5*(6), 6156–6164. https://doi.org/10.1016/j.jece.2017.11.045

Kennedy, K. J., & van den Berg, L. (1982). Stability and performance of anaerobic fixed film reactors during hydraulic overloading at 10–35°C. *Water Research*, *16*(9), 1391–1398. https://doi.org/10.1016/0043-1354(82)90223-8

Kenneth Ndyabawe, & William S. Kisaalita (2014). Validity of the Africa-wide Lang factor of 2.63 for estimating small biogas plant installation costs in Uganda. *International Journal of Agricultural and Biological Engineering*, *7*(1), 70–76. https://doi.org/10.25165/ijabe.v7i1.880

Khanal, S. K. (2008). *Anaerobic biotechnology for bioenergy production: Principles and applications*. Wiley-Blackwell.

Kim, M., Kim, B.-C., Choi, Y., & Nam, K. (2017). Minimizing mixing intensity to improve the performance of rice straw anaerobic digestion via enhanced development of microbe-substrate aggregates. *Bioresource Technology*, *245*(Pt A), 590–597. https://doi.org/10.1016/j.biortech.2017.09.006

Kim, W., Hwang, K., Shin, S. G., Lee, S., & Hwang, S. (2010). Effect of high temperature on bacterial community dynamics in anaerobic acidogenesis using mesophilic sludge inoculum. *Bioresource Technology*, *101 Suppl 1*(1), S17-22. https://doi.org/10.1016/j.biortech.2009.03.029

Koch, K., Hafner, S. D., Weinrich, S., & Astals, S [Sergi] (2019). Identification of Critical Problems in Biochemical Methane Potential (BMP) Tests From Methane Production Curves. *Frontiers in Environmental Science*, *7*, Article 178. https://doi.org/10.3389/fenvs.2019.00178

Koch, K., Hafner, S. D., Weinrich, S., Astals, S [Sergi], & Holliger, C. (2020). Power and Limitations of Biochemical Methane Potential (BMP) Tests. *Frontiers in Energy Research*, *8*, Article 63. https://doi.org/10.3389/fenrg.2020.00063

Kondusamy, D., & Kalamdhad, A. S. (2014). Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. *Journal of Environmental Chemical Engineering*, *2*(3), 1821–1830. https://doi.org/10.1016/j.jece.2014.07.024

Korres, N., O'Kiely, P., Benzie, J. A., & West, J. S. (2013). *Bioenergy production by anaerobic digestion: Using agricultural biomass and organic wastes*. Earthscan from Routledge.

Kouas, M., Torrijos, M [Michel], Sousbie, P., Steyer, J.-P., Sayadi, S., & Harmand, J. (2017). Robust assessment of both biochemical methane potential and degradation kinetics of solid residues in successive batches. *Waste Management (New York, N.Y.)*, *70*, 59–70. https://doi.org/10.1016/j.wasman.2017.09.001

Kougias, P. G., & Angelidaki, I [Irini] (2018). Biogas and its opportunities—A review. *Frontiers of Environmental Science & Engineering*, *12*(3), 1–12. https://doi.org/10.1007/s11783-018-1037-8

Kroiss, H. (1985). *Anaerobe Abwasserreinigung* (Vol. 62). Techn. Univ. Wien, Inst. für Wassergüte und Landschaftswasserbau; Wien. https://repositum.tuwien.at/handle/20.500.12708/513

Kunze, C., & Lehmann, P. (2019). The Myth of the Dark Side of the. In *The European Dimension of Germany's Energy Transition* (pp. 255–263). Springer, Cham. https://doi.org/10.1007/978-3-030-03374-3_14

L.E. López de la Maza, L. Zumalacárregui de Cárdenas, & O. Pérez Ones (2019). Análisis de componentes principales aplicado a la fermentación alcohólica. *Revista Científica de la UCSA*, *6*(2), 11–19. https://revista.ucsa-ct.edu.py/ojs/index.php/ucsa/article/view/13

Labatut, R. A., Angenent, L. T., & Scott, N. R. (2011). Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource Technology*, *102*(3), 2255–2264. https://doi.org/10.1016/j.biortech.2010.10.035

Lahav, O., & Morgan, B. E. (2004). Titration methodologies for monitoring of anaerobic digestion in developing countries?a review. *Journal of Chemical Technology & Biotechnology*, *79*(12), 1331–1341. https://doi.org/10.1002/jctb.1143

Lalman, J., & Bagley, D. M. (2002). Effects of C18 long chain fatty acids on glucose, butyrate and hydrogen degradation. *Water Research*, *36*(13), 3307–3313. https://doi.org/10.1016/s0043-1354(02)00014-3

Lauer, M., Hansen, J. K., Lamers, P., & Thrän, D. (2018). Making money from waste: The economic viability of producing biogas and biomethane in the Idaho dairy industry. *Applied Energy*, *222*, 621–636. https://doi.org/10.1016/j.apenergy.2018.04.026

Lauer, M., Leprich, U., & Thrän, D. (2020). Economic assessment of flexible power generation from biogas plants in Germany's future electricity system. *Renewable Energy*, *146*, 1471–1485. https://doi.org/10.1016/j.renene.2019.06.163

Lauer, M., & Thrän, D. (2018). Flexible Biogas in Future Energy Systems—Sleeping Beauty for a Cheaper Power Generation. *Energies*, *11*(4), 761. https://doi.org/10.3390/en11040761

Lebuhn, M., Munk, B., & Effenberger, M. (2014). Agricultural biogas production in Germany - from practice to microbiology basics. *Energy, Sustainability and Society*, *4*(1), 1–21. https://doi.org/10.1186/2192-0567-4-10

