sustainability

MDPI

Article

Effects of Aloe Vera and Algae-Derived Biochars and Hydrochars on Mesophilic Anaerobic Digestion of Food Waste

Angeliki Maragkaki [1,*], Pelagia Panteli [1], Thrassyvoulos Manios [1] and Dimitrios Kalderis [2]

[1] Department of Agriculture, School of Agricultural Science, Hellenic Mediterranean University, 71401 Crete, Greece; pelapanteli@gmail.com (P.P.); tmanios@hmu.gr (T.M.)
[2] Laboratory of Environmental Technologies and Applications, Department of Electronics Engineering, Hellenic Mediterranean University, 73100 Chania, Greece; kalderis@hmu.gr
* Correspondence: amaragkaki@hmu.gr

Abstract

Biochar (BC) and hydrochar (HC) are carbon-rich materials derived from organic wastes through pyrolysis/gasification and hydrothermal carbonization (HTC), respectively, offering promising pathways for waste valorization and resource recovery within a circular economy framework. Owing to their porous structure and surface functionality, these materials have gained attention as additives in anaerobic digestion (AD), where they may enhance the microbial activity, improve the buffering capacity, and facilitate direct interspecies electron transfer (DIET), resulting in greater process stability and higher methane (CH_4) yields. This study evaluated the effect of BC and HC derived from aloe vera leaves and algae on methane production during the AD of food waste (FW). Batch experiments were conducted under mesophilic conditions (37 °C) for 60 days, using a 1:1 inoculum-to-substrate ratio and a dosage of 10 g L^{-1} of each carbonaceous material. The results show that adding BC increased cumulative biogas production by 10–14%, while HC led to an 18–35% increase compared with the control. Additionally, dissolved chemical oxygen demand (d-COD) removal improved by up to 30%, and volatile solids (VSs) removal rose by 31% in the FW and aloe HC reactors, highlighting the greater enhancement potential of HCs in methane production. Overall, the findings demonstrate that integrating carbonaceous materials derived from organic residues into AD systems can enhance bioenergy recovery while reducing environmental impacts, supporting more sustainable and circular waste-to-energy processes.

Keywords: circular economy; waste valorization; anaerobic digestion; biochar; hydrochar; biogas production; pyrolysis; food waste treatment; DIET

Check for updates

Academic Editor: Agostina Chiavola

Received: 27 February 2026
Revised: 21 March 2026
Accepted: 3 April 2026
Published: 10 April 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Because of the growing global energy demand, environmental concerns about greenhouse gas emissions, and the limited supply of energy from non-renewable fossil fuels, many academics have concentrated on discovering sustainable options for fuel production in recent decades [1]. On the other hand, technologies that use renewable raw materials and are based on the biological carbon cycle can help achieve carbon neutrality, which is when the amount of carbon emitted and absorbed by carbon sinks is equal. For instance, the creation of hydrochar and biochar can help achieve this objective because they are made from renewable organic waste and have useful applications in the environment. Among its many uses, biochar and hydrochar can be used as fuel, adsorbents, soil conditioners, AD and composting additives, and catalytic precursors [2].

It is important to note that not all bacterial and archaeal species contribute to methane production in equal proportions. Methanogens and fermentative bacteria that are known to take part in DIET are displayed in summaries of microbial community analysis. For the archaeal community, these are *Methanosaeta*, *Methanobacterium*, *Methanospirillum*, and *Methanosarcina*, while for bacterial analysis, these are *Firmicutes*, *Proteobacteria*, *Synergistetes*, *Spirochetes*, and *Bacteroidetes* [3]. *Methanothrix* is a substantial contributor to worldwide methane emissions and is widely distributed in anoxic habitats, both natural and manmade. It is one of only two genera that when combined with exoelectrogens, may produce methane from DIET [4]. But there are still a lot of unidentified bacteria that are thought to promote DIET, and there are very few identifiable co-cultures that do so. Certain materials and boosters have favorable properties that have been found to increase methane production, reduce lag phase, and facilitate inhibitor adsorption through increased DIET. These properties include the specific surface area (SSA), cation exchange capacity (CEC), presence of functional groups (FGs), and electrical conductivity (EC) [3]. The conclusion is that one could improve the efficiency of AD by simply increasing DIET through additives that facilitate cell-to-cell electrical connections [5].

For this purpose, activated carbon, biochar, carbon cloth, carbon fiber, graphite, graphite felt, graphene, and carbon nanotubes are the most intriguing and thoroughly studied carbon-based materials [6]. Activated carbon appears to yield the greatest outcomes out of all of these. Activated carbon is characterized by its high conductivity, large specific surface area, and small particle size. By buffering the pH, enriching methanogenic bacteria, and increasing the rate at which CH_4 is produced, these features improve the efficacy of AD [7]. Zhang et al. (2019) [8] observed that pH values in digesters with biochar supplementation increase due to the alkaline nature of the biochar. Since the optimal pH for anaerobic digestion is reported to be 6.6–7.6 [9], digesters without biochar experience significant inhibition of methanogenic activity when the pH drops below 6.6, resulting in lower methane yields. Therefore, the presence of biochar maintains a more favorable pH range for microbial activity, enhancing the VFA degradation and improving the overall reactor stability. For instance, Zhang et al. [10] observed that the addition of activated carbon under high-temperature conditions enhances the abundance of *Methanosarcina* and boosted the methane generation by 150% when utilizing food waste as a substrate. In simple terms, activated carbon not only increases DIET but also affects the microbiological ecology in the bioreactor. However, activated carbon is a high-cost material, while recovery will also be needed, which raises, among others, an issue of complexity.

The solid carbonaceous substance known as BC is produced by thermochemically treating biomass without air or with little air. A variety of feedstocks, such as wood, herbaceous crops, agro-industrial waste, animal manure, and biosolids, can be used to create BC [11,12] using various technologies (pyrolysis, gasification, hydro-thermal carbonization) [12]. BC can be created with a variety of physicochemical properties and customized for particular uses by regulating the biomass feedstock, thermo-chemical operating conditions, and further activation. Since BC has the ability to improve soil quality and has a major impact on reducing greenhouse gas (GHG) emissions through carbon sequestration, there was initially a lot of interest in its agronomical applications. Other BC uses have recently been suggested, including as a supplement for AD or composting, a precursor for BC-based products or energy storage applications, and an adsorbent for pollutants in soil, water, and gaseous streams [13].

HTC at 180–250 °C produces HC, a carbon-based substance. With notable benefits like low energy usage, high yield, and low ash content, this process has recently become a very competitive product [14]. The polar surface functional groups, low porosity and

specific surface area of hydrochar have been reported to indicate a low adsorption ability, especially for non-polar organic matter [15].

Different physicochemical characteristics of biochar and hydrochar have a big impact on their possible uses. As the biomass feedstock goes through intricate chemical reactions like degradation, dehydration, and repolymerization under various reaction conditions (e.g., temperature, heating rate, duration, and pressure), they provide various chemical compositions and porosity features. As a result, they are easily differentiated. Reaction circumstances have a substantial impact on the yield and properties of biochar and hydrochar [16].

Because it affects which reaction mechanism predominates, the reaction temperature has major effects on the physicochemical characteristics, as well as the yields of hydrochar and biochar. Because hot water is available during the reaction, hydrothermal carbonization is carried out at a lower temperature than the pyrolysis process used to produce biochar, resulting in higher solid product yield, higher heating value (HHV) and lower carbon conversion [17,18]. Consequently, hydrochar exhibits higher atomic H/C and O/C ratios than biochar, reflecting its less thermally transformed and more oxygen-rich chemical structure [1].

Biochar may improve the AD performance by speeding up the DIET efficiency. According to Wang et al. [12], adding biochar enhances the highest rate of CH_4 generation from 4.0 mL d^{-1} to 10.4–13.9 mL d^{-1}. It is believed that these physicochemical properties—particularly surface area, conductivity, and functional groups, as described above—may contribute to the improvement in DIET efficiency when biochar is added [6]. Enough attachment sites are provided by the porosity and rough surface shape, which further reinforces microbial adhesion and indirectly supports DIET. Lü et al. [19] found that the electrical conductivity of biochar can increase *Methanosarcina's* attraction to the biochar's surface. Furthermore, the rate of AD is determined by the effectiveness of electron transfer between acidogenic and methanogenic bacteria. Although the basic mechanism of DIET via biochar is still unknown, the addition of biochar can speed up the process. According to some research, biochar may be employed as an electronic catheter to sustain the symbiosis between methanogenic and acidogenic bacteria, hence influencing the community of microorganisms [19]. According to other studies, the addition of oil sludge biochar enriches potential syntrophic microbes like the archaea *Methanothrix* and *Methanospirillum*, as well as the bacteria *Comamonas*, *Petrimonas*, and *Syntrophomonas*. This was likely due to the biochar's large surface area and microelements like iron, which improve the methane production [20]. In simple terms, the exact mechanism of the positive biochar addition and DIET increase, including the alteration of the microbial community structure, is still being investigated, while there is no clear correlation between all the physiochemical characteristics of the biochar and its effect on AD. Finally, one should bear in mind that the addition of such materials is always a potential risk. The main inhibitors found are a high organic loading rate (OLR), pH shocks, rapid accumulation and comparatively slow breakdown of volatile fatty acids (VFAs), and the presence of toxins and heavy metals.

