Article

Effect of Alfalfa-Derived Biochar on Anaerobic Digestion of Dairy Manure

Shengquan Zeng 1, Riley Harris 2 and Eunsung Kan 1,3,*

1 Texas A&M AgriLife Research Center, Department of Biological and Agricultural Engineering, Texas A&M University, College Station, TX 77843, USA; shengquan.zeng@ag.tamu.edu
2 Department of Engineering and Computer Science, Tarleton State University, Stephenville, TX 76401, USA; riley.harris@go.tarleton.edu
3 Department of Wildlife, Sustainability, and Ecosystem Sciences, Tarleton State University, Stephenville, TX 76401, USA
* Correspondence: eunsung.kan@ag.tamu.edu; Tel.: +1-254-968-4144; Fax: +1-254-968-3759

Abstract: Biochemical methane potential (BMP) tests were conducted for investigating the effects of alfalfa-derived biochar (AF-BC) on anaerobic digestion (AD) of dairy manure under various loading of AF-BC (0–10 g/L). BMP tests were performed at mesophilic temperature (37 °C) with the addition of AF-BC. Biogas and methane volumes and concentrations, water quality parameters (i.e., COD (chemical oxygen demand)), and volatile fatty acids (VFAs) were measured during the AD process. The addition of 1 and 5 g/L of AF-BC increased the biogas yields by 15.51% and 26.09% and methane yields by 14.61% and 26.88% compared with the control without addition of AF-BC. Additionally, the addition of AF-BC (1–10 g/L) decreased the lag phase by 7.14–22.45% and the CO2 content of biogas by 13.60–32.48%, while increasing the COD removal efficiency by 19.19–35.94% in the AD of dairy manure. Moreover, the addition of AF-BC also decreased total VFAs and acetic acid concentrations in the AD process. The increase in AD performance was mainly owing to the improvement of buffering ability of the AD system and direct interspecies electron transfer (DIET) among AD microorganisms resulting from the addition of AF-BC. In contrast, the addition of 10 g/L AF-BC did not show any obvious improvement in biogas and methane yields in the AD of dairy manure, possibly because of toxic effects from excessive addition of AF-BC toward the AD microorganisms. Therefore, this study supported practical feasibility of AF-BC-enhanced AD of dairy manure.

Keywords: alfalfa; biochar; anaerobic digestion; dairy manure; biogas and methane production

1. Introduction

Anaerobic digestion (AD) has been widely used as a sustainable route to convert various waste to biogas and digestate as fuel and biofertilizer while mitigating environmental problems and greenhouse gas emission [1–4]. A large quantity of animal manures (about 132 million metric tons of dried manure per year in United States) has been used as the major feedstock for AD [5,6]. Dairy manure, among the animal manures, is generated by up to 9.4 million dairy cows each year [7] and has caused increasing pollution in soil, water and air when it has been applied to farms as a fertilizer [8]. Therefore, effective AD of dairy manure has been considered as a sustainable remediation process for production of renewable energy (either cleaned biogas or renewable natural gas), effective disposal of manure, and reduction of manure-derived greenhouse gas emission [9]. To date, a substantial number of works about AD of dairy manure have been reported. Li et al. [5] conducted AD tests with diary manure under various substrate concentrations and achieved a methane yield up to 270 mL/g VS. Zheng et al. [10] evaluated the impact of co-digestion ratios on the performance of AD in mixtures of dairy manure and switchgrass, and found that the optimal methane production (155.1 mL/g VS) was observed under a 2:2 mixture ratio of manure to grass. In addition, Zeng et al. [11] applied mechanical refining pretreatment to
the process of AD of dairy manure and improved biogas and methane yields by 6% and 8%, respectively.

However, AD of dairy manure suffers from inconsistent and fluctuating performance, low yields of biogas, and the need to dispose of a large volume of undigested sludge after AD [12,13]. For enhancing AD performance, a variety of carbon additives have been applied for the AD process, including activated carbon, carbon nanotube, graphene, and carbon cloth [14]. Carbon nanotubes have improved electrical conductance of substrates and have accelerated the substrate consumption and methane production rates in the AD of granular sludge [15]. Carbon cloth enhanced the AD of incineration leachate by lowering VFAs level and preventing a significant drop in pH [16]. Graphene was also used for increasing direct interspecies electron transfer (DIET) between electron-active bacteria and methanogens, increasing methane production in the AD of ethanol [17]. Similarly, activated carbon (AC) also improved methane production through enriching the Methanosaeta/Methanosarcina species on AC and accelerating the DIET in the AD of activated sludge [18]. Nevertheless, these carbon additives required their disposal after use and high production costs, limiting their practical application. Thus, development of cost-effective and environmentally friendly additives applied in the AD process is highly recommended.

