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ANÁLISIS DEL CICLO DE VIDA DE LA CODIGESTIÓN ANAERÓBICA DE SARGAZO CON RESIDUOS DE AVES (LIFE CYCLE ASSESMENT OF SARGASSUM ANAEROBIC CO-DIGESTION WITH POULTRY WASTE)

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I want to dedicate this thesis to "God" for giving me life, patience, and lifting me up on difficult days. To my parents, Ofelia and Lucano, for everything they did throughout my life; without them, I wouldn't have been able to get this far. To my siblings for being the example that things can be achieved with work, dedication, and effort. To my friends and colleagues.

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RESUMEN GENERAL

Análisis del ciclo de vida de la codigestión anaeróbica de sargazo con residuos de aves

El incremento continuo en la generación de residuos, asociado a las actividades antropogénicas, representa un desafío ambiental prioritario. Ante esta problemática, las estrategias de conversión de residuos en bioenergía han cobrado relevancia en los sectores económico, científico y social. En este contexto, la digestión anaeróbica se posiciona como una alternativa biotecnológica prometedora para el tratamiento sustentable de residuos agropecuarios, permitiendo la producción simultánea de biogás y digestato con valor agronómico. El objetivo de esta investigación fue evaluar los impactos ambientales asociados a la producción de biogás a través del análisis de cinco escenarios: monodigestión de pollinaza (línea base), codigestión de pollinaza con sargazo, codigestión de pavaza con sargazo, y los respectivos sistemas con recuperación de metano residual. Los ensayos se llevaron a cabo en reactores semicontinuos bajo condiciones mesofílicas (37 °C), alimentados con 3% de sólidos totales y un tiempo de retención hidráulica de 30 días. Se recopilaron datos experimentales de cada proceso unitario y se realizó un Análisis de Ciclo de Vida siguiendo el enfoque "de la cuna a la puerta", considerando 17 categorías de impacto mediante el método ReCiPe 2016. Los resultados mostraron que las estrategias de codigestión incrementaron la producción de biogás y redujeron los impactos ambientales en 15 de las 17 categorías analizadas, en comparación con la línea base. Además, la implementación de sistemas con recuperación de metano residual permitió una reducción del 97% en el Potencial de Calentamiento Global, destacando su valor como estrategia de mitigación climática. En conclusión, la combinación de codigestión con recuperación de metano residual no solo mejora la eficiencia energética del proceso, sino que también contribuye a un perfil ambiental más favorable, promoviendo modelos sostenibles de manejo de residuos dentro del marco de la economía circular.

Palabras clave: Bioenergía, Impactos ambientales, pollinaza, pavaza, ReCiPe.

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GENERAL ABSTRACT

Life cycle analysis of the anaerobic co-digestion of sargassum with poultry waste

The continuous increase in waste generation, associated with anthropogenic activities, has become a pressing environmental challenge. In response, strategies for converting waste into bioenergy have gained relevance across economic, scientific, and social sectors. In this context, anaerobic digestion has emerged as a promising biotechnological alternative for the sustainable treatment of agro-industrial residues, enabling the simultaneous production of biogas and nutrient-rich digestate. The aim of this study was to evaluate the environmental impacts associated with biogas production by analyzing five scenarios: poultry litter monodigestion (baseline), poultry litter-sargassum co-digestion, turkey litter-sargassum co-digestion, and the respective systems with residual methane recovery. Experiments were carried out in semi-continuous reactors under mesophilic conditions (37 °C), fed at 3% total solids, and operated with a 30-day hydraulic retention time. Experimental data from each unit process were collected, and a Life Cycle Assessment was conducted using a cradle-to-gate approach. Seventeen impact categories were assessed using the ReCiPe 2016 method. Results showed that co-digestion strategies increased biogas production and reduced environmental impacts in 15 out of the 17 categories compared to the baseline. Additionally, systems with residual methane recovery achieved a 97% reduction in Global Warming Potential, highlighting their contribution to climate change mitigation. In conclusion, the combination of co-digestion and methane recovery not only enhances the energy efficiency of the process but also results in a more favorable environmental profile. This approach supports sustainable waste management strategies and contributes to the development of circular economy models.

Keywords: Bioenergy, Environmental impacts, Chicken litter, Turkey litter, ReCiPe.

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1 GENERAL INTRODUCTION

Annually, approximately 2.01 million tons of waste is generated, and it is estimated that this figure will increase to 3.4 million tons by 2050 (Kaza et al., 2018). Inadequate management of this waste contributes to environmental impact, mainly through the emission of greenhouse gases (GHG). Therefore, it is essential to implement alternatives that favor its reduction and promote the generation of bioenergy and organic fertilizers in an efficient, economical, and environmentally friendly way (Ahmed et al., 2020). Anaerobic digestion (AD) is a technology with great potential for the treatment of organic waste and biomass (Tawfik et al., 2023), thanks to its multiple ecological benefits. These include odor reduction, reduction of gas emissions and its contribution to the promotion of a circular economy (Duan et al., 2025). To ensure optimal AD performance, it is essential to control parameters such as pH, temperature, alkalinity, carbon to nitrogen ratio (C/N), substrate, organic loading rate (OLR), and hydraulic retention time (HRT) (Gyadi et al., 2024). To optimize the process, anaerobic co-digestion is employed, which involves combining two or more substrates with the objective of improving nutrient availability, balancing inhibitory conditions, optimizing the C/N ratio, and enhancing the synergy of microorganisms. This enables the production of higher yields in biogas and methane production (Liu et al., 2023; Pérez et al., 2024). In this context, organic wastes and residual biomass, such as wastewater, plant material, agricultural products, and manure, have become key elements for the development of clean technologies (Jameel et al., 2024). End products include biofuels, biohydrogen, and biogas, the latter with the potential to replace fossil fuels in heat and power generation (Duarah et al., 2022; Ghaedi & Rahimpour, 2024).

Sargassum and poultry waste represent an environmental challenge and an opportunity for their utilization as biomass through anaerobic co-digestion, becoming key elements in the transition to a sustainable economy (Al-Zoubi et al., 2024). Poultry litter is identified as a source of organic waste, including feathers, droppings, and straw. If not managed properly, it can lead to eutrophication and water pollution, resulting in serious environmental issues.

On the other hand, Sargassum is a marine macroalgae that proliferates in the Caribbean Sea and whose presence has increased significantly in recent years (Maldonado-Rodríguez & Méndez-Reyes, 2023). Its massive arrival has negative effects on marine ecosystems, beaches, and tourism activities (Chávez et al., 2020; Rodriguez-Martinez et al., 2021). However, their accumulation has opened new opportunities for their utilization. Like poultry waste, sargassum can be processed to generate sustainable products and serve as an alternative source of renewable energy (Thompson et al., 2020).

1.1 General objective

This study evaluated the environmental impacts of biogas production from various anaerobic digestion configurations using three organic matter-rich substrates: turkey litter, chicken litter, and Sargassum. It compared five scenarios against the baseline of chicken litter monodigestion without methane recovery through a life cycle assessment methodology. The findings could significantly influence decision-making for sustainable biogas production systems, which are vital for climate change mitigation and the implementation of circular economy principles, particularly in areas such as the Yucatán Peninsula.

1.2 Specific objectives

- To develop the mass and energy balance for the chicken litter monodigestion (baseline), and the 5 different scenarios: co-digestion chicken litter with Sargassum (Sce1), co-digestion turkey litter with Sargassum (Sce2), chicken litter monodigestion with recovery of methane residual (Sce3), co-digestion chicken litter with Sargassum and recovery of methane residual (Sce4), co-digestion turkey litter with Sargassum and recovery of methane residual (Sce5), data were collected for each unit process within the defined system boundaries to build the life cycle inventory.

- To quantify the environmental impacts associated with biogas production based on baseline and different scenarios, by applying the ReCiPe 2016 Midpoint methodology, to identify the most relevant impact categories.
- To compare the environmental and energy sustainability of co-digestion of sargassum with poultry waste by applying the Energy Return on Investment (EROI) index and Principal Component Analysis (PCA) to identify the most sustainable configurations.

1.3 Hypotheses

The arrival of Sargassum and poultry waste can be utilized as biomass through anaerobic co-digestion, serving as a technological alternative to increase biogas and methane production compared to monodigestion. Also, co-digestion of these substrates will improve the energy return on investment compared to the baseline approach, and life cycle assessment will determine the environmental impacts. Methane recovery will also contribute to improving energy efficiency.

1.4 Thesis chapters

The present work is divided into four chapters as follows:

Chapter 1. This chapter provides a general introduction to the research problem, reviewing key concepts related to the topics addressed in the following sections. Additionally, the most relevant general and specific objectives that inform the hypothesis of this work are presented.

Chapter 2. The state of the art is presented, and each of the most relevant topics within the current literature is detailed. These chapters include data on biomass residues, anaerobic co-digestion, the most relevant physicochemical parameters of the process, bioenergy, biogas, and digestate. With an environmental and sustainable approach.

Chapters 3 and 4. This section focuses on the analysis of the life cycle in anaerobic digestion. The main objective of this chapter is to evaluate and compare the environmental impacts associated with the production of biogas from Sargassum, turkey litter, and chicken litter, considering three different strategies: monodigestion, co-digestion, and residual methane recovery. For this purpose, Life Cycle Assessment is used to identify the most environmentally and energetically sustainable options.

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2 LITERATURE REVIEW

2.1 Poultry production in Mexico

The national poultry industry is one of the most important sectors within the Mexican food industry. According to the Union Nacional de Avicultores (UNA, 2021), Mexico ranks fifth worldwide in chicken production, and nationally, the state of Yucatán ranks fourth and fifth in chicken and egg production. According to data obtained by the Servicio de Información Agroalimentaria y Pesquera (SIAP, 2023), in the Yucatán Peninsula, chicken production increased 23% between 2018 to 2023 (Figure 1), driven by the growing demand for meat. Annual chicken consumption in Mexico is 33.5 kg per capita (UNA, 2021). Williams (2024) estimates that 1000 chickens generate between 1.1 and 2.4 tons of excrement accompanied by straw and wood shavings, during their entire production cycle until they obtain meat for sale. The generation of excrement from this production system generates 98.67 tons of residual biomass per year in Yucatán. On the other hand, turkey production (Figure 1) has increased by 33% (SIAP, 2023), and it is estimated that 1000 turkeys generate between 7.3 and 12.7 tons of excrement accompanied by straw and wood chips.

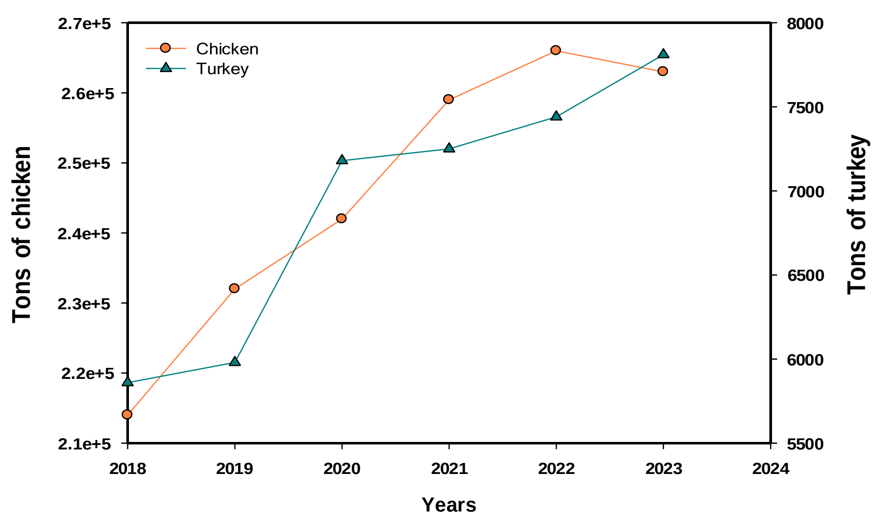

Figure 1. Tons of chicken and turkey production in the Yucatán Peninsula from 2018 to 2023.

Source: Servicio de Información Agroalimentaria y Pesquera SIAP (2023).

In this case, the generation of excrement during the production process reaches 18.44 tons of residual biomass per year in Yucatán (Williams, 2024).The accumulation of chicken and turkey droppings not only poses economic challenges, but also generates serious environmental problems as eutrophication and water pollution.

2.2 Sargassum

Pelagic Sargassum is a brown macroalgae of the genus Sargassum, named after the Sargasso Sea in the North Atlantic. This biomass is found on the surface of the beaches of the Mexican Caribbean (Thompson et al., 2020).In the Yucatán Peninsula, three species have been identified (Figure 2): *Sargassum pelagicus* and *Sargassum natans*, which are characterized by smooth shafts, linear leaves and spherical vesicles with spines. *Sargassum fluitans* has spiny axes, ovoid leaves and smooth vesicles (Vazquez-Delfin et al., 2021).

Figure 2. *Sargassum Pelagicus* from the Mexican Caribbean (A-B), *Sargassum natans* (C-D), and *Sargassum fluitans* (E-F).

Source: Vazquez-Delfin et al. (2021).

The components of Sargassum (Table 1) include macro and micro elements, as well as complex molecules such as carbohydrates and lipids. Their composition varies according to the genus and the environment in which they are found (Nava-Jiménez et al., 2022).

Table 1. Elemental analysis of Sargassum in percentage (%) of dry weight.

Species	C	H	N	S	C / N
Sargassum sp.	29.73±0.01	4.32±0.01	3.13±0.15	1.13±0.08	-
S. consortium	31.87±1.82	4.92±0.09	1.16±0.02	0.98±0.03	27.51
S.natans and S.fluitans	30.1±1.2	-	1.1±0.1	-	26.9±1.2

Source: Azcorra-May et al. (2022).

Sargassum arrivals to the Mexican Caribbean coasts between 2018 and 2023 (Figure 3), has reported an annual average of 197,708 tons (Maldonado-Rodríguez & Méndez-Reyes, 2023).

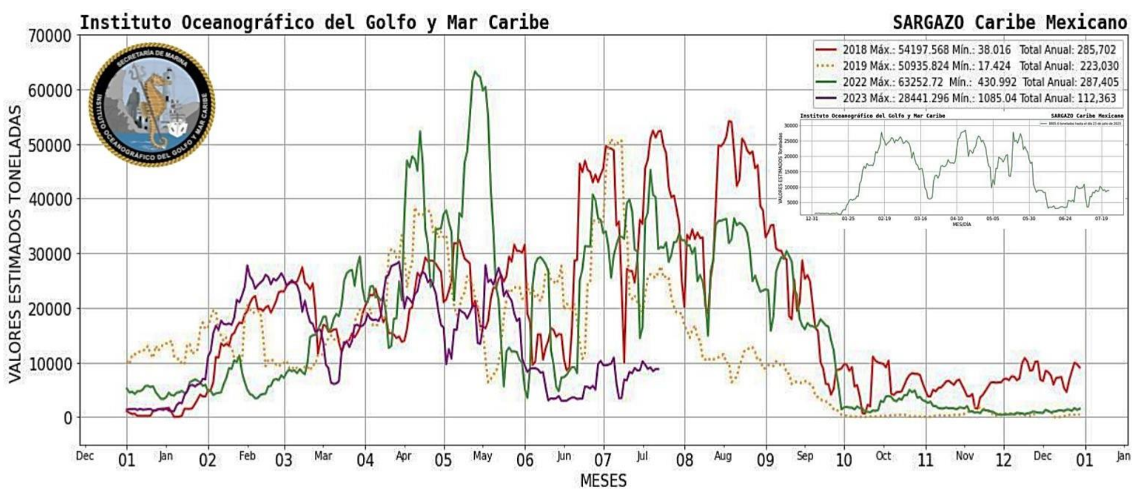

Figure 3. Prevalence of Sargassum in the Yucatán Peninsula.

Source: Maldonado-Rodríguez and Méndez-Reyes (2023).

The effects associated with Sargassum arrivals on ecosystems (Figure 4) can hinder access to sea turtle nesting areas (Rodriguez-Martinez et al., 2021), produce leachate, debris, bad odors, contamination of subway rivers, and negative visual impact on beaches (Chávez et al., 2020).

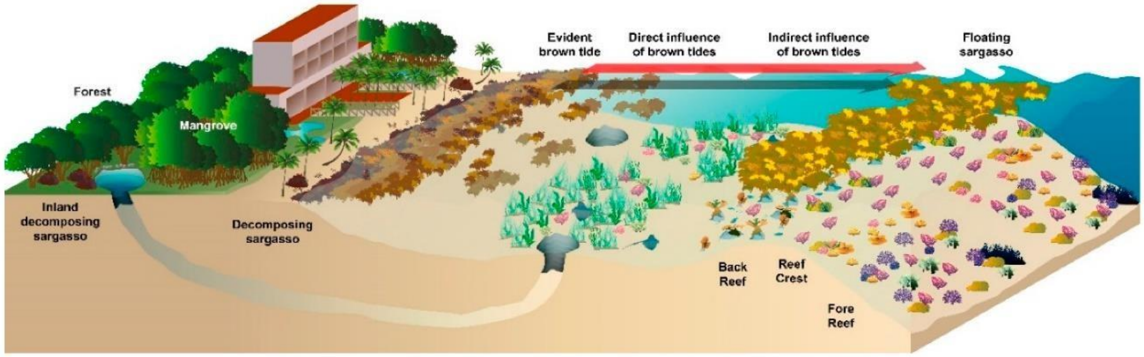

Figure 4. Effects associated with Sargassum accumulation.

Source: Chávez et al. (2020).

2.3 Anaerobic digestion

Waste is constantly generated worldwide, approximately 683 million tons of waste is produced by high-income countries, and this figure is expected to increase by 19% by 2050 (Table 2). In contrast, low-income countries account for only 5 % of total global waste, this amount is projected to triple by 2050 (Kaza et al., 2018).

Table 2. Annual waste generation potential by region.

Region	Waste generation by millions of tons per year			Waste generation per capita (kg/capita/day)		
	2016	2030	2050	2016	2030	2050
Middle East and North Africa	129	177	255	0.81	0.9	1.06
Sub-Saharan Africa	174	269	516	0.46	0.5	0.63
Latin America and the Caribbean	231	290	396	0.99	1.11	1.3
North America	289	342	396	2.21	2.37	2.5
South Asia	334	466	661	0.52	0.62	0.79
Europe and Central Asia	334	466	490	1.18	1.3	1.45
East Asia and the Pacific	468	602	714	0.56	0.68	0.81

Source: Kaza et al. (2018).

Many types of waste can be recycled, reused, or recovered to support a circular economy (Duan et al., 2025).

Among the various waste treatment methods, anaerobic digestion (AD) (Figure 5) is considered an ideal process due to its ecological benefits, reduction of unpleasant odors, pathogen accumulation, and greenhouse gas emissions.

Furthermore, it offers social and economic advantages, as well as energy savings (Duan et al., 2025). AD generates digestate and biogas, which accounts for more than 35% of the bioenergy produced from waste biomass sources (Fardin et al., 2018). Biogas is a gas mixture composed primarily of methane and carbon dioxide. Methane can be used as a direct source of heat, power, and electricity (Nguyen et al., 2021).