Therefore, while biochar can facilitate DIET and enrich methanogenic species, its inorganic fraction must be evaluated carefully. Certain metals commonly retained in biochar, such as Fe, Ni, Co, and Mo, serve as essential micronutrients for methanogenic enzymes and support the activities of *Methanosarcina*, *Methanothrix*, and hydrogenotrophic methanogens [21]. However, other heavy metals, including Cd, Pb, Cr, and excessive Ni, are known to impair cell integrity, inhibit electron transport, and reduce methanogenic activity, ultimately suppressing the methane yield [22]. Thus, although biochar's conductive surface and catalytic minerals can accelerate DIET, its metal composition must be assessed to balance nutritional benefits with potential toxicity risks [23].

However, the limited published research on the impact of using BC and HC to increase biogas output is really interesting, despite a significant amount of work on mixing digestate with biochar and applying it to different cultivations, such as celery [24]. This could be due to the fact that small amounts are added or that most work is conducted in small-scale lab bioreactors. Qi et al. [25] present an interesting review on the subject but the information on organic pollutants like antibiotics is limited. According to Cheng et al. [26], adding biochar at a rate of 0.5 g L^{-1} can improve the amount of sulfonamide antibiotics removed in AD of synthetic swine effluent by more than 30%. Such enhancement may possibly be related to increased microbial activity with biofilm formation on the surface of biochar, in addition to adsorption, while also adsorbing sulfonamides and reducing their toxicity. In this context, synthetic swine effluent refers to a laboratory-prepared wastewater formulated to mimic the organic and nutrient composition of real swine manure, allowing experiments to be conducted under controlled and reproducible conditions. HC is a reasonably priced carbonaceous material that has been shown to boost DIET and increase the rate at which sludge and glucose produce methane [27,28]. According to Ren et al. [28], HC made from sewage sludge increases the rate at which glucose produces methane by 37%. Thus, it may be concluded that HC has the ability to facilitate propionate methanation.

This study aims to investigate the effects of BC and HC derived from aloe vera leaves and algae on the anaerobic digestion of food waste, exploring their potential to stabilize the process, enhance methane generation, and reduce organic waste streams. It was hypothesized that both materials would enhance the AD performance by improving microbial activity and facilitating electron transfer, whereas differences in performance between materials may be related to their distinct physicochemical properties. The specific objectives were as follows: (1) evaluation of the overall impact of BC and HC addition on the AD performance based on batch experiments, and (2) examination of how the physicochemical characteristics and production conditions of each material influence the biogas yield and quality.

2. Materials and Methods

2.1. Raw Materials, Substrates and Inoculum

The current study used FW that was collected from the student restaurant at the Hellenic Mediterranean University in Heraklion, which provides daily meals to a large student population using a standardized weekly menu. To account for temporal variability, FW samples were collected on multiple non-consecutive days during regular academic operation. The sampling was planned and carried out based on the international specifications as reflected in the standard ASTM D5231-92 (2003) ("Standard Test Method for Determination of the Composition of Unprocessed Municipal Solid Waste") [29]. The FW was not sterilized prior to processing in order to preserve its native physicochemical and microbial characteristics and to better reflect realistic conditions. The FW composition consisted of 62% cooked meals (on a wet-weight basis), 26% vegetables and salads, and 12% bread and bakery. The FW (about 4.0 mm) was homogenized using a mechanical mixer. The inoculum was obtained from the anaerobic digester of the sewage treatment plant (STP) in Heraklion, Greece, which has a population of approximately 200,000. The STP of Heraklion has an average flow of 150 m^3 day^{-1}, an OLR of 1.0 kg VSs m^{-3} d^{-1}, mesophilic conditions (35 °C) and a hydraulic retention time (HRT) of 22 days. The average mix of FW and raw inoculum is summarized in Table 1.

Table 1. FW and inoculum composition.

Parameters	Inoculum	FW
pH	7.0 ± 0.0	4.8 ± 0.1
TSs—Total Solids (g kg^{-1})	17.17 ± 0.17	240.53 ± 5.85
VSs (g kg^{-1})	10.48 ± 0.13	228.23 ± 5.77
T-COD—Total Chemical Oxygen Demand (g L^{-1})	16.71 ± 1.00	155.2 ± 5.30
N—Nitrogen (g L^{-1})	-	2.22 ± 0.05

2.2. Biochar and Hydrochar Production

Aloe vera (*Aloe barbadensis Mill.*) processing waste (leaves) and algae (*Spirulina* spp.) were used as the feedstock material for the biochars and hydrochars. For each biomass, hydrochars were produced at 220 °C with a 4 h residence time in a stainless steel Parr 4748 reactor (200 mL capacity, Parr Instrument Company, Moline, IL, USA). Twenty grams of biomass and eighty milliliters of distilled water (with a pH of 6.8) were introduced to the reactor for the HTC process. This amount of water was required to completely submerge the biomass while also providing sufficient space inside the reactor for safety. After the end of each run, the reactor was allowed to cool down to room temperature and the solid hydrochar was separated from the wastewater and recovered by vacuum filtration. The hydrochar was dried at 105 °C overnight. Biochar production was performed on a Carbolite Gero ELF6 furnace (Carbolite Gero Ltd., Hope Valley, UK) at 400 °C with a 1 h residence time in a nitrogen atmosphere. Following production, the biochars were allowed to cool down and were then stored in air-tight containers. The properties of the aloe vera waste have been described in our earlier publication [30].

2.3. Biogas—Methane Potential Experiments

Batch tests were carried out in triplicate at a mesophilic temperature (37 °C) to determine the effects of hydrochars and biochars on the methane potential of fresh FW. To determine which substrates were most successful at producing the largest methane yield, several substrates were evaluated, including FW, aloe's biochar (BA), algae's biochar (Bal), FW + BA, FW + Bal, aloe's hydrochar (HA), algae's hydrochar (Hal), FW + HA, and FW + Hal. Ten grams of char per liter were introduced to batch reactors. This dosage was selected based on literature evidence, as Osman et al. (2022) [31] reported that an optimal biochar loading of approximately 10 g L^{-1} relative to the substrate can enhance the anaerobic digestion performance. An approach based on Angelidaki & Sanders [32] was used to carry out the digestion. The 1:1 (VSs) inoculum (64 mL) to substrate ratio (ISR) (FW, 1.35 gr for biochar batches and 1.47 gr for hydrochar, respectively) was investigated. Eighty milliliters (inoculum, 64 mL and aqueous solution, 16 mL) was the working volume used in the bottles; the remaining forty milliliters was headspace volume. The investigation was conducted using 120 mL serum bottle reactors. In order to establish anaerobic conditions, the reactors were flushed with a gas mixture of 70% nitrogen and 30% carbon dioxide as soon as the inoculum and substrates were added. Serum vials were then sealed using rubber septa and aluminum crimp closures. The amount and composition of biogas in each bottle were regularly checked for over two months (about sixty days). Biochar and hydrochar were added to the bottles at a final concentration of 10 g L^{-1}, according to the earlier studies [28,33], and the control group (blind) was conducted without char or FW. The biogas yields reported for all treatments were corrected by subtracting the gas production of the blind (inoculum control). Two separate inoculum-only controls (blanks) were used, one for the biochar experiments and one for the hydrochar experiments, as the tests were conducted at different times. Although identical substrates and operating conditions were applied, each blank was used to correct the corresponding experimental

batch. Biogas production was first corrected using the respective blank, and increments were calculated relative to the corresponding FW control within each set.

2.4. Analytical Methods

2.4.1. pH, COD, TSs and VSs

The pH was determined using a pH meter in compliance with APHA [34] (from Crison, Alella, Spain; model GLP21). Spectrophotometry (DR 2800, Hach-Lange, Düsseldorf, Germany) and standard test kits (Hach-Lange) were used to measure the T-COD and d-COD. The TSs and VSs were measured gravimetrically in accordance with APHA (2005) [34] criteria and using suitable laboratory ovens (Memmert UF 30, Memmert GmbH + Co. KG, Schwabach, Germany and Protherm PC442, Protherm Furnaces, Ankara, Turkey). Each analysis of a single sample was carried out three times.

