

# Biochar-synergy in anaerobic digestion of animal wastes for total pollution control and bioenergy production: A sustainable integrated perspective

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## ABSTRACT

Organic waste disposal and treatment are key public and environmental health issues contributing to pollution reduction and minimizing the spread of diseases from agricultural setups. Current treatment methods of animal waste often generate odors and greenhouse gases, which become catastrophic downstream, including algae blooms and groundwater contamination. Anaerobic digestion (AD) using bioreactors has been an economic resource utilization strategy for organic waste treatment with ecological integrity for environmental justice. To enhance the effectiveness of AD, the addition of biochar has been shown to improve treatment efficiency by amplifying bacterial activity and aiding in the breakdown of complex organic materials for biofuel production. We reviewed the integration of biochar in the AD of animal waste material as a cost-effective bio-carrier to enhance treatment for environmental protection and bioenergy production. We discussed the current relationship between pyrolysis conditions and feedstock types used in the AD process and evaluated the ecological functions of microbial activities and their interaction with biochar-based biomass in AD engineering designs. A comprehension of the technological advances to improve the AD performances associated with microbial biomass and biochar addition and potential areas for future research and their limitations toward a zero-waste paradigm for sustainable development in farm management systems was reviewed.

## 1. Introduction

The demand for sustainable energy and wastewater treatment solutions is increasing, and this has led to growing interest in the anaerobic digestion (AD) of animal wastes for bioenergy production at local scales. However, challenges such as slow digestion rates and low methane yields limit its efficiency. Biochar (BC), a carbon-rich material derived from the pyrolysis of biomass, has emerged as a promising additive to enhance the AD process. This synergy improves microbial metabolic activity, enhances biogas production, and stabilizes the digestion process.

Anaerobic digestion has emerged as a promising biotechnology for sustainable waste management and renewable energy production, offering the dual benefits of waste treatment and bioenergy generation (Chen et al., 2008). This biological process involves the degradation of

organic matter by microorganisms in oxygen-free conditions, resulting in the production of biogas – a mixture primarily composed of methane (50–75 %) and carbon dioxide (25–45 %) (Wang et al., 2012). The increasing global energy demand, coupled with environmental concerns about fossil fuel consumption, has intensified interest in optimizing AD processes for enhanced bioenergy production, particularly from animal waste. The AD process occurs through four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, complex organic compounds are broken down into simpler molecules by hydrolytic bacteria. These simple molecules are then converted into volatile fatty acids (VFAs) during acidogenesis. In the acetogenesis phase, VFAs are transformed into acetic acid, hydrogen, and carbon dioxide. Finally, methanogenic archaea convert these products into methane and carbon dioxide in the methanogenesis stage (Li et al., 2022).). In principle, Eqs. (1) and (2) explain the AD process for general decomposition and Acetogenesis of VFAs into acetic acid ( $\text{CH}_3\text{COOH}$ ),

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### Nomenclature

AD	Anaerobic digestion
BC	Biochar
QS	Quorum sensing
AHLs	Acyl-homoserine lactones
CHP	Combined heat and power
DIET	Direct interspecies electron transfer
VFAs	Volatile fatty acids

hydrogen (H<sub>2</sub>), and CO<sub>2</sub>.



**Organic Matter+Water→Biogas+Digestate (Residual Solids/Liquids)**



Several key factors influence the efficiency and stability of the AD process. Temperature plays a crucial role, with most industrial-scale digesters operating in either mesophilic (35–40 °C) or thermophilic (50–55 °C) conditions (Ibrahimi et al., 2021). pH maintenance is equally critical as methanogens are particularly sensitive to pH fluctuations, with optimal performance typically observed between pH 6.8 and 7.2. The carbon-to-nitrogen (C/N) ratio significantly affects microbial growth and metabolism, with optimal ratios ranging from 20:1 to 30:1 (Phan et al., 2023). Animal waste, primarily consisting of manure from livestock operations, represents a significant environmental challenge worldwide. Anaerobic digestion offers a viable solution by converting organic matter into biogas (primarily methane) while producing nutrient-rich digestate suitable for fertilization.

Biochar, a carbon-rich material produced through the pyrolysis of biomass under oxygen-limited conditions, possesses unique physico-chemical properties that can address many of these limitations. Its porous structure, high specific surface area, and functional groups make it an effective adsorbent for potentially inhibitory compounds in the AD process. Additionally, biochar can serve as a supporting matrix for microbial colonization, enhancing the retention of key microorganisms involved in methanogenesis (Wang et al., 2023). Microbial communities that are widely distributed and enriched within the pores of biochar may enhance methane production during anaerobic digestion. (Sirohi et al., 2024). The synergistic integration of biochar into AD systems creates a cascade of beneficial effects. First, biochar's capacity to absorb ammonia and hydrogen sulfide reduces inhibitory effects on methanogenic archaea, improving process stability (Shen et al., 2015). Second, its conductive properties facilitate direct interspecies electron transfer between syntrophic bacteria and methanogens, potentially accelerating the rate-limiting steps in AD (Zhao et al., 2016). Third, BC-enriched digestate demonstrates enhanced agronomic value, with improved nutrient retention capacity and reduced greenhouse gas emissions when applied to soil (B. Chen et al., 2021).

From a life cycle perspective, this integrated approach creates a circular economy model where animal wastes are transformed into multiple valuable products: biogas for energy generation, BC for process enhancement, and nutrient-rich digestate for soil amendment. Such integration not only maximizes resource utilization but also minimizes environmental impacts across multiple dimensions (He et al., 2023). Recent technological advances have further expanded the potential applications of this integrated system. Co-pyrolysis of digestate can produce BC with tailored properties specifically suited for AD enhancement (Wambugu et al., 2019). Meanwhile, innovative reactor designs incorporating fixed-bed BC systems have demonstrated significant improvements in methane production rates and biogas quality (J. Pan et al., 2019). BC addition improved methane production as

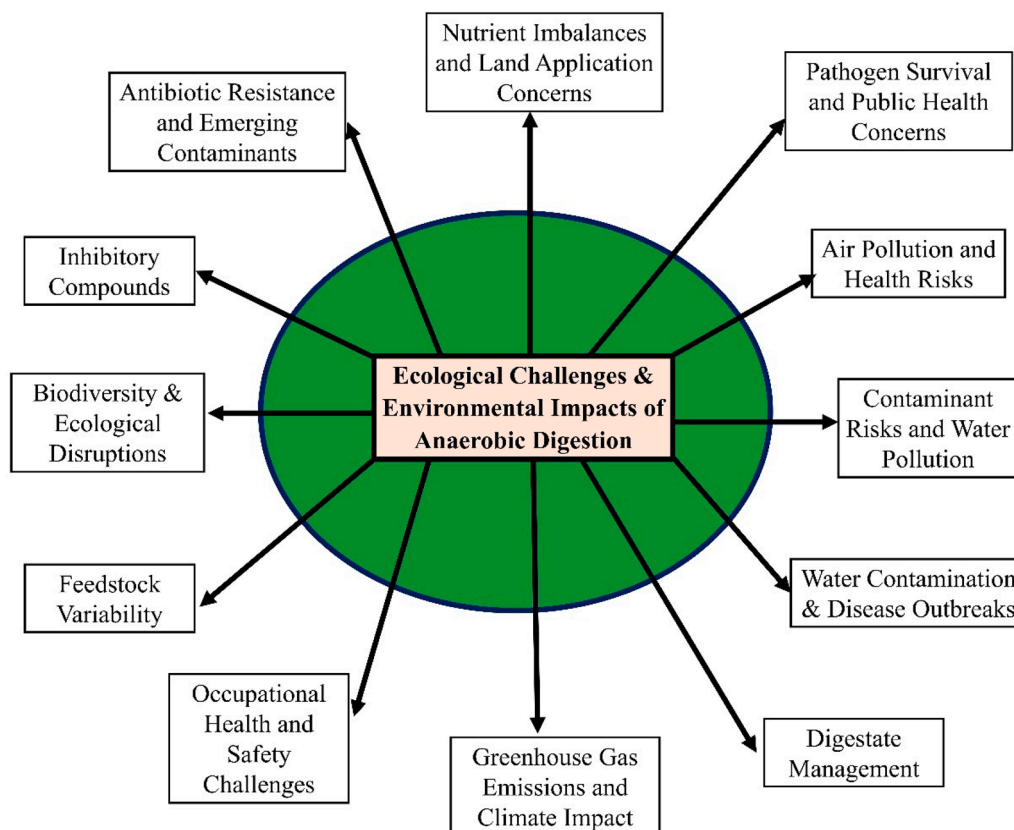
measured by biochemical methane potential testing. Of the three BC types tested, potato straw BC demonstrated the greatest enhancement of methane generation compared to the control group without BC. The study found strong correlations between anaerobic digestion microbiota structure and biochar properties (pH, specific surface area, cation exchange capacity, and carbon-to-nitrogen ratio). Combining BC with anaerobic digestion technology offers an improved method for converting agricultural waste into clean energy (Liu et al., 2023c).

Despite the widespread application of AD technology, several challenges persist, including process instability, incomplete degradation of organic matter, and suboptimal methane yields (Fig. 1). These limitations have prompted researchers to explore various enhancement strategies, among which biochar integration has shown promising results (Obileke et al., 2023). The synergistic effects of biochar in AD systems present an innovative approach to addressing these challenges while potentially improving the overall system efficiency and stability. This review explores the potential of integrating BC with AD as a sustainable approach, role highlights, function, and contribution to optimizing bioenergy recovery while promoting ecological benefits, including valorization and carbon sequestration. Additionally, we intend to highlight and understand the inter-relationship between pyrolysis conditions and feedstock types used in AD process, evaluate the mechanisms of microbial activities and their interaction with biochar based on the engineering designs in AD, comprehend the technological advances to improve the AD performances associated with microbial biomass and BC addition, and review potential areas for future research and their limitations towards a zero-waste paradigm for sustainable development. Therefore, we highlighted and considered these interdisciplinary perspectives where we integrate microbial ecology, their processes, engineering capabilities, and the circular economy. Moreover, the review addressed critical analysis on how specific feedstock properties influence AD operations and display inconsistencies in literature. We also identify underexplored challenges and limitations including strategies to mitigate such challenges for optimum operations. By enhancing process efficiency, improving digestate quality, and creating a circular economy model, this integrated system addresses multiple environmental challenges while generating renewable energy. As this research serves as a roadmap for future research and practical biochar application in AD, further research and development in this field could lead to significant advances in sustainable waste management and bioenergy production technologies, bridging the gap between theory and mechanisms for real-world implementation.

#### 1.1. Operational challenges affecting environmental performance in anaerobic digestion

The application of AD is a widely adopted biological process for organic waste treatment and renewable energy production. Despite its benefits, various operational challenges can significantly affect its environmental performance. These challenges include feedstock variability, process instability, methane leakage, digestate management, and energy efficiency concerns. Addressing these issues is essential to maximizing AD's environmental benefits while minimizing its ecological footprint.

Firstly, the composition and quality of feedstock used in AD systems play a critical role in maintaining stable operation. Variations in organic content, moisture levels, and contamination with non-biodegradable materials can lead to process inefficiencies (Ye et al., 2018). High levels of lignocellulosic materials, fats, or industrial waste may cause imbalances in microbial activity, reducing methane yields and increasing the risk of process failure (Kamperidou and Terzopoulou, 2021). Pre-treatment methods, such as hydrolysis and co-digestion, can help mitigate these effects by improving feedstock consistency and biodegradability. Moreover, AD requires optimal environmental conditions, including temperature, pH, and organic loading rates (OLR), to maintain microbial activity. Temperature fluctuations and sudden



**Fig. 1.** Presents several ecological challenges and environmental impacts that must be carefully managed to ensure sustainability and implementation of anaerobic digestion systems.

changes in feedstock composition can disrupt microbial communities, leading to process inhibition and decreased biogas production (Angelidaki et al., 2011). Additionally, the accumulation of toxic compounds such as ammonia, hydrogen sulfide, and volatile fatty acids (VFAs) can further inhibit methanogenesis and reduce system efficiency (Harirchi et al., 2022). Regular monitoring and adaptive control strategies, such as trace element supplementation and gradual OLR adjustments, are crucial to maintaining stable AD operations.