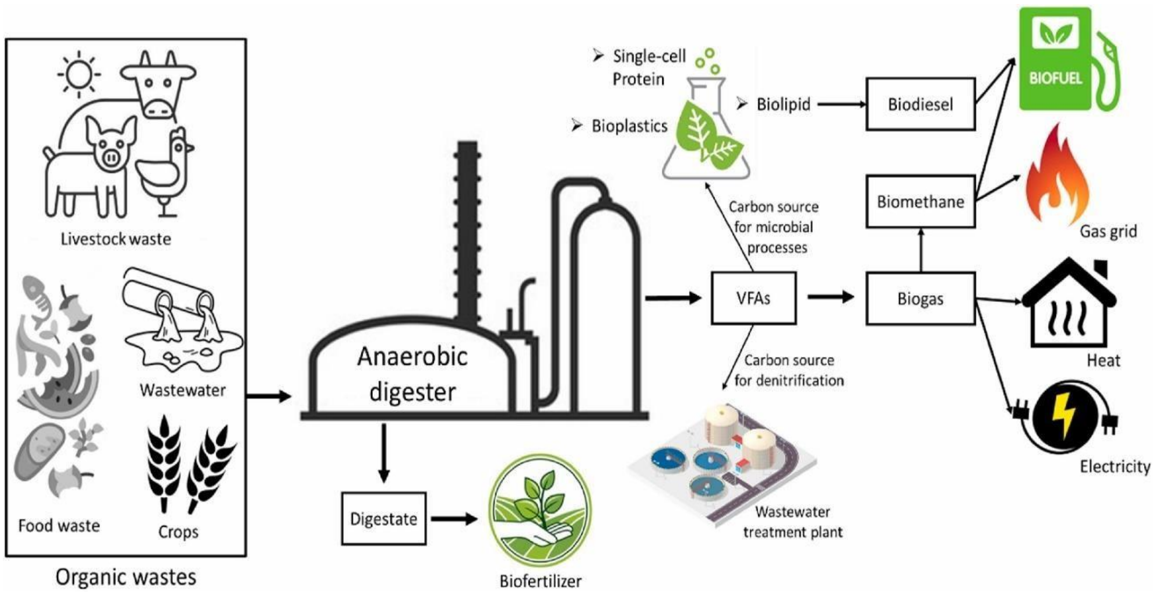

Figure 5. Benefits of using anaerobic digestion for organic residues and/or waste.

Source: Duan et al. (2025).

In addition to producing methane during the AD process, another advantage is the production of various carbon sources, specifically volatile fatty acids (VFAs) such as acetic acid, propionic acid, butyric acid, valeric acid and caproic acid. This can be achieved by inhibiting methane-producing microorganisms (Harirchi et al., 2022).

VFA generated through AD have the potential to contribute to the development of a circular economy by supporting microbial processes that lead to the production of biofuels, polymers and biomass (Ramos-Suarez et al., 2021). Digestate, or AD residue, is a nutrient-rich material produced during the biogas fermentation process, which recirculates to the soil as a biofertilizer. Digestate can be found in different forms: liquid (with less than 4 % dry matter), semi-solid (10-20 % dry matter) and slurry (4-20 % dry matter) (Dahlin et al., 2017). The composition of digestate includes 30-50 % organic matter, 10-20 % microorganisms and various macro- and micronutrients. The nutrients present in digestate, specifically nitrogen (N: 0.5-1.5 %), phosphorus (P: 0.3-0.7 %) and potassium (K: 0.4-1.3 %), improve soil quality when used as an organic fertilizer. Compared to raw manure, digestate is more homogeneous in nature and contains a higher amount of inorganic nitrogen, which helps balance the nitrogen-phosphorus (N-P) cycle. This application has been shown to increase soil carbon stocks and improve pH (Ogunwo, 2022). This makes it a viable substitute for traditional fertilizers in agricultural applications. AD has become a widely used technology in many European countries (Edwards et al., 2015), and is gaining interest in other regions (Moustakas et al., 2020), particularly in developing countries. The major attraction of AD is that it generates renewable energy through organic waste management (Khalid et al., 2020) and digestate suitable for use as a better quality organic fertilizer.

2.3.1 Anaerobic digestion process

The AD process is highly complex consisting of simultaneous phases of hydrolysis, acidogenesis, acetogenesis, and methanogenesis involving biochemical, chemical, and physicochemical conversion processes seen in (Figure 6). The biochemical processes in the liquid phase relate to several steps of biochemical metabolism (Xu et al., 2015), including disintegration, hydrolysis (Kegl, 2022), acidogenesis, acetogenesis, and methanogenesis (Van et al., 2020), which are driven by the coordinated, sequential, and collaborative activities of diverse microbial communities under anaerobic conditions (Kegl & Kralj, 2020). Chemical processes occurring in a bioreactor mainly involve liquid-liquid interactions, including dissociation-association processes, complex formation processes facilitated by synthetic chelating agents, adsorption, and redox reactions (Kegl & Kralj, 2020). In contrast, physicochemical processes are mainly associated with liquid-solid interactions, such as precipitation and dissolution, and liquid-gas mass transfer processes (Vaneeckhaute et al., 2018).

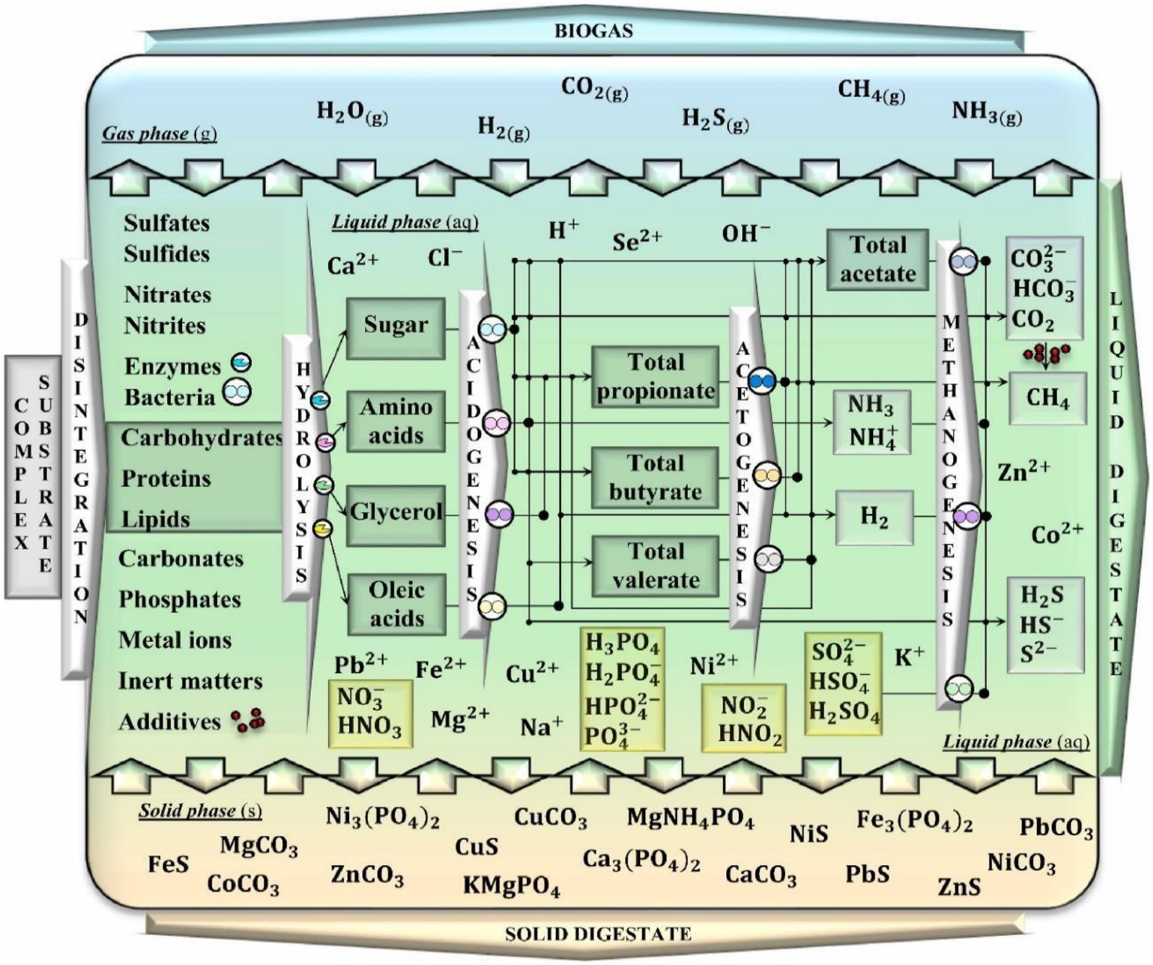

Figure 6. Conversion processes occurring in anaerobic digestion.

Source: Kegl et al. (2025).

2.3.1.1 Hydrolysis

During the disintegration step of the AD process, organic matter is broken down into biodegradable macro components that are solid substrate particles, such as: carbohydrates, proteins represented by collagen and lipids (Kegl & Kralj, 2022). The subsequent hydrolysis step involves further degradation of these macro components to monomers which are soluble components: carbohydrates are converted into sugars; proteins into gelatin consisting of various amino acids including taurine, hydroxyproline, aspartic acid, threonine, serine, glutamic acid, proline, lanthion, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, hydroxylysine, histidine, ornithine, lysine, arginine, and tryptophan; and lipids are transformed into glycerol and various long-chain fatty acids, such as oleic, linoleic and palmitic acid (Kegl & Kralj, 2022).

The products of the hydrolytic reactions are further converted into carbon dioxide (CO_2), ammonia (NH_3), hydrogen sulfide (H_2S) and short-chain volatile fatty acids (SCFAs) (Angelidaki et al., 1999), such as acetic acid ($C_2H_4O_2$), propionic acid ($C_3H_6O_2$), butyric acid ($C_4H_8O_2$) and valeric acid ($C_5H_{10}O_2$) (Mackie & Bryant, 1981) by specific acidogenic bacteria, which grow rapidly and can thrive in extreme conditions (Emebu et al., 2022).

The disintegration and hydrolysis steps depend on the activity of extracellular enzymes such as lipases, proteases, celluloses and amylases (Kegl & Kralj, 2022) that are synthesized and secreted by acidogenic bacteria (Zhang et al., 2024). In general, hydrolysis is a relatively slow process, although it can be accelerated through appropriate pretreatment methods (Lauwers et al., 2013).

2.3.1.2 Acidogenesis

Involving SCFAs carried out by acetogenic bacteria, these reactions produce a small amount of methane (CH_4) (Mackie & Bryant, 1981). Numerous enzymes are involved in this metabolic step (Feng et al., 2022) and the hydrolyzed monomers undergo several enzymatic reactions (Zhang et al., 2024). First, pyruvate formate lyase (PFL) catalyzes the conversion of pyruvate to Acetyl-CoA. After this, a series of key enzymes, including phosphotransacetylase (PTA), acetate kinase (AK), propionyl-CoA transferase (PCT), phosphotransbutyrylase (PTB) and butyrate kinase (BK), sequentially convert acetyl-CoA to acetic acid, propionic acid and butyric acid (Feng et al., 2009).The enzyme oxaloacetate trans carboxylase (OAATC) plays a crucial role in propionic acid synthesis by catalyzing the conversion of pyruvate to oxaloacetic acid and methylmalonyl-CoA to propionyl-CoA. In addition, pyruvate can be converted to lactic acid by NAD-dependent lactate dehydrogenases (LDH), whereas NAD-independent lactate dehydrogenases (ILDH) catalyze the reverse reaction. Therefore, lactic acid intermediates, acetyl-CoA and succinate can be used for propionic acid production (Zhou et al., 2018). This process also generates hydrogen (H_2) and carbon dioxide (CO_2). Several hydrolytic bacteria and acidogenic bacteria are involved in the process. Their combined actions, together with bacterial secreted enzymes, regulate the concentration and distribution of different organic acids (Zhang et al., 2024).

2.3.1.3 Acetogenesis

The process of acetogenesis is a vital biochemical pathway that transforms acidification products into valuable compounds such as acetate, hydrogen (H_2) and carbon dioxide (CO_2) (Zhang et al., 2024). This intricate process is crucial to keep the process balanced. It can only take place under specific conditions characterized by low hydrogen (H_2) partial pressure, which is necessary to facilitate the effective conversion of acidogenesis substrates (Keskin et al., 2018), contributing to energy recovery (Yang & Wang, 2018).

2.3.1.4 Methanogenesis

Several conventional biosynthetic pathways for methane (CH_4) formation have been identified based on different substrates such as acetate ($C_2H_4O_2$), hydrogen (H_2), carbon dioxide CO_2, and methyl compounds (Zhang et al., 2024). Acetotrophic methanogens ferment acetic acid ($C_2H_4O_2$) into methane (CH_4) and carbon dioxide (CO_2), whereas hydrogenotrophic methanogens use hydrogen (H_2) to convert carbon dioxide (CO_2) into methane (CH_4) (Zhao et al., 2016). Methanogens are particularly sensitive to environmental conditions and exhibit the slowest growth rates among all microbial consortia involved in AD (Van et al., 2020). Furthermore, sulfate-reducing bacteria contribute to the formation of hydrogen sulfide (H_2S) from hydrogen (H_2), acetic acid ($C_2H_4O_2$), and sulfate (SO_4^{2-}) (Kegl & Kralj, 2022).

2.4 Factors affecting the anaerobic digestion process

Critical parameters during anaerobic digestion include pH, alkalinity, temperature, feedstock, Organic Loading Rate (OLR), Hydraulic Retention Time (HRT), Volatile Fatty Acids (VFAs), sulfide, ammonia, and carbon/nitrogen (C/N) ratio (Figure 7). Each of these factors plays a fundamental role in the efficiency and stability of the process. They are classified into four groups (Figure 8): (1) waste pretreatment parameters, (2) feedstock composition and feed strategy parameters, 3) condition parameters, and (4) additive parameters. To ensure optimal AD performance, it is critical to determine the optimal values for these key parameters (Issah et al., 2020).

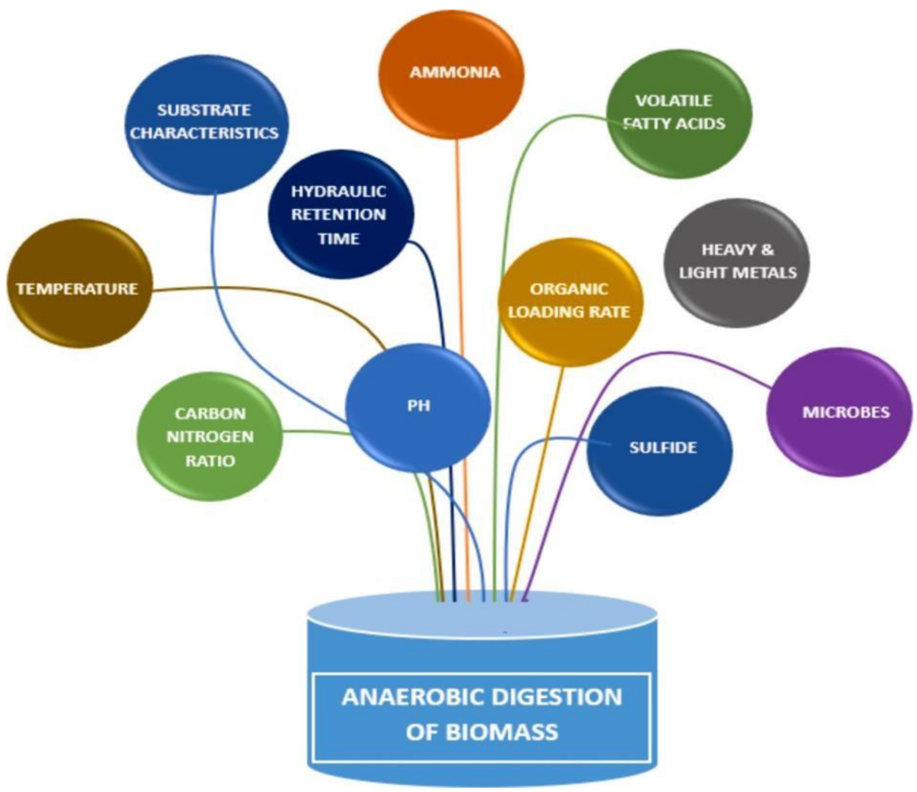

Figure 7. Various factors affecting anaerobic digestion.

Source: Gyadi et al. (2024).

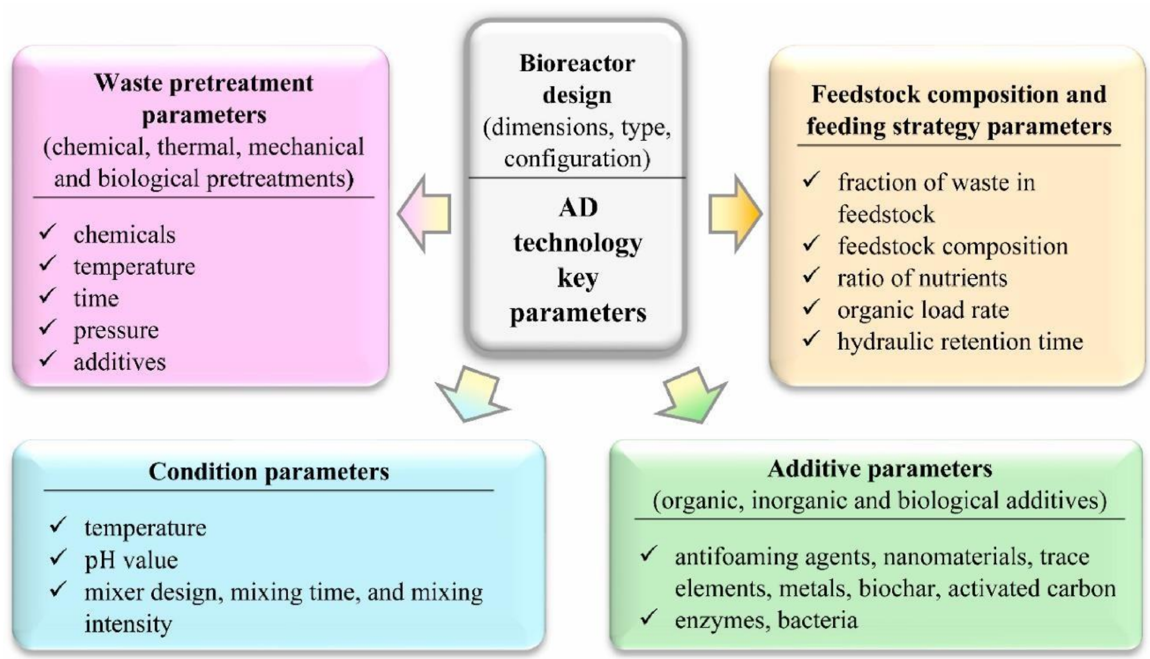

Figure 8. Classification of key anaerobic digestion parameters for good performance.

Source: Kegl et al. (2025).

2.4.1 pH

Each microbial group has an optimal pH range for each stage of AD processes; pH values outside this range can be detrimental to anaerobic bacteria and their activity (Issah et al., 2020). A pH range of 5.5-6.5 is most favorable for hydrolysis and acidogenesis. Acetogenesis and Methanogenesis thrive best within the pH range of 7.0-8.5. Furthermore, pH plays a crucial role in minimizing the inhibitory effects of VFAs and ammonia (Kegl & Kralj, 2020).

2.4.2 Alkalinity

Alkalinity is defined as the ability of an aqueous solution to neutralize acids. Substrates with high alkalinity can help maintain a pH of 7, which is optimal for methanogenesis, counteracting the carbon dioxide (CO_2) produced during AD, which forms carbonic acid in the liquid phase and VFAs (Neshat et al., 2017).

