

I. ENVIRONMENTAL BIOTECHNOLOGY

ANAEROBIC DIGESTION AND CO-DIGESTION OF POULTRY WASTE: CHALLENGES, PROCESS LIMITS, AND EMERGING OPPORTUNITIES

Ivan Simeonov¹, Venelin Hubenov^{1,2}

¹ The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

² Competence Center “Clean Technologies for a Sustainable Environment – Waters, Wastes, Energy for Circular Economy”, Sofia, Bulgaria

¹ Contact details of corresponding author

e-mail: issim@abv.bg; issim@microbio.bas.bg

Abstract. The poultry industries are growing rapidly due to its availability of lean meat with low cholesterol content. However, the problems also coming along with the poultry manure production as a waste from poultry industries. The increase in the demand of poultry products have led to fast and concentrated growth of the poultry industry, which deliver superabundant manure supplies. Poultry waste is one of the best organic manure and also much valuable resource. At present the poultry farming supply vital sprig of animal protein and also the most freely reared livestock species amongst the all livestock species. In spite of its huge benefits like production of meat, eggs and job generations it frequently creates some environmental risks that adversely affect both humans and animals, because the poultry farming is the main cause of offensive odor, water, soil, and air pollution. The conversion of poultry waste into biogas and biofertilizer is considered one of the most effective waste management and recycling strategies.

The aim of this manuscript is to deliver a comprehensive overview of the state of the art, the technical feasibility and some perspectives of the anaerobic digestion and anaerobic co-digestion of poultry waste for biogas production including our own results in this field.

Keywords: poultry waste, environmental pollution, chicken manure, AD, AcoD, complex mixtures, biogas production, biofertilizer

Abbreviations

AD – anaerobic digestion
AcoD – anaerobic co-digestion
AR – acidogenic reactor
BMP – biochemical methane potential
BR – bioreactor
BPR – biogas production rate
DMY – daily methane yield
EC – electrical conductivity
CMY – cumulative methane yield
CSTR – continuous stirred tank reactor
SBR – sequencing batch reactor
VFAs – volatile fatty acids
LCFA – long chain fatty acids
OLR – organic loading rates
VS – volatile solids
TS – total solids
SHW – slaughterhouse waste
SMA – specific methanogenic activities
SOB – sulfur-oxidizing bacteria
FAN – free ammonium nitrogen
TAN – total ammonium nitrogen
TDS – total dissolved solids
CHP – Combined Heat and Power (co-generation)
HRT – Hydraulic Retention Time
COD – Chemical Oxygen Demand
BOD5 – biochemical oxygen demand
BMP – biomethane potential
GHG – greenhouse gas
PL – poultry litter

PSE – poultry slaughter-house effluent

RES – renewable energy sources

TSAD – two-stage anaerobic digestion

UASB – upflow anaerobic sludge blanket reactor

EGSB – expanded granular sludge bed reactor

I. INTRODUCTION

The current world population of 8.2 billion is expected to reach 10.3 billion in the coming decades. This growth, combined with increased demand for food and the generation of industrial waste, is exacerbating environmental impacts. Therefore, providing food and sustainable energy resources for overpopulation are the main challenges for current and future generations (Coêlho et al., 2025). The world is now seeking renewable energy sources (RES) that are both economical and environmentally friendly. Purified biogas is one essential RES that can act as a substitute for fossil fuels. In general, one cubic meter of biogas has the equivalent of 0.7 m³ of natural gas, 0.7 kg of fuel oil, 0.6 kg of kerosene, 0.53 L of petrol, 3.5 kg of wood, 4 kWh of electrical energy etc.

Poultry farming is an agro-industrial activity with a major environmental impact. Global consumption of chicken meat, estimated at 103,046 tons in 2024 (Karumanchi et al., 2026) which is associated with the generation of approximately 68



doi.org/10.32006/eeep.2026.1.0529

This article is distributed under the terms of the Creative Commons Attribution License

billion tons of poultry waste annually (Coêlho et al., 2025), has boosted the generation of waste by the poultry industry, especially poultry litter (PL) and poultry slaughter-house effluent (PSE). Poultry is the second most consumed meat worldwide. To meet the growing demand, global poultry meat production increased from 9 to 132 million tons between 1961 and 2019, and egg production went from 15 to 90 million tons for the same period. Therefore, tremendous quantities of poultry production waste were generated over the past decade. For instance, in France in 2008, the total production of poultry waste was estimated at about 5.6 million tons/per year, including 2.5 million tons of manure, 2.5 million tons of slurry and 0.6 million tons of droppings per year (Mansour et al., 2023). In China, there are around 2.5 billion layer chickens, producing annually about 155 million tons of chicken manure (Alipoursarbani et al., 2026). If this waste is not treated well then it causes environmental pollutions and create problems to environment and humans. Poultry waste can aspect the spread of diseases and also pollute the soil and groundwater resources if not properly handled (Verma et al., 2020). The requirement and utilization of chicken manure has overtaken the use of other animal manure (e.g. pig manure, cattle manure) because of its high content of nitrogen, phosphorus and potassium (Schlegel, 1992; Warman, 1986). The poultry industry generates huge quantity of waste that includes solid wastes consisting of feathers, feed, hatchery wastes, bedding material and excreta. Even, chicken manure is prime between any other animal manure due to its high concentration of macro-nutrients (Duncan, 2005; Warman, 1986). Adding of poultry manure to soils not only aid to overcome the disposal issue but also increase the physical, chemical and biological prolificity of soils (Friend et al., 2006; McGrath et al., 2010). Chicken manure contains less salt and free of the weed seeds that can appear in the waste of other animals. Nevertheless, manure by-products may also have other possibly toxic trace elements, namely Copper (Cu), Zinc (Zn) and Arsenic (As) that are not noticed seriously till now ((Bolan et al., 1992; Epstein & Moss, 2006; Jackson et al., 2003; Toor & Haggard, 2009). Whether the waste of poultry such as chicken feathers, chicken poop and hatchery wastes are not used in an appropriate form then they cause immense pollution, unpleasant odors and attract insects, potentially serving as vectors for various diseases, thereby impacting human and animal health (Kyakuwaire et al., 2019; Zhang et al., 2019).

Depletion of water sources, soil nutrient imbalances, pathogen spread, and the release of greenhouse gases (GHG) such as methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3), and nitrous oxide (N_2O) are additional concerns associated with inadequate and unscientific disposal of broiler litter (Moore, Jr et al., 2006).

Being good sources of keratin proteins and amino acids the chicken feathers can be modified into useful products such as poultry manure which can be used as organic fertilizer. The other products which can be made by it are biodegradable plastic, feather meal and bio diesel. Out of all animal manures like vermicompost, horse, cow or steer manure, the nutritional value of poultry manure is high because it has highest amount of nitrogen, phosphorous & potassium. Soil microorganisms have potential to convert poultry farming waste into ecofriendly clean fertilizer which increases the productions of agricultural products. Poultry waste is rich in organic matter, which allows its treatment by the process of anaerobic digestion (AD) to reinforce economic and environmental green strategies. Chicken manure is composed of easily degradable organic matter that led, under anaerobic conditions to the production of $296 - 377 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$, this value being much higher than that reported, for example, for cow manure (Bi et al., 2019). In most of the developed countries, poultry litter (PL) is typically removed after each flock and replaced with fresh bedding material. In contrast, some countries reuse the same bedding across multiple production cycles. The amount of litter produced per bird during a production cycle is reported to range between 1.5 and 5.7 kg (Jeswani et al., 2019).

During the last three decades our team (in the frame of The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Sciences) accumulates a lot of knowledge and extensive experimental experience in the field of AD and anaerobic co-digestion (AcoD) of different organic wastes – PL, activated sludge, cattle dung, milk whey, etc. During the last two years, our studies have been focused mainly on the AD and AcoD of poultry waste.

The aim of this manuscript is to deliver a comprehensive overview of the state of the art and some perspectives of the AD and AcoD of poultry waste for biogas production. Consequently, we aim to summarise the research to recognise fields where the AD and AcoD of poultry waste have been effectively embraced, and where it has not been possible to do so, and to identify the difficulties emerging out of

utilisation of AcoD processes. Our own results in this field are presented as well. Also the present manuscript studied the research prospects and challenges of AD and AcoD of poultry waste, the contributions of different methods in biogas generation studies. Finally, this review describes a design and development framework to incorporate various aspects to enhance biogas production.

II. COMPOSITION, CHARACTERIZATION AND APPLICATIONS OF POULTRY WASTE

2.1. Composition and characterization of poultry waste

An example of poultry waste composition mixture is - 65% broiler litter, 25% sawdust and 10% hog fuel on wet weight basis (Verma et al., 2020). Materials used as litter include straw, sawdust, wood shavings, shredded paper and peanut or rice hulls. The collected manure is mixed with litter. Broiler chickens digest approximately 85 to 90% of the dry substance of the feed and ingest about 2.5 to 3.0 kg of dry matter up to 35 days of age and 5 to 6 kg of dry material up to 49 days of age (Verma et al., 2020). On the basis of the dried material digestibility of the feed (87.5%), it is concluded that 0.34 and 0.63 kg of solid is spit out by a 35 and 49 day old bird, respectively. How much nutrient excreted can be calculated by the difference between amount of feed taken and amount digests by the chicken. It has been calculated that broiler chickens produce about 80% of the total K, 70% of the P and 55% of the total N (Verma et al., 2020).

Ammonia exists either as a gas (NH_3) or in its ionized, water-soluble form, ammonium (NH_4^+). NH_3 can be released into the atmosphere, while NH_4^+ can be transformed by microorganisms into nitrate through a process known as nitrification. The presence of ammonium ions also contributes to a high pH that creates and disposal, storage and handling problems. The minimization of ammonia content is favored for any treatment of poultry waste. Gaseous losses of N have great concern because it not only reduces the fertilizer value of poultry waste, but also cause atmospheric pollution, thus decay the environmental health.

2.1.1. Applications of poultry waste

The main areas of application are (Verma et al., 2020):

1. Fuel as a source of biogas - AD of poultry waste produce biogas which is a combustible gas composed of almost 60% methane, 38% carbon

dioxide, small amounts of water vapors, ammonia and hydrogen sulphide. The remains such as post anaerobic digested solid and burnt ash material utilize in the form of fertilizer and animal feed supplement (Blake et al., 2007; Liedl et al., 2006).

2. Poultry waste can be ignited straight as a fuel source to produce heat energy. The only one main problem to use poultry waste is its approximately high moisture concentration. The moisture level of poultry waste should be less than 15% to obtain the maximal heat energy during combustion.

3. Electricity production - the burning of poultry waste contributes the main attention as a method to yield heat and electricity at major facilities (Kelleher et al., 2002; Turnell et al., 2007). To make electric power from PL, renewable 'green' sources there are a number of states in the US have turned to thermal modification of biomass. There is a best example that shows the best use of poultry waste to produce electricity, Minnesota generate above two million tons of broiler and turkey waste, the fuel for 'poultry power.' Nevertheless, it is possible to utilize the litter as a fuel source to produce hot water, which will be useful for a poultry processing unit.

4. As a good soil amendment - the addition of urea and poultry manure enhances the dry matter production of the maize crop in both cultivated and the grazing soils.

5. As an animal feed - poultry manure singly or when mixed with feed grains, seem to be a significant feed for fish and cattle.

6. Enhances soil fertility.

Broiler litter possesses a lignocellulosic composition with a tough and crystalline structure, making it resistant to microbial degradation (Mohsenzadeh et al., 2012; Wei et al., 2020). Consequently, prior pre- treatment before anaerobic digestion becomes necessary to enhance the biodegradability of lignocellulose. This pretreatment involves reducing the material's crystallinity and breaking down its network structure, including amorphous components (Salehian et al., 2013). NaOH concentration plays a significant role in enhancing the chemical kinetics of biogas production during the anaerobic digestion of organic waste, including poultry manure. NaOH acts by breaking down lignocellulosic bonds, increasing hydrolysis rates, and improving substrate availability for microbes. It accelerates the digestion process but must be carefully dosed to avoid microbial inhibition (Thakur et al., 2025).

III.AD AND AcoD DESCRIPTION

3.1. Mono digestion description

AD has been recognized as a biotechnological method of biogas generation that can convert organic waste into a sustainable source of energy (Evans & Furlong, 2003; Forester & John Wase, 1990; Price & Cheremisinoff, 1981; Sawyerr et al., 2019). This is a very complicated process consisting of the following main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Deublein, 2010; Rawoof et al., 2021) (Fig. 1), AD is a long-established technology for the valorization of diverse organic wastes with concomitant generation of valuable resources. Normally, AD processes are designed for a single type of substrate. However, mono-digestion (i.e., AD using one feedstock) suffers from challenges associated with feedstock characteristics.