Recently, biochar (BC), made from pyrolyzing the biomass in oxygen-limited conditions, has been considered as an effective carbon additive for improving AD performance [19–21]. BC can offer a benign environment for microbial attachment and growth, resulting in the improvement of activity of AD microorganisms [22]. In addition, BC could relieve drastic pH drops from VFA accumulation and reduce ammonia inhibition during the AD process [23]. Moreover, BC can act as a microbial support to immobilize microbial cells and act as a conductive medium for facilitating the DIET between VFAs-oxidative bacteria and methanogens [19]. To date, considerable research for the effects of various BCs on AD has been reported. For example, Pan et al. [4] found that, during AD of chicken manure, fruitwood biochar increased the methane yield by 69% while reducing the ammonia concentration and improving the buffering capacity. Wang et al. [24] added vermicompost-derived BC into the AD of easily acidified substrates and found that BC can offer excellent buffering capacity to hold back a pH drop and cause a significant increase in methane production. In addition, Jang et al. [25,26] showed that the BC derived from dairy manure achieved a 24.90% increase in methane production and a 36.84% decrease in the lag phase during the mesophilic AD of dairy manure.

Conversely, grass alfalfa (AF), one of the extensively grown forage grasses in the US (about 56 million tons per year), is commonly used as a hay at dairy farms [27]. In addition, because of its high forage nutritive value and dry matter yield, AF is also widely planted and important for forage production around the world [28]. However, the molds are easily generated at the surface of hay during storage, and approximately 20% of hay is commonly moldy and discarded to avoid harmful effects on animal health [29]. Therefore, AF is a viable option as feedstock for BC production due to high output of the AF, especially discarded ones available at dairy farms. Several studies have focused on the production of bio-oil and BC by pyrolyzing AF and used AF-derived BC for treatment of organic contaminants [27,30–32]. However, no research to date has focused on producing and applying AF-derived BC for enhancing AD of dairy manure. Recycling of discarded AF to AD of dairy manure could enhance environmental and agricultural sustainability at dairy farms.

To the best of our knowledge, for the first time, this study evaluated the feasibility for the application of AF-derived BC for enhancing the AD of dairy manure. In the present work, the effects of AF-derived BC with various loading rates on biogas and methane production were examined along with monitoring of AD metabolites and water quality in the AD of dairy manure. On the basis of changes of biogas production, metabolites, and water quality, possible roles of AF-derived BC in the AD process were discussed.
2. Materials and Methods

2.1. Substrate and Inoculum

Dairy manure utilized in the present work was obtained from Southwest Dairy Center at Tarleton State University (Stephenville, TX, USA). Before AD experiments, the manure was dried at 65 °C, ground, and sieved (below 500 µm). The elemental compositions of dried dairy manure were C (16.6%), H (2.3%), O (31.0%), N (1.1%), S (0.2%), and ash (48.8%) [25]. The inoculum sludge was obtained from the lagoon bottom at Tarleton dairy farm. The collected sludge was activated via cultivating with dairy manure at mesophilic and anaerobic conditions for about a month [25]. Then, the activated sludge was utilized as the inoculum to conduct AD tests for further. The major properties of the inoculum obtained in the present work included pH at 7.72 ± 0.07, total solids (TS) at 29.92 ± 0.16 g/L, volatile solids (VS) at 14.55 ± 0.16 g/L, total COD (TCOD) at 17.50 ± 2.12 g/L, soluble COD (SCOD) at 0.53 ± 0.01 g/L, total VFAs (TVFAs) at 199 ± 9 mg/L, and total alkalinity (TA) at 939 ± 53 mg CaCO₃/L.

2.2. Preparation and Characterization of BC

The grass alfalfa (AF), obtained from a local hay vendor (Stephenville, TX, USA), was used as the feedstock for BC. After being dried, ground, and sieved (<500 µm), 10 g of dry AF was placed into a quartz-tube furnace (MTI corporation, Richmond, VA, USA) for pyrolysis. In order to lower the production cost of BC, AF was pyrolyzed at a low pyrolysis temperature. The pyrolysis conditions were set at 350 °C with the heating rate of 10 °C/min and heating time of 120 min under continuous flow of nitrogen gas (2 L/min). The resulting BC was named as “AF-BC”. Then, AF-BC was milled and sieved until the particle size was less than 106 µm. The analysis of elemental and mineral compositions of AF and AF-BC was conducted at Robert Microlit Lab (Ledgewood, NJ, USA) and the Soil, Forage and Water Testing Lab at Texas A&M AgriLife (College Station, TX, USA), respectively. Proximate analysis, including fixed carbon, volatile carbon, and ash, was determined according to ASTM D7582-12 [33]. The surface functional groups of AF-BC were determined via a FTIR Spectrometer (Bruker Optik GmbH, Ettlingen, Germany). The surface area of AF-BC was evaluated by a surface area analyzer (Particle Technology Lab, IL, USA).