Methane is a potent greenhouse gas, and unintentional emissions from AD facilities can significantly impact the overall environmental performance of the system. Methane leaks can occur during biogas storage, upgrading, and utilization stages, particularly in poorly maintained infrastructure (Pöschl et al., 2010). Implementing robust leak detection systems, using advanced gas capture technologies, and ensuring proper maintenance of digesters and biogas pipelines can help mitigate methane emissions and enhance sustainability (Traven, 2023). On the other hand, the digestate produced as a byproduct of AD contains valuable nutrients, including nitrogen and phosphorus, making it a useful biofertilizer. However, improper storage, handling, and land application can lead to nutrient leaching, water contamination, and eutrophication of aquatic ecosystems. Effective nutrient management strategies, such as controlled application rates, composting, and advanced treatment methods, can reduce environmental risks and enhance digestate utilization efficiency (Lamolinara et al., 2022). Lastly, AD systems require significant energy inputs for feedstock preparation, mixing, heating, and biogas upgrading. Inefficient energy use can reduce net energy gains and environmental performance (Browne et al., 2013). Implementing energy recovery techniques, optimizing heat integration, and utilizing combined heat and power (CHP) systems can improve the overall energy efficiency of AD plants (Sarpong et al., 2020).

Therefore, operational challenges in anaerobic digestion can significantly impact its environmental performance. Feedstock variability,

process instability, methane leakage, digestate management, and energy efficiency must be addressed to enhance AD sustainability. Future advancements in monitoring technologies, process optimization, and integrated waste management strategies will be crucial in ensuring the long-term viability of anaerobic digestion as a sustainable waste treatment solution. These challenges underscore the need for improved technologies and management strategies in AD systems treating animal waste. Future developments should focus on enhancing process stability, reducing environmental impacts, and ensuring public health protection while maintaining economic viability. This requires a holistic approach that considers both technical solutions and regulatory frameworks to guide the sustainable implementation of AD technology. Despite its potential, the anaerobic digestion of animal waste faces significant challenges in practical applications, ecological sustainability, and public health safety. Addressing these issues through technological advancements and regulatory frameworks is crucial for maximizing the benefits of AD while minimizing its negative impacts.

## 2. Pyrolysis of biochar and its properties for the AD process

The production of BC through pyrolysis generates high-energy-density by-products, including BC under different degradation conditions. Heating temperature, retention time, biomass granulometry, and feedstock types influence the intrinsic physicochemical attributes of BC, including polarity, porosity, functionality of the surface, aromaticity, carbon structure, and ash content. The yield of BC is based on temperature and heating time. Any heating below 400°C results in low carbon content and a higher yield of BC than moderate temperatures between 400°C and 600°C, whereas above 600°C, the yield keeps decreasing (Gotore et al., 2024a). These physical and chemical variations and changes have different effects on the performance of AD. BC exhibits an alkaline nature after pyrolysis, which offers multiple benefits to AD,



including the alkalinity and buffering ability, which is helpful to mitigate inhibition of VFAs degradation caused by the pH drop (Meng et al., 2020). Carboxylic ( $-\text{COOH}$ ) and phenolic ( $\text{X-OH}$ ) surface functional groups of biochar interact simultaneously under alkaline conditions, giving the buffer environment in AD (D. Wang et al., 2017).

For instance, Vayena et al. (2024) reiterated that wheat straw-based BC heated at 400°C produced adequate methane compared to BC from higher pyrolysis. The reason is that low heating BC contains reasonable biodegradable organic materials for microbials to fully stabilize the carbon in AD. Bamboo and Olive tree BC pyrolyzed at 650°C to enhance AD performance in antibiotic removal from cow dairy wastes. The findings suggested that after 30 days, bamboo produced over 1000 mL/g VS, while olive tree BC produced over 800 mL/g VS (You et al., 2024). After pyrolysis, the material composition of the two BC exhibited similar surface functional groups, with substantial porosity (W. Zhao et al., 2021), however, surface characteristics were different based on lignin content giving a disparity in biogas yield (You et al., 2024), of which the particle sizes of BC contribute significantly during AD as shown in Fig. 2. Incomplete charring of biomass materials is associated with lower heating temperatures. Higher heating temperatures of over 600°C usually result in more organized carbon surface, skeleton structures, and graphene-like morphologies compared to almost original feedstock with purer surface characteristics in lower production temperatures (Gotore et al., 2024a; Tomczyk et al., 2020).

AD can rely on microbial extracellular electron transfer and the decomposition and degradation of animal wastes based on microbial

metabolic activities (Xiao et al., 2021). The carbonized BC has an outstanding ability to transfer electron from the surface physiochemical functional groups accompanied by the conjugated  $\pi$ -electron on its aromatic interface (Wang et al., 2018). The carbonization process influences the electronic transfer capacity in BC, as this ability is significantly related to increasing temperatures. In summary, at higher pyrolysis temperatures, amorphous sheets of carbon and conductive graphite-like networks forms, carbon content increases, conjugated  $\pi$ -electron forms, and aromaticity increases due to formation, destruction, and recombination of oxygen-containing functional groups significantly increases the electron conductivity and transfer in BC (Thakur et al., 2024; Dang et al., 2022; Chacón et al., 2020; Zhang et al., 2019; Gabhi et al., 2017; Klüpfel et al., 2014). Higher carbonization temperatures of feedstocks from agricultural wastes biomass have high electron transferring ability from the combination of functional groups and graphitized structures (Nzediegwu et al., 2021; Zhang et al., 2019), that enhances the function of an AD as shown in Fig. 2. Agriculturally based BC produced at 600°C demonstrated a cumulative methane yield (319.44 vs. 282.77 mL/g VS) in AD from the degradation of cow manure (Shen et al., 2020). Moreover, AD of chicken manure by 550°C corn straw nano- $\text{Fe}_3\text{O}_4$  BC, which yielded over 62 % methane production aided by the immobilized and enriched microorganisms, occupied the enhanced porous structure and surface properties of the BC (Di et al., 2022).

The effects of biomass type and its contribution to AD are still limited, and it is recommended to conduct comprehensive research using various origins of BC feedstocks and pyrolysis conditions to

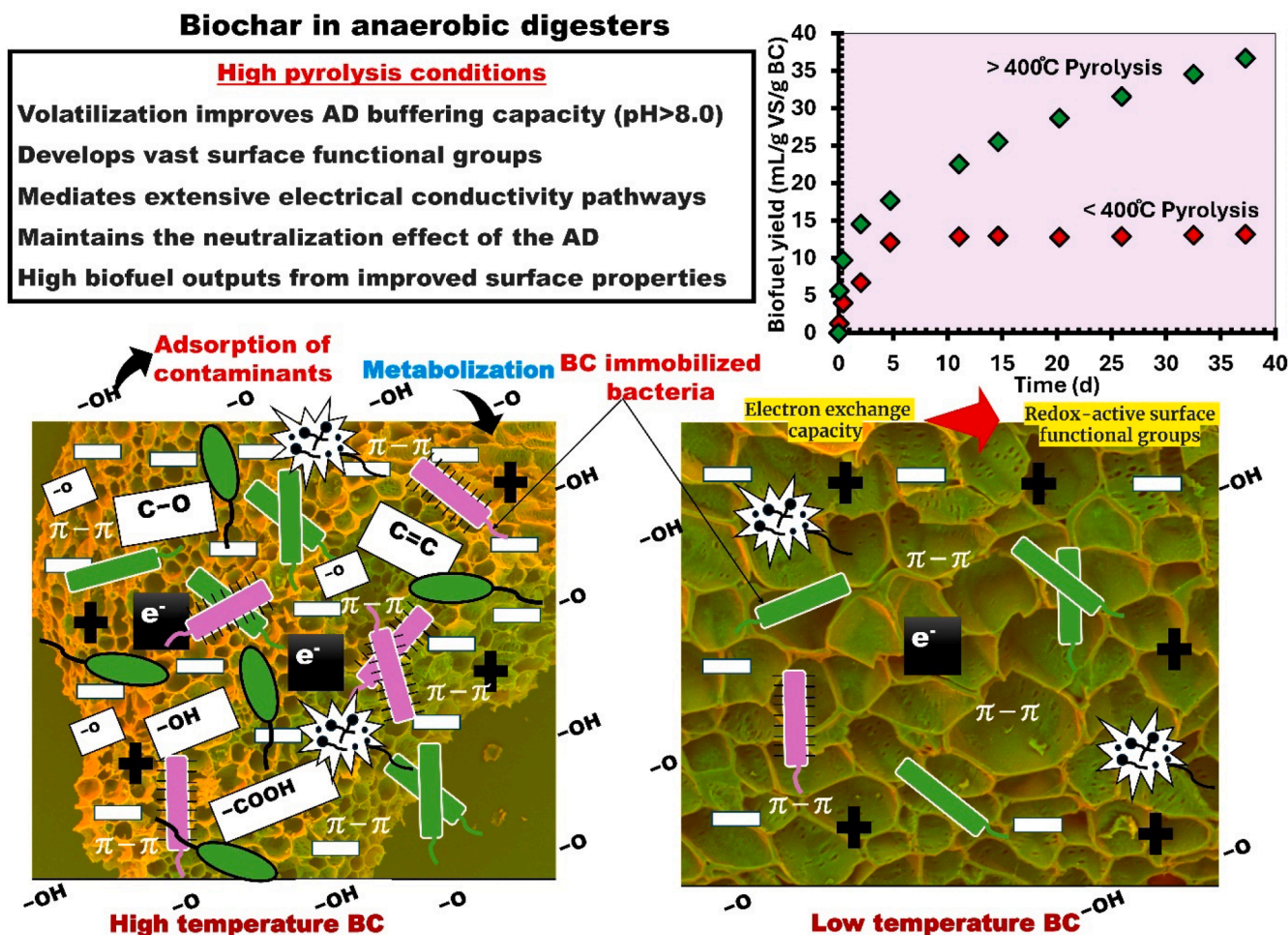


Fig. 2. The surface complexity and morphology of biochar produced from different carbonization conditions and its contribution in anaerobic digestion process of animal wastes. Presence of oxygen-containing functional groups significantly upsurges the electron conductivity and transfer abilities, and bacterial growth in agriculturally based biomass converted materials under different environments.



address this effect. Considering the functional groups in BC, they influence the conductivity and activity of the biomass, and this becomes a fundamental role in microbial adhesion and electrical transfer which directly impact the efficiency of AD. Oxygen containing functional groups such as -COOH, -OH, C = O enhances hydrophilicity and establishes negatively charged surface which improves bacterial attachment. Nitrogen and sulfur groups such as -NH<sub>2</sub> and -SH tend to promote electrostatic interactions with microbe's membranes and aid in the binding effect of enzyme. This enhances higher retention of biomass and biofilm stability which fuels up the degradation of animal wastes and minimizes the washing out of microbes during reactor flows/mixing. When BC is added, microbial community tends to rely on the functional groups for direct interspecies electron transfer among other redox reactions as discussed above. For practical and pilot scale, BC modification, reactor design and optimization of waste type and source need to be considered for effective treatment of AD.