During the process of hydrolysis (Fig. 1), specialized enzymes secreted by hydrolytic microorganisms help to break down complex particulate organic materials. This extracellular process converts carbohydrates, proteins, and lipids into monosaccharides, amino acids, and long-chain fatty acids, respectively. Hydrolysis is facilitated by exo-enzymes produced by acidogenic bacteria and serves as a crucial step in transforming complex organic substances into accessible soluble substrates for further

fermentation. The main exo-enzymes involved in catalyzing the hydrolysis of cellulose, proteins, and lipids are cellulases, peptidases, and lipases.

Fermentation, also known as acidogenesis, is the biological process of converting hydrolysis products into VFAs, alcohols, and other organic acids. Fermentation involves a diverse group of microorganisms, both hydrolytic and non-hydrolytic, with only a small fraction of known bacteria (about 1%) being facultative fermenters.

In the process of acetogenesis, fermentation products undergo further biological conversion to acetate through anaerobic oxidation, using H^+ or HCO_3^- as electron acceptors. The resulting H_2 and formate carry electrons for use in interspecies transfer by hydrogenotrophic methanogens, which helps lower H_2 concentrations necessary for most acetogenic reactions to occur.

The final stage of traditional AD, methanogenesis, converts a restricted range of substrates (such as acetate, H_2 , and formate) into CH_4 (Moletta, 2015). Under mesophilic conditions, around 70% of CH_4 production occurs through the direct cleavage of acetate by specialized methanogenic archaea. This process is known as aceticlastic methanogenesis. The remaining CH_4 production is attributed to the hydrogenotrophic methanogenesis pathway, which utilizes H_2 or formate as a substrate.

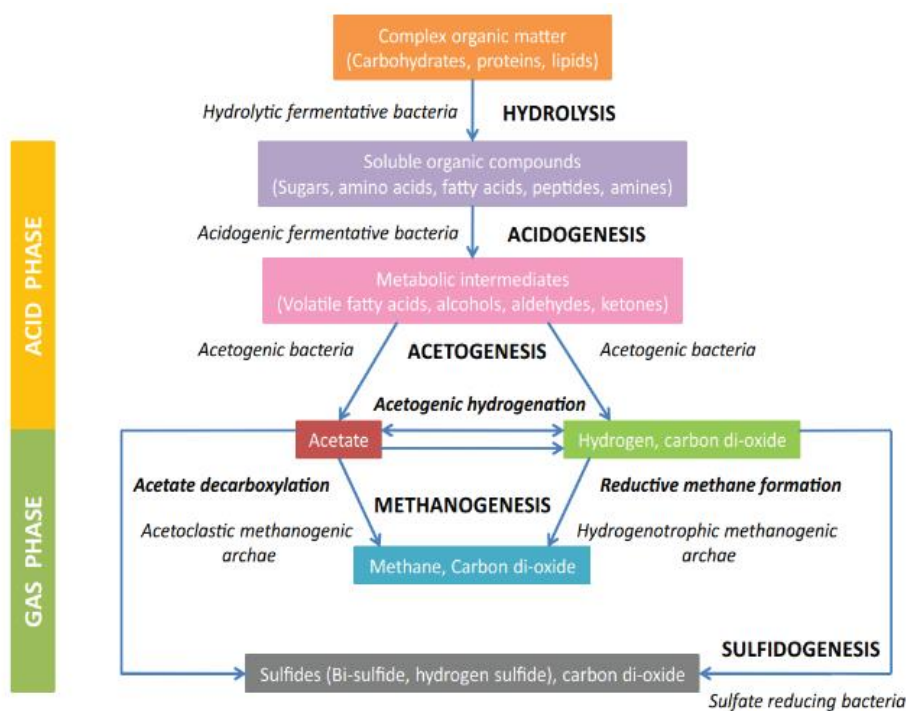


Fig. 1. Schematic representation of the sub-processes occurring during the anaerobic biodegradation of complex organic feedstock materials (Rawoof et al., 2021)

Table 1: Biogas composition (Sawyerr et al., 2019)

Component	Concentration, (%)
Methane (CH ₄)	55–60
Carbon dioxide (CO ₂)	35–40
Hydrogen (H ₂)	2–7
Hydrogen sulphide (H ₂ S)	0–2
Ammonia (NH ₃)	0–0.05
Nitrogen (N)	0–2

AD plays an important role in the circular bioeconomy by converting organic waste into renewable methane and nutrient-rich fertilizer. AD performances depend on specific substrate characteristics, heat integration, and control complexity. Optimizing the C:N ratio, buffering capacity, and trace-element supplementation, while simultaneously diluting toxic inhibitors, makes co-digestion an effective and adaptable approach to enhancing anaerobic digestion processes and overall process stability (Siddique & Wahid, 2018). Additives like carbon, iron nanoparticles, enzymes, and buffers can optimize digestion, but their performance is highly dependent on dosage and substrat (Bi, Qiao, et al., 2020a).

An estimated 50 million micro-digesters ranging from 2 to 10 m³ are currently in 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 (Kadam et al., 2024; Karki et al., 2021).

Utilizing AD for livestock manure offers various advantages, including:

1. Waste stabilization;
2. Odor control;
3. Energy generation;
4. The reduction of pathogenic organisms;
5. The preservation of biogenic elements;
6. The inactivation of weed seeds;
7. Adherence to evolving legal regulations;
8. Societal approval.

The use of biogas derived from livestock manures should be viewed as climate-neutral given that any fugitive GHG emissions are appropriately controlled. 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.

The selection of reactor type is primarily based on the dry matter content:

- with continuously stirred tank reactors (CSTR) commonly used for manure with high dry matter content;
- upflow anaerobic sludge blanket (UASB) reactors preferred for diluted streams;
- expanded granular sludge bed (EGSB) reactors preferred for diluted streams.

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.

Elevated levels of NH₃ pose a significant challenge in the digestion of livestock manure particularly in chicken manure. The combined effects of substrate-to-inoculum ratio, inoculum origin and ammonia accumulation have also been shown to strongly influence the stability and methane yield of poultry manure digestion (Mansour et al., 2024). Supplementation with trace elements such as selenium has also been shown to mitigate ammonia inhibition and improve methane production during the anaerobic digestion of chicken manure (Molaey et al., 2018). High ammonia concentrations remain one of the major limitations in the anaerobic digestion of chicken manure, and recent technological developments in Europe and China highlight several effective strategies to mitigate ammonia inhibition (Fuchs et al., 2018). Bioaugmentation with methanogenic cultures has been shown to enhance methane production and improve process stability during the anaerobic

digestion of chicken manure (Linsong et al., 2022). Lowering the organic loading rate has been demonstrated as an effective strategy to overcome ammonia-induced inhibition during the anaerobic digestion of chicken manure (Mahdy et al., 2020). Furthermore, prolonged manure storage leads to higher NH_3 content, necessitating prompt waste management or efficient removal of this harmful substance. Storage conditions have been shown to significantly influence the chemical composition and biochemical methane potential of livestock manures, with notable differences between in-house and outdoor stored materials (Hilgert et al., 2023).

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. 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. Lignin notably contributes to the challenges in AD. 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.

In essence, the lignocellulosic material hinders the hydrolysis process, acting as a barrier or shield that inhibits the action of microorganisms in substrate degradation.

The aim of the 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 the removal of lignocellulosic materials, encompassing physical, chemical, physicochemical, and biological procedures.

Physical and physiochemical treatments, including thermal and hydrothermal processes, have proven highly effective, enhancing CH_4 yield by 15–206.9%, with the highest improvement observed in pig manure. Similarly, mechanical treatments, specifically microwave treatment, demonstrate the ability to increase CH_4 yield by 15–20%. Chemical pretreatments have shown potential, as evidenced in the literature, boosting CH_4 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

CH_4 yield from cattle manure, a 15–292% improvement in pig manure, and notably, the most effective enhancement in chicken manure, with a CH_4 yield increase of 168%.

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. 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. 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. Energy insecurity and environmental contamination are perhaps the greatest challenges mankind has ever faced in this century. Organic wastes generation is intricately associated with population growth and economic development. Alleviation of carbon dioxide (CO_2) discharge and associated global warming drive the search for sources of energy alternatives to fossil fuels.

The AD system for treatment of PL is shown in Fig. 2 (Omar et al., 2020) and include operation of the biogas plant (transport of litter, digester and combined heat and power (CHP) operation, digestate storage and use (Levasseur et al., 2017)), the processes displaced by the two co-products (biogas and digestate) and the processes displaced by the initial use of the PL (land spreading). Broiler production is excluded from the system boundaries. The integration of CHP and waste-heat recovery systems significantly increases the overall efficiency of biogas installations, as demonstrated in the multigeneration models (Arslan & Yılmaz, 2022).

3.2. Anaerobic co-digestion description

AcoD can be considered as the instantaneous digestion of two or more substrate and co-substrate mixtures. Co-digestion is a powerful way to enhance AD because it can address several constraints simultaneously: it corrects nutrient imbalances, improves alkalinity and pH buffering, provides essential trace elements, and dilutes inhibitory compounds (Manono & Ming'ate, 2026). AcoD is considered as pragmatic method to resolve the difficulties related to substrate properties and system optimisation in single-substrate digestion processes (Tsigkou et al., 2021).

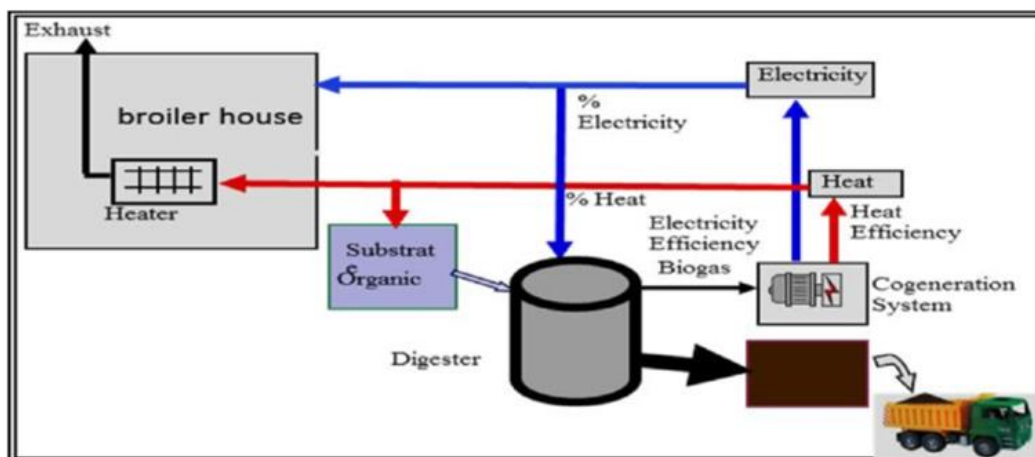


Fig. 2. Schematic diagram of the AD of poultry waste system (Omar et al., 2020)

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;
- the dilution of toxic compounds including heavy metals;
- the production of a safe and higher quality digestate for agricultural use;
- the reduction of antibiotic resistance genes and antibiotic resistance bacteria.

Co-digestion of chicken manure with the organic fraction of municipal solid waste has been shown to substantially improve methane yield and overall process performance (Matheri et al., 2017) which is consistent with findings that food waste (Phillip et al., 2024) is a highly biodegradable substrate suitable for enhancing methane production in anaerobic digestion systems (Mirmohamadsadeghi et al., 2019). The positive synergistic effects of co-digesting poultry manure with lignocellulosic materials such as straw have been experimentally demonstrated, showing improved methane yields and higher process stability under both mesophilic and thermophilic conditions (Babae et al., 2013). Similar improvements in methane yield have been reported when poultry droppings were co-digested with alkali-pretreated wheat

straw under both mesophilic and thermophilic conditions (Rahman et al., 2018). Synergistic improvements have been reported when chicken manure is co-digested with other lignocellulosic residues, such as spent poppy straw, resulting in higher methane yields and reduced ammonia inhibition (Bayrakdar et al., 2017). Co-digestion of poultry manure with readily biodegradable, carbon-rich substrates such as fruit and vegetable waste has also been shown to enhance methane production and improve digestate quality under semi-continuous operation (Bres et al., 2018). Co-digestion of chicken manure with oxidative-cleaved wheat straw has also been shown to enhance methane production, improve process stability, and provide favorable kinetic performance (Hassan et al., 2017). AcoD offers several merits such as better digestibility and process stability while enhancing methane yield due to synergistic effects. Operation of an efficient AcoD system, however, requires full comprehension of important operational parameters, such as co-substrates ratio, their composition, volatile fatty acids/alkalinity ratio, organic loading rate, and solids/hydraulic retention time (HRT). Recent advances highlight the growing role of artificial intelligence tools in optimizing anaerobic co-digestion processes and improving predictive accuracy for system performance (Khan et al., 2023).