Lee, Y.-S., & Han, G.-B. (2012). Pig slurry treatment by a hybrid multi-stage unit system consisting of an ATAD and an EGSB followed by a SBR reactor. *Biosystems Engineering*, *111*(3), 243–250. https://doi.org/10.1016/j.biosystemseng.2011.11.014

Leitão, R. C., Santaellla, S. T., van Haandel, A. C., Zeeman, G [G.], & Lettinga, G [G.] (2011). The effect of operational conditions on the hydrodynamic characteristics of the sludge bed in UASB reactors. *Water Science and Technology*, *64*(9), 1935–1941. https://doi.org/10.2166/wst.2011.628

Lens, P., van den Bosch, M. C., Hulshoff Pol, L. W., & Lettinga, G [G.] (1998). Effect of staging on volatile fatty acid degradation in a sulfidogenic granular sludge reactor. *Water Research*, *32*(4), 1178–1192. https://doi.org/10.1016/s0043-1354(97)00323-0

Lettinga, G [G.], van Velsen, A. F. M., Hobma, S. W., Zeeuw, W. de, & Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, *22*(4), 699–734. https://doi.org/10.1002/bit.260220402

Levenspiel, O. (1999). *Chemical reaction engineering* (3. ed.). Wiley. http://www.loc.gov/catdir/description/wiley033/97046872.html

Lewis, A. E. (2010). Review of metal sulphide precipitation. *Hydrometallurgy*, *104*(2), 222–234. https://doi.org/10.1016/j.hydromet.2010.06.010

Li, Y [Yue], Chen, Y [Yinguang], & Wu, J. (2019). Enhancement of methane production in anaerobic digestion process: A review. *Applied Energy*, *240*, 120–137.

Liebetrau, J., O'Shea, R., Wellisch, M., Lyng, K.-A., Bochmann, G., McCabe, B. K., Harris, P. W., Lukehurst, C., Kornatz, P., & Murphy, J. D. (2021). *Potential and utilization of manure to generate biogas in seven countries*. IEA Bioenergy. https://www.ieabioenergy.com/wp-content/uploads/2021/07/potential-utilization_web_end_new.pdf

Lissens, G., Vandevivere, P., Baere, L. de, Biey, E. M., & Verstraete, W. (2001). Solid waste digestors: process performance and practice for municipal solid waste digestion. *Water Science and Technology*, *44*(8), 91–102. https://doi.org/10.2166/wst.2001.0473

Liu, S. (2020). *Bioprocess engineering: Kinetics, sustainability, and reactor design* (Third edition). Elsevier.

Liu T. (1998). Anaerobic digestion of solid substrates in an innovative two-phase plug-flow reactor (tppfr) and a conventional single-phase continuously stirred-tank reactor. *Water Science and Technology*, *38*(8-9). https://doi.org/10.1016/s0273-1223(98)00723-9

Londoño, Y. A., Castrillón, L. V., Pino, N. J., Chica, E. L., & Peñuela, G. A. (2019). Analysis and Modeling of the Hydraulic Behavior of EGSB Reactors with Presence and Absence of Granular Biomass at Different Hydraulic Retention Times. *Ingeniería E Investigación*, *39*(1). https://doi.org/10.15446/ing.investig.v39n1.76753

López, I., & Borzacconi, L [Liliana] (2011). Modelling of an EGSB treating sugarcane vinasse using first-order variable kinetics. *Water Science and Technology*, *64*(10), 2080–2088. https://doi.org/10.2166/wst.2011.697

Lou, S. J., Tartakovsky, B., Zeng, Y., Wu, P., & Guiot, S. R. (2006). Fluorescence-based monitoring of tracer and substrate distribution in an UASB reactor. *Chemosphere*, *65*(7), 1212–1220. https://doi.org/10.1016/j.chemosphere.2006.03.045

M., J., J., A., Pedersen, L [Lene], & G., S. (2013). Characteristics of Animal Slurry as a Key Biomass for Biogas Production in Denmark. In M. D. Matovic (Ed.), *Biomass now.* InTech. https://doi.org/10.5772/54424

MacCarty P.L. (1964). Anaerobic waste treatment fundamentals, Part III, Toxic materials and their control. *Public Works*, *95*, 91–94. https://ci.nii.ac.jp/naid/10020360060/

Madsen, M., Holm-Nielsen, J. B [Jens Bo], & Esbensen, K. H. (2011). Monitoring of anaerobic digestion processes: A review perspective. *Renewable and Sustainable Energy Reviews*, *15*(6), 3141–3155.

Mahmoud, N., Zeeman, G [Grietje], Gijzen, H., & Lettinga, G [Gatze] (2003). Solids removal in upflow anaerobic reactors, a review. *Bioresource Technology*, *90*(1), 1–9. https://doi.org/10.1016/S0960-8524(03)00095-6

Mahnert, P., Heiermann, M., & Linke, B. (2005). Batch- and Semi-continuous Biogas Production from Different Grass Species. *1682-1130*. https://ecommons.cornell.edu/handle/1813/10439

Majer, S., Kornatz, P., Daniel-Gromke, J., Rensberg, N., Brosowski, A., Oehmichen, K., & Liebetrau, J. (Eds.). (2019). *Stand und Perspektiven der Biogaserzeugung aus Gülle*. Deutsches Biomasseforschungszentrum gemeinnützige GmbH. https://edocs.tib.eu/files/e01fn21/1770081690.pdf

Mamun, M. R. A., & Torii, S. (2015). Removal of Hydrogen Sulfide (H2S) from Biogas Using Zero-Valent Iron. *Journal of Clean Energy Technologies*, *3*(6), 428–432. https://doi.org/10.7763/jocet.2015.v3.236

Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, *45*, 540–555. https://doi.org/10.1016/j.rser.2015.02.032

Marañón, E., Castrillón, L., Vázquez, I., & Sastre, H. (2001). The influence of hydraulic residence time on the treatment of cattle manure in UASB reactors. *Waste Management & Research*, *19*(5), 436–441. https://doi.org/10.1177/0734242X0101900508