2.4.3 Temperature

Temperature is one of the most important factors in AD, due to its effect on microbial growth and activity, process kinetics, and stability (Khalid et al., 2011). There are three temperature ranges commonly used in AD: psychrophilic (0-25°C), mesophilic (20-45°C), and thermophilic (>50°C). The most commonly used temperatures are mesophilic and thermophilic. New insights have been found into the interaction of raw material composition and temperature in psychrophilic conditions to improve AD performance, with application to temperate and cold regions of developing countries (Gabbanelli et al., 2024).

2.4.4 Feedstock/Substrates

Raw material can contain a wide variety of simple and complex organic compounds. Depending on their sources, such as agricultural waste, municipal waste, food waste, or industrial waste, certain organic compounds may predominate, potentially affecting the AD process.

Song et al. (2021) mentioned that high levels of carbohydrates can lead to a rapid accumulation of VFAs in the reactor, generating a decrease in pH and suppressing methanogenesis. In contrast, protein-rich substrates tend to be energy-dense and produce a relatively high amount of (CH_4). However, high ammonia concentrations can inhibit microbial growth and activity, which can lead to process instability. Furthermore, the presence of fat-rich organic compounds can cause microbial inhibition and problems with mass transfer. Therefore, maintaining an appropriate balance of carbohydrate-rich, protein-rich, and fat-rich organic materials is crucial (Hagos et al., 2017).

This balance helps achieve the necessary nutrient levels, promotes beneficial microbial populations, reduces inhibitor buildup, and improves the stability and efficiency of biogas production and CH_4 yield.

2.4.5 Organic Loading Rate

The Organic Loading Rate (OLR) refers to the amount of organic solids loaded into the system per unit volume per time and significantly influences the viability and optimal activity of microorganisms (Neshat et al., 2017). The introduction of a high concentration of OLR into the system can cause disturbances that lead to hyperactivity of hydrolyzed and acidogenic bacteria. This results in the accumulation of VFAs in the medium, which can inactivate methanogenic microorganisms due to acidification and lead to an inefficient AD process.

2.4.6 Hydraulic Retention Time

Hydraulic Retention Time (HRT) represents the average duration that the organic substrate remains within the reactor (Kainthola et al., 2019). HRT directly influences AD efficiency. Lower HRTs increase the risk of active biomass leaching. Conversely, a higher HRT can lead to the need for larger reactor volumes, resulting in a design that may be less economical and efficient (Achinas et al., 2020).The optimal HRT is not a one-size-fits-all measurement; it varies depending on several key factors. These include the specific bioreactor design and construction, the type of feedstock being processed, and various operating parameters, such as temperature and pH. Importantly, the influence of HRT on AD process efficiency is highly dependent on the nature of the substrate being used. Therefore, determining the ideal HRT is essential for each biogas plant and is typically unique to its specific operating conditions and objectives.

2.4.7 Volatile Fatty Acids

Hydrolytic and acidogenic microorganisms can be inhibited by high concentrations of VFAs and free ammonia (Van et al., 2020). VFAs deionize at low pH, making them more toxic to the cell membrane (Amani et al., 2010). It has been reported that VFA concentrations between 40 and 50 g COD L^{-1} at low pH levels such as 5.0-5.5 can halt the hydrolysis process (Veeken & Hamelers, 2000).

2.4.8 Sulfide and Ammonia

Sulfide and ammonia can inhibit the AD process. Koster and Lettinga (1988) reported that acidogenesis is significantly affected by ammonia concentrations ranging from 4,051 to 5,734 mg NH_{3-N} L^{-1}.

2.4.9 Carbon/Nitrogen (C/N) Ratio

Microorganisms require carbon and nitrogen in any growth environment. The optimal C/N ratio for microbial growth varies; however, a range of 15-30 (Risberg et al., 2013) or 10-40 (Van et al., 2020) is ideal. Suitable substrates for AD contain carbon (C) and nitrogen (N). However, high levels of N in waste can negatively affect digester performance due to ammonia buildup (Tsapekos et al., 2015). Therefore, achieving a balanced C/N ratio is essential for effective biogas production. An optimum C/N ratio of 30/1 or 20/1 is recommended for efficient digester performance without inhibiting the process (Sasse, 1998). Deviation from this optimum C/N ratio can negatively affect biogas production. Higher C/N ratios can lead to nitrogen deficiency, which hinders microbial growth and ultimately results in lower methane production due to the inhibition of methanogens (Kayhanian, 1999). Conversely, lower C/N ratios can restrict microbial growth due to insufficient carbon, leading to the accumulation of ammoniacal nitrogen and VFAs in the digester (Parkin & Owen, 1986).

2.5 Co-digestion

Co-digestion is the mixing of two or more substrates aimed at balancing environmental conditions and physicochemical parameters and/or maintaining an ideal C/N ratio (Issah et al., 2020) as seen in Figure 9 (Pérez et al., 2024). Co-digestion can improve methane production by 25-400 % (Shah et al., 2015) and is a means of complete optimization of the AD process. By introducing one or more co-substrates, the AD process can be optimized due to the increased availability of macro- and micronutrients (Liu et al., 2023).

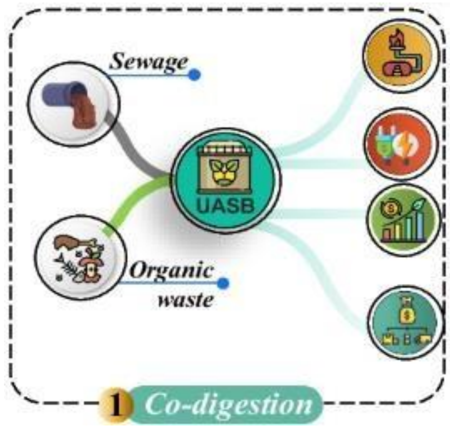

Figure 9. Anaerobic co-digestion.

Source: Pérez et al. (2024).

Figure 10 shows the advantages of anaerobic co-digestion (AcoD) compared to single-substrate AD (Karki et al., 2021). AcoD improves biogas production and energy production by volume by increasing nutrient availability for microbial metabolism and optimizing organic load (Liu et al., 2022).Furthermore, AcoD helps mitigate the effects of inhibitory chemicals and reduces the presence of recalcitrant compounds by diluting them through the incorporation of substrates (Ren et al., 2023).

Figure 10. Advantages of anaerobic co-digestion (AcoD) over single-substrate anaerobic digestion.

Source: Pérez et al. (2024).

2.6 Final products of anaerobic digestion

2.6.1 Biogas

Biogas is the end product of biomass decomposition through AD. Its main content is 50-70% CH_4, 30-50% CO_2 and other gases such as hydrogen sulfide (H_2S) (Jameel et al., 2024). Optimizing the physicochemical parameters of temperature, pH, C/N ratio and substrate are critical in the stability of the system to improve the production of methane-rich biogas (Gyadi et al., 2024). Waste management for biogas production promotes the renewable energy sector and reduces climate change (Archana et al., 2024).

2.6.2 Digestate

Digestate is a byproduct of AD and is rich in elements such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and potassium (K), playing an important role in crop growth. Its pH ranges between 7 and 8.5, indicating a slightly alkaline nature (Cao et al., 2019; Chiew et al., 2015). With appropriate recovery techniques, they could be transformed into potential renewable resources and close nutrient cycles, focusing on an environmental context and a circular economy (Kovalev et al., 2021). Digestate is usually separated into solid and liquid fractions using solid-liquid separation techniques. The liquid fraction is usually used for algae cultivation. The solid fraction is used as biofertilizer, biostimulation, bioremediation, and when treated by thermochemical processes, biofuels, cogeneration, and biochar are extracted (Figure 11) (Habchi et al., 2024; Wang & Lee, 2021).

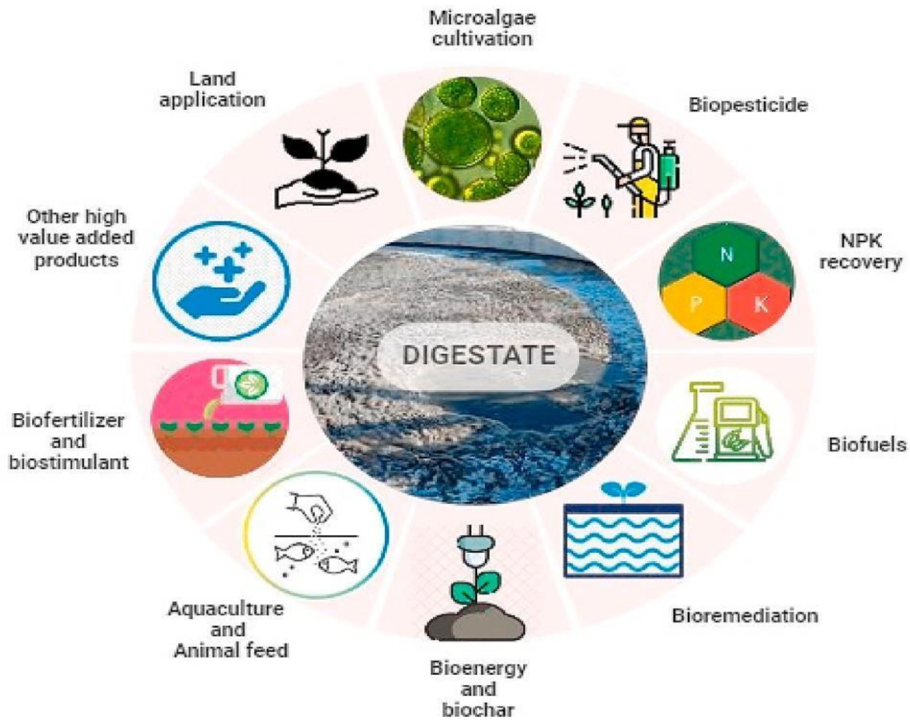

Figure 11. Digestate valorization routes.

Source: Habchi et al. (2024).

2.7 Bioenergy

Energy generation derived from fossil fuels constitutes one of the main sources of carbon dioxide (CO_2) emissions, being responsible for approximately 75% of greenhouse gases, which contributes significantly to climate change (Osman et al., 2022). Faced with this scenario, there is a need to develop sustainable energy alternatives that allow reducing dependence on fossil fuels. In this sense, renewable energy sources, such as solar, wind, hydraulic, geothermal and biomass-based, represent viable options to mitigate environmental impact and renewable energy (Figure 12) (Nazir et al., 2020).

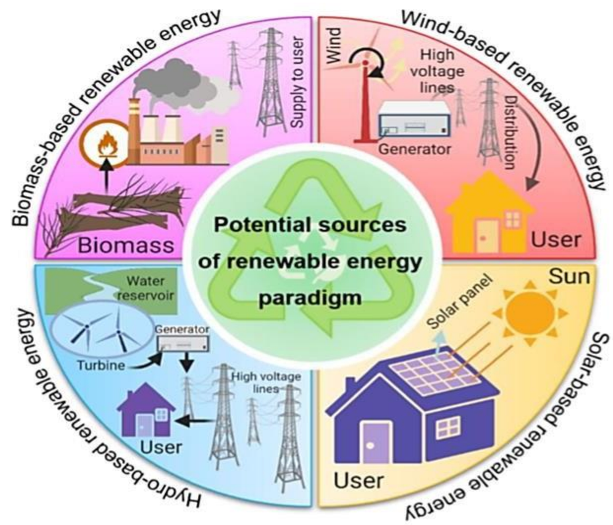

Figure 12. Natural sources: wind, solar, hydrothermal, and biomass.

Source: Nazir et al. (2020).

Among natural renewable energy sources, biomass stands out for its potential for bioenergy generation. The types of biomass used for this purpose come from a variety of sources (Figure 13), including liquid waste (municipal and industrial wastewater) and organic solid waste such as agricultural waste, food waste, and animal manure (Ahmed et al., 2020).

Figure 13. Different biomass sources.

Source: Gyadi et al. (2024).

The main techniques for transforming biomass into bioenergy are presented in (Figure 14). These are divided into three main categories: direct use of biomass, thermochemical processes, and biochemical processes. Thermochemical conversion consists of the decomposition of organic compounds in biomass through the application of heat. It includes processes such as pyrolysis, gasification, liquefaction, and direct combustion (Ghaedi & Rahimpour, 2024; Mancuso et al., 2024). On the other hand, biochemical conversion uses microorganisms or enzymes to transform biomass into energy, through processes such as fermentation and AD (Archana et al., 2024; Osman et al., 2024). Bioethanol can be obtained through fermentation, while transesterification allows the production of biodiesel from vegetable oils or animal fats. Both biofuels can be used in internal combustion engines to generate energy. Biomass can also be converted into gaseous forms of bioenergy, such as biohydrogen, biomethane, and biogas, which can be used to generate heat and electricity (Ghaedi & Rahimpour, 2024). Depending on the type of conversion and end use, the resulting bioenergy can be classified into three main applications: heat production, biofuel production, and electricity generation (Duarah et al., 2022).

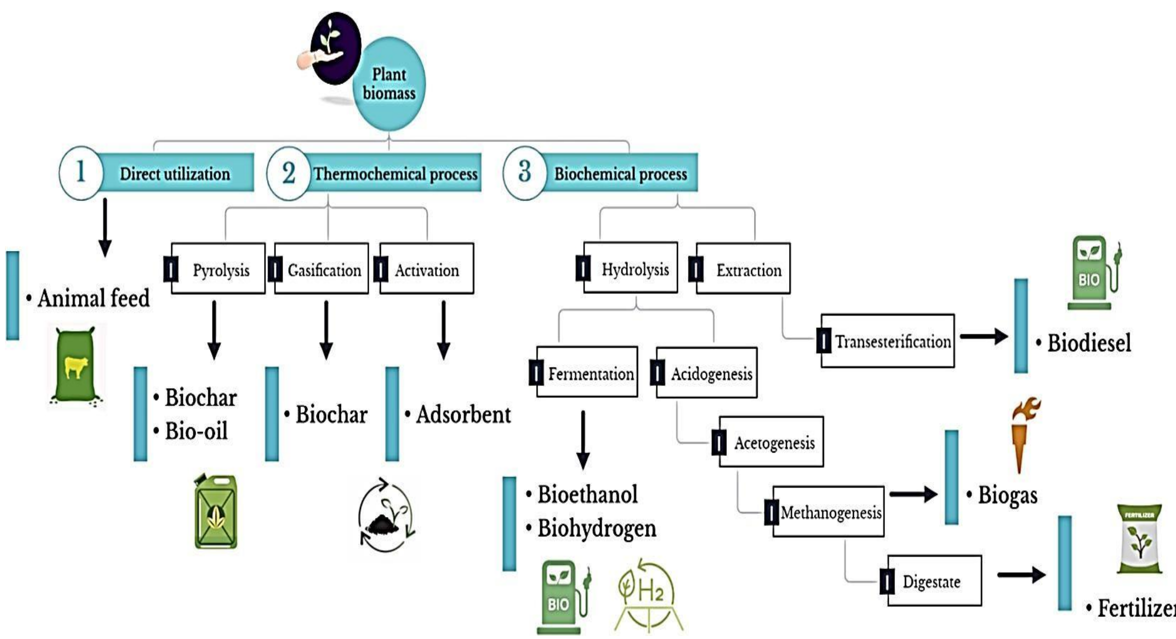

Figure 14. Natural biomass-based energy to be transformed into bioenergy, conversion routes, and final products.

Source: Mancuso et al. (2024).

2.8 Life Cycle Assessment in anaerobic digestion.

Life Cycle Assessment (LCA) and anaerobic digestion have become fundamental tools in contemporary approaches to environmental sustainability, particularly in the waste management and energy production sectors. AD, specifically, is a biological process by which microbial communities decompose organic matter under anoxic conditions, resulting in the generation of biogas, a renewable energy source, and digestate, which can be used as agricultural fertilizer, thus closing the nutrient cycle and contributing to the circular economy (Chew et al., 2021). Life Cycle Assessment (LCA) is a methodological tool that allows quantifying the environmental impacts associated with all stages of the life cycle of a product, process, or service, using various approaches (Mulya et al., 2022). In recent years, LCA has established itself as one of the most recognized and rigorous methods for comprehensively assessing the potential environmental impacts associated with AD (Ahmed et al., 2020). Below is a description of the fundamental concepts established by the ISO 14040 (ISO, 2016) standard:

Product system: collection of materially and energetically connected unit processes which performs one or more defined functions

Functional unit: quantified performance of a product system for use as a reference unit in a life cycle assessment study

Input: material or energy which enters a unit process

Output: material or energy which leaves a unit process

System boundary: interface between a product system and the environment or other product systems

Unit process: smallest portion of a product system for which data are collected when performing a life cycle assessment

Waste: any output from the product system which is disposed of

Process: set of mutually related or interacting activities, which transform input elements into results

Coproduct: Any product between two or more products coming from the same unit process or product system.

Raw material: Primary or secondary material used to make a product.

Elementary flow: Matter or energy entering the system under study, extracted from the environment without prior human transformation, or matter or energy leaving the system under study, released into the environment without further human transformation.

Energy flow: Input or output of a unit process or product system, expressed in energy units.

Intermediate flow: Flow of product, matter, or energy that occurs between unit processes of the product system under study.

Intermediate product: Output of a unit process that is an input to other unit processes and requires further transformation within the system.

2.8.1 Stages Life Cycle Assessment

LCA is a methodology standardized by the International Standards Organization ISO 14040 (ISO, 2016). Figure 15 presents the four LCA stages: a) objective and scope definition, b) life cycle inventory (LCI), c) life cycle impact assessment (LCIA), and d) interpretation.

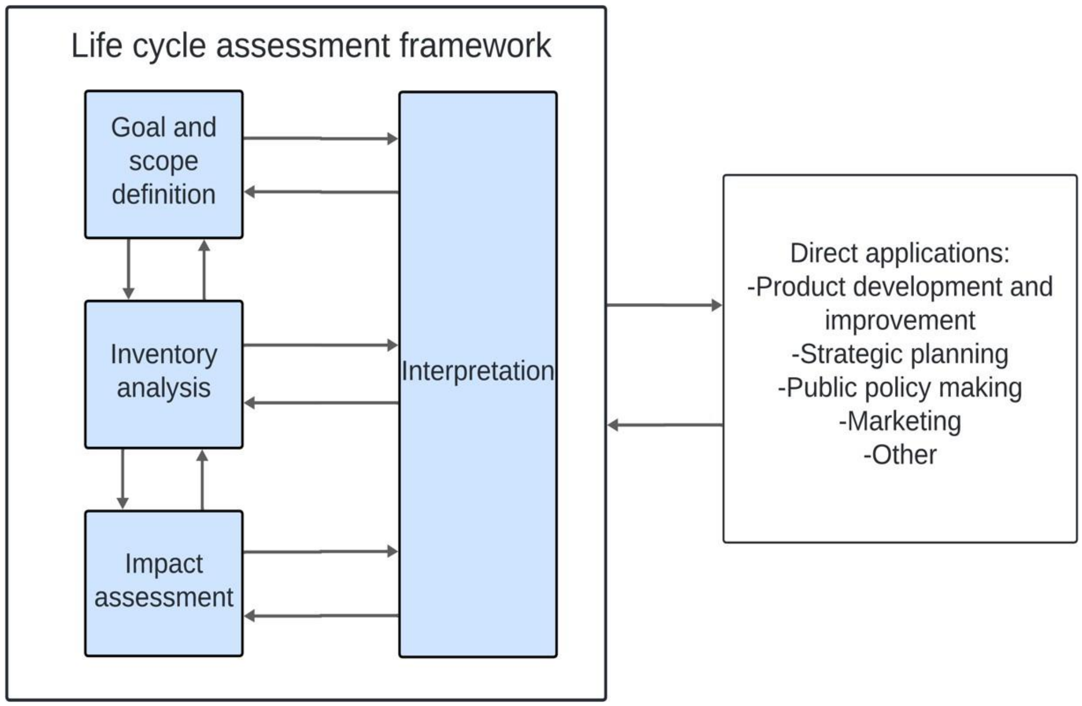

Figure 15. Stages of Life Cycle Assessment.

