

THESIS TITLE: Anaerobic Digestion of Organic Waste for Biogas Generation: State of the Art, Research Trends and Experimental Investigations

PhD Thesis – Summary

submitted for the award of the PhD degree at

Politehnica University Timișoara

in the doctoral field of Chemical Engineering

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Summary of PhD thesis:

Brief overview

This thesis aims to provide an overview of anaerobic digestion (AD) as a technology for energy production by integrating a detailed state-of-the-art analysis with experimental findings obtained from laboratory-scale investigations. In the context of the high demand for multiple energy streams and the need to alleviate pressure arising from the constraints of fossil fuel dependency, AD represents one of several alternatives for renewable energy production. The versatility of this technology resides in the fact that, in addition to its widespread implementation, it offers a wide range of development opportunities, making it potentially adaptable to a wide range of substrate streams, various scales, configurations, and operational requirements. It can also be combined with complementary technologies, leading to hybrid systems for further advancement, such as CH₄ upgrading. Therefore, the approaches for advancing AD are diverse and are presented in this thesis through a systematic discussion of both standard and cutting-edge strategies, including convergent reactor configurations and artificial intelligence (AI) - based mathematical modeling of the system.

Prior to the comprehensive literature survey, the relevant theoretical aspects of AD are presented, including biochemical transformation phases, factors determining process stability and performance, conventional reactor configurations, and biogas utilization, which are essential for system design. The experimental research focused on the utilization of available resources, including food waste (FW), in continuous stirred-tank reactor (CSTR) configurations operated under wet conditions. The results contribute to existing data in the field, considering the specific experimental configuration employed [1]. The full potential of AD is further emphasized by presenting theoretical aspects regarding its compatibility with circular bioeconomy principles and the future development of biorefineries based on AD.

Summary of the main scientific objectives of the PhD thesis

As per the brief overview introduced above, the main scientific objectives of the PhD thesis, which were detailed across the first four chapters, will be summarized below.

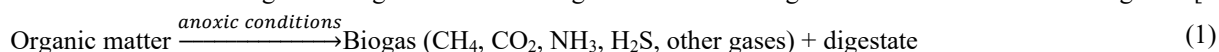
The first chapter entitled as *ANAEROBIC DIGESTION TECHNOLOGY* provides a necessary introduction to the technological and mechanistic foundations of AD as an engineered process. It aims to define the AD process, through documentation on the four main metabolic stages and the associated microbial communities, as well as relevant redox transformations. Key operational parameters and considerations for process design and biogas utilization, followed by an overview of common reactor configurations and an evaluation of the advantages and limitations of the technology are also discussed.

Therefore, the AD is a natural occurring process that was subsequently observed, studied and exploited by the humans since ancient times for energy production. Nowadays, it is utilized as a controlled engineering system for optimized energy recovering from biodegradable organic matter in the form of biogas [2], [3], [4], [5]. AD technology represents an important waste-to-energy solution that combines knowledge from multiple scientific and engineering

disciplines and offers benefits across energetic, environmental, and economic dimensions [6], [7]. Decrease of greenhouse gas emissions, production of biofertilizer as a secondary product, mitigation of water, soil, and air pollution and contribution to improved waste management represent the main environmental benefits [6], [7], [8], [9]. One of the most important energetic advantages refers to the production of renewable energy and its potential utilization as a replacement for natural gas, which is a fossil fuel energy source [8]. The diversity of organic waste that can be implemented within AD technology and the multiple use of biogas emphasizes the energetic importance of AD technology [6], [8], [9], [10]. Additionally, the technological-economic benefits support the attractiveness of technology and include a wide diversity of digester configurations and technological operating schemes, implementation at different scales, valorization of both products of technology (biogas and digestate), and possibility of innovating the technology through hybridization [6], [8], [10], [11], [12].

This process undergoes through four subsequent stages of biochemical transformations (hydrolysis, acidogenesis, acetogenesis and methanogenesis), metabolized by specific microbial consortiums under oxygen-free and controlled conditions within a digester [9]. From 40% to 90% of the initially fed organic material (on volatile solids, VS, basis) is converted into biogas and the remaining fraction that accounts for 10% to 60% is represented by the digestate - the organic by-product, with general application as a biofertilizer [13], [14], [15], [16]. Biogas consists mainly of methane (CH_4 , 55–70%), which represents the energetic component, and carbon dioxide (CO_2 , 30–45%), accompanied by amounts of lower concentration of water vapor (H_2O , 0–10%, up to saturation), nitrogen gas (N_2 , 0–8%), hydrogen sulfide (H_2S , 0–3%), ammonia (NH_3 , 0–1%), carbon monoxide (CO , 0–1%), hydrogen gas (H_2 , 0–1%), oxygen gas (O_2 , 0–2%), and traces of siloxanes and halogenated hydrocarbons [9], [17], [18], [19], [20], [21].

The general empirical equation of AD is depicted by equation (1), whereas the schematic representation of the biotransformation stages that organic matter undergo towards final biogas formation is illustrated in figure 1 [22].



Understanding the underlying chemico-biological mechanism of each metabolic pathway and their interconnectedness is of paramount important in the subsequent stages regarding process design and optimization. Disintegration is the first transformation of organic matter and is characterized by a mechanical preparation of the substrate to make it more soluble and susceptible to the subsequent transformation phases [23]. Therefore, during the hydrolysis stage, the pretreated substrate found in the form of complex organic compounds (carbohydrates, proteins, and lipids) are hydrolyzed by hydrolytic microorganism into simpler molecules (mono- and disaccharides, amino acids, glycerol and long-chain fatty acids [LCFAs]). Optimal pH values spanning from 5 to 8 and mesophilic and thermophilic temperatures are attributed to this phase [24], [25], [26]. The products of this phase are further converted into namely volatile fatty acids (VFAs), alcohols, H_2 , and CO_2 during acidogenesis, which is also referred as the fermentation stage. The involved microorganism are termed acidogens, and the pH of this phase is lower than that of the precedent phase, while the operating temperature domain is mesophilic and thermophilic, as in the case of hydrolysis stage [24], [25], [26].

A common approach is to couple the hydrolysis and fermentation stages in two-stage AD for the optimization of process parameters, operability, and gas yields [27]. The precursors for CH_4 formation are mostly resulted during acetogenesis, the phase following acidogenesis. These precursors mainly include acetate, CO_2 and H_2 [24], [25], [28]. The syntrophic relationship between acetogenesis and methanogenesis determines the thermodynamic equilibrium of CH_4 formation and the functionality of each phase, as the H_2 consumed during methanogenesis favors the conversion of VFAs during acetogenesis [24], [26], [28], [29].