Marchaim, U., & Krause, C. (1993). Propionic to acetic acid ratios in overloaded anaerobic digestion. *Bioresource Technology*, *43*(3), 195–203. https://doi.org/10.1016/0960-8524(93)90031-6

Martín-González, L., Font, X., & Vicent, T. (2013). Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. *Biochemical Engineering Journal*, *76*, 1–5. https://doi.org/10.1016/j.bej.2013.03.016

Mata-Alvarez, J [J.], Dosta, J [J.], Romero-Güiza, M. S., Fonoll, X., Peces, M., & Astals, S [S.] (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, *36*, 412–427. https://doi.org/10.1016/j.rser.2014.04.039

Mata-Alvarez, J [Joan], Dosta, J [Joan], Macé, S., & Astals, S [Sergi] (2011). Codigestion of solid wastes: A review of its uses and perspectives including modeling. *Critical Reviews in Biotechnology*, *31*(2), 99–111. https://doi.org/10.3109/07388551.2010.525496

Méndez-Acosta, H. O., Palacios-Ruiz, B., Alcaraz-González, V., González-Álvarez, V., & García-Sandoval, J. P. (2010). A robust control scheme to improve the stability of anaerobic digestion processes. *Journal of Process Control*, *20*(4), 375–383. https://doi.org/10.1016/j.jprocont.2010.01.006

Mišík, M. (2022). The EU needs to improve its external energy security. *Energy Policy*, *165*, 112930. https://doi.org/10.1016/j.enpol.2022.112930

Møller, S. F., Frese, J. von, & Bro, R. (2005). Robust methods for multivariate data analysis. *Journal of Chemometrics*, *19*(10), 549–563. https://doi.org/10.1002/cem.962

Monteith, H. D., & Stephenson, J. P. (1978). Mixing Efficiencies in Full-Scale Anaerobic Digesters by Tracer Methods. *Water Quality Research Journal*, *13*(1), 135–148. https://doi.org/10.2166/wqrj.1978.011

Mortezaei, Y., Amani, T., & Elyasi, S. (2018). High-rate anaerobic digestion of yogurt wastewater in a hybrid EGSB and fixed-bed reactor: Optimizing through response surface methodology. *Process Safety and Environmental Protection*, *113*, 255–263. https://doi.org/10.1016/j.psep.2017.10.012

Mosquera, J., Varela, L., Santis, A., Villamizar, S., Acevedo, P., & Cabeza, I. (2020). Improving anaerobic co-digestion of different residual biomass sources readily available in Colombia by process parameters optimization. *Biomass and Bioenergy*, *142*, 105790. https://doi.org/10.1016/j.biombioe.2020.105790

Murray, M. (2022, April 28). Energy prices bump German inflation to four-decade high. *Reuters Media*(Carrel, Paul; Evans, Catherine). https://www.reuters.com/world/europe/energy-prices-bump-german-inflation-four-decade-high-2022-04-28/

Nallamothu, R. B., Teferra, A., & Rao, A. (2013). Biogas purification, compression and bottling.

Nations, Food and Agriculture Organization of the United. (1997). *Renewable biological systems for alternative sustainable energy production*. *FAO Agricultural services bulletin: Vol. 128*. Food and Agriculture Organization of the United Nations.

Neshat, S. A., Mohammadi, M., Najafpour, G. D., & Lahijani, P. (2017). Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renewable and Sustainable Energy Reviews*, *79*, 308–322. https://doi.org/10.1016/j.rser.2017.05.137

Nielsen, H. B., Mladenovska, Z., Westermann, P., & Ahring, B. K [B. K.] (2004). Comparison of two-stage thermophilic (68 degrees C/55 degrees C) anaerobic digestion with one-stage thermophilic (55 degrees C) digestion of cattle manure. *Biotechnology and Bioengineering*, *86*(3), 291–300. https://doi.org/10.1002/bit.20037

Nkuna, R., Roopnarain, A., Rashama, C., & Adeleke, R. (2021). Insights into organic loading rates of anaerobic digestion for biogas production: A review. *Critical Reviews in Biotechnology*, 1–21. https://doi.org/10.1080/07388551.2021.1942778

Obiukwu, O. O., & Nwafor, M. O. (2016). Comparative evaluation of batch and continuous process biogas production from animal wastes. *International Journal of Ambient Energy*, *37*(1), 29–35. https://doi.org/10.1080/01430750.2013.874369

Oehmichen, K., & Thrän, D. (2017). Fostering renewable energy provision from manure in Germany – Where to implement GHG emission reduction incentives. *Energy Policy*, *110*, 471–477. https://doi.org/10.1016/j.enpol.2017.08.014

Ohemeng-Ntiamoah, J., & Datta, T. (2019). Perspectives on variabilities in biomethane potential test parameters and outcomes: A review of studies published between 2007 and 2018. *The Science of the Total Environment*, *664*, 1052–1062. https://doi.org/10.1016/j.scitotenv.2019.02.088

Oleszkiewicz, J. A., & Sharma, V. K. (1990). Stimulation and inhibition of anaerobic processes by heavy metals—A review. *Biological Wastes*, *31*(1), 45–67. https://doi.org/10.1016/0269-7483(90)90043-r

Oliveira, L. G. de, Paiva, A. P. de, Balestrassi, P. P., Ferreira, J. R., da Costa, S. C., & da Silva Campos, P. H. (2019). Response surface methodology for advanced manufacturing technology optimization: Theoretical fundamentals, practical guidelines, and survey literature review. *The International Journal of Advanced Manufacturing Technology*, *104*(5-8), 1785–1837. https://doi.org/10.1007/s00170-019-03809-9

