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The role of Biochar in anaerobic digestion to enhance the biogas production by focusing on Methanogenic pathway: A review

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ABSTRACT

Optimizing the biogas generation process (BGP) during the anaerobic digestion (AD) route is a challenging goal to be achieved through the potential *valence* of the biochar (BC) application. Anaerobic digestion has become a favored technique aimed at producing clean energy and effectively handling organic waste. Nonetheless, the reactor performance is hindered by the buildup of ammonia, acids and nutrients, resulting in inhibition and instability. Due to its versatility, biochar (BC) has attracted significant attention in biogas production and can be produced by carbonizing biomass and waste materials. Incorporating BC into the anaerobic digestion (AD) process could provide several advantages, such as reducing toxic inhibition, shortening the methanogenic lag phase, immobilizing functional bacteria, and improving the electron transfer rate between methanogenic and acetogenic microorganisms. However, there is still a need for a deeper understanding of the complex role of BC and its detailed mechanisms in biogas production within the AD process. The present brief review paper intends to explore the main role and mechanisms of the BC application as an additive in the AD process, by focusing on how the characteristics of BC affect the AD processes during *methanogenic pathways* and processes and how they effectively address significant challenges.

KEYWORDS

Biochar, Anaerobic Digestion, Biogas Production, Organic Waste Management, Methanogenesis

HOW TO CITE THIS ARTICLE

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The conversion of organic waste into clean, renewable energy is facilitated by the economical use of anaerobic digestion (AD), which is widely used worldwide (Masebinu *et al*., 2019; Li *et al*., 2020; Yang *et al*., 2021). AD is commonly applied for the treatment of organic waste and production of biogas: it is pragmatically well-known as a key bioengineering technology (Vayena *et al*., 2024). Through the conversion of organic carbon into biogas, AD can convert wastewater or solid waste like livestock wastewater and manure. The composition of this biogas typically consists of approximately 40-70% methane (CH_4) and 30-60% carbon dioxide (CO_2), along with smaller quantities of gaseous water (H_2O), hydrogen sulfide (H_2S), and ammonia (NH_3) (Sahota *et al*., 2018). As highlited by (Arif *et al*., 2018), due to its high calorific value (21-25 MJ/m^3), biogas presents an attractive alternative to conventional fossil fuels. The biological process of anaerobic digestion is typically classified into four specific stages, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. By activating microbial extracellular hydrolytic enzymes, the breakdown of complex organic materials into their soluble monomers is accomplished through hydrolysis. Fatty acids, hydrogen, and carbon dioxide (CO_2) are produced during the acidogenesis phase, when fermentative bacteria convert the soluble monomers obtained from hydrolysis.

Acetate is produced through a cooperative process between anaerobic bacteria and methanogens through acidogenesis, which then undergoes further processing to produce the compound. The final step is characterized by methanogenesis: it is a critical stage and serves as a rate-limiting factor since it is the slowest biochemical reaction and is essential for converting all the earlier compounds into methane (Arif *et al*., 2018; Patel *et al*., 2021). As argued by (Sahoo *et al*., 2023), although anaerobic digestion (AD) offers distinctive benefits for converting organic waste into biogas, it also is characterized by certain drawbacks. For instance, biogas, that is rich in methane (CH_4) and carbon dioxide (CO_2), is classified as a greenhouse gas (GHG), and uncontrolled releases trigger significant environmental hazards and consequent issues. In terms of usage, the biogas generated can either be used for energy production, such as electricity or heat, or it can be converted to CH_4 for vehicles fuel.

Nevertheless, their effectiveness in biogas extraction is partially limited by various challenges, such as low CH_4 production, pH imbalance, unstable performance, and the breakdown of unwanted recalcitrant substances (Avagyan, 2018; Zhao *et al*., 2021b). Recent research works have identified that the direct interspecies electron transfer (DIET) mechanism, along with the addition of carbon-based conductive materials to the Anaerobic co-digestion (ACoD) system, can significantly enhance both the stability of the system and the yield of CH_4. The improvement in biogas production through ACoD with conductive materials has been the focus of numerous studies. The integration of conductive materials is expected to affect the properties of the digestate during the ACoD process by changing the characteristics of the co-digestate, promoting organic degradation, influencing enzyme activities, and modifying the microbial community and structure (Liu *et al*., 2021). Within this match, it is possible to involve the biochar as an additive to generate a better methane-rich biogas. Before dealing with the mechanisms involved into the whole AD processing match, it is relevant to remember that biochar, a material originally known as Terra Preta (Bezerra *et al*., 2019), is formed by the carbonization of waste biomass in an oxygen-free environment or restricted oxygen conditions through specific thermochemical approaches under a wide range of temperature (180-1500°C) (Liu *et al*., 2015), such as pyrolysis (Mumme *et al*., 2014), gasification (Indren *et al*., 2020), hydrothermal carbonization (HTC) Choe *et al*., 2019), and hydrothermal liquefaction (Ren *et al*., 2020). However, the present review paper deals with the pyrolytic biochar with a brief note on hydrochar (from HTC process). Biochar is a typical carbon-rich solid material, specifically it is a conductive carbonaceous. It possesses several significant characteristics that are similar to those of other carbon-based additives, such as activated carbon– (Chiappero *et al*., 2020). As stated by (Sanei *et al*., 2020), the biochar production was initially created and suggested mainly as an additive for soil enhancement aimed at capturing carbon. However, its beneficial properties quickly led to investigations into its possible uses in composting, anaerobic digestion (AD), soil cleanup, and treating wastewater, among other areas (Osman *et al*., 2022).