2.3. Anaerobic Digestion Experiments

BMP (Biochemical Methane Potential) tests were performed for evaluating the impacts of AF-BC on the AD of dairy manure. Briefly, 130 mL of inoculum and dry dairy manure with a ratio of 1 (TS basis) was loaded into a 280 mL serum bottle. The AF-BC was added into the serum bottle with four loading rates (0, 1, 5, and 10 g of BC/L of manure). After inoculation, each serum bottle was sealed with a rubber plug and a screw cap. Then, N₂ gas was flushed into each bottle for removing oxygen and ensuring anaerobic condition in each bottle. All bottles, in replicate, were incubated at mesophilic temperature (37 °C) and manually mixed every day. In this study, the BMP tests were carried out for 36 d. The experiment sets are referred to as control, A1, A5, and A10. The control represents the experiment group without addition of AF-BC, while A1, A5, and A10 represent the addition of 1, 5, and 10 g of AF-BC/L of manure. The experiment setup is shown in Figure S1.

2.4. Analytical Methods

TS, VS, pH, TCOD, SCOD, NH₃-N, PO₄³⁻, and TA were analyzed via APHA standard methods [34] and commercial test kits (Hach Company, Loveland, CO, USA). The biotoxicity tests of leaching solution from the addition of various concentrations of AF-BC (0–10 g/L) into DI water was conducted using a Toxi-ChromoTest™ kit (Environmental Bio-Detection Products Inc., Mississauga, ON, Canada) [35]. The biogas volume was evaluated using a 60 mL syringe, and methane and carbon dioxide contents of biogas were determined through the gas chromatograph (GC) (GC-2014, Shimaza Corp., Kyoto, Japan) connected with a packed column, a thermal conductivity detector (TCD) and a flame
ionization detector (FID). The concentrations of VFAs were also determined by the GC as previously described [25]. The FID temperature was 250 °C, and the carrier gas was helium.

2.5. Modified Gompertz Model

For examining the impact of AF-BC addition on lag phase, maximum production potential and production rate from AD of dairy manure in the current work, the methane production data from the AD tests were fitted to a modified Gompertz model shown in the following equation [4,25]:

\[
M(t) = P \times \exp \left\{ -\exp \left[ \frac{R_{\text{max}} \times e^{\lambda - t}}{P} + 1 \right] \right\}
\]

where \(M(t)\) represents the methane yield in a time \(t\) (mL/g VS\(_{\text{removed}}\)), \(P\) represents the maximum methane potential (mL/g VS\(_{\text{removed}}\)), \(R_{\text{max}}\) represents the maximum methane production rate (mL/g VS\(_{\text{removed}}\)·d), \(\lambda\) represents the lag phase (d), and \(e\) is the Euler’s constant (2.7183). In the present study, the fitting precision of the model was determined through the determination coefficient \((R^2)\):

\[
R^2 = 1 - \frac{\sum (M_e - M_c)^2}{\sum (M_e - M_{\text{mean}})^2}
\]

where \(M_e\), \(M_c\), and \(M_{\text{mean}}\) represent the experimental value, calculated value, and mean of experimental value, respectively.

3. Results and Discussion

3.1. Effects of AF-BC Addition on Methane and Biogas Production

Biogas and methane production from the AD of dairy manure with various loading rates of AF-BC are displayed in Figure 1. The highest cumulative biogas and methane production in the AD of dairy manure was achieved with the addition of 5 g/L (A5) of AF-BC (Figure 1a,b). However, compared to the control, the addition of 10 g/L (A10) of AF-BC to the AD of dairy manure showed negative effects on cumulative biogas and methane volumes, probably due to the potential biotoxicity from AF-BC addition, which is described in Section 3.2. Conversely, the biogas yields in A1 and A5 were improved by 15.51% and 26.09%, respectively, compared to the control (625.99 mL/g VS\(_{\text{removed}}\)) (Figure 1c). Similarly, the methane yields in A1 and A5 increased by 14.61% and 26.88%, respectively, compared to the control (300.01 mL/g VS\(_{\text{removed}}\)) (Figure 1d). Therefore, compared to the control, the addition of AF-BC (1–5 g/L) to the AD of dairy manure resulted in effectively enhancing biogas and methane production. As shown in Table S1, these results were remarkably consistent with the positive impacts of BC on methane and biogas production reported by previous studies [4,36]. Wei et al. [37] evaluated the impacts of corn stover-derived BC on the AD of primary sludge and found that BC significantly improved the methane yield by 8.6–17.8%. Pan et al. [4] conducted the AD of chicken manure with the addition of nine different kinds of BCs, and the results showed that all BCs significantly improved methane yields, and the addition of fruitwood-derived BC achieved a methane yield of 294 mL/g VS. Moreover, the AD of sewage sludge and orange peels carried out by Martínez et al. [36] indicated that 10 g/L of vineyard pruning-derived BC improved the methane yield by 33% in the batch digestion system.
However, the addition of 10 g/L of AF-BC (A10) showed no obvious improvement of methane and biogas yields compared with the control. The biogas and methane yields of A10 were 641.70 mL/g VS removed and 301.68 mL/g VS removed (Figure 1), which were lower than those of A1 and A5, with almost no difference from the control, indicating that a high loading rate of AF-BC was not favorable for the AD of dairy manure. Similar results have also been observed by Shen et al. [2], Shen et al. [38], and Sunyoto et al. [39]. Shen et al. [2] found that CH$_4$ yield from the AD of mixtures of straw and cattle manure increased from 267.55 to 281.48 mL/g VS with the addition of coconut shell-derived BC at a 2% loading rate. However, the CH$_4$ yield was reduced to 271.5 mL/g VS with 4% loading of the BC, mainly because of the accumulation of higher concentrations of acidic intermediates resulting in the imbalance of the AD system. Shen et al. [38] also reported that the high loading rates of wood-derived BC to the AD of primary sludge showed no significant difference for methane production compared to the control due to possible inhibition of microbial activities among AD microorganisms. Moreover, Sunyoto et al. [39] investigated the two-phase AD process using food waste with the addition of sawdust-derived BC. The results indicated that increasing loading rates of the BC ranging from 8.3–33.3 g/L caused a decrease in methane production. It was found that the addition of BC at high loading rates caused a significant accumulation of propionic acid, which could inhibit methane production [39].