Onodera, T., Sase, S., Choeisai, P., Yoochatchaval, W., Sumino, H., Yamaguchi, T., Ebie, Y., Xu, K., Tomioka, N., Mizuochi, M., & Syutsubo, K. (2012). Evaluation of Process Performance and Sludge Properties of an up-flow staged Sludge Blanket (USSB) reactor for Treatment of Molasses Wastewater. *International Journal of Environmental Research*, *6*(4), 1015–1024. https://doi.org/10.22059/ijer.2012.572

Organisation for Economic Co-operation and Development OECD. (2020). *World Energy Outlook 2020*. OECD. https://doi.org/10.1787/557a761b-en

Pagés-Díaz, J., Pereda-Reyes, I., Sanz, J. L., Lundin, M., Taherzadeh, M. J., & Horváth, I. S. (2018). A comparison of process performance during the anaerobic mono- and co-digestion of slaughterhouse waste through different operational modes. *Journal of Environmental Sciences (China)*, *64*, 149–156. https://doi.org/10.1016/j.jes.2017.06.004

Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M. J., Sárvári-Horváth, I., & Lundin, M. (2014a). Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: synergistic and antagonistic interactions determined in batch digestion assays. *Chemical Engineering Journal*, *245*, 89–98.

Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M. J., Sárvári-Horváth, I., & Lundin, M. (2014b). Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: Synergistic and antagonistic interactions determined in batch digestion assays. *Chemical Engineering Journal*, *245*, 89–98. https://doi.org/10.1016/j.cej.2014.02.008

Panichnumsin, P., Nopharatana, A., Ahring, B. K [B. K.], & Chaiprasert, P. (2012). Enhanced biomethanation in co-digestion of Cassava pulp and pig manure using a two-phase anaerobic system. http://jseejournal.com/media/120/attachment/enhanced%20biomethanation%20pp.%2073-79.pdf

Paulose, P., & Kaparaju, P [Prasad] (2021). Anaerobic mono-digestion of sugarcane trash and bagasse with and without pretreatment. *Industrial Crops and Products*, *170*, 113712. https://doi.org/10.1016/j.indcrop.2021.113712

Pereira, M. A., Pires, O. C., Mota, M., & Alves, M. M. (2005). Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnology and Bioengineering*, *92*(1), 15–23. https://doi.org/10.1002/bit.20548

Pérez-Pérez, T., Correia, G. T., Kwong, W. H., Pereda-Reyes, I., Oliva-Merencio, D., & Zaiat, M. (2017). Effects of the support material addition on the hydrodynamic behavior of an anaerobic expanded granular sludge bed reactor. *Journal of Environmental Sciences (China)*, *54*, 224–230. https://doi.org/10.1016/j.jes.2016.02.011

A perspective on the state of the biogas industry from selected member countries of IEA Bioenergy Task 37 | Bioenergy. (2022, April 20). https://www.ieabioenergy.com/blog/publications/a-perspective-on-the-state-of-the-biogas-industry-from-selected-member-countries-of-iea-bioenergy-task-37/

Peters, M. S., & Timmerhaus, K. D. (1991). *Plant design and economics for chemical engineers* (4. ed.). *McGraw-Hill chemical engineering series*. McGraw-Hill. http://worldcatlibraries.org/wcpa/oclc/20853353

Pobeheim, H., Munk, B., Müller, H., Berg, G., & Guebitz, G. M. (2010). Characterization of an anaerobic population digesting a model substrate for maize in the presence of trace metals. *Chemosphere*, *80*(8), 829–836. https://doi.org/10.1016/j.chemosphere.2010.06.011

Prapaspongsa, T., Poulsen, T. G., Hansen, J. A., & Christensen, P. (2010). Energy production, nutrient recovery and greenhouse gas emission potentials from integrated pig manure management systems. *Waste Management & Research*, *28*(5), 411–422.

Preißler, D. (2009). *Die Bedeutung der Spurenelemente bei der Ertragssteigerung und Prozessstabilisierung*. https://scholar.google.com/citations?user=nu97c2iaaaaj&hl=es&oi=sra

Rabii, A., Aldin, S., Dahman, Y., & Elbeshbishy, E. (2019a). A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration. *Energies*, *12*(6), 1106. https://doi.org/10.3390/en12061106

Rabii, A., Aldin, S., Dahman, Y., & Elbeshbishy, E. (2019b). A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. *Energies*, *12*(6), 1106.

Rahman, M. A., Saha, C. K., Ward, A. J., Møller, H. B., & Alam, M. M. (2019). Anaerobic co-digestions of agro-industrial waste blends using mixture design. *Biomass and Bioenergy*, *122*, 156–164. https://doi.org/10.1016/j.biombioe.2019.01.036

Rajendran, K. (2015a). *Industrial Bioprocess Developments for Biogas and Ethanol Production* [, Borås, Högskolan i.]. EndNote.

Rajendran, K. (2015b). *Industrial Bioprocess Developments for Biogas and Ethanol Production* [, Högskolan i Borås]. www.diva-portal.org. http://www.diva-portal.org/smash/record.jsf?pid=diva2:848465

Rajendran, K., Kankanala, H. R., Martinsson, R., & Taherzadeh, M. J. (2014). Uncertainty over techno-economic potentials of biogas from municipal solid waste (MSW): A case study on an industrial process. *Applied Energy*, *125*, 84–92.