In the realm of AD, the application of biochar has been linked to numerous benefits: it enhances pH-buffering capacity, aids in the adsorption and breakdown of harmful substances, supports microbial biofilm development, fosters interspecies electron transfer, promotes interrelationships among anaerobic microbes, allows for in situ biogas purification through the capture of CO_2 and H_2S, facilitates the biological transformation of CO_2 into CH_4, and enhances the quality of the digestate used as fertilizer (Chiappero *et al*., 2020; Kumar *et al*., 2021; Nie *et al*., 2024; Zhao *et al*., 2021). These processes, when combined together, can enhance the stability and efficiency of the anaerobic digestion (AD) system, leading to higher methane production and purity, while also decreasing reliance on biogas post-treatments. In the next work-sections a brief literature review will be presented by focusing on the main role and mechanisms behind biochar enhancement methane-rich biogas production in anerobic digestion (AD) process. The present work has been divided into four main sections, as follows:

- **Biochar**: Production, properties and application
- **Role of biochars on methanogenic pathway during the AD process**: Biochar effect on methane yield
- Impact of biochar on methanogenic archae and direct electron transfer (DIET)
- Conclusions and future outlooks.

Biochar

Biochar Production

As stated by (Kather *et al*., 2024 and Kan *et al*., 2016), by undergoing carbon-rich conversion processes, such as pyrolysis, biochar is created from biomass and is considered to be a highly carbonated material that breaks down organic materials at high temperatures (300-700 °C) in an oxygen-free environment. The nature of the feedstock, operational conditions (such as temperature and heating rate), along with other factors, can affect the yield and characteristics of biochar through various factors including feedstock type (e.g., moisture level or particle size) and the processing atmosphere (type and flow rate of the carrier gas) (Tripathi *et al*., 2016). The temperature during the pyrolysis process is strategic to shaping the physical and chemical properties of the final biochar product. Generally, as the temperature rises, the surface area and porosity of biochar also increase due to the breakdown of aliphatic alkyl and ester groups (Bonelli *et al*., 2007; Chen and Chen, 2009). At elevated temperatures, the amount of volatile substances released diminishes, resulting in biochar that typically has a higher pH, increased ash content, enhanced porosity, and reduced volatile matter these features are due to significant organic matter decomposition (Shaaban *et al*., 2014; Tomczyk *et al*., 2020).

Recently, new methods for producing carbon-rich materials have been developed, including the creation of hydrochar via hydrothermal carbonization (HTC). The creation of hydrochar occurs under low temperature (180-250 °C) and in humid conditions where the pressure is self-generated (Cao *et al*., 2021; Lu *et al*., 2012). Unlike traditional pyrolysis, hydrothermal carbonization (HTC) presents several benefits, such as lower energy use, decreased emissions, and the elimination of the necessity to dry the feedstock before processing. This approach results in a higher product count with lower energy consumption (Kambo *et al*., 2015). Temperature and time of reaction, as well as pressure or temperature alone, are the main determinants of HTC, along with feedstock ratio and catalyst usage (Sharma *et al*., 2020). As demonstrated by (Fang *et al*., 2018), the resulting hydrochar can be utilized as an economical adsorbent to eliminate heavy metals, organic contaminants, phosphates, and pathogens from wastewater. Fig 1 shows the main thermochemical processes aimed at producing biochar and its characteristics.

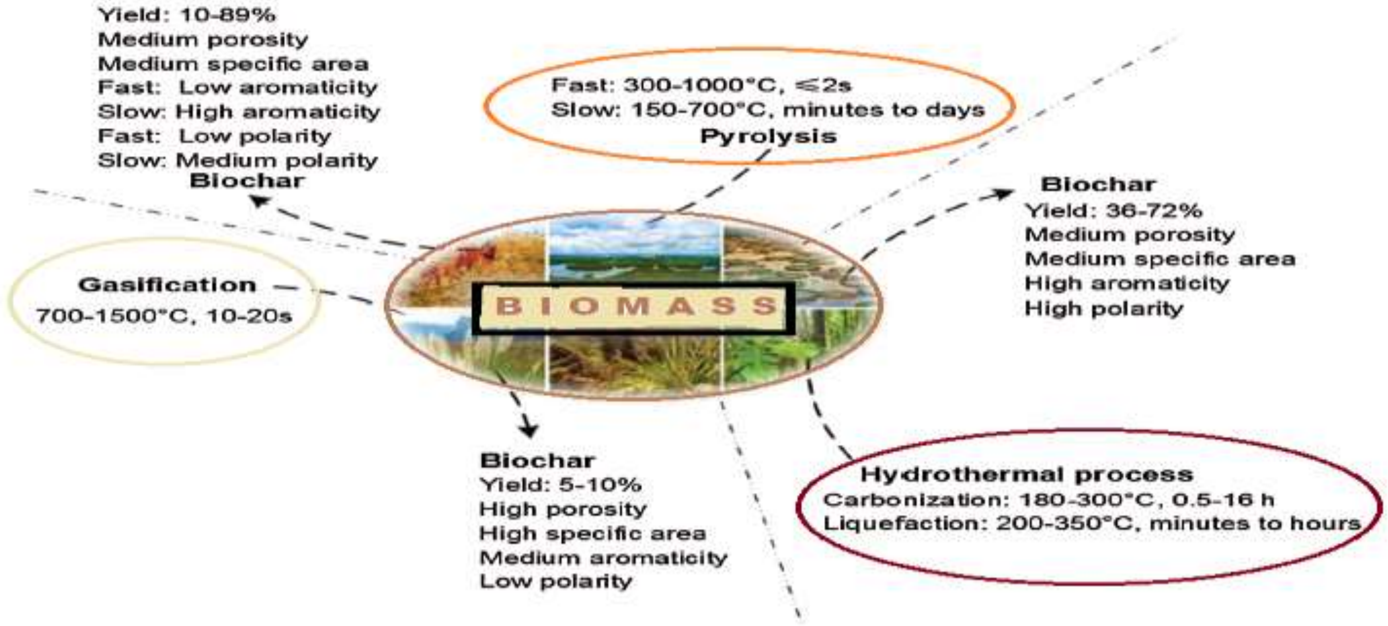


Fig 1. Biochar production through the main thermochemical processes and its characteristics (Adapted from Igalavithanaa *et al*., 2018)

