

Review

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Review

A Review on Anaerobic Co-Digestion of Livestock Manures in the Context of Sustainable Waste Management

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Abstract: As the worldwide demand for meat per person is continuously increasing, there is a corresponding rise in the number of livestock animals, leading to an increase in livestock manure. Selecting appropriate treatment technologies for livestock manures is still a complex task and considerable debates over this issue persist. To develop a more comprehensive understanding of the manure treatment framework, this review was undertaken to assess the most utilized manure management technologies and underscore their respective challenges. Anaerobic digestion has become a commercial reality for treating livestock manures. However, the mono-digestion of single substrates comes with certain drawbacks associated with manure characteristics. Anaerobic co-digestion, involving the utilization of multiple feedstocks, holds the potential to overcome these limitations. Extensive research and development have underscored numerous intrinsic benefits of co-digestion. These include improved digestibility resulting from synergistic effects of co-substrates and enhanced process stability. This review underscores the limitations associated with the mono-digestion of livestock manures and critically evaluates the advantages of their co-digestion with carbon-rich substrates. Additionally, this review delves into key livestock manure management practices globally, emphasizing the significance of co-digesting livestock manures while addressing the progress and challenges in this field.

Keywords: anaerobic co-digestion; livestock manures; sustainability; synergistic effects; biogas

1. Introduction

Livestock manure disposal stands as a prominent contributor to global atmospheric pollution and emissions of greenhouse gases (GHG), including methane (CH_4), nitrous oxide (N_2O), carbon dioxide (CO_2), hydrogen sulfide (H_2S), ammonia (NH_3), methyl mercaptan (CH_3SH), di- and trimethyl sulfide, volatile organic compounds and endotoxins [1]. Forecasts predict that to accommodate the needs of the growing global population, the worldwide meat production will surge from 330 million tons in 2017 to 465 million tons by 2050, consequently driving an expansion in intensive livestock farming. The rapid expansion of livestock production has led to a substantial increase in the volume of livestock manure, estimated at approximately 13 billion tons annually on a global scale. This poses potential environmental hazards if left untreated [2,3]. Comprising a blend of feces, urine, and flush water, livestock manure is inherently biodegradable. Mismanaged, however, it can inflict severe contamination upon soil, air, water, and even result in the proliferation of hazardous microorganisms within eco-systems. Stringent environmental regulations have presented a challenge for dairy proprietors in effectively conducting their livestock production and management activities.

Historically, livestock manure has been a traditional choice for enriching soil fertility in agricultural practices, providing essential nutrients for crop growth. An average cow dung contains 10 lb of nitrogen (N), 5 lb of phosphate (PO_4^{3-}) and 10 lb of potash (K) per ton [4]. Moreover, manure hosts microorganisms that can enhance soil structure and biological activity. However, its application as a fertilizer has dwindled due to stringent regulations, transportation expenditure and availability of synthetic fertilizers. Several countries employ open storage as a manure management strategy, yet this approach can result in a 70% N loss within 24 hours, primarily through NH_3 volatilization and

nitrate leaching (NO_3^-) leaching, particularly in humid tropical regions with high rainfall [5]. Alternatively, some nations implement lagoon method, where treated manure is released into surface water, causing complete loss of organic matter and nutrients, leading to water body eutrophication and metal toxicity [6]. Additionally, livestock manure is a major contributor to environmental pollution and GHG emissions, accounting for 14.5% of total anthropogenic emissions including CH_4 , CO_2 and N_2O . More than 1.4 billion tons of CO_2eq is attributed to livestock manures, with cattle manure being the largest contributor and having the highest share in total manure generation [7]. The environmental consequences from livestock manure generation and contemporary livestock manure disposal have prompted authorities to seek sustainable alternatives for livestock farming.

Anaerobic digestion (AD) involves series of biochemical processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Over the last few decades, the application of AD to livestock manures has been prompted to mitigate GHG emissions linked to traditional manure disposal techniques. If the currently exploitable amount of manure were used for energy production through AD, it is possible to prevent the release of 159 kt of CO_2eq , in contrast to the emissions associated with current manure management practices [8]. However, its feasibility of AD for livestock manure is now being reconsidered due to several factors. Livestock manure displays low organic load coupled with a high N concentration, which hampers the activity of methanogenic microorganisms. Ruminant manure, like that from cows, plays a pivotal role in the initial stages as it harbors essential methanogenic bacteria. These bacteria facilitate the breakdown of easily biodegradable components while leaving biomass with high cellulose content undigested. The complex nature of cellulose presents a challenge for microorganisms to efficiently decompose it, thereby making hydrolysis the rate limiting step [9]. Nevertheless, this results in a reduced CH_4 yield owing to moderate anaerobic biodegradability of 45-50% [10]. Mono-digestion of pig manure leads to comparatively low CH_4 productivity because of the slow degradation rate of its solid fraction and the high levels of NH_3 water content [11]. Similarly, poultry droppings contain a notable concentration of NH_3 acting as an inhibitor. The AD of N-rich manure is frequently hindered by the elevated levels of NH_3 generated during breakdown of proteins and uric acid. This heads to the buildup of VFAs and diminishes CH_4 production [12]. Despite potential improvements in digestion outcomes, biogas production often falls short of ensuring profitability due to the need for larger reactor volumes, heightened water consumption and substantial slurry volume generation [3,13].

Anaerobic co-digestion (AcoD) emerged as a solution to mitigate the limitations of mono-digestion by concurrently digesting multiple substrates. The simultaneous digestion of livestock manure with other substrates like food waste, organic fractions of municipal solid waste, agricultural residues, sewage sludge, and slaughterhouse effluents significantly enhances CH_4 production [14–18]. The improved CH_4 yield can be attributed to role of manure as carrier for dryer substrates due to high moisture, manure has high buffering capacity, which maintains pH of digester, manure is nutrient rich, which is necessary for microbes to thrive, manure has the requisite anaerobic microbes from the rumen to initiate the degradation process [19]. The synergy including, livestock manure driving the digestion process while co-substrates enhancing CH_4 yield due to higher biodegradability underscores the unique attributes of this approach.

The noticeable surge in publications concerning AcoD in recent years underscores its practicality and suitability for livestock manure management. The objective of this review is to present recent advancements in AcoD involving livestock manure and various co-substrates. Furthermore, it aims to offer valuable insights into the compatibility of diverse co-substrates and the resultant synergistic effects.

2. Types of livestock manure

Over the years, there has been a substantial increase in global livestock production to cater need of a growing population. Globally, the poultry production has seen sharp rise in the last few decades with an overall global increase of 73% since 1995. Goat production has also increased rapidly (+46%) during this period. In contrast, global production of pigs, cattle and sheep has increased at much slower pace with increase of 11%, 9.5% and 7.8% since 1995 [20]. The substantial escalation in worldwide

livestock production has led to the generation of a substantial volume of manure as a byproduct of animal husbandry. The production of livestock manure has experienced a notable increase, with cattle manure comprising the largest proportion at 53 kg/head/day, followed by 4.5 kg/head/day for pig manure and 0.3 kg/head/day for chicken manure [21,22]. The cattle industry serves as a substantial source of manure production globally, particularly in countries such as Brazil, India, China, Argentina, and Australia, which are among the leading cattle producers. Typically, feedlot cattle can generate manure equivalent to about 5-6% of their body weight, a significantly higher proportion to compared to other animals. This elevated contribution from cattle manure makes it primary contributor to the overall manure generation. Following closely, pig manure stands as the second largest contributor to total manure production, reaching nearly 1.7 billion tons annually. This figure is expected to rise further, given the anticipated 22% increase in global pork consumption by 2050. Despite the substantial growth in the poultry breeding industry, global chicken manure generation remains comparatively lower, estimated at around 20,708 million tons, when compared to cattle and pig manure [23,24]. Livestock manure represents a type of organic waste that typically includes animal feces and urine. Beyond these components, it may encompass other organic materials like straw, remnants of fodder, and various body parts of animals such as hairs, bristles, and feathers. Additionally, the composition may involve foreign materials such as lime, sawdust, and other substrates, the presence of which largely influenced by the type of bedding materials used in livestock farms. As per dry matter content, livestock manure is categorized as solid, liquid, and slurry. Solid manure is commonly collected from animal farms using scrapers and stored in open pits. Liquid manure is typically a byproduct of the water used during the cleaning of animal farms, while slurry is usually collected using vacuum pumps and stored in designated tanks [25]. Understanding the characteristics of livestock manure is essential for addressing concerns related to its management. Table 1 represents the characteristics of livestock manure reported in previous studies.

Table 1. Characteristics of cattle, pig, and chicken manure reported in previous studies [26–28].