Raju, K. S. N. (2010). *Fluid mechanics, heat transfer, and mass transfer: Chemical engineering practice*. Wiley. https://doi.org/10.1002/9780470909973

Ramos-Suárez, J. L., Arroyo, N. C., & González-Fernández, C. (2015). The Role of Anaerobic Digestion in Algal Biorefineries: Clean Energy Production, Organic Waste Treatment, and Nutrient Loop Closure. In *Algae and Environmental Sustainability* (pp. 53–76). Springer, New Delhi. https://doi.org/10.1007/978-81-322-2641-3_5

Raposo, F [F.], Fernández-Cegrí, V., La Rubia, M. A. de, Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B [B.], Fernández-Polanco, M., Frigon, J. C., Ganesh, R., Kaparaju, P [P.], Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M [M.], Uellendahl, H., . . . Wilde, V. de [V.] (2011). Biochemical methane potential (BMP) of solid organic substrates: Evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, *86*(8), 1088–1098. https://doi.org/10.1002/jctb.2622

Rapp, B. E. (Ed.). (2017). *MicroNano Technologies Series*. *Microfluidics: Modeling, mechanics, and mathematics*. William Andrew.

Rastogi, G., Ranade, D. R., Yeole, T. Y., Patole, M. S., & Shouche, Y. S. (2008). Investigation of methanogen population structure in biogas reactor by molecular characterization of methyl-coenzyme M reductase A (mcrA) genes. *Bioresource Technology*, *99*(13), 5317–5326. https://doi.org/10.1016/j.biortech.2007.11.024

Ratanatamskul, C., & Siritiewsri, T. (2015). A compact on-site UASB–EGSB system for organic and suspended solid digestion and biogas recovery from department store wastewater. *International Biodeterioration & Biodegradation*, *102*, 24–30. https://doi.org/10.1016/j.ibiod.2015.04.002

Regalado, R. E. H., Weide, T., Baumkötter, D., Wettwer, L., Häner, J., Brügging, E., Tränckner, J., & Wetter, C. (2021). Optimization of the conventional agricultural biogas plant by the integration of a high-rate reactor in the treatment scheme. In M. Nelles (Ed.), *Schriftenreihe Umweltingenieurwesen: Band 105, 15. Rostocker Bioenergieforum: Am 16./17. Juni 2021 online als Videokonferenz : Tagungsband* (pp. 229–240). Universität Rostock Agrar- und Umweltwissenschaftliche Fakultät Professur Abfall- und Stoffstromwirtschaft; Universitätsbibliothek. https://www.researchgate.net/publication/354697870_Optimization_of_the_conventional_agricultural_biogas_plant_by_the_integration_of_a_high-rate_reactor_in_the_treatment_scheme

Rico, C [Carlos], Montes, J. A., & Rico, J. L [José Luis] (2017). Evaluation of different types of anaerobic seed sludge for the high rate anaerobic digestion of pig slurry in UASB reactors. *Bioresource Technology*, *238*, 147–156. https://doi.org/10.1016/j.biortech.2017.04.014

Rico, C [Carlos], Muñoz, N., Fernández, J., & Rico, J. L [José Luis] (2015). High-load anaerobic co-digestion of cheese whey and liquid fraction of dairy manure in a one-stage UASB process: Limits in co-substrates ratio and organic loading rate. *Chemical Engineering Journal*, *262*, 794–802. https://doi.org/10.1016/j.cej.2014.10.050

Rico, C [Carlos], Rico, J. L [José Luis], & Lasa, C. (2012). Anaerobic digestion of the liquid fraction of dairy manure separated by screw pressing and centrifugation in a upflow anaerobic sludge blanket reactor at 25 °C. *Biosystems Engineering*, *112*(4), 344–351. https://doi.org/10.1016/j.biosystemseng.2012.05.006

Rico, J. L [J. L.], García, H., Rico, C [C.], & Tejero, I. (2007). Characterisation of solid and liquid fractions of dairy manure with regard to their component distribution and methane production. *Bioresource Technology*, *98*(5), 971–979. https://doi.org/10.1016/j.biortech.2006.04.032

Rincón, B., Sánchez, E., Raposo, F [F.], Borja, R., Travieso, L., Martín, M. A., & Martín, A. (2008). Effect of the organic loading rate on the performance of anaerobic acidogenic fermentation of two-phase olive mill solid residue. *Waste Management*, *28*(5), 870–877. https://doi.org/10.1016/j.wasman.2007.02.030

Rintala, J., & LEPISTO, S. (1992). Anaerobic treatment of thermomechanical pulping whitewater at 35–70°C. *Water Research*, *26*(10), 1297–1305. https://doi.org/10.1016/0043-1354(92)90124-m

Rocamora, I., Wagland, S. T., Villa, R., Simpson, E. W., Fernández, O., & Bajón-Fernández, Y. (2020). Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. *Bioresource Technology*, *299*, 122681. https://doi.org/10.1016/j.biortech.2019.122681

Rodríguez-Reinoso, F. (Ed.). (2002). *Studies in Surface Science and Catalysis: vol. 144*. *Characterization of porous solids VI: Proceedings of the 6th International Symposium on the Characterization of Porous Solids (COPS-VI), Alicante, Spain, May 8-11, 2002*. Elsevier.

Rodriguez-Verde, I., Regueiro, L., Carballa, M., Hospido, A., & Lema, J. M. (2014). Assessing anaerobic co-digestion of pig manure with agroindustrial wastes: The link between environmental impacts and operational parameters. *The Science of the Total Environment*, *497-498*, 475–483. https://doi.org/10.1016/j.scitotenv.2014.07.127

Rosato, M. A. (2018). *Managing biogas plants: A practical guide*. *Green chemistry and chemical engineering*. CRC Press Taylor & Francis Group.

Rosenwinkel, K.-H., Kroiss, H., Dichtl, N., Seyfried, C.-F., & Weiland, P. (2015). *Anaerobtechnik: Abwasser-, Schlamm- und Reststoffbehandlung, Biogasgewinnung*. Springer-Verlag.