Biochar Properties and Characteristics

Biochar generally contains more than 60% carbon, in addition to other important elements such as nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) (Yuan *et al*., 2011). Moreover, as greatly outlined by (Ahmad *et al*., 2014), it is known for its high porosity and large surface area, which allow it to absorb and hold water, nutrients, and pollutants effectively. Variations in the source materials and pyrolysis temperature lead to significant changes in the physical and chemical characteristics of biochar, impacting its surface area, pore distribution, and elemental makeup (Pariyar *et al*., 2020). In addition, as shown by (Waheed *et al*., 2023) high-quality biochar is often produced from agricultural residues, which are abundant and commonly used sources. The elemental composition of biochar is altered by the temperature at which pyrolysis takes place, with an increase in magnesium (Mg), calcium (Ca), and phosphorus (P), while decreasing carbon and nitrogen (N) levels (Mendez *et al*., 2013; Yuan *et al*., 2014). These alterations have a direct impact on the potential uses of biochar in both agriculture and environmental settings. The internal structure of biochar is made up of three primary types of pores, as follows:

- micropores (50 nm)
- mesopores (2-50 nm)
- macropores (>50 nm)

As reported by (Chen *et al*., 2017), the role of macropores is to facilitate the diffusion of substances, while mesopore are used as channels for mass transfer, with micropore pores providing sites for adsorption of small molecules and ions. The amount of biochar produced is significantly influenced by the method of pyrolysis used. Fast pyrolysis, which has a retention time of approximately 0.1 to 0.3 seconds and a heating rate between 10 and 200°C per second, usually results in 15 to 20% biochar yield. In contrast, slow pyrolysis, characterized by a retention time of 15 to 30 minutes and a heating rate of 0.1 to 1°C per second, can generate up to 35% biochar. As still shown by (Rangabhashiyam *et al*., 2022), although slow pyrolysis is capable of yielding more biochar, higher temperatures often lead to a decrease in overall yield due to the loss of water, the thermal breakdown of organic materials, and the development of stable aromatic structures.

Biochar Application

As highlited by (Bridgwater *et al*., 1999), biochar is commonly utilized in farming because of its ability to bind substances. Being very porous, it lowers soil weight, improves air flow, and increases water holding capacity in the soil. When added to the ground, biochar enhances soil health by aiding in the natural capture of carbon (Jha *et al*., 2016). Thanks to its large surface area and the capacity to exchange cations, biochar can absorb both organic and inorganic materials (Ali *et al*., 2017). This property makes biochar a cost-effective and efficient material for cleaning water and wastewater. Additionally, making biochar helps with waste recovery and reduces harmful environmental effects, like greenhouse gas emissions (GHGs) (Khaters *et al*., 2024).

As a soil improvement agent, biochar provides an eco-friendly way to enhance soil characteristics, particularly in poor and damaged soils (Yan *et al*., 2024). Its use promotes sustainable growth and fits within the concept of a circular economy (Roberts *et al*., 2009). The mechanisms related to adsorption of biochar aimed at adsorbing organic and inorganic pollutants are diverse and rely on its physical and chemical traits, such as the amount used, the temperature during production, and the acidity of the environment. The methods for removing heavy metals include attractions through electric charges, exchanging ions, filling pores, creating stable compounds, and transformations that lead to adsorption (Inyang *et al*., 2016). Besides heavy metals, biochar is also capable of adsorbing organic waste. As stated by (Ambaye *et al*., 2011), this occurs through processes like filling pores, interactions based on water-repelling properties, partitioning, electric charge interactions, and electron donor-acceptor (EDA) interactions.

Role of Different Biochars on Methanogenic Pathway during the AD Process

Biochar's Feedstock Sources and Dosage Effect on Methane (CH_4) Yield

Several experimental studies demonstrated that biochar produced from different materials, such as tree branches, straw, coconut shells, and sewage sludge was applied to improve the anaerobic digestion of wastewater from liquor, leading to a major increase in methane (CH_4) production (from

286.23 mL to between 1305.12 and 1585.54 mL) and in the efficiency of removing soluble chemical oxygen demand (sCOD) (which went up from 23.11% to between 65.88% and 91.92%) (He *et al*., 2024; Li *et al*., 2022). The amount of lignin in the feedstock influenced both the surface area of the biochar and its ability to give up electrons, which were strongly linked to the total methane produced ($r = 1$, $p < 0.05$) (Qin *et al*., 2020). A higher surface area encouraged the growth of microorganisms, and the electron-donating ability (ECA) helped with the transfer of electrons between different species (Qin *et al*., 2020). The biochar increased the amounts of Firmicutes, Synergistota, and Proteobacteria, which play roles in breaking down substances and producing acids, improving metabolic processes like breaking down fatty acids and aromatic compounds. Synergistota and Proteobacteria contain bacteria that can transfer electrons and may enhance direct interspecies electron transfer (DIET) with Methanosaeta and Methanobacterium, supporting the DIET process (Wang *et al*., 2025). For example, biochar modified with iron (Fe), produced from discarded tea leaves, resulted in a 21.9 % increase in methane generation (He *et al*., 2024).