As displayed in Figure 2, the first daily methane yield peak appeared on the fourth day of the AD process for all experiment groups, which might have been caused by the
consumption of easily degradable organic matter by anaerobic microorganisms [4]. The maximum daily methane yields in A1 (26.84 mL/g VSremoved), A5 (34.35 mL/g VSremoved), and A10 (27.45 mL/g VSremoved) were obviously higher than that in the control (23.29 mL/g VSremoved), which means that AF-BC could enhance utilization efficiency of organic substrates in the initial stage of AD. In addition, the second and third peaks in A5 (17th and 24th day) appeared earlier than those in the control (18th and 26th day), revealing that the addition of AF-BC to the AD process can accelerate the degradation of complex intermediates in the middle and later stages, thus increasing methane production overall [4]. Pan et al. [4] also observed three daily methane yield peaks during the AD of chicken manure and indicated that the addition of BC produced methane earlier than the control without the addition of BC.

![Figure 2. Daily methane yield from AD of dairy manure with the addition of different concentrations of AF-BC.](image)

In the present study, the experimental data were also fitted to the Gompertz model to develop a microbial kinetic model for the AD of dairy manure with the addition of AF-BC. Table 1 shows that A1 and A5 achieved significantly higher \( R_{\text{max}} \) (mL CH₄/g VSremoved·d) and \( P \) (mL CH₄/g VSremoved) than the control. Both \( R_{\text{max}} \) and \( P \) increased by 17.70% and 13.89% in A1, and 30.86% and 25.60% in A5, respectively, compared with the control. The results highly agree with positive impacts of BC addition on the methane production rate and yield \((R_{\text{max}} \text{ and } P)\) during the AD process [1,25,40]. For example, Wei et al. [37] showed that both \( R_{\text{max}} \) and \( P \) were improved by 53.79% and 13.72% when the corn stover-derived BC was applied to the AD of primary sludge obtained from the WWTP. However, there were no significant differences between the control and A10 for \( R_{\text{max}} \) (15.20 vs. 15.51 mL/g VSremoved·d) and \( P \) (295.78 vs. 297.47 mL/g VSremoved). Shen et al. [38] indicated that a high concentration of wood-derived BC (4.97 g BC/g dry sludge) caused the lower \( R_{\text{max}} \) and \( P \) than a low concentration of BC (2.49 g BC/g dry sludge). Moreover, Wang et al. [1] also reported that \( R_{\text{max}} \) and \( P \) significantly increased during the AD of sludge and food waste with increasing loading of the sawdust-derived BC up to 6 g/L. However, the \( R_{\text{max}} \) and \( P \) markedly decreased with high loading of BC (more than 6 g/L). In addition, as noted in Table 1, the lag phase (\( \lambda \)) in AD was shortened after the addition of AF-BC. Compared to the control, \( \lambda \) was reduced by 7.14%, 22.45%, and 12.24% under the addition of 1, 5, and 10 g/L of AF-BC, possibly owing to faster adaptation and communication of microorganisms...
with the addition of AF-BC. Similarly, Fagbohungbe et al. [41] applied three kinds of BCs (coconut shell BC, rice husk BC, and wood BC) in the process of AD of citrus peel and indicated that all BCs could lower the lag phase of AD. Wang et al. [42] also found that Douglas fir-derived BCs pyrolyzed at 500 and 600 °C led to the decrease in lag phase by 14.29% and 9.52% during AD of wastewater sludge.