Modified from Mehmeti et al. (2016).

2.8.1.1 Definition of objective and scope

According to ISO 14040 (ISO, 2016), the definition of the objective in a life cycle assessment (LCA) study establishes the purpose of the analysis, its intended application, the intended audience, and the dissemination of results. The scope defines the product system to be assessed, defines the system boundaries, the impact categories to be considered, the assessment methodology to be used, and the selection of the functional unit (Nieuwlaar, 2004).The functional unit is a key element of LCA, as it represents the reference unit to which the inputs and outputs within our system are related, based on which all system inputs and outputs are quantified. This ensures the consistency of the analysis and the comparability of the results with other similar studies. The system boundary is used to define the unit processes to be included in the study, as well as the system inputs and outputs, final waste disposal, maintenance activities, and other relevant elements.

Depending on the objective of the study, system boundaries can be adjusted to include or exclude certain life cycle stages (ISO, 2016). Figure 16 shows the different approaches to system delimitation, among which the following stand out: the first "cradle-to-grave" approach covers all stages of the product life cycle, including the extraction of raw materials, transportation, production, use, and finally, its disposal or disposal. The second, "cradle-to-gate" approach covers only the phases until the product leaves the production plant, excluding the use and final disposal stages. Finally, the "gate-to-gate" approach is limited to the analysis of a specific stage within the production process (Mulya et al., 2022; Osman et al., 2024).

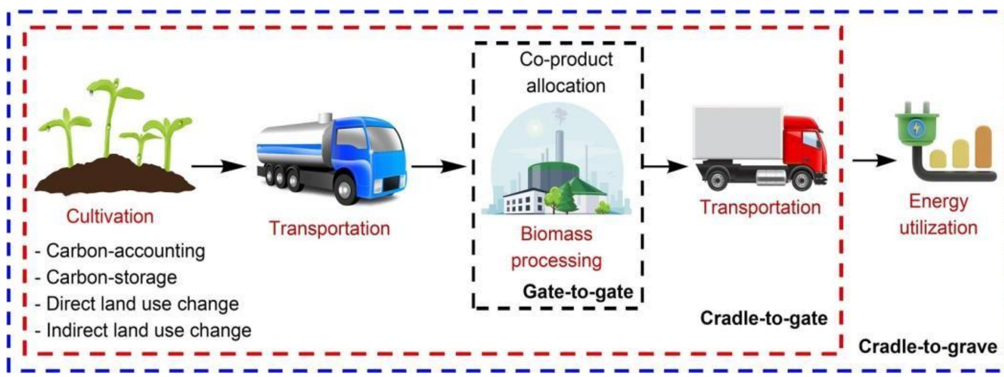

Figure 16. Assessments of gate-to-gate and cradle-to-grave processes.

Source: Osman et al. (2024).

2.8.1.2 Life Cycle Inventory Analysis

Inventory analysis consists of the systematic collection and quantification of relevant input and output data associated with a product system. This process is iterative in nature, as it requires continuous review and adjustment of the data as the study progresses. Data for each unit process, within the defined boundaries of the system, can be classified into general categories, such as: raw materials, energy, auxiliary inputs, final products, by-products, waste, as well as emissions to air, water, and soil throughout the product life cycle (Figure 17) (ISO, 2016; Mehmeti et al., 2016).

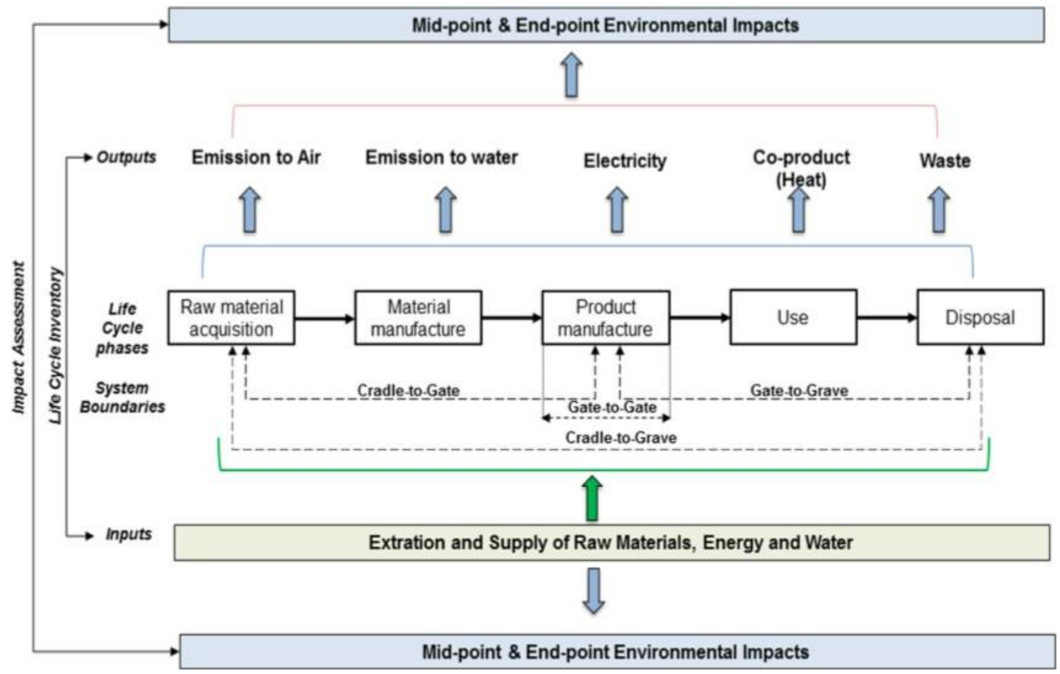

Figure 17. Inpunts,outputs,products,by-products and emissions.

Source: Mehmeti et al. (2016).

2.8.1.3 Life Cycle Impact Assessment

This impact assessment phase aims to determine the magnitude and significance of potential environmental impacts based on the results obtained from the process inventory. This stage involves the collection, evaluation, and interpretation of potential environmental effects associated with the product, through the categorization and characterization of flows. Data are associated with specific impact categories and their respective indicators to identify opportunities for improvement and propose solutions within the assessed system (Capaz & Seabra, 2016; ISO, 2016). There are various methods for impact assessment in LCA, including CML, Eco-Indicator 99, and ReCiPe 2016. The CML method, developed by Leiden University (Netherlands) in 2001, focuses on midpoint impacts and is divided into two approaches: baseline and non-baseline, the latter being the most widely used in LCA studies. Eco-Indicator 99 assesses impacts at the endpoint level, considering three main categories: human health, ecosystem quality, and resources. This method incorporates three different cultural perspectives or "archetypes": H (hierarchist (default)), I (individualist), and E (Egalitarian) (Acero, 2016). The main objective of the ReCiPe method is to integrate the CML method and Eco-Indicator 99, offering an updated and more comprehensive version for assessing environmental impacts. ReCIPe distinguishes two levels of indicators: midpoint (H) and endpoint (E) (Table 3) (Acero, 2016; Huijbregts et al., 2016).

Table 3. Midpoint impact categories and related indicators.

Midpoint impact category	Indicator	CFm	Unit
Climate change	Infrared radiative forcing increase	Global warming potential (GWP)	kg CO_2-eq to
Ozone depletion	Stratospheric ozone decrease	Ozone depletion potential (ODP)	kg CFC-11-eq to air
Ionising radiation	Absorbed dose increase	Ionising radiation potential (IRP)	kBq Co-60-eq to air
Fine particulate matter formation	PM2.5 population intake increase	Particulate matter formation potential (PMFP)	kg PM2.5-eq to air
Photochemical oxidant formation: terrestrial ecosystems	Tropospheric ozone increase	Photochemical oxidant formation potential: ecosystems (EOFP)	kg NOx-eq to air
Photochemical oxidant formation: human health	Tropospheric ozone population intake increase	Photochemical oxidant formation potential: humans (HOFP)	kg NOx-eq to air
Terrestrial acidification	Proton increase in natural soils	Terrestrial acidification potential (TAP)	kg SO_2-eq to air
Freshwater eutrophication	Phosphorus increase in freshwater	Freshwater eutrophication potential (FEP)	kg P-eq to freshwater
Human toxicity: cancer	Risk increase of cancer disease incidence	Human toxicity potential (HTPc)	kg 1,4-DCB-eq to urban air
Human toxicity: non-cancer	Risk increase of non-cancer disease incidence	Human toxicity potential (HTPnc)	kg 1,4-DCB-eq to urban air
Terrestrial ecotoxicity	Hazard-weighted increase in natural soils	Terrestrial ecotoxicity potential (TETP)	kg 1,4-DCB-eq to industrial soil

Freshwater ecotoxicity	Hazard-weighted increase in freshwaters	Freshwater ecotoxicity potential (FETP)	kg 1,4-DCB-eq to freshwater
Marine ecotoxicity	Hazard-weighted increase in marine water	Marine ecotoxicity potential (METP)	kg 1,4-DCB-eq to marine water
Land use	Occupation and time-integrated land transformation	Agricultural land occupation potential (LOP)	m^2 × yr annual cropland-eq
Water use	Increase of water consumed	Water consumption potential (WCP)	m^3 water-eq consumed
Mineral resource scarcity	Increase of ore extracted	Surplus ore potential (SOP)	kg Cu-eq
Fossil resource scarcity	Upper heating value	Fossil fuel potential (FFP)	kg oil-eq

Source: Huijbregts et al. (2016).

2.8.1.4 Interpretation

The findings obtained from the interpretation of the inventory analysis and the impact assessment are integrated to formulate conclusions and recommendations, consistent with the previously defined objective and scope of the study. This final phase allows the results to be translated into concrete actions, and its direct applications include product development and improvement, strategic planning, and marketing activities (ISO, 2016).

2.8.2 Review of scientific studies on LCA in anaerobic digestion

In this section, a review of the scientific literature related to LCA and AD was conducted. Table 4 presents a summary of the main characteristics considered in the reviewed studies, including aspects such as the raw materials used, the functional unit, the databases employed, as well as the method and software applied in each case.

LCA has become an increasingly used and necessary tool for assessing the potential environmental impacts associated with products, processes, or services throughout their life cycle. Several countries and regions have contributed significantly to the development and application of this methodology. Among the continents with the greatest research activity on LCA are Europe, North America, Asia, Latin America, and Africa. Europe leads the number of publications, with countries such as Germany, Italy, Denmark, and Spain standing out. In North America, the main contributions come from the United States, Canada, and Mexico. In Asia, the most notable are China and Saudi Arabia; in Latin America, Brazil; and in Africa, South Africa (Esteves et al., 2019).

The most commonly studied systems approaches are "cradle to grave," followed by "cradle to gate." Regarding the raw materials evaluated in these studies, food waste, wastewater, microalgae, macroalgae, and animal manure predominate.

The functional units used in the different articles reviewed vary depending on the study's focus. The most common are the kilogram (kg) or the ton of raw material. In studies related to energy generation, the units used include the megajoule (MJ) and the kilowatt-hour (kWh) of energy produced, which are converted using the calorific value of methane. 1 MJ of biogas produced through anaerobic digestion and 1 MJ of purified methane are also identified as functional units. In studies that prioritize volume, units such as 1 cubic meter (m^3) of biogas or 1 m^3 of treated water are used.

Regarding the database, the most widely used is Ecoinvent, in its different versions. Regarding environmental impact assessment methods, the most widely used are ReCiPe (in its 2008 and 2016 versions, both at the midpoint and endpoint levels), followed by the CML 2001 method. The most important categories used in LCA are global warming (98%), eutrophication/acidification (79%), toxicity (57%), energy/resources (52%), ozone depletion (33%), and land use (12%) (Mayer et al., 2019). The main software used in LCA studies are SimaPro, followed by GaBi and openLCA. Their main function is to track the most significant factors contributing to environmental impacts, as well as to apply cutoff criteria to focus the analysis on the most relevant processes (Dastjerdi et al., 2021).

Table 4. Different raw materials, functional unit, database and methodology used in life cycle assessment.

Feedstock	Functional unit (FU)	Database	Methods	Software used	Reference
Sewage treatment	1 m^3 of sewage treated	Ecoinvent 3.2	ReCiPe 2016 Endpoint (E) CML-2000 (baseline)	SimaPro 8	Alvarado et al. (2019)
Microalgae grow in municipal waste water	1 m^3 treated waste water 1MJ CNG	Ecoinvent 2.0	CML 2001	GaBi 6.0	Maga (2016)
Waste water	1kWh of produced energy	Ecoinvent 3.8	CML-2013 (baseline) ReCiPe 2016 Midpoint (H)	SimaPro 9.4.2.0	Karolinczak et al. (2024)
Energy crops Macroalgae	1 kg of feedstock 1 MJ of energy produced	Ecoinvent 2.2	ReCiPe 2016 Midpoint (H)	SimaPro 7.3.2	Ertem et al. (2017)
Agroindustrial waste	1 MJ of energy produced	Ecoinvent 3.6	ReCiPe 2016 Midpoint (H)	SimaPro 9.1	Ugwu et al. (2022)
Microalgae	1 MJ of purified mehane	Ecoinvent 3.9	CML-2016 (baseline)	OpenLCA	Xu et al. (2024)
Food waste	1 ton on food waste	Ecoinvent 2.0	ReCiPe 2016 Midpoint (H)	GaBi 10.0	Tian et al. (2023)

Food waste	1 ton on food waste	Ecoinvent 3.5	ReCiPe 2016 Midpoint (H) and (E)	GaBi 8.7	(Zhang et al., 2020)
Pig manure	1-ton substrate (fresh matter)	Ecoinvent 2.2	ReCiPe 2016 Midpoint (H)	Microsoft Excel	De Vries et al. (2012)
Microalgae	1 MJ of energy produced	Ecoinvent 2.2	ReCiPe 2008 Midpoint (H) and (E)	Umberto 5.6	(Shimako et al., 2016)
Livestock manure	1 MJ of biogas energy	Ecoinvent 2.0	CML- 2002	OpenLCA 1.10.3	Hossain et al. (2023)
Landfill biogas	1 Nm^3 of biomethane	Ecoinvent 2.0	ReCiPe 2016 Midpoint (H) and (E)	SimaPro 9.4.0.1	Godoy et al. (2024)

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3 LIFE CYCLE ASSESSMENT OF BIOGAS PRODUCTION USING POULTRY WASTE AND SARGASSUM: EVALUATING CO-DIGESTION AND METHANE RECOVERY STRATEGIES

3.1 Abstract

Anaerobic digestion is an effective technology for waste management and renewable energy production through biogas generation. However, environmental and energy performance challenges still need to be addressed. This study aimed to assess and compare the environmental impacts and energy efficiency of biogas production using six scenarios: monodigestion of chicken litter (baseline), co-digestion of chicken litter and Sargassum (Sce1), co-digestion of turkey litter and Sargassum (Sce2), and the same configurations with methane recovery (Sce3; Sce4, Sce5). A Life Cycle Assessment was conducted from cradle-to-gate using the ReCiPe 2016 method, along with an Energy Return on Investment (EROI) analysis, to evaluate the environmental and energy performance of each scenario. Results show that co-digestion strategies improved biogas production efficiency by optimizing the carbon-to-nitrogen ratio, increasing EROI by 27%-34% compared to monodigestion. Methane recovery from digestate storage reduced the Global Warming Potential (GWP) by up to 97% compared to non-recovery systems. Co-digestion with turkey litter and Sargassum combined with methane recovery (Sce 5) achieved the highest energy efficiency and the lowest GWP among all scenarios. Additionally, co-digestion strategies showed reductions in other impact categories such as Terrestrial Acidification Potential (TAP) and Particulate Matter Formation Potential (PMFP). The study highlights the environmental and energetic advantages of integrating Sargassum into co-digestion processes while contributing to circular economy practices and climate change mitigation. These findings provide insights for optimizing AD systems and support sustainable bioenergy production strategies.

Keywords: Anaerobic co-digestion, Bioenergy, Sargassum, Poultry manure.

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3.2 Introduction

Anaerobic digestion (AD) has become a key technology in organic waste management, enabling biogas production as a renewable and sustainable energy source. This biological process, mediated by microbial communities, decomposes organic matter in the absence of oxygen, primarily generating methane (CH_4) and carbon dioxide (CO_2), along with by-products such as digestate, which can have agricultural applications (Nghiem et al., 2017). Growing concerns over the energy crisis and the environmental impact of fossil fuel use have driven the adoption of this technology worldwide, highlighting its contribution to reducing greenhouse gas (GHG) emissions and its role in the circular economy (Dasgupta, 2025). However, process efficiency and environmental impact depend on various factors, including substrate composition, digester type, C/N radio, and operating conditions, leading to new strategies to optimize performance and minimize environmental externalities (Chew et al., 2021; Mao et al., 2015).

Anaerobic co-digestion (AcoD) has been regarded as one of the more promising options for increasing biogas production because of its better nutrient balance and energy production (Mao et al., 2015). C/N ratio is crucial to ensure the microbiological stability of the system, as an imbalance can inhibit methanogenic microorganisms and reduce biogas production efficiency. The combination of nitrogen-rich substrates, such as animal manures, are often co-digested with other biodegradables carbon-rich substrates (Kunatsa & Xia, 2022).

In the Yucatan Peninsula, chicken litter, turkey litter, and sargassum represent three abundant and underutilized organic residues with significant potential for AcoD. The region is a leading chicken and turkey production hub in Mexico, generating large volumes of nitrogen-rich wastes such as chicken litter and turkey litter, which pose environmental risks due to improper disposal or open-air storage, contributing to nutrient leaching and greenhouse gas emissions (Seidavi et al., 2019). Simultaneously, the Mexican Caribbean coastal areas face a growing challenge with the recurring arrival of Sargassum blooms, negatively impacting marine ecosystems, tourism, and fisheries (Rodríguez-Martínez, 2020).

However, the high organic matter and carbon content of Sargassum, combined with the nitrogen richness of chicken litter or turkey litter, offer a synergistic opportunity to balance the carbon-to-nitrogen ratio in AD processes. Valorizing these residues through co-digestion supports regional waste management strategies, contributes to sustainable energy generation, and mitigates local environmental issues. In this context, integrating chicken litter, turkey litter, and Sargassum into biogas production systems could strengthen the circular economy model in the Yucatan Peninsula while addressing waste challenges specific to the livestock and coastal sectors.

Evaluating the life cycle of AD processes is essential to comprehensively understanding their environmental performance and identifying potential trade-offs beyond energy generation. Life Cycle Assessment (LCA) methodology provides a holistic perspective, quantifying direct and indirect environmental impacts such as greenhouse gas emissions, acidification, eutrophication, and fossil resource depletion (Huijbregts et al., 2016). This methodology can act as a support tool for decision-making in the area of waste management (Chiu & Lo, 2016). Through LCA, the environmental impacts of different anaerobic digestion improvement strategies can be systematically quantified and compared. This approach allows for an objective evaluation of alternative configurations—such as monodigestion, co-digestion, and methane recovery-by considering the entire life cycle of the system, from feedstock collection to energy production. By identifying environmental hotspots and trade-offs, LCA supports the selection of the most sustainable design options, promoting anaerobic digestion systems that not only optimize biogas yields but also minimize greenhouse gas emissions and other environmental burdens.