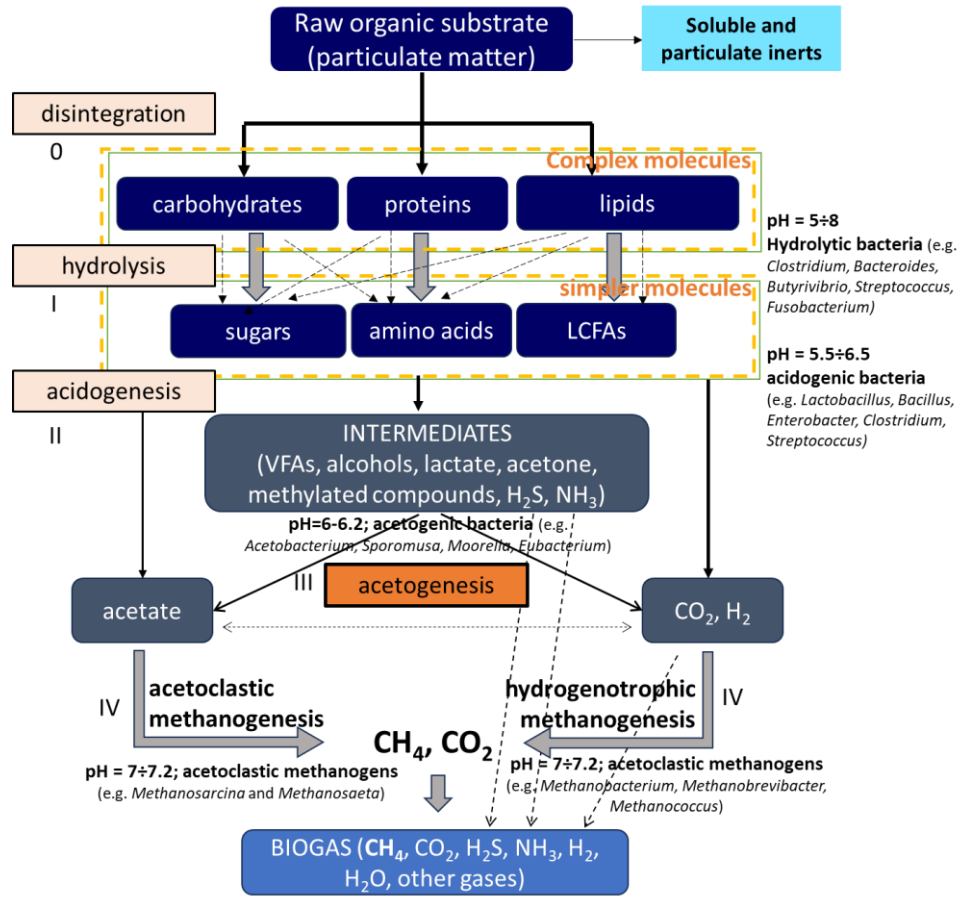


Figure 1. Schematic representation of AD stages (the diagram was constructed based on selected relevant information concerning each stage adapted from sources [30], [31], [32], [33], [34])

Therefore, CH_4 is obtained via two pathways: acetoclastic (equation 2) and hydrogenotrophic methanogenesis (equation 3) mediated by acetoclastic methanogenic archaea and hydrogenotrophic methanogens respectively [24], [26], [28], [29].



The mesophilic or thermophilic temperature regimes are favorable for both stages, whereas the pH of acetogenesis is slightly acidic and the pH of methanogenesis is neutral to slightly alkaline. Acetogenesis coupled with methanogenesis represent the second phase of two-stage AD [24], [25], [26], [28], [29].

While the conversion reactions are mostly regarded as chemo-biological processes, redox chemistry is an important aspect of these reactions, not only from perspective of the theoretical understanding of the process but also as a means of process control, particularly in hybrid technologies such as microbial electrolysis cells for gas yield improvement. Methane formation is a reduction process while the acidogenesis and acetogenesis are predominantly oxidation processes. Redox reactions occur intracellularly catalyzed by enzymes and electron carriers. Electron transfer occurs via intracellular redox couples and extracellularly through small soluble diffusible carriers - H_2 and formate —and through electrogenic bacteria [24], [30], [31], [35], [36], [37], [38], [39].

From the perspective of an engineering system, it is necessary to efficiently control and operate the AD process in order to maintain process stability and obtain satisfactory yields of CH_4 . Hence, the relevant operating and design parameters of AD adjusted during process optimization include temperature, pH, organic loading rate, substrate-to-inoculum (S/I) ratio, hydraulic retention time (HRT), substrate characteristics, nutrient balance, mixing conditions, and reactor configuration [9], [11], [26], [28], [29], [40].

Although research in this field is extensive, it should be mentioned that AD is a technically mature and commercially available technology. There are multiple digester types in which AD can be commercially operated, encompassing covered lagoon digesters, plug-flow digesters, completely mixed digesters, induced-bed reactor digesters, fixed-film digesters, anaerobic sequencing batch reactor digesters, high-solids anaerobic digesters, upflow anaerobic sludge blanket (UASB) reactors, expanded granular sludge bed reactors, and internal circulation reactors [6], [9], [11], [12], [29], [41], [42], [43], [44]. The typical components of an AD system are: the inlet of the influent, wherein the feedstock supply comes from a storage or mixing tank; the digestion vessel (the digester itself); a biogas accumulation dome with pipelines connected to external biogas infrastructure; and outlets for treated feedstock/digestate, usually connected to a storage space. Usually, the reactors involve various feeding regimes: batch operation, continuous operation, or semi-continuous operation. Digesters can be categorized as single-phase or multi-phase reactors depending on the operation of the AD stages, and as wet (with a moisture content higher than 85%) or dry (with a solids content >15%), depending on the moisture/solids content of the substrate [6], [9], [29], [45], [46].

Once obtained during AD and prior to being utilized as a fuel, biogas requires preliminary preparation, ranging from conventional pretreatment steps to additional upgrading stages [10], [47]. After pretreatments such as dewatering and the removal of H_2S and other trace contaminants, biogas containing varying CH_4 levels (e.g., 50–70%) can be directly combusted in boilers, burners, and furnaces for heat production and used in industrial heating, steam production, cooking, or hot water supply. Biogas can also be used for electricity generation in combined heat and power units and as a fuel for gas engines and turbines to produce heat, mechanical energy, and electricity. The quality requirements for biogas are defined by the final application [10], [45], [48], [49].

Chapter 2 of the PhD thesis, under the title *PROGRESS IN ANAEROBIC DIGESTION PROCESS DEVELOPMENT: A SCIENTIFIC LITERATURE-BASED ANALYSIS*, aims to continue the documentation on AD technology and to delve into current research trends aimed at improving AD performance.

Because AD processes are technically mature, commercially available, well documented, and constantly studied in the laboratory, research in this field is largely application-oriented. Herein, pretreatment of biomass/waste prior to the AD process, supplementation with additives, and the combination of multiple substrates in the anaerobic co-digestion process can be considered traditional research developments aimed at improving overall process performance through several actions, such as enhancement of feedstock conversion potential, process efficiency and stability, and biogas and CH_4 outputs. Hybrid AD systems and the integration of AI models into the mathematical optimization of the process pave the way for advanced research on this topic [9], [10], [25], [27], [50], [51], [52], [53].