Ruile, S., Schmitz, S., Mönch-Tegeder, M., & Oechsner, H. (2015). Degradation efficiency of agricultural biogas plants--a full-scale study. *Bioresource Technology*, *178*, 341–349. https://doi.org/10.1016/j.biortech.2014.10.053

Sahm, H. (1981). Biology of methane formation, *53:11*, 854–863. https://www.osti.gov/etdeweb/biblio/5136214

Sakar, S., Yetilmezsoy, K., & Kocak, E. (2009). Anaerobic digestion technology in poultry and livestock waste treatment--a literature review. *Waste Management & Research*, *27*(1), 3–18. https://doi.org/10.1177/0734242X07079060

Salehiyoun, A. R., Di Maria, F., Sharifi, M., Norouzi, O., Zilouei, H., & Aghbashlo, M. (2020). Anaerobic co-digestion of sewage sludge and slaughterhouse waste in existing wastewater digesters. *Renewable Energy*, *145*, 2503–2509. https://doi.org/10.1016/j.renene.2019.08.001

Sang, N. N., Soda, S., Inoue, D., Sei, K., & Ike, M. (2009). Effects of intermittent and continuous aeration on accelerative stabilization and microbial population dynamics in landfill bioreactors. *Journal of Bioscience and Bioengineering*, *108*(4), 336–343. https://doi.org/10.1016/j.jbiosc.2009.04.019

Saravanan, V., & Sreekrishnan, T. R. (2006). Modelling anaerobic biofilm reactors--a review. *Journal of Environmental Management*, *81*(1), 1–18. https://doi.org/10.1016/j.jenvman.2005.10.002

Schiemenz, K., & Eichler-Löbermann, B. (2010). Biomass ashes and their phosphorus fertilizing effect on different crops. *Nutrient Cycling in Agroecosystems*, *87*(3), 471–482. https://doi.org/10.1007/s10705-010-9353-9

Schievano, A., Tenca, A., Scaglia, B., Merlino, G., Rizzi, A., Daffonchio, D., Oberti, R., & Adani, F. (2012). Two-stage vs single-stage thermophilic anaerobic digestion: Comparison of energy production and biodegradation efficiencies. *Environmental Science & Technology*, *46*(15), 8502–8510. https://doi.org/10.1021/es301376n

Schlüter, A., Bekel, T., Diaz, N. N., Dondrup, M., Eichenlaub, R., Gartemann, K.-H., Krahn, I., Krause, L., Krömeke, H., Kruse, O., Mussgnug, J. H., Neuweger, H., Niehaus, K., Pühler, A., Runte, K. J., Szczepanowski, R., Tauch, A., Tilker, A., Viehöver, P., & Goesmann, A. (2008). The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *Journal of Biotechnology*, *136*(1-2), 77–90. https://doi.org/10.1016/j.jbiotec.2008.05.008

Seghezzo, L., Zeeman, G [Grietje], van Lier, J. B., Hamelers, H. V., & Lettinga, G [Gatze] (1998). A review: the anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology*, *65*(3), 175–190.

Sentürk, E., Ince, M., & Engin, G. O. (2010). Treatment efficiency and VFA composition of a thermophilic anaerobic contact reactor treating food industry wastewater. *Journal of Hazardous Materials*, *176*(1-3), 843–848. https://doi.org/10.1016/j.jhazmat.2009.11.113

Sentürk, E., Ince, M., & Onkal Engin, G. (2010). Kinetic evaluation and performance of a mesophilic anaerobic contact reactor treating medium-strength food-processing wastewater. *Bioresource Technology*, *101*(11), 3970–3977. https://doi.org/10.1016/j.biortech.2010.01.034

Seyfried, C. F. (1990). *Anaerobe Verfahren zur Behandlung von Industrieabwässern.*

Shen, F., Tian, L., Yuan, H., Pang, Y., Chen, S [Shulin], Zou, D., Zhu, B., Liu, Y., & Li, X. (2013). Improving the mixing performances of rice straw anaerobic digestion for higher biogas production by computational fluid dynamics (CFD) simulation. *Applied Biochemistry and Biotechnology*, *171*(3), 626–642. https://doi.org/10.1007/s12010-013-0375-z

Shen, F., Yuan, H., Pang, Y., Chen, S [Shulin], Zhu, B., Zou, D., Liu, Y., Ma, J., Yu, L., & Li, X. (2013). Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. Two-phase. *Bioresource Technology*, *144*, 80–85. https://doi.org/10.1016/j.biortech.2013.06.099

Singh, B., Szamosi, Z., & Siménfalvi, Z. (2019). State of the art on mixing in an anaerobic digester: A review. *Renewable Energy*, *141*, 922–936. https://doi.org/10.1016/j.renene.2019.04.072

Singhania, R. R. (2009). Cellulolytic Enzymes. In *Biotechnology for Agro-Industrial Residues Utilisation* (pp. 371–381). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-9942-7_20

Sinnott, R. K., & Towler, G. P. (2020). *Chemical engineering design: Si Edition* (Sixth edition). *Coulson & Richardson's chemical engineering series*. Butterworth-Heinemann an imprint of Elsevier.

Smith, P., Beaumont, L., Bernacchi, C. J., Byrne, M., Cheung, W., Conant, R. T., Cotrufo, F., Feng, X., Janssens, I., Jones, H., Kirschbaum, M. U. F., Kobayashi, K., LaRoche, J., Luo, Y., McKechnie, A., Penuelas, J., Piao, S., Robinson, S., Sage, R. F., . . . Long, S. P. (2022). Essential outcomes for COP26. *Global Change Biology*, *28*(1), 1–3. https://doi.org/10.1111/gcb.15926

Šolcová, O., & Schneider, P. (2002). Transport characteristics of porous solids derived from chromatographic measurements. In F. Rodríguez-Reinoso (Ed.), *Studies in Surface Science and Catalysis: vol. 144. Characterization of porous solids VI: Proceedings of the 6th International Symposium on the Characterization of Porous Solids (COPS-VI), Alicante, Spain, May 8-11, 2002* (Vol. 144, pp. 475–482). Elsevier. https://doi.org/10.1016/S0167-2991(02)80170-7

Sommer, S. G [Sven G.], Christensen, M. L., Schmidt, T., & Jensen, L. S. (2013). *Animal Manure Recycling: Treatment and Management*. Wiley.