The main objective of this study is to evaluate and compare the environmental impacts associated with biogas production from different anaerobic digestion configurations, including mono-digestion and co-digestion strategies with turkey litter, chicken litter and sargassum, with and without methane recovery. The expected contribution of this research is to provide a comprehensive understanding of how co-digestion and methane recovery strategies influence the environmental performance of anaerobic digestion systems, highlighting their potential to reduce greenhouse gas emissions and other environmental burdens. Ultimately, this study aims to support decision-making processes for designing more sustainable biogas production systems aligned with climate change mitigation goals and circular economy principles, particularly in regions with abundant organic residues, such as the Yucatan Peninsula.

3.3 Materials and methods

3.3.1 Experimental data

This section describes the experimental trials conducted to evaluate biogas production through anaerobic digestion using three strategies: monodigestion, co-digestion, and residual methane recovery. The residual methane potential (RMP) was subsequently assessed using tests in anoxic microcosms to determine the amount of methane that can still be produced from the previously digested material. The following are detailed:

3.3.1.1 Anaerobic digestion

The study was conducted using anaerobic digesters that had operated for over nine years, and six additional digesters were later established. Of these, three were used to treat chicken litter (CL), while the other three treated turkey litter (TL). Each digester had a working volume of 10 L and operated under mesophilic conditions (37 ± 2 °C), with a Hydraulic Retention Time (HRT) of 30 days. The digesters were maintained under mesophilic conditions for four HRT periods to ensure their stabilization. Once stabilized, the digesters were fed in a co-digestion regime, using mixtures of chicken litter-sargassum (CL-S) and turkey litter-sargassum (TL-S). The mixtures were prepared with a total solids (TS) ratio of 3% and were subjected to an operation time of five HRT periods (Figure 18).

At the end of the co-digestion period, analyses were performed to determine the physicochemical parameters of the digestates. TS and volatile solids (VS) were measured according to standard procedures APHA (2005). Biogas production was measured using a tilting flowmeter, which utilized volume displacement and a digital counter (LA8N-BN, Autonics, Korea). The methane percentage was obtained by injecting 5 µL of biogas from each digester into a gas chromatograph (GC) (Clarus 500, Perkin Elmer, USA), equipped with a flame ionization detector (FID) under the following operating conditions: nitrogen as the carrier gas at a flow rate of 14 mL min^{-1}, at 14 psi, a 100 °C injector port, a 70 °C oven, and 220 °C detector. The retention time was 4 minutes, and the column used was Elite-FFAP, with a length of 30 m and a diameter of 0.32 mm (Perkin Elmer, USA). The percentage of methane was determined using a calibration curve with pure methane (HDSP No. P-4618-F, Praxair, Mexico). Biogas volume and methane concentration were monitored daily. Methane produced is reported under normal temperature and pressure conditions. The methane yield and the percentage of methane produced were calculated using data collected between the third and fifth HRT of operation.

3.3.1.2 Evaluation the residual methane potential

At the end of the five Hydraulic Retention Times (HRT), digestate samples were collected directly from the outlet of each digester. For each digestion system, three anoxic microcosms were prepared in serological flasks, using a working volume of 250 mL of effluent. These microcosms were incubated under mesophilic conditions (37 ± 1 °C) for a period of 71 days. The experimental systems were coded as follows: PD-CL: Post-digestion of chicken litter., PD-CL-S: Post-digestion of chicken litter in co-digestion with Sargassum, PD-TL-S: Post-digestion of turkey litter in co-digestion with Sargassum (Figure 18). At the end of the incubation period, the physicochemical parameters of the samples were determined, including total solids TS, VS according to the APHA (2005). The RMP was obtained from the parameters resulting from the fitting of the experimental Accumulated Methane Yield (AMY) (mL CH_4 g_{VSfed}^{-1}) to the modified Gompertz Model (Zwietering, 1990).

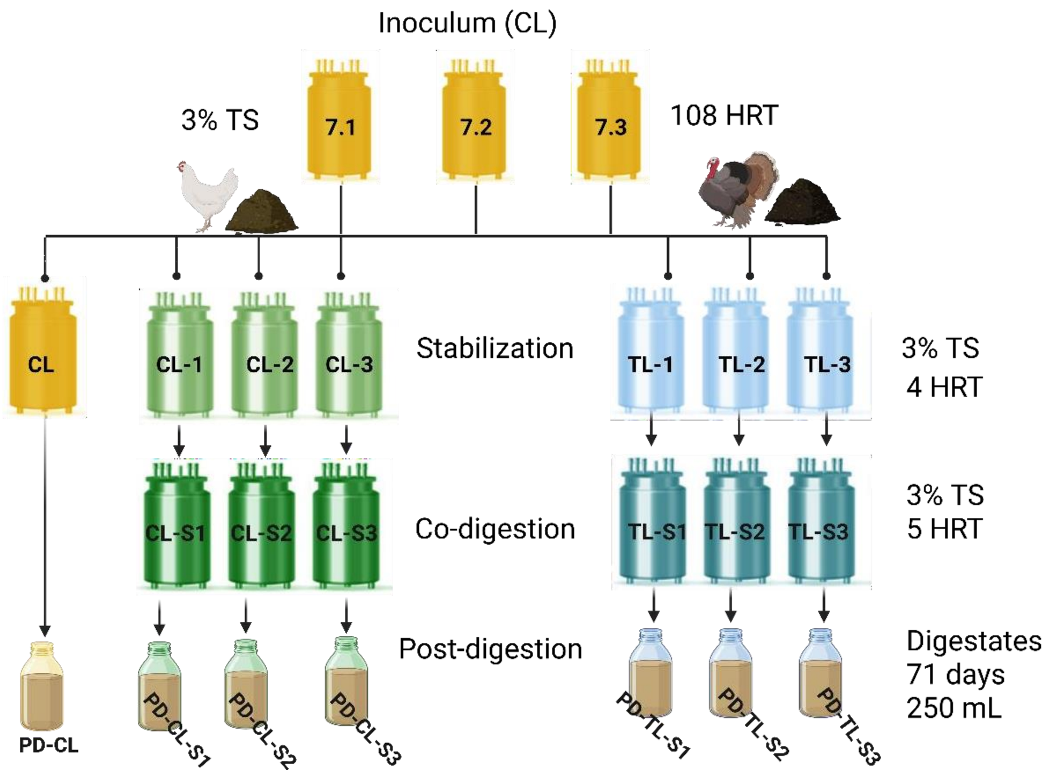

Figure 18. Stabilization, co-digestion and post-digestion of the different systems. **PD:** Post-digestion.

3.3.2 Life Cycle Assessment

The standard LCA methodology was applied to assess the environmental aspects and potential impacts of energy production through monodigestion, co-digestion, and methane recovery. The study followed the procedures established by ISO 14040 (ISO, 2016), which consists of four stages: (a) goal and scope definition, (b) life cycle inventory analysis, (c) life cycle impact assessment, and (d) interpretation of results. The evaluation was conducted using OpenLCA 2.0.0, an open-source software widely used for LCA studies and sustainability assessments.

3.3.2.1 Goal and scope definition

The analysis aims to identify the critical stages of the process, quantify emissions and resource consumption, and provide insights to support decision-making toward more sustainable biogas production. The scope of the study covers the system boundary from substrate collection to biogas production ready for energy use, following a cradle-to-gate approach. The processes considered include substrate collection, transportation, pretreatment, anaerobic digestion, and the digestate storage process. Co-digestion and methane recovery were implemented as a strategy to enhance biogas production efficiency (Figure 19). The system boundaries exclude the production, installation, and use of equipment and infrastructure, labor, as well as the transport and distribution of the final biogas. The defined functional unit (FU) was 1 MJ of bioenergy produced and contained in the biogas as biomethane. The system boundaries include raw material inputs (chicken litter, turkey litter, and Sargassum), the energy consumption of the process, and the generation of waste and emissions.

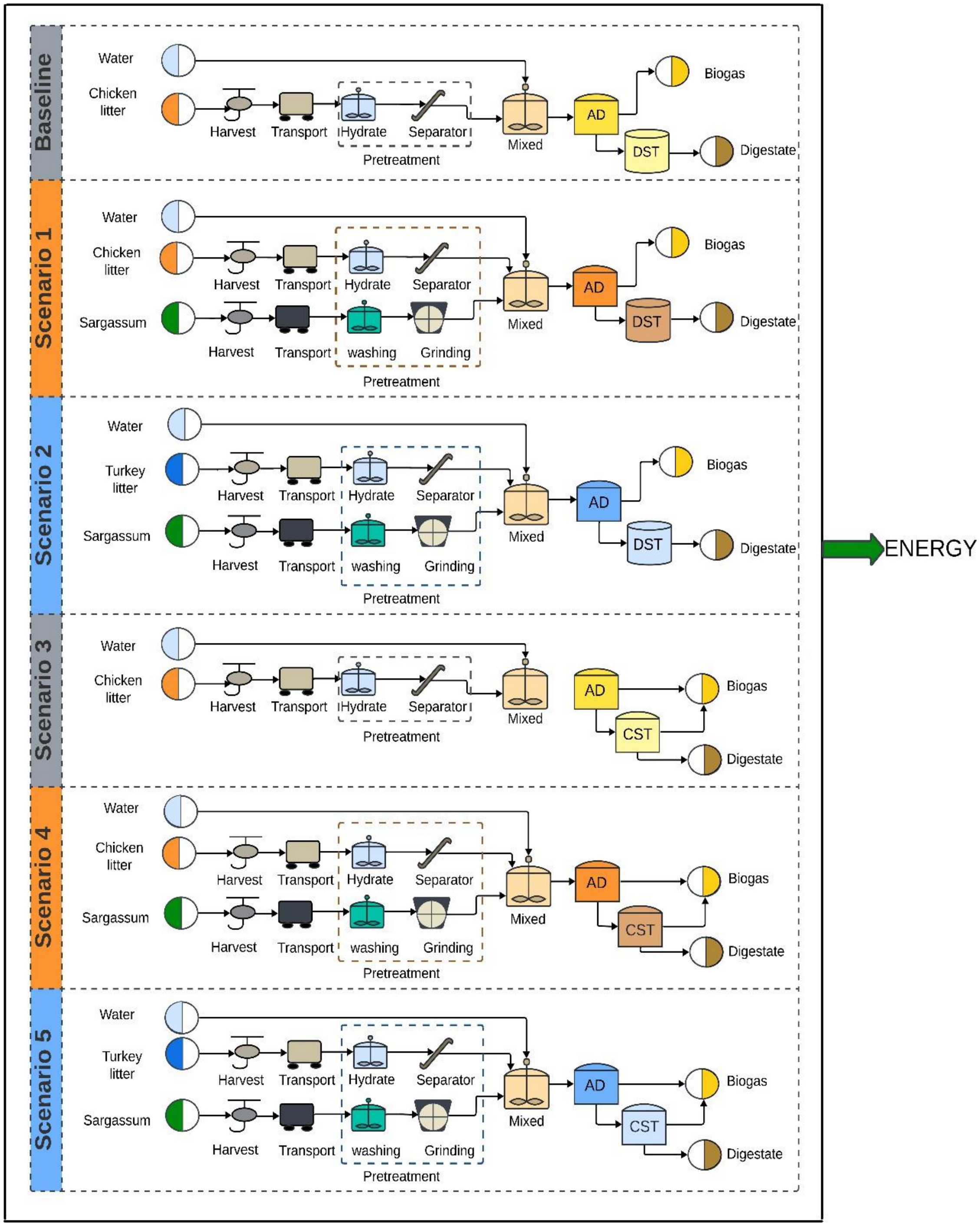

Figure 19. Scenarios and system boundaries. It shows substrate input (chicken litter, turkey litter, Sargassum), unit processes: **AD:** anaerobic digestion, **CST:** close storage tank, **DST:** digestate storage tank, and final products (biogas and digestate).

3.3.2.2 Process description

Based on the experimental data described in section 3.3.1, a baseline and five different scenarios were modeled. The different scenarios in this study are presented in Figure 19. The baseline corresponds to biogas production through the monodigestion of chicken litter. Five additional scenarios were proposed to improve the energy efficiency and sustainability of the process, considering both co-digestion with alternative substrates and the recovery of residual methane. The AD process operating parameters for LCA were scaled to 300 m^3 with experimental results obtained from a 10-liter pilot-scale anaerobic digester. For this study, the digester was strategically located on the Yucatán Peninsula, taking advantage of the abundant availability of poultry litter and Sargassum as feedstock. The selection of these substrates was based on their high generation in the region and their geographic distribution, which facilitates their collection and minimizes transportation distances. This not only optimizes process logistics but also reduces emissions associated with the transport of materials, contributing to the project's sustainability. The main characteristics of each scenario are described below.

Baseline (BL): Biogas production through monodigestion of chicken manure without methane recovery. This scenario serves as a benchmark for the comparative analysis. The material was collected from a poultry farm and transported 14.2 km to the anaerobic digestion plant. Upon arrival, the chicken manure was hydrated and processed using a gravity separation system to remove inorganic materials and poorly degradable components, such as straw and wood chips. The concentrated substrate was transferred to a mixing tank, where it was diluted with water to adjust the total solids content to 3%. The resulting effluent was fed to a 300 m^3 covered plug-flow lagoon digester with a hydraulic retention time (HRT) of 30 days. In addition, 50% of the digester's liquid volume was recirculated daily to improve process stability. The resulting digestate was discharged into a 300 m^3 uncovered storage tank, where it was stored until its final application on agricultural fields.

Scenario 1 (Sce1): This scenario involved biogas production through co-digestion of chicken litter with Sargassum. This strategy sought to balance the carbon-nitrogen ratio and improve biogas production compared to monodigestion. Chicken litter collection, transportation, and pretreatment are carried out in the same manner as in the BL until the mixing stage. In parallel, Sargassum is collected from the coastal areas of the Mexican Caribbean and transported 43.1 km to the anaerobic digestion plant. For conditioning, the Sargassum is washed to remove excess salts and undergoes a grinding process to reduce particle size. The pretreated Sargassum is subsequently transferred to the mixing tank, where it is combined with the chicken manure and diluted with water to adjust the total solids content to 3%. The resulting mixture is fed to the anaerobic digester, maintaining the same operating conditions described in the BL.

Scenario 2 (Sce2): This scenario produces biogas through the co-digestion of turkey litter with Sargassum. The unit operations–including collection, transportation, pretreatment, mixing, and anaerobic digestion–are carried out in a similar manner to those previously described in BL and Sce1.

Scenario 3 (Sce3): Is an improvement on the baseline; the digestate was incorporated into a covered lagoon system for digestate storage. This strategy has a dual purpose: first, it reduces fugitive methane emissions during storage; second, it improves the overall energy efficiency of the system by recovering residual methane through post-digestion, thereby increasing total biogas production.

Scenario 4 (Sce4): Is an improvement on scenario 1; the digestate was incorporated into a covered lagoon system for digestate storage. The residual methane was recovered through post-digestion, thereby increasing total biogas production.

Scenario 5 (Sce5): Is an improvement on scenario 2; the digestate was incorporated into a covered lagoon system for digestate storage. The residual methane was recovered through post-digestion, thereby increasing total biogas production.

3.3.3 Life cycle inventory

For the life cycle inventory, the input and output data for each system were scaled to a volume of 300 m^3. The experimental physicochemical properties of the substrates, the resulting digestates, the biogas yield, and the methane content in the biogas are shown in the results in section 4.4.1. Background data on substrate collection, transportation, water use, and electricity generation were sourced from the Ecoinvent 3.2 database (Wernet et al., 2016). The energy consumption of the equipment used in the process was estimated based on their technical specifications, as detailed in Table 5.

Table 5. Characteristics of the equipment used.

Process	Equipment	Capacity	Power	Other
Pumps	Industrial pump	0.9 $m^3 \cdot min^{-1}$	3.7 kW	
Mixed	Paddle mixer	2 m^3	1.5 kW	
Washing	Washer LMNP-300	300 $kg \cdot h^{-1}$	1.5 kW	Mass loss 0,5 [%]
Grinding	Industrial mill	29 $kg \cdot min^{-1}$	3.7 kW	Mass loss 1 [%]

The inputs and outputs of each unit process were determined through mass and energy balances specific to each system (see **Appendix A**). Table 6 presents the simplified inventory corresponding to each scenario analyzed.

Table 6. Material and energy inventory of inputs and outputs per functional unit.

Parameter	Unit	Scenarios					
		BL	1	2	3	4	5
Inputs							
Chicken litter	kg[W.B]	2.397	0.521		2.397	0.521	
Turkey litter	kg[W.B]			0.388			0.388
Sargassum	kg[W.B]		1.067	1.014		1.067	1.014
Water	kg	18.706	7.325	6.680	18.706	7.325	6.680

Electricity	MJ	0.076	0.060	0.057	0.061	0.056	0.051
Outputs							
Biogas	kg	0.084	0.061	0.060	0.084	0.061	0.060
Residual biogas	kg				0.021	0.004	0.007
Digestate	kg	14.823	6.423	6.101	14.802	6.418	6.093

3.3.3.1 Life cycle environmental impact assessment

The environmental performance of the evaluated systems was compared at the midpoint level (H) using the ReCiPe 2016 method (Huijbregts et al., 2016). A total of 18 midpoint impact categories were considered: Global Warming Potential (GWP), Fossil Fuel Potential (FFP), Freshwater Ecotoxicity Potential (FETP), Freshwater Eutrophication Potential (FEP), Particulate Matter Formation Potential (PMFP), Human Toxicity Potential, cancer effects (HTPc), Human Toxicity Potential, non-cancer effects (HTPnc), Ionizing Radiation Potential (IRP), Agricultural Land Occupation Potential (LOP), Marine Ecotoxicity Potential (METP), Marine Eutrophication Potential (MEP), Mineral Resource Scarcity Potential (SOP), Photochemical Oxidant Formation Potential - human health (HOFP), Photochemical Oxidant Formation Potential - ecosystems (EOFP), Ozone Depletion Potential (ODP), Terrestrial Acidification Potential (TAP), Terrestrial Ecotoxicity Potential (TETP), and Water Consumption Potential (WCP).

3.3.4 Principal Components Analysis

To reduce the number of variables for analyzing the potential values of the impact categories from the LCA, Principal Component Analysis (PCA) was applied to the 18 categories using JMP Pro version 18.0.2 (https://www.jmp.com/,2025).

3.3.5 Energy balance

The energy balance of the process is reported in terms of the Energy Return on Investment (EROI) (**Equation 1**), a concept developed by Hall et al. (Hall et al., 2009). This indicator assessed the system's energy efficiency by determining the relationship between the energy generated from methane and the energy consumption required for its production throughout the entire system. In systems with recovery, the methane recovered during post-digestion was considered an additional source of generated energy, taking into account its energy content. The energy content of the biogas was calculated based on the methane (CH_4) concentration and its lower heating value (LHV). According to the Mexican Ministry of Energy (SENER, 2015), the LHV of methane is estimated at 37.52 MJ•m³ under standard temperature and pressure conditions. This value was used to quantify the available energy in the biogas produced. EROI is a key metric for assessing bioenergy processes, as it enables the comparison of the energy viability of different technologies and system configurations.

$$EROI = \frac{Renewable\ energy\ produced}{Total\ energy\ consumed} \tag{1}$$

3.4 Results and discussion

3.4.1 Physicochemical parameters of substrates and digestates

The physicochemical characteristics of the substrates and digestates used in the life cycle inventory are presented in Table 7 . Among the substrates evaluated, turkey litter showed the highest concentration of total solids and volatile solids, followed by chicken litter. Sargassum, meanwhile, had a high carbon-nitrogen ratio. By combining these substrates in a co-digestion process, we sought to optimize this ratio, thereby improving the efficiency of the anaerobic digestion process.