## 2.1. Effects of different BC feedstocks and types of animal waste on AD

Anaerobic digestion remains a cornerstone technology in sustainable waste management and renewable energy production, transforming diverse organic substrates—such as animal manure, agricultural residues, and food waste—into valuable biogas and nutrient-rich digestate. However, challenges like ammonia inhibition, process instability, and low methane yields hinder their efficiency, particularly when treating nitrogen-rich animal wastes such as poultry litter or swine manure (Karim et al., 2005; Ahlberg-Eliasson et al., 2021; Ngo et al., 2023). BC, a carbon-rich material produced via pyrolysis, has emerged as a promising additive to enhance AD performance by mitigating inhibitors, improving microbial activity, and facilitating electron transfer (Zhang et al., 2019; Achi et al., 2024). Recent advancements in machine learning further enable predictive optimization of biochar properties and AD outcomes, enhancing bioenergy recovery (Wang et al., 2020a).

The efficacy of BC in AD systems depends on feedstock type and pyrolysis conditions (Table 1). Wood-derived BC (pyrolyzed at 500 °C)

**Table 1**

Key bacterial community structure observed from the anaerobic digestion process based on different biomass and pyrolysis conditions contributing to biogas production.

Biochar Feedstock	Animal Waste treated	Pyrolysis Temperature	Treatment Capacity	Key Microbial Communities	Observed Effects	Reference
Corn straw, coconut shell, sewage sludge	Sewage sludge	400–600 °C	Batch reactors (1–5 L)	<i>Methanosarcina</i>	↑ Methane yield (15–25 %); reduced VFA accumulation	(Zhang et al., 2019)
Wood biochar and acid-alkali-treated wood biochar	Chicken manure	550 °C	Batch reactor (2–4 gVS/L/d)	<i>NH<sub>3</sub>-sensitive Methanosaetaceae and Methanosarcinaceae families</i>	↑ Methane by 30 %; alleviated NH <sub>3</sub> inhibition	(Ngo et al., 2023)
Rice straw	Poultry manure	600 °C	Lab-scale reactors	<i>Firmicutes, Bacteroidetes, Tenericutes, Synergistetes, Chloroflexi, and Proteobacteria</i>	Enhanced syntrophy; ↑ CH <sub>4</sub> by 20 %	(Wang et al., 2022b)
Sawdust	Food waste	700 °C	automatic biomethane potential testing system	<i>Bathyarchaeia, Methanobacterium, Methanolinea, Methanoculleus, and Methanospirillum</i>	increase the cumulative methane production by 15.70–128.38 %	(Pei et al., 2024)
Multiple (wood, straw)	Cattle manure	400–600 °C	Semi-continuous reactors	<i>Methanosarcinaceae, Synergistaceae Methanoculleus receptaculi, Syntrophobacter fumaroxidans</i>	Feedstock-dependent δ <sup>13</sup> C—CH <sub>4</sub> ; enhanced DIET	(Lv et al., 2019)
Wood, crop residues	Inoculum (cellulose–peptone–swine inoculum (CPI) and swine manure inoculum (SMI))	400–600 °C	Batch assays	<i>Bacteroidetes and Clostridiales, enriched the relative abundance of hydrogenotrophic methanogens Methanobrevibacter and Methanobacterium.</i>	Improved startup time	(Ding et al., 2024)
Corn stover	Cow manure + cassava wastewater	500 °C	Semi-continuous reactors (2 L)	<i>Methanogens</i>	↑ Biogas by 18 %; balanced C/N	(Achi et al., 2024)
Corn straw (CS), <i>Dicranopteris dichotoma</i> (DD), bamboo (B), KW, tea residue (TR), mushroom cultivation waste (MW), cassava lees (CL), <i>Chlorella</i> (C), and sargassum (S)	kitchen waste	300–800 °C	Batch reactors	<i>Hydrogenotrophic methanogens (e.g., Methanobacteriaceae, Methanomicrobiaceae, and/or Methanomassiliococcaceae)</i>	High-temp BC (>600 °C) promotes DIET	(Wu et al., 2024)
Wood-derived biochar	Cow manure + food waste	500 °C	Batch reactor	<i>Methanosaeta</i>	70:30 mix; ↑ CH <sub>4</sub> by 35 %	(Oghoghorie et al., 2024)
Wood	Pig/cow manure	800–900 °C	CSTR (OLR 1–4 kgVS/m <sup>3</sup> /d)	<i>Methanosaeta Methanosarcina</i>	High OLR leads to decreased CH <sub>4</sub> yield	(Shao et al., 2019)
Woody-derived biochar	Cow manure + food waste	450–500 °C	Lab-scale reactors	<i>Methanosaeta</i>	↑ CH <sub>4</sub> by 10 %	(Quintana-Najera et al., 2023)
Wood waste biochar	Food waste	750 °C	Batch experiment	<i>Methanosaeta, Romboutsia, and norank_f_Anaerolineaceae</i>	↑ Methane by 21.5 %	(Zhang et al., 2022)
FeCl <sub>3</sub> -modified food-waste-derived biochar	Food waste	550 °C	Pilot-scale reactors	<i>Syntrophomonas, Methanofollis, Methanoculleus and Methanosarcina</i>	↑ Biogas yield (22.50 % and 12.79 %); improved process stability	(Li et al., 2022)

significantly reduced ammonia toxicity in chicken manure digesters by adsorbing free  $\text{NH}_3$ , enriching ammonia-tolerant methanogens like *Methanosarcina* (Ngo et al., 2023). Conversely, rice straw BC (450 °C) enhanced syntrophic interactions between *Bacteroidetes* and *Methanoculleus* in mixed manure systems, increasing methane production by 20 % (Lv et al., 2019; Wang et al., 2022b). High-temperature BC (>600 °C), such as wheat straw BC, demonstrated superior conductivity, promoting direct interspecies electron transfer (DIET) in co-digestion systems with cow manure and cassava wastewater (Achi et al., 2024). Similarly, Wu et al. (2024) observed that biochar derived from kitchen waste at varying pyrolysis temperatures differentially influenced hydrolysis and acidogenesis stages, highlighting the need to tailor BC properties (e.g., porosity, surface functionality) to specific substrates. Machine learning models have further advanced the prediction of optimal BC characteristics, enabling precise customization for AD enhancement (Ghatak and Ghatak, 2018; Cheon et al., 2022).

Animal waste-specific responses to BC augmentation vary considerably. For chicken manure, high ammonia concentrations (3–5 g/L) often inhibit methanogenesis. Wood BC (500 °C) reduced free  $\text{NH}_3$  by 40 %, favoring *Methanosarcina* dominance and increasing methane yield by 30 %, while enriching *Syntrophomonas* to oxidize volatile fatty acids (VFAs) under ammonia stress (Ngo et al., 2023; Zhang et al., 2024; Shinde et al., 2025). In cow manure systems, co-digestion with food waste (70:30 ratio) and wheat straw BC addition improved the carbon-to-nitrogen (C/N) ratio, boosting methane production by 35–40 % (Oghoghorie et al., 2024). BC also supported hydrolytic bacteria (*Bacteroidetes*) and acetoclastic methanogens (*Methanosaeta*), enhancing substrate degradation (Shen et al., 2020; Ding et al., 2024). For swine manure, high organic loading rates (>4 kgVS/m<sup>3</sup>/d) reduced methane yields by 20 %, but iron-modified BC stabilized pH, reduced VFA accumulation, and enhanced DIET, increasing methane production by 22.5 % (Ahlberg-Eliasson et al., 2021; Li et al., 2022). Pei et al. (2024) similarly reported that biochar addition modulated microbial pathways in food waste digestion, underscoring its versatility across substrates. In addition, BC addition in AD enhances both acetogenesis and methanogenesis by improving the activity of microbial ecology, electron transfer, and process stability of these reduces contaminant accumulation such as VFAs and lowers the partial pressure of diatomic hydrogen, making acetogenesis thermodynamically favorable. Biochar tends to boost the conversion of acetate or methylated compounds into  $\text{CH}_4$  hence methanogenesis.

BC significantly reshapes microbial consortia, favoring syntrophic bacteria (e.g., *Syntrophomonas*, *Clostridium*) and hydrogenotrophic methanogens (*Methanoculleus*). Wood BC in chicken manure systems enriched *Syntrophomonas* (VFA oxidizers) and *Methanosarcina*, enabling resilience under ammonia stress. Rice straw biochar increased *Bacteroidetes* abundance, enhancing hydrolysis of complex organics in cow manure (Wang et al., 2022b). Additionally, BC facilitates DIET by enriching electroactive bacteria like *Geobacter* and *Methanobacterium*, which drive methane production (Shao et al., 2019; Zhang et al., 2022). Ding et al. (2024) further demonstrated that BC modulates microbial communities differently depending on inoculum sources, emphasizing the need for tailored applications. Russell et al. (2020) noted feedstock contamination risks in AD systems, though BC's role in mitigating such issues remains underexplored.

Anaerobic digestion can be applied to a wide range of animal waste types, each presenting unique challenges and opportunities. Animal waste is rich in organic materials, including proteins, fats, carbohydrates, and lignocellulose, all of which are biodegradable under anaerobic conditions. The specific type of animal waste influences the microbial community composition and the biogas yield. Cow manure is composed of a high proportion of fibrous materials and has a high lignocellulosic content that requires efficient microbial breakdown for optimal methane production (Pan et al., 2024). Similar to cow manure, sheep and goat manure has a relatively high lignocellulosic content but is often easier to digest due to its lower fibrous nature (Holaj-Krzak et al.,

2024). Rich in nitrogenous compounds, pig manure can lead to high ammonia concentrations, which inhibit microbial activity in AD (Yenigun and Demirel, 2013).

Biochar can absorb ammonia, helping to reduce toxicity and enhance microbial viability. Horse manure contains high amounts of cellulose and lignin, making it more difficult to degrade, but its high nutrient content is beneficial for biogas production. Poultry manure consists of a mixture of chicken waste and bedding materials, including sawdust, rice husks, and wheat straw (Molaey et al., 2018). It is characterized by a high content of proteins and lipids, making it a good substrate for methane production, but its high nitrogen content can also pose challenges (Hassan et al., 2023). This high percentage of N results in the build-up of  $\text{NH}_3$ , which leads to the inhibition of AD and, consequently, the inhibition of biogas production.

The microbial community involved in AD is diverse and plays a central role in the breakdown of organic substrates and the production of biogas. This process involves four major stages as shown in Fig. 2 and Fig. 3.

### 3. Enhancing microbial viability in anaerobic digestion with biochar for increased biofuel production

Anaerobic digestion is a well-established biological process for the treatment of organic waste, such as animal manure, agricultural residues, and municipal solid waste (B. Chen et al., 2021). This process not only reduces the volume of waste but also produces valuable by-products, such as biogas, which can be converted into biofuels (Pan et al., 2024). Biogas is a mixture of  $\text{CH}_4$ , carbon dioxide ( $\text{CO}_2$ ), and trace gases. The methane in biogas can be captured and used as a renewable energy source for heating, electricity generation, or as vehicle fuel. The amount of biogas produced is influenced by the type of substrate, the efficiency of microbial activity, and the operational conditions of the digester. By enhancing microbial viability with biochar, biogas yield and quality can be significantly increased (Gahane et al., 2022).

The efficiency of AD depends on the activity and viability of the microorganisms involved in the breakdown of organic matter. However, one of the key challenges in AD is the inhibition of microbial activity due to factors such as environmental stress, high concentrations of toxic compounds, and sub-optimal operational conditions. Recently, BC has emerged as a promising material to enhance microbial activity and increase biogas production in AD processes. Biochar, a carbon-rich material produced through the pyrolysis of organic matter, has been shown to improve the physical and chemical properties of the anaerobic digester environment (Fu et al., 2019). Its porous structure and large surface area provide a habitat for microorganisms, enhance microbial attachment, and improve nutrient retention. Additionally, biochar can mitigate toxic byproducts, such as ammonia, that typically inhibit microbial growth (Gahane et al., 2022). This section explores the role of biochar in enhancing microbial viability during AD, specifically concerning the digestion of various animal waste types and its impact on biofuel production.