In addition, as stated by (Wu *et al*., 2023) and demonstrated by (Wang *et al*., 2025), biochar - that was co-modified with S-N - greatly improved the anaerobic digestion of potent landfill leachate, resulting in a 40 % boost in methane output and a 31.6 % rise in chemical oxygen demand removal effectiveness. The chemical makeup of the starting materials influences the physical and chemical characteristics of the resulting biochar, thereby affecting its performance in anaerobic digestion applications. Qin *et al*. (2020) found out that wood-derived biochars provided a greater increase in methane output compared to the ones from herbage in anaerobic digestion systems. Likewise, (Indren *et al*., 2020) noted a 32% rise in methane yield from wood pellet biochar, while biochars from wheat straw and sheep manure limited biomethane production (Sun *et al*., 2022; Li *et al*., 2022). Zhang *et al*. (2019) examined three types of biochars (those from corn straw, coconut shells, and sludge) within anaerobic digestion (AD) systems. The outcomes of their research confirmed a notable improvement ranking: biochar from coconut shells exhibited the most significant impact ($p < 0.05$), surpassing both biochars from corn straw and sludge (Jiang *et al*., 2022). Together, these investigations reveal the enhanced effectiveness of lignocellulosic biochars obtained from woody or herbaceous resources. These substances encompass considerable amounts of lignin, cellulose, and hemicellulose elements that are largely lacking in non-lignocellulosic substances such as sewage sludge. This difference in composition implies that the presence of lignocellulose plays a crucial role in the performance of biochar. As stated by (Chiappero *et al*., 2022), the integration of biochar into anaerobic digestion improves the efficiency of oxygen use and shortens the lag phase of methanogenesis, ultimately resulting in a higher methane output. Although there are some existing challenges, utilizing biochar as a pre-treatment option helps mitigate these issues and further enhances methane (CH_4) production.

The benefits of integrating biochar into an anaerobic digestion system are evident in its characteristics, which include a large surface area conducive to biofilm formation, the ability to adsorb inhibitory substances like ammonium, and a capacity for pH buffering (Fagbohungbe *et al*., 2017). An interesting research work by (Zhou *et al*., 2020) demonstrated that biochar derived from cornmeal resulted in a 26.2% increase in methane (CH_4) production. The findings indicated that methane output could be substantially elevated (218.45 L per kg of volatile solids) when using biochar sourced from corn straws. Biochar is stable and serves as a nutrient source for methanogens throughout the anaerobic digestion process, which subsequently leads to higher methane (CH_4) production (Jang *et al*., 2017). However, the observed increase in methane yield should be assessed from various perspectives, including the type of biochar employed, temperature of pyrolysis and dosage. Besides the biochar type, the amount of biochar introduced into the anaerobic digestion system (ADS) significantly influences the results (Zhang *et al*., 2019). Research indicates that elevated doses of biochar (beyond 10 g/L) may have inhibitory effects, resulting in a decrease in methane (CH_4) yield. The possible explanation for this phenomenon is that a moderate application of biochar can effectively reduce the buildup of volatile fatty acids (VFAs), thereby enhancing methanogenic activity.

Conversely, excessive biochar may result in the accumulation of propionic acid, which can disrupt system stability and decrease CH_4 production, as shown in fig 2. Specifically, when propionic acid levels exceed 937.1 mg/L, the rate of propionic acid acetogenesis declines, leading to reduced methane output (Amani *et al*., 2011). For example, (Torri and Fabbri, 2014) identified that the ideal amount for boosting methane (CH_4) production in anaerobic digestion is 10 g/L.