Pretreatment methods are applied to the substrate prior to the onset of the AD process, usually to improve biodegradability and solubility, remove toxic compounds, and consequently improve process performance. These methods are applied to overcome some limitations associated with the substrates and to meet substrate-related performance requirements or simply to boost gas yields and quality [25], [33], [54], [55]. Additionally, pretreatment methods enhance process flexibility, enabling the use of a broader range of substrate types and combinations, and improve the accessibility of the substrate to microorganisms [25], [51]. The appropriate selection of a pretreatment method is conditional on the type of substrate and its inherent limitations and compositional characteristics. The maturity of pretreatment methods is revealed by the full-scale stage of development documented for most of the methods [25], [33], [51], [56].

Based on the literature survey, pretreatment strategies are broadly grouped into three main categories: physical, chemical, and biological. By integrating two or more methods belonging to the aforementioned types, a fourth category is defined, regarded as hybrid methods. Each principal category is subdivided into several other subcategories, with the classification conducted based on the internal mechanism and the involved process conditions and equipment. Figure 2 illustrates the principal categories and subcategories of the pretreatment methods [25], [54], [55].

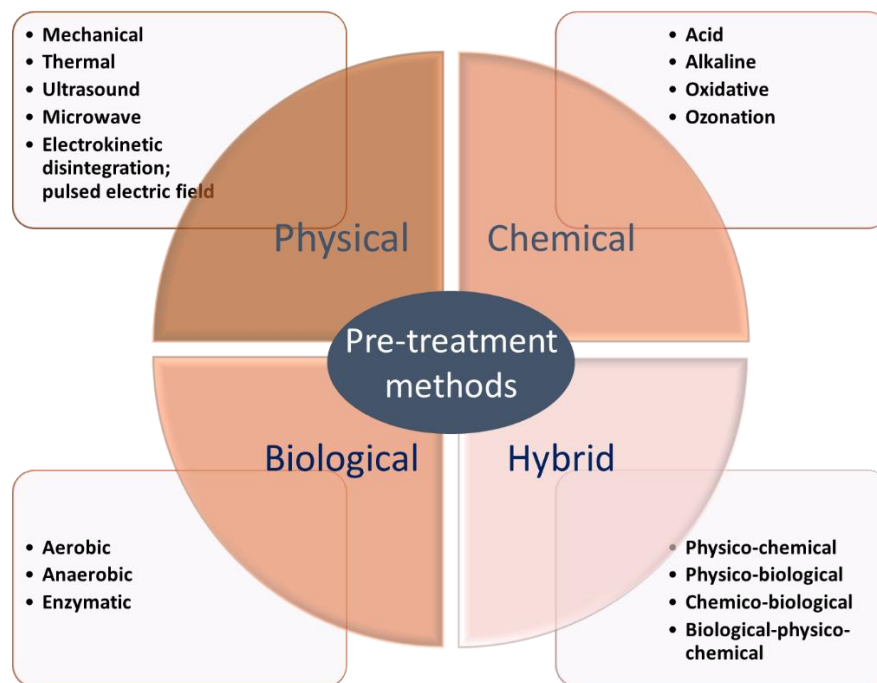


Figure 2. Classification of pretreatment methods that can be performed on biomass waste prior to the AD process.

The use of physical pretreatments aims to achieve particle size reduction and increase the surface area of the feedstock, as well as to improve contact between microorganisms and the feedstock, thus enabling enhanced process homogeneity through the application of physical forces. The application of physical treatments also facilitates the release of intracellular contents into the surrounding environment and reduces the degree of polymerization, thereby enhancing the rate-determining stage: hydrolysis. Chemical methods focus on the use of chemicals to treat the feedstock by breaking down complex molecular structures, releasing intracellular materials into the environment, and increasing their solubility and susceptibility to microbial action [28], [54].

The application of chemical pretreatment technologies requires specific operational parameters and the use of various chemical agents. These include a diverse array of substances, namely acids, alkalis, oxidizing agents, as well as ionic liquids, trace metals, and essential nutrients [28], [52], [54], [57], [58].

Biological pretreatment techniques employ microorganisms to treat the substrate prior to AD, thereby accelerating the hydrolysis stage. These methods are considered advantageous in terms of efficiency and mode of action because they do not induce extreme alterations in the substrate structure and solubilize organic matter with minimal energy input under mild operational conditions. From an environmental perspective, they are favorable because, unlike chemical methods, they do not generate toxic by-products. Under the action of enzymes, complex structures are broken down, and biopolymers are converted into simpler compounds. Usually, combined methods are operated simultaneously or sequentially, leading to systems that are less expensive in terms of lower energy requirements and lower implementation and operating costs, and that show enhanced efficiency (with respect to enhanced hydrolysis and organic matter solubilization, biogas and CH₄ production, and ease of operation) compared to each individual pretreatment procedure [25], [54], [55], [59].

According to the literature survey, the proper selection of the pretreatment method is conducted based on data related to the composition of the feedstock and with respect to the common pretreatment techniques tested for each category of feedstock classified by its source. Figure 3 schematically correlates the type of feedstock with the corresponding pretreatment methods utilized [51], [60].

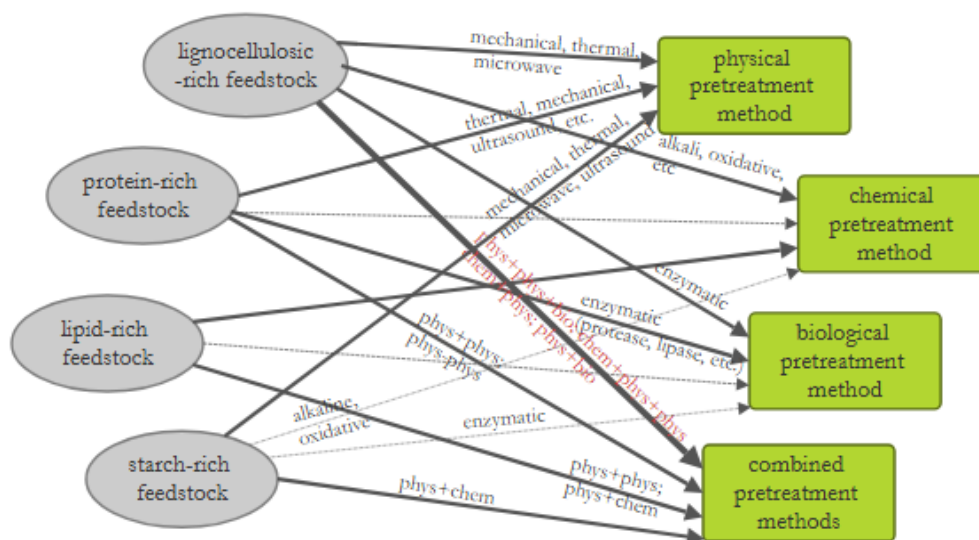


Figure 3. Schematic representation of summarized pretreatment techniques applied for various substrate types; the classification was based on the most efficient/common techniques, while the feedstocks were categorized based on its dominant component; the bolded lines indicate the most used techniques, while dotted lines symbolize the less common techniques; the graphic was designed based on studies [51], [60]

Additive supplementation is another promising strategy for the advancement of biogas production through the AD process. Broadly, the incorporation of additives serves to stabilize the digestion environment, facilitate key biochemical transformations, optimize microbial metabolic pathways, and enhance overall process efficiency [28], [61], [62], [63]. To support these outcomes, additives perform several critical functions, including promoting microbial proliferation and enzymatic activity, maintaining the system buffering capacity, adsorbing inhibitory compounds, facilitating direct interspecies electron transfer, and enhancing biochemical transformations during specific stages such as hydrolysis or methanogenesis [64], [65], [66]. There is a broad variety of additives, which can be mainly classified into the following groups: (i) biological additives (e.g., enzymes, microorganisms, fungi); (ii) inorganic additives (e.g., micro- and macronutrients, functional nanomaterials, carbon-containing materials); and (iii) chemical additives (e.g., pH-buffering materials) [63], [67].