However, with the increased use of AcoD, the complexity of the process also increases. AcoD is essential to prevent certain problems, such as a lack of nitrogen, excess nitrogen, the risk

of acidification due to high biodegradability, a lack of microelements and the presence of long chain fatty acids (LCFAs).

Significant research and development efforts have highlighted several inherent merits of co-digestion, including enhanced digestibility due to synergistic effects of co-substrates, better process stability, and higher nutrient value of the produced co-digestate (Abouelenien et al., 2016). The co-substrate should be carefully chosen whilst taking into account its composition and effect on the main substrate, and the mixture ratio should also be optimized. Poultry droppings can be successfully treated with whey, fruit and vegetable waste, organic fractions of municipal solid waste, rice straw, digested sludge and even other types of manure, including cattle slurry, buffalo manure and sheep manure.

Reported methane gains are often large, but direct comparison across studies is difficult because baseline mono-digestion conditions vary widely. The more transferable insight is mechanistic: co-digestion works when mixing shifts the composite feed toward operating windows that better match microbial demand increasing micronutrient supply, and reducing the effective burden of free ammonia or other inhibitors per unit reactor volume, a mechanistic framework well-established in foundational AD literature (Ruggeri et al., 2015), for example by moving the C:N ratio toward approximately 20–30:1, improving alkalinity, increasing micronutrient supply (Molaey et al., 2019), and reducing the effective burden of free ammonia or other inhibitors per unit reactor volume. For optimal AD of lignocellulosic residues, co-digestion with manure or sludge is highly effective because it provides buffering, inoculation, and dilution of inhibitory intermediates. However, a pretreatment step is still required to break down the complex, recalcitrant structure of many crop residues. This reinforces a broader point: co-digestion is not a universal substitute for pretreatment, but a compatibility tool that improves biochemical resilience when blending is matched to substrate chemistry and reactor constraints. A persistent gap is that many co-digestion studies optimize

methane yield while underreporting logistical constraints such as seasonal feedstock availability, preprocessing requirements, transport distance, and mixture-specific ammonia or LCFA risk. These factors often determine whether co-digestion remains advantageous outside laboratory or pilot settings.

IV. AD OF POULTRY WASTE

4.1. Mono digestion of poultry waste

Our own studies of AD of PL started in 2006 (Simeonov et al., 2006). This paper deals with laboratory studies of batch and continuous processes of AD of PL in mesophilic temperatures (24°C and 34°C). The influence of some basic variables upon the biogas production has been studied. These basic variables were: pH, concentrations of VFAs, chemical oxygen demand (COD), total and soluble proteins, glucose and total glucose concentrations, ammonia ions concentrations and dry matter concentrations. The results obtained had been analyzed in view of future optimization of the process. In (Petkov, 2008, 2009) the results of a large-scale experimental work with laboratory BRs on AD of PL in continuous and sequencing batch modes at mesophilic temperature are presented. Their advantages and disadvantages are compared. Hubenov et al. (2026) investigated AD processes for the production of biogas from PL in a CSTRs was performed. The BR was supplied by electrical energy from a proper photovoltaic system. To obtain the maximum amount of solar energy, the photovoltaic system consisting of four panels, inverter and a battery with smart control was designed to collect, store, and bring energy to the reactor system collector and connected to the laboratory bioreactor, conveying the biogas production process. Several HRT were tested for optimizing biogas production, reaching a maximum of 575.49 NmL CH₄/dm³ at an HRT of 13.3 days for the first bioreactor and 278.7 NmL CH₄/g VS_{add} at an HRT of 120 days for the whole system. The energy balance made, reporting meteorological data, showed the economic feasibility for small farms to meet

their own electricity needs (Hubenov et al., 2026).

In (Dalkılıç & Ugurlu, 2015) the biogas production from chicken manure at different organic OLRs, in a mesophilic-thermophilic two-stage anaerobic digestion (TSAD) system was investigated. TSAD system used in this study consisted of glass vessels with working volumes of 0.5 L (acidogenic) and 2.5 L (methanogenic) respectively (1:5). The acidogenic reactor (AR) was operated under mesophilic conditions ($37 \pm 2^\circ\text{C}$) and BR was operated under thermophilic ($53 \pm 2^\circ\text{C}$) conditions. Heating and the mixing of the reactors were achieved by using the hot plate magnetic stirrers. Reactors operated under 12 days of HRT that of 2 days for BR1 and 10 days for BR2. The system was operated on semi continuous mode under different OLRs (from 1.9 g VS/L.d to 4.7 g VS/L.d) and TS contents 3.0-8.25%. The system was operated on semi continuous mode by feeding and decanting once a day. No external chemicals were used to control pH values of the reactors. The chicken manure was diluted in order to maintain a TS content ranging between 3.0% and 8.25%. It was observed that the anaerobic bacteria acclimatized to high total ammonia nitrogen concentration ($>3000 \text{ mg/L}$) originated as a result of the degradation of chicken manure. High volatile fatty acid concentrations were tolerated by the system due to high pH in the reactors. The maximum average biogas production rate was found as $554 \text{ mL/g VS}_{\text{feed}}$ while feeding 2.2 g VS/L.d (2.3% VS - 3.8% TS) to the system. Average methane content of produced biogas was 74% during the study. The biogas productions per liter of volume of BR1-BR2 two stage system were 0.86, 1.2, 1.19, 1.6 and 1.99 L/L for the five sets, respectively. Two-stage fermentation of chicken manure has also been shown to improve methane production and process performance compared to single-stage digestion (Liu et al., 2018).

Comparative studies have shown that the performance and resilience of chicken manure anaerobic digestion are strongly influenced by operating temperature, with significant differences in microbial community dynamics between mesophilic and thermophilic systems

(Niu et al., 2015). In (Bi et al., 2019) mesophilic and thermophilic AD of chicken manure under two OLR (1.6 and $2.5 \text{ kg VS m}^3 \text{ d}^{-1}$) were compared. A 180-day continuous study was carried out to analyze the effects of OLR on biogas production, specific methane production and process mass balances. The results demonstrated that the mesophilic reactor yield methane at 252 and $245 \text{ mL CH}_4 \text{ g}^{-1} \text{ TS}$ at the two OLRs. However, in the thermophilic reactor, the methane yield was lower ($220 \text{ mL CH}_4 \text{ g}^{-1} \text{ TS}$ for OLR of $1.6 \text{ kg VS m}^3 \text{ d}^{-1}$ and $94 \text{ mL CH}_4 \text{ g}^{-1} \text{ TS}$ for OLR of $2.5 \text{ kg VS m}^3 \text{ d}^{-1}$). High free ammonium nitrogen (FAN) concentration, particularly at a high OLR, led to a high concentration of VFAs which negatively affected specific methane production, while under mesophilic condition the VFAs was lower and specific methane production was not affected. Principal component analysis indicated that OLR, total ammonium nitrogen (TAN), and FAN have an effect on biogas production. It was concluded that mesophilic and thermophilic reactors could work comparatively well at a low OLR, but that the thermophilic condition had significantly deleterious effects on the efficiency of anaerobic digestion above all at the higher OLR (Wang et al., 2019). Results of current long term experiment ascertained that methane yield under mesophilic conditions was considerably higher than that under thermophilic conditions when higher OLR was used. The accumulation of TAN inhibiting hydrolytic, acidogenic and methanogenic processes caused a decreased methane yield (Song et al., 2024). AD depression and the subsequent accumulation of VFAs were more rapid in the thermophilic reactors than in the mesophilic ones (Sun et al., 2016). The specific methanogenic activity (SMA) under thermophilic conditions was also lower than that under mesophilic conditions (Song et al., 2023). Therefore, the mesophilic digestion of chicken manure was the more efficient process.

Very interesting study of chicken manure mono AD is presented in (Jurgutis et al., 2020). Laboratory biochemical methane potential (BMP) tests performed in BR with total volume of 500 mL (working volume was 400 mL) at

mesophilic conditions ($35 \pm 1^\circ\text{C}$) indicated the biogas potential of methane is $508 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ added with methane content of $61.5 \pm 1.6\%$. This result was compared to biogas yields obtained in full scale AD plant with a working volume of 2500 m^3 operated continuously at $35 \pm 1^\circ\text{C}$ with four mixers stirring once every hour for 15 min mixing interval. The duration of the experiment was 217 days. As a result, the performance of biogas plant was able to deliver 93% of chicken manure biogas potential with an OLR of $3.14 \text{ kg VS L}^{-1}\text{d}^{-1}$ and TAN concentration of 5.5 g L^{-1} .

In (Omar et al., 2020) a study of the proportions of renewable energy that can be produced from poultry waste and use it inside poultry farms is presented. A mathematical model consisting of algebraic equations was developed and processed by MATLAB software package in four stages. The first stage is the calculation of the broiler house heating required according to recommended inside air temperature, outside air temperature and heat loss through structure and ventilation. The second stage is the estimation of the broiler litter waste produced and the calculation of its biogas production m^3/day . The third stage is the calculation of the electricity generated from biogas and the recovery of heat of exhaust gas from the generator. Finally, the calculation of the energy saved by energy production from wastes to the energy required of the broiler house. Mathematical model predicted the energy of operating a digester at a specified temperature of 38°C and the energy generated by used biogas produced as a fuel in the 3.7 kW engine. The daily flow rate of substrate was $0.375 \text{ m}^3\text{day}^{-1}$ which produces $13.2 \text{ m}^3\text{day}^{-1}$ biogas. The results show that the electricity, thermal energy production, energy requirement for biogas production presses and the net energy production were 847.36, 1355.78, 811.86 and 1391.28 kWh for the total period of broiler cycle production. These results show good agreement between predicted and measured values where about $19.9 \text{ kWh day}^{-1}$ of electricity can be generated from the broiler waste and $32.3 \text{ kWh day}^{-1}$ of waste heat energy is recovered from generator exhaust gas. The

conclusion showed that energy losses by transmission were 49%. Therefore, the thickness should be increased to reduce this percent. The moving insulating wall saved 200% and 100% of energy in the first and second stages of the breeding cycle respectively. About 44% of the lost energy was due to ventilation. Therefore, the ventilation process should be carried out through heat exchangers to keep the energy. AD of farm waste to produce biogas and use it in the farm met about 49% of the farm's energy needs.

In (Beausang et al., 2020) several scenarios where biogas produced from PL is used to generate heat and electricity or is upgraded to biomethane which can substitute natural gas were examined. The net environmental impact depended mainly on the processes displaced by the use of the two co-products, biogas and digestate. The AD system in this study is based on operational data from an existing facility in Ireland. The plant parameters were modified for the reference flow of 20,000 tonnes of PL being considered in this study. The proposed plant is fed with PL from contracted farms in the region. Digestion takes place in a two-stage process. The digesters operate at mesophilic temperatures of 37°C and the total digestion time is 34 days. The biogas consists of 56% methane. Fugitive emissions are assumed to be 2.4% of methane produced. Following digestion, the digestate is pasteurised at 70°C for one hour to satisfy "Animal By-Product Regulations of the European Union". The biogas produced is used for CHP production. The biogas engine has 42% efficiency for heat and 42% efficiency for electricity. All of the heat produced is used in the digestion and pasteurisation processes. The electricity generated is exported to the grid. The results of this study indicate that AD is a suitable disposal route for PL which may lead to reduced environmental impacts.

In (Jurgutis et al., 2020) the biogas production from chicken manure as a sole feedstock before spreading the digested chicken manure biomass on the fields as an organic biofertilizer at different organic loading rates in a mesophilic full scale anaerobic digestion plant, was studied. Laboratory BMP tests

performed indicated the biogas potential of methane – 508 mL CH₄ g⁻¹ VS added and this result was compared to biogas yields obtained in full scale anaerobic digestion plant using manure as a core substrate. Fermentation process data analysis of anaerobic digestion performance indicates that the system is able to adapt even if the same operational conditions, such as organic loading rate and retention time are applied. Once adapted, the performance of biogas plant bioreactor was able to deliver 93% of chicken manure biogas potential with an OLR of 3.14 kg VS L⁻¹d⁻¹ and TAN concentration of 5.5 g L⁻¹. The results indicated that it is possible to digest chicken manure as a stand-alone feedstock if ammonium concentration was carefully controlled via optimized fresh water dilution process. The results obtained from the full scale anaerobic digestion plant from the 217 days' operational period of chicken manure digestion as a mono substrate indicated that there is time required for adaptation phase of the anaerobic digestion process. This adaptation can be related to ammonium concentration in the fermentation media causing partial inhibition, which is reflected in biogas volume as well as energy content reduction. However, the data analysis of anaerobic digestion performance indicates that the system is able to adapt even if the same operational conditions, such as organic loading rate and retention time are applied. Authors concluded that it would be useful to examine the shift of anaerobic population during such adaptation and the response of process efficiency to such microbial population changes. This controlled population shift strategies could lead to improved digestion of high ammonium content substrates under maximized biogas production quality and rates.