### Table 1. Parameter values of modified Gompertz model fitted with the experimental data.

| Biochar Addition | Lag Phase, λ (d) | \( R_{\text{max}} \) (mL CH\(_4\)/g VS\(_{\text{removed}}\)) | \( P \) (mL CH\(_4\)/g VS\(_{\text{removed}}\)) | \( R^2 \) | \( p \) Value |
|------------------|------------------|---------------------------------|---------------------------------|--------|-------------|
| Control          | 0.98 ± 0.01      | 15.20 ± 0.12                    | 295.78 ± 11.71                  | 0.99   | <0.001      |
| A1 (1 g/L)       | 0.91 ± 0.17      | 17.89 ± 0.39                    | 336.85 ± 11.87                  | 0.99   | <0.001      |
| A5 (5 g/L)       | 0.76 ± 0.08      | 19.89 ± 0.48                    | 371.51 ± 9.30                   | 0.99   | <0.001      |
| A10 (10 g/L)     | 0.86 ± 0.03      | 15.51 ± 0.24                    | 297.47 ± 3.21                   | 0.99   | <0.001      |

### 3.2. Potential Roles of AF-BC in AD

#### 3.2.1. COD, Ammonia, and Phosphate

The impacts of AF-BC addition on COD, ammonia, and phosphate removal in the AD process are presented in Figure 3. A1, A5 and A10 achieved COD removal efficiencies of 56.64%, 64.60%, and 62.39% (Figure 3a), which were higher than that of the control (47.52%). This supported that the addition of AF-BC improved COD reduction from the AD of dairy manure. It was thought that microbial activity of AD can increase with the addition of AF-BC to AD, resulting in a higher consumption of organic compounds in the AD of dairy manure. The result was consistent with that reported by Shanmugam et al. [43], showing that switchgrass-derived BC could significantly improve the efficiency of COD removal by 16% during the AD of glucose. Moreover, Choe et al. [44] added the bamboo-derived hydrochar into the AD of fish processing waste while significantly enhancing COD reduction. However, the COD removal efficiency in A10 (Figure 3a) was comparable to that in A5, revealing that high loading of AF-BC was not favorable for further COD reduction. One assumption could be that AF-BC might contain some toxic compounds, which were produced during the pyrolysis process, resulting in the negative effects on AD microorganism activity due to the high loading of AF-BC. Lyu et al. [45] also reported that toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) and dioxin-compounds were generated during the BC production by pyrolyzing the sawdust at 300 to 700 °C, with a higher toxicity of BC at lower temperatures. In addition, Smith et al. [46] indicated that toxicity of pine wood-derived BCs was mainly from lignin-derived phenolic compounds in BCs, and the toxicity of BCs was higher at a low pyrolysis temperature (300–400 °C). Moreover, in this study, the biotoxicity of leaching solution of AF-BC showed an increasing trend with an increase in AF-BC concentration from 1 to 10 g/L (Table S2). Therefore, high loading of AF-BC, produced from pyrolysis of grass alfalfa (containing 13% lignin) at low temperature (350 °C), in this study, might contain possible toxic compounds such as PAHs and phenolic compounds, which would be detrimental to AD microorganisms and negatively affect the AD process. Thus, this could explain the decrease in methane production with high loading of AF-BC (10 g BC/L, A10 in Figure 1).
As shown in Figure 3b, after AD, ammonia concentrations significantly increased in all experiment groups, from 96.2–102.4 to 773–803 mg/L NH$_3$-N, which resulted from effective degradation of nitrogen-containing organic matters by AD microorganisms. Moreover, the final ammonia concentrations in A1 and A5 showed no significant difference with that in the control. However, compared to the control, a high dosage of AF-BC (A10) increased the final ammonia concentration from 777 to 803 mg/L. Similarly, Figure 3c indicates that the AD with AF-BC also enhanced phosphate concentration due to the enhanced hydrolysis of organic phosphate into inorganic phosphate. The high ammonium and increased phosphate concentrations, after the AD of manure, could be valuable sources of liquid biofertilizer when properly irrigated to agricultural farms.

3.2.2. CO$_2$ Content

Biogas, produced from the AD process, largely consists of methane, carbon dioxide, and other impurities. CO$_2$, considered as a greenhouse gas, can cause climate change, while higher CO$_2$ concentrations result in the lower energy value of biogas [47]. Therefore, possibly low amounts of CO$_2$ in biogas are beneficial for high energy values of biogas and have a low impact on climate change. Figure 4 shows that, after 36 days of AD, the CO$_2$ content of biogas in A1, A5, and A10 decreased from 48.59% to 41.98%, 38.57%, and 32.81% compared to the control, respectively, implying that AF-BC addition was favorable for the reduction of the CO$_2$ content in biogas. In addition, it is obvious that the CO$_2$ content in biogas decreased with an increased loading rate of AF-BC. It is well known that BC has...
the adsorption capacity for CO$_2$ in biogas via physisorption (e.g., Van der Waals’ force), which could be enhanced by chemical interactions between acidic CO$_2$ and basic nitrogen functional groups of BC [48]. Creamer et al. [49] also mentioned that nitrous groups in the BC played an important role in CO$_2$ adsorption by the BC. As seen in Figure S2, AF-BC contained some nitrous functional groups (C-N and N-O), which was beneficial for CO$_2$ capture in the AD of dairy manure. Similarly, Baltrėnas et al. [47] conducted the AD of chicken manure with the addition of wood BC and indicated that the addition of wood BC did not result in enhancement for biogas production, but CO$_2$ content decreased from 47.5% to 33.1%.

**Figure 4.** Change of CO$_2$ concentration during AD of dairy manure with the addition of different concentrations of AF-BC.