2.4.2. Biogas Volume and Composition

Biogas volumes were determined through manual headspace extraction with a gas-tight syringe inserted through the rubber septum, ensuring no atmospheric leakage during sampling. The composition of the biogas was assessed using a gas chromatograph (Agilent 6890N GC System, HP Agilent, Santa Clara, CA, USA). Before the needles were transferred to the gas chromatograph, they were sealed with butyl rubber stoppers after being drawn from gas-tight syringes. A 20 µL gas sample was injected for the analysis of carbon dioxide and methane (CH_4). A thermal conductivity detector (TCD) and a capillary column (GS Carbonplot, Agilent Technologies, Santa Clara, CA, USA, 30 m × 0.32 mm, 3 µm) were used. The column was working at an isothermal temperature of 80 °C, whilst the detector port was operating at 150 °C. Helium was the carrier gas, which moved at 15 mL min^{-1}. All samples were analyzed in triplicate.

2.4.3. Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectroscopy (SEM-EDS)

The surface morphology of the BCs and HCs was analyzed by scanning electron microscopy (SEM), while the surface elemental composition and distribution was determined using energy-dispersive X-ray spectroscopy (EDS), both of which were performed on a Tescan Mira S6123 (TESCAN, Brno, Czech Republic) scanning electron microscope. The detected surface elements are presented as weight percentages (wt.%). Additionally, elemental maps (C, O, Ca, Cl, Na, Mg, P, K, Cu) were generated to characterize the spatial distribution of elements on the biochar surface.

2.4.4. X-Ray Diffraction (XRD)

X-Ray diffraction (XRD Cu Kα graphite, λ = 1.5406 Å, Rigaku Europe SE-Hugenottenallee, Neu-Isenburg, Germany) was utilized to observe the crystal composition and structure. The XRD experiments were carried out with a range of 5–80°.

2.5. Statistical Analysis

The data and conclusions of this study were statistically analyzed using Origin 9 (OriginLab, Northampton, MA, USA) (analysis of average values, variance, and standards deviation). The results of the co-digesting FW and different chars were compared in terms of the biogas generation, d-COD removal, and VSs removal using one-way analysis of variance (ANOVA). To identify statistically significant ($p < 0.05$) differences in the average values of the normally distributed variables, Tukey analysis was employed.

3. Results and Discussion

3.1. Influence of Biochar and Hydrochar on the Anaerobic Digestion of Food Waste

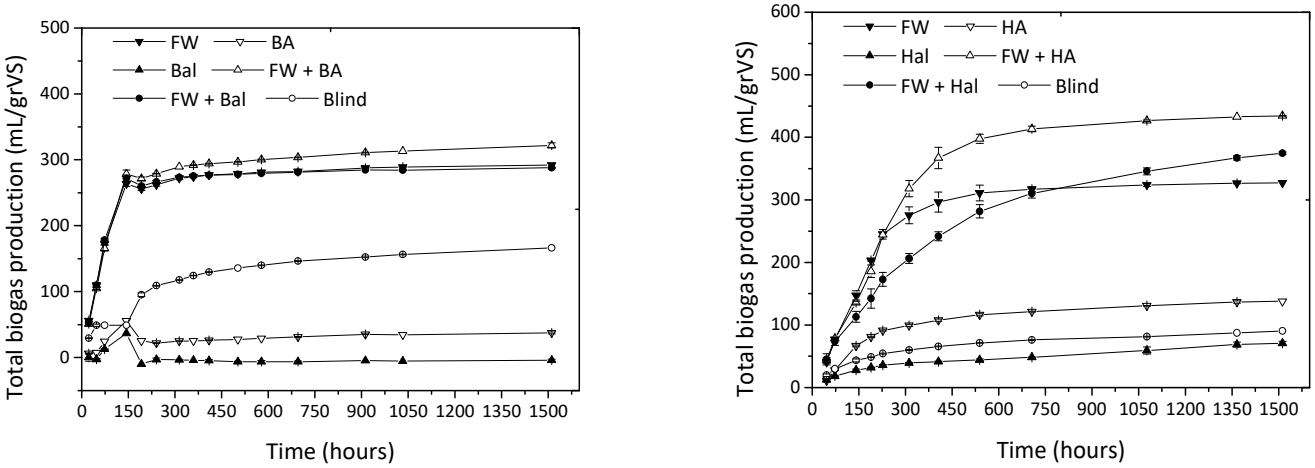
The biogas output and the corresponding increases relative to the FW substrate are presented in Table 2. The table also summarizes the d-COD and VS removal efficiencies, as well as the methane content (CH_4) of the produced biogas. The results clearly suggest that different BCs and HCs exhibit distinct effects on biogas production.

Table 2. Production of biogas and biomethane, biogas composition, d-COD and VSs removal for various substrates.

Parameter	FW-B	FW + BA	FW + Bal	FW-H	FW + HA	FW + Hal
Total biogas production (mL gr $^{-1}$ VSs)	292 ± 19.3	322 ± 4.9	288 ± 22.9	327 ± 59.2	434 ± 34	375 ± 34
Biogas increment (mL gr^{-1} VSs) & (%)	-	30 (10%)	-	-	107 (33%)	47 (14%)
Biogas composition (%) CH_4	69.2 ± 1.8	71.4 ± 2.1	69.1 ± 3.4	68.4 ± 1.1	70.1 ± 2.6	70.7 ± 3.1
Total biomethane production (mL gr^{-1} VSs)	202 ± 15	230 ± 4	199 ± 16	224 ± 22	304 ± 16	265 ± 15
d-COD removal (%)	25.5	31.2	13.6	32.7	42.5	37.2
VSs removal (%)	27.8	30.5	32.4	28.2	37.1	23.7

Food waste (FW-B), food waste and aloe's biochar (FW + BA), food waste and algae's biochar (FW + Bal), food waste (FW-H), food waste and aloe's hydrochar (FW + HA), and food waste and algae's hydrochar (FW + Hal).

A careful examination of the patterns in biogas generation in the six tests—FW-B, FW + BA, FW + Bal, FW-H, FW + HA and FW + Hal, which include blank and blank with 10 g L^{-1} of aloe or algae biochar and hydrochar, respectively—yields unique results (Figure 1a,b). In particular, in some cases, the addition of biochar derived from aloe and algae of hydrochar to the FW and inoculum mixture increases the amount of methane produced (Figure 2).



(a) (b)

Figure 1. Total biogas production for different substrates: (**a**) biochars and (**b**) hydrochars.

However, methane production in the algae biochar reactor was comparable with the control, indicating limited enhancement effect, suggesting that this carbonaceous material had no appreciable impact on the digestion process.

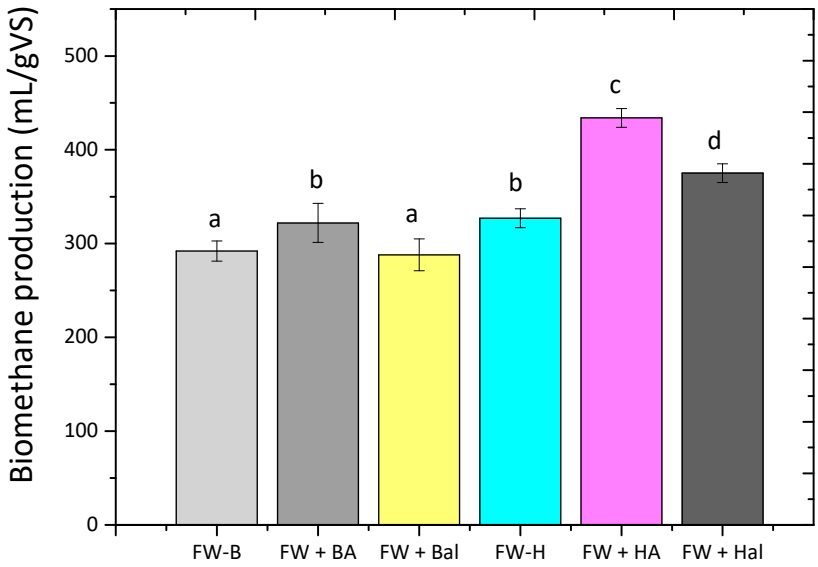


Figure 2. Production of biomethane (mL gr^{-1} VSs) for various substrates. Significant differences are shown by different letters when the *p*-value is 0.05. The standard deviations of the biogas production are displayed by the error bars.

Furthermore, co-digestion with biochars resulted in 1.1 times the amount of biogas produced, while hydrochars showed 1.1–1.3 times. The amount of methane in the biogas ranged from 69.1% to 71.4% for BCs and from 70.1 to 70.7% for HCs. With the addition of FW and BA, the methane percentage of biogas peaked at 71.4%, while with Hal, it increased to 70.7%. The FW + HA substrate produced the greatest increase in biogas generation, at around 33%, whereas the FW + Hal substrate produced an improvement of about 14% and FW + BA about 10%. Additionally, each substrate's total biomethane production is displayed in Figure 2, with FW + HA yielding the highest output (304 mL gr^{-1} VSs). Significant differences are shown by different letters when the *p*-value is 0.05. Another study found that adding bamboo hydrochar at a 1:2 ratio (dry mass ratio of fish processing waste to hydrochar) enhanced the biogas generation in the digestion of fish processing waste by 64% [35]. Regarding AD, Jang et al., 2018 [36] found that adding 10 g/L of biochar at different temperatures increased the CH_4 output by 27.7% to 35.7%. In a two-phase AD system, Sunyoto et al., 2017 [37] similarly found that adding biochar increased CH_4 generation by 23–41.6% and 1.9–9.6%.