An interesting paper (Manogaran et al., 2022) reviews the technical and practical aspects of different techniques (composting, pyrolysis, gasification, anaerobic digestion, hydrothermal liquefaction and torrefaction) able to convert chicken manure to value-added products in terms of operation, performance and limitations. This paper also examines the preferential treatment techniques in relation to

the product outputs with good market potential while being environmentally sustainable. It was shown that applying most of these treatment strategies require an energy consuming pre-drying stage. AD on the other hand adapts well with chicken manure (feedstock of relatively high-water content) to harness renewable energy in the form of biogas that is rich in CH₄.

A review paper for dry AD of chicken manure is presented in (Shapovalov et al., 2020). Dry anaerobic digestion has some advantages such as reduced reactor volume, higher volumetric methane yield, lower energy consumption for heating, less wastewater production and lower logistic costs for fertilizers. Different dry anaerobic digestion technologies, the features of dry anaerobic digestion, laboratory studies on chicken manure dry anaerobic digestion and methods of reducing inhibitors' effects are analyzed in this paper. The main problems of dry AD systems of municipal solid waste, agricultural waste, and food waste are mixing and transportation, but not biochemical constraints. One of the principal disadvantages of dry AD is the absence of dilution of inhibitors. Dry anaerobic digestion of chicken manure is a prospective way to decrease excess effluent production, however its valorization is characterized by low stability of the process, and therefore it is necessary to optimize the process. AcoD, ammonia removal, and adaptation have been used to decrease the effect of ammonia inhibition (Abouelenien et al., 2010). AcoD studies are still characterized by low stability of the process (methane yield ranged from 2 to 406 mL/g VS). It is shown in this paper, that the most effective way to decrease ammonium content is vacuuming, which provided 80% efficiency of ammonium content removal, but led to reducing the economic efficiency of the technology. The perspective way to regulate ammonia is through absorption without volatile acid located in the reactor. It decreases ammonia content during wet anaerobic digestion by 33% and has a positive economic effect. Finally, authors concluded that dry AD of chicken manure is possible, but there is still

no efficient way to provide it and additional research is needed.

In (Mansour et al., 2023) authors assessed the AD process in batch mode to determine the methanogenic potential (BMP) of different poultry waste, such as viscera, droppings, sieving and sludges recovered from wastewater treatment after the slaughtering process. The highest BMP was obtained with substrates rich in fat (such as viscera and sieve potential). The effect of ammonia concentration was evaluated on methane yield and assumed that the inoculum used had the capacity to tolerate high ammonia concentration since no inhibition was observed at $[N-NH_3] = 6.06 \text{ g.L}^{-1}$. However, when the reactor is fed with an input characterized by high TS, problems related to lab pumps being clogged by solids or the sedimentation of solids in the tubes may occur. In this case, the bioreactor cannot be fed constantly. It will thus be fed periodically. The methanogenic potential of poultry droppings is studied in batch mode and assessed in semi-continuous mode using a 12 L CSTR. A production of $0.270 \text{ Nm}^3 \text{ CH}_4 \cdot \text{g}^{-1} \text{VS}$ was obtained, indicating an efficiency of 82% in comparison to the yield obtained in the previous experiment using batch mode ($0.320 \text{ Nm}^3 \text{ CH}_4 \cdot \text{g}^{-1} \text{VS}$).

In (Bofinger et al., 2024) information about the dilution, solid/liquid separation, mass balance, and reuse of the biofertilizer (recycle) to dilute PL and to promote water saving while generating an organic fertilizer richer in nutrients is presented. Five dilutions (1:4, 1:6, 1:8, 1:10, and 1:12) were submitted to mass balance test followed or not by solid/liquid separation on a sieve. The fractions of the dilutions 1:6 to 1:10 (PL: water, w:v) were subjected to a test to determine the B. The volume of the produced biogas was recorded daily for a period of 34 days. The biogas composition (H_2 , CH_4 , and CO_2) was determined using a gas chromatograph. The temperature was maintained constant ($35 \pm 1^\circ\text{C}$) using a heating plate with nickel-chromium resistors. The highest potential biogas production was obtained in the liquid fraction of the dilution 1:6, reaching $179.4 \text{ L CH}_4 \text{ kg}^{-1}$

VS_{added} . This condition was subjected to a semi-continuous test in plug flow reactors (maximum 5% TS). In this test, one reactor was conducted only by diluting PL with water and the other reactor was conducted using 40% of the biofertilizer to dilute PL in substitution for water (recycle). A HRT of 25 days was assumed for all treatments. The daily feeding volume of 2.4 L was defined based on the reactor volume of 60 L and HRT of 25 days. The results showed that the replacement of 40% of the dilution water by the biofertilizer had no inhibitory effect on the anaerobic digestion process, reaching values of 236 and $226 \text{ L CH}_4 \text{ kg}^{-1} VS_{\text{added}}$ without and with the recycle, respectively. The decrease in the use of water and an increase in nutrients contained in the effluent (biofertilizer) is reflected in a 66% larger area for corn production. A detailed study of economic feasibility is essential for decision-making on investment and its recovery (payback). Thus, the liquid fraction of the dilution 1:6 with the recycle allowed a 40% saving in the use of clean water for PL dilution, providing a biofertilizer with higher amounts of nitrogen, one of the nutrients most demanded by most crops. Samples of gas (instantaneous chromatographic analyses – one sample per week) and biofertilizer (subsequent physicochemical analyses – daily) were collected from the biogas production stabilization for a period of 25 days (HRT). The specific methane production per reactor volume and kg of added VS was statistically equal ($p \leq 0.05$) between treatments. Methane contents did not differ statistically between treatments, resulting in values between 55 and 65%. Thus, the use of recycle was beneficial because it did not harm biogas production and provided a 40% reduction in the use of clean water for PL dilution, thus reducing production costs and providing a more concentrated biofertilizer. The use of recycle also provides the return of a range of adapted microorganisms to the reactor, as well as alkalinity, which has an important buffering role to decrease the risk of the system acidification. The use of recycle in the semi-continuous test at a 40% proportion does not negatively influence biogas production, thus

making it a viable option for reducing the amount of clean water used in PL dilution and providing a biofertilizer with a higher amount of nutrients.

In (Al-Zoubi et al., 2024) determination of optimal conditions for biogas generation from poultry manure is presented. This study was conducted in laboratory batch bioreactor with working volume of 2 L at different conditions with respect to pH, C/N ratio, and temperature. A full factorial design is designed to analyze the impact of the three parameters and their interactions. Experiments are replicated four times to ensure the reproducibility of data. Results indicate that temperature is the only influencing parameter on biogas production from poultry manure within a pH range of 6–8 and a C/N ratio range of 10/1 to 40/1. Moreover, there is no interaction between the three parameters, and mesophilic conditions are found to be the optimum conditions for biogas production. The maximum biogas yield of 285 ml/g VS is found in mesophilic conditions at 37 °C with a retention time of 20 days. As a result, temperature seems to be the controlling parameter for biogas production from poultry manure, and mesophilic conditions are the most effective.

In (Tzenos et al., 2025) a study investigated a combined strategy involving two modes of pretreatment of poultry manure (mild thermal hydrolysis pretreatment and bioaugmentation with ammonia-acclimatised inoculum) to enhance methane production and process stability under ammonia-stressed conditions is presented. Batch AD efficiency assays were first conducted to evaluate the effect of different hydrolysis conditions (55–70 °C, 30–60 min) on methane yields and biodegradability. The optimal condition (70 °C for 60 min) increased methane potential by 8.7% compared to the untreated substrate. In addition, a mesophilic CSTR experiment was conducted using both non-hydrolysed and thermally hydrolysed poultry manure under HRT of 25 and 30 days ($D = 0.033 \div 0.04$), across four operational phases: steady-state, ammonia toxicity, bioaugmentation recovery, and increased organic loading rate. CSTRs (three identical lab-

scale CSTR reactors with a total and operating volume of 2 L and 1.5 L, respectively) were subjected to ammonia stress (6500 mg $\text{NH}_4^+-\text{N L}^{-1}$) to assess the effectiveness of an acclimatised bioaugmentation inoculum. Methane yields recovered up to 93% and 100% of pre-inhibition and ammonia-toxicity levels, respectively, accompanied by process stability while reaching 7280 mg $\text{NH}_4^+-\text{N L}^{-1}$. The synergistic application of hydrolysis and bioaugmentation significantly improved substrate conversion and overall AD robustness. This integrated approach provides a viable and scalable strategy for optimising AD performance of nitrogen-rich feedstocks, enabling its future application in AD plants.

In (Thakur et al., 2025) alkali pretreatment of PL with the objective of utilizing broiler litter as a substrate for biogas production was carried out to produce a higher quantity of biogas without any other co-substrate. This study was taken without co-digestion with any other substrates. The experimental set-up consists of three biogas BRs, each consisting of a digester and a gas collector. Experiments were constructed within an enclosed room at the poultry farm. The capacity of each digester was 0.2 m³ and all three along with their components were constructed utilizing the easily available, local, and inexpensive materials. Three holes, each about 1.25 in. in diameter, were drilled into each drum for connecting different components like inlet, outlet, and water level. Biogas samples were collected every 15 days in a locally sourced rubber pillow and transported to the Avian Waste Management Laboratory for analysis. The composition of the biogas was analyzed using an automated portable biogas analyzer. This device measures various components of biogas within specific ranges: CO₂ from 0 to 100 % (v/v), CH₄ from 0 to 100 % (v/v), O₂ from 0 to 25 %, and H₂S from 0 to 9999 ppm. Daily methane yield was calculated as methane yield (m³)/day; methane yield (m³)/day/kg of fresh broiler litter added; methane yield (m³)/day/kg TS and methane yield (m³)/day/kg of VS. The physicochemical characteristics of the slurry, including pH, electrical conductivity (EC), and total dissolved

solids (TDS), were evaluated every week. These properties were measured using a digital pH meter, EC meter and TDS meter. The pH and EC meters were directly immersed into the slurry samples, and the readings were recorded accordingly. Pretreatment with NaOH at different concentrations followed by conditioning techniques like balancing C:N ratio, maintaining optimal pH, addition of methanogens in the beginning, were employed for anaerobic digestion of broiler litter. Three treatments were given as no alkali pretreatment (B0), alkali pretreatment with 0.1 mol/L (B1), and 0.2 mol/L (B2). The initial and daily OLR was kept at 25.40 gVS/L and 1.06 gVS/L, respectively, in all treatments due to a similar dilution rate of 1:10 and digester volume. Results showed that HRT was lowest in B1 (28 days) followed by B2 (40 days) and then in control (B0) (51 days). B1 had the highest average daily biogas yield (0.0186 m³), followed by B2 (0.0105 m³), and then B0 (0.0013 m³). The composition of biogas also varied significantly between days as well as treatments with the highest methane percent in B2, but the average daily methane yield was significantly higher in B1. Analysis of digested poultry litter slurry revealed significant ($P < 0.01$) differences in physico-chemical characteristics and proximate composition across treatments and days. It was concluded that alkali pretreatment with 0.1 mol/L enhances the biogas production potential of PL and slurry obtained along can be utilized as fertilizer. This study offers a sustainable solution for the poultry industry by enabling efficient waste management and renewable energy generation. The results of the study showed successful production of biogas from broiler litter by applying alkali pretreatment followed by conditioning techniques. The best results were obtained when pretreatment was given with 0.1 mol/L with initial and daily OLR of 25.40 g.VS/L and 1.06 g.VS/L, respectively. In the B1 treatment, the average methane content of produced biogas was 54.02 % with a calorific value of 21,510.76 KJ/m³. Also, the slurry obtained after anaerobic digestion had N, P, K content of 1.67 %, 1.16 %, 2.29 % respectively.

Therefore, this study showed that broiler litter after pretreatment with alkali can enhance the efficiency of biogas production from broiler litter and in addition to biogas, slurry having handsome manurial value was obtained which can serve as a source of fertilizer.