### 3.2.3. VFAs Analysis

VFAs accumulation during AD processes often causes drastic pH drops and inhibition of microbial activity [19]. Figure 5 indicates that all experiments in the control, A1, A5 and A10 exhibited a similar trend of total VFAs concentration variation. Total VFAs concentration at 7 d was the highest and then rapidly decreased in all experiments. This is due to the rapid hydrolysis of the easily degradable organic matter in the manure by AD microorganisms at the initial stage. Compared to the control, total VFAs concentration was significantly reduced with the addition of AF-BC. For instance, in the seventh day, total VFAs concentrations in A1, A5, and A10 (124.89, 94.64, and 153.69 mg/L) were lower than the control (214.66 mg/L). Wang et al. [24] and Sunyoto et al. [39] found that the addition of vermicompost BC and sawdust BC to the AD of chicken manure and white bread can significantly reduce VFAs accumulation and lead to an increase in methane production. In contrast, Pan et al. [23] reported that excess BC could cause an excessive acceleration of hydrolysis and acidogenesis-acetogenesis to an unbearable extent, resulting in an imbalance to the AD system and the accumulation of intermediates, which finally reduced methane production. Therefore, in the present study, total VFAs concentration in A5 was less than that in A10, which supported the result that high concentrations of AF-BC were not favorable for methane production.
nally reduced methane production. Therefore, in the present study, total VFAs concentration in A5 was less than that in A10, which supported the result that high concentrations of AF-BC were not favorable for methane production. Figure 5.

As seen in Figure 5, acetic acid was the primary VFA from the AD of dairy manure under all experimental conditions. Acetic acid is one of the main precursors for methane production and could be directly utilized by acetotrophic methanogens for methane production [19]. Figure 5 also indicates that the addition of AF-BC led to the decrease in the concentration of acetic acid, implying that AF-BC could facilitate the transformation of acetic acid to methane and finally improve the methane yield. Lately, DIET has been considered as an effective pathway for enhancing methane production in the AD process [19, 23]. Figure S2 shows that AF-BC possessed various functional groups, including C-H, C-O, C-N, N-O, and C=C, which are usually related with quinone, phenazine, and hydroquinone moieties [50, 51]. In addition, these moieties can act as electron shuttles, accepting electrons from microorganisms and donating electrons to microorganisms, which are favorable for the DIET [50, 51]. Thus, it can be inferred that AF-BC can enhance the metabolism of acetic acid via the DIET between acetogens and methanogens on the surface of AF-BC, which improved the efficiency of conversion of acetate to methane.

3.2.4. Total Alkalinity and pH

Figure 6a indicates that TA in A1 was comparable to that in the control; however, TAs in A5 and A10 were much higher than that in the control during the AD process. A higher TA concentration with the addition of AF-BC was thought to exist because AF-BC contained various alkali and alkaline-earth metals such as K (1 g/kg BC), Ca (14 g/kg BC), and Mg (3 g/kg BC) (Table S3), which endowed the AF-BC with alkalinity properties and buffering abilities in the AD system [4, 24]. Moreover, macronutrients such as N (49 g/kg)
and P (2 g/kg) in AF-BC (Table S3) also played an important role as buffering agents in the AD system [3,4]. Therefore, Figure 6b also shows that the pH values in the AD of dairy manure with the addition of AF-BC increased compared to the control. Wang et al. [24] also mentioned that vermicompost BC increased the buffering capacity to avoid drastic reduction of pH owing to a high accumulation of VFAs and maintaining high activity of AD microorganisms during the AD of kitchen wastes and chicken manure, while resulting in an increase in AD performance and methane production. In this study, the increased TA concentration resulted in higher pH and OH$^-$ ions, which reacted with CO$_2$ to produce CO$_3^{2-}$ or HCO$_3^-$ . Both CO$_2$ and CO$_3^{2-}$/HCO$_3^-$ could act as the electron acceptors and were reduced to methane by hydrogenotrophic methanogens [52,53]. Therefore, the increase in TA and pH values due to the addition of AF-BC could facilitate the maintenance of CO$_2$ in the form of CO$_3^{2-}$/HCO$_3^-$ in aqueous solution, which enhanced the CO$_2$ utilization for methane production [25,52]. Thus, based on the results from this study, the addition of AF-BC at a moderate loading rate (or appropriate loading rate) could significantly improve the performance of AD of dairy manure. Future works will focus on analyzing microbial communities and the understanding of possible mechanisms associated with the AD of dairy manure with the addition of AF-BC.