The two carbonaceous products that are a part of the AD process can enhance the chemical reactions between the microorganisms and their substrates, according to the trends that have been observed. On the other hand, these products might act as process enhancers or catalysts because they lack specific production techniques. In particular, hydrochar's conductivity and other characteristics might affect how molecular bonds are broken, which could improve the process efficiency. The effects of these two carbonaceous materials can be directly compared based on the experiments conducted under the two scenarios involving hydrochar and biochar addition. In the case of biochar, the maximum methane production peak was reached more rapidly, as illustrated in the corresponding graphs. Notably, the methane production achieved with hydrochar is also higher (14–33%) than that observed in the case of using biochar (10%). External factors that could influence methane production include the substrate composition, pH, temperature, and microbial community dynamics, all of which were controlled and maintained consistently across the experiments to isolate the effects of the carbon materials. Therefore, the observed differences in methane production may be mainly attributed to the properties and type of char added.

One important metric for assessing the AD biodegradation process of wastes is the rate at which organic matter is removed. Following the addition of biochars and hydrochars, high overall VS removal efficiencies (ranging from 30.5% to 37.1%) were achieved, as illustrated in Figure 3. HA addition showed the largest decrease in VSs (37.1%) across the various chars, which is in line with one of the highest methane outputs. The FW + Hal reactor's VSs removal efficiency rose 8.9% in comparison with the FW reactor; however, the FW + Hal reactor saw a little decrease. These data are in line with other researchers. Organic matter is removed in AD through sequential microbial processes, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where complex polymers are converted into methane and carbon dioxide. The addition of biochar may have enhanced the microbial activity and stability by providing surfaces for biofilm formation, buffering the pH, and facilitating electron transfer, which contributed to faster and higher methane production. These mechanisms may help explain the observed improvements in VSs removal and methane yield in reactors containing carbonaceous amendments [38]. According to Chen et al. [39], by significantly increasing the conversion efficiency of organic matter, functional biochar can improve the removal of organic matter and optimize the dissolving and hydrolysis processes during the AD of sludge. For example, the degradation rate of T-COD increased by 34.93% and the degradation efficiency of organic matter was improved by adding 5.0 g g^{-1} VSs BC [40]. Additionally, Mangerino Bortoloti Sicchieri et al. [41] found that the mean VS removals in the control and biochar systems were 61% and 66%, respectively, with an OLR of 0.75 g VSs L^{-1} d^{-1}.

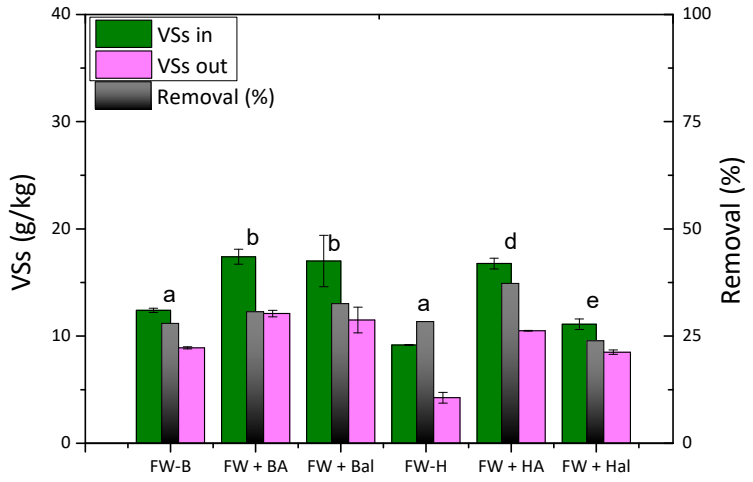


Figure 3. VS concentrations and removal in the influent (in) and effluent stages (out). With a *p*-value of 0.05, different letters represent significant differences. The standard deviations are indicated by the error bars.

Measurements of d-COD were conducted for both the feeding substrate and the digestate. The additions of BA, HA and Hal resulted in significant dissolved COD removal efficiencies (between 13.6% and 42.5%), as seen in Figure 4. The addition of HA resulted in the best d-COD (43%) elimination effectiveness. Nevertheless, the effectiveness of organic matter removal improved with the addition of HA. The addition of BA resulted in a 31% elimination of d-COD, which was higher than the control's 25% removal. Regarding the HCs, the additions of HA and Hal resulted in 42.5% and 37% eliminations of d-COD, respectively, higher than the control's 33% removal. These outcomes are linked to increased methane and biogas production that is facilitated by the addition of biochar. According to Jin et al. [42] and Kobayashi & Kuramochi [43], biochar increases the effectiveness of COD removal. This may be attributed to the physicochemical properties of biochar discussed above, particularly its surface area, conductivity, and porous structure, which

likely facilitates microbial colonization and electron transfer, reducing the lag phase of methanogenesis and improving VFA degradation [3,44]. According to Zhuang et al. [45], biochar plays a significant role in the removal of COD, improvement in CH_4 yield, and VFA reduction by 37.5% relative to the control. The biochar demonstrated the ability to increase DIET among electrogenic archaea and microorganisms, improving the granular sludge's COD removal and electron transfer characteristics, as well as the CH_4 output. Finally, during the AD of sludge, Wang et al. [46] found that magnetic biochar can remove up to 87.80% of the COD. Cooney et al. examined the influence of biochar on the anaerobic digestion of grease-laden wastewater and reported that biochar enhances the attachment and growth of acidogenic, acetogenic, and methanogenic microorganisms, leading to a COD removal efficiency of 69% [47]. Comparable findings were reported by Sharma, who demonstrated that biochar facilitates microbial biofilm development, thereby improving the COD removal performance [48].

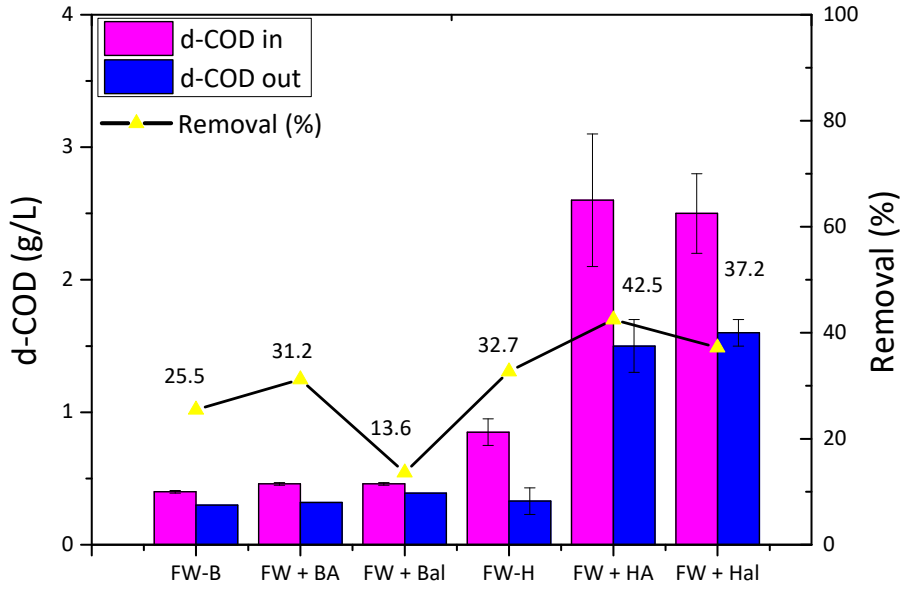


Figure 4. Concentrations of d-COD removal in the influent (in) and effluent stages (out). The standard deviations are indicated by the error bars.

The production of biogas and biomethane, biogas composition, and d-COD and VS removals for various substrates are summarized in Table 2.

3.2. Characterization of BCs and HCs

The influences of BC and HC on DIET are largely determined by their ability to facilitate electron transfer; BCs or HCs with higher electron-conducting capacity promote more efficient DIET. The higher methane yield achieved with HA relative to Hal may be explained by a combination of physicochemical characteristics that enhance microbial activity and may facilitate direct interspecies electron transfer (DIET). BA exhibits higher electrical conductivity (7.23 mS cm^{-1}) in relation to Bal (5.62 mS cm^{-1}), and the same trend is observed for HA compared with Hal. HA demonstrated substantially greater electrical conductivity (8.52 mS cm^{-1}), approximately 3.1 times higher than that of Hal (2.72 mS cm^{-1}), which may be attributed to its higher ash content (27.81%) and elevated concentrations of elements such as Ca (4.61%). EC is a key BC and HC property influencing electron transfer among microbial cells [3]. Kato et al. [49] reported that enhanced conductivity significantly increases methanogenesis rates while reducing lag phases. Furthermore, Li et al. [50] demonstrated that DIET does not occur in non-conductive carbon materials, indicating that microbial DIET is strongly dependent on the conductive nature of the added

materials. Ca^{2+} promotes microbial attachment via ionic bridging mechanisms [51]. Collectively, these properties may enhance the ionic mobility and electron transfer, thereby strengthening the DIET efficiency and leading to enhanced methane production in HA compared with Hal.