Parameter	Cattle manure	Pig manure	Chicken manure
TS (g/kg)	310	70.6	132-171
VS(g/kg)	235.3	56.5	55-75
TSS (g/kg)	280	58.5	NA
VSS (g/kg)	230	46.8	NA
pH	8.0	7.6	6.9-7.4
TCOD (g/kg)	290.8	134.5	87-130
SCOD(g/kg)	22.5	39.4	32-97
TVFAs (g COD/kg)	1.5	22.3	10-50
TN (g/kg)	9.1	6.1	4
TP (g/kg)	1.0	1.8	8.1
C	38.8	46.4	30.92
H	4.9	5.9	3.89
O	29.3	34.5	32.08
N	1.6	2.1	7.74
Alkalinity (g caCO ₃ /kg)	32.7	13.8	NA

(TS: Total solids; VS: Volatile solids; TSS: Total suspended solids; VSS: Volatile suspended solids; TCOD: Total chemical oxygen demand; SCOD: Soluble chemical oxygen demand; TVFAs: Total volatile fatty acids; TN: Total nitrogen; TP: Total phosphorous.).

The literature review highlights a discernible variation in the characteristics of diverse livestock manures. The quality of manure is predominantly influenced by the quality of feed, a factor that significantly impacts the choice of manure management technology. Feces and urine serve as the primary source of carbon (C) and other nutrients in manure, with common nutrients including nitrogen, phosphorus, potassium, and others. The nutrient content can vary based on factors such as

region, feeding patterns, animal physiology, external mineral elements, and use of antibiotics. Feeding patterns and type of feed exert a significant influence on the nutrient composition of livestock manures. For example, when animals are fed low-quality grass, especially from tropical regions, that excreta would exhibit lower nutrient content [29]. Manure with low nutrient levels is unsuitable for energy generation and may not be economically viable. Conversely, chicken manure is often rich in organics, ammonia-nitrogen, pathogens, and microorganisms due to the use of nutrient rich feed and supplements for accelerated growth. Consequently, chicken manure emerges as a suitable substrate for bioenergy production, given its high total solids content and increased biodegradability. However, challenges may arise due to the higher NH_3 concentration and low C/N ratio in chicken manure, stemming from its elevated protein and fat content [30]. Effectively managing livestock manure necessitates consideration of its physiochemical characteristics. Moreover, experiences from various countries emphasize that regulations and protocols lacking simultaneous consideration of technical, agronomic, economic, environmental, and social/health safety aspects are likely to fall short in delivering optimal manure management strategies.

3. Environmental impact of livestock manure generation

Livestock manure holds a significant potential for environmental contamination and the release of GHG emissions. For example, the CH_4 emissions potential from cattle, pig, and chicken manure in Asia are 25.57, 4, and 0.2 kg CH_4 head⁻¹ yr⁻¹, respectively. The N_2O emissions potential from cattle, pig, and chicken manure are 0.07, 0.007, and 0.001 kg N_2O -N (nitrogen excreted) [31]. In 2018, the global number of livestock unit was 1.9 billion and this figure is projected to rise considerably by 2030, leading to rapid increase in the production of livestock waste and associated gases [2]. With rise in livestock population, the global production of livestock manure reached 125 million tons of N, a 23% increase since 1990. Out of this total, approximately 88 million tons of N were left on pasture, showing a 43 % increase since 1990. Around 34 million tons of N were treated in management systems, with 7 million tons of N lost primarily as NH_3 and 27 million tons of N applied to soils. A small portion, approximately 3 million tons of N utilized for other purposes such as heating and construction. These categories of manure management have remained relatively unchanged as of 2023 [32] which is adversely affecting ecosystem. Figure 1 depicts global distribution of N excretion from livestock manure, including land applications and management losses.

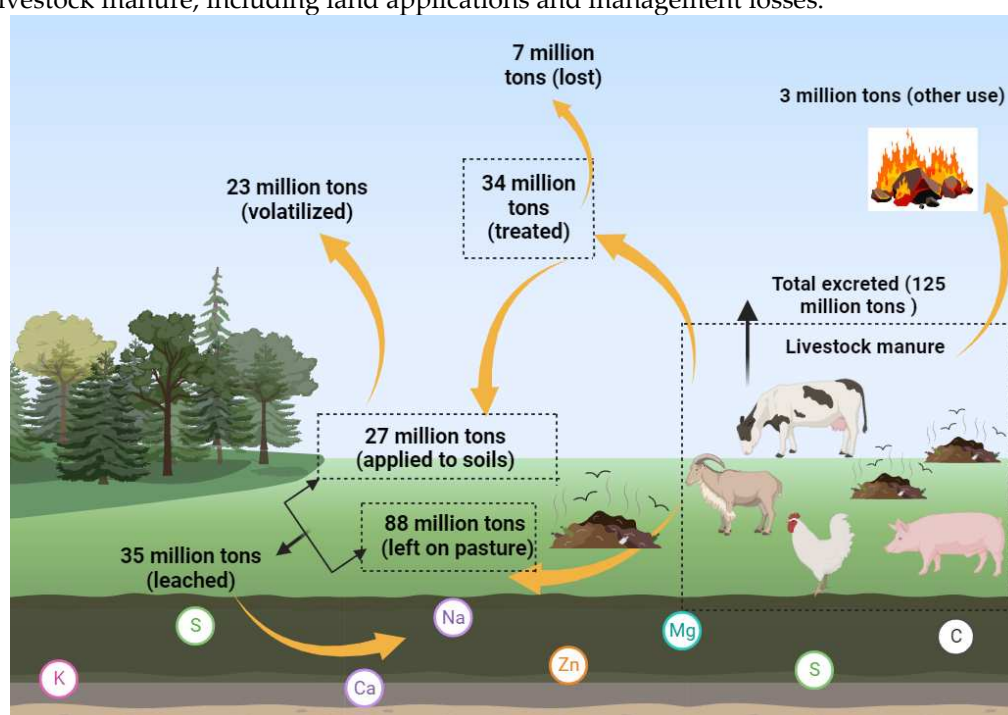


Figure 1. The worldwide flow of N excretion from livestock manure to land application and losses during management (in million tons of N) (source: FAOSTAT, 2020).

Regarding climate effects, livestock manure was responsible for over 1.4 billion tons CO₂eq emissions. Specifically, manure left on pasture accounted for 875 million tons CO₂eq, while manure applied to soils produced 190 million tons CO₂eq as N₂O gas, and 347 million tons CO₂eq resulted from CH₄ lost in manure management systems. When looking at regional distribution, Asia and America are the leading contributors with annual emissions surpassing 1 billion tons of CO₂eq. These emissions were primarily driven by a combination of enteric fermentation and manure processes. Notably, Africa experienced the highest growth rate in GHG emissions since 1990 with an average annual increase of 2.4% [32]. Livestock manure has a substantial impact on GHG emissions, constituting 14.5% of the total anthropogenic emissions. The emissions originating from livestock manure are comprised of 50% CH₄, 24% N₂O, and 26% CO₂. Within this spectrum, cattle emerge as the primary contributors, contributing to 62% of the overall GHG emissions from livestock [7]. This underscores the immediate necessity to alter the strategy for managing cattle manure with the goal of safeguarding aquatic environments and mitigating GHG emissions. The practices, including composting, vermicomposting, gasification and combustion, and pyrolytic carbonization, are frequently employed for the management of livestock manures.

4. Global livestock manure management practices

For centuries, manure has served as a bio-fertilizer in both developed and developing nations. With a comprehension of the connection between the application of manure and its associated environmental consequences, many countries have established specific and, in certain instances, stringent regulations concerning the recycling of manure and the utilization of nutrients derived from animal waste in agricultural production. For instance, in European Union (EU), stringent regulations are in place to prevent the field application of manure. Commonly enforced laws and regulations within EU encompass “Common Agriculture Policy (1992)”, Nitrate act (91/676/EC), and “Good Agricultural Practices”. In USA, the Department of Agriculture (USDA) proposed “Comprehensive Nutrient Management Planning (CNMP)” as a significant measure to address environmental pollution stemming from intensive livestock farming [33]. Likewise, in Korea, the Ministry of Environment has instituted stringent regulations on practices related to manure management, prompted by the highest N balance (222 kg/ha) [34]. Given the circumstances, the livestock manure undergoes diverse management practices in both developed and developing nations. Figure 2 depicts the common global livestock manure management practices.