#### 3.1. Effect of biochar treatment on total microbial biomass in anaerobic digestion for enhanced biofuel production

Biochar treatment has been proposed to enhance microbial viability, accelerate organic matter degradation, and ultimately improve biofuel production from various waste types. This section explores the effect of BC treatment on microbial biomass, biofuel type, and production volume across different organic wastes, BC dosages, and other key variables. The total microbial biomass in anaerobic digestion is a key determinant of the system's performance, particularly concerning biogas production. The primary microbial groups involved in AD include hydrolytic, acidogenic, acetogenic, and methanogenic microorganisms. These microbes work synergistically to break down complex organic matter into methane, which is the main biofuel produced during the

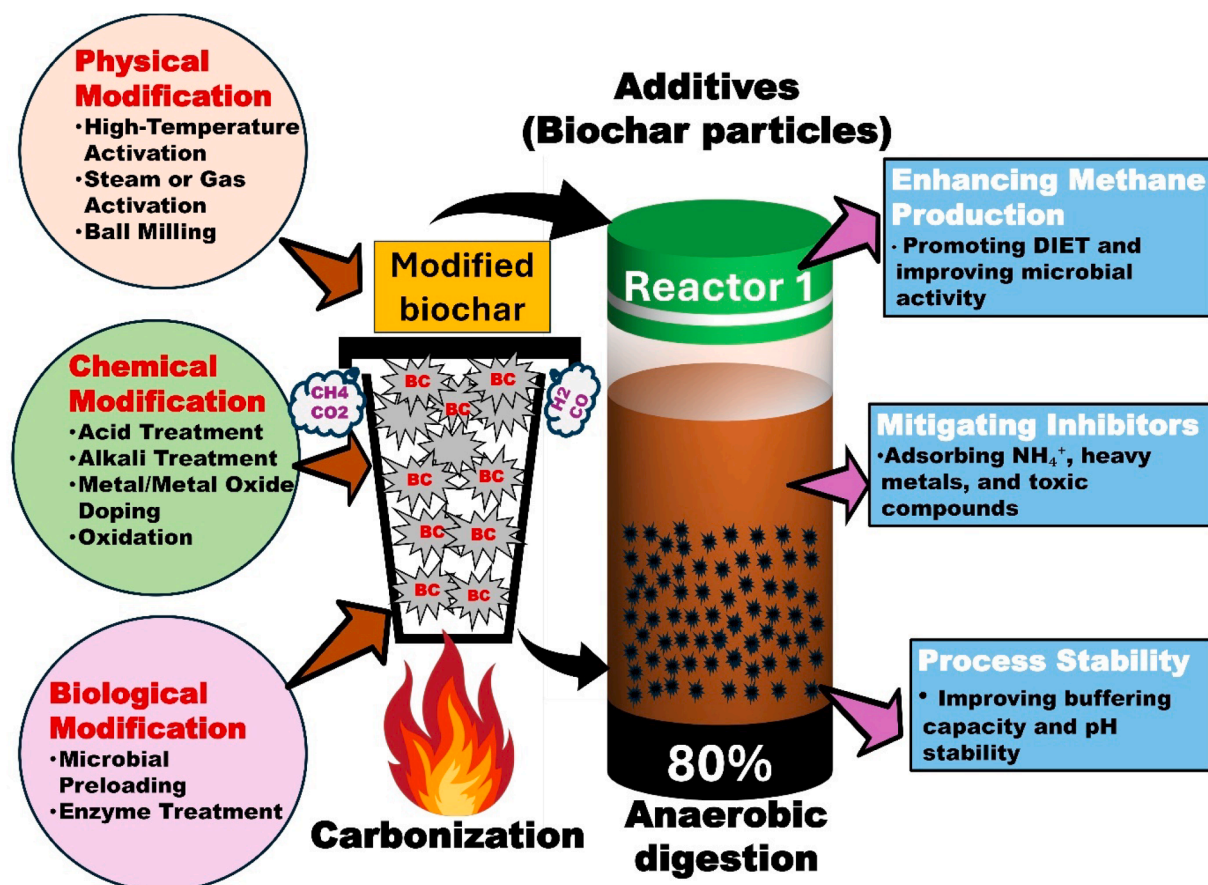


Fig. 3. Application of modified and uncustomed biochar in anaerobic digestion based on different treatment conditions and respective outcomes of animal waste degradation.

process. The quantity and activity of these microbial communities can be influenced by a variety of factors, including the composition of the feedstock, the operational parameters of the digester, and the addition of additives. A key mechanism behind biochar's efficacy is its influence on total microbial biomass, which drives the efficiency of organic waste conversion. According to Zhang et al. (2021), modified biochar from cattail was investigated with particle sizes <1 mm and revealed that it was most conducive to methane production, increasing the biogas production by 18.3–20.1 %. They further discussed that the increased buffer capacity, nutrients released by the biochar, enhanced electron transfer, and better aggregation function of small particles may contribute to the improved methane production in AD. Moreover, they also concluded that the use of BC doped with 2 M  $\text{MgCl}_2$  and calcined at 800 °C resulted in the highest specific methane productivity of  $83.2 \pm 4.5 \text{ mL/g}$  initial VS, 21.1 % higher compared with AD without biochar.

On the other hand, the dosage of BC used in AD plays a significant role in determining its impact on microbial biomass and biogas production. Generally, too little BC may not provide sufficient surface area for microbial colonization or buffering capacity, while too much biochar could potentially lead to changes in the microbial community structure or limit the availability of nutrients. Studies have shown that the optimal biochar dosage varies depending on the type of organic waste and the specific goals of the AD process (for example, maximizing biogas yield, enhancing microbial growth, or reducing toxicity). Xu et al. (2018) found that methane yield decreased with the increase of hydrochar dosage (4–8 g/L). Similarly, Dudek et al. (2019) confirmed that the high amount of BC addition (20 to 50 %) decreased methane production during AD of brewers' spent grain. The study conducted by Cheng et al. (2018) showed a substantial decrease in methane production with the increased dosage of powdered and granular BC.

Wang et al. (2022a) reiterated on the direct interspecies electron transfer (DIET) participating and dominating the digestion process; however, the adsorption and detoxification effects of biochar was suggested for investigation. Their results revealed that the total pore volume and adsorption capacity have influenced BC functions. It has been shown that microbial analysis also indicated mediated interspecies electron transfer as it remained the primary mechanism rather than DIET, including the participation of several microbes such as *Thermovirga* and *Methanosaeta*, whereas a suppressive biochar addition shifted the dominant microbes to *Asaccharospora*, *Clostridium*, and *Methanobacterium*. It is therefore noted that biochar treatment can significantly enhance total microbial biomass in AD by improving the habitat, availability of nutrients, and removal of toxicities, leading to high bio-fuel yields. Further research should be conducted focusing on customizing biochar properties for specific animal waste streams to maximize treatment and production of biofuel.

#### 4. Engineering applications of bioreactor designs on AD of animal wastes

The use of biogas in the power industry and heating as a constituent for the bio-based chemical industry has been increasing over the past years (Qian et al., 2025; Htm1 et al., 2020). Nevertheless, the uses and application of biogas depend on the amount of  $\text{CH}_4$  gas. Biogas is a mixture of gases that consists of 50–70 %  $\text{CH}_4$  and 30–50 %  $\text{CO}_2$ , with small constituencies of  $\text{H}_2\text{S}$ ,  $\text{O}_2$ ,  $\text{H}_2$ , and  $\text{N}_2$ . (Qian et al., 2025). The production and quantity of biogas in the process of AD depends on several factors, which consist of types of raw materials and engineering designs of reactors. This section takes into consideration how types of reactors and substrate affect  $\text{CH}_4$  production. Understanding these



factors is crucial for optimization of reaction parameters in increasing CH<sub>4</sub> yield in biogas (Qian et al., 2025).

#### 4.1. Bioreactor designs and treatment efficiencies

Choosing a suitable reactor type is crucial in enhancing the production of biogas and improving CH<sub>4</sub> levels in AD operation. Various reactor designs and their mode of operation depend on the metabolism of microorganisms and the constituents of the ultimate biogas (Qian et al., 2025; Lin et al., 2024). Choosing an appropriate reactor based on types of specific raw materials, the procedure needed, and cost can be effective in maximizing CH<sub>4</sub> levels of biogas. This section entails the integration of biochar with biogas systems to attain high concentrations of CH<sub>4</sub>, better process stability, and reduced operational costs, thereby paving the way for their broader application in sustainable energy production and carbon neutrality efforts (Qian et al., 2025; Hmtl et al., 2020). Precisely, adding onto a stabilized hydrolysis, acidogenesis, and methanogenesis of AD could be attained through biochar inclusion (Tang et al., 2024). The characteristics of biochar, methods of preparation, feedstock used, and reactor design may influence the performance of an AD reactor and the production of biofuels (Tang et al., 2024; Manga et al., 2023). Biochar can enhance biogas production and CH<sub>4</sub> levels in AD, and its mode of operation consists of various aspects. Biochar can act as a microbial bearer, enhancing a conducive environment for the growth of anaerobic bacterial communities and stabilizing microbial community structure (Tang et al., 2024).

The design of an AD reactor highly impacts the method of how raw material is changed. The recent modifications of AD reactors consist of different changes in the procedures and reactor structure and raw material that suit the required product (Kumar et al., 2024). There are two types of digesters: continuous and batch. Batch digesters are easy to use, need fewer parts, and are cheap. Nevertheless, generally, they are used to obtaining the CH<sub>4</sub> capacity of substrates because they need a longer time of AD until the theoretical maximum is attained. Continuous digestion systems are known for generating gas continuously and, thus, are more suitable in real-life situations. The AD techniques can be grouped as single and multiple stage digestors (Kumar et al., 2024).

Several studies revealed that integrating biogas technologies with biochar enhances CH<sub>4</sub> production. Sunyoto et al. (2019) studied the temporary behavior of a pilot-scale two-phase anaerobic digestion process demonstration unit in the treatment of wastes with the addition of biochar. The fed-batch operation allowed sufficient time for microbial enrichment and adaptation. The authors observed a peak H<sub>2</sub> of 49 % and 46 L/kgVS with a semi-continuous operation process while the production of CH<sub>4</sub> reached up to 59 %, and 301 L/kgVS yield was obtained during the second stage. Nevertheless, the inclusion of biochar in the start-up was involved in the start-up positively as it facilitated the enrichment of microbial growth and enhanced the biofilm formation. They further concluded that adding BC decreases the levels of bases (NH<sub>3</sub>) needed to alter the pH in an appreciative span for H<sub>2</sub> and CH<sub>4</sub> production in all the reactors. The inclusion of BC to a wet batch-fed AD with cow manure and corn straw in which the biogas slurry was fed with a continuously stirred tank reactor was found to improve the levels of CH<sub>4</sub> and employ the substrate effectively (Shen et al., 2017). Moreover, they used wet AD (TS = 8 %), therefore, the inhibitors and AD operation guide, such as VFA and NH<sub>3</sub> concentrations, were not observed at the actual time. The inclusion of BC in AD was observed to reduce the lag phase of the AD of dairy manure. Indren et al. (2020) observed a notable increase of daily CH<sub>4</sub> yield by 136 % in AD of poultry waste using BC made from wood pellets.

Additionally, BC was observed to raise the CH<sub>4</sub> levels to 25 %, as well as 37 % during CH<sub>4</sub> formation in a year of semi-continuous AD (Shen et al., 2017). Biochar has been applied in reactors being fed with animal waste and other agricultural waste such as grass. Ning et al. (2023) mixed cattle slurry and grass silage together in single-stage and two-stage AD reactors with and without BC. The authors showed that

with continuous experiments, biochar enhanced the change of easily broken constituents from the initial-stage H<sub>2</sub> digester and showed collective impacts on biomethane formation. Thus, integrating biochar supplementation could enhance biohydrogen and biomethane formation through two-stage anaerobic digestion systems. AD of chicken manure alone in a continuously stirred tank reactor operating within a mesophilic range (35 ± 1 °C) showed an increase in CH<sub>4</sub> yields by 33 %, 36 %, and 32 % when BC was added at various loads (J. Pan et al., 2019). Biogas and CH<sub>4</sub> showed the highest concentrations during AD of chicken manure with pristine and recovered BC (Ngo et al., 2024). These results showed that rectifying reactors with new pristine biochar just as keeping a few of the BC already in the container can be a bright technique for not only reducing functional cost but enhancing treatment performance.