Figure 5 shows the SEM images of biochar and hydrochar samples at the magnifications of 1000× and 10,000×. BA exhibits a fragmented and irregular structure (Figure 5(a1,a2)), with rough surfaces and limited structural uniformity. These microcracks and rigid surfaces enhance microbial adhesion and create stable microhabitats for methanogens. Although Bal (Figure 5(b2)) has good porosity, its fragile and folded structure collapses easily, reducing long-term stability as a microbial support material. HA (Figure 5(c2)) shows a smoother, more coherent microstructure with characteristic spherical formations produced during hydrothermal carbonization. These features support improved microbial attachment [52] and increased methanogenic community diversity [53], ultimately facilitating better substrate accessibility and conversion efficiency. Although BA possessed a higher carbon content (70.12%) compared with HA, a characteristic that favors long-term carbon sequestration, its relatively low porosity and reduced electrical conductivity likely limited microbial interactions during anaerobic digestion. Overall, the combined effects of higher conductivity, enhanced porosity, and richer surface functionality may have created a more favorable environment for methanogenesis in HA, which resulted in a significantly higher methane yield compared with Hal.

The EDS analysis (Figure 6a–d) indicated that carbon and oxygen were the dominant elements, with minor contributions from calcium and other trace minerals (Table 3). These compositional characteristics enhance the material's suitability for applications such as anaerobic digestion, where surface-mediated interactions play a key role [54].

The crystallinity of different BC and HCs samples was investigated via XRD analysis, as depicted in Figure 7. The XRD patterns of the BA and Bal revealed clear differences in their inorganic mineral compositions. The XRD pattern of the aloe biochar was dominated by reflections at 2θ = 29.38° and 28.39°, corresponding to the (104) plane of calcite ($CaCO_3$) and the (200) plane of KCl, together with a peak at 31.54°, which may be attributed to NaCl-type halide salts. The presence of calcite suggests a calcium-rich ash fraction with notable buffering capacity, while the moderate NaCl content indicates limited soluble salt loading. In contrast, Bal exhibited a larger number of intense peaks at 2θ at around 28–35° and 40.72°. The prominent reflections at 28.39° and 40.72° may be attributed to KCl, indicating a high potassium-chloride mineral content typical of algal feedstocks. This mineralogical distinction aligns with the observed performance in anaerobic digestion: BA may have enhanced the biogas production, which was likely due to its buffering the $CaCO_3$ content and lowering the ionic stress, whereas Bal's high KCl concentration may have contributed to microbial inhibition and reduced methane yield. The improved biogas production observed with BA is consistent with Meng et al. (2020) [55], who noted that biochar enhances anaerobic digestion through buffering effects, a mechanism further supported here by the $CaCO_3$-rich ash of BA, which stabilizes pH and promotes methanogenesis. The HCs showed broad low-angle reflections (8–15° 2θ) characteristic of partially ordered organic structures, accompanied by weak peaks at 24–30° indicating the early presence of minor inorganic phases. This partially ordered organic structure, together with the limited mineral crystallinity, may explain the relatively good biogas performance of HA, as hydrochars with low inorganic stress and high functional group availability often support more favorable conditions for anaerobic digestion. HA gave higher biogas yields than BA because its lower mineral content and richer organic functionality provided more favorable conditions for methanogenic microbes.

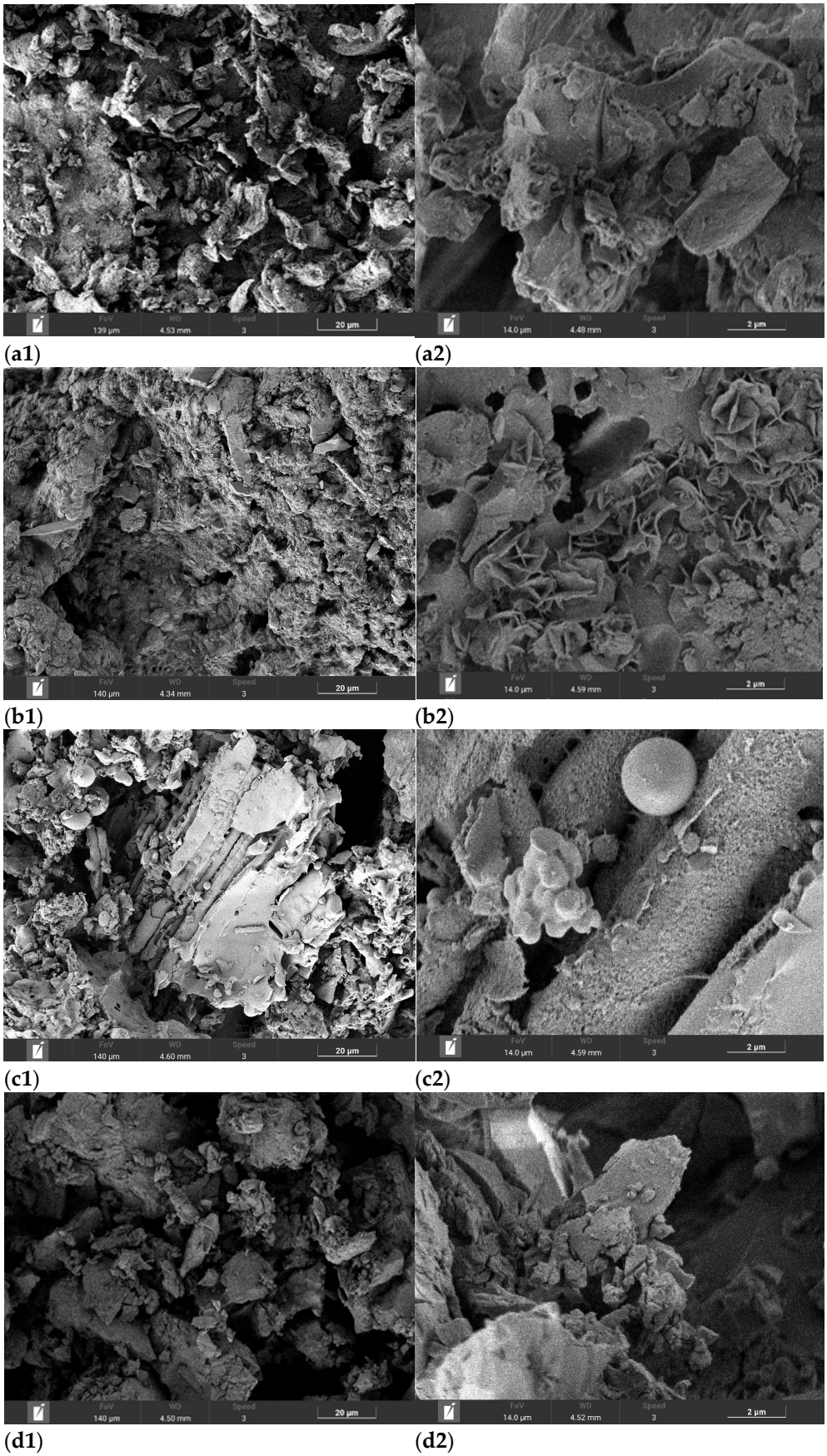


Figure 5. SEM images at 1000× (1) and 10,000× (2): (**a1**,**a2**) aloe's biochar (BA), (**b1**,**b2**) algae's biochar (Bal), (**c1**,**c2**) aloe's hydrochar (HA) and (**d1**,**d2**) algae's hydrochar (Hal).

Table 3. HC and BC characteristics [30].

Parameters	BA	HA	Bal	Hal
Ash (%)	38.29	27.81	25.83	18.41
pH	9.91	10.90	6.3	6.92
EC (mS cm^{-1})	7.23	8.52	5.62	2.72
Carbon (%)	70.12	60.13	64.25	72.51
Oxygen (%)	24.38	31.91	19.77	23.43
Calcium (%)	4.42	4.61	-	-
Chlorine (%)	0.55	1.63	2.71	
Sodium (%)	0.54	1.74	3.32	
Magnesium (%)	-	-	1.15	1.63
Phosphorus (%)	-	-	2.33	2.45
Potassium (%)	-	-	4.94	
Copper (%)	-	-	1.54	

- not detected.