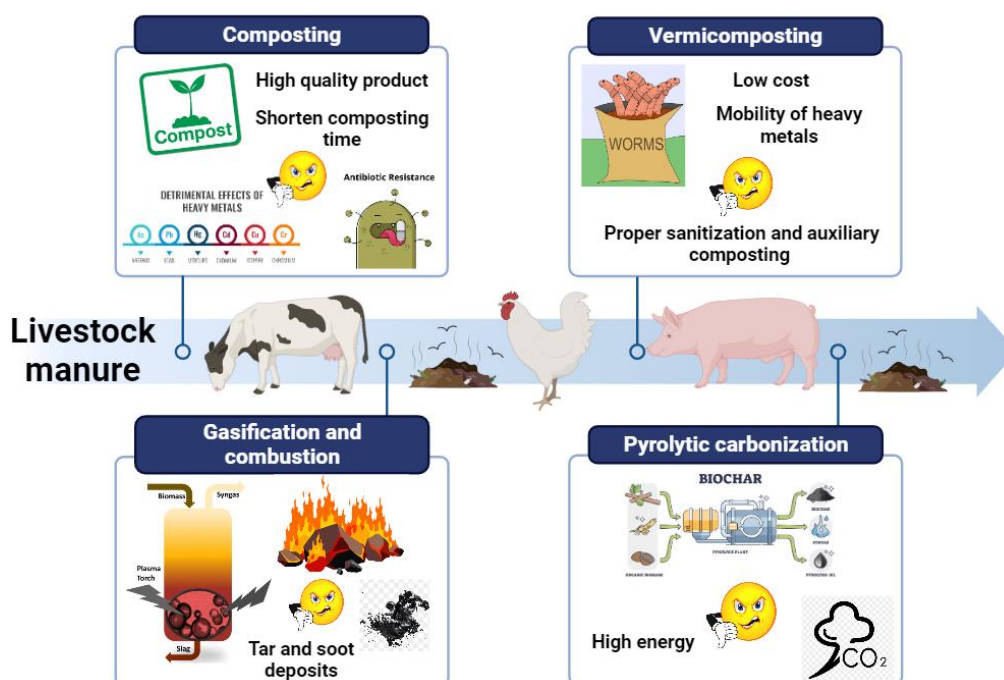


Figure 2. Graphical overview of livestock manure management practices commonly used all over globe.

4.1. Composting

Composting is considered an aerobic, thermophilic, microbial-mediated solid-state fermentation process through which organic wastes including livestock manures are converted into more stable compounds, free of phytotoxicity and pathogens and with certain humic properties [35]. In the initial phase, microorganisms easily mineralize and metabolize simple organic C compounds, generating CO₂, NH₃ and H₂O, organic acids, and heat. Composting is an inherently spontaneous biological decomposition process involving organic materials within predominantly aerobic environment. Throughout the process, bacteria, fungi, and other microorganisms, facilitate the breakdown of organic materials into stable, usable organic substances referred to as compost. Additionally, composting entails volume reduction of wastes and the elimination of weed seeds and pathogenic microorganisms. Composting offers significant advantages, such as enhancing soil structure, mobilizing nutrients in soil, improving soil fertility, facilitating microbial remediation, and mitigating soil desertification. Nevertheless, the composting of livestock manures comes with certain drawbacks that might impede the industrialization of composting technology. These drawbacks encompass the loss of N during the composting process, the heightened bio-availability of heavy metals, the diminished humus content of organic matter, the residue of antibiotics, and GHG emissions [36]. During composting, the transformation of substrates is influenced by the degradability of organic matter, a factor affecting decomposition rate, gas emissions, process duration, and oxygen requirements. Labile organic compounds, including simple carbohydrates, fats, amino acids, undergo rapid degradation in the initial composting stage. In contrast, more resistant substrates like cellulose, hemicellulose, and lignin are only partially degraded and transformed at a slower rate. Composting thus entails a partial mineralization of the organic substrate, resulting in C losses throughout the process. During composting of livestock manures, organic C losses can be as high as 67% in cattle manure, 52% in chicken manure, and 72% in pig manure [35]. Due to the reduction in material dry weight during composting, the concentration of mineral elements tends to rise, provided leaching is minimal or controlled. Typically, there is an increase in total N concentration during composting due to the concentration effect. Composting leads to the mineralization of organic compounds, resulting in the formation of NH₄⁺-N which is highest during the thermophilic phase. In this phase, organic matter degradation and aeration demand are at their maximum, while pH is usually above 7.5 which promotes NH₃ volatilization [37]. The loss of N during the composting process has negative effects on both compost quality and the environment. It leads to a decrease in nutrient concentration and can cause health and environmental issues. N losses can occur through NH₃ volatilization, leaching and denitrification. Manure composting experiences significant N losses due to high initial NH₄⁺-N concentration and the presence of easily mineralized compounds, such as uric acid found in chicken manure and slurry [38]. Additives can improve the composting process and end-product quality, while reducing associated problems. For example, a mixture of zeolite, wood vinegar, and biochar can reduce NH₃ volatilization, GHG emissions and N loss [39]. Superphosphate can decrease NH₃ emissions and enhance the nutritional content of final product. Biochar additives and bacterial inoculation can also decrease N and C loss by inhibiting gaseous emissions and improving carbon and N sequestration during manure composting [40]. However, the inclusion of additives in the composting process could escalate production cost, potentially jeopardizing the economic feasibility of composting. It is crucial to strike a balance between economic performance and environmental impacts to find an optimal solution. In addition to biological, chemical, and physical additives, other effective approaches for reducing gaseous emissions include controlled aeration, negative pressure aeration and forced aeration [41].

4.2. Vermicomposting

Vermicomposting is a natural process that involves earthworms and microorganisms working together to break down organic materials. It enhances the decomposition process, leading to the stabilization of organic matter and significant changes in its physical and biochemical characteristics. Vermicomposting has gained popularity in numerous countries because of its simple and effective technical approach. It is widely adopted in waste management and farming due to its ease of

implementation [42]. While microorganisms are responsible for producing the enzymes that initiate the biochemical decomposition of organic matter, earthworms play a pivotal role in driving the process forward. By fragmenting and consuming organic matter, earthworms stimulate and enhance biological activity, leading to an increased surface area for microorganisms to work on. Essentially, functioning as mechanical blenders, earthworms break down organic matter and gradually alter its physical and chemical characteristics by reducing C/N ratio and increasing the surface area available for microorganisms to thrive. Vermicompost is a finely textured substance that resembles peat. It has a low C/N ratio and boasts excellent structure, porosity, aeration, drainage, and moisture retention capabilities. Additionally, it provides a balanced mineral composition, enhances plant nutrient availability, and can serve as granules for complex nutrient sourcing. Previous literature review has suggested that vermicomposting is a cost-effective approach for converting livestock manure, into nutrient rich bio-fertilizer using various species of earthworms [33,43]. While vermicomposting has been found to reduce the mobility and availability of heavy metals present in livestock manure [44], there is limited information on the impact of vermicompost on sustainable nutrient management and crop sustainability in intensive agricultural practices. Further research is needed in this area. Furthermore, the commercial application of vermicompost is not yet widely adopted. Therefore, it is crucial to gain a deeper understanding of the composition of immature vermicompost and the reasons behind its application failures. Additionally, determining appropriate vermicompost concentrations under typical soil-water plant microclimatic conditions is necessary for successful implementation [42]. In comparison to traditional composting, vermicomposting offers the potential for higher returns and reduced annual costs. However, for scaling up and commercialization purposes, a comprehensive economic evaluation is necessary. Typically, vermicomposting requires lower capital investment and less space compared to composting facilities. However, it may not effectively sanitize materials, requiring the use of auxiliary composting methods.

4.3. Gasification and combustion

Gasification is a well-established technology that has been utilized for many centuries to convert biomass into energy. This process involves the use of high temperature (800-1000°C) to partially oxidize and dissociate the biomass molecules, effectively transforming the stored energy into a clean and usable form known as syngas [45]. The primary components of syngas, which is derived from biomass gasification are carbon monoxide (CO), and hydrogen (H₂). This composition makes syngas suitable for various applications. It can be utilized as fuel in single or combined mode gas turbines to generate electricity. Additionally, syngas can be used in the production of hydrocarbon-based chemicals using the Fischer-Tropsch process. In recent times, the gasification of poultry litter has gained significant popularity. While many studies have focused on bench-scale reactors or simulation-based approaches, there is growing recognition of chicken manure as a promising feedstock for gasification [46]. Gasification offers the advantage of fuel flexibility, allowing to produce heat and power using clean biomass. The resulting syngas can serve as a valuable chemical building block. This technology proves to be effective in supporting cleaner energy strategies by generating hydrogen-rich syngas with a higher heating value ranging from 15-20 MJ/Nm³. Furthermore, gasification plays a crucial role in the decarbonization of the entire energy system [47]. Gasification technology shows great promise for the treatment of livestock manure due to its versatility in processing different feedstocks and generating valuable products. However, significant drawbacks of gasification are the presence of tar and other contaminants such as soot in the syngas. These impurities necessitate the use of specialized treatment methods and costly equipment for their removal. Soot formation can also result in the accumulation of solid deposits in gas engines [33,45]. Combustion, as an alternative method for treating livestock manure, utilizes the combustion of biomass in a boiler to generate heat and electricity. The combustion process produces hot gases containing CO₂ and H₂O. Steam is generated during this process and can be utilized to drive a steam turbine for the production of electricity [45]. The gases emitted during the combustion process, namely CO₂ and water vapor, are exhaust gases that are released into the atmosphere. Like gasification, combustion processes are also characterized by the creation of carbonaceous deposits.