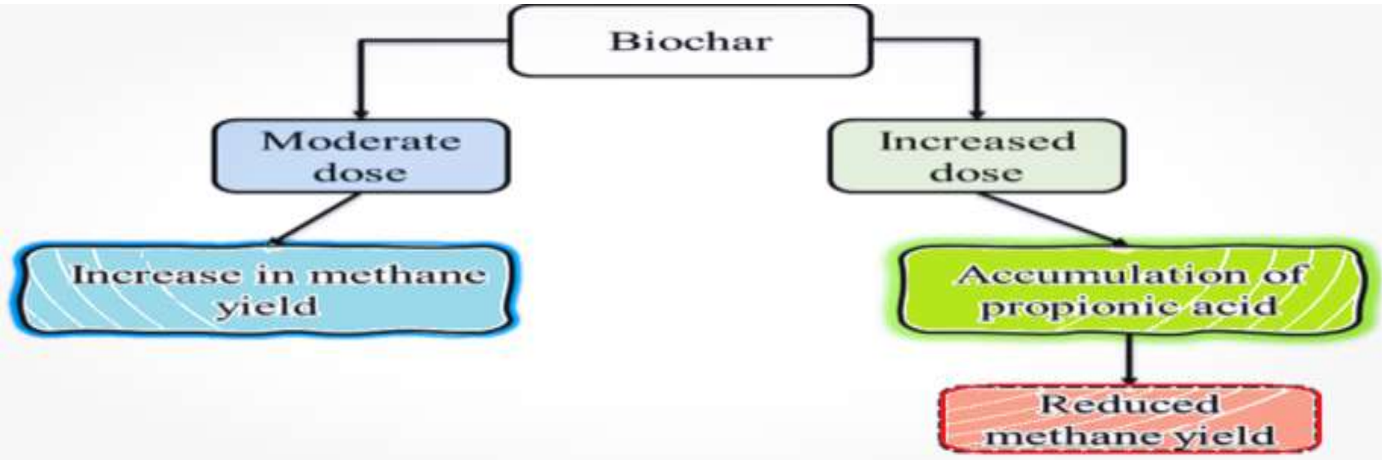
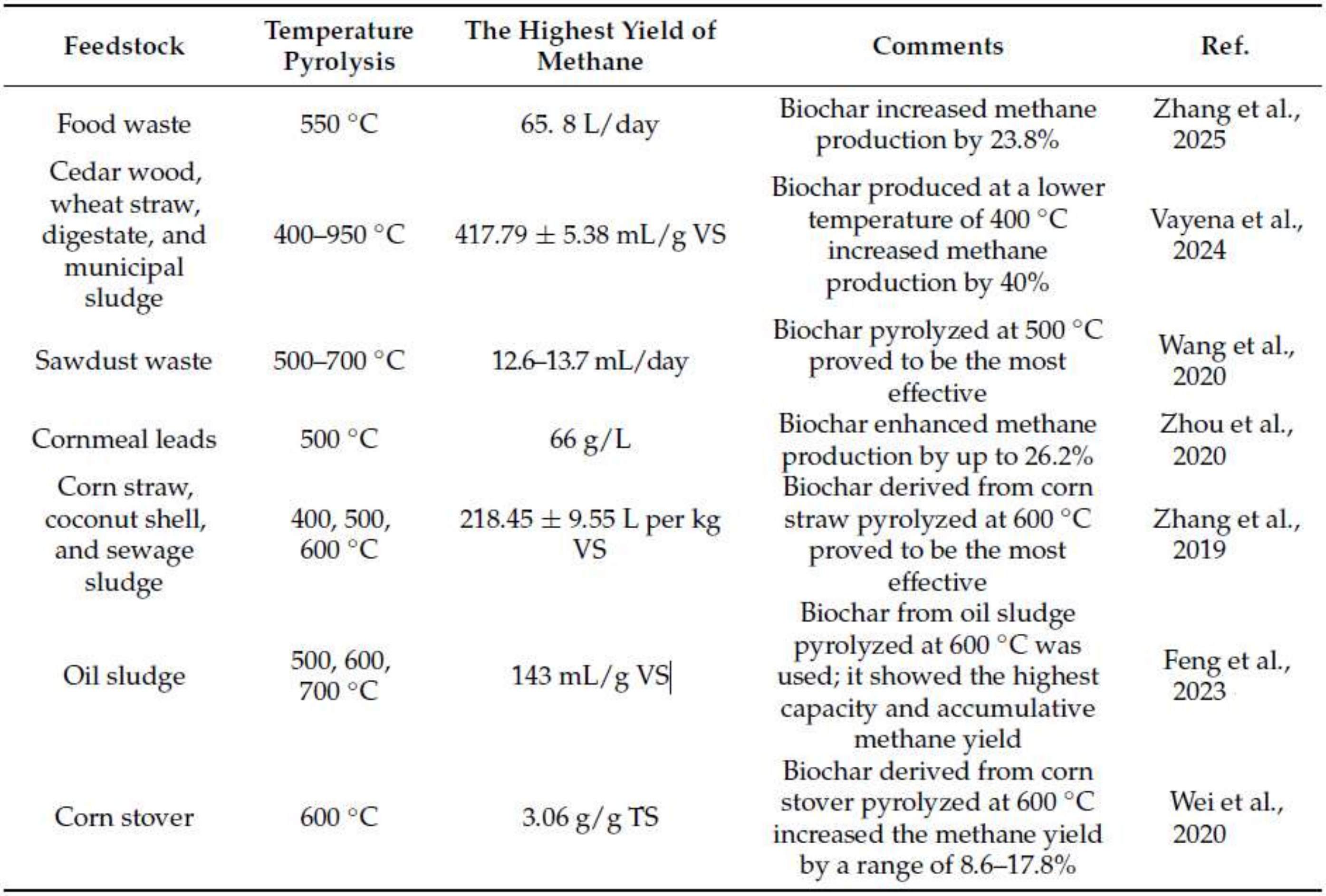


Fig 2. Effect of different biochar dosages on CH4 yield [Adapted from Zhang *et al*., 2019]

Table 1. The main correlation between biochar feedstock sources and pyrolysis temperature to reach the highest methane (CH_4) yield. [Adapted from Zhang *et al*., 2025; Vayena *et al*., 2024; Wang *et al*., 2020; Zhou *et al*., 2020; Zhang *et al*., 2019; Feng *et al*., 2023; Wei *et al*., 2020]



Feedstock	Temperature Pyrolysis	The Highest Yield of Methane	Comments	Ref.
Food waste	550 °C	65. 8 L/day	Biochar increased methane production by 23.8%	Zhang et al., 2025
Cedar wood, wheat straw, digestate, and municipal sludge	400–950 °C	417.79 ± 5.38 mL/g VS	Biochar produced at a lower temperature of 400 °C increased methane production by 40%	Vayena et al., 2024
Sawdust waste	500–700 °C	12.6–13.7 mL/day	Biochar pyrolyzed at 500 °C proved to be the most effective	Wang et al., 2020
Cornmeal leads	500 °C	66 g/L	Biochar enhanced methane production by up to 26.2%	Zhou et al., 2020
Corn straw, coconut shell, and sewage sludge	400, 500, 600 °C	218.45 ± 9.55 L per kg VS	Biochar derived from corn straw pyrolyzed at 600 °C proved to be the most effective	Zhang et al., 2019
Oil sludge	500, 600, 700 °C	143 mL/g VS	Biochar from oil sludge pyrolyzed at 600 °C was used; it showed the highest capacity and accumulative methane yield	Feng et al., 2023
Corn stover	600 °C	3.06 g/g TS	Biochar derived from corn stover pyrolyzed at 600 °C increased the methane yield by a range of 8.6–17.8%	Wei et al., 2020

While moderate biochar applications aid in methane generation, overly large amounts may adversely affect the system. Additionally, as illustrated by (Shi *et al*., 2022), increased biochar quantities can impede the conversion of organic matter into butyrate, resulting in a 32.5% reduction in methanogenesis efficiency.