Song, M., Shin, S. G., & Hwang, S. (2010). Methanogenic population dynamics assessed by real-time quantitative PCR in sludge granule in upflow anaerobic sludge blanket treating swine wastewater. *Bioresource Technology*, *101 Suppl 1*(1), S23-8. https://doi.org/10.1016/j.biortech.2009.03.054

Sperling, M. von. (2007). *Basic principles of wastewater treatment*. IWA Publishing.

Stamatelatou, K., Vavilin, V., & Lyberatos, G. (2003). Performance of a glucose fed periodic anaerobic baffled reactor under increasing organic loading conditions: 1. Experimental results. *Bioresource Technology*, *88*(2), 131–136. https://doi.org/10.1016/s0960-8524(02)00276-6

Tallou, A., Haouas, A., Jamali, M. Y., Atif, K., Amir, S., & Aziz, F. (2020). Review on Cow Manure as Renewable Energy. In S. Patnaik, S. Sen, & M. S. Mahmoud (Eds.), *Modeling and Optimization in Science and Technologies. Smart Village Technology: Concepts and Developments* (1st ed.

2020, pp. 341–352). Springer International Publishing. https://doi.org/10.1007/978-3-030-37794-6_17

Tang, Y.-Q., Fujimura, Y., Shigematsu, T., Morimura, S., & Kida, K. (2007). Anaerobic treatment performance and microbial population of thermophilic upflow anaerobic filter reactor treating awamori distillery wastewater. *Journal of Bioscience and Bioengineering*, *104*(4), 281–287. https://doi.org/10.1263/jbb.104.281

Theuerl, S., Herrmann, C., Heiermann, M., Grundmann, P., Landwehr, N., Kreidenweis, U., & Prochnow, A. (2019). The Future Agricultural Biogas Plant in Germany: A Vision. *Energies*, *12*(3), 396. https://doi.org/10.3390/en12030396

Thiruselvi, D., Kumar, P. S., Kumar, M. A., Lay, C.-H., Aathika, S., Mani, Y., Jagadiswary, D., Dhanasekaran, A., Shanmugam, P., Sivanesan, S., & Show, P.-L. (2021). A critical review on global trends in biogas scenario with its up-gradation techniques for fuel cell and future perspectives. *International Journal of Hydrogen Energy*, *46*(31), 16734–16750. https://doi.org/10.1016/j.ijhydene.2020.10.023

Thorin, E., Olsson, J., Schwede, S., & Nehrenheim, E. (2017). Biogas from Co-digestion of Sewage Sludge and Microalgae. *Energy Procedia*, *105*, 1037–1042. https://doi.org/10.1016/j.egypro.2017.03.449

Thrän, D. (2015). *Smart Bioenergy: Technologies and Concepts for a More Flexible Bioenergy Provision in Future Energy Systems*. Springer International Publishing AG. https://ebookcentral.proquest.com/lib/kxp/detail.action?docID=2094494

Thrän, D., Schaubach, K., Majer, S., & Horschig, T. (2020). Governance of sustainability in the German biogas sector—adaptive management of the Renewable Energy Act between agriculture and the energy sector. *Energy, Sustainability and Society*, *10*(1). https://doi.org/10.1186/s13705-019-0227-y

Treichel, H., & Fongaro, G. (2019). *Improving Biogas Production: Technological Challenges, Alternative Sources, Future Developments*. *Biofuel and Biorefinery Technologies: Vol. 9*. Springer International Publishing. https://doi.org/10.1007/978-3-030-10516-7

Trommler, M., Barchmann, T., Dotzauer, M., & Cieleit, A. (2017). Can Biogas Plants Contribute to Lower the Demand for Power Grid Expansion? *Chemical Engineering & Technology*, *40*(2), 359–366. https://doi.org/10.1002/ceat.201600230

tttStrompreise. (2021, June 19). https://www.stromauskunft.de/strompreise/

Van, D. P., Fujiwara, T., Leu Tho, B., Song Toan, P. P., & Hoang Minh, G. (2020). A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environmental Engineering Research*, *25*(1), 1–17. https://doi.org/10.4491/eer.2018.334

van der Last, A. R. M., & Lettinga, G [G.] (1992). Anaerobic Treatment of Domestic Sewage under Moderate Climatic (Dutch) Conditions Using Upflow Reactors at Increased Superficial Velocities. *Water Science and Technology*, *25*(7), 167–178. https://doi.org/10.2166/wst.1992.0149

van Lier, J. B [J. B.], Boersma, F., Debets, M. M. W. H., & Lettinga, G [G.] (1994). High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. *Water Science and Technology*, *30*(12), 251–261. https://doi.org/10.2166/wst.1994.0621

Vavilin, V. A., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management*, *28*(6), 939–951. https://doi.org/10.1016/j.wasman.2007.03.028

Vavilin, V. A., Rytov, S. V., & Lokshina, L. (1996). A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresource Technology*, *56*(2-3), 229–237. https://doi.org/10.1016/0960-8524(96)00034-X

Vázquez-Fernández, A., Suárez-Ojeda, M. E., & Carrera, J. (2022). Review about bioproduction of Volatile Fatty Acids from wastes and wastewaters: Influence of operating conditions and organic composition of the substrate. *Journal of Environmental Chemical Engineering*, *10*(3), 107917. https://doi.org/10.1016/j.jece.2022.107917

Verma, A. K., Bhunia, P., & Dash, R. R. (2014). Carbonaceous organics removal kinetics in an upflow anaerobic sludge blanket (UASB) reactor treating physico-chemically pre-treated textile wastewater. *Desalination and Water Treatment*, 1–12. https://doi.org/10.1080/19443994.2014.888687

Vo, T. T., Wall, D. M., Ring, D., Rajendran, K., & Murphy, J. D. (2018). Techno-economic analysis of biogas upgrading via amine scrubber, carbon capture and ex-situ methanation. *Applied Energy*, *212*, 1191–1202. https://doi.org/10.1016/j.apenergy.2017.12.099

Vrieze, J. de, Colica, G., Pintucci, C., Sarli, J., Pedizzi, C., Willeghems, G., Bral, A., Varga, S., Prat, D., & Peng, L. (2018). Resource recovery from pig manure via an integrated approach: A technical and economic assessment for full-scale applications. *Bioresource Technology*.