These deposits can pose challenges to the efficient operation of the system. Similarly, the efficiency of gasification and combustion is largely influenced by various factors such as the type, design, and size of the system, as well as the characteristics of livestock manures. Livestock manures, contains a high amount of ash compared to the other biomass materials like wood and straw. During combustion this can result in technical challenges such as agglomeration and fouling, which negatively impacts process efficiency [48]. Additionally, a high moisture content in the feedstock (above 20%) is detrimental to the burning rate, as it lowers the flame temperature and create cold spots. This ultimately leads to decreased process efficiency [45]. While pretreatment can address issues related to the heterogeneity and high ash content of manure feedstock, other significant problems remain unsolved. The high levels of incombustible constituents and increased concentration of alkali and chlorine are major concerns in gasification and combustion of livestock manure. Gasification and combustion technologies for livestock manure are offered to users in various scales, including small, medium, and large systems. However, to optimize and advance these processes, it is crucial to possess extensive knowledge and deep understanding of their operating conditions.

4.4. *Pyrolytic carbonization*

Pyrolysis or devolatilization is a process that involves the thermal decomposition of carbonaceous materials. This occurs at moderate temperatures, typically above 300°C, in an environment free of oxygen. The resulting products are non-condensable gases such as CO₂, CH₄, CO and H₂, and other light hydrocarbons, as well as bio-oil and solids, including biochar [47]. Biochar is a C rich substrate that is produced through the process of pyrolytic carbonization. It is commonly used in agriculture for soil improvement and has various eco-friendly applications. The characteristics of biochar are closely linked to the type of feedstock used, the specific pyrolysis conditions, and other operational factors in the process. Livestock manures is commonly used feedstock for biochar production, However, the properties of the manure and specific operating conditions used during biochar production can significantly impact the characteristics of final product. Biochar derived from manure exhibits various physiochemical traits that are influenced by factors such as the carbonization process, combustion temperature, process duration, and feedstock type. When produced under low-temperature conditions, biochar typically has a higher yield and lower density, while experiencing fewer losses of C and N [49]. Biochar derived from pyrolysis of livestock manures is widely employed as a soil amendment to improve soil fertility. Its porous structure and nutrient rich composition make it an effective soil additive. Additionally, biochar finds applications as a water and air pollutant adsorbent, a C sequestration material, a catalyst for pyrolysis, and an energy storage supercapacitor. During the pyrolysis process, biochar can help prevent the accumulation of heavy metals from manure, thus minimizing environmental pollution [50]. Numerous studies have investigated and illustrated the vast potential of utilizing biochar derived from livestock manure as a soil amendment. For instance Shakoore et al. [51] demonstrated the effectiveness of using biochar derived from livestock manure as an organic amendment. This application showed promising results in increasing soil organic C content, improving soil fertility, and boosting crop yield. Furthermore, livestock manure derived biochar proved to be more efficient in reducing N₂O emissions compared to raw manure. Similarly, Kiran et al. [52] demonstrated that cattle manure derived biochar exhibited superior performance compared to raw manure, resulting in a significant decrease in soil Cr content by 34.3% to 69.9%. Furthermore, biochar produced from livestock manure holds immense potential in safeguarding ecosystems through various means such as GHG mitigation, waste management and soil enhancement.

Livestock manure management practices, such as composting, vermicomposting, gasification, combustion, and pyrolytic combustion are commonly used. Composting and vermicomposting are widely used for recycling nutrients and creating soil amendment. However, it is essential to acknowledge that each of these methods comes with limitations and environmental concerns. For example, composting is not without drawbacks, including N loss, leaching of heavy metals, residue of antibiotics, and low humus content in organic matter. During the composting of livestock manures,

approximately 932 g kg⁻¹ of CO₂ and 0.4 g kg⁻¹ of N₂O are emitted until the process is completed [53]. A significant concern associated with composting livestock manure is the presence of antibiotic resistance genes. The composting process has been reported as an ineffective solution for addressing issue related to antibiotic resistance genes. Similarly, vermicomposting is an efficient method for recycling organic waste and generating beneficial organic amendments. Despite its effectiveness, the widespread commercial application of vermicompost remains poor [26]. Efficiency plays a pivotal role in thermochemical conversion technologies such as gasification and combustion, typically ranging from 50% for gasification and 45-55% for combustion [33]. This efficiency is contingent upon factors such as the type, design, and size of the conversion system, along with the specific characteristics of the livestock manure being processed. At last, pyrolytic carbonization necessitates higher operating temperatures, demanding increased energy input. Additionally, several environmental concerns associated with pyrolysis carbonization impede its widespread commercialization on a large scale. AD of livestock manures is a cost effective and sustainable energy option, but high N content makes it challenging to achieve ideal C/N ratio. AcoD of livestock manures addresses the limitations of mono-digestion by concurrently digesting multiple feedstocks, leading to improved system stability, CH₄ yield, diverse microbial community, better nutrient balance and reducing overall GHG emissions from livestock manures management [54].

5. Anaerobic digestion of livestock manures

AD involves the production of biogas from biodegradable materials in the absence of oxygen. The composition of the biogas depends on the materials used and conditions of digestion, typically consisting of CH₄, CO₂, and small quantities of other gases such as N, H₂, H₂S and NH₃. The production of biogas is a result of the activity of different microorganisms in a series of steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [55]. AD, a leading method for treating livestock manure, has become widely embraced for organic waste management, as it allows for energy generation through biogas production and the recovery of nutrients in the form of digestate. An estimated 50 million micro-digesters ranging from 2 to 10 m³ are currently in the use globally to produce biogas for cooking, heating, and lighting. Moreover, there are approximately 132,000 small (<1000 m³), medium (1000-10,000 m³), and large scale (>10,000 m³) digesters in operation worldwide [56]. Utilizing AD for livestock manure offers various advantages, including waste stabilization, odor control, energy generation, reduction of pathogenic organisms, preservation of biogenic elements, inactivation of weed seeds, adherence to evolving legal regulations, and societal approval. The C present in livestock manures is part of the renewable carbon cycle, meaning that CO₂ produced from burning waste biogas does not contribute to additional GHG emissions unlike traditional waste management methods where C from waste is converted to CO₂ [57]. As a result, the use of biogas derived from livestock manures should be viewed as climate-neutral given that any fugitive GHG emissions are appropriately controlled. Moreover, in the process of AD, the organic N from substrate is transformed into NO₃ and NH₃, which are retained in digester residue. This digestate has lower levels of pathogens and related odors compared to untreated livestock manure, and it contains nutrients that can be easily utilized by plants [3]. The primary design parameters for manure-based AD can vary significantly depending on substrate characteristics, temperature, reactor type, and decisions made by biogas plant operators. For instance, cattle manure-based AD reactors typically operate with an organic loading rate (OLR) of 1.6-6.75 kg VS m⁻³ day⁻¹, a retention time of 15-30 days, an organic matter removal efficiency of 36-85%, and CH₄ yield of 0.16-0.39 m³ kg⁻¹ VS. On the other hand, pig manure-based AD plants have reported OLR of 0.8-4.0 kg VS m⁻³ day⁻¹, retention time of 15-60 days, organic matter removal efficiency of 44-77%, and a CH₄ yield of 0.16-0.32 m³ kg⁻¹ VS [54,58]. The selection of reactor type is primarily based on the dry matter content with continuous stirred tank reactors (CSTR) commonly used for manure with high dry matter content, and upflow anaerobic sludge blanket (UASB) or expanded granular sludge bed (EGSB) reactors are preferred for diluted streams. AD of manure is most frequently applied under mesophilic (30-40°C) and thermophilic (50-60°C) conditions [59]. It's important to note that the mesophilic process requires a longer retention time due to slower microbe growth, while thermophilic process has higher heating

demands, leading to additional operational costs that should be considered for assessing process sustainability. Likewise, factors such as pH, levels of ammonia and C/N ratio play a crucial role in ensuring biogas production. Organisms involved in various stages of the AD process exhibit varying pH tolerances. However, it is considered optimal to maintain a pH range of 6.8 to 7.5 for their proliferation. Livestock manure is known for its relatively high pH, which can even reach up to 10, along with a substantial buffer capacity [60]. Elevated levels of NH_3 pose a significant challenge in the digestion of livestock manure particularly in chicken manure. Furthermore, prolonged manure storage leads to higher NH_3 content, necessitating prompt waste management or efficient removal of this harmful substance [61]. Maintaining the appropriate ratio of elements, such as C and N, in the substrate is crucial for AD process. In the context of livestock manure, this ratio is often inadequate for efficient digestion. The optimum C/N ratio of cattle, pig, and chicken manure is 2.3-5.2, 7.4-12.96, and 10.1 respectively [62–64]. Numerous studies have examined the AD process, with particular focus on effectively processing livestock manures while ensuring optimal biogas production Table 2 illustrates the AD performance of three significant types of livestock manure at respective operation conditions.