#### 4.2. Configurations of bioreactor and biochar integration for AD

Treatment processes developed for wastewater treatment for both industrial and domestic applications demand adequate survey with special reference to biological treatment including design, operational parameters, and configuration of bioreactors. Continuous Stirred-Tank Reactors (CSTRs) of a cylindrical container using a motor driven central shaft are common reactors that suit biochar-amended anaerobic digestion of animal wastes especially for onsite applications. As operation parameters, impeller speed should be adjusted for optimum, if not minimum mixing (~80 rpm in > 150 L containers) of organic material (Chitsi and Moo-Young 2006), that enhances bacterial attachment on biochar incorporated in the reactor. Higher speeds may discourage and limit bacterial attachment, growth, and activity on the integrated biochar due to the abrasion effect. The operation of a bioreactor is basically characterized by optimum temperature, organic loading rate and substrate retention time which also determines the amount of biofuel produced and quality of the effluent. Configuration can either be horizontal or vertical, domed or flat, underground or above ground, cylindrical or any form, however, cylindrical are more convenient (Baltrėnas and Baltrėnaitė 2018). In CSTRs, mostly liquid or slurry-based animal wastes are used, and BC can be either mixed or placed in a retention mesh to prevent washing out (Fig. 3 and 5). This enhances microbial immobilization since BC particles provide biofilm carriers hence increasing the retention of microbial ecology. Additionally, mixing efficiency from 30 to 80 rpm can balance contact time and minimizes shear stress. Moreover, as noted in recent research, methane yield tends to increase up to 40 % due to enhanced DIET.

Fixed bed or plug flow systems are configurations where biochar can be packed in static column bed and animal wastes will have to pass through the biochar concentrated zone for biotreatment. This tends to enhance longer microbial retention in a stationary biocarrier environment ensuring slow-developing methanogens. This also excludes short-circuiting, thereby improving the hydraulic retention time especially for high-solid animal wastes. Considering the treatment of nutrients, biochar layers reduce ammonia inhibition through adsorption especially for cow manure higher in fiber content.

For start-up experiments, sequential batch reactors are efficient in establishing relevant bacterial communities and different BC adjustments can be controlled with biochar-to-waste ratio adjustments. Gotore et al. (2024b, 2025) conducted start-up experiments using batch reactors (27 %) for mine drainage treatment where the establishment of associated bacterial community for manganese removal developed within two weeks compared to continuous flow reactor (11 %), similar to Petracchini et al. (2018) when treating food wastes and cow manure with 80 days of reactor stability. Batch reactors enhance faster start-up with minimum lag phase and immediate microbial colonization, offers process stability, buffering pH fluctuations between batches and they are flexible with vast types of biochar type. In addition to the above, biochar in membrane bioreactors, and biochar in several stages AD systems or hybrid are other configurations and designs under investigation for a unified standard for pilot-scale research.

## 5. Advances in AD for environmental protection and technology development

Recent advances in AD have significantly enhanced its role in environmental protection and sustainable waste management through various technological innovations. The development of novel pretreatment methods has improved the biodegradability of recalcitrant animal wastes, with ultrasonic pretreatment emerging as an efficient technique for cell wall disruption, enhancing hydrolysis rates and subsequent biogas production (Espinoza et al., 2022).

### 5.1. Thermal pretreatment and artificial intelligence in AD

Thermal hydrolysis processes operating at high temperatures and pressures have demonstrated significant improvements in organic matter solubilization and pathogen reduction, addressing both efficiency and safety concerns in AD systems (Chang et al., 2020). The integration of advanced monitoring systems utilizing artificial intelligence (AI) and machine learning has revolutionized AD process control. Real-time sensors coupled with predictive algorithms enable early detection of process instabilities and automated adjustment of operational parameters, significantly improving process stability and biogas yield while reducing operational costs (Khan et al., 2022). Enhanced understanding of substrate synergies has led to optimized co-digestion protocols, where the strategic mixing of animal wastes with complementary substrates has shown to improve process stability and methane yields while addressing nutrient imbalances (González et al., 2022), of which AI-enabled process control can help predict the BC dosage and anticipated biofuel production among other fundamental ancillary parameters. Advanced biogas-upgrading technologies have improved the quality and utilization potential of AD products.

#### 5.1.1. Membrane technology and microalgae cultivation systems

Membrane separation technologies and biological upgrading methods have enhanced methane content and removed harmful compounds, making the biogas suitable for injection into natural gas grids (Gkotsis et al., 2022). These developments have expanded the market potential for biogas and improved their economic viability. Innovation in digestate processing has transformed waste management approaches, with advanced nutrient recovery systems selectively extracting and concentrating valuable components from digestate, producing high-quality fertilizers while reducing environmental impacts (Vaneckhaute et al., 2016).

The implementation of two-stage AD systems has enhanced process efficiency and stability by separating the acidogenic and methanogenic phases. These systems provide optimal conditions for different microbial groups, resulting in improved degradation rates and biogas yields (Holl et al., 2022). The combination of AD with complementary technologies has created more comprehensive waste treatment solutions. Integration with microalgae cultivation systems has shown promise in enhancing nutrient recovery and carbon dioxide utilization, while the coupling of AD with solid-state fermentation has improved the treatment of high-solids waste streams while producing valuable bioproducts (Uggetti et al., 2014). Recent developments in membrane filtration systems have enabled water recovery and reuse, addressing water scarcity concerns in AD operations (Wu and Kim, 2019). These technological advancements, coupled with an improved understanding of microbial communities and their interactions, have led to more robust and efficient AD systems. The integration of these various technological improvements has resulted in AD systems that not only provide improved waste treatment and energy recovery but also contribute significantly to environmental protection through reduced emissions, improved resource recovery, and enhanced process stability (Archana et al., 2023).

### 5.2. Employing modified biochar for practical adoption

Employing biomass-based carbon in AD is a promising technology for converting organic waste into biogas, contributing to renewable energy production and sustainable waste management. However, several challenges, such as process instability, low methane yield, and the accumulation of inhibitory compounds, limit their efficiency and widespread adoption. Recent advances have explored the use of biochar, a carbon-rich material derived from biomass pyrolysis, as an additive to enhance AD performance. However, BC produced through pyrolysis often exhibits certain limitations, including low porosity, poor electrical conductivity, and a limited variety and quantity of surface functional groups (Xie et al., 2022). These characteristics can hinder its broader application and reduce its effectiveness in various sectors. The performance of BC is largely influenced by its physicochemical properties, such as pH, cation exchange capacity, specific surface area, and pore structure. Consequently, increasing attention has been directed toward developing suitable activation and functionalization techniques to improve these properties and further enhance AD performance (Chen et al., 2024; Nie et al., 2024).

Modified BC, obtained through chemical, physical, or biological treatments (Fig. 3), offers improved physicochemical properties that enhance AD processes. Modifications such as acid treatment, metal impregnation, or microbial inoculation increase BC's surface area, porosity, and functional groups, promoting microbial attachment and direct interspecies electron transfer (DIET). These enhancements lead to better substrate degradation, improved methanogenesis, and higher biogas yields. Additionally, BC acts as a buffer, stabilizing pH fluctuations and mitigating toxic compounds such as ammonia and sulfides, which can otherwise inhibit microbial activity.

Table 2 presents the effectiveness of modified biochar from various materials and modification methods in enhancing anaerobic digestion efficiency. Modified BC can significantly enhance the AD process by promoting anaerobic sludge aggregation and improving reactor performance. Biochar's surface modifications, particularly with metal modifications, enable it to adsorb and retain multivalent cations like calcium (II), iron (II), and magnesium (II). These cations help bridge the negatively charged microbial cells and extracellular polymeric substances (EPS), which are vital for sludge particle formation. Additionally, BC encourages the secretion of EPS by microbes, acting as a biological adhesive that binds cells together, facilitating sludge granulation and strengthening the sludge structure, which in turn enhances settling properties (Chen et al., 2024; Chiang et al., 2025).

Several studies have confirmed that modified BC accelerates key stages of anaerobic digestion, such as hydrolysis and acidogenesis. Metal-modified biochar, especially those with iron or cobalt, facilitates electron transfer between microbial species, speeding up the breakdown of complex organic matter. This enhances the conversion of volatile fatty acids (VFAs) into methane, reducing the accumulation of VFAs that could lower pH and inhibit methanogenesis (Ma et al., 2024; Valentin et al., 2023). Gao et al. (2024) found that HNO<sub>3</sub>-modified BC and nZVI-biochar improved methane production by 90 % and 204.84 %, respectively, while also accelerating the decomposition of VFAs. Organic matter removal efficiency is also improved, with Wang et al. (2021) reporting that magnetite-contained BC derived from Fenton sludge achieved 87.8 % COD removal from dairy wastewater. Similarly, Zhong et al. (2023) observed a 15 % increase in sCOD removal with 5 g/L of nitrogen-doped BC-supported magnetite, while Zhang and Wang (2021) noted a 34.93 % improvement in tCOD removal during anaerobic digestion of sewage sludge and food waste with nZVI-modified BC.

A significant advantage of modified biochar is its ability to mitigate common inhibitors in anaerobic digestion. High ammonia concentrations can hinder bacterial activity, particularly methane production. Biochar helps stabilize the bacterial environment by adsorbing excess ammonia (Wang et al., 2023). Deng et al. (2022) showed that iron-modified corn cob BC had a higher ammonium adsorption capacity

**Table 2**

The use of modified biochar derived from various feedstock to enhance biochar- amended anaerobic digestion efficiency for the treatment of different substrates and reactor operations,.