Figure 6. Changes of TA (a) and pH (b) during the AD of dairy manure with the addition of different concentrations of AF-BC.
4. Conclusions

The effects of AF-BC on the AD of dairy manure were examined in the present study. The AD of dairy manure with the addition of AF-BC at 1–5 g/L showed a significant enhancement of biogas and methane production compared to the control. The biogas and methane yields during the AD of dairy manure increased by 15.51% and 14.61%, with the addition of 1 g/L AF-BC, and 26.09% and 26.88% with the addition of 5 g/L AF-BC. The main reasons for the increase in AD performance were that the addition of AF-BC improved the buffering ability of the AD system and DIET between AD microorganisms. However, the addition of AF-BC at 10 g/L was not favorable for methane production, possibly owing to toxicity of AF-BC at high loading. Besides, the addition of AF-BC also reduced the lag phase and VFAs concentrations while increasing total alkalinity in the AD process. Overall, this study provides valuable information for application of AF-BC in the enhancement of AD of dairy manure. This sustainable reuse of various wastes (waste hay, manure) at dairy farms through interaction of biochar and AD could significantly enhance environmental and agricultural sustainability at dairy and other animal farms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12040911/s1, Table S1: Summary of effects of various biochars on methane production during anaerobic digestion [54,55]; Table S2: The biotoxicity of leaching solutions of AF-BC at various concentrations; Table S3: The physicochemical characteristics of AF and AF-BC; Figure S1: Experiment set-up for anaerobic digestion of dairy manure with the addition of AF-BC; Figure S2: FT-IR spectrum of AF-BC.

Author Contributions: S.Z. carried out all experiments, analyzed the experimental data, and wrote the original manuscript. R.H. participated in the measurement of biogas during the AD experiments. E.K. provided the novel concepts, methodologies, and funding for this research work and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Texas A&M University Chancellor Research Initiative Fund (435680) and by the US Department of Agriculture (TEX09764, 1022440).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data reported in this study are contained within the article.

Acknowledgments: We would like to thank the financial support from Texas A&M University Chancellor Research Initiative Fund, grant number 435680, and the US Department of Agriculture, grant number TEX09764.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Wang, G.; Li, Q.; Gao, X.; Wang, X.C. Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: Performance and associated mechanisms. Bioresour. Technol. 2018, 250, 812–820. [CrossRef] [PubMed]
2. Shen, R.; Jing, Y.; Feng, J.; Luo, J.; Yu, J.; Zhao, L. Performance of enhanced anaerobic digestion with different pyrolysis biochars and microbial communities. Bioresour. Technol. 2020, 296, 122354. [CrossRef] [PubMed]
3. Romero-Güiza, M.S.; Vila, J.; Mata-Alvarez, J.; Chimenos, J.M.; Astals, S. The role of additives on anaerobic digestion: A review. Renew. Sustain. Energy Rev. 2016, 58, 1486–1499. [CrossRef]
4. Pan, J.; Ma, J.; Liu, X.; Zhai, L.; Ouyang, X.; Liu, H. Effects of different types of biochar on the anaerobic digestion of chicken manure. Bioresour. Technol. 2019, 275, 258–265. [CrossRef] [PubMed]
5. Li, K.; Liu, R.; Sun, C. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. Bioresour. Technol. 2015, 198, 133–140. [CrossRef] [PubMed]
6. Dolliver, H.; Kumar, K.; Gupta, S. Sulfamethazine uptake by plants from manure-amended soil. J. Environ. Qual. 2007, 36, 1224–1230. [CrossRef] [PubMed]
7. MacDonald, J.M.; O’Donoghue, E.J.; McBride, W.; Nehring, R.; Sandretto, C.L.; Mosheim, R. Profits, Costs, and the Challenging Structure of Dairy Farming/ERR-47; USDA ARS Rep; USDA ERS: Washington, DC, USA, 2018.
8. Hill, D.; Morra, M.J.; Stalder, T.; Jechalke, S.; Top, E.; Pollard, A.T.; Popova, I. Dairy manure as a potential source of crop nutrients and environmental contaminants. *J. Environ. Sci.* 2021, 100, 117–130. [CrossRef]

9. Flores-Orozco, D.; Patidar, R.; Levin, D.B.; Sparling, R.; Kumar, A.; Cicek, N. Effect of mesophilic anaerobic digestion on the resistosome profile of dairy manure. *Bioren. Technol.* 2020, 315, 123889. [CrossRef]

10. Zheng, Z.; Liu, J.; Yuan, X.; Wang, X.; Zhu, W.; Yang, F.; Cui, Z. Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion. *Appl. Energy* 2015, 151, 249–257. [CrossRef]

11. Zeng, S.; Jang, H.M.; Park, S.; Park, S.; Kan, E. Effects of mechanical refining on anaerobic digestion of dairy manure. *ACS Omega* 2021, 6, 16934–16942. [CrossRef]

12. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioren. Technol.* 2008, 99, 4044–4064. [CrossRef] [PubMed]

13. Yenigün, O.; Demirel, B. Ammonia inhibition in anaerobic digestion: A review. *Process Biochem.* 2013, 48, 901–911. [CrossRef]

14. Zhang, J.; Zhao, W.; Zhang, H.; Wang, Z.; Fan, C.; Zang, L. Recent achievements in enhancing anaerobic digestion with carbon-based functional materials. *Bioren. Technol.* 2018, 266, 555–567. [CrossRef] [PubMed]

15. Li, L.L.; Tong, Z.H.; Fang, C.Y.; Chu, J.; Yu, H.Q. Response of anaerobic granular sludge to single-wall carbon nanotube exposure. *Water Res.* 2015, 70, 1–8. [CrossRef]