Table 2. Operational performance of AD of three prominent livestock manures (cattle, pig, and chicken manure).

Livestock manure	Reactor type	Temperature (°C)	OLR (kg VS $\text{m}^{-3} \text{day}^{-1}$)	HRT (d)	CH_4 yield ($\text{m}^3 \text{kg}^{-1} \text{VS}$)	Biogas yield ($\text{m}^3 \text{kg}^{-1} \text{VS}$)	CH_4 content (%)	Reference
Cattle	Batch	53	NA	17	0.184	0.159	65	[65]
Cattle	UASB	37	2.35	22.5	NA	0.200	64	[66]
Cattle	CSTR	55	3	15	0.202	NA	NA	[67]
Cattle	TPAD	35	5.8	14	0.210	NA	60	[68]
Pig	Stirred batch	35	12.39	0.9-3.6	0.176	NA	50	[69]
Pig	Batch	25	NA	20	0.317	NA	44	[70]
Pig	ASBR	20	1.1	15	0.266	NA	75	[71]
Pig	Batch	22.6	NA	80	NA	0.207	22	[72]
Pig	batch	35	NA	15	0.548	NA	70	[73]
Chicken	batch	35	NA	33	0.473	NA	41	[73]
Chicken	UASB	34	2.9	13.2	0.26	NA	NA	[74]
Chicken	Batch	55	NA	10	0.195	NA	67	[75]

OLR: Organic loading rate, HRT: Hydraulic retention time, NA: Not available, UASB: Upflow anaerobic sludge blanket, CSTR: Continuous stirred tank reactor, ASBR: Anaerobic sequencing batch reactor, TPAD: Temperature phased anaerobic digestion.

Despite being well-established treatment method, the AD of manure often experiences low process efficiency for several reasons. Livestock manure presents limitations due to its low C/N ratio, minimal volatile solids, and the presence of challenging-to-degrade materials such as lignocellulosic biomass, resulting in unsatisfactory biogas production. These challenges stem from the inclusion of considerable number of lignocellulosic materials in the diet of cattle, particularly from pasture residues. The hydrolysis stage is particularly constrained in AD due to the arduous degradation of lignocellulosic materials, which are composed of cellulose, hemicellulose, and lignin, and various inorganic materials [76]. Cellulose accounts for 40-50%, hemicelluloses for 25-35%, and lignin for 15-20% of these materials, all of which exhibit high resistance to enzymatic digestion. The conversion of lignocellulosic biomass residues into biofuels is intricate, especially in livestock manures. Lignin notably contributes to the challenges in digestion [77]. Research interest has surged in recent years to enhance the AD processes for livestock manures. The average content of cellulose, hemicellulose, and lignin in cattle manure is 23.5%, 12.8%, and 8% respectively. For pig manure, the average content is 15.9%, 16.7%, and 1.8%. Chicken manure has an average content of 44%, 11.8%, and 1.7% for cellulose,

hemicellulose, and lignin [78,79]. The transformation of cellulose and hemicellulose into energy is characterized by low efficiency in biogas production, attributed to the intra and intermolecular hydrogen bonds within hydroxyl groups. This results in a supramolecular structure with a high degree of polymerization [80]. Consequently, the hydrogen bonding induces cellulose crystallinity, posing a challenge for enzymatic hydrolysis. In essence, the lignocellulosic material hinders the hydrolysis process, acting as a barrier or shield that inhibits the action of microorganisms in substrate degradation [77]. Historically, one technique employed to address the hydrolysis limitations involves the solubilization and degradation of the hemicellulosic and lignin components of substrate. The aim of pretreatment process is to eliminate lignin and hemicelluloses, thereby reducing the quantity of crystalline cellulose and enhancing the porosity of lignocellulosic materials. Several types of pretreatments exist for removal of lignocellulosic materials, encompassing physical, chemical, physicochemical, and biological procedures. Table 3 presents effects of various pretreatments on cattle, pig, and chicken manure.

Table 3. The impact of different pretreatments on methane yield enhancement from AD of livestock manures.

Livestock manure	Type of pretreatment	CH ₄ (mL/g VS)	CH ₄ enhancement (%)	Reference
Cattle	Physical (maceration and pressurized at 100 atm. pressure)	276	20	[81]
	Chemical (peracetic acid)	182.4	39	[82]
	Biological (Incubation with B4 bacteria)	300	30	[81]
Pig	Physiochemical (100°C) for 1 hour	237.5	28	[83]
	Chemical, Ca (OH) ₂ , 1h (70°C)	345	72	[83]
	Biological (cell biocatalyst)	98.7	93.2	[84]
	Physiochemical (High pressure and temperature)	518	54.6	[85]
Chicken	Chemical, Ca (OH) ₂ , at 90°C and 1.27 bar pressure	137	NA	[86]
	Biological (Clostridium <i>saccharolyticum</i> and <i>Clostridium thermocellum</i> as bioaccumulation strains)	102	15	[86]

Physical and physiochemical treatments including thermal and hydrothermal processes, have proven highly effective, enhancing CH₄ yield by 15-206.9%, with the highest improvement observed in pig manure. Similarly, mechanical treatments, specifically microwave treatment, demonstrate the ability to increase CH₄ yield by 15-20%. Chemical pretreatments as evidenced in literature, have shown potential, boosting CH₄ yield by 26-155% for cattle manure and 17-72% for pig manure. Lastly, biological pretreatments also exhibit positive effects on livestock manure, leading to a 30% increase in CH₄ yield from cattle manure, a 15-292% improvement in pig manure, and notably, the most

effective enhancement in chicken manure, with a CH₄ yield increase of 168% [77]. However, each pretreatment method comes with its inherent advantages and disadvantages, influenced by factors such as the biomass source, utilized methods, and the lignocellulosic composition. The efficacy of applying pretreatment is linked to the manure's characterization. Consequently, the primary challenge in pretreating manures lies in aligning the ideal substrate composition with the most suitable pretreatment technique. Assessing the impact of pretreatment on enhancing AD faces a significant disparity between laboratory results and those obtained at pilot and industrial scales; most literature studies are conducted on a smaller scale. As of now, the pretreatment of livestock manures for biogas production has not received as much attention as other organic substrates. In general, only a limited number of pretreatment methods have been explored, and most of them have been evaluated solely in laboratory biochemical methane potential tests. Ultimately, the critical consideration in pretreatment methods is the operating cost, a factor that directly impacts the economic feasibility of biogas production. Every pretreatment approach demands a substantial amount of energy input and chemical inputs, leading to a notable increase in operational costs and GHG emissions resulting from energy expended. Consequently, there is an urgent need to explore and assess alternative methods for managing livestock manures that are more economically and environmentally sustainable.

6. Anaerobic co-digestion of livestock manures

In rural areas, AD has been recognized as a significant cost-effective option, particularly as a sustainable energy source. The high level of N in livestock manure creates a challenge in achieving the ideal C/N ratio for AD. To solve this, it is necessary to raise the C content of the livestock manure before beginning the AD process. Additionally, livestock manures are frequently linked to low CH₄ yields. The co-digestion of livestock manures with other substrates has been utilized as a cost-efficient solution to enhance process efficiency and ultimately make facilities financially viable [87]. AcoD offers the potential to address the limitations of mono-digestion by concurrently digesting two or more feedstocks. The key advantages of AcoD include improved system stability and CH₄ yield, achieved through the synergistic effects of fostering a more diverse microbial community, better nutrient balance (proper C/N ratio and trace element supplement), enhanced buffering capacity, dilution of toxic compounds including heavy metals, production of safe and higher quality digestate for agricultural use, and reduction of antibiotic resistance genes (ARG) and antibiotic resistance bacteria (ARB) [56,87]. Figure 3 depicts significance of AcoD of livestock manures over its mono-digestion.