The addition of enzymes has demonstrated a significant impact on AD, functioning as catalytic agents that convert complex molecules into simpler intermediates, with a consequent improvement in overall productivity [63].

Inorganic additives in AD consist primarily of micronutrients and macronutrients, both of which are integral to the biochemical stability and metabolic activity of microbial communities. pH-buffering additives play a critical role in maintaining system stability in AD by regulating pH fluctuations and preventing process failures. These fluctuations are primarily attributed to the accumulation of VFAs during acidogenesis [68].

The pH-control additives include both neutralizing agents—acidic and alkaline compounds such as KOH, NaOH, H₂SO₄, and HCl—and buffering agents, such as NaHCO₃, HCO₃⁻, and iron-based materials. As in the case of pretreatment methods, the application of additives has reached full-scale maturity from the perspective of technological readiness level [63], [69], [70].

Anaerobic co-digestion (AcoD) is another widely implemented strategy to enhance AD effectiveness. Therefore, this method can be regarded as an advanced alternative to anaerobic mono-digestion, in which two or more substrate types—referred to as co-substrates—are processed within a single facility to enhance process stability and promote higher biogas and CH₄ yields. The overall purpose is to mitigate the constraints of anaerobic mono-digestion and to achieve improved technical performance resulting from the synergistic effects of combined substrates, while also obtaining economic and environmental benefits [71], [72], [73]. To quantify the synergistic effect, researchers have introduced the co-digestion performance index (CPI), defined as the ratio of the biochemical methane potential (BMP) achieved through co-digestion to the weighted average of the BMPs obtained from mono-digestion of each respective feedstock [73]. The level of development of this technology has reached full-scale application, with sewage sludge co-digested with organic fraction of municipal solid waste, FW, or other sludge types being among the most

common examples [73], [74]. The co-substrates have to be compatible and are usually selected to be complementary from the point of view of chemical composition and physical characteristics [73]. Herein, one of the most important compositional characteristics is represented by the C/N ratio, which is identified as being optimally adjusted between 20 and 30 [29], [73], [75]. Other nutrients (C, N, P, S) and trace metals are additional compositional parameters carefully configured during the substrate selection stage. This is justified by the fact that a balanced nutrient composition is closely linked to process performance, as it prevents the formation of toxic compounds and alterations in buffering capacity and is further reflected in the overall microbial activity. Trace metals are essential for supporting enzyme synthesis and activity, as well as maintaining synergistic and syntrophic microbial associations [25], [73], [75], [76].

The overview of research strategies adopted for AD improvement continues with the discussion of cutting-edge approaches represented by hybrid technologies, AD reactors, and the integration of AI models into process control and optimization.

The improved technologies refer to modified versions of conventional configurations, as well as integrated technologies resulting from a combination of AD technology and other complementary technologies, such as membrane and fuel cell technologies. The synergy of hybrid technologies results in higher gas production rates, as well as enhanced economic and operational performance compared to conventional AD systems. In this regard, two-stage AD (figure 4(a)), anaerobic membrane bioreactors (figure 4(b)), hydrogen injection technology (figure 5(a)), and microbial electrolysis cells (MEC) (figure 5(b)) represent several advanced technologies under investigation [27], [77]. Biogas enriched in CH_4 , the possibility of exploiting other value-added products (VFAs, H_2), and the maximization of resource utilization are significant benefits of these technologies [27], [77], [78], [79].

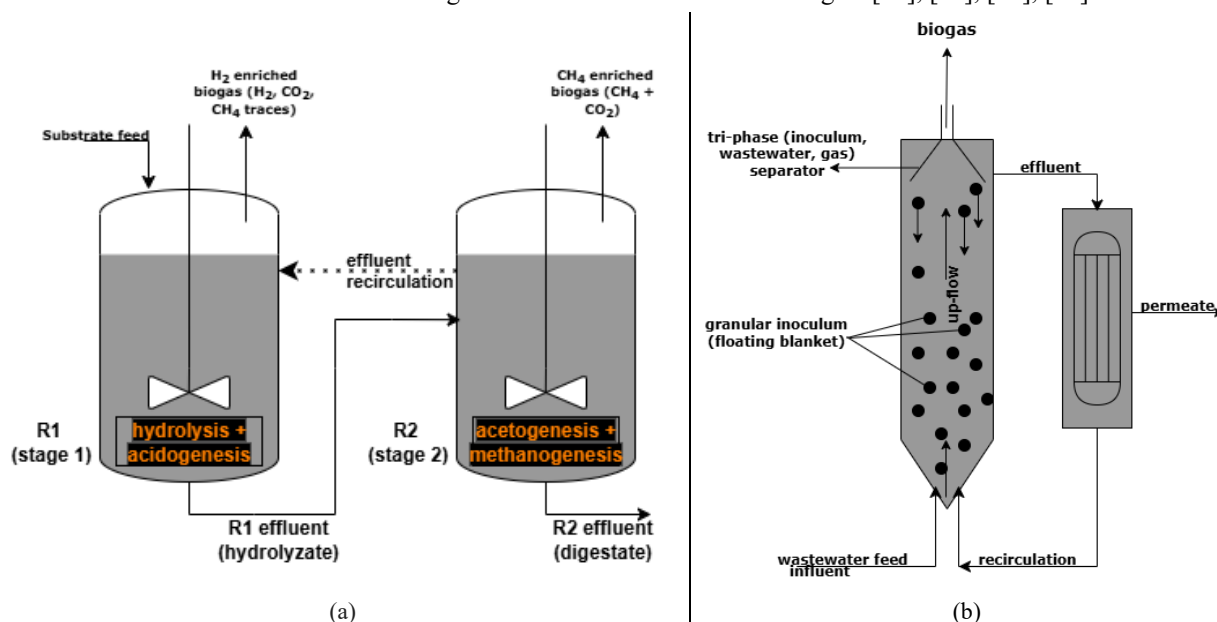


Figure 4. Schematic representation of (a) the two-stage AD system (CSTR–CSTR combination); (b) the AnMBR system (UASB–AnMBR, external submerged configuration) for wastewater treatment.