Table 7. Physicochemical parameters of substrate and digestates.

	TS	**VS**	**C/N**
	g_{TS}•kg[W.B]	g_{VS}•kg [D.B]	[-]
Chicken litter	907.2	626.78	4.31
Turkey litter	914.5	669.32	6.28
Sargassum	93.0	46.88	9.51
Digestate CL	11.8	0.025	6.68

Digestate CL-S	11.1	0.024	8.77
Digestate TL-S	10.3	0.020	8.99

TS: total solids; **VS:** volatile solids; **WB:** wet basis; **DB:** dry basis.

Source: Data obtained experimentally.

Table 8 presents the methane yield results for the non-recovery systems and those with residual methane recovery. In addition, the methane concentration in each system is shown. The results indicated that the co-digestion of turkey litter and Sargassum achieved the highest methane yield and percentage compared to the monodigestion system, followed by the co-digestion of chicken litter and Sargassum. In the systems with recovery, the co-digestion of turkey litter and sargassum maintained the highest methane yield, followed by the monodigestion system, and finally, the co-digestion of chicken litter and sargassum. These results highlight the efficiency of the turkey litter-sargassum co-digestion system in optimizing biogas production.

Table 8. Methane yield, residual methane potential and methane concentration.

	Methane yield	**RMP**	**CH_4**
	mL CH_4•g_{VS} fed^{-1}	mL CH4•gvs fed^{-1}	% [v/v]
CL	85.65	-	44.78
CL-S	192.42	-	55.58
TL-S	218.33	-	55.82
PD-CL	-	60.39	50.20
PD-CL-S	-	49.37	50.14
PD-TL-S	-	84.19	52.11

RMP*: Residual methane potential

Source: Data obtained experimentally.

3.4.2 Energy analysis

The EROI compares the input and output energies within the biogas production system, as defined in Figure 19. The energy efficiency results for the baseline and alternative scenarios are presented in Figure 20. The functional unit for this analysis was set to 1 MJ of energy produced across the different scenarios. For

BL, the total energy consumption throughout the process was 0.076 MJ, resulting in an EROI of 13.12. In the co-digestion scenarios (Sce1 and Sce2), energy increased by 27% and 34%, respectively, compared to the BL, raising the EROI to 16.68 and 17.59. Notably, the co-digestion of turkey litter and Sargassum (Sce2) showed the highest EROI increase, with a 34% improvement over the BL. Rivera-Hernández et al. (2022) reported similar trends, where the co-digestion of pig manure and Sargassum improved biogas production by 79.5% and up to 160.4% compared to monodigestion.

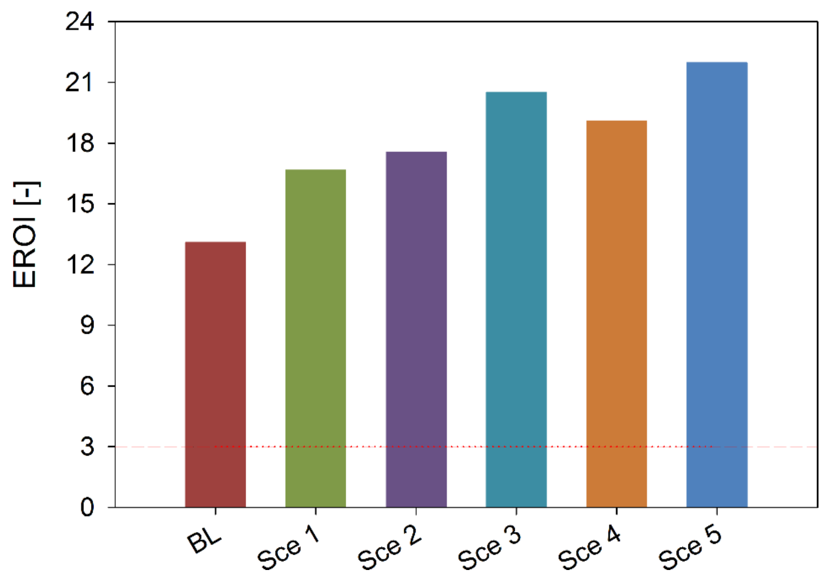

Figure 20. Energy Return on Investment (EROI) for the baseline and alternative scenarios. Comparison of energy efficiency from monodigestion, co-digestion, and methane recovery systems.

In the methane recovery scenario (Sce3), energy recovery increased by 43% compared to the system without recovery (BL). For co-digestion scenarios with methane recovery (Sce4 and Sce5), the energy gain increased by approximately 7% and 12%, respectively, compared to the non-recovery systems (Sce1 and Sce2). Among the recovery scenarios, Sce5 yielded the highest biogas production, followed by Sce3 and then Sce4. This improvement in biogas production increased the EROI to 22, 20.54 and 19.13, respectively, as a result of the additional energy recovered of post-digestion from digestate. It is important to highlight that all scenarios achieved EROI values above 3, which is widely considered a threshold for energy sustainability (Hall et al., 2009).

Sharno and Hiloidhari (2022) evaluated the EROI of biofuel production via pyrolysis using various feedstock, including wheat straw, jute, rice, and potato residues, reporting values ranging from 1.6 to 1.78. Similarly, Ramírez-Arpide et al. (2018) analyzed the co-digestion of prickly pear (nopal) and manure, reporting EROI values between 8.14 and 12.41, and highlighted that residual biogas recovery increased the EROI by 16.5%. In addition, Blengini et al. (2011) estimated EROI values between 3 and 5 for the co-digestion of manure with maize and sorghum crops. They also emphasized that for LCA development in bioenergy systems, reliable inventory data is critical to ensure comparability and serve as a basis for future research.

3.4.3 Environmental impact assessment

The environmental impacts of the six evaluated scenarios were assessed across 18 midpoint categories using the ReCiPe 2016 method. The results, summarized in Table 9, reveal that the baseline scenario (BL) exhibited the highest environmental burdens across most impact categories, (GWP), (FEP), and (TAP). In contrast, co-digestion scenarios (Sce1 and Sce2) showed notable reductions in several categories, particularly in (PMFP) and (HOFP), due to improved process efficiency and substrate synergy. Moreover, the implementation of methane recovery systems in scenarios 3, 4, and 5 resulted in significant additional reductions in GWP, achieving up to a 97% decrease compared to the baseline scenario.

Table 9. Impact categories of the different scenarios per functional unit.

Categories	Unit [kg eq]	Scenarios					
		BL	1	2	3	4	5
GWP	CO_2	7.46E-1	7.12E-1	7.10E-1	2.72E-2	2.09E-2	1.86E-2
FFP	oil	1.08E-2	7.48E-3	6.98E-3	8.62E-3	6.98E-3	6.22E-3
FETP	1,4 DCB	6.60E-4	3.77E-4	3.48E-4	5.27E-4	3.52E-4	3.11E-4
FEP	P	7.46E-6	3.71E-6	3.46E-6	5.95E-6	3.46E-6	3.09E-6
PMFP	PM2.5	7.10E-5	4.25E-5	3.97E-5	5.67E-5	3.96E-5	3.54E-5
HTPc	1, 4 DCB	1.22E-3	5.47E-4	5.07E-4	9.76E-4	5.11E-4	4.52E-4
HTPnc	1,4 DCB	2.72E-2	1.14E-2	1.05E-2	2.17E-2	1.06E-2	9.37E-3
IRP	kBq Co-60	2.68E-3	1.54E-3	1.44E-3	2.14E-3	1.44E-3	1.28E-3
LOP	m^2 a crop	3.84E-4	3.52E-4	3.24E-4	3.06E-4	3.28E-4	2.89E-4
METP	1,4 -DCB	9.76E-4	6.08E-4	5.62E-4	7.79E-4	5.67E-4	5.01E-4
MEP	N	6.29E-6	3.23E-6	3.00E-6	5.02E-6	3.02E-6	2.68E-6
SOP	Cu	2.74E-4	7.49E-5	6.89E-5	2.19E-4	6.99E-5	6.15E-5
HOFP	NO_x	1.53E-4	9.37E-5	8.70E-5	1.22E-04	8.75E-5	7.75E-5
EOFP	NO_x	1.57E-4	9.55E-5	8.86E-5	1.25E-4	8.91E-5	7.90E-5
ODP	CFC-11	1.43E-8	8.68E-9	8.11E-9	1.14E-8	8.10E-9	7.23E-9
TAP	SO_2	1.74E-4	1.10E-4	1.03E-4	1.39E-4	1.03E-4	9.21E-5
TETP	1,4-DCB	1.90E-1	2.22E-1	2.05E-1	1.51E-1	2.08E-1	1.83E-1
WCP	m^3	2.21E-1	1.49E-1	1.40E-1	1.77E-1	1.39E-1	1.25E-1

3.4.4 Principal component analysis

The potential values of the impact categories from the LCA results (Table 9) of the different systems, monodigestion, co-digestion and methane recovery were analyzed using PCA. Figure 21a on the X axis represents component 1, which explains 86.5% of the variance, while the Y axis corresponds to component 2 and represents 12.1%. Figure 21b shows that 15 categories, all highly correlated, have the main effect on component 1, while GWP, TETP, and LOP affect component 2, which contributed to the differentiation of the scenarios.

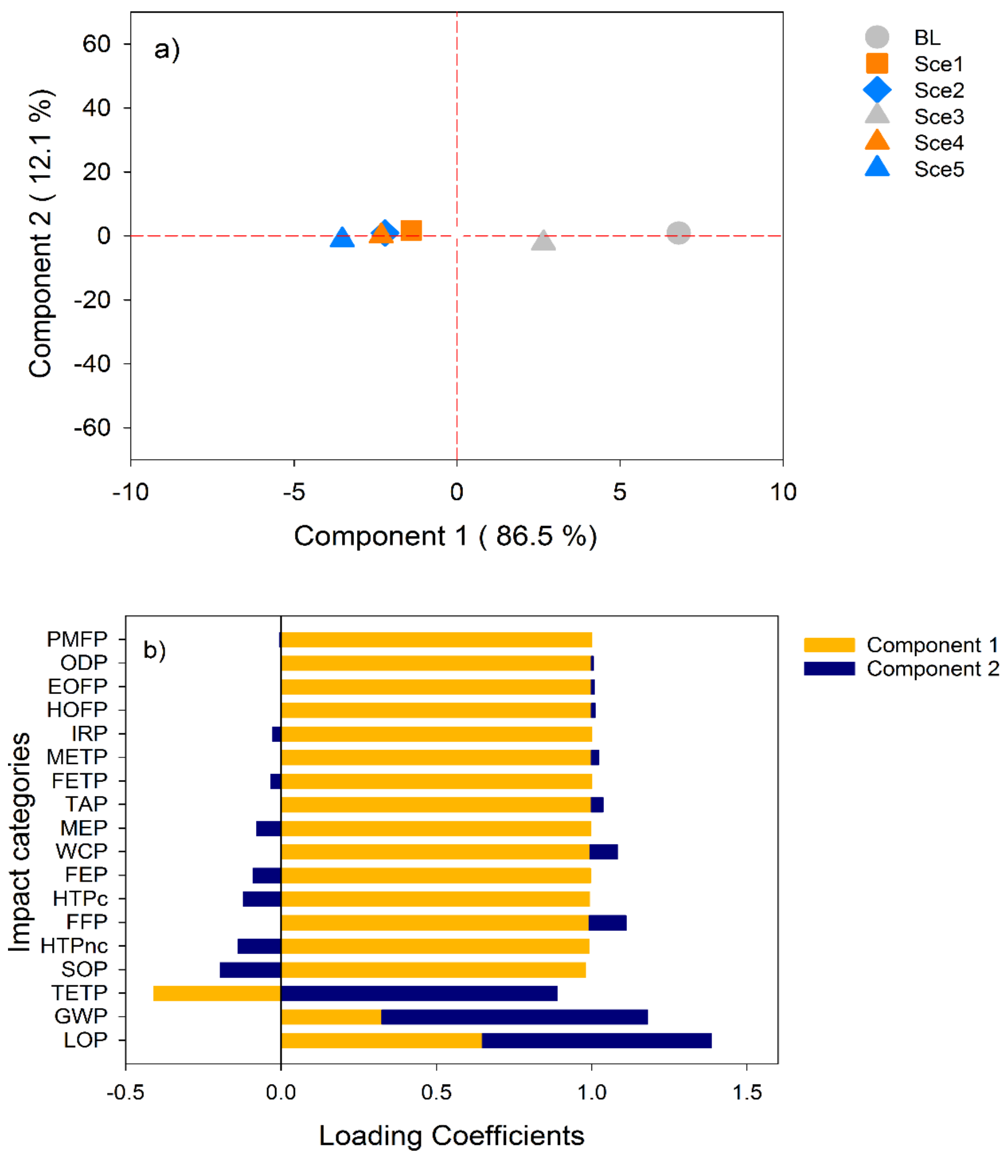

Figure 21. Principal Component Analysis (PCA) of the evaluated scenarios and their associated environmental impact categories.

Scenarios in component 1 have the greatest influence on the 15 categories on the right, while scenarios in component 2 have the least effect on the remaining categories. Furthermore, scenarios located in the component 2 are observed to have a positive impact on (GWP), (TETP), and (LOP). In contrast, scenarios in component 1 show negative effects on these indicators. This analysis indicates that it is possible to show a trend in the different scenarios. Following the counter-clockwise pattern and starting with the first group, BL, followed by Sce1 and Sce2, form a group representing the treatments without methane recuperation. The second group formed by Sce3, Sce4, and Sce5 correspond to the scenarios with methane recovery. In general, it is possible to consider the formation of four groups:

- Based on monodigestion of chicken litter, BL presented a clustering pattern associated with higher environmental loads.
- Sce1 and Sce2, both in co-digestion, slightly shifted toward lower impacts but remained grouped within higher component 2 scores.
- Sce4 and Sce5, both in co-digestion with methane recovery, presented the lowest environmental loads of the 18 categories.
- SCE 3 has a clustering pattern associated with higher environmental loads in component 2 but the lowest environmental loads in GWP, TETP, and LOP.

The following sections provide a detailed analysis of individual impact categories, focusing on those that most differentiate the evaluated scenarios, specifically (GWP), (TETP), and (LOP). Additionally, four categories strongly associated with component 1, which captures the cumulative environmental burdens according to the PCA, are discussed in-depth: (FEP), (PMFP), and (TAP).

3.4.5 Environment impact assessment by category

3.4.5.1 Global warning potential

In Table 9, the baseline scenario reports 7.46E-1 kg CO_2 eq emissions. Sce1 and Sce2 showed a similar reduction of approximately 5% compared to BL. In contrast, Sce3, Sce4, and Sce5, where digestate is subjected to methane recovery, achieved emissions of 2.72E-2, 2.09E-2, and 1.86E-2 kg CO_2 eq, respectively, representing a 96% to 97% reduction compared to non-recovery scenarios. Benalia et al. (2021) evaluated the co-digestion of wastewater and digestate at different concentrations, reporting emissions of 2.22–3.12E-1 kg CO_2 eq. Similarly, Ertem et al. (2017) analyzed the co-digestion of microalgae and poultry manure, obtaining emissions of 1.39E-2 kg CO_2 eq and 1.6E-1 kg CO_2 eq, depending on the operational conditions. Differences in these results can be attributed to the absence of digestate storage in some systems; however, methane recovery results are comparable.

Figure 22a shows the contribution of each unit process per functional unit. In the BL scenario, the digestate storage tank (DST) is the major contributor, accounting for approximately 96% of GWP emissions, followed by substrate collection (1%) and mixing (0.89%). In co-digestion scenarios (Sce1 and Sce2), DST remains the dominant contributor (97%) due to digestate storage. The second largest contributor is transportation (1%), followed by pretreatment (0.89%).

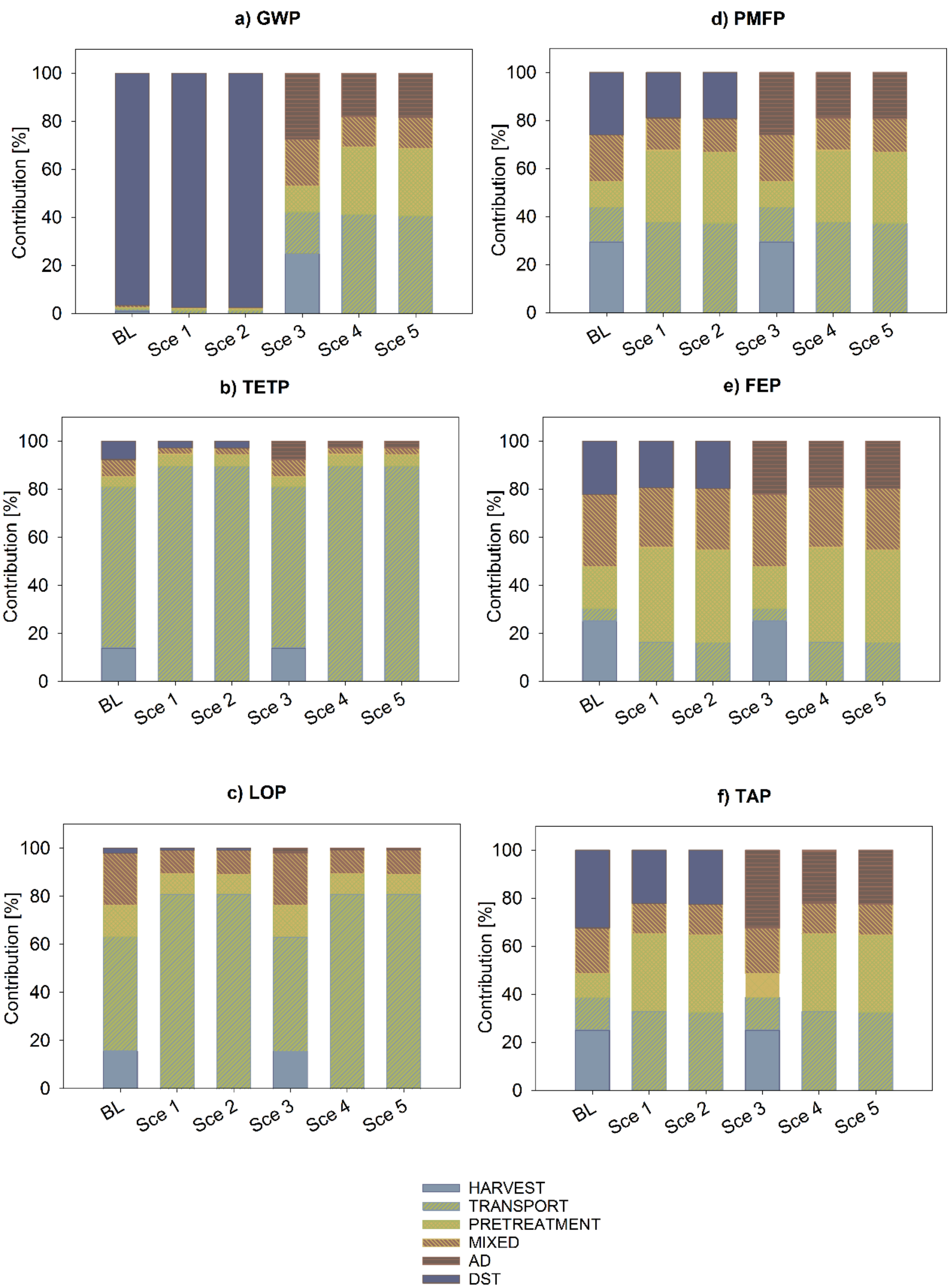

Figure 22. Impact potentials of the biogas production system of the different scenarios.