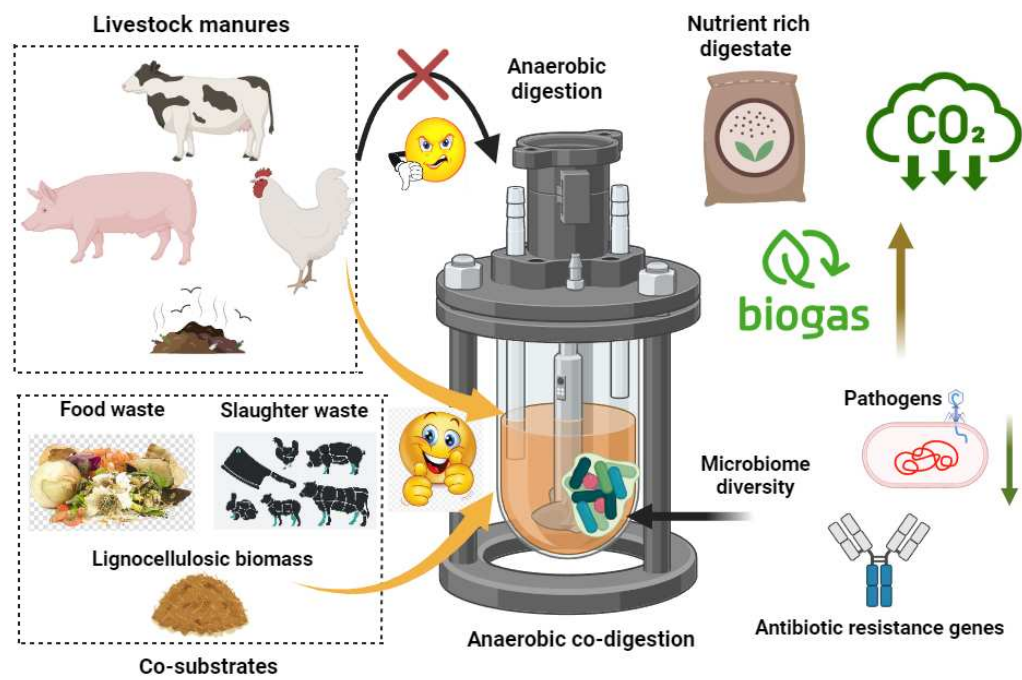


Figure 3. Graphical illustration depicting significance of co-digestion of livestock manures compared to their mono-digestion.

Most biogas plants utilizing manure typically incorporate agricultural residues for co-digestion. For instance, energy crops (corn, grass, and cereal silages) and livestock slurries make up 92% of biogas substrate in Germany. Alternatively, co-digestion with municipal biowaste is proposed due to the mutual benefits for both substrates. Municipal biowaste can enhance CH₄ production, while manure provides effective buffering capacity to prevent pH decrease and reactor acidification [33]. These co-substrates are known for their high C/N ratio, limited buffer capacity, and based on their biodegradability, the potential to generate significant amounts volatile fatty acids (VFAs). In contrast, livestock manures have high buffer capacities and low C/N ratios, with NH₃ concentrations often exceeding the needs for microbial growth and potentially becoming inhibitory for methanogens [87]. Agro-industrial wastes serves as the most suitable co-substrate for manures. However, addressing their seasonal availability and enhancing CH₄ production in digesters has sparked interest in biodegradable industrial wastes and other substrates abundant in biodegradable organic material. Table 4 summarizes AcoD of livestock manures from previously published literature.

Table 4. Anaerobic co-digestion of livestock manures with various co-substrates in previously published literature.

Livestock manure	Co-substrate	Reactor configuration	Biogas or CH ₄ production increase	Methane (%)	Reference
Cattle	Wheat straw	Batch	Sp. CH ₄ yield 0.460 m ³ /kg VS _{add} (+24.6%)	53 (1.3%)	[88]
	Food waste	CSTR	Sp. CH ₄ yield 0.6-0.8 m ³ /kg VS _{add} +88.6%)	61.34- 65.89(+4.7%)	[89]
	Maize straw	Batch	Sp. CH ₄ yield	51.21-58.66 (+39.5%)	[90]

			0.534-0.614 m ³ /kg VS _{add} (39.8%) Sp. CH ₄ yield 0.290 m ³ /kg VS _{add} (+31.82%) CH ₄ yield 220 (mL/g VS _{add}) At PM:CS- 70:30		
	Corn straw	Batch		NA	[91]
Pig	Corn straw	Batch			[92]
	Sewage sludge	Batch	Biogas yield 410 mL/g VS _{add} at TS 2%	65% at TS 2%	[93]
	Glycerol	CSTR	Biogas production 5.44-5.58 L/g VS _{add}	NA	[94]
	Cassava pulp	Semi- continuous reactor	Sp. Methane yield 380 mL/g VS _{add}	NA	[95]
Chicken	Agricultural waste	500 mL anaerobic vials	CH ₄ yield 695 mL/g VS	NA	[14]
	Rice straw	Batch	Sp. CH ₄ yield 0.123-270 m ³ /kg VS _{add}	NA	[96]
	Corn cob	Batch	Sp. CH ₄ yield 0.131- 0.291m ³ /kg VS _{add}	NA	[96]
	Sugar cane bagasse	Batch	Sp. CH ₄ yield 0.140-230 m ³ /kg VS _{add}	NA	[96]

(CSTR: Continuously stirred tank reactor; TS: Total solids; NA: Not available.).

Fox example, rice straw and maize straw, being the most abundant agricultural waste globally, presents an intriguing resource for biogas generation. Silvestre, Gomez et al. [97] incorporated 1, 2, and 5% rice straw (based on mass) into the digestion of cattle manure. The study revealed that the most substantial rise in biogas production compared to controls (mono-digestion of cattle manure), reaching 54%, was attained with a 5% inclusion of rice straw. Similarly, according to findings from Han et al. [98], introduction of 4.6 kg of wheat straw per ton of cattle manure resulted in a 10% enhancement in biogas yield. Additional co-substrates employed for co-digesting cattle manure include food and distillery waste. As indicated in reports, 37-55% of municipal solid wastes consist of kitchen wastes, posing significant challenges for waste management. These materials have a high C content, making them suitable substrates for co-digestion with livestock manures. For instance, Li et al. [99] conducted a study where they co-digested kitchen waste with cattle manure in a laboratory-scale batch reactor and observed a 44% increase in CH₄ production compared to sole digestion of kitchen waste. Salix, commonly known as willow, possess a high C content and thrives in diverse soil conditions. With its high productivity, yielding 35,000 kg of stem/ha annually, it is often referred to as an energy crop. However, its high lignocellulose content may be viewed as a drawback for AD.

Nevertheless, appropriate treatment can render it a suitable substrate for co-digestion. Estevez et al. [13]. Reported that co-digestion of *Salix* with cattle manure resulted in an 18% increase in CH₄ yield compared to the sole digestion of cattle manure. Switchgrass, an energy grass with a high C content and minimal requirements for pest control and fertilization, can thrive on marginal lands and serves as an excellent substrate for co-digestion. According to Lehtomäki et al. [100] the incorporation of energy crops with cattle manure for co-digestion resulted in a 16-65% increase in CH₄ production per digester volume, with the crops ratio to manure at approximately 1:3. In addition to lignocellulosic biomass, other organic waste such as cheese whey have the potential to serve as substrate for co-digestion of livestock manures. Cheese whey is characterized by high COD, protein, lactose, low alkalinity, and very high biodegradability. Rico et al. [101] evaluated the co-digestion of cheese whey with cattle manure in a UASB reactor and achieved a COD removal efficiency of 95.1% at an HRT of 1.3 days. When dealing with pig manure, known for its high N concentration, co-digestion can be achieved using energy crops residues. Cuetos, Fernandez et al. [102] employed maize, rapeseed, and sunflower residues for this purpose and concluded, based on their findings, that the most favorable outcomes were achieved with maize as co-substrate. Cotton stalk with a lignin content of approximately 21.6%, exhibits poor degradability in AD. In a study by Cheng and Zhong [103], when co-digested with pig manure in a laboratory scale batch reactor, they noted an increase of about 1.9 times in biogas production and 1.8 times production rate compared to the sole digestion of cotton stalk. Algae, commonly utilized for biodiesel production has emerged as a favorable substrate for co-digestion with livestock manures due to its versatility in growth and adaptability to various environmental conditions throughout the year. In a study by Astals et al. [94], the co-digestion of algae with pig manure resulted in an approximately 29-37% increase in CH₄ yield compared to the sole digestion of algae. Sugar beet byproduct, comprising mainly of pulp and molasses, is the residual material left after sugar extraction from the sugar beet plant. Its high C content makes it a suitable material for anaerobic co-digestion with livestock manures. In a study by Aboudi et al. [104], sugar beet byproduct and pig manure were co-digested in a semi-continuous stirred tank reactor under mesophilic conditions, resulting in a highest CH₄ production yield of 57.5%. Much of the research is centered in chicken manure due to its ability to yield the highest CH₄ production per kilogram of dry matter compared to other types of manure. Common agricultural wastes such as corn stover can be utilized as co-substrates for chicken manure. Bayrakdar, Molaey et al. [105] conducted the initial co-digestion of chicken manure with used poppy straw, which has an annual production of approximately 20,000 tons in Turkey. The study yielded a CH₄ yield of 0.36 L/g VS when the total N concentration remained below 4000 mg/L. Abouelenien et al. [14] investigated the co-digestion of a blend of agricultural wastes, including cassava waste, coconut waste, and coffee grounds with chicken manure. They noted a significant 93% increase in CH₄ production yield compared to the sole digestion of chicken manure. Cocoa pod husk, a by-product of cocoa production, can be utilized for AD of chicken manure but its decomposition is challenging due to the presence of lignin components. Dahunski et al. [106] recommended pre-treating cocoa pod husks with alkaline hydrogen peroxide before co-digestion. Whey generated during the precipitation and extraction of casein from cheese, is distinguished by its elevated organic matter content and biodegradability, considered as a substrate for processing chicken manure. Wang et al. [107] reported noteworthy findings on chicken manure processing. They co-digested cattle manure with wheat straw, strategically incorporating it to optimize the C/N ratio. They achieved the peak CH₄ potential at mixing ratio of 40.3:59.7 by weight and a C/N ratio of 27.2:1. The suggested co-digestion serves as a promising model for establishing a circular bioeconomy, utilizing biogas as an energy source and digestate as fertilizer. This proposed method has the potential to play a significant role in closing the loop between major cities (municipal biowaste) and agriculture (manures) and vice versa in the future. However, the potential obstacle may arise from the characterization of digestate. Pollutants such as microplastics and metals/metalloids present in municipal biowaste could hinder the spread to agricultural land for organic farming if regulatory requirements are not met.