No	Raw materials	Modification conditions	Modified biochar	Substrate	Reactor	AD operation conditions	AD performance	References
1	Fenton sludge	Pyrolysis 400 °C, 2 h; Mixed with 20 % KH <sub>2</sub> PO <sub>4</sub> solution, 24h	Magnetite-contained biochar	Dairy wastewater	Semicontinuous	37 °C, pH = 7.2, COD = 5000 mg/L, seed sludge: TS = 70,000 g/L, VS = 35,000 g/L 35 ± 1 °C, 30 d, pH = 7.2, seed sludge: tCOD = 236,432 ± 3042 mg/L, TS = 35,400 mg/L, VS = 23,200 mg/L	COD removal: 86.7 % Increase in CH <sub>4</sub> : 38.1 % VFAs: 51.4 % VS removal: 37.8 % COD removal: 62.7 % Increase in CH <sub>4</sub> : 56.3 % VFAs: 35.4 % Increase in CH <sub>4</sub> : 55.9 %	<a href="#">Wang et al., 2021</a>
2	Reed straw	Pyrolysis 600 °C, 4 h; Mixed with ethylene glycol, FeCl <sub>3</sub> ·6 H <sub>2</sub> O, ethylene diamine, and CH <sub>3</sub> COONa·3 H <sub>2</sub> O, autoclave at 200 °C, 2h	Magnetic biochar	Pigment sludge	Batch			<a href="#">Ruan et al., 2023</a>
3	Corn straw	Pyrolysis 600 °C, 3 h; Mixed with FeSO <sub>4</sub> ·7H <sub>2</sub> O, KMnO <sub>4</sub> , NaOH, and deionized water; dried 35 °C, 36 h.	MnFe <sub>2</sub> O <sub>4</sub> -biochar	Sewage sludge	Batch	35 ± 1 °C, 150 rpm, seed sludge: TS = 9.1 %, VS = 47.3 % of TS		<a href="#">Zhang and Wang, 2020</a>
4	Corn straw	Mixed with 1 M FeCl <sub>3</sub> , 36 h; dried at 70 °C, 30 h; pyrolysis 600 °C, 1 h; washed with 0.1 M HCl; dried 80 °C	Magnetic biochar	Sludge	Batch	35 ± 2 °C, 30 d, seed sludge: sCOD = 200.67 ± 49.15 mg/L, TS = 43,000 mg/L, VS = 11,330 mg/L	VFAs: 48.38 % COD removal: 31.44 % Increase in CH <sub>4</sub> : 9.1 %	<a href="#">Zhang et al., 2023</a>
5	Corn stover	Pyrolysis 300 °C, 500 °C, 700 °C, 2 h; stored at 4 °C	Redox-active biochar	Waste activated sludge	Batch	37 ± 1 °C, 60 rpm, seed sludge: sCOD = 2290.8 ± 25.8 mg/L, TS = 2.09 %, VS = 1.19 %	VFAs: 9.5 % COD removal: 12.3 % Increase in CH <sub>4</sub> : 46.9 %	<a href="#">Shen et al., 2021</a>
6	Coconut shell	Washed with 0.1 M HCl, 5 M HNO <sub>3</sub> ; pyrolysis 700 °C, 2 h; added with Fe <sup>2+</sup> and Fe <sup>3+</sup> ; dried 105 °C, 24h	Nitrogen-doped biochar supported magnetite	Waste activated sludge	Batch	35 ± 1 °C, 22 d, 180 rpm, seed sludge: sCOD = 1700 mg/L, TSS = 79,780 mg/L, VSS = 34,563 mg/L	VFAs: 42 % COD removal: 81 % Increase in CH <sub>4</sub> : 80.8 %	<a href="#">Zhong et al., 2023</a>
7	Corn stover	Pyrolysis 300 °C, 2 h; Milled and sieved < 300 µm	Redox-active biochar	Waste activated sludge	Batch	37 ± 1 °C, 80 rpm, pH = 7.2, seed sludge: TS = 19.5 g/kg, VS = 11.1 g/kg	VFAs: 72.4 % Increase in CH <sub>4</sub> : 46.9 %	<a href="#">Lü et al., 2020</a>
8	Sewage sludge	Pyrolysis 550 °C; milling, 0.5 h, mixed with distilled water, centrifuged at 4000 rpm, 5 mins; dried 105 °C	Nano biochar	Orange peel waste	Semicontinuous	55 °C, 85 d, seed sludge: pH = 7.61, TS = 1.71 wt %, VS = 1.22 wt%	Increase in CH <sub>4</sub> : 80.8 %	<a href="#">Zhang et al., 2022</a>
9	Rice straw	Ball milling, FeCl <sub>3</sub> ·6H <sub>2</sub> O; Pyrolysis 850 °C, 2 h.	Magnetic straw-based biochar	Waste activated sludge	Batch	37 ± 1 °C, 100 rpm, seed sludge: sCOD = 960 mg/L, TS = 2.51 %, VS = 1.24 %	VFAs: 14.13 % Increase in CH <sub>4</sub> : 45.36 %	<a href="#">Liu et al., 2022</a>
10	Fruitwood	Pyrolysis 500 °C, 2 h; mixed with FeSO <sub>4</sub> ·7H <sub>2</sub> O and FeCl <sub>3</sub> ·6H <sub>2</sub> O, 60 °C; 0.5 h; added with NaOH, 1 h; dried 105 °C	Recycled magnetic biochar	Waste activated sludge	Batch	35 ± 1 °C, 120 rpm, seed sludge: TSS = 1.49 %, VSS = 0.80 %	Increase in CH <sub>4</sub> : 14.3 %	<a href="#">Jin et al., 2024</a>
11	Fruitwood and corn straw	Pyrolysis 500 °C, 2 h; mixed with 1 M HCl	Acid washing biochar	Waste activated sludge	Batch	35 ± 1 °C, 105 rpm, 35 d, seed sludge: TSS = 1.49 %, VSS = 0.80 %	Increase in CH <sub>4</sub> : 15.7 %	<a href="#">Jin et al., 2022</a>
12	Corn stover	Pyrolysis 550 °C, 2 h; rinsed with 1 M HCl; mixed with 1 M FeCl <sub>3</sub> ; added with 0.12 M NaBH <sub>4</sub>	nZVI-biochar	Sewage sludge	Batch	37 ± 1 °C, 170 rpm, 34 d, seed sludge: TS = 9.6 %, VS = 49.1 % of TS	Increase in CH <sub>4</sub> : 115.39 %	<a href="#">Zhang et al., 2019</a>
13	Bagasse	Mixed with 0.1 M FeCl <sub>3</sub> , 24 h; pyrolysis 350 °C, 2 h; mixed with NaOH, 2h	Fe-Mg-chitosan bagasse biochar	Kitchen waste	Batch	The upward flow rate of 0.3, 0.6, 1.2 m/h, HRT of 24 h, room temperature, 45d, seed sludge: VSS = 720 mg/L, TSS = 24,000 mg/L	COD removal: 96 % NH <sub>3</sub> -N removal: 83 %	<a href="#">Wang et al., 2023</a>
14	Alligator weed	Mixed with FeSO <sub>4</sub> ·7H <sub>2</sub> O and urea, 1 h; sealed, 24 h, dried 60 °C; pyrolysis 300, 500, and 700 °C	Fe-N co-modified biochar	Alternanthera philoxeroides and cow manure	Batch	37 °C, seed sludge: TS = 6.53 ± 0.06 %, VS = 4.44 ± 0.07 %	Increase in CH <sub>4</sub> : 42.37 %	<a href="#">Fan et al., 2024</a>
15	Corn straw	Pyrolysis 600 °C, 2 h; soaked with KMnO <sub>4</sub> , 24 h; pyrolysis 400 °C, 500	MnO <sub>2</sub> -modified biochar	Food waste	Batch	37 °C, 90 rpm, seed sludge: TS = 2.73 ± 0.02 %, VS = 1.50 ± 0.04 %	VFAs: 9.09 % Increase in CH <sub>4</sub> : 5.83- 24.32 %	<a href="#">Li et al., 2024</a>

(continued on next page)



Table 2 (continued)

No	Raw materials	Modification conditions	Modified biochar	Substrate	Reactor	AD operation conditions	AD performance	References
16	Coconut shell	°C, 600 °C, 2 h; dried 80 °C, 4 h. Mixed with FeCl <sub>3</sub> ·6H <sub>2</sub> O, 24 h dried 80 °C, 72 h; pyrolysis 600 °C, 4h	Iron-modified biochar	Sulfamethoxazole pharmaceutical wastewater	Batch	35 ± 1 °C, HRT of 24 h, seed sludge: VSS = 18,630 mg/L	VFAs: 75.00 % Increase in COD removal: 34.00 % Increase in SMX removal: 40.00 % Increase in CH <sub>4</sub> : 81.07mL/gCODremoved	Ni et al., 2023
17	Peanut blight	Pyrolysis 600 °C, 3 h; mixed with FeCl <sub>3</sub> ·6H <sub>2</sub> O and MnCl <sub>2</sub> ·4H <sub>2</sub> O; 200 °C, 12 h; pyrolysis 800 °C, 1h	Fe/Mn modified biochar	Sludge	Batch	37 °C, 120 rpm, 35 d, seed sludge: pH = 6.92±0.2, sCOD = 146.21±8.7 mg/L, TS = 18,030±900 mg/L, VS = 7210±200 mg/L, 35 °C, 60 d, seed sludge: pH = 7.628, TS = 4.102 %, VS = 1.622 %	VFAs: 47.3 % Increase in CH <sub>4</sub> : 10.06 %	Wu et al., 2023
18	Wheat stalk	Pyrolysis 500 °C, 4 h; Mixed with 20 % KH <sub>2</sub> PO <sub>4</sub> , 24h	KH <sub>2</sub> PO <sub>4</sub> -modified biochar	Swine manure	Batch	35 ± 1 °C, 170 rpm, 42 d, seed sludge: pH = 7.09 ± 0.17, tCOD = 23,759.8 ± 612.3 mg/L, TS = 31,250 ± 970 mg/L, VS = 9950 ± 230 mg/L	Increase in CH <sub>4</sub> : 7.21–43.52 %	Yang et al., 2021
19	Corn straw	Pyrolysis 550 °C, 2 h; rinsed with HCl; mixed with 1 M FeCl <sub>3</sub> , 12 h, added with 0.12 M NaBH <sub>4</sub>	nZVI-biochar	Sewage sludge and food waste	Batch		Increase in CH <sub>4</sub> : 43.37 % Increase in tCOD removal: 34.93 % Increase in VSS removal: 11.44 % Increase in TSS removal: 13.96 %	Zhang and Wang, 2021
20	Corn straws	Pyrolysis 600 °C, 2 h; mixed with KMnO <sub>4</sub> ; pyrolysis 600 °C, 1h	Manganese oxide-modified biochar	Sewage sludge	Batch	35 °C, seed sludge: pH = 6.9 ± 0.1, tCOD = 80,529 ± 1176.9 mg/L, TS = 69,057 ± 1207.3 mg/L	Increase in CH <sub>4</sub> : 121.97 %	Li et al., 2019
21	Waste sewage sludge	Mixed with melamine; hydrothermal 150 °C, 5 h; pyrolysis 700 °C, 3 h	Novel nitrogen doped sludge carbon	Coal gasification wastewater	UASB reactors	37 ± 2 °C, HRT of 24 h, 180 d, seed sludge: pH = 6.9 ± 0.1, TSS = 18,500 ± 300 mg/L, VSS/TSS = 0.75	VFAs: 37.5 % Increase in COD removal: 25.4 % Increase in CH <sub>4</sub> : 68.1 %	Zhuang et al., 2018
22	Woody	Pyrolysis 200–300 °C, Size of 100–250 µm; mixed with <i>Methanosarcina thermophila</i> (2 g/L)	Biochar plus bioaugmentation	Food waste	Batch	55 °C, seed sludge: TS = 9.8 %, VS = 4.5 %	Increase in CH <sub>4</sub> : 32 %	Lee et al., 2022
23	Fresh pigeon manure	Pyrolysis 550 °C, 2 h; mixed with C <sub>8</sub> H <sub>12</sub> ClNO <sub>2</sub> and FeCl <sub>3</sub> ·6H <sub>2</sub> O, 10 h; pyrolysis 900 °C, 0.5h	Surface electron-polarized biochar	Pig manure	Batch	35 °C	Increase in CH <sub>4</sub> : 35.4 %	Xu et al., 2023

(2.92 to 6.35 mg/g) than pure biochar (2.01 to 4.96 mg/g). Additionally, KH<sub>2</sub>PO<sub>4</sub>-modified BC and MnFe<sub>2</sub>O<sub>4</sub>-modified BC enhance detoxification of heavy metals such as copper (II) and lead (II), which would otherwise harm microbial communities (Yang et al., 2021; Zhang and Wang, 2020). Modified BC also absorbs inhibitory organic pollutants like phenols and antibiotics, reducing their toxicity (Ni et al., 2023). Modified BC also contributes to in-situ biogas upgrading by adsorbing CO<sub>2</sub> and hydrogen sulfide (H<sub>2</sub>S). Zhang et al. (2022) reported that N-doped biochar enhanced CO<sub>2</sub> adsorption from 0.52 to 2.43 mmol/g. In certain cases, BC supports direct interspecies electron transfer (DIET), facilitating the conversion of CO<sub>2</sub> into methane and further boosting methane yield (Ning et al., 2023).