Correlation among Biochar Feedstock Sources, Pyrolysis Temperature and CH_4 Yield

Parameters such as heat transfer, temperature, and retention time affect the properties of biochar during pyrolysis, which can impact anaerobic digestion. Temperature is regarded as a crucial factor that influences the properties of biochar (Vayena *et al*., 2024). Both the temperature of pyrolysis and the type of feedstock biomass can greatly influence the production of methane during anaerobic digestion. Numerous experiments have been carried out to explore the relationship between pyrolysis temperature and the properties of biochar. In this regard, table 1 indicates the main correlation between biochar feedstock sources and pyrolysis temperature aimed at assessed the highest methane (CH_4) yield with specific comments. The findings reported in table 1 indicate that an increase in temperature results in a greater specific surface area of biochar, a lower cation exchange capacity, an elevated pH, a decrease in yield, and an increase in carbon fractions (Luo *et al*., 2015). Biochar produced at various pyrolysis temperatures exhibits different levels of carbonization, with a highly ordered structure emerging when the pyrolysis temperature surpasses 400°C (Mumme *et al*., 2014).

The influence of pyrolysis temperature on biochar properties can be observed from multiple perspectives. At lower temperatures (300-600°C) than usual, biochars with redox-active surface functional groups exhibit increased electron exchange. On the other hand, as demonstrated by (Devi and Eskicioglu, 2024), electrical conductivity plays a major role in electron exchange in biochars that are exposed to higher temperatures (400-700°C) Therefore, it is essential to establish a relationship between the pyrolysis temperature of biochar and methane (CH_4) yield. In the research conducted by (Wang *et al*., 2018), biochar pyrolyzed at various temperatures (300-700°C) was examined, with biochar pyrolyzed at 500°C demonstrating the highest effectiveness.

Among the samples, biochar produced at 500°C was found to be the most effective. While biochar produced at 700°C exhibited higher electrical conductivity, it was less efficient compared to sweep biochar. This can be attributed to the presence of numerous redox-active functional groups in the biochar produced at 500°C (Wang *et al*., 2018). High pyrolysis temperatures may result in the emission of toxic substances that can hinder anaerobic digestion, whereas lower pyrolysis temperatures may not adequately stabilize the biomass, resulting in biochar that promotes methane production due to residual biodegradable organic matter. This is further supported by findings from a study indicating that wood-based biochar (BCW800), which underwent high-temperature gasification, reduced the anaerobic digestion process by 52% in terms of methane production at an addition rate of 15 g/L, accompanied by an 18% decrease in methanogens and microbial diversity.

In cases where biochar still contained residual biodegradable organic matter owing to incomplete pyrolysis (BC400), an increase in biogas yield reaching 417.79 ± 5.38 mL/g VS was recorded (Vayena *et al*., 2024). Through an examination of the existing research literature, various raw materials suitable for biochar creation were identified. Nonetheless, there are some discrepancies in the effectiveness of anaerobic digestion. Wood-derived biochar has shown to be more effective at generating methane in comparison to biochar that comes from agricultural waste (Devi and Eskicioglu, 2024). The biochar produced from wood demonstrates characteristics such as an elevated specific surface area of 253.39 m^2/g and an electron-donor capability measured at 0.019 ± 0.0002 μS/cm (Devi and Eskicioglu, 2024). Conversely, biochar generated from agricultural residues is rich in nutrients that are essential for the growth of microorganisms (Chen *et al*., 2023). It is important to take into account both the type of feedstock and the temperature of pyrolysis collectively, as they influence the physical and chemical traits of the biochar produced. In a specific research investigation, the production of methane (CH_4) was assessed using biochar derived from various types of biomass, including corn straw, coconut shell, and sewage sludge, which were subjected to pyrolysis at varying temperatures.

Findings revealed that the origins of biomass and the specific temperatures involved in biochar creation significantly enhanced methane generation. The highest methane (CH_4) output was recorded at 218.45 ± 9.55 liters per kilogram of volatile solids (VS) for the CS600 sample, followed by SS500, CS500, CS400, CCS600, SS600, SS400, CCS400, and CCS500, with control groups showing cumulative methane (CH_4) production of 207.49 ± 7.29, 195.77 ± 6.92, 184.12 ± 9.69, 174.44 ± 7.72, 165.85 ± 8.02, 155.86 ± 8.19, 143.85 ± 8.92, 125.50 ± 9.36, and 117.36 ± 8.96 liters per kilogram of VS, respectively. The CS600 treatment was identified as the most effective, enhancing yield by 86.14%. Among all types of biochars, the one generated from sewage sludge exhibited the most significant increase in ash content, reaching 42.2%.