7. Synergistic effects of anaerobic co-digestion of livestock manures

AcoD has the potential to generate synergistic interactions by balancing nutrients, supplementing trace elements, diluting toxic and inhibitory compounds, and promoting microbial diversity. Previous research indicates that achieving a balanced C/N ratio through the co-digestion of different feedstocks can prevent VFAs accumulation, despite a higher OLR [56]. For instance, co-digestion of food waste with trace element rich piggery wastewater can prevent VFA accumulation, leading to process stability and improved CH₄ production rates. Since food waste is deficient in trace elements crucial for activating enzymes needed for the growth of syntrophic bacterial communities and methanogens (carbon monoxide dehydrogenase, co-enzyme M-methyltransferase complex, and complex F430), co-digestion with piggery wastewater, rich in trace elements like Fe, Ni, and Co, can enhance microbial diversity, enzyme activities, and support symbiotic and syntrophic associations [108]. In the fermentation process, the ratios of co-substrates play a crucial role, with particular focus on C/N ratio. Ensuring the establishment and maintenance of an appropriate C/N ratio stands out as a key factor in the successful implementation of AcoD of livestock manures. Wastes abundant in proteins typically have a high N content, while the majority of biomass consists of C-rich material. The inhibition resulting from a low C/N ratio can potentially be mitigated by augmenting the dose of straw as a C source. Numerous studies have documented that co-digestion of diverse biomasses with protein rich substrates has the effect of elevating the C/N ratio and subsequently enhancing biogas production. A decrease in inhibitory compounds including total ammonia nitrogen (TAN), lignin derivatives like phenolic acids and eugenol, and furan, has been noted due to the dilution effect of co-digestion in laboratory scale experiments. Nevertheless, the impact of implementing co-digestion to reduce the concentration of inhibitory compounds in full-scale applications requires further investigation [56]. Similarly, co-feedstocks containing microbial populations pertinent to AD play a continuous role in sustaining diverse microbial communities over extended periods of co-digestion, potentially addressing issues related to microbial washout. In a study involving the co-digestion of pig manure was associated with an elevated Shannon diversity index for methanogenic populations, particularly noticeable at shorter retention times [109]. The microbial community in AD stands out as a crucial factor influencing the bioconversion of various feedstocks in AD processes. In comparison to mono-digestion, co-digestion systems typically foster microbial communities with increased diversity, given the continuous introduction of diverse microorganisms through co-feedstocks. The stability of microbial communities can be markedly improved by co-digesting complementary feedstocks, achieving microbial supplementation through the incorporation of co-feedstocks. In conventional AD, the majority of bacteria are categorized into *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* [56,110]. Nevertheless, the type of manure significantly influences the structure of the microbial community. The proportions of various manures and their biodegradability also contribute to shaping the microbial community structure. The most common genera found in pig manure belong to the *Firmicutes* phylum, including *Clostridium*, *Turicibacter*, *Streptococcus*, and *Lactobacillus*, as well as *Corynebacterium* from the *Actinobacteria* phylum. Additionally, *Bacteroides*, *Megasphaera*, and *Propionibacterium* are also reported to be present in fresh pig manure. [111]. The dominant phyla reported in chicken manure are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Spirochaetes*, *Actinobacteria*, and *Methanogens* [112]. In cattle manure, the microbial composition differs from the rumen microbiome. The manure is often dominated by *Firmicutes*, *Bacteroidetes*, *Lentisphaerae*, *Proteobacteria*, *Verrucomicrobia*, and *Methanocorpusculum*. On the other hand, the rumen is mostly dominated by *Bacteroidetes*, *Fibrobacteres*, *Firmicutes*, *Lentisphaerae*, *Methanobrevibacter*, and *Methanoplasma* [113]. Cattle manure is often seen as a suitable for initiating AD due to diverse microbial community, which can readily adjust to varying operational circumstances. Therefore, co-digestion of livestock manures with other substrates has been shown to enhance the microbial community in digesters, leading to improved co-digestion performance. This approach allows for more diverse microbial population, better adaptation to operational changes, and increased biogas production efficiency.

Furthermore, the digestate produced during AcoD poses fewer environmental problems such as heavy metal accumulation, phytotoxicity, and ecotoxicity [114]. The incorporation of at least 30%

sweet potato (on a dry weight basis) into dairy cattle manure resulted in an increase from 13.5% to 22.9% in N and from 5.8% to 8.3% in potassium K in the co-digestate [115]. Additionally, Kataki et al. [116] found that co-digestate exhibited higher concentrations of calcium (Ca), sulfur (S), copper (Cu), molybdenum (Mo), nickel (Ni), manganese (Mn), and zinc (Zn) compared to mono-digestate. The variability in the nutrient composition of digestates poses a significant challenge in accurately predicting the quality of digestate as a soil amendment, emphasizing the need for further research in this area. The presence of ARG and ARB poses a serious threat to both the environment and human health [117]. The rise in ARGs and ARB abundance in effluents and mono-digestate is primarily attributed to horizontal gene transfer facilitated by mobile genetic elements like plasmids, integrons, and transposons. Given that *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* are closely linked to ARGs, a shift in their relative abundances could contribute to the reduction of ARGs. As previous studies have indicated substantial effects of manures on microbial community shifts [117,118], the strategic selection of appropriate co-feedstocks emerges as a potential strategy to mitigate ARGs and ARB levels. However, further research is needed to comprehensively understand the influence of co-feedstocks and the underlying mechanisms supporting the reduction of ARGs and ARB from livestock manures. In addition to essential micronutrients like carbon, nitrogen, phosphorus, and sulfur, microbes engaged in AD also necessitate trace elements as growth factors, albeit at lower concentrations. Numerous studies have explored the significance of trace elements in the context of AD. Many earlier investigations have consistently indicated that the addition of trace elements yields positive effects on the AD process. These benefits primarily encompass prolonged digester stability, enhanced organic matter degradation, reduced VFA generation, and increased biogas yield over the long term [62,119]. Rather than introducing micronutrients directly into the reactor, it is advisable to effectively utilize the trace elements inherent in the substrates. The co-digestion performance index (CPI) or synergistic index serves as a metric for assessing antagonistic ($CPI < 1$), additive ($CPI = 1$), and synergistic ($CPI > 1$) interactions in co-digestion scenarios. The CPI is calculated as the specific methane yield (SMY) derived from co-digestion, divided by the weighted average of SMYs obtained from the mono-digestion of each respective feedstock [120]. While a high CPI value utilized as a performance indicator, it does not ensure the attainment of a maximum SMY [121]. Previous literature has demonstrated synergistic indices across various types of livestock manures. For example, co-digestion of cattle manure with food waste and meat and bone meal exhibited maximum synergistic indices of 1.41 and 1.69, respectively [122,123]. In the case of pig manure, co-digestion with corn straw and wheat straw resulted in synergistic indices of 2.09 and 1.24, respectively [124,125]. Similarly, for chicken manure, co-digestion with wheat and corn straw showed synergistic indices of 1.49 and 1.22, respectively [126,127]. The AcoD holds significant potential for enhancing the digestibility of livestock manures, offering benefits in waste management and bioenergy generation.