Additional approaches have been proposed to improve the anaerobic digestion of poultry manure under ammonia-stressed conditions. Pan et al., (2019) reported that biochar amendment enhanced methane production and promoted process stability, highlighting the potential of conductive materials as additives for improving the digestion of nitrogen-rich substrates.

4.2. AcoD of poultry waste

In (Nwosu-Obieogu et al., 2020) a study of the AcoD of poultry manure and banana peels as a co-substrate is presented. The experiments were carried out in a 20 liter biodigester and incubated for 50 days at mesophilic temperature of 34°C and pH of 6.8. Comparison of first and second order kinetics to test a liner and exponential models for biogas production from poultry manure and banana peels was made.

In (Lohani et al., 2025) a study of AcoD of food waste (FW), sewage sludge (SS), and PL is presented. These organic waste were co-digested at ratios (SS: PL: FW: 3:2:1, 2:1:1, 1:1:1) with 8% TS content at ambient temperature in summer and winter seasons. Biogas yield was highest with mixing ratio of 2:1:1 with the values of 640 L/kgVS in summer while it gave extremely low biogas yield of 106 L/ kgVS in winter. The 2:1:1 mixture also had the highest methane composition of nearly 65% as well as the highest VS removal efficiency of 60%, making it the most viable option for increasing biogas production. The experimental biogas production results were used to establish regression models for biogas production prediction. Mathematical modeling results using Gompertz model and first order model predicted well with R² value ranging from 0.91 to 0.98, which upheld the experimentally obtained values. Findings from this study suggest that co-digestion substrates (SS:PL:FW) mixing ratio of 2:1:1 is an optimized ratio among the studied co-substrate ratio for enhanced biogas

production. Furthermore, winter biogas yield is almost one sixth of summer biogas yield, which means use of temperature enhancement techniques to anaerobic digesters operating at ambient conditions would be essential to increase the biogas yield during winter.

In (Dhungana et al., 2022) AcoD of FW with livestock manure at ambient temperature was studied. FW, cow manure (CM), PL and goat manure (GM) were co-digested at mixing ratios (FW:PL:CM) of 2:1:1, 2:2:1, 1:1:2, 1:1:1 (wt/wt) and FW:PL:GM at mixing ratios of 2:1:1 and 1:1:2, at an organic loading rate of 1 g volatile solid (VS)/L/day, and 8% total solids. A maximum methane yield was obtained from co-digestion of FW:PL:GM at a mixing ratio of 2:1:1 in autumn-to-winter conditions, 21–10 °C, while the mixing ratio of FW:PL:CM at 2:2:1, showed negligible methane production under the same temperature condition. This study suggests that co-digestion of FW and PL with GM yields more biogas than other substrate combinations.

In (Mansour et al., 2023) to optimize the AD process a co-digestion method was applied mixing poultry droppings with physico-chemical sludge in a 60/40 ratio. As a result, an enhanced methane production by 54% was obtained without affecting process stability.

In (Paranhos et al., 2020) and poultry manure was successfully co-digested with lignocellulosic biomass such as agricultural residues and wheat straw. Different micro-aeration (MA) strategies for AD of PL and wheat straw (WS) were examined (Zhan, Zhu, Xiao, Schrader, et al., 2023a). MA at different stages (pretreatment, middle, pretreatment plus middle, and daily) in batch AD of WS showed that daily MA had the highest increase (16.5 %) of the cumulative methane yield (CMY) compared to the control BR. Batch AcoD of WS and PL with daily MA obtained an improved (15.1 %) CMY of 225.44 N mL CH₄/g vS added. Continuous AcoD of PL and WS experiments (with a weight ratio of 1:1; TS of 4 %; HRT of 16 days daily loading weight of 16.72 g; loaded substrate of 0.75 L daily; OLR of 1.72 g VS/day) in anaerobic sequencing batch reactor (SBR) were performed. Two

identical cylindrical SBRs were used for. The total volume of each reactor was 18 L with a full working volume of 16 L and 2 L headspace for maintaining enough biogas partial pressure. The SBR showed an increased (with 21.5 %) daily methane yield (DMY) when 0.5 h/d MA. To examine the effect of MA on the kinetic parameters of AD process, five kinetic models (the first-order model; the modified Gompertz model; the Cone model, the transfer model and the Chen and Hashimoto model) were used to fit the methane yield from both the mono AD and AcoD batch experiments in this study. The modified Gompertz model and Cone model were good in fitting the methane yield kinetics of MA engaged AD process. Daily MA shortened the lag phase of AcoD by 3.4 %. With micro-aeration, the ASBR for continuous AcoD of WS and PL achieved an enhanced performance with an increase in DMY by 21.5 %. The results provided support for the application of micro-aeration in the AD of agricultural wastes.

In (Zhan, Zhu, Xiao, Wu, et al., 2023b) AcoD laboratory experiments of PL with wheat straw in the daily anaerobic SBRs are performed considering operation parameters of C/N ratio (11.6 to 28.4), TS (2.6 to 9.4%), and HRT (7.6 to 24.4 day). The inoculum with a diverse microbial community structure included 2% of methanogens (*Methanosaeta*) was chosen in two anaerobic SBRs. Experimental performance by central composite design showed continuous methane production with the highest biogas production rate (BPR) obtained at C/N = 20, TS = 6%, and HRT = 7.6 day, being (1.18 ± 0.14 L/LR.day). A modified quadratic model for predicting BPR was built. The operation parameters and process stability both affected the release of nitrogen, phosphorus, and magnesium in the effluent. Inhibitions due to COD accumulation were relieved by employing the center values of parameters (C/N = 20, TS = 6%, HRT = 16d). The concentration of nitrogen, phosphorus, and magnesium in the effluent was affected by the C/N ratio, TS, HRT, and process stability. The results provided new support for the novel reactor operations for efficient bioenergy production from PL and agricultural wastes.

In (Bist et al., 2024) AcoD of PL with slaughterhouse waste (SHW) was studied. This study explores AD as an optimal solution for poultry SHW management, aiming to produce energy-rich biogas. Four distinct runs of anaerobic digestion were performed, each utilizing varying substrate compositions with SHW to CL ratios of 1:0, 4:1, 1:1, and 1:4. Throughout the process, essential parameters (TS, VS, BOD₅, pH, temperature, biogas generation) were measured. The volume of gas produced from different runs were determined on daily basis. The pH trends were systematically investigated, and it was observed that maintaining the appropriate pH range within the batch type reactor was crucial for sustaining the microbial community's stability and activity during the AcoD process. The cumulative biogas production for each run was as follows: 14.87 liters and a biogas yield of 88.73 ml/gVS with a 17.39 % VS reduction for Run 1, 25.98 liters and a biogas yield of 147.21 ml/gVS with a 28.64 % VS reduction for Run 2, 78.32 liters and a biogas yield of 314.69 ml/gVS with a 54.32 % VS reduction for Run 3, and 89.195 liters and a biogas yield of 344.36 ml/gVS with a 63.05 % VS reduction for Run 4. Notably, as the proportion of CL increased in the mixture from 4:1 to 1:1, a considerable VS reduction was observed. Furthermore, when the ratio of SHW to CL reached 1:1 and 1:4, a significant BOD₅ reduction of 50 % and 63.13 % was achieved, respectively, surpassing previous runs. The results reveal that the addition of CL in an appropriate ratio effectively manages poultry SHW, with the optimum SHW:CL ratio for significant biogas yield lying between 1:1 and 1:4. The study's outcomes offer valuable guidance for the practical implementation of co-digestion strategies, fostering a more sustainable waste management paradigm and facilitating the adoption of renewable energy solutions in the poultry industry.

In (Coêlho et al., 2025) AcoD of PL and poultry slaughterhouse effluent (PSE) was compared with anaerobic mono-AD of both, using BMP tests at 35°C and 180 rpm. An anaerobic SBR operated in fed-batch mode

under the best condition identified in the BMP tests, with a 24-hour cycle, without mechanized agitation, and temperature controlled at 30°C ±2°C, with an increasing OLR (0.2–1.6 gVS L⁻¹ d⁻¹). The total volume of the reactor was 20 liters, of which 10 liters was the useful volume, with the remainder destined for the gas phase (headspace) to collect the biogas and keep the partial pressures of the biogas constituents low. The start was with an OLR of 0.2 g VS L⁻¹ d⁻¹. A gradual increase was achieved by doubling the OLR every 30 days, reaching a final operating load of 1.6 g VS L⁻¹ d⁻¹. The OLR rate increased throughout the experiment based on the operational stability observed in the reactor, as assessed by the VS concentrations. The experiments were conducted in triplicates, with additional controls, totaling 18 reactor flasks which were monitored for 60 days. The substrate was mixed manually during sample collection and after feeding the reactor (24-hour cycle). The 24-hour cycle was chosen based on practical and operational criteria. The optimal mixing ratio of 25 % PL and 75 % PSE was obtained in BMP tests for generating 223.5 NmL CH₄ gCOD⁻¹, with biodegradability of 64 %. The Cone and Gompertz kinetic models fitted well with the experimental data; according to the latter, the 25 % PL:75 % PSE mixing condition resulted in the shortest lag phase (2.18 days) and the highest methane production rate (11.0 NmL CH₄ gCOD⁻¹ d⁻¹). During SBR operation, with an OLR of 0.8 gVS L⁻¹ d⁻¹, the system produced biogas with the highest average methane content (46.1 %) and had the highest average methane yield (170.7 NmL CH₄ gVS_{add}⁻¹). By increasing the OLR to 1.6 gVS L⁻¹ d⁻¹, there was a reduction in the methane content (42.5 %) and an increase in the concentration of total volatile fatty acids (VFAs), reaching a value of 1466.4 mg L⁻¹. The sludge and anaerobic digestate exhibited compatible chemical characteristics for use as biofertilizers. The operation of the SBR, with the 25%PL:75%PSE mixing ratio, produced gradually higher methane levels as the OLR increased to 0.8 gVS L⁻¹ d⁻¹, rising to 46.1% with maximum methane production of 170.69±5.38 NmL CH₄ gVS_{add}⁻¹ (125.22±3.94 NmL CH₄

gCOD_{add}⁻¹). However, when the OLR was increased to 1.6 gVS L⁻¹d⁻¹, there was a reduction in methane content to 42.5% and greater instability of AcoD. The sludge generated in the process met the microbiological criteria for classification as class B biosolids, reinforcing its potential for agricultural use. However, additional stability measures are recommended to ensure greater safety and comply with regulatory standards. In conclusion, the AcoD of PL and PSE proved to be a sustainable and economically viable solution for managing agro-industrial waste, combining environmental and economic benefits.

In (Hossain et al., 2025) the potential of AcoD of cow dung and PL as a sustainable energy solution for Bangladesh's garment industry was studied. The optimal co-digestions for biogas production were determined by investigating several process parameters, including the feedstock ratio, stirring rate, pH, and temperature. Laboratories experiment in a 25-liter BR were performed. The BR automatic control system included a temperature controller, thermocouple, electric heater, magnetic contactor, electric motor and regulator for automatic speed-controlled stirring. In this paper it was determined that a 1:1:1 mixture of cow dung, PL, and water, combined with a temperature of 35 °C and a stirring rate of 120 rpm, yielded the best results. Biogas for the optimum input condition is composed of methane (61.40 %), CO₂ (50 %), H₂S (391 ppm), and O₂ (2.90 %). Analyzing digestate obtained for these optimal conditions it was find that the moisture content was found to be 60.3 %, organic carbon 24.25 %, phosphorus 0.42 %, potassium 0.67 %, sulfur 0.20 %, zinc 0 %, and the pH value was found to be 6.0. Under these conditions, the biogas production reached a cumulative total of 14.97 L over a 21-day HRT. Additionally, regression models for biogas yield and fertilizer output were developed using machine learning to predict biogas output under different conditions. The techno-economic analysis of the industrial plant project revealed a net present value of USD 8,861.88 million, an internal rate of return of 24 %, and an investment payback period of 3.09 years over 20 years.