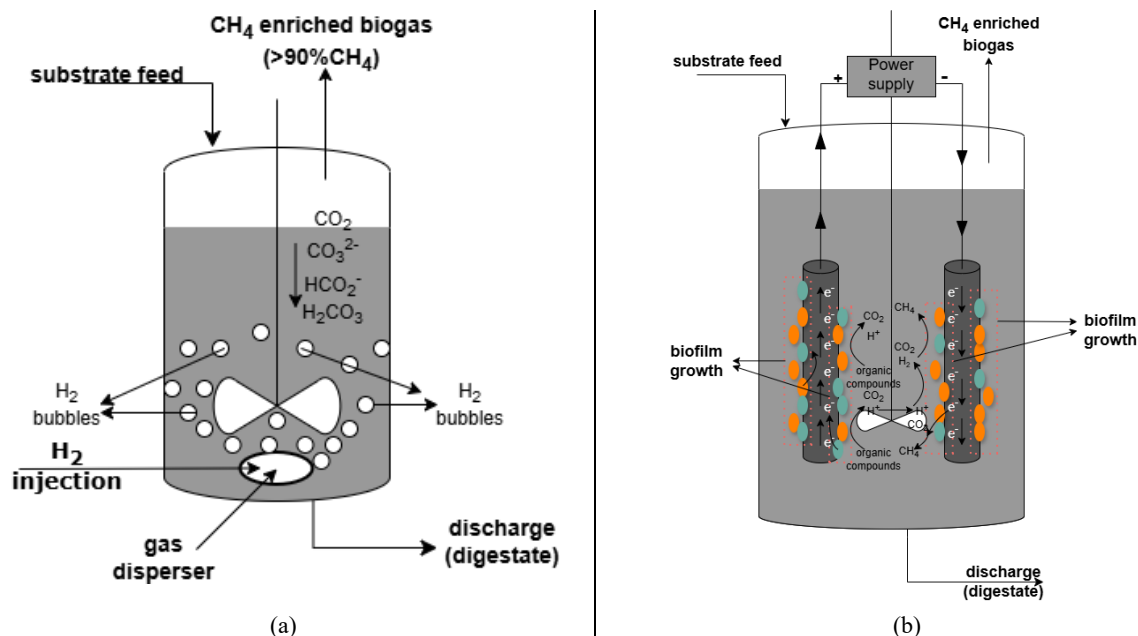


Figure 5. Schematic representation of (a) the H₂ injection AD system (CSTR-type digester); (b) the AD-MEC system: in-situ, single-chamber configuration (CSTR reactor).

Two-stage AD, abbreviated as TSAD, is a process configuration in which AD is conducted in two units rather than in a single reactor, as in conventional systems. In this arrangement, the first digester handles the hydrolysis and acidogenesis stages, while the second digester performs the subsequent metabolic phases, namely acetogenesis and methanogenesis. The effluent discharged from the first unit is directed to the second reactor (figure 4(a)). This configuration enables improved optimization of each stage, reducing overall reactor size, increasing gas production and process stability, enhancing buffering capacity, enabling better control over microbial communities, reducing microbial competition and inhibition, and ultimately increasing overall process productivity [27], [80], [81].

An anaerobic membrane bioreactor (AnMBR) is a hybrid technology in which AD is conducted in an anaerobic reactor coupled with a membrane system. The membrane retains biomass and essential microorganisms within the reactor, while the liquid permeate passes through the membrane and is subsequently collected. This configuration enables short HRTs and long solid retention times (SRTs) (figure 4(b)) [77], [82], [83]. This technology is especially applicable to substrates with elevated water content (e.g., sludge, wastewater, animal slurries, sewage sludge, landfill leachate) and is unsuitable for substrates with high solids content, as these promote membrane fouling through pore blocking caused by increased concentrations of particles, colloids, and biological substances that adhere to or penetrate the membrane surface, forming a cake layer and increasing hydraulic resistance [77], [83], [84], [85].

Hydrogen injection technology, also known as biomethanation, is a biogas upgrading technology in which CO₂ is converted into CH₄ as a result of H₂ injection through the hydrogenotrophic methanogenic pathway during AD (figure 5(a)). This reaction is known as the Sabatier reaction. This process has a direct impact on the energetic value of biogas due to the high CH₄ percentage—more than 95% of the total biogas—which represents one of the principal benefits of the technology [77]. Hydrogen is supplied as a surplus from other processes, such as water electrolysis, and can be added in situ inside the AD reactor or ex situ in an adjacent bioreactor [77], [86]. Direct H₂ supplementation can be easily integrated into existing plants, requires a small installation footprint, and therefore reduces costs. Moreover, this configuration provides endogenous precursors—VFAs and CO₂ produced during hydrolysis and acidogenesis—and microbial communities, such as H₂-producing and H₂-consuming microorganisms, required to initiate and sustain the biomethanation reaction [77], [86].

AD coupled with a MEC is a bioelectrochemical system, a hybrid technology in which electrodes integrated into the system provide an electrostimulated environment to facilitate microbial metabolism, increase the reaction rates of organic matter conversion, and achieve higher CH₄ yields and superior biogas quality [39], [87]. The integration of the electrolysis cell can be accomplished in situ by embedding the electrodes inside the AD reactor, or ex situ by connecting the AD digester to an external MEC and circulating the material to be digested in a loop between

the two component units (figure 5(b)) [77].

Full-scale demonstration has been attested for all four technology models, validating the feasibility of innovative solutions in the field [83], [84], [88], [89], [90]. Innovation is extended to numerous other prototypes and case studies, and herein the following can be mentioned: two-stage processes (liquid-solid AD systems), in situ methane enrichment, AD paired with algal cultivation, biofilm-based AD systems, and vacuum-assisted AD. Optimization of AD process metabolic phases, shifting the thermodynamic equilibrium of CH_4 formation, resource leverage, and implementation of modified biofilms are among the enhancements that these technologies bring about [77], [78], [79], [91], [92], [93].

AD process optimization and automation are performed through the integration of mathematical modelling and specialized engineering software. Therefore, the outputs of AD mathematical modelling encompass biogas information, substrate degradation efficiency, process stability parameters, digestate quality outputs, and information about microbial populations. The main subcategories of mathematical modelling frameworks commonly used to characterize AD systems are ADM1 and its modified forms, kinetic models, data-driven models, and machine learning (ML) approaches [23], [34], [94], [95], [96].

One of the most popular mathematical model is ADM1, which aims to address the need for a generally applicable mathematical framework capable of comprehensively describing the AD process, namely the conversion of biodegradable organic matter into biogas (CH_4 and CO_2) and inert intermediate compounds. It is suitable for any biodegradable organic substrate that can be fractionated into carbohydrates, proteins, and lipids and is intrinsically formulated for a single-stage CSTR reactor. To extend its applicability, several modified versions of ADM1 have been developed for different operating conditions as well as alternative reactor and process configurations, thereby addressing the inherent limitations of the original model [23], [34], [94], [97], [98], [99].