8. Progress, challenges, and future direction

When managed and utilized effectively, livestock manure can be transformed into valuable resources for energy production and nutrient recovery, without posing a risk to the environment and ecosystem. In addition to energy production, the AcoD of livestock manures holds significant potential for reducing GHG emissions. The overall reduction in GHG emissions from ACOD of livestock manure ranged from 65 to 105 kg CO₂eq ton⁻¹ [128]. The composition of biomass plays a crucial role in determining the extent of GHG reduction. Notably, higher reduction can be observed when agricultural residues (such as straw) and grass are co-digested with livestock manures. Moreover, implementing measures such as minimizing CH₄ leaks from biogas installations, covering digestate storage, and utilizing low NH₃ emission technology for field applications can further contribute to emission reduction. AcoD is commonly used in rural areas to enhance biogas production from digesters. Agricultural waste and organic fraction of municipal solid waste are frequently used as co-substrates for manure-based digesters. However, the availability of easily biodegradable substrates is limited, leading to consideration of more complex waste. This has sparked interest in pretreatments and the use of additives to improve biogas production. Utilizing

both inorganic and biological additives can enhance the effectiveness of process [129]. Nanoparticles are increasingly employed in commercial products for large-scale industrial applications. The addition of 3 g of magnetite and 1 g of natural zeolite to the co-digestion of chicken manure and wheat straw resulted in significant increase in CH_4 production, reaching a maximum of 52.01% and 51.01% respectively [130]. Microorganism and enzymes are frequently employed as effective substitutes for physiochemical pretreatments. Biological additives enhance microbial activity to boost CH_4 production. Enzymes can be added directly to the digester or utilized for pretreating organic substrates [131,132]. Microorganisms face difficulty in degrading the complex structures of cellulose, hemicellulose, and lignin. Therefore, pretreatment is necessary to convert these substances into biodegradable compounds. Pretreatments such as thermo-alkaline, thermal, ultrasonic chemical, microwave irradiation and biological treatments has capability to enhance to further biogas production by 25 to up to 80% [133–136]. Similarly, microbial fuel cells (MFC) can break down the remaining organic materials in the anaerobically digested sludge. Therefore, incorporating post-treatment with MFC into AcoD system can improve both energy recovery and pollution control from substrates [137]. The AcoD system consist of four stages and improving the overall co-digestion in one-stage reactor is challenging due to varying metabolic characteristics, nutritional requirements, and growth rates. To mitigate this challenges, a two-stage system is employed. [138]. A two-phase system offers numerous advantages over single-phase reactors, including enhanced process stability, increased energy recovery, higher biogas production, reduced lag phase, improved VS removal, and grater energy recovery [139]. Nevertheless, the two-stage reactor presents certain drawbacks, such as inhibition of acid forming bacteria, technical complexity, and higher initial cost. Addressing the elevated operational expenses and technical intricacies of the two-phase AcoD process requires additional research investigation [129,140]. Figure 4 visualizes the advancements and obstacles in the AcoD of livestock manures.

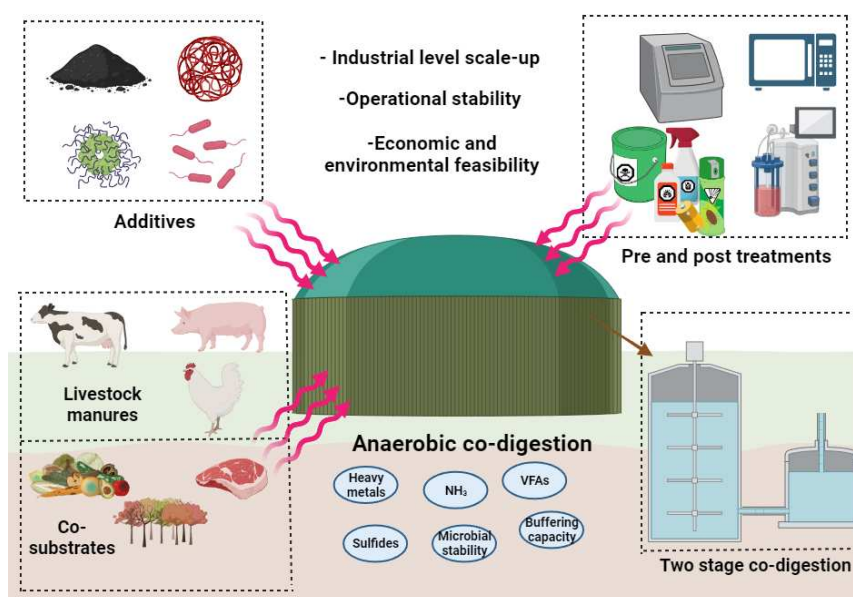


Figure 4. A visual representation depicting progress and challenges pertaining to AcoD of livestock manures.

Although co-digestion offers advantages, there is a significant challenge in achieving successful outcomes. The process may result in not only positive synergistic effects but also antagonistic interactions, leading to reduced biogas productivity. The growing use of AcoD for combining livestock manures with diverse feedstocks demonstrates the potential of utilizing different types of feedstocks in AcoD of livestock manures. Nevertheless, several challenges must be addressed to transition from laboratory-scale production to industrial implementation. AcoD presents operational risks related to continuous feedstock availability, complexities arising from varying biodegradability rates, and safety concerns for agricultural applications, particularly when utilizing feedstocks like

livestock manures. Additionally, determining the ideal ratio for different feedstocks is challenging, as it is influenced by factors such as feedstock type, composition, trace elements content, and biodegradability. In addition to substrate feasibility, it is essential to optimize temperature, OLR, HRT and other factors that influence AcoD. Creating ideal physicochemical conditions in AcoD can enhance functional microorganisms that develop the optimal microbial community. Further investigation using advanced sequencing technologies is required to thoroughly categorize any unidentified microbes and comprehend their specific functions in the intricate AcoD process. Investigating the community's reaction to the introduction of co-substrates can lead to a tailored community structure by modifying co-substrates and their composition, ultimately improving livestock manure management. Similarly, efforts are needed to explore the use of co-substrates in the disintegration and hydrolysis stages. Additional research is needed to establish the relationships between biogas yields from fiber and non-fiber components. Ultimately, future studies should include an analysis of the economic and environmental feasibility of scaling up the industrialization of AcoD of livestock manures. For instance, an essential requirement for commercializing AcoD of livestock manures is to situate livestock farms and co-substrates sources as close as possible to the digestion facility, ensuring the overall process is economically viable. Hence, it is crucial to investigate all these issues to establish a comprehensive model for the AcoD of livestock manures.

9. Conclusion

Currently, there is worldwide focus on the environmental implications of livestock manures due to their significant content of CH₄ and CO₂. The release of these gases into the atmosphere significantly contributes to the detrimental “global warming” phenomenon, with CH₄ being a potent GHG. When considering the various methods for managing livestock manure, including composting, gasification and combustion, the selection of AD is debated not only for its efficacy in generating alternative energy but also for its potential to yield high-quality fertilizer and recover valuable elements. Nevertheless, the distinctive characteristics of livestock manures, such as low C/N ratio and NH₃ inhibition can disrupt the process, resulting in reduced biogas production. In this scenario, pretreatments may offer a solution, but primarily, co-digestion of livestock manures with carbon-rich substrates emerges a viable option. Livestock manures owing to their high buffer capacity, can be effectively decomposed in conjunction with other raw materials like agricultural residues, food waste, and slaughter waste. Co-digestion of livestock manures is a financially and environmentally viable approach for biogas generation, applicable at both laboratory and industrial scales. The primary challenge associated with AcoD of livestock manures include assessment of limiting factors and steps, calibrating, and characterizing parameters, understanding the dynamic behavior of microbial community, and characterizing organic materials. Further research is necessary to develop a systematic framework that ensures the effective implementation of livestock manures management, considering technical, environmental, agronomic, economic, and social perspectives. As technology advances, the AcoD of livestock manures should persist in enhancing biogas production.

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Abbreviations

The following abbreviations are used in this manuscript.

GHG: Greenhouse gas; **CH₄:** Methane; **N₂O:** Nitrous oxide; **CO₂:** Carbon dioxide; **H₂S:** Hydrogen sulfide; **NH₃:** ammonia; **N:** Nitrogen; **PO₄³⁻:** Phosphate; **K:** Potassium; **NO₃⁻:** Nitrate; **AD:** Anaerobic digestion; **AcoD:** Anaerobic co-digestion; **C/N:** Carbon to nitrogen ratio; **CO:** Carbon monoxide; **H₂:** Hydrogen; **OLR:** Organic

loading rate; **CSTR**: Continuously stirred tank reactor; **UASB**: Upflow anaerobic sludge blanket; **EGSB**: Expanded granular sludge bed; **C**: Carbon; **ASBR**: Anaerobic sludge blanket reactor; **HRT**: Hydraulic retention time; **VFA**s: Volatile fatty acids; **ARG**: antibiotic resistance genes; **ARB**: Antibiotic resistance bacteria; **CPI**: Co-digestion performance index; **SMY**: Specific methane yield.

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