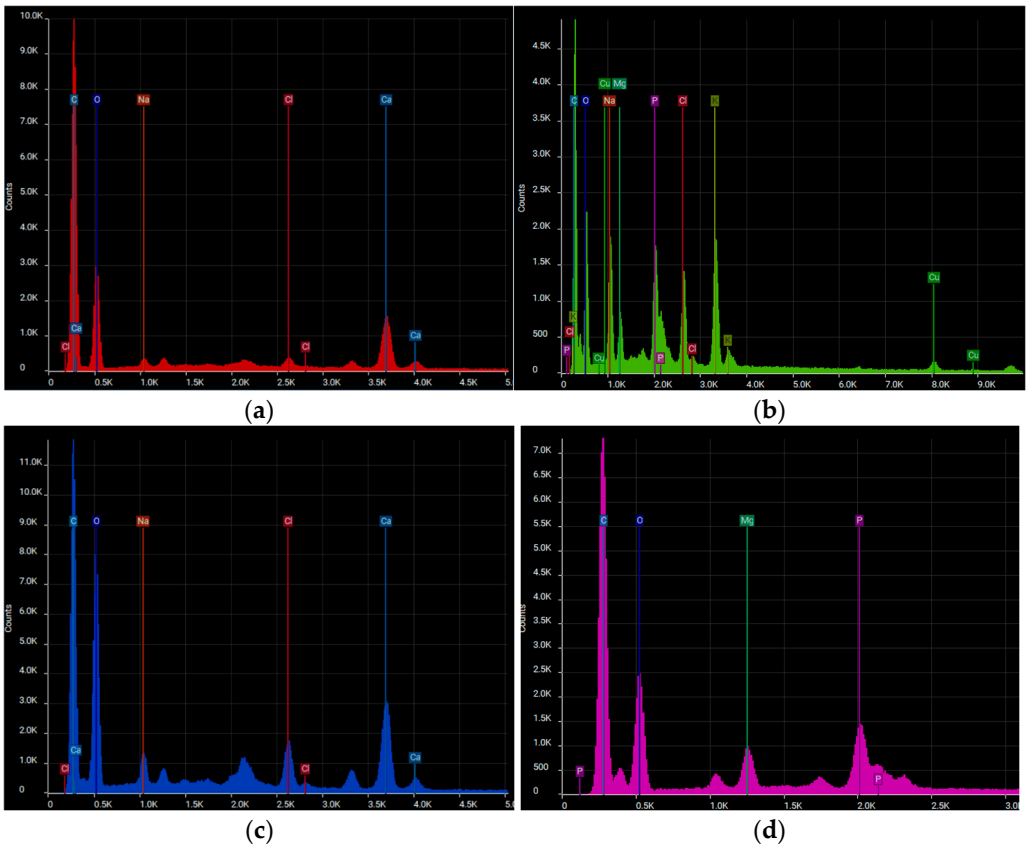


Figure 6. EDS images: (**a**) aloe's biochar (BA), (**b**) algae's biochar (Bal), (**c**) aloe's hydrochar (HA) and (**d**) algae's hydrochar (Hal).

To facilitate a clearer understanding of the relationship between material properties and anaerobic digestion performance, a summary of the key physicochemical characteristics of the biochars and hydrochars and their effects is presented in Table 4. The relationships presented in Table 4 are based on observed trends and literature-supported interpretations.

Overall, these characterization results, spanning conductivity, morphology, mineral composition, and crystallinity, collectively support the observed differences in AD performance between the four materials and highlight the importance of feedstock selection and production methods when choosing carbonaceous additives for anaerobic digestion.

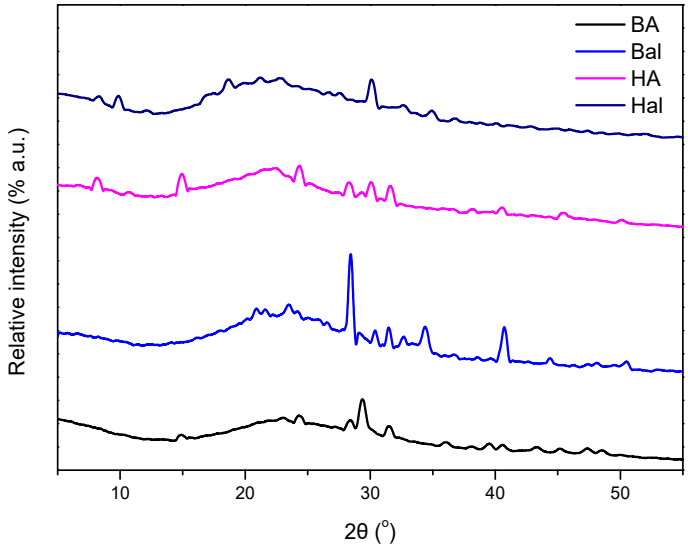


Figure 7. XRD analysis results for BCs and HCs.

Table 4. Summary of physicochemical properties of BCs and HCs and their potential effects on anaerobic digestion performance.

Material	BA	Bal	HA	Hal
EC (mS cm^{-1})	7.23	5.62	8.52	2.72
Ash (%)	38.29	25.83	27.81	18.41
pH	9.91	6.30	10.90	6.92
Carbon content (%)	70.12	64.25	60.13	72.51
Biogas increase (%)	10	0	33	14
Dominant mineral phase (XRD)	$CaCO_3$, KCl, NaCl	KCl (high)	Amorphous organic	Amorphous organic
SEM morphology	Fragmented, rough, rigid	Porous, fragile/folded	Smooth, spherical formations	Irregular, lower coherence
Potential mechanistic interpretation	$CaCO_3$ buffering may enhance pH stability and methanogenesis	High KCl may cause ionic stress and inhibit performance	High conductivity and porosity may enhance DIET and microbial colonization	Low conductivity may limit DIET efficiency

As shown in Table 4, materials with higher electrical conductivity and favorable surface characteristics may be associated with improved anaerobic digestion performance.

4. Conclusions

This study demonstrated that both BC and HC derived from aloe vera leaves and algae positively influenced the anaerobic digestion of food waste under mesophilic conditions, though to varying degrees depending on the feedstock and material type. HC consistently outperformed BC, increasing the cumulative biogas production by 18–35% compared with 10–14% for biochar. The FW and HA reactor showed the highest process efficiency, with the VSs removal rising by 31% and d-COD removal improving by up to 30%. In contrast, Bal showed no appreciable enhancement in biogas production compared with the control, likely due to its high KCl content, which may have induced ionic stress and limited the microbial activity. The superior performance of HC may be attributed to its higher electrical conductivity, porosity and surface functionality, which may enhance microbial colonization and organic matter degradation. Characterization results across conductivity,

morphology, mineral composition, and crystallinity collectively support these performance differences, with HA's amorphous organic structure and elevated Ca content appearing to contribute to its buffering capacity and DIET facilitation. These findings highlight the importance of carbonaceous additive type and feedstock selection. Incorporating HC or BC at 10 g L^{-1} may improve the methane yield, stabilize digestion, and optimize the microbial performance in food waste anaerobic digestion systems.

This study has some limitations. The proposed mechanisms are based on observed trends and literature evidence, as no direct microbial or electrochemical analyses were performed. Future studies should confirm the underlying mechanisms, including microbial community analysis and electrochemical characterization. Future work should also investigate the long-term stability of biochar and hydrochar in continuous systems, as well as the effect of different dosages and feedstock variability on anaerobic digestion performance. Additionally, scale-up studies are needed to evaluate the practical applicability of these materials under real operating conditions.

Author Contributions: Methodology, A.M., T.M. and D.K.; software, A.M.; validation, A.M., T.M. and D.K.; formal analysis, A.M. and P.P.; investigation, P.P. and A.M.; data curation, A.M.; writing—original draft preparation, A.M. and D.K.; supervision, T.M. and D.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded by the EU LIFE+ project with name "Sustainable Transformation of Waste to Biomethane, through Innovative Upgraded Anaerobic Digestion and Gasification", project number 101213273 and project acronym "LIFE24-CCM-EL-LIFE-W2B".

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of 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.