Additionally, it was noted that as the pyrolysis temperature rose, so did the ash content. Biochar produced from corn may contain a greater abundance of nutrients, which can foster the growth of microorganisms that promote anaerobic digestion (AD). An elevation in pyrolysis temperature leads to a rise in the specific surface area of biochar, the elimination of volatile compounds, and a growth in biochar's pore structure (Zhang *et al.*, 2019). Moreover, a greater specific surface area of biochar supports the proliferation of microorganisms (Luo *et al.*, 2015). In a separate investigation involving corn stover as biomass subjected to pyrolysis at 600°C, CH_4 production was found to increase between 8.6% and 17.8% (Wei *et al.*, 2020). Sewage sludge contains a significant quantity of inorganic materials, which are transformed into biochar throughout the pyrolysis process (Agrafioti *et al.*, 2013; Qambarani *et al.*, 2017).

In a study conducted by (Feng *et al.*, 2023), biochar derived from oil sludge pyrolyzed at 600°C demonstrated the highest efficiency, yielding a total methane (CH_4) output of 143.96 mL for each gram of VS. Consequently, the source of biomass as well as the pyrolysis temperature plays crucial roles in determining the characteristics of biochar, including its specific surface area and ash content. From the information provided, it can be inferred that a pyrolysis temperature of 600°C along with food waste biomass is optimal for generating biochar aimed at enhancing anaerobic digestion (AD).

Impact of Biochar on Methanogenic Pathways

Impact of Biochar on Functional Microbial Activity and Structures

Earlier research showed that biochar significantly improved the activity of microbes involved in methane production by boosting functional bacteria and methanogens in anaerobic digestion (Li *et al.*, 2018; Qin *et al.*, 2017). Zhang *et al.* (2017) noticed that biochar encouraged the release of extracellular polymeric substances, which play a crucial role in helping microorganisms stick to surfaces during biofilm development (Sheng *et al.*, 2010). Therefore, adding biochar supported the growth of attached microbes, resulting in faster sludge granulation and potentially solving the problem of methanogen loss in anaerobic digesters. Similarly, (Sun *et al.*, 2016) confirmed that biochar served as an effective carrier that notably increased the number of microbes in anaerobic digestion.

Specifically, (Dang *et al.*, 2016) discovered that biochar increased the population of Sporanaerobacter and Enterococcus, which can break down fermentable materials and pass electrons to Methanosarcina species that also increased with biochar addition. Indren *et al.* (2020) noted that Methanosaetaceae preferentially attached to biochar, establishing a biological interaction that could shorten the lag time. These findings were in line with research that suggested biochar aided in enhancing methanogens by enriching Methanosarcinales (Lü *et al.*, 2016). At the level of the genus, Methanosarcina was the most common type of methanogen, followed closely by Methanosaeta, Methanobacterium, and Methanospirillum (Shanmugam *et al.*, 2018). The bacteria Methanosarcina and Methanosaeta are known for breaking down acetate to produce methane (CH_4). In samples that included biochar, Methanosarcina made up a larger percentage, ranging from 65.97% to 75.93%, whereas in the control group, this percentage grew from 57.92% to 64.4% over a period of 34 days (Wang *et al.*, 2018). The significance of Methanosarcina is highlighted by its various methanogenic pathways, which enable it to generate methane (Liu *et al.*, 2012). An interesting research work by (Sugiarto *et al.*, 2021) demonstrated that biochar with iron plays a crucial role in biogas production, particularly by fostering the growth of Clostridia.

Higher growth rates of Clostridia help convert more substance into acetic acid and butyric acid, which increases biogas production. Additionally, there was a rise in the number of Methanosaeta. Methanosaeta is a methanogen that converts acetic acid into methane. This statement is backed by a study conducted by (Qi *et al*., 2021), which found that adding biochar resulted in a four-fold rise in Methanosaeta species compared to the control. Furthermore, it was observed that more Methanobacterium was present in the control group than in the biochar sample, suggesting that adding biochar encourages the growth of more acetoclastic methanogens than those that rely on hydrogen. This increase is connected to their attachment to the biochar particles, which are very porous and support biofilm development (He *et al*., 2024). Wang *et al*. (2018) indicated that an increased dosage of hydrochar (carbon-rich material derived from Hydrothermal Carbonization (HTC)) is beneficial for the attachment of methanogenic bacteria. This finding supports the observation that hydrochar promotes the growth of the acetoclastic methanogen Methanosaeta (Ren *et al*., 2020). Consequently, adding hydrochar could facilitate the conversion of volatile fatty acids (VFAs) into methane by helping to immobilize methanogens (Xu *et al*., 2018).

Furthermore, biochar also improved the production of VFAs by promoting biofilm development in the early stages of anaerobic digestion (AD) (Yin *et al*., 2019). This observation further suggests that biochar has the potential to enhance AD through the enrichment of functional microorganisms. Conversely, a previous study carried out by (Aragón-Briceño *et al*., 2017) noted that hydrochar may also have an inhibitory effect that could suppress methanogenic metabolism. The impact of biochar on microbial growth is highly dependent upon its physical properties. Biochar possesses a high surface area (greater than 300 m^2/g), making it an excellent medium for microbial growth and settlement (Zhang *et al*., 2017). As reported by (Cruz Viggi *et al*., 2017), the increase in microorganisms on biochar has resulted in improved anaerobic processing of organic waste. Reports indicate that biochar features pore sizes ranging from 1 to 40 μm, suggesting that both micro and macropores can accommodate 2 to 10 methanogenic cells (Huggins *et al*., 2016; Lü *et al*., 2016).