In (Zhan et al., 2024) amendment AcoD of PL and agricultural straw using biochar has been studied. This study conducted two sequential experiments to evaluate the effect of adding alkaline biochar on improving the batch AcoD of PL and wheat straw. Experiments identified the advantages of adding biochar by the improved CMYo (mL CH₄/g VS_{substrate}) of 9.7%; the increased removal rates of the substrate TS_{substrate} and VS_{substrate} of 15.2% and 14.2%, respectively; the enhanced abundance of both hydrolytic bacteria and methanogens, including hydrogenotrophic *Methanobacterium* and, especially, the acetolactic *Methanosaeta*; and the 37.3% higher abundance of the methanogenic pathways, compared to the control. The interaction experiment showed that biochar dosage (BD) interacted with the initial substrate C/N ratio and TS level. The developed mathematical models for CMYo and VS_{substrate} removal by response surface methodology were significant, which predicted the optimal conditions being initial substrate C/N ratio 29.93 and TS 6.98%, and BD 9.98% substrate. The optimized CMYo and VS_{substrate} removal were 17.7% and 22.1% higher, respectively, than those of the Control. These results support the utilization of biochar in improving the AcoD of agricultural wastes.

In (Karumanchi et al., 2026) assessed PL AD is presented at 10 %, 30 %, and 60 % TS under three setups: (i) PL alone, (ii) co-digestion with food waste hydrolysate (PL +FH) to improve C/N ratio, and (iii) in situ desulfurization (St-PL) using sulfur-oxidizing bacteria (SOB) with micro-aeration to reduce H₂S. This study demonstrated that AD of PL at high solids concentrations (up to 30 % TS) correlates positively with biogas yield, achieving 0.31 L/gVS_{reduced} (PL +FH 30 %), 0.28 L/gVS_{reduced} (PL-30 %), 0.3 L/gVS_{reduced} (St-PL 30 %). The highest biogas yields were obtained at 30 % TS: 12.4 L (PL +FH), 9.9 L (PL), and 8.9 L (St-PL). The improved C/N ratio from 8.9 (PL) to 29.2 (PL +FH), contributed to the overall increase in biogas yield. Methane content was highest in St-PL (68 %) with H₂S removal of 80–92 %. However, slight sulfate accumulation was detected in the digestate, attributed to the

metabolic activity of sulfur oxidizing bacteria (SOB). The integration of SOB and micro-aeration effectively reduced H_2S levels in the biogas, enhancing its suitability as a biofuel. Microbial analysis revealed the dominance of *Firmicutes*, *Bacteroidetes*, *Methanosaeta*, and *Methanobacterium*. Metagenomic analysis revealed that microbial diversity and composition were influenced by solid concentrations and bioreactor parameters, highlighting their critical role in biogas generation efficiency. Additionally, the nutrient-rich digestate offers potential as a sustainable fertilizer alternative. Overall, the findings highlight the dual benefits of AD of PL for efficient waste management and renewable energy production, offering a viable and sustainable approach to address environmental challenges while contributing to the circular bioeconomy.

In (Coêlho et al., 2025) AcoD of PL and PSE was compared with mono AD of both, using BMP tests at 35°C and 180 rpm. An anaerobic SBR operated in fed-batch mode (feeding the reactor with 24-hour cycle) under the best condition identified in the BMP tests, with a 24-hour cycle, without mechanized agitation, and temperature controlled at $30^\circ\text{C} \pm 2^\circ\text{C}$, with an increasing OLR ($0.2\text{--}1.6 \text{ gVS L}^{-1} \text{ d}^{-1}$). The total volume of the reactor was 20 liters, of which 10 liters was the useful volume. The experiments were conducted in triplicates, with additional controls, totaling 18 reactor flasks which were monitored for 60 days. Among the mixing proportions evaluated, the 25 % PL:75 % PSE mixture stood out in BMP tests with the best performance, reaching an accumulated production of $223.5 \text{ NmL CH}_4 \text{ gCOD}^{-1}$ in BMP tests, as well as a lower accumulation of VFAs (around 300 mgL^{-1}) and biodegradability of 64 %. The Cone and Gompertz kinetic models fitted well with the experimental data; according to the latter, the 25 % PL:75 % PSE mixing condition resulted in the shortest lag phase (2.18 days) and the highest methane production rate ($11.0 \text{ NmL CH}_4 \text{ gCOD}^{-1} \text{ d}^{-1}$). The kinetic analysis revealed that the Cone model was more accurate for estimating methane production than the modified Gompertz model. During

anaerobic SBR operation, with an OLR of $0.8 \text{ gVS L}^{-1} \text{ d}^{-1}$, the system produced biogas with the highest average methane content (46.1 %) and had the highest average methane yield ($170.7 \text{ NmL CH}_4 \text{ gVS}_{\text{add}}^{-1}$). By increasing the OLR to $1.6 \text{ gVS L}^{-1} \text{ d}^{-1}$, there was a reduction in the methane content (42.5 %) and an increase in the concentration of total VFAs, reaching a value of 1466.4 mg L^{-1} . The sludge and anaerobic digestate exhibited compatible chemical characteristics for use as biofertilizers.

V. CHALLENGES, FUTURE PROSPECTS AND ECONOMIC FEASIBILITY

5.1. Current Limitations to Large-Scale Implementation

The primary challenge for AD and AcoD of poultry waste is not lab-scale efficiency, but ensuring consistent, economic performance in real-world, variable-feedstock applications. Capital Expenditure remains a major barrier when pretreatment, advanced reactors, and upgrading are combined, (Bi, Westerholm, et al., 2020b) particularly in farm-scale and decentralized systems. Operational complexity is equally important. Intensified systems increase the number of interacting control variables, which narrows the margin for error and raises demands on operator expertise. As a result, technologies that perform well in pilot studies may underperform when maintenance, staffing, or feedstock quality management is inconsistent. Process instability remains a persistent limitation, especially under variable loading, ammonia stress, or poor feedstock conditioning. Future performance gains will depend as much on control architecture and monitoring as on any single pretreatment, additive, or reactor innovation.

5.2. Future Research Directions

Future AD research is moving toward integrated, digitally enabled process optimization, but the immediate goal should be validated hybrid control rather than algorithm proliferation. AI and machine learning will be most useful where they are linked to interpretable features, mechanistic models, and

plant-relevant datasets rather than deployed as isolated black boxes. A second priority is truly coupled techno-economic analysis (TEA) with life-cycle assessment (LCA). Performance gains from severe thermal or oxidative pretreatment, specialized additives, or H_2 - dependent upgrading are not automatically beneficial once parasitic energy demand, material-input burdens, methane slip, and capital intensity are counted. For example, pretreatments that improve methane yield can still worsen net energy or climate performance when waste-heat recovery is absent, and biological methanation can become environmentally or economically unattractive when H_2 is not both low-carbon and reliably available. A third priority is biorefinery integration, where biomethane is co-optimized with digestate valorization, CO_2 conversion, and other product streams. In this framework, profitability depends on value stacking rather than methane yield alone, and future claims of superiority should increasingly be supported by long-duration pilot or full-scale validation.

5.3. Economic Analysis and Feasibility Pathways

Economic viability in AD increasingly depends on portfolio economics. Biomethane revenue remains central, but digestate valorization, CO_2 utilization, tipping fees, and policy incentives are often decisive for project bankability. Digestate is particularly important because it links AD to agricultural nutrient management and can create stable local value if product quality is standardized. Policy and market design strongly influence outcomes. Feed-in tariffs, renewable-gas standards, carbon pricing, and permitting frameworks frequently determine whether technically viable projects are financially attractive. A strong synthesis across economic studies is that AD should be evaluated as an integrated waste–energy–nutrient–carbon system. Projects that combine reliable methane recovery with digestate and CO_2 valorization often outperform simpler waste-treatment alternatives, but only when feedstock logistics, uptime, and local market conditions are explicitly considered. Integrated biogas projects which combine reliable methane recovery with digestate and CO_2 valorization

often outperform simpler waste-treatment methods. However, their success hinges on careful planning regarding feedstock logistics, system uptime, and local market conditions.

VI. CONCLUSION

The continuous increase in the Earth's population leads to an increase in the number of chickens raised (for meat and eggs), and hence - in waste from poultry farms and slaughterhouses. The best method for solving the problem of this waste is AcoD, which produces biogas and natural fertilizer. However, poultry waste has a certain specificity - it is very rich in ammonia, which can lead to inhibition of AD processes.

The net environmental impact depended mainly on the processes displaced by the use of the two co-products, biogas and digestate. Upgrading biogas to biomethane had the highest GHG emissions savings when assuming 1:1 substitution of natural gas. The results for the eutrophication impact category varied according to the energy pathway biogas is used in and the fuel mix that is displaced, leading to a net impact in this category in some scenarios. However, for all other impact categories, there is a potential net reduction in environmental burdens.

Recent advances show that methane production from AD is improved most reliably when process intensification, additive-enabled control, and biomethane upgrading are evaluated as an integrated design problem rather than as isolated unit operations. Pretreatment and reactor innovations can reduce hydrolysis and phase-instability constraints, co-digestion can improve biochemical compatibility, and additives can provide targeted support for electron transfer, hydrolysis, or methanogenic stability. However, the strength of evidence differs across interventions: co-digestion, staged reactors, and established physicochemical upgrading routes are comparatively mature, whereas AI-assisted control, and some biological or hybrid upgrading pathways remain promising but still under-validated at long duration and full scale. Future progress will

depend on reproducible comparative reporting, hybrid ADM1–ML monitoring, and linked demonstration studies that determine when higher methane yield translates into better net energy, climate, and economic performance.

The results indicate that AD is a suitable disposal route for PL which can lead to reduced environmental impacts. The increasing trends of the feasibility and applicability of biogas production from poultry waste indicate the potential for using different kinds of feedstock waste in the production of biogas through the AcoD process. However, some challenges need to be confronted to convert laboratory-scale production to the industrial level. Many aspects must be maintained, such as buffering capacity, the production rate of biogas, nutritional balance, and microbiological stability. Dry AD of chicken manure is possible, but there is still no efficient way to provide it and additional research is needed.

ACKNOWLEDGEMENTS: This research was funded by the Bulgarian National Science Fund, Grant KII-06-H77/2. This study was supported by Center of competence: Clean technologies for sustainable environment—water, waste, energy for circular economy (Clean&Circle) No BG16RFPR002-1.014-0015-C01, financed by the Operational Programs “Science and Education for Smart Growth” 2014–2020 and “Research, Innovation and Digitalization for Smart Transformation” 2021–2027, cofounded by the European Union through the European structural and investment funds.

REFERENCES

1. Abouelenien, F., Fujiwara, W., Namba, Y., Kosseva, M., Nishio, N., & Nakashimada, Y. (2010). Improved methane fermentation of chicken manure via ammonia removal by biogas recycle. *Bioresource Technology*, 101(16), 6368–6373. <https://doi.org/10.1016/j.biortech.2010.03.071>
2. Abouelenien, F., Namba, Y., Nishio, N., & Nakashimada, Y. (2016). Dry Co-Digestion of Poultry Manure with Agriculture Wastes. *Applied Biochemistry and Biotechnology*, 178(5), 932–946. <https://doi.org/10.1007/s12010-015-1919-1>
3. Alipoursarbani, M., Tideman, J., López, M., & Abendroth, C. (2026). Bioaugmentation in anaerobic digesters: A systematic review. *Biotechnology for Biofuels and Bioproducts*, 19(1), 25. <https://doi.org/10.1186/s13068-026-02746-6>
4. Al-Zoubi, A. I., Alkhamis, T. M., & Alzoubi, H. A. (2024). Optimized biogas production from poultry manure with respect to pH, C/N, and temperature. *Results in Engineering*, 22, 102040. <https://doi.org/10.1016/j.rineng.2024.102040>
5. Arslan, M., & Yılmaz, C. (2022). Design and optimization of multigeneration biogas power plant using waste heat recovery System: A case study with Energy, Exergy, and thermoeconomic approach of Power, cooling and heating. *Fuel*, 324, 124779. <https://doi.org/10.1016/j.fuel.2022.124779>
6. Babaei, A., Shayegan, J., & Roshani, A. (2013). Anaerobic slurry co-digestion of poultry manure and straw: Effect of organic loading and temperature. *Journal of Environmental Health Science and Engineering*, 11(1), 15. <https://doi.org/10.1186/2052-336X-11-15>
7. Bayrakdar, A., Molaey, R., Sürmeli, R. Ö., Sahinkaya, E., & Çalli, B. (2017). Biogas production from chicken manure: Co-digestion with spent poppy straw. *International Biodeterioration & Biodegradation*, 119, 205–210. <https://doi.org/10.1016/j.ibiod.2016.10.058>
8. Beausang, C., McDonnell, K., & Murphy, F. (2020). Anaerobic digestion of poultry litter – A consequential life cycle assessment. *Science of The Total Environment*, 735, 139494. <https://doi.org/10.1016/j.scitotenv.2020.139494>
9. Bi, S., Qiao, W., Xiong, L., Mahdy, A., Wandera, S. M., Yin, D., & Dong, R. (2020a). Improved high solid anaerobic digestion of chicken manure by moderate in situ ammonia stripping and its relation to metabolic pathway. *Renewable Energy*, 146, 2380–2389. <https://doi.org/10.1016/j.renene.2019.08.093>
10. Bi, S., Qiao, W., Xiong, L., Ricci, M., Adani, F., & Dong, R. (2019). Effects of organic loading rate on anaerobic digestion of chicken manure under mesophilic and thermophilic conditions. *Renewable Energy*, 139, 242–250. <https://doi.org/10.1016/j.renene.2019.02.083>
11. Bi, S., Westerholm, M., Qiao, W., Xiong, L., Mahdy, A., Yin, D., Song, Y., & Dong, R. (2020b). Metabolic performance of anaerobic digestion of chicken manure under wet, high solid, and dry conditions. *Bioresource Technology*, 296, 122342. <https://doi.org/10.1016/j.biortech.2019.122342>
12. Bist, S., Nepal, R., Regmi, T., & Sharma, R. K. (2024). Poultry slaughterhouse waste management through anaerobic digestion with