Abbreviations

The following abbreviations are used in this manuscript:

AD	anaerobic digestion
HTC	hydrothermal carbonization
DIET	interspecies electron transfer
FW	food waste
d-COD	dissolved chemical oxygen demand
VSs	volatile solids
SSA	specific surface area
CEC	cation exchange capacity
FGs	functional groups
EC	electrical conductivity
BC	biochar
GHG	greenhouse gas
HC	hydrochar
HHV	higher heating value
OLR	organic loading rate
VFAs	volatile fatty acids
STP	sewage treatment plant
HRT	hydraulic retention time
FW + BA	food waste and aloe's biochar
FW + Bal	food waste and algae's biochar
FW + HA	food waste and aloe's hydrochar
FW + Hal	food waste and algae's hydrochar

References

1. Masoumi, S.; Borugadda, V.B.; Nanda, S.; Dalai, A.K. Hydrochar: A Review on Its Production Technologies and Applications. *Catalysts* **2021**, *11*, 939. [CrossRef]
2. Cavali, M.; Libardi Junior, N.; Mohedano, R.d.A.; Belli Filho, P.; da Costa, R.H.R.; de Castilhos Junior, A.B. Biochar and Hydrochar in the Context of Anaerobic Digestion for a Circular Approach: An Overview. *Sci. Total Environ.* **2022**, *822*, 153614. [CrossRef] [PubMed]
3. Valentin, M.T.; Luo, G.; Zhang, S.; Białowiec, A. Direct Interspecies Electron Transfer Mechanisms of a Biochar-Amended Anaerobic Digestion: A Review. *Biotechnol. Biofuels Bioprod.* **2023**, *16*, 146. [CrossRef] [PubMed]
4. Zhou, J.; Smith, J.A.; Li, M.; Holmes, D.E. Methane Production by *Methanothrix thermoacetophila* via Direct Interspecies Electron Transfer with *Geobacter metallireducens*. *mBio* **2023**, *14*, e0036023. [CrossRef]
5. Baek, G.; Kim, D.; Choi, Y.; Cha, J.; Lee, C. Challenges in Engineering Direct Interspecies Electron Transfer for Enhanced Methanogenesis. *Renew. Sustain. Energy Rev.* **2023**, *183*, 113503. [CrossRef]
6. Kong, T.; Zhang, W. Enhanced Anaerobic Digestion Using Conductive Materials through Mediation of Direct Microbial Interspecies Electron Transfer: A Review. *Fermentation* **2023**, *9*, 884. [CrossRef]
7. Jiang, Q.; Zhang, C.; Wu, P.; Ding, P.; Zhang, Y.; Cui, M.-H.; Liu, H. Algae Biochar Enhanced Methanogenesis by Enriching Specific Methanogens at Low Inoculation Ratio during Sludge Anaerobic Digestion. *Bioresour. Technol.* **2021**, *338*, 125493. [CrossRef]
8. Zhang, M.; Li, J.; Wang, Y.; Yang, C. Impacts of Different Biochar Types on the Anaerobic Digestion of Sewage Sludge. *RSC Adv.* **2019**, *9*, 42375–42386. [CrossRef]
9. Feng, Q.; Song, Y.C.; Yoo, K.; Kuppanan, N.; Subudhi, S.; Lal, B. Bioelectrochemical Enhancement of Direct Interspecies Electron Transfer in Upflow Anaerobic Reactor with Effluent Recirculation for Acidic Distillery Wastewater. *Bioresour. Technol.* **2017**, *241*, 171–180. [CrossRef]
10. Zhang, L.; Lim, E.Y.; Loh, K.C.; Ok, Y.S.; Lee, J.T.E.; Shen, Y.; Wang, C.H.; Dai, Y.; Tong, Y.W. Biochar Enhanced Thermophilic Anaerobic Digestion of Food Waste: Focusing on Biochar Particle Size, Microbial Community Analysis and Pilot-Scale Application. *Energy Convers. Manag.* **2020**, *209*, 112654. [CrossRef]
11. Ghodake, G.S.; Shinde, S.K.; Kadam, A.A.; Saratale, R.G.; Saratale, G.D.; Kumar, M.; Palem, R.R.; AL-Shwaiman, H.A.; Elgorban, A.M.; Syed, A.; et al. Review on Biomass Feedstocks, Pyrolysis Mechanism and Physicochemical Properties of Biochar: State-of-the-Art Framework to Speed up Vision of Circular Bioeconomy. *J. Clean. Prod.* **2021**, *297*, 126645. [CrossRef]
12. Wang, G.; Gao, X.; Li, Q.; Zhao, H.; Liu, Y.; Wang, X.C.; Chen, R. Redox-Based Electron Exchange Capacity of Biowaste-Derived Biochar Accelerates Syntrophic Phenol Oxidation for Methanogenesis via Direct Interspecies Electron Transfer. *J. Hazard. Mater.* **2020**, *390*, 121726. [CrossRef]
13. Chiappero, M.; Fiore, S.; Berruti, F. Impact of Biochar on Anaerobic Digestion: Meta-Analysis and Economic Evaluation. *J. Environ. Chem. Eng.* **2022**, *10*, 108870. [CrossRef]
14. Zhang, Z.; Zhu, Z.; Shen, B.; Liu, L. Insights into Biochar and Hydrochar Production and Applications: A Review. *Energy* **2019**, *171*, 581–598. [CrossRef]
15. Nizamuddin, S.; Baloch, H.A.; Griffin, G.J.; Mubarak, N.M.; Bhutto, A.W.; Abro, R.; Mazari, S.A.; Ali, B.S. An Overview of Effect of Process Parameters on Hydrothermal Carbonization of Biomass. *Renew. Sustain. Energy Rev.* **2017**, *73*, 1289–1299. [CrossRef]
16. Dieguez-Alonso, A.; Funke, A.; Anca-Couce, A.; Rombolà, A.G.; Ojeda, G.; Bachmann, J.; Behrendt, F. Towards Biochar and Hydrochar Engineering—Influence of Process Conditions on Surface Physical and Chemical Properties, Thermal Stability, Nutrient Availability, Toxicity and Wettability. *Energies* **2018**, *11*, 496. [CrossRef]
17. Cao, X.; Peng, X.; Sun, S.; Zhong, L.; Sun, R. Hydrothermal Conversion of Bamboo: Identification and Distribution of the Components in Solid Residue, Water-Soluble and Acetone-Soluble Fractions. *J. Agric. Food Chem.* **2014**, *62*, 12360–12365. [CrossRef]
18. Kim, D.; Lee, K.; Park, K.Y. Upgrading the Characteristics of Biochar from Cellulose, Lignin, and Xylan for Solid Biofuel Production from Biomass by Hydrothermal Carbonization. *J. Ind. Eng. Chem.* **2016**, *42*, 95–100. [CrossRef]
19. Lü, F.; Luo, C.; Shao, L.; He, P. Biochar Alleviates Combined Stress of Ammonium and Acids by Firstly Enriching Methanosaeta and Then Methanosarcina. *Water Res.* **2016**, *90*, 34–43. [CrossRef]
20. Feng, L.; He, S.; Gao, Z.; Zhao, W.; Jiang, J.; Zhao, Q.; Wei, L. Mechanisms, Performance, and the Impact on Microbial Structure of Direct Interspecies Electron Transfer for Enhancing Anaerobic Digestion-A Review. *Sci. Total Environ.* **2023**, *862*, 160813. [CrossRef] [PubMed]
21. Thauer, R.K. Methyl (Alkyl)-Coenzyme M Reductases: Nickel F-430-Containing Enzymes Involved in Anaerobic Methane Formation and in Anaerobic Oxidation of Methane or of Short Chain Alkanes. *Biochemistry* **2019**, *58*, 5198–5220. [CrossRef]
22. Kadam, R.; Khanthong, K.; Jang, H.; Lee, J.; Park, J. Occurrence, Fate, and Implications of Heavy Metals during Anaerobic Digestion: A Review. *Energies* **2022**, *15*, 8618. [CrossRef]
23. Paritosh, K.; Yadav, M.; Chawade, A.; Sahoo, D.; Kesharwani, N.; Pareek, N.; Vivekanand, V. Additives as a Support Structure for Specific Biochemical Activity Boosts in Anaerobic Digestion: A Review. *Front. Energy Res.* **2020**, *8*, 541499. [CrossRef]