Kinetic models represent a class of mathematical frameworks applied to study and simulate the kinetic behavior of biochemical processes in AD, including microbial growth, substrate degradation, and product formation reactions [94]. The development of kinetic models is aimed at achieving diverse functionalities, enabling their independent use or integration into more comprehensive modelling frameworks for enhanced process evaluation [94]. Kinetic models usually describe time-dependent processes and typically account for microbial biomass growth or decay. The number of kinetic models investigated is substantial, and herein, the following can be mentioned: (i) microbial growth models (e.g., McCarty, Powell, Grau, etc.); (ii) microbial growth models with substrate inhibition effects (e.g., Holzberg et al., Bazua and Wilke et al., Aiba et al., etc.); (iii) kinetic growth models accounting for pH and temperature effects; (iv) modified kinetic models: variants of the first-order, Monod, Gompertz, and Chen–Hashimoto models; (v) hydrolysis kinetic models: dual-pooled first-order and surface-based approaches; (vi) CH_4 prediction models for continuous systems: Chen–Hashimoto, modified Stover–Kincannon, and Deng models; and (vii) cumulative gas production kinetic models: Logistic, Cone, Schnute, Richard, and Fitzhugh models [34], [94], [100], [101], [102], [103], [104], [105].

Data-driven models establish mapping relationships between system inputs—such as organic loading rate, substrate physicochemical properties, pH, temperature, and C/N ratio—and outputs, including gas yield and VS removal. These models describe system behavior without relying on mechanistic knowledge of the process [34], [95], [106]. The advantages of data-driven models lie in the fact that they do not require detailed biochemical process data, which may be time-intensive and costly to obtain; in their versatility, as they are suitable for predicting results for a wide variety of digested feedstocks; in their capability to identify data trends, including complex patterns; and in their efficiency in identifying uncertain parameters and supporting process optimization [107], [108]. Some commonly encountered data-driven models in AD include equation-based data-driven models, classical statistical models, artificial neural networks, and fuzzy logic models [30], [34], [98], [108], [109].

ML is a field focused on developing predictive models using algorithms and statistical techniques that learn patterns from data, enabling the mapping of inputs to outputs and the generation of predictions for new, unseen data without explicit programming [110], [111], [112]. In AD, ML algorithms are proving to be efficient tools for modelling nonlinear processes, forecasting process efficiency, and supporting automated control, operation, and maintenance. The inputs incorporated into ML model training encompass feedstock characteristics (e.g., substrate composition, content of specific compounds, total solids, VS, chemical oxygen demand, C/N), operating conditions (e.g., temperature, pressure, HRT, pH), system design variables (e.g., reactor shape), and process state variables (e.g., VFAs, total ammonia nitrogen, and H_2 contents)[96]. The application of ML in laboratory-scale studies is therefore expected and has also been validated in full-scale implementations for biogas production prediction using several ML algorithms, including principal component analysis, random forest, support vector machine, k-nearest neighbor, and

artificial neural networks [113].

Chapter 3, entitled EXPERIMENTAL RESEARCH AT LABORATORY SCALE, describes the original experimental work conducted within the PhD thesis, providing new insights into AD processes using FW predominantly composed of fruit and vegetable wastes (FWW) as the substrate, in combination with a granular biomass inoculum, and continuing the research on the AD process through experimental investigation [1].

The principal experimental investigation focused on the laboratory-scale study of AD using FW as the substrate and industrial granular inoculum as the microbial source. The main objective was to examine how key operational parameters—particularly temperature and the S/I ratio—affect the performance of the digestion process. Process performance was evaluated based on indicators such as biogas production, gas composition, conversion yields, and the kinetics of the underlying biochemical processes [1].

The laboratory-scale AD setup is schematically illustrated in figure 6, and the experimental configurations of the AD tests are presented in table 1[1].

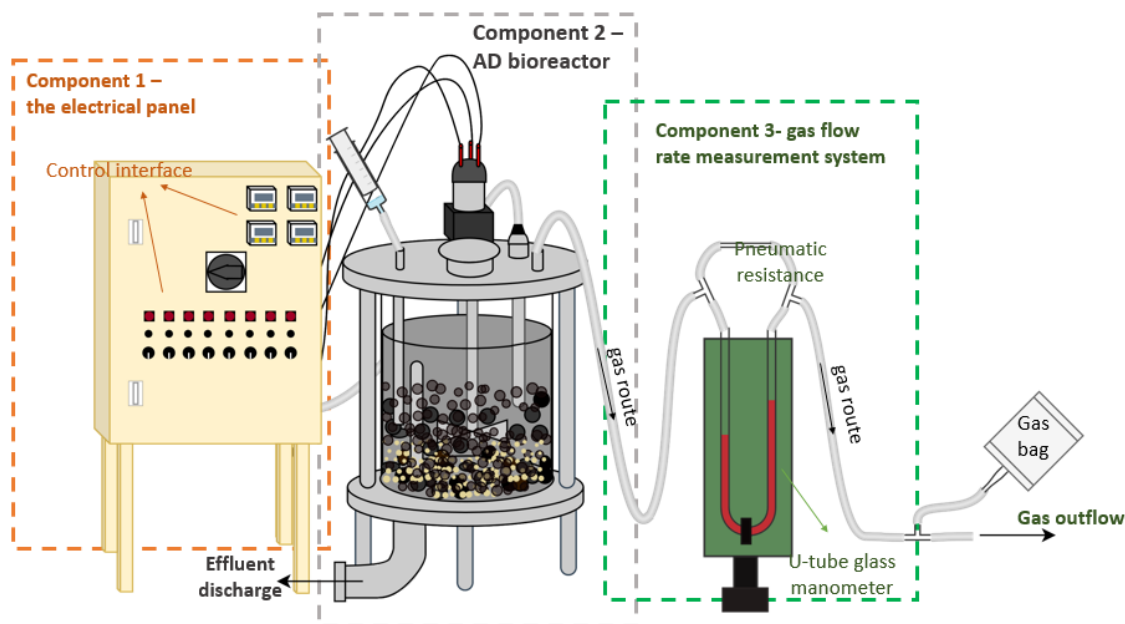


Figure 6. Schematic representation of the laboratory-scale AD setup illustrating the three main components of AD studies (component 1 – electrical panel, component 2 – CSTR bioreactor and component 3 - gas flow rate measurement system) [1]

Table 1. Experimental configurations of the batch AD experiments [1]

Experiment No.	Experiment label	Operating temperature (°C)	S/I ratio (w/w)
1	AD33SI11	33	1:1
2	AD37SI11	37	1:1
3	AD41SI11	41	1:1
4	AD45SI11	45	1:1
5	AD37SI32	37	3:2
6	AD37SI21	37	2:1

An important aspect of the experimental design is that the tests were performed in medium-scale laboratory CSTRs made of glass. This configuration allowed direct visual observation of the digestion slurry and facilitated the measurement of gas flow rates. In addition, parameters such as pH, electrical conductivity, and biogas composition were continuously monitored. The digestion experiments were conducted over short periods, ranging from 4 to 10 days, under controlled temperature and mixing regimes [1]. The analyzers for biogas, pH, and electrical conductivity are shown in figure 7 [1].