Lin et al. (2021) proposed three biogas production scenarios from cassava processing and reported that digestate storage contributed to 38% of GWP emissions. Hou et al. (2017) reported emissions ranging from 104 to 8,513 kg CO_2 eq per year during manure storage in a 450 m^3 reactor, attributing this variability to factors such as substrate source, quantity, and traditional uncovered lagoons. These factors exacerbate GHG emissions and increase the negative environmental impact.

For recovery systems, in Sce3, AD, collection, and mixing contributed 27%, 25%, and 19% to GWP, respectively. On the other hand, for Sce4 and Sce5, the major contributors shifted to transportation (41%), pretreatment (28%), and AD (18%).

Ertem et al. (2017), who reported that in AcoD of manure and macroalgae, AD contributed 44% and digestate storage accounted for 38% of emissions. Likewise, van den Oever et al. (2021) found that in the monodigestion of municipal organic waste and manure, AD and synthetic fertilizer production were the dominant contributors, assuming no methane fugitive emissions from the AD process.

Overall, the reduction in GWP is attributed to the benefits of co-digestion, as reported by (Ugwu et al., 2022). The use of digestate in a two-stage anaerobic digestion system prevents methane from being released into the atmosphere, mitigating its negative climate impact. Biomass waste management thus emerges as a viable option for green energy production within the framework of a circular economy (Subbarao et al., 2023).

3.4.5.2 Terrestrial ecotoxicity

In this category, the highest values were recorded in the co-digestion scenarios (Sce1 and Sce2), followed by the monodigestion BL. Unlike other impact categories, Sce3 achieved a 20% reduction in terrestrial TETP compared to the BL. Those were followed by the turkey litter and sargassum co-digestion with recovery (Sce5), which showed an 11% decrease, and Sce4, which reduced approximately 7%. These values are lower than those reported by Godoy et al. (2024) who indicated a value of 2.50E-1 kg 1,4 DCB eq for this category in the production 1Nm^3 of biomethane from landfill gas. Across all scenarios, transportation emerged as the dominant contributor, accounting for 67% to 90% of the impact, followed by collection activities (14%) in the BL. In co-digestion scenarios (Sce1 and Sce2), pretreatment contributed around 5%, while digestate storage (DST) contributed 3% (Figure 22b).

Although our results show a noticeable improvement in reducing TETP, they differ from the findings of Hossain et al. (2023), who reported reductions ranging from 28% to 35% during co-digestion compared to monodigestion. Such discrepancies may be attributed to variations in substrate biodegradability and the system boundaries considered in each study.

3.4.5.3 Land occupation potential

In Table 9, the BL recorded a LOP of 3.84E-4 m^{2}a crop eq. In the co-digestion scenarios (Sce1 and Sce2), approximately 8% and 16% reductions were observed, respectively, compared to the BL. The reductions for the methane recovery scenarios (Sce3, Sce4, and Sce5) were approximately 20%, 7%, and 11%, respectively, compared to their non-recovery counterparts.

Figure 22c shows the contribution of each process to the overall LOP impact. In the baseline scenario, transportation accounted for approximately 47% of the total land use impact, followed by pretreatment (21%) and collection (16%). Transportation became the dominant contributor in co-digestion scenarios (Sce1 and Sce2), with approximately 81% of the total impact. These results are consistent with those reported by Ertem et al. (2017), who evaluated the co-digestion of microalgae and poultry manure and reported land occupation values of 1.00E-3 m^2 attributable to transportation and 2.03E-4 m^2 to pretreatment in the total impact of this category. These results are similar to those reported by Benalia et al. (2021), where transportation accounts for 65%, followed by electricity with approximately 32%.

The findings highlight the significant role of logistics in land use impacts, especially in systems involving multiple biomass sources.

3.4.5.4 Particulate matter formation potential

This impact category is associated with human health risks due to the formation of particulate matter and its subsequent inhalation by the population. The highest total PMFP value was observed in the BL, reaching 7.10E-5 kg PM2.5 eq. In the methane recovery system (Sce3), this value was reduced by 20% compared to BL. This impact was mainly attributed to the feedstock collection stage (30%), followed by digestate storage (DST) at 26% and mixing at approximately 19% (Figure 22d).

In Sce1 and Sce2, PMFP values were reduced by 40% to 44% compared to BL. Furthermore, recovery scenarios (Sce4 and Sce5) showed an additional 7% and 11% reduction compared to their non-recovery counterparts (Table 9). In the co-digestion and methane recovery systems, transportation was the largest contributor to PMFP (37%-38%) due to the movement of poultry litter and sargassum, followed by pretreatment (30%) and DST (approximately 19%) (Figure 22d).

These results align with findings by Kumawat et al. (2024), who reported a PMFP of 4.13E-4 kg PM2.5 eq from a biogas plant fed with manure for energy generation, where feedstock supply, including transportation, accounted for 41% of the impact, followed by digestate processing (41%).

Since few studies have thoroughly examined this category, it is crucial to incorporate PMFP into the LCA of bioenergy production to evaluate better its potential impacts on human health and the environment. Our results are consistent with literature indicating that fine particulate formation is primarily driven by diesel usage, road dust during collection and transportation activities, and agricultural and industrial operations (Thangavel et al., 2022).

3.4.5.5 Freshwater eutrophication

Eutrophication impacts are estimated based on phosphorus (P) emissions. In this context, BL exhibited a value of 7.46E-6 kg P e. At the same time, co-digestion scenarios (Sce1 and Sce2) ranged between 3.71E-6 and 3.46E-6 kg P eq, representing a reduction of 50% and 54%, respectively, compared to BL. Sce3 showed a 20% reduction relative to BL. Similarly, co-digestion scenarios with methane recovery (Sce4 and Sce5) presented additional reductions of 7% and 11%, respectively, compared to their non-recovery counterparts.

Buonocore et al. (2018) reported eutrophication values between 3.4E-1 and 8.4E-1 kg P eq per functional unit (1,000 m^3 of treated wastewater), while Lin et al. (2021) documented values of 1.0E-4 and 1.1E-4 kg P eq per kilogram of treated matter. The values obtained in this study are notably lower than those previously reported, primarily due to differences in functional units and system boundaries. This shows the importance of standardizing functional units to ensure comparability across LCA studies.

In BL, the highest FEP contributions were associated with the mixing stage (30%), followed by collection (25%) and digestate storage (22%). This finding is consistent with (Ertem et al., 2017), who reported elevated eutrophication impacts during AD and collection stages. In contrast, for Sce1 and Sce2, the most impactful stages were pretreatment (39% and 38%, respectively), followed by mixing (25%) and digestate storage (19%) (Figure 22e).

Lijó et al. (2017) reported that in the co-digestion of energy crops with animal manure, the most impactful processes in the FEP category were feedstock cultivation and collection (75%), followed by bioenergy production (AD and CHP processes) and electricity consumption (each contributing 7.5%). While these studies did not include the mixing process, the negative impact observed in our mixing stage could be related to water usage and energy consumption required during this phase (Arbour et al., 2024; Payen et al., 2021).

3.4.5.6 Terrestrial acidification

In this category, the highest impact was recorded in the BL with 1.74E-4 kg SO2 eq, whereas the lowest impact occurred in Sce2, with 1.03E-4 kg SO2 eq. Sce3 achieved a 20% reduction compared to BL. Sce4 presented a 41% decrease relative to BL and a 6% reduction compared to its non-recovery equivalent. Lastly, Sce5 demonstrated the most significant reduction, with a 47% decrease compared to the BL and an 11% reduction relative to the non-recovery system.

In BL, the main contributors to TAP were DST (32%), collection (25%), and mixing (19%). In the co-digestion scenarios (Sce1 and Sce2), the key contributors were transportation (33%), pretreatment (32%), and DST (22%) (Figure 22f).

The collection and transportation stages were major drivers of acidification due to gasoline and diesel consumption, which directly influences TAP formation. These findings are consistent with Canabarro et al. (2023), who reported TAP values of 1.8E-4 kg SO_2 eq and 4.9E-5 kg SO_2 eq per functional unit (1 MJ) in biodiesel production systems.

The co-digestion of poultry litter and sargassum improved TAP by 41% compared to the monodigestion BL. (Subbarao et al., 2023) emphasized that the appropriate selection of feedstocks is crucial in managing acidification potential within AD systems.

3.5 Conclusions

This study assessed the environmental and energy performance of biogas production systems using monodigestion, co-digestion, and methane recovery strategies based on Life Cycle Assessment and Energy Return on Investment analyses. The results demonstrate that co-digestion with Sargassum and poultry or turkey litter improves biogas production efficiency by optimizing the carbon-to-nitrogen (C/N) ratio and reducing environmental impacts compared to mono-digestion. Implementing methane recovery from digestate storage significantly decreased greenhouse gas emissions, achieving up to 97% reductions in Global Warming Potential compared to non-recovery systems. Moreover, energy efficiency, reflected by the EROI, improved by 25% to 34% in co-digestion and recovery scenarios, confirming the system's energy viability, with EROI values above 3 in all cases. The study identified that transportation and digestate storage are key contributors to environmental impacts in several categories, highlighting the importance of improving logistics and biogas recovery systems for residual methane. Additionally, integrating Sargassum as a co-substrate presents an opportunity to address coastal environmental issues while contributing to renewable energy generation and circular economy models. This research confirms that combining co-digestion with methane recovery offers a promising solution for sustainable waste management and bioenergy production in line with climate change mitigation and sustainable development goals. Future studies should further explore the optimization of substrate mixtures and investigate additional recovery strategies to reduce the environmental footprint of anaerobic digestion systems.

3.6 Acknowledgments

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4. GENERAL CONCLUSION

Anaerobic digestion is a viable alternative for waste management. Co-digestion of substrates improved methane yield compared to monodigestion. Furthermore, energy efficiency improved by 25% to 34% in the co-digestion and waste methane recovery scenarios. Life cycle Assessment is a key tool for quantifying the impacts associated with biogas production from cradle to gate. The lowest environmental impacts were observed in the co-digestion of turkey litter and Sargassum, in most categories, with the exception of Terrestrial Ecotoxicity Potential. Furthermore, the recovery systems managed to reduce global warming potential by up to 97%. These findings confirm that the combination of substrates and methane recovery represents a viable strategy for bioenergy generation and sustainable waste management.

5. APPENDIX A

Flow diagrams and material balance analysis under different experimental scenarios

Figure A1 below, ilustrates the baseline flow diagram.The process entails several key stages, including raw material reception, entailed unit processes, and final products (biogas and digestate). Table A1 reflects the flows, solid fraction, and material balance analysis for each of the inputs and resulting outputs.

Table A1. Material balance analysis of inputs and outputs for the baseline.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	1608.25	0.907		
2	1608.25	0.907		
3	1608.25	0.907		
4	12548.50	0.000		
5	4824.75	0.000		
6	6433.00	0.265		
7	6433.00	0.265		
8	4156.85	0.338		
9	2276.15	0.132		
10	7723.75	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9943.82	0.012		
16	56.08		12.77	43.31

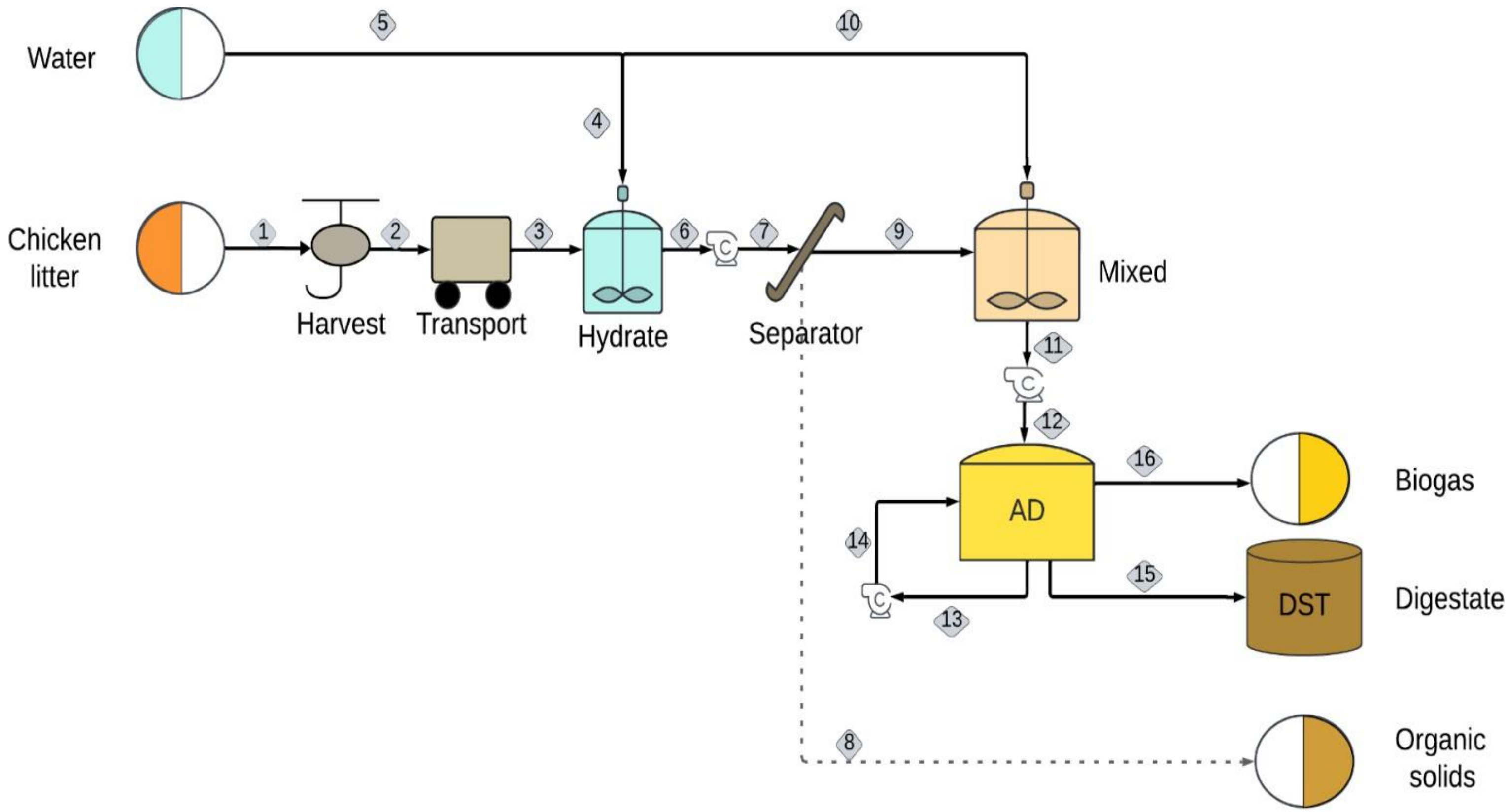

Figure A1. Monodigestion flow diagram (baseline). **AD:** anaerobic digestion, **DST:** digestate storage tank.

Figure A2 depicts the flow diagram associated with scenario 1.Thise process encompasses several key stages, including raw material reception, entailed unit processes, and final products (biogas and digestate). Table A2 provide information on the flows, solid fraction and material balance analysis for each of the inputs and resulting outputs.

Table A2. Material balance analysis of inputs and outputs associated with scenario 1.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	804.13	0.907		
2	804.13	0.907		
3	804.13	0.907		
4	2412.38	0.000		
5	9652.60	0.000		
6	3216.51	0.265		
7	3216.51	0.265		
8	2078.43	0.338		
9	1138.08	0.132		
10	7240.22	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9906.02	0.011		
16	93.89		29.36	64.52
17	1646.22	0.093		
18	1646.22	0.093		
19	1646.22	0.093		
20	3292.43	0.000		
21	1637.99	0.093		
22	3300.66	0.002		
23	1621.61	0.093		
24	16.38	0.093		

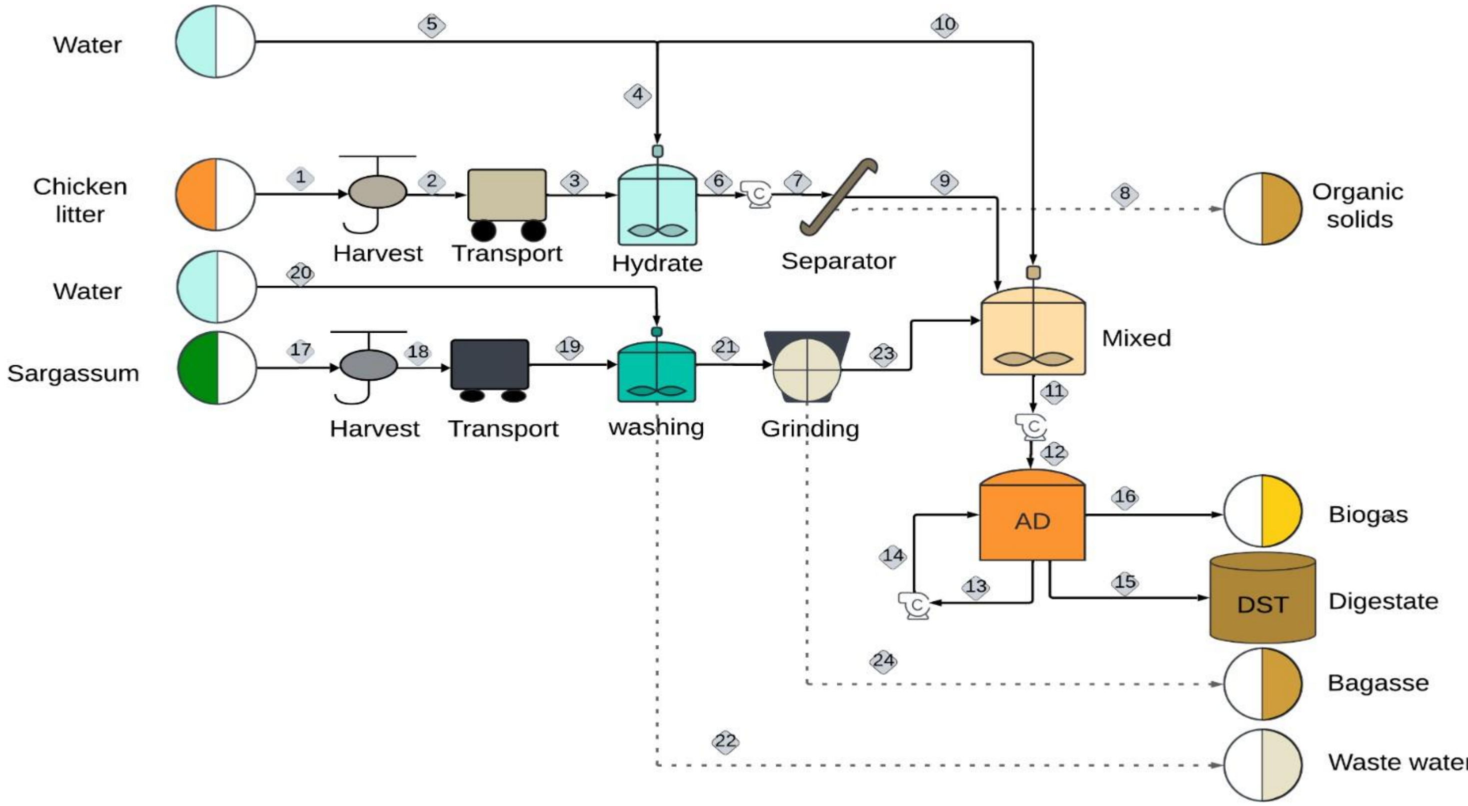

Figure A2. Scenario 1 (Co-digestion of Chicken litter and Sargassum). **AD:** anaerobic digestion,**DST:** digestate storage tank.