Another key advantage of modified biochar is its impact on enzyme activity. Previous studies demonstrated that magnetic straw-based biochar increased enzymatic hydrolysis rates, accelerating the degradation of organic matter (Chen et al., 2022; Liu et al., 2022). Modified BC also enriches the microbial community, which is essential for stable and efficient anaerobic digestion. Its porous structure creates an ideal environment for microbial colonization, promoting the growth of functional microorganisms such as hydrolytic bacteria, acidogenic

bacteria, and methanogens. Studies using N-doped biochar (Zhong et al., 2023), nano BC (Zhang et al., 2022), magnetic straw-based BC (Liu et al., 2022), and recycled magnetic BC (Jin et al., 2024) have enriched methanogens like *Methanosarcina*, *Methanosaeta*, and *Methanobacterium*, resulting in increased CH<sub>4</sub> production. Modified BC selectively promotes the growth of microbes involved in DIET, improving metabolic efficiency (Liu et al., 2022; Wang et al., 2020b). It also enhances microbial community stability by supporting biofilm formation, helping the community resist environmental fluctuations and toxic shocks (Wang et al., 2024).

Although the use of modified BC in anaerobic digestion has demonstrated notable efficiency improvements, most research to date has been limited to small-scale batch experiments. To facilitate practical adoption, further investigations at a larger, pilot or full-scale level are necessary. Economic and operational factors must also be carefully considered. Producing BC from agricultural residues, industrial by-products, or other low-cost biomass sources can enhance feasibility and align with circular economy principles (Chen et al., 2024; Nie et al., 2024). Furthermore, optimizing modification methods tailored to specific anaerobic digestion conditions is essential to maximizing

performance. Future research should prioritize large-scale applications, long-term BC stability, and comprehensive techno-economic assessments to ensure sustainable implementation.

### 5.3. Influence of QS in promoting biofuel production with BC in AD

Anaerobic digestion is a widely utilized biological process for biofuel production, primarily generating methane in the form of biogas. The efficiency of AD depends on microbial interactions, metabolic pathways, and environmental conditions (Karakashev et al., 2005; Mutungwazi et al., 2021; Subramanian and Suresh, 2024). Among the various strategies to enhance AD performance, quorum sensing (QS) and biochar have attracted significant attention due to their synergistic effects in promoting methane production. QS is a microbial communication mechanism that regulates gene expression based on cell population density, playing a crucial role in coordinating key AD processes, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Anburajan et al., 2023). QS signaling molecules, such as acyl-homoserine lactones (AHLs) in bacteria and autoinducer-2 (AI-2) in both bacteria and archaea, influence microbial behavior by promoting biofilm formation, enhancing enzyme secretion, and facilitating electron transfer. This ultimately improves the metabolic efficiency of methanogenic consortia and increases biogas production (Chen et al., 2018; Markowska et al., 2024; Ziegert et al., 2024). Biochar, a carbon-rich material derived from biomass pyrolysis, is a highly effective additive in AD due to its high surface area, porosity, and redox-active properties. It provides a conducive environment for microbial colonization, enhances microbial aggregation, and facilitates electron exchange between microbial species (Ding et al., 2024; Htmel et al., 2020; Zhao et al., 2024). Additionally, BC adsorbs QS signaling molecules, increasing their localized concentration and strengthening QS-mediated microbial interactions (Ma et al., 2024). The integration of BC into AD systems promotes direct interspecies electron transfer (DIET), accelerating syntrophic relationships between fermentative bacteria and methanogenic archaea (Valentin et al., 2023; Zhang et al., 2023). This improved electron transfer enhances the conversion of VFAs into methane, preventing the accumulation of intermediate by-products that can inhibit methanogenesis (Devi et al., 2024; Htmel et al., 2020). Furthermore, biochar stabilizes pH fluctuations and adsorbs toxic compounds, reducing stress on microbial communities and maintaining optimal AD efficiency (Devi et al., 2024).

The combined effects of QS and BC contribute to enhanced microbial aggregation, increased hydrolytic and methanogenic enzyme production, improved DIET, and better environmental stability, ultimately optimizing methane production in AD systems.

Several recent studies have demonstrated the efficacy of integrating QS molecules and BC in AD systems. Li et al. (2024a) investigated the roles of AHLs in the AD of waste-activated sludge aided by BC. Their results demonstrated that the co-addition of BC and AHLs significantly enhanced methane production, with the maximal methane yield reaching 154.7 mL/g volatile suspended solids—an increase of 51.9 % compared to the control group. This enhancement was attributed to the promotion of the hydrolysis and acidification stages, improved conversion of organic matter, and optimized accumulation of acetate acid. Additionally, microbial electron transfer activity and coenzyme F420 levels were notably increased, indicating enhanced microbial interactions facilitated by QS molecules in the presence of BC.

Li et al. (2024b) further evaluated the impact of BC and QS molecules on methane production in single-stage and two-stage anaerobic digestion systems. The findings revealed that the single-stage digestion system with the addition of BC and AHLs achieved a methane yield of 134.9 mL/g volatile suspended solids, which was 41.7 % higher than the control group. Mechanistic studies indicated that this strategy promoted solubilization, hydrolysis, acetogenesis, and methanogenesis stages, improving the conversion and utilization of organic matter and enhancing syntrophic metabolism between microorganisms.

Furthermore, electron transfer activity between microbial species increased by 54.7 %, strengthening syntrophic relationships between methanogens and electroactive bacteria.

Gao et al. (2024) investigated the effect of BC on methane production in food waste anaerobic digestion and its interaction with QS. Their study found a 20 % increase in methane yield with 700C BC, reaching 298.8 mL/g volatile suspended solids. This improvement was linked to elevated QS signaling molecule levels, particularly N-acyl-homoserine lactones, including 3OC6-HSL. Biochar-enriched electroactive microbes such as DMER64 and *Methanospirillum* boosted coenzyme F420 activity by 33 %. Enhanced carbon metabolism, extracellular electron transfer, and QS-related gene expression suggested that BC enhances methane production by improving QS-mediated microbial interactions and electron transfer pathways. Overall, the integration of QS and BC into AD systems has shown substantial potential in optimizing methane production. The synergistic effects of enhanced microbial communication, improved electron transfer, and environmental stabilization provide a promising strategy for increasing biogas yields and advancing sustainable biofuel production.

### 5.4. Effects of additives on AD performance and microbial community structure

Additives are commonly used in AD to improve its performance, especially when digesting complex substrates. These additives include nutrients, trace elements, external carbon sources, pH regulators, and solid materials like biochar. Nutrient supplementation, such as the addition of nitrogen, phosphorus, or trace elements like iron and magnesium, helps to support microbial activity and overcome nutrient limitations. Organic or inorganic carbon sources can serve as electron donors, promoting enhanced biogas production, particularly in systems with carbon-deficient substrates (Dhull et al., 2024; Paritosh et al., 2020; Pilarska et al., 2024).

Among solid additives, biochar has been explored for its potential to enhance AD performance and positively influence microbial communities (Manga et al., 2023; Pilarska et al., 2024). Biochar has been shown to positively influence AD performance in various ways (Fig. 4). It can enhance biogas production, particularly methane yield, by providing a surface for microbial attachment and facilitating electron transfer between microorganisms (Manga et al., 2023; Devi and Eskicioglu, 2024). In addition, BC's ability to absorb inhibitory compounds, such as ammonia or volatile fatty acids, helps to mitigate the negative effects of high concentrations of these substances, which often limit AD performance (Manga et al., 2023; Valentin et al., 2023).

Several studies have demonstrated that BC supplementation can enhance the overall efficiency of anaerobic digestion by promoting the degradation of organic matter and improving biogas production, even when digesting challenging substrates such as food waste or livestock manure. For instance, Pei et al. (2024) reported that the addition of BC at a concentration of 10.0 g/L to food waste digestion increased the cumulative methane yield by 128 %, significantly enhancing daily methane production. Similarly, Shin et al. (2022) found that incorporating BC derived from food waste at a dosage equivalent to 1 % of the digester volume resulted in approximately a 10 % increase in total biogas production and a 4 % improvement in methane content. Supporting these findings, Silva et al. (2025) observed that BC addition under mesophilic conditions in a continuously stirred-tank reactor treating pig slurry resulted in notable improvements in both biogas and methane production.

The increase in methane production is attributed to both the enhanced microbial activity facilitated by BC's surface area and the adsorption of inhibitors. Additionally, the biochar's capacity to buffer pH and provide a stable environment further contributes to improved system stability. Table 3 summarizes numerous studies that have compared AD performance and microbial community structures between systems with and without BC. The impact of BC on microbial

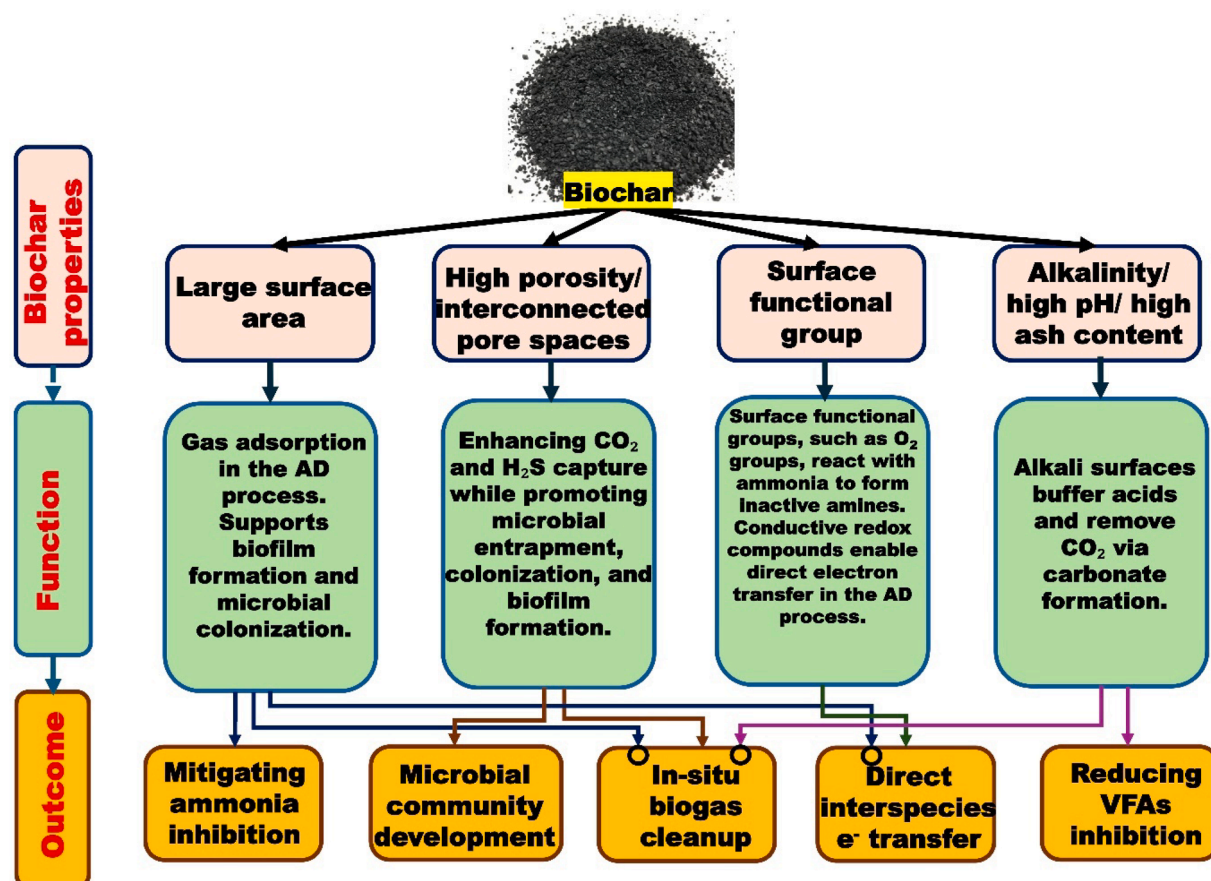


Fig. 4. The role of biochar in an anaerobic digestion system associated with its physiochemical properties, modified from Manga et al. (2023).

community structure in AD systems is an important consideration. As illustrated in Fig. 5, BC can enhance microbial activity by providing a favorable habitat that supports microbial growth and increases diversity. Notably, biochar promotes the proliferation of methanogens, which play a critical role in methane production. It also fosters the development of hydrolytic bacteria and acidogenic microorganisms involved in the initial stages of organic matter breakdown, thereby contributing to a balanced and efficient microbial community (Kumar et al., 2021; Zhao et al., 2024).