Figure 7. Portable equipment used for periodic measurements of gas composition, electrical conductivity, and pH: a) biogas analyzer and b) portable meter[1].

A noteworthy element of the current research is the integrated use of a defined FW mixture, medium-scale laboratory CSTRs equipped with temperature regulation and mechanical stirring, and a wider temperature range (33 °C, 37 °C, 41 °C, and 45 °C). The substrate consisted mainly of fruit and vegetable residues, which enabled the evaluation of AD performance specific to this type of feedstock and provided additional experimental data for comparable substrate combinations [1]. Before digestion, the fruit residues were mechanically homogenized to obtain a suspension. Another characteristic of the experimental setup is the significant addition of water to the feed mixture, reflecting conditions typical of wet AD systems. These experimental conditions were selected to explore the potential use of FW as a readily available substrate for AD, particularly in combination with industrial waste streams such as wastewater, while employing granular biomass as an inoculum source in wet AD systems [1].

To further characterize the process, analyses of both the substrate and the inoculum were performed and complemented by mathematical modeling. Specific modeling approaches were selected to characterize the biochemical system, combining a mechanistic (stoichiometric), steady-state model for the estimation of the maximum theoretical gas potential with data-driven dynamic kinetic models to simulate cumulative gas production over time [1]. A sigmoidal growth model, specifically the Zwietering-modified Gompertz model, was applied to fit the experimental data and to estimate kinetic parameters describing cumulative biogas and CH₄ production [1], [114].

The model parameters were estimated using an optimization algorithm implemented in MATLAB. The conversion yields were calculated relative to the theoretical gas potential. Additionally, the classification profile of microorganisms in the industrial inoculum was determined to provide insight into the microbial community involved in the digestion process [1].

Methane yield increased significantly with operating conditions, showing an approximately 8.3-fold increase at the highest temperature compared to the lowest and a 3.5-fold increase under the highest substrate loading conditions. Although increased acidity may exert inhibitory effects on methanogenesis and potentially shorten effective digestion periods, biogas production continued to increase with higher substrate loading [1]. Notably, the composition of the biogas remained relatively consistent under these conditions. The trends in biogas and CH₄ production, together with the assessment of CH₄ conversion yield and the mechanistic interpretation of the modified Gompertz model parameters, showed that the described AD system had not reached its production capacity limits, opening up possibilities for extending the experimental investigations on the same system for a more comprehensive evaluation [1].

Total gas production obtained from the measurements and calculations conducted in the AD experiments is presented in table 2, and the conversion yields can be explored in table 3. Furthermore, the modified Gompertz fitting is presented in figure 8 and figure 9 [1].

Table 2. Total gas production resulting from the conducted measurements and calculations in the AD experiments [1]

Referenced experiment	Total gas volume (NL kg ⁻¹ _{VS,add})					
	biogas	CH ₄	CO ₂	N ₂	H ₂ O	H ₂ S
AD33SI11	69.88	24.08	13.08	28	0.350	43.23 × 10 ⁻³
AD37SI11	130.7	67.19	35.85	17.31	8.18	62.01 × 10 ⁻³
AD41SI11	214.43	60.97	43.83	75.91	16.64	193.56 × 10 ⁻³
AD45SI11	465.37	203.03	160.44	64.62	44.9	698.36 × 10 ⁻³
AD37SI32	286.25	147.2	69.29	51.93	16.92	93.03 × 10 ⁻³
AD37SI21	468.59	233.95	168.37	77.4	29.84	298.41 × 10 ⁻³

Table 3. Conversion yields for CH₄ and organic carbon under laboratory conditions as defined in the experimental section [1]

Referenced experiment	η _{CH₄} (%)	η _{organic carbon} (%)	CH ₄ _{th} (NL kg ⁻¹ _{VS,add})	CO ₂ _{th} (NL kg ⁻¹ _{VS,add})
AD33SI11	3.98	4.15	604.82	291.22
AD37SI11	11.11	11.5		
AD41SI11	10.08	11.69		
AD45SI11	33.57	40.56		
AD37SI32	22.91	24.15	642.38	253.89
AD37SI21	36.42	44.89	655.47	240.78

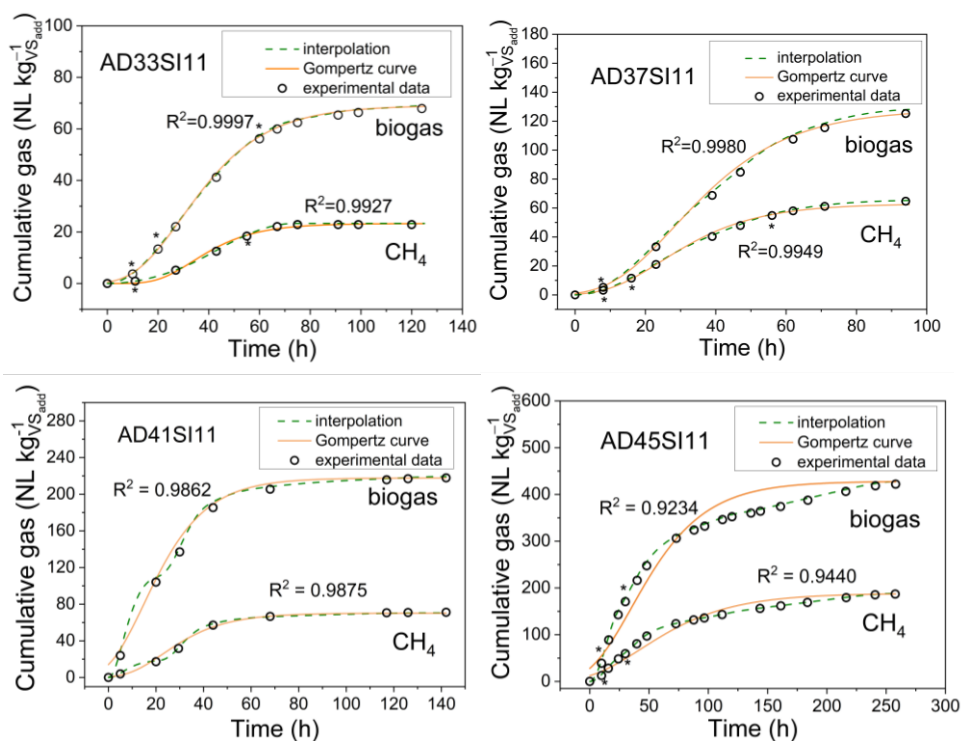


Figure 8. Gompertz model curves obtained for AD test systems: AD33SI11, AD37SI11, AD41SI11, AD45SI11; data points marked with an asterisk symbolize values generated by interpolation algorithm [1]