Figure A3 presents the flow diagram of scenario 2 .Thise process encompasses several key stages, including raw material reception, unit processes and final resulting products (biogas and digestate). Table A3 shows the flows, solid fraction and material balance analysis for each of the inputs and resulting outputs.

Table A3. Material balance analysis of inputs and outputs associated with scenario 2.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	630.10	0.915		
2	630.10	0.915		
3	630.10	0.915		
4	1890.31	0.915		
5	9195.65	0.000		
6	2520.41	0.260		
7	2520.41	0.260		
8	1447.46	0.349		
9	1072.95	0.140		
10	7305.34	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9901.75	0.010		
16	98.15		30.90	67.25
17	1646.22	0.093		
18	1646.22	0.093		
19	1646.22	0.093		
20	3292.43	0.000		
21	1637.99	0.093		
22	3300.66	0.002		
23	1621.61	0.093		
24	16.38	0.093		

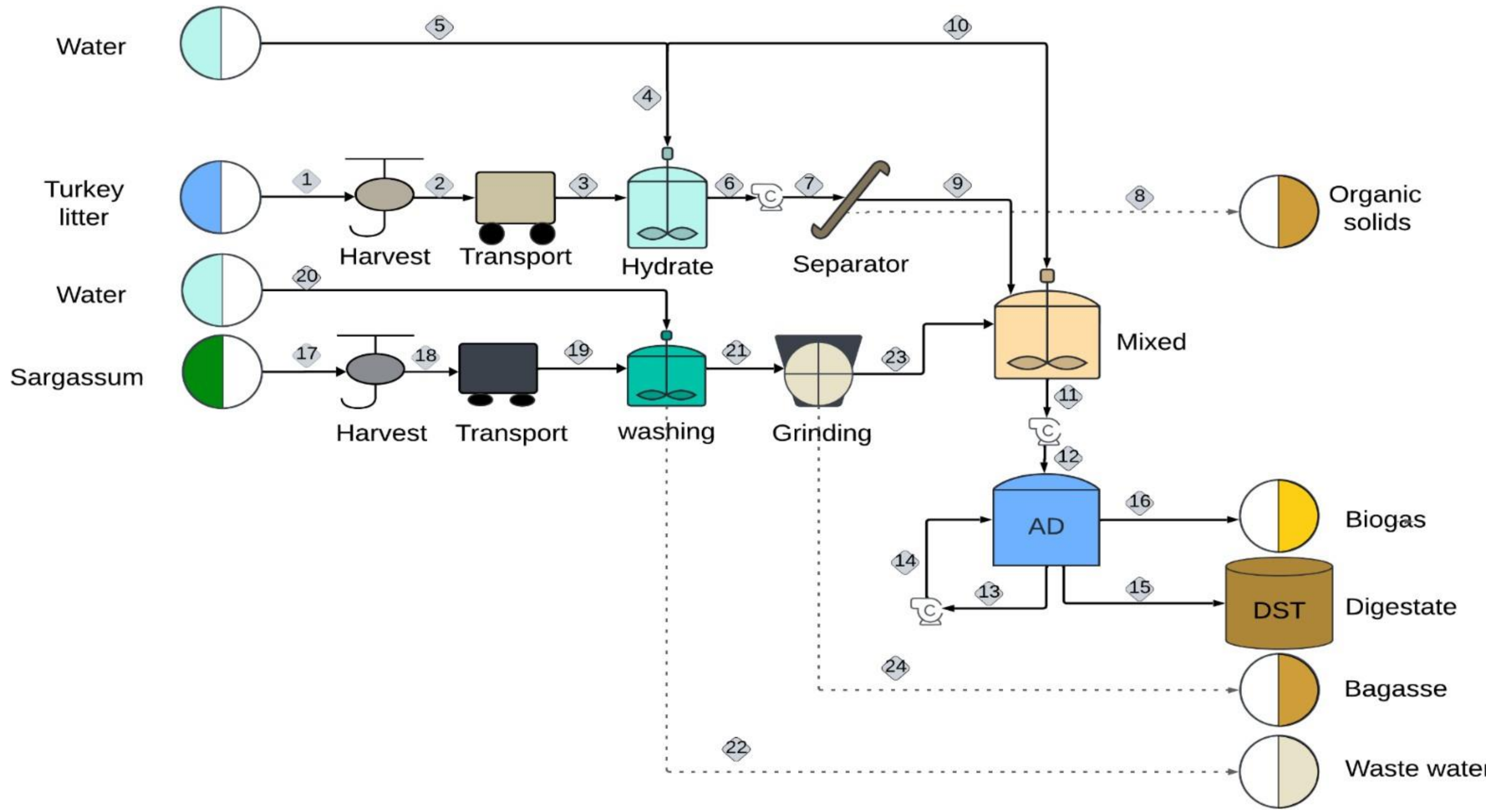

Figure A3. Scenario 2 (Co-digestion of Turkey litter and Sargassum). **AD:** anaerobic digestion. **DST:** digestate storage tank.

Figure A4 shows the flow diagram for scenario 3. Scenario 3 is an alternative to the baseline as it, , introduces a secondary system. Under this scenario the digestate storage tank (DST) is replaced by close storage tanks (CST). Table A4 reflects the flows, the solid fraction, and the balance of matter for each of the utilized inputs and resulting outputs.

Table A4. Material balance of the inputs and outputs for the scenario 3.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	1608.25	0.907		
2	1608.25	0.907		
3	1608.25	0.907		
4	12548.50	0.000		
5	4824.75	0.000		
6	6433.00	0.265		
7	6433.00	0.265		
8	4156.85	0.338		
9	2276.15	0.132		
10	7723.75	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9943.82	0.012		
16	56.08		12.77	43.31
17	14.20		3.23	10.96
18	70.28		16.00	54.28
19	9929.62	0.010		

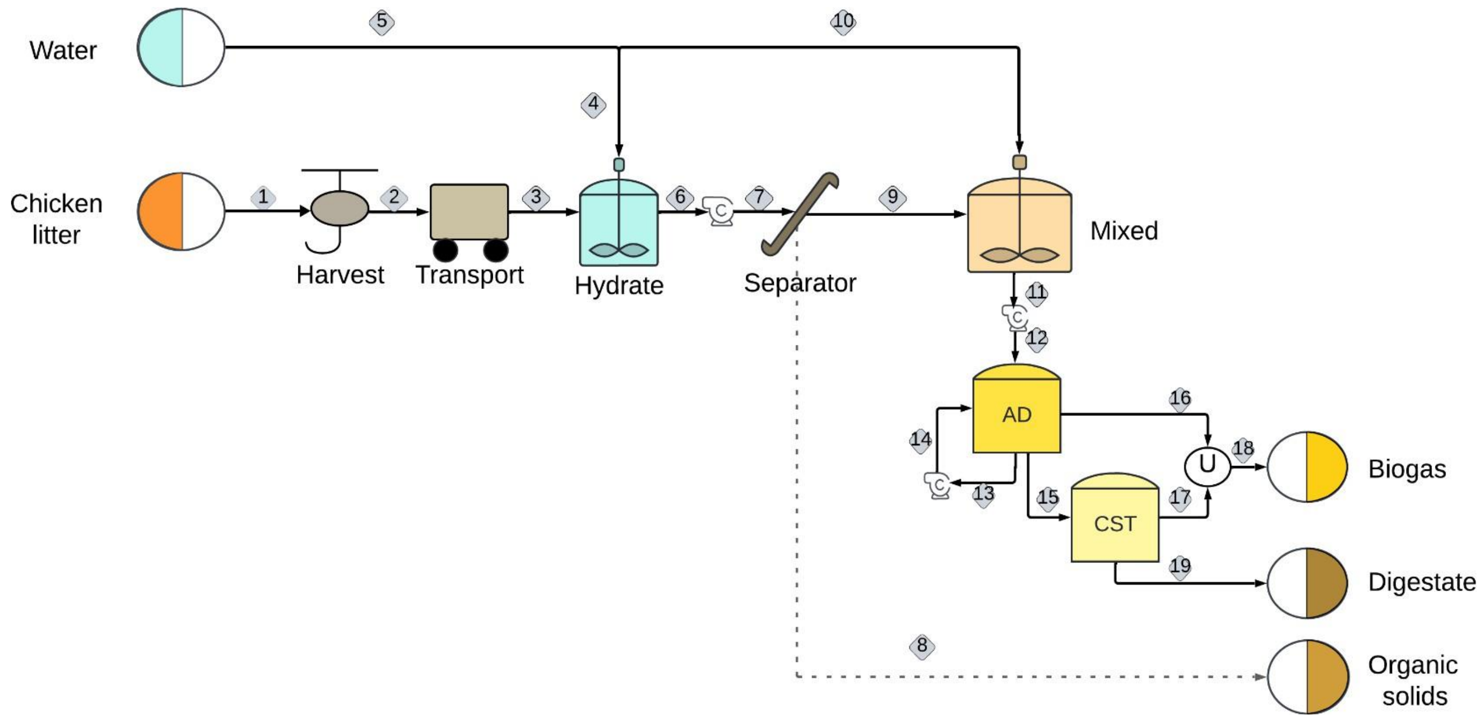

Figure A4. Scenario 3 baseline with recovery. **AD:** anaerobic digestion. **CST:** close storage tank.

Figure A5 shows the flow diagram associated with scenario 4. Scenario 4 is an alternative to scenario 1, in that it introduces a secondary system. Under this scenario the digestate storage tank (DST) is replaced by close storage tank (CST). Table A5 shows the flows, the solid fraction, and the balance of matter for each of the inputs and outputs.

Figure A5. Material balance of inputs and outputs for scenario 4.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	804.13	0.907		
2	804.13	0.907		
3	804.13	0.907		
4	2412.38	0.000		
5	9652.60	0.000		
6	3216.51	0.265		
7	3216.51	0.265		
8	2078.43	0.338		
9	1138.08	0.132		
10	7240.22	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9906.02	0.011		
16	93.89		29.36	64.52
17	1646.22	0.093		
18	1646.22	0.093		
19	1646.22	0.093		
20	3292.43	0.000		
21	1637.99	0.093		
22	3300.66	0.002		
23	1621.61	0.093		
24	16.38	0.093		
25	6.71		2.098	4.610
26	100.59		31.459	69.134
27	9899.31	0.008		

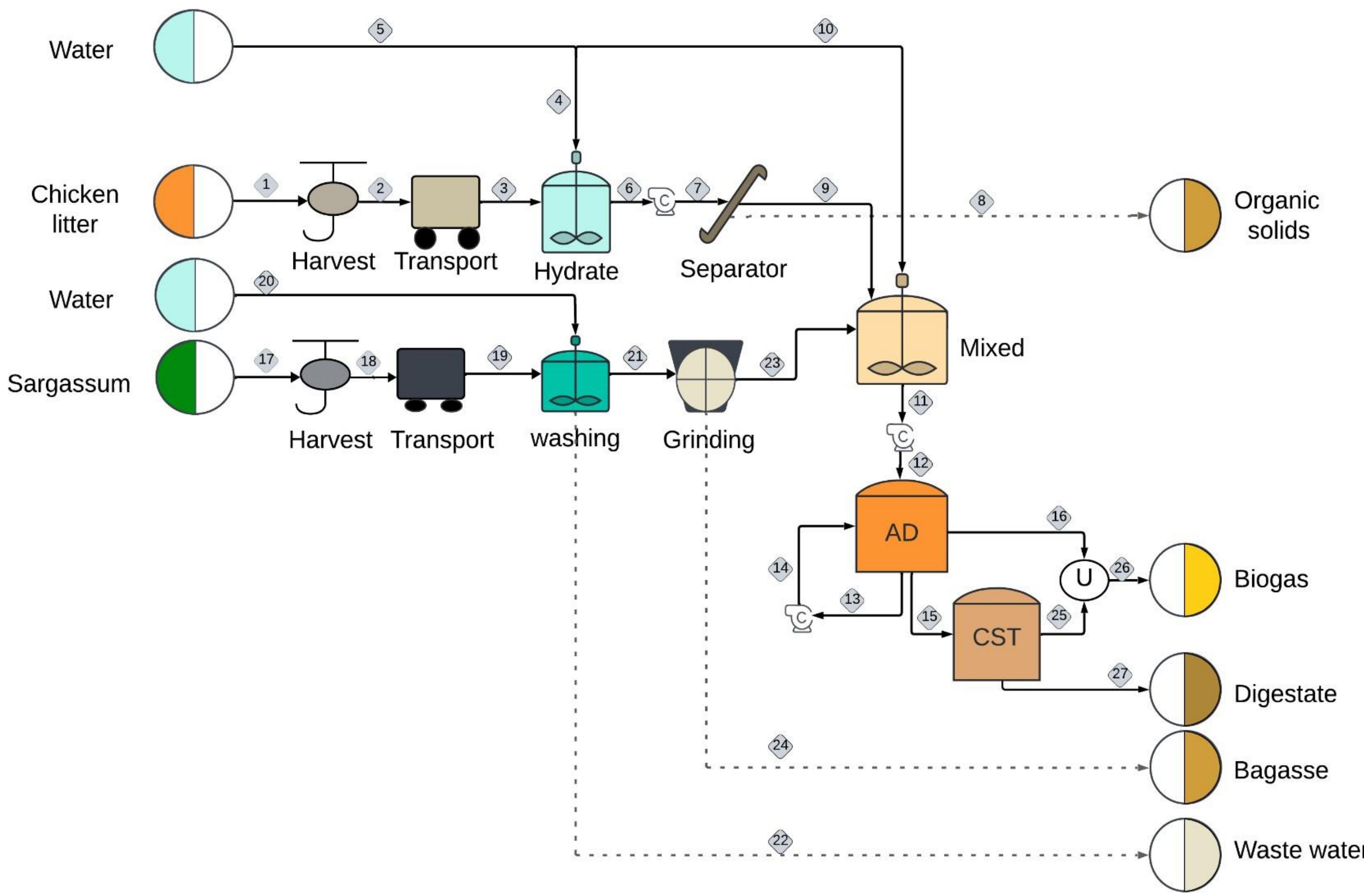

Figure A5. Scenario 4, similar to scenario 1, including the addition of a second methane recovery tank.

Figure A6 depicts scenario 5´s flow diagram. Scenario 5 is an alternative to the scenario 2, which introduces the implementing a secondary system. In this new scenario the digestate storage tank (DST) is replaced by close storage tank (CST). Table A6 reflects the flows, the solid fraction, and the balance of matter analysis for each of the inputs and outputs.

Table A6. Material balance for inputs and outputs associated with scenario 5.

Flow	Mass flow [kg d^{-1}]	Solids fraction %	CH_4 Mass flow [kg]	CO_2 Mass flow [kg]
1	630.10	0.915		
2	630.10	0.915		
3	630.10	0.915		
4	1890.31	0.915		
5	9195.65	0.000		
6	2520.41	0.260		
7	2520.41	0.260		
8	1447.46	0.349		
9	1072.95	0.140		
10	7305.34	0.000		
11	9999.90	0.030		
12	9999.90	0.030		
13	4999.95	0.030		
14	4999.95	0.030		
15	9901.75	0.010		
16	98.15		30.90	67.25
17	1646.22	0.093		
18	1646.22	0.093		
19	1646.22	0.093		
20	3292.43	0.000		
21	1637.99	0.093		
22	3300.66	0.002		
23	1621.61	0.093		
24	16.38	0.093		
25	11.94		3.76	8.18
26	110.09		34.66	75.43
27	9889.81	0.006		

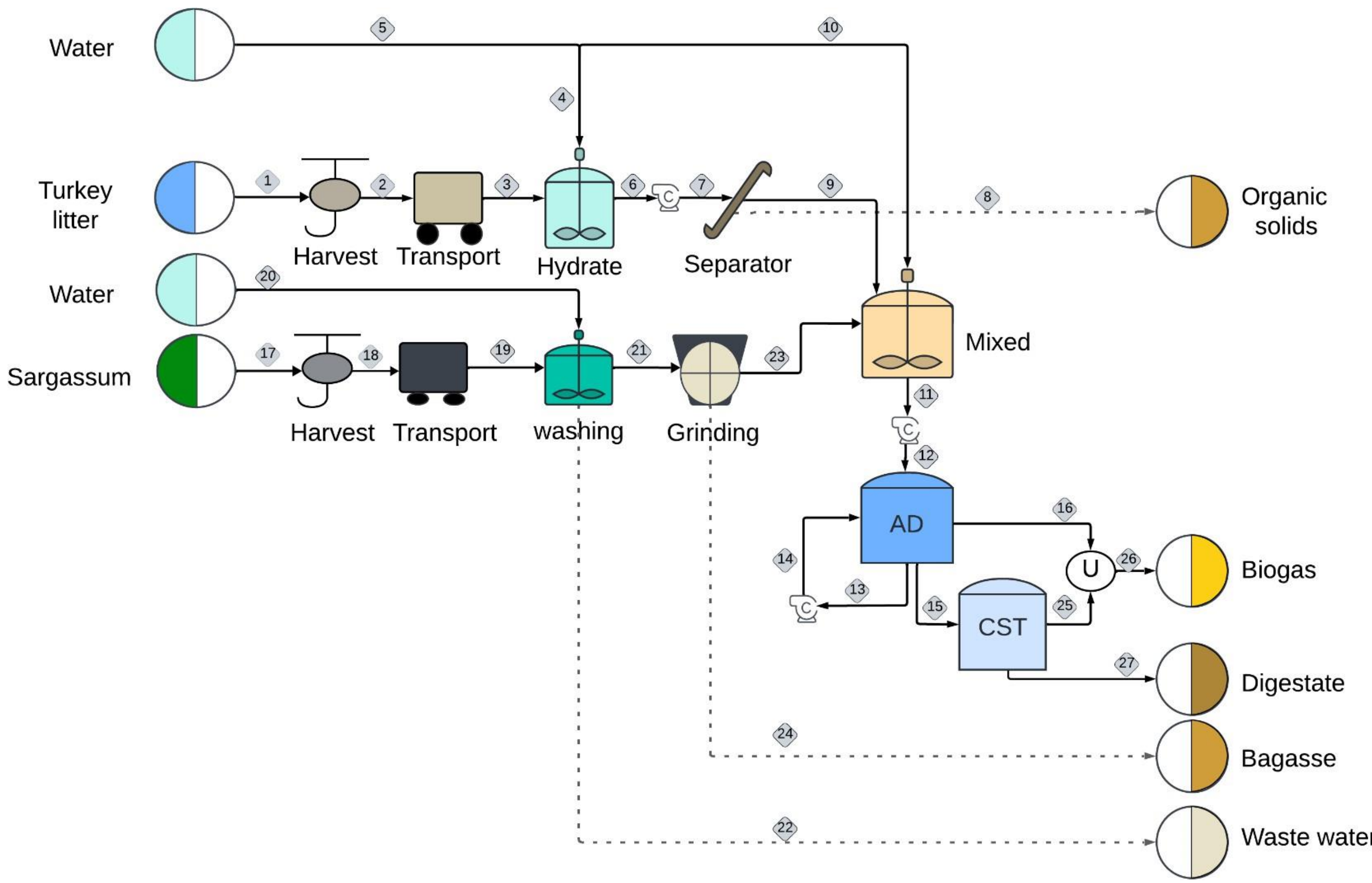

Figure A6. Scenario 5, system similar to scenario 2, including the addition of a second methane recovery tank.