- varying proportions of chicken litter. *Environmental Challenges*, 17, 101035. <https://doi.org/10.1016/j.envc.2024.101035>
13. Blake, J. P., Hess, J. B., & Bock, B. R. (2007). Nutritional and Economic Value of Poultry Litter Ash as a Feed Supplement for Broilers. *International Symposium on Air Quality and Waste Management for Agriculture*, 16-19 September 2007, Broomfield, Colorado. *International Symposium on Air Quality and Waste Management for Agriculture*, 16-19 September 2007, Broomfield, Colorado. <https://doi.org/10.13031/2013.23818>
14. Bofinger, J., Damaceno, F. M., Restrepo, J. C. P. S., Costa, L. A. de M., De Lucas Junior, J., & Costa, M. S. S. de M. (2024). Anaerobic digestion of poultry litter: Technical aspects to improve process performance and water saving. *DELOS: Desarrollo Local Sostenible*, 17(53), e1308. <https://doi.org/10.55905/rdelosv17.n53-010>
15. Bolan, N. S., Mahimairaja, S., & Hedley, M. J. (1992). Improving the fertilizer value of poultry manures. *Proceedings of the Seminar on 'Eggs '92-Winning in a Deregulated Environment,' Egg Producers Federation of New Zealand*. 1-15.
16. Bres, P., Beily, M. E., Young, B. J., Gasulla, J., Butti, M., Crespo, D., Candal, R., & Komilis, D. (2018). Performance of semi-continuous anaerobic co-digestion of poultry manure with fruit and vegetable waste and analysis of digestate quality: A bench scale study. *Waste Management*, 82, 276–284. <https://doi.org/10.1016/j.wasman.2018.10.041>
17. Coêlho, Y. R., Silva, S. de Q., & Aquino, S. F. de. (2025). Anaerobic co-digestion of poultry litter and poultry slaughterhouse effluent in an anaerobic sequencing fed-batch reactor. *Journal of Environmental Chemical Engineering*, 13(5), 118677. <https://doi.org/10.1016/j.jece.2025.118677>
18. Dalkılıç, K., & Ugurlu, A. (2015). Biogas production from chicken manure at different organic loading rates in a mesophilic-thermophilic two stage anaerobic system. *Journal of Bioscience and Bioengineering*, 120(3), 315–322. <https://doi.org/10.1016/j.jbiosc.2015.01.021>
19. Deublein, D. (Ed.). (2010). *Biogas from waste and renewable resources: An introduction* (3. Nachdr.). Wiley-VCH-Verl.
20. Dhungana, B., Lohani, S. P., & Marsolek, M. (2022). Anaerobic Co-Digestion of Food Waste with Livestock Manure at Ambient Temperature: A Biogas Based Circular Economy and Sustainable Development Goals. *Sustainability*, 14(6), 3307. <https://doi.org/10.3390/su14063307>
21. Duncan, J. (2005). *Composting chicken manure*. WSU Cooperative Extension.
22. Epstein, E., & Moss, E. H. (2006). A comparison of characteristics of manures, biosolids, and mineral fertilizers. 3, 35–42.
23. Evans, G. M., & Furlong, J. C. (2003). *Environmental biotechnology— theory and application*. John Wiley&Sons Ltd, Chichester.
24. Forester, Ch. F., & John Wase, D. A. (1990). *Environmental biotechnology*. Ellis Horwood. New York.
25. Friend, A. L., Roberts, S. D., Schoenholtz, S. H., Mobley, J. A., & Gerard, P. D. (2006). Poultry Litter Application to Loblolly Pine Forests: Growth and Nutrient Containment. *Journal of Environmental Quality*, 35(3), 837–848. <https://doi.org/10.2134/jeq2005.0244>
26. Fuchs, W., Wang, X., Gabauer, W., Ortner, M., & Li, Z. (2018). Tackling ammonia inhibition for efficient biogas production from chicken manure: Status and technical trends in Europe and China. *Renewable and Sustainable Energy Reviews*, 97, 186–199. <https://doi.org/10.1016/j.rser.2018.08.038>
27. Hassan, M., Umar, M., Ding, W., Mehryar, E., & Zhao, C. (2017). Methane enhancement through co-digestion of chicken manure and oxidative cleaved wheat straw: Stability performance and kinetic modeling perspectives. *Energy*, 141, 2314–2320. <https://doi.org/10.1016/j.energy.2017.11.110>
28. Hilgert, J. E., Herrmann, C., Petersen, S. O., Dragoni, F., Amon, T., Belik, V., Ammon, C., & Amon, B. (2023). Assessment of the biochemical methane potential of in-house and outdoor stored pig and dairy cow manure by evaluating chemical composition and storage conditions. *Waste Management*, 168, 14–24. <https://doi.org/10.1016/j.wasman.2023.05.031>
29. Hossain, Md. S., Emon, A. S., Mhamud, R., Robin, H. M., Mourshed, M., & Rahman, Md. A. A. (2025). Anaerobic co-digestion of cow dung and poultry litter for sustainable biogas production: A green energy solution for Bangladesh's garment industry. *Energy*, 339, 139144. <https://doi.org/10.1016/j.energy.2025.139144>
30. Hubenov, V., Stoyanov, L., Stoychev, S., Simeonov, I., Milenov, V., Bachev, I., & Kabaivanova, L. (2026). Utilization of Poultry Litter from a Small Farm in Anaerobic Digestion for Energy Production Supported with Photovoltaics. *Energies*, 19(6), 1428. <https://doi.org/10.3390/en19061428>

31. Jackson, B. P., Bertsch, P. M., Cabrera, M. L., Camberato, J. J., Seaman, J. C., & Wood, C. W. (2003). Trace Element Speciation in Poultry Litter. *Journal of Environmental Quality*, 32(2), 535–540. <https://doi.org/10.2134/jeq2003.5350>
32. Jeswani, H. K., Whiting, A., Martin, A., & Azapagic, A. (2019). Environmental and economic sustainability of poultry litter gasification for electricity and heat generation. *Waste Management*, 95, 182–191. <https://doi.org/10.1016/j.wasman.2019.05.053>
33. Jurgutis, L., Slepeliene, A., Volungevicius, J., & Amaleviciute-Volunge, K. (2020). Biogas production from chicken manure at different organic loading rates in a mesophilic full scale anaerobic digestion plant. *Biomass and Bioenergy*, 141, 105693. <https://doi.org/10.1016/j.biombioe.2020.105693>
34. Kadam, R., Jo, S., Lee, J., Khanthong, K., Jang, H., & Park, J. (2024). A Review on the Anaerobic Co-Digestion of Livestock Manures in the Context of Sustainable Waste Management. *Energies*, 17(3), 546. <https://doi.org/10.3390/en17030546>
35. Karki, R., Chuenchart, W., Surendra, K. C., Shrestha, S., Raskin, L., Sung, S., Hashimoto, A., & Kumar Khanal, S. (2021). Anaerobic co-digestion: Current status and perspectives. *Bioresource Technology*, 330, 125001. <https://doi.org/10.1016/j.biortech.2021.125001>
36. Karumanchi, B., Begum, S., Anupoju, G. R., & Pramanik, B. K. (2026). Micro-aeration strategy for improved biogas yield and in-situ hydrogen sulfide mitigation during high-solids co-digestion of poultry litter. *Bioresource Technology*, 439, 133399. <https://doi.org/10.1016/j.biortech.2025.133399>
37. Kelleher, B. P., Leahy, J. J., Henihan, A. M., O'Dwyer, T. F., Sutton, D., & Leahy, M. J. (2002). Advances in poultry litter disposal technology – a review. *Bioresource Technology*, 83(1), 27–36. [https://doi.org/10.1016/S0960-8524\(01\)00133-X](https://doi.org/10.1016/S0960-8524(01)00133-X)
38. Khan, M., Chuenchart, W., Surendra, K. C., & Kumar Khanal, S. (2023). Applications of artificial intelligence in anaerobic co-digestion: Recent advances and prospects. *Bioresource Technology*, 370, 128501. <https://doi.org/10.1016/j.biortech.2022.128501>
39. Kyakuwair, M., Olupot, G., Amoding, A., Nkedi-Kizza, P., & Ateenyi Basamba, T. (2019). How Safe is Chicken Litter for Land Application as an Organic Fertilizer?: A Review. *International Journal of Environmental Research and Public Health*, 16(19), 3521. <https://doi.org/10.3390/ijerph16193521>
40. Levasseur, P., Toudic, A., Bonhomme, S., & Lorinquer, E. (2017). Gestion et traitement des digestats issus de méthanisation [Technical guide / Brochure]. IFIP – Institut du Porc.
41. Liedl, B. E., Bombardiere, J., & Chatfield, J. M. (2006). Fertilizer potential of liquid and solid effluent from thermophilic anaerobic digestion of poultry waste. *Water Science and Technology*, 53(8), 69–79. <https://doi.org/10.2166/wst.2006.237>
42. Linsong, H., Lianhua, L., Ying, L., Changrui, W., & Yongming, S. (2022). Bioaugmentation with methanogenic culture to improve methane production from chicken manure in batch anaerobic digestion. *Chemosphere*, 303, 135127. <https://doi.org/10.1016/j.chemosphere.2022.135127>
43. Lohani, S. P., Shakya, S., Gurung, P., Dhungana, B., Paudel, D., & Mainali, B. (2025). Anaerobic co-digestion of food waste, poultry litter and sewage sludge: Seasonal performance under ambient condition and model evaluation. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 47(2), 1887976. <https://doi.org/10.1080/15567036.2021.1887976>
44. Mahdy, A., Bi, S., Song, Y., Qiao, W., & Dong, R. (2020). Overcome inhibition of anaerobic digestion of chicken manure under ammonia-stressed condition by lowering the organic loading rate. *Bioresource Technology Reports*, 9, 100359. <https://doi.org/10.1016/j.biteb.2019.100359>
45. Manogaran, M. D., Shamsuddin, R., Mohd Yusoff, M. H., Lay, M., & Siyal, A. A. (2022). A review on treatment processes of chicken manure. *Cleaner and Circular Bioeconomy*, 2, 100013. <https://doi.org/10.1016/j.clcb.2022.100013>
46. Manono, B. O., & Ming'ate, F. L. M. (2026). Recent Advances and Prospects in Methane Production from Anaerobic Digestion: Process Intensification, Additives, and Biogas Upgrading. *Methane*, 5(2), 13. <https://doi.org/10.3390/methane5020013>
47. Mansour, M.-N., Lendormi, T., Drévilion, L., Naji, A., Louka, N., Maroun, R. G., Hobaika, Z., & Lanoisellé, J.-L. (2024). Influence of substrate/inoculum ratio, inoculum source and ammonia inhibition on anaerobic digestion of poultry waste. *Environmental Technology*, 45(10), 1894–1907. <https://doi.org/10.1080/09593330.2022.2157754>
48. Mansour, M.-N., Lendormi, T., Louka, N., Maroun, R. G., Hobaika, Z., & Lanoisellé, J.-L. (2023). Anaerobic Digestion of Poultry Droppings in