Consequently, the appropriate pore size of biochar is essential for the development of biofilms and granulation (Zhang *et al*., 2017). Biochar can enhance the presence of Methanosaeta, which plays a crucial role at the beginning stages of methanogenesis (Lü *et al*., 2016). Methanosaeta, which prefer areas associated with biochar, can utilize acids that diffuse into the biochar pores, thereby continuing the acid degradation process initiated by Methanosarcina (Lü *et al*., 2013). Furthermore, the ability of methanogens to access the pores in biochar is associated with the structure of the microorganisms (Lü *et al*., 2016). Biochar encourages the colonization of Methanosaeta and Methanosarcina on the outer layer, while smaller Methanoculleus predominantly colonize the inner pore regions of the biochar matrix.

The conductive properties of biochar may further aid in the selective increase of microbial populations. A research activity conducted by (Yu *et al*., 2015) observed that biochar served as an electron acceptor for microbial extracellular respiration and growth, indicating that biochar's role as an electron acceptor for various electroactive microorganisms may be a common characteristic. It is believed that biochar enhances the presence of electro-active microbial communities and fosters a more effective metabolic interaction between bacterial and archaeal groups, leading to improved performance in anaerobic digestion (AD) (Martinez *et al*., 2018).

This type of enrichment of syntrophic microbes, such as Anaerolineaceae and Methanosaeta, through the addition of biochar was also documented by (Wang *et al*., 2018), which in turn enhanced the degradation of volatile fatty acids (VFAs) and increased methane production during the AD process involving complex organic waste, specifically a mixture of dewatered activated sludge and food waste. Li *et al*. (2018) indicated that this phenomenon might have been caused by the selective succession of bacteria and methanogens that were shown to be involved in direct interspecies electron transfer (DIET). Consequently, the introduction of biochar facilitated the formation of biofilms, which enhanced methane (CH_4) production. These observations further highlighted the benefits of biochar in improving anaerobic digestion (AD).

However, it is essential to note that drawing definitive conclusions regarding the effects of biochar conductivity on microbial enrichment during AD remains challenging. Cheng *et al*. (2018) discovered that the conductivity of biochar had a weak correlation with methane (CH_4) production in swine wastewater AD. Sunyoto *et al*. (2017) suggested that biochar could aid microbial metabolism and growth through its carbon-rich biodegradable content, thereby fostering the development of methanogenic biofilms during the AD of food waste. These findings illustrated that factors other than biochar conductivity such as nutrient content, adsorption capacity, pH level, and surface characteristics significantly accounted for its impact on microbial enrichment in short-term batch AD under specific conditions.

Impact of Biochar on Methanogenic Archae and Direct Interspecies Electron (DIET) Acceleration

Biochar, while it has lower conductivity than granular activated carbon (GAC), serves as a habitat for the growth of beneficial microbes, such as those involved in direct interspecies electron transfer (DIET). This can lead to enhanced formation of aggregates and improved electron transfer properties (Wang *et al*., 2018). Syntrophic microorganisms, such as Geobacter metallireducens and Geobacterios sulfurreducents (which contain high levels of c-type cytochrome), are capable of producing aggregates that allow for electron transfer in anaerobic digestion systems lacking carbon-rich materials. However, it was noted that Geobacter metallireducens tends to adhere closely to conductive materials and does not aggregate as effectively in the presence of these materials when compared to environments without carbon-rich inputs, where microorganisms achieve aggregation and establish cellular connections to facilitate electron transfer (Summers *et al*., 2010; Baek *et al*., 2018). This indicates that conductive materials, including biochar, may be beneficial for promoting electron transfer (Baek *et al*., 2018). The electron transfer processes catalyzed by biochar may involve two types of redox structures: quinone-hydroquinone units and/or the conjugation of π electron systems linked to the non-bonded aromatic structures present in biochar (Wang *et al*., 2018; Klupfel *et al*., 2014; Xu *et al*., 2013).

As outlined by (Zhang *et al*., 2018), quinine moieties, phenolic moieties, and arene rings present in biochar may serve as redox-active components that contribute to its ability to accept electrons. Biochar produced at lower pyrolysis temperatures contains a greater amount of redox-active elements capable of engaging in the electron transfer process. Specific studies indicate that the conductivity of biochar does not correlate with its influence on the metabolic activities of Geobacter metallireducens and Geobacter sulfurreducens co-cultures; rather, the electron transfer relies on the charging and discharging processes of the biochar's surface. The surface functional groups of biochar can reversibly accept and donate electrons through charge and discharge cycles. Therefore, it can be concluded that incorporating biochar may enhance direct interspecies electron transfer (DIET) between syntrophic acetogenic and methanogenic communities, mitigate system acidification, and facilitate methane production (He *et al*., 2018). One particular study found that the addition of hydrochar encourages the growth of microorganisms that are conducive to direct interspecies electron transfer, including Peptococcaceae, Methanosaeta, and Methanobacterium (Wu *et al*., 2019).

Conclusions and Future Outlooks

It was observed that biochar is characterized by a huge potential to be applied as an AD additive in order to enhance the AD processes efficiency by overcoming toxic compound inhibitions, facilitating DIET and enriching strong microbial communities. In addition, from a biorefinery perspective, integration between the AD system and the thermochemical conversion of biochar could result a great strategy enable to efficiently manage organic waste and, at the same time, become a source enable to generate biofuels as well: these dynamics could offer a greater improving of the AD processes in its own whole system. Such a valuable and sustainable perspective, biochar can represent an efficient linkage between thermochemical and biochemical conversions and transformations as well. An interesting future outlook may focus on the biochar design and production with preferred and selected properties and characteristics by specific activation technologies and approaches with targeted synergistic tools and systems aimed at obtaining and reaching particular enhancement with other additives and compounds.

Moreover, targeted investigations on the microbial metabolic mechanisms with the biochar as an additive, and the optimization and scaling-up of biochar incorporated into an AD reactor for large-scale continuous conditions are required.

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