24. Bian, X.; Wang, K.; Gong, H. Biochar-Enhanced Agricultural Application of Liquid Digestate from Food Waste Anaerobic Digestion for Celery Cultivation. *Sci. Total Environ.* **2023**, *869*, 161562. [CrossRef]
25. Qi, Z.; Qi, Y.; Le, Z.; Han, F.; Li, F.; Yang, H.; Zhang, T.; Feng, Y.; Liu, R.; Sun, Y. The Interactions Between Antibiotic Resistance Genes and Heavy Metal Pollution Under Co-Selective Pressure Influenced the Bio-Enzyme Activity. *Front. Chem.* **2021**, *9*, 691565. [CrossRef]
26. Cheng, N.; Wang, B.; Wu, P.; Lee, X.; Xing, Y.; Chen, M.; Gao, B. Adsorption of Emerging Contaminants from Water and Wastewater by Modified Biochar: A Review. *Environ. Pollut.* **2021**, *273*, 116448. [CrossRef] [PubMed]
27. Luo, G.; Shi, Z.; Campanaro, S.; Usman, M.; Treu, L.; Basile, A.; Angelidaki, I.; Zhang, S. Genome-Centric Metatranscriptomics Analysis Reveals the Role of Hydrochar in Anaerobic Digestion of Waste Activated Sludge. *Environ. Sci. Technol.* **2021**, *55*, 8351–8361. [CrossRef]
28. Ren, S.; Usman, M.; Tsang, D.C.W.; O-Thong, S.; Angelidaki, I.; Zhu, X.; Zhang, S.; Luo, G. Hydrochar-Facilitated Anaerobic Digestion: Evidence for Direct Interspecies Electron Transfer Mediated through Surface Oxygen-Containing Functional Groups. *Environ. Sci. Technol.* **2020**, *54*, 5755–5766. [CrossRef] [PubMed]
29. *ASTM D5231-92(2003)*; Test Method for Determination of the Composition of Unprocessed Municipal Solid Waste. ASTM International: West Conshohocken, PA, USA, 2003. [CrossRef]
30. Kalderis, D.; Stavroulakis, G.; Tsubota, T.; Çalhan, S.D. Valorization of Aloe Vera Waste for the Production of Ca and P-Rich Hydrochars. *Sustain. Chem. Environ.* **2024**, *5*, 100057. [CrossRef]
31. Osman, A.I.; Fawzy, S.; Farghali, M.; El-Azazy, M.; Elgarahy, A.M.; Fahim, R.A.; Maksoud, M.I.A.A.; Ajlan, A.A.; Yousry, M.; Saleem, Y.; et al. Biochar for Agronomy, Animal Farming, Anaerobic Digestion, Composting, Water Treatment, Soil Remediation, Construction, Energy Storage, and Carbon Sequestration: A Review. *Environ. Chem. Lett.* **2022**, *20*, 2385–2485. [CrossRef] [PubMed]
32. Angelidaki, I.; Sanders, W. Assessment of the Anaerobic Biodegradability of Macropollutants. *Rev. Environ. Sci. Biotechnol.* **2004**, *3*, 117–129. [CrossRef]
33. Shi, Z.; Usman, M.; He, J.; Chen, H.; Zhang, S.; Luo, G. Combined Microbial Transcript and Metabolic Analysis Reveals the Different Roles of Hydrochar and Biochar in Promoting Anaerobic Digestion of Waste Activated Sludge. *Water Res.* **2021**, *205*, 117679. [CrossRef] [PubMed]
34. APHA. *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; APHA: Washington, DC, USA, 2005.
35. Choe, U.; Mustafa, A.M.; Lin, H.; Xu, J.; Sheng, K. Effect of Bamboo Hydrochar on Anaerobic Digestion of Fish Processing Waste for Biogas Production. *Bioresour. Technol.* **2019**, *283*, 340–349. [CrossRef] [PubMed]
36. Jang, H.M.; Choi, Y.K.; Kan, E. Effects of Dairy Manure-Derived Biochar on Psychrophilic, Mesophilic and Thermophilic Anaerobic Digestions of Dairy Manure. *Bioresour. Technol.* **2018**, *250*, 927–931. [CrossRef]
37. Sunyoto, N.M.S.; Zhu, M.; Zhang, Z.; Zhang, D. Effect of Biochar Addition and Initial PH on Hydrogen Production from the First Phase of Two-Phase Anaerobic Digestion of Carbohydrates Food Waste. *Energy Procedia* **2017**, *105*, 379–384. [CrossRef]
38. Zhao, W.; Yang, H.; He, S.; Zhao, Q.; Wei, L. A Review of Biochar in Anaerobic Digestion to Improve Biogas Production: Performances, Mechanisms and Economic Assessments. *Bioresour. Technol.* **2021**, *341*, 125797. [CrossRef]
39. Chen, B.; Zeng, H.; Yang, F.; Yang, Y.; Qiao, Z.; Zhao, X.; Wang, L.; Wu, F. Functional Biochar as Sustainable Precursors to Boost the Anaerobic Digestion of Waste Activated Sludge from a Circular Economy Perspective: A Review. *Biochar* **2024**, *6*, 60. [CrossRef]
40. Zhang, M.; Wang, Y. Impact of Biochar Supported Nano Zero-Valent Iron on Anaerobic Co-Digestion of Sewage Sludge and Food Waste: Methane Production, Performance Stability and Microbial Community Structure. *Bioresour. Technol.* **2021**, *340*, 125715. [CrossRef]
41. Mangerino Bortoloti Sicchieri, I.; Camila Fernandes de Quadros, T.; Aparecido Bortoloti, M.; Teixeira de Souza, A.; Pereira Nogueira, H.; Araki, K.; Fernandes, F.; Kiyomi Kuroda, E. Biochars from Waste as an Additive Material in Anaerobic Co-Digestion: Characterization and Application in Batch and Semi-Continuous Systems. *Fuel* **2025**, *381*, 133342. [CrossRef]
42. Jin, H.Y.; Yang, L.; Ren, Y.X.; Tang, C.C.; Zhou, A.J.; Liu, W.; Li, Z.; Wang, A.; He, Z.W. Insights into the Roles and Mechanisms of a Green-Prepared Magnetic Biochar in Anaerobic Digestion of Waste Activated Sludge. *Sci. Total Environ.* **2023**, *896*, 165170. [CrossRef] [PubMed]
43. Kobayashi, T.; Kuramochi, H. Catalytic Pyrolysis of Biomass Using Fly Ash Leachate to Increase Carbon Monoxide Production and Improve Biochar Properties to Accelerate Anaerobic Digestion. *Bioresour. Technol.* **2023**, *387*, 129583. [CrossRef]
44. Wang, C.; Liu, Y.; Gao, X.; Chen, H.; Xu, X.; Zhu, L. Role of Biochar in the Granulation of Anaerobic Sludge and Improvement of Electron Transfer Characteristics. *Bioresour. Technol.* **2018**, *268*, 28–35. [CrossRef]
45. Zhuang, H.; Zhu, H.; Shan, S.; Zhang, L.; Fang, C.; Shi, Y. Potential Enhancement of Direct Interspecies Electron Transfer for Anaerobic Degradation of Coal Gasification Wastewater Using Up-Flow Anaerobic Sludge Blanket (UASB) with Nitrogen Doped Sewage Sludge Carbon Assisted. *Bioresour. Technol.* **2018**, *270*, 230–235. [CrossRef] [PubMed]
46. Wang, M.; Zhao, Z.; Zhang, Y. Magnetite-Contained Biochar Derived from Fenton Sludge Modulated Electron Transfer of Microorganisms in Anaerobic Digestion. *J. Hazard. Mater.* **2021**, *403*, 123972. [CrossRef] [PubMed]

47. Cooney, M.J.; Lewis, K.; Harris, K.; Zhang, Q.; Yan, T. Start up Performance of Biochar Packed Bed Anaerobic Digesters. *J. Water Process Eng.* **2016**, *9*, e7–e13. [CrossRef]
48. Sharma, P.; Melkania, U. Impact of Heavy Metals on Hydrogen Production from Organic Fraction of Municipal Solid Waste Using Co-Culture of *Enterobacter aerogenes* and *E. coli*. *Waste Manag.* **2018**, *75*, 289–296. [CrossRef]
49. Kato, S.; Hashimoto, K.; Watanabe, K. Methanogenesis Facilitated by Electric Syntrophy via (Semi)Conductive Iron-Oxide Minerals. *Environ. Microbiol.* **2012**, *14*, 1646–1654. [CrossRef]
50. Li, H.; Chang, J.; Liu, P.; Fu, L.; Ding, D.; Lu, Y. Direct Interspecies Electron Transfer Accelerates Syntrophic Oxidation of Butyrate in Paddy Soil Enrichments. *Environ. Microbiol.* **2015**, *17*, 1533–1547. [CrossRef]
51. De Kerchove, A.J.; Elimelech, M. Calcium and Magnesium Cations Enhance the Adhesion of Motile and Nonmotile Pseudomonas Aeruginosa on Alginate Films. *Langmuir* **2008**, *24*, 3392–3399. [CrossRef]
52. Jia, Y.L.; Hu, W.; Tang, C.C.; Zhou, A.J.; Liu, W.; Chen, Z.; Ren, Y.X.; He, Z.W. Biochar Derived from Straw Regulate Anaerobic Digestion: Insights into the Roles of Vegetative Organs. *Renew. Energy* **2025**, *253*, 123554. [CrossRef]
53. Bu, J.; Wang, Y.; Gao, Y.; Zhao, Q.; Luo, Y.; Tiong, Y.W.; Lam, H.T.; Zhang, J.; He, Y.; Wang, C.H.; et al. Enhancing Anaerobic Digestion of Food Waste with Chemically Vapor-Deposited Biochar: Effective Enrichment of Methanosarcina and Hydrogenotrophic Methanogens. *Bioresour. Technol.* **2025**, *424*, 132225. [CrossRef] [PubMed]
54. Capone, A.; Panico, A.; Travaglino, A.; Zaccariello, L.; Ageorges, H.; Morrone, B. The Role of Hydrochar as Anaerobic Digestion Process Additive and CO_2 Adsorbent. *Chem. Eng. Trans.* **2025**, *117*, 487–492. [CrossRef]
55. Meng, L.; Xie, L.; Suenaga, T.; Riya, S.; Terada, A.; Hosomi, M. Eco-Compatible Biochar Mitigates Volatile Fatty Acids Stress in High Load Thermophilic Solid-State Anaerobic Reactors Treating Agricultural Waste. *Bioresour. Technol.* **2020**, *309*, 123366. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.