- Semi-Continuous Mode and Effect of Their Co-Digestion with Physico-Chemical Sludge on Methane Yield. *Sustainability*, 15(7), 5997. <https://doi.org/10.3390/su15075997>
49. Matheri, A. N., Ndiweni, S. N., Belaid, M., Muzenda, E., & Hubert, R. (2017). Optimising biogas production from anaerobic co-digestion of chicken manure and organic fraction of municipal solid waste. *Renewable and Sustainable Energy Reviews*, 80, 756–764. <https://doi.org/10.1016/j.rser.2017.05.068>
50. McGrath, S., Maguire, R. O., Tracy, B. F., & Fike, J. H. (2010). Improving Soil Nutrition with Poultry Litter Application in Low-Input Forage Systems. *Agronomy Journal*, 102(1), 48–54. <https://doi.org/10.2134/agronj2009.0198>
51. Mirmohamadsadeghi, S., Karimi, K., Tabatabaei, M., & Aghbashlo, M. (2019). Biogas production from food wastes: A review on recent developments and future perspectives. *Bioresource Technology Reports*, 7, 100202. <https://doi.org/10.1016/j.biteb.2019.100202>
52. Mohsenzadeh, A., Jeihanipour, A., Karimi, K., & Taherzadeh, M. J. (2012). Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production. *Journal of Chemical Technology & Biotechnology*, 87(8), 1209–1214. <https://doi.org/10.1002/jctb.3695>
53. Molaey, R., Bayrakdar, A., Sürmeli, R. Ö., & Çalli, B. (2018). Anaerobic digestion of chicken manure: Mitigating process inhibition at high ammonia concentrations by selenium supplementation. *Biomass and Bioenergy*, 108, 439–446. <https://doi.org/10.1016/j.biombioe.2017.10.050>
54. Molaey, R., Bayrakdar, A., Sürmeli, R. Ö., & Çalli, B. (2019). Anaerobic digestion of chicken manure: Influence of trace element supplementation. *Engineering in Life Sciences*, 19(2), 143–150. <https://doi.org/10.1002/elsc.201700201>
55. Moletta, R. (2015). La méthanisation (3^e éd). Lavoisier-Médecine sciences.
56. Moore, Jr, P. A., Joern, B. C., Edwards, D. R., Wood, C. W., & Daniel, T. C. (2006). EFFECTS OF MANURE AMENDMENTS ON ENVIRONMENTAL AND PRODUCTION PROBLEMS. *Animal Agriculture and the Environment*, National Center for Manure & Animal Waste Management White Papers;   Animal Agriculture and the Environment, National Center for Manure & Animal Waste Management White Papers;   <https://doi.org/10.13031/2013.20271>
57. Niu, Q., Takemura, Y., Kubota, K., & Li, Y.-Y. (2015). Comparing mesophilic and thermophilic anaerobic digestion of chicken manure: Microbial community dynamics and process resilience. *Waste Management*, 43, 114–122. <https://doi.org/10.1016/j.wasman.2015.05.012>
58. Nwosu-Obieogu, K., Aguele, F. O., Onyenwoke, A., & Adekunle, K. (2020). Kinetic Model Comparison for Biogas Production from Poultry Manure and Banana Peels. *European Journal of Sustainable Development Research*, 4(2). <https://doi.org/10.29333/ejosdr/7595>
59. Omar, M. N., Samak, A. A., Keshek, M. H., & Elsis, S. F. (2020). Simulation and validation model for using the energy produced from broiler litter waste in their house and its requirement of energy. *Renewable Energy*, 159, 920–928. <https://doi.org/10.1016/j.renene.2020.06.049>
60. Pan, J., Ma, J., Liu, X., Zhai, L., Ouyang, X., & Liu, H. (2019). Effects of different types of biochar on the anaerobic digestion of chicken manure. *Bioresource Technology*, 275, 258–265. <https://doi.org/10.1016/j.biortech.2018.12.068>
61. Paranhos, A. G. D. O., Adarme, O. F. H., Barreto, G. F., Silva, S. D. Q., & Aquino, S. F. D. (2020). Methane production by co-digestion of poultry manure and lignocellulosic biomass: Kinetic and energy assessment. *Bioresource Technology*, 300, 122588. <https://doi.org/10.1016/j.biortech.2019.122588>
62. Petkov, Y. (2008). Anaerobic digestion of poultry litter in sequencing batch mode of operation. 4, 28–35.
63. Petkov, Y. (2009). Optimization of methane fermentation from poultry manure and ecotoxicological assessment of biosludge. *The University of Forestry – Sofia*.
64. Phillip, A., Bhatt, S. M., Sharma, N., & Poudel, B. (2024). Enhanced Biogas Production Using Anaerobic Co-digestion of Animal Waste and Food Waste: A Review. *Journal of Scientific Research and Reports*, 30(8), 761–781. <https://doi.org/10.9734/jsrr/2024/v30i82297>
65. Price, E. C., & Cheremisinoff, P. N. (1981). *Biogas Production & Utilization*. Ann Arbor Science Publishers, Michigan.
66. Rawoof, S. A. A., Kumar, P. S., Vo, D.-V. N., & Subramanian, S. (2021). Sequential production of hydrogen and methane by anaerobic digestion of organic wastes: A review.

- Environmental Chemistry Letters, 19(2), 1043–1063. <https://doi.org/10.1007/s10311-020-01122-6>
67. Ruggeri, B., Tommasi, T., & Sanfilippo, S. (2015). *BioH₂ & BioCH₄ Through Anaerobic Digestion: From Research to Full-scale Applications*. Springer London. <https://doi.org/10.1007/978-1-4471-6431-9>
68. Salehian, P., Karimi, K., Zilouei, H., & Jeihanipour, A. (2013). Improvement of biogas production from pine wood by alkali pretreatment. *Fuel*, 106, 484–489. <https://doi.org/10.1016/j.fuel.2012.12.092>
69. Sawyerr, N., Trois, C., Workneh, T., & Okudoh, V. (2019). An Overview of Biogas Production: Fundamentals, Applications and Future Research. *International Journal of Energy Economics and Policy*, 9(2), 105–116.
70. Schlegel, A. J. (1992). Effect of Composted Manure on Soil Chemical Properties and Nitrogen Use by Grain Sorghum. *Journal of Production Agriculture*, 5(1), 153–157. <https://doi.org/10.2134/jpa1992.0153>
71. Shapovalov, Y., Zhadan, S., Bochmann, G., Salyuk, A., & Nykyforov, V. (2020). Dry Anaerobic Digestion of Chicken Manure: A Review. *Applied Sciences*, 10(21), 7825. <https://doi.org/10.3390/app10217825>
72. Siddique, Md. N. I., & Wahid, Z. Ab. (2018). Achievements and perspectives of anaerobic co-digestion: A review. *Journal of Cleaner Production*, 194, 359–371. <https://doi.org/10.1016/j.jclepro.2018.05.155>
73. Simeonov, I., Petkov, Y., Baykov, B., Mihajlova, S., & Mirkov, A. (2006). Laboratory studies upon the possibility of biogas production from poultry litter. (3–4), 88–97.
74. Song, Y., Qiao, W., Westerholm, M., Zhou, Y., & Dong, R. (2024). High rate methanogenesis and nitrogenous component transformation in the high-solids anaerobic digestion of chicken manure enhanced by biogas recirculation ammonia stripping. *Chemical Engineering Journal*, 498, 155744. <https://doi.org/10.1016/j.cej.2024.155744>
75. Song, Y., Qiao, W., Zhang, J., & Dong, R. (2023). Process Performance and Functional Microbial Community in the Anaerobic Digestion of Chicken Manure: A Review. *Energies*, 16(12), 4675. <https://doi.org/10.3390/en16124675>
76. Sun, C., Cao, W., Banks, C. J., Heaven, S., & Liu, R. (2016). Biogas production from undiluted chicken manure and maize silage: A study of ammonia inhibition in high solids anaerobic digestion. *Bioresource Technology*, 218, 1215–1223. <https://doi.org/10.1016/j.biortech.2016.07.082>
77. Thakur, R., Chandras, Tarafdar, A., Yadav, S., Godara, R., Singh, M., Deo, C., & Narayan, R. (2025). From litter to energy: Effect of alkali pretreatment on biogas production from poultry litter and slurry characteristics. *Energy for Sustainable Development*, 88, 101799. <https://doi.org/10.1016/j.esd.2025.101799>
78. Toor, G. S., & Haggard, B. E. (2009). Phosphorus and trace metal dynamics in soils amended with poultry litter and granulates. *Soil Use and Management*, 25(4), 409–418. <https://doi.org/10.1111/j.1475-2743.2009.00235.x>
79. Tsigkou, K., Zagklis, D., Tsafrakidou, P., Zapanti, P., Manthos, G., Karamitou, K., Zafiri, C., & Kornaros, M. (2021). Expired food products and used disposable adult nappies mesophilic anaerobic co-digestion: Biochemical methane potential, feedstock pretreatment and two-stage system performance. *Renewable Energy*, 168, 309–318. <https://doi.org/10.1016/j.renene.2020.12.062>
80. Turnell, J. R., Faulkner, R. D., & Hinch, G. N. (2007). Recent advances in Australian broiler litter utilisation. *World's Poultry Science Journal*, 63(2), 223–231. <https://doi.org/10.1017/S0043933907001420>
81. Tzenos, C. A., Lithourgidis, A. A., Pitsikoglou, D. S., Tsitsimpikou, M.-A., Kalamaras, S. D., Firfiris, V. K., Fotidis, I. A., & Kotsopoulos, T. A. (2025). Combined Mild Thermal Pretreatment and Bioaugmentation of Ammonia-Acclimatised Inoculum to Enhance Biomethanation of Poultry Manure. *Energies*, 18(24), 6622. <https://doi.org/10.3390/en18246622>
82. Verma, R., Kumar, V., Singh, J., & Sharma, N. (2020). Poultry Manure and Poultry Waste Management: A Review. *International Journal of Current Microbiology and Applied Sciences*, 9(6), 3483–3495. <https://doi.org/10.20546/ijcmas.2020.906.410>
83. Wang, F., Pei, M., Qiu, L., Yao, Y., Zhang, C., & Qiang, H. (2019). Performance of Anaerobic Digestion of Chicken Manure Under Gradually Elevated Organic Loading Rates. *International Journal of Environmental Research and Public Health*, 16(12), 2239. <https://doi.org/10.3390/ijerph16122239>
84. Warman, P. R. (1986). The effect of fertilizer, chicken manure and dairy manure on timothy yield, tissue composition and soil fertility. *Agricultural Wastes*, 18(4), 289–298. [https://doi.org/10.1016/0141-4607\(86\)90074-0](https://doi.org/10.1016/0141-4607(86)90074-0)

85. Wei, Y., Wachemo, A. C., Yuan, H., & Li, X. (2020). Enhanced hydrolysis and acidification strategy for efficient co-digestion of pretreated corn stover with chicken manure: Digestion performance and microbial community structure. *Science of The Total Environment*, 720, 137401. <https://doi.org/10.1016/j.scitotenv.2020.137401>
86. Zhan, Y., Zhu, J., Xiao, Y., Schrader, L. C., Xiao Wu, S., Aka Robinson Jr, N., & Wang, Z. (2023a). Employing micro-aeration in anaerobic digestion of poultry litter and wheat straw: Batch kinetics and continuous performance. *Bioresource Technology*, 368, 128351. <https://doi.org/10.1016/j.biortech.2022.128351>
87. Zhan, Y., Zhu, J., Xiao, Y., Wu, S., & Ndeddy Aka, R. J. (2023b). Efficient methane production from anaerobic co-digestion of poultry litter with wheat straw in sequencing batch reactor: Effects of carbon-to-nitrogen ratio, total solids, and hydraulic retention time. *Bioresource Technology*, 381, 129127. <https://doi.org/10.1016/j.biortech.2023.129127>
88. Zhan, Y., Zuo, B., Cao, X., Xiao, Y., & Zhu, J. (2024). Biochar enhanced anaerobic co-digestion of poultry litter and wheat straw: Performance, microbial analysis, and multiple factors' interaction. *Renewable Energy*, 231, 120907. <https://doi.org/10.1016/j.renene.2024.120907>
89. Zhang, L., Loh, K.-C., & Zhang, J. (2019). Jointly reducing antibiotic resistance genes and improving methane yield in anaerobic digestion of chicken manure by feedstock microwave pretreatment and activated carbon supplementation. *Chemical Engineering Journal*, 372, 815–824. <https://doi.org/10.1016/j.cej.2019.04.207>

Assoc. Prof. Ivan Simeonov, PhD

Institute of Microbiology, BAS,
Acad. G. Bonchev St., Bl. 26
Sofia 1113, Bulgaria
E-mail: issim@microbio.bas.bg;
issim@abv.bg

Assoc. Prof. Venelin Hubenov, PhD

Institute of Microbiology, BAS,
Acad. G. Bonchev St., Bl. 26
Sofia 1113, Bulgaria
Competence Center “Clean & Circle”,
1 James Boucher Blvd.
Sofia 1164, Bulgaria
E-mail: vhubenov@microbio.bas.bg