Microbial community analysis, typically using techniques like 16S rRNA sequencing or PCR-based methods, has shown that BC supplementation modifies microbial composition in AD systems. Biochar addition generally enhances microbial diversity and stability, encouraging the growth of key functional microorganisms across different stages of AD (Pei et al., 2024; Sugiarto et al., 2021; Zhao et al., 2024). Biochar boosts the abundance of methanogens, as well as bacteria responsible for fermentation and hydrolysis, leading to improved overall digestion performance. Previous studies have documented the enrichment of both bacteria and archaea under BC-amended conditions (Table 3). Ding et al. (2024) reported that BC addition in AD systems stimulated the growth of *Bacteroidetes* and *Clostridiales* bacteria while enriching hydrogenotrophic methanogens, such as *Methanobrevibacter* and *Methanobacterium*. This microbial modulation contributed to the stability and efficiency of the digestion process. Similarly, Pei et al. (2024) investigated the impact of poplar sawdust-derived BC on the AD of food waste. Their results indicated that BC significantly increased the relative abundance of dominant AD bacteria (by 85.54–25.30 %) and facilitated the degradation of recalcitrant organic matter. Enrichment of *Bathyarchaeia* and hydrogenotrophic methanogens was also observed following BC supplementation. Further supporting evidence comes from Shen et al. (2022), who assessed low-cost BC produced from agricultural

waste, such as corn straw, in a pilot-scale continuous-flow AD system. Their findings demonstrated a positive correlation between specific bacterial and archaeal groups, indicating that BC can positively influence microbial community interactions and enhance AD system performance.

## 6. Areas of future studies, prospects, and limitations

The integration of BC into AD for animal waste biodegradation holds immense potential for advancing sustainable biogas production alongside waste management. Even though there are several interests and progress in this perspective, there are research gaps that need to be filled for effective implementations of field and large-scale operations. The optimization of BC properties and mechanistic understanding of biochar-anaerobic digestion synergy requires further investigation. This entails the influence of BC biomass, the carbonization process, functional groups, and the involved physiochemical and biological interaction between microbes and BC in AD. The effectiveness of biochar is dependent on feedstock nature; therefore, variability can lead to inconsistent performance of the AD as some biochar by-products may enhance metabolic activity by bacteria while others may have negligible and/or inhibitory outcomes. Moreover, the effects of pyrolysis remain a challenge especially when comparing low and high temperatures because low pyrolysis tend to release inhibitory compounds (phenolic compounds) whereas high temperatures may limit the transfer of electrons between bacteria and the biochar surface which limits AD performances. On the other hand, clogging is a common challenge in bioreactors and AD is no exception. High dosage of biochar influences the treatment performance of AD as it may increase the viscosity and reduces the mixing efficiency whereas inadequate biochar limits the colonization sites which lead to less microbial ecology. Therefore,



**Table 3**  
Performance of amended and unamended anaerobic digestion process and the influence of enriched microorganisms for bioenergy production under different treatment conditions.

AD performance			
Parameter	Without Biochar	With Biochar	Reference
<b>Methane yield</b>	<b>Baseline</b>	<b>Increased</b>	
	308.0 (L/kgVS)	381.9 (L/kgVS)	Kaur et al., 2020
	94.2–120.5(L/kgTS)	144.33–151.29 (L/kg TS)	Ding et al., 2024
	36.37 (L/kgVS)	175.02 (L/kgVS)	Ramírez et al., 2024
	340.2 (L/kgVS)	465–543 (L/kgVS)	Zhang et al., 2020
	117.36 (L/kgVS)	125.50- 218.45 (L/kgVS)	Zhang et al., 2019
<b>VS removal</b>	89.1 (l/kg COD)	172.3 (l/kg COD)	Wang et al., 2020a
	242.7 (L/kgVS)	275.8–394.6 (L/kgVS)	Zhang et al., 2020
	<b>Lower</b>	<b>Higher</b>	
	33.84 %	41.62 %	Kaur et al., 2021
<b>COD removal</b>	18.00 %	55.70 %	Sánchez et al., 2021
	<b>Baseline</b>	<b>Increased</b>	
	13 %	88 %	Ramírez et al., 2024
	76 %	90 %	Su et al., 2019
<b>Process stability</b>	89 %	94 %	Wang et al., 2025
	70 %	89 %	W. Zhao et al., 2021
	Variable	Enhanced	Devi and Eskicioglu, 2024; Hu et al., 2023;
			Kumar et al., 2021
<b>Inhibition control</b>	Limited	Improved	Devi and Eskicioglu, 2024
<b>DIET efficiency</b>	Lower	Higher	Devi and Eskicioglu, 2026
<b>Microbial aggregation</b>	Less Pronounced	Enhanced	
		Bacteria (% increased)	
		Clostridiales (10.33–21.19 %), Bacteroidetes (12.11- 34.97 %)	Archaea (% increased)
		Clostridia (25 %)	Methanobacterium and Methanobrevibacter (34.17–119 %)
		Syntrophobacter (3.14 %)	Methanosaeta (7.2 %)
		Proteinclasticum (46.2 %)	Methanosaeta (40.4 %)
		Bacteroides (23.3 %)	Methanosaeta (3.0 %)
		Paraclostridium (13.2 %)	Methanosaeta (10.4 %)
		Synergistia (28.5 %)	Methanosarcina (6.6 %)
		Petrimonas (6.23 %)	Methanoculleus (7.7 %)
		Coprothermobacter (12 %)	Methanotherix (15.8 %)
		Synergistaceae (4.94 %)	Methanosarcina (5.60 %)
		Synergistaceae (3.08 %)	Methanosarcina (36.4 %)
		Clostridia (1.64 %)	Methanosaeta (12 %)
			Methanotherix (15.85 %)

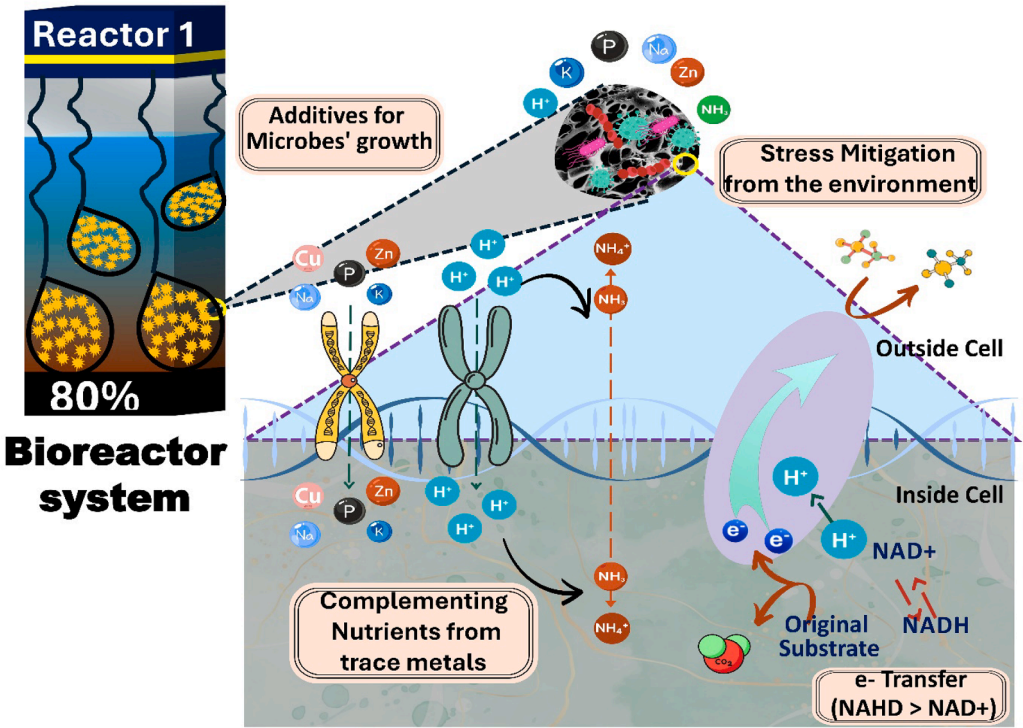


Fig. 5. Metabolic activities of bacterial community structures in biochar-amended anaerobic digestion system, modified from Zhao et al. (2024).

optimum biochar should be determined based on solids' amount and the type of animal wastewater to be treated as well as the specific design of the reactor. Additionally, long-term stability and recycling sustainability and performance of BC overtime alongside economic and environmental feasibility for pilot and large-scale implementation. By exploring the potential of biochar in AD, there is a potential to unlock new opportunities for improving the efficiency, stability, and environmental sustainability of AD processes.

The future of AD amended with BC has the potential to significantly increase biogas yields, making bioenergy production more efficient and economically viable, especially in underserved communities in developing countries. Moreover, this approach reduces greenhouse gas emissions and combats climate change. The post-treatment of animal waste produces nutrient digestate, which can be used to improve soil health and fertility, supporting sustainable agriculture. However, the high initial costs of establishing AD and the production logistics of BC could hinder the adoption of this system, especially in low-income countries. There are no universal standards for BC properties optimized for use in AD, which then offers variability in performance across treatment systems and their respective applications. In addition, this also raises challenges related to reactor design, dosing of BC, and process control for long or short-term. So, realizing the potential of BC-amended AD, these challenges need to be addressed for long-term application and data collection. Addressing these limitations is crucial to ensure the sustainability and viability of BC-enhanced AD systems as they technically hinder the widespread adoption of biochar-enhanced AD systems. This review has shown that biochar-amended anaerobic digestion systems can play a pivotal role in achieving a circular economy and a more sustainable future.

## 7. Conclusion

The integration of biochar into the anaerobic digestion of animal waste presents a promising pathway for enhancing bioenergy production and lowering greenhouse gases while addressing environmental challenges. Biochar's unique properties, such as its high surface area, porosity, and ability to stabilize microbial communities and growth, significantly improve the efficiency of the AD process. From a sustainability perspective, this integrated approach offers a dual benefit: it converts animal waste, a significant environmental burden, into valuable clean energy while simultaneously producing nutrient-rich digestate as a fertilizer. Furthermore, the carbon sequestration potential of biochar aligns with global efforts to reduce greenhouse gas emissions and combat climate change. However, challenges such as optimizing biochar properties, scaling up technology, locally available and efficient additives, and ensuring economic viability remain critical areas for future research. The use of quorum-sensing molecules as additives has been reviewed for AD, and the mechanisms of biochar-enhanced methanogenesis and extracellular electron transfer have been discussed. Different reactor designs have been observed to perform differently on whether co-addition of biochar and quorum sensing molecules or QS-loaded biochar as this brings different bioenergy production levels and treatment efficiencies. There is a need for further investigation of such methodologies to verify these differences. This review reiterated the synergy between biochar and anaerobic digestion to represent a transformative opportunity for sustainable waste management and renewable energy production. By harnessing this innovative approach, we can move closer to achieving a circular economy and a greener, more sustainable future using locally available materials.

## CRediT authorship contribution statement

**Obey Gotore:** Writing – review & editing, Writing – original draft, Visualization, Validation, Data curation, Conceptualization. **Thuong Thi Nguyen:** Writing – review & editing, Writing – original draft, Conceptualization. **Tirivashe Philip Masere:** Writing – review &

editing, Writing – original draft. **Albert Shumba:** Writing – review & editing. **Albert Gumbo:** Writing – review & editing, Writing – original draft. **Prattakorn Sittisom:** Writing – review & editing, Writing – original draft, Conceptualization. **Mufwankolo Apingien Heritier:** Writing – review & editing. **Tomoaki Itayama:** Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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