Wang, G. (2010). *Biogas production from energy crops and agriculture residues*. RIS. https://www.osti.gov/etdeweb/biblio/1001402

Wang, Y., Yang, L., Li, Y [Yangyang], Fu, L., Yuan, C., Li Yao, & Luo, J. (2019). Reactor performance and economic evaluation of singular, binary, and ternary mixing of feedstocks for anaerobic digestion. *Environmental Technology*, 1–11. https://doi.org/10.1080/09593330.2019.1627424

Weide, T., Baquero, C. D., Schomaker, M., Brügging, E., & Wetter, C. (2020). Effects of enzyme addition on biogas and methane yields in the batch anaerobic digestion of agricultural waste (silage, straw, and animal manure). *Biomass and Bioenergy*, *132*, 105442. https://doi.org/10.1016/j.biombioe.2019.105442

Weide, T., Hernández Regalado, R. E., Brügging, E., Wichern, M., & Wetter, C. (2020). Biohydrogen Production via Dark Fermentation with Pig Manure and Glucose Using pH-Dependent Feeding. *Chemical Engineering & Technology*, *43*(8), 1578–1587. https://doi.org/10.1002/ceat.201900678

Weiland, P. (2010). Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology*, *85*(4), 849–860. https://doi.org/10.1007/s00253-009-2246-7

Weinrich, S. (2018). *Value of batch tests for biogas potential analysis: Method comparison and challenges of substrate and efficiency evaluation of biogas plants* (J. D. Murphy, Ed.). IEA Bioenergy.

Weinrich, S., & Nelles, M. (2021a). *Basics of Anaerobic Digestion: Biochemical Conversion and Process Modelling*. https://www.openagrar.de/receive/openagrar_mods_00069296

Weinrich, S., & Nelles, M. (2021b). Systematic simplification of the Anaerobic Digestion Model No. 1 (ADM1) - Model development and stoichiometric analysis. *Bioresource Technology*, *333*, 125124. https://doi.org/10.1016/j.biortech.2021.125124

Wetter, C., Brügging, E., & Weide, T. (2017). *Optimierung von Hochlastreaktor*. https://www.hb.fh-muenster.de/opus4/frontdoor/index/index/docId/8844

Wilkinson, K. G. (2011). A comparison of the drivers influencing adoption of on-farm anaerobic digestion in Germany and Australia. *Biomass and Bioenergy*, *35*(5), 1613–1622. https://doi.org/10.1016/j.biombioe.2011.01.013

Winterbottom, J. M., & King, M. (1999). *Reactor design for chemical engineers* (1st publ). Thornes.

Xie, S., Hai, F. I., Zhan, X., Guo, W., Ngo, H. H., Price, W. E., & Nghiem, L. D. (2016). Anaerobic co-digestion: A critical review of mathematical modelling for performance optimization. *Bioresource Technology*, *222*, 498–512. https://doi.org/10.1016/j.biortech.2016.10.015

Yenigün, O., & Demirel, B [Burak] (2013). Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry*, *48*(5-6), 901–911. https://doi.org/10.1016/j.procbio.2013.04.012

Yiridoe, E. K., Gordon, R., & Brown, B. B. (2009). Nonmarket cobenefits and economic feasibility of on-farm biogas energy production. *Energy Policy*, *37*(3), 1170–1179. https://doi.org/10.1016/j.enpol.2008.11.018

Yousefi, Z., Behbodi, M., & Ali Mohammadpour, R. (2018). Slaughterhouse wastewater treatment by combined anaerobic baffled reactor and anaerobic filter: Study of OLR and HRT optimization in ABR/AF reactors. *Environmental Health Engineering and Management*, *5*(3), 137–142. https://doi.org/10.15171/EHEM.2018.19

Yu, H., Wilson, F., & Tay, J.-H. (1998). Kinetic analysis of an anaerobic filter treating soybean wastewater. *Water Research*, *32*(11), 3341–3352. https://doi.org/10.1016/s0043-1354(98)00102-x

Yu, L., & Wensel, P. C. (2013). Mathematical Modeling in Anaerobic Digestion (AD). *Journal of Bioremediation & Biodegradation*, *s4.* https://doi.org/10.4172/2155-6199.s4-003

Zhang, C., Su, H., & Tan, T. (2013). Batch and semi-continuous anaerobic digestion of food waste in a dual solid-liquid system. *Bioresource Technology*, *145*, 10–16. https://doi.org/10.1016/j.biortech.2013.03.030

Zoetemeyer, R. J., Arnoldy, P., Cohen, A., & Boelhouwer, C. (1982). Influence of temperature on the anaerobic acidification of glucose in a mixed culture forming part of a two-stage digestion process. *Water Research*, *16*(3), 313–321. https://doi.org/10.1016/0043-1354(82)90191-9

Zumdahl, S. S., Zumdahl, S. L., & DeCoste, D. J. (2007). *World of chemistry*. McDougal Littell. http://worldcatlibraries.org/wcpa/oclc/74906191

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