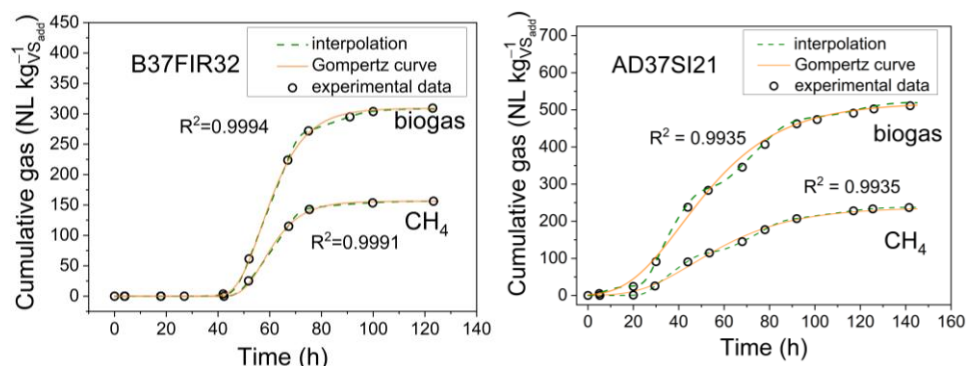


Figure 9. Gompertz model curves obtained for AD test systems: AD37SI11, AD37SI32, AD37SI21; data points marked with an asterisk symbolize values generated by interpolation algorithm [1]

Finally, Chapter 4, under the title *ANAEROBIC DIGESTION: A PATHWAY TOWARD THE CIRCULAR BIOECONOMY AND BIOREFINERY DEVELOPMENT*, concludes the scientific exploration of AD by discussing the full potential of AD technology, reflected through the alignment of AD with circular bioeconomy principles and the integration of AD into AD-based biorefinery systems.

Circular bioeconomy is a multidimensional system that focuses on the use of renewable resources (biological resources and organic waste), minimizing unusable waste and negative environmental impacts while promoting the long-term and sustained production of energy and valuable products. This framework incorporates economic, technological, environmental, as well as policy dimensions [115], [116], [117]. For AD systems, this circularity comprises the following stages: collection and preparation of organic waste (e.g., agricultural residues, industrial waste, FW), fermentation of waste through AD to obtain biogas and products such as digestate or derived chemicals and bioproducts, reintroduction of nutrients into ecosystems, and regeneration of new biomass, thus initiating a new material flow loop (figure 10) [115], [116], [118], [119].

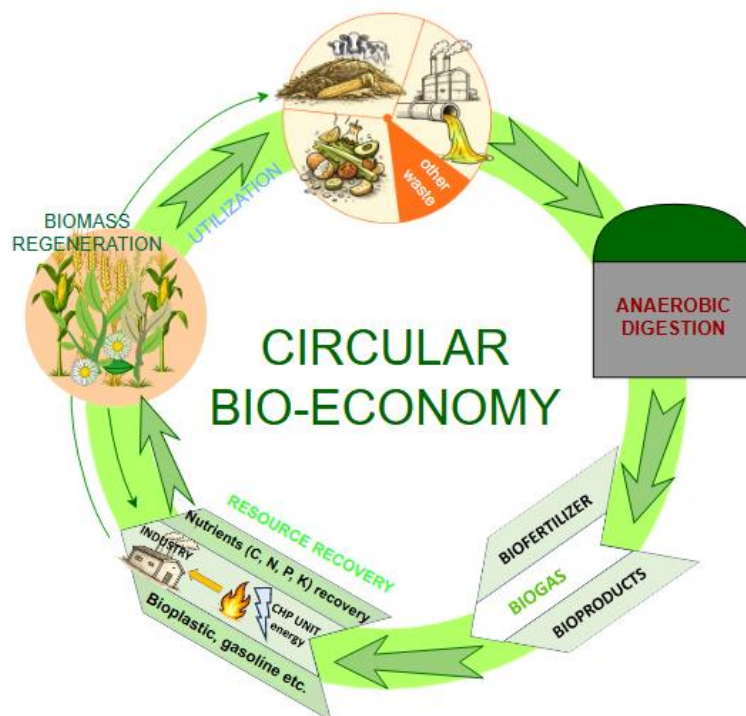


Figure 10. Schematic drawing of material flow in an AD-based circular bioeconomy framework [115], [119], [120].

AD-based biorefineries have the AD process as their core pillar while simultaneously developing multi-stream processes to intentionally generate, aside from CH₄/biogas, other valuable products such as digestate—which is very common—VFAs, biohydrogen, and their corresponding derivative chemicals and products. These derivatives comprise a range of carboxylic acids—such as acetic, propionic, butyric, valeric, and caproic acids—various alcohols, NH₃, upgraded gaseous fuels including biomethane and syngas, and bioliquid and biosolid fractions. Furthermore, the applications of these chemicals are diversified and include pharmaceuticals, laboratory chemicals, polymers, textiles, etc. [118], [121], [122].

In an attempt to emphasize the versatility of the AD process both as an independent technology for renewable energy production and as a potential replacement for fossil fuel-based processing technologies, this thesis summarizes research trends by integrating the literature on the technological developments and mechanistic aspects of AD with state-of-the-art and experimental findings obtained from laboratory-scale batch tests. The extent to which state-of-the-art advancement strategies provide real improvements is illustrated through examples from laboratory-scale studies on the AD of FW and agricultural residues. Moreover, the technological readiness level is addressed throughout the state-of-the-art chapter. The experimental part of this thesis provided new insights into wet AD systems, particularly those employing FW mixtures and granular biomass as inoculum. Methane yield increased significantly with operating conditions, showing an approximately 8.3-fold increase at the highest temperature compared to the lowest and a 3.5-fold increase under the highest substrate loading conditions. Although increased acidity may exert inhibitory effects on methanogenesis and potentially shorten effective digestion periods, biogas production continued to increase with higher substrate loading [1]. The methane yields obtained are consistent with values reported in the literature for similar systems. Finally, the potential transition of AD technology to a more advanced stage is emphasized through the discussion of the compatibility between AD and circular bioeconomy principles and the possible future integration of AD within AD-based biorefineries [1].

Software Resources: The schematic diagrams presented in this summary (Figures 1–6 and Figure 10) were created using Microsoft PowerPoint and Microsoft Word, as well as the draw.io and chemix.org applications. The data used to generate Figures 8 and 9 were obtained using MATLAB (version R2024b, The MathWorks, Inc.) and from experimental investigations, respectively, and were graphically represented using Origin software (OriginPro 2026 SR1, OriginLab Corporation). The same tools were used to produce the corresponding figures in the PhD thesis.

Declaration on the use of generative artificial intelligence tools. ChatGPT (OpenAI, GPT-4o–GPT-5.6 Sol models, accessed during 2025–2026) was used in the preparation of the summary and the thesis exclusively for the following purposes: (i) generating illustrative graphical elements containing no data, included in Figure 10 of the summary and Figure 41 of the thesis; (ii) graphical editing of the image on the right-hand side of Figure 19 of the thesis (digital photograph); and (iii) linguistic editing, namely grammar correction and improvement of clarity of expression, without modifying the scientific content. The tool was not used for the generation, processing, or interpretation of experimental data; the development of mathematical models; or the formulation of working hypotheses, conclusions, or original contributions. All resulting content was verified by the author, who assumes full responsibility for the scientific accuracy and originality of the work.

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