16. Lei, Y.; Sun, D.; Dang, Y.; Chen, H.; Zhao, Z.; Zhang, Y.; Holmes, D.E. Stimulation of methanogenesis in anaerobic digesters treating leachate from a municipal solid waste incineration plant with carbon cloth. *Bioren. Technol.* 2016, 222, 270–276. [CrossRef]

17. Lin, R.; Cheng, J.; Zhang, J.; Zhou, J.; Chen, K.; Murphy, J.D. Boosting biomethane yield and production rate with graphene: The potential of direct interspecies electron transfer in anaerobic digestion. *Bioren. Technol.* 2017, 239, 345–352. [CrossRef] [PubMed]

18. Yang, Y.; Zhang, Y.; Li, Z.; Zhao, Z.; Quan, X.; Zhao, Z. Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. *J. Clean. Prod.* 2017, 149, 1101–1108. [CrossRef]

19. Qiu, L.; Deng, Y.F.; Wang, F.; Davaritouchae, M.; Yao, Y.Q. A review on biochar-mediated anaerobic digestion with enhanced methane recovery. *Renew. Sustain. Energy Rev.* 2019, 115, 109573. [CrossRef]

20. Zeng, S.; Kan, E. Chemical activation of forage grass-derived biochar for treatment of aqueous antibiotic sulfamethoxazole. *ACS Omega* 2020, 5, 13793–13801. [CrossRef]

21. Sharma, B.; Suthar, S. Enriched biogas and biofertilizer production from Eichhornia weed biomass in cow dung biochar-amended anaerobic digestion system. *Environ. Technol. Innov.* 2021, 21, 101201. [CrossRef]

22. Wang, G.; Li, Q.; Gao, X.; Wang, X.C. Sawdust-derived biochar much mitigates VFAs accumulation and improves microbial activities to enhance methane production in thermophilic anaerobic digestion. *ACS Sustain. Chem. Eng.* 2018, 7, 2141–2150. [CrossRef]

23. Pan, J.; Ma, J.; Zhai, L.; Luo, T.; Mei, Z.; Liu, H. Achievements of biochar application for enhanced anaerobic digestion: A review. *Bioren. Technol.* 2019, 292, 122058. [CrossRef] [PubMed]

24. Wang, D.; Ai, J.; Shen, F.; Yang, G.; Zhang, Y.; Deng, S.; Zhang, J.; Zeng, Y.; Song, C. Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermiconpost. *Bioren. Technol.* 2017, 227, 286–296. [CrossRef]

25. Jang, H.M.; Choi, Y.K.; Kan, E. Effects of dairy manure-derived biochar on psychrophilic, mesophilic and thermophilic anaerobic digestions of dairy manure. *Bioren. Technol.* 2018, 250, 927–931. [CrossRef] [PubMed]

26. Jang, H.M.; Brady, J.; Kan, E. Succession of microbial community in anaerobic digestion of dairy manure induced by manure-derived biochar. *Environ. Eng. Res.* 2021, 26, 138–155. [CrossRef]

27. Jang, H.M.; Kan, E. Engineered biochar from agricultural waste for removal of tetracycline in water. *Bioren. Technol.* 2019, 284, 437–447. [CrossRef]

28. McDonald, I.; Baral, R.; Min, D. Effects of alfalfa and alfalfa-grass mixtures with nitrogen fertilization on dry matter yield and forage nutritive value. *J. Anim. Sci. Technol.* 2021, 63, 305–318. [CrossRef]

29. Casteel, S.W. Liver disease in cattle induced by consumption of moldy hay. *Vet. Hum. Toxicol.* 1995, 37, 248–251.

30. Boateng, A.A.; Mullen, C.A.; Goldberg, N.; Hicks, K.B.; Jung, H.-J.G.; Lamb, J.F. Production of bio-oil from alfalfa stems by fluidized-bed fast pyrolysis. *Ind. Eng. Chem. Res.* 2008, 47, 4115–4122. [CrossRef]

31. Wang, S.; Gao, B.; Zimmerman, A.R.; Li, Y.; Ma, L.; Harris, W.G.; Migliaccio, K.W. Physicochemical and sorptive properties of biochars derived from woody and herbaceous biomass. *Bioresour. Technol.* 2018, 250, 138–155. [CrossRef]

32. Jang, H.M.; Kan, E. Effects of pyrolysis temperature on the physicochemical properties of alfalfa-derived biochar for the adsorption of bisphenol A and sulfamethoxazole in water. *Bioresour. Technol.* 2019, 218, 741–748. [CrossRef] [PubMed]

33. ASTM D7582-12; Standard Test Methods for Proximate Analysis of Coal and Coke by Macro Thermogravimetric Analysis. ASTM: West Conshohocken, PA, USA, 2012.

34. APHA. *Standard Methods for the Examination of Water and Wastewater*; APHA: Washington DC, USA, 2005.

35. Zeng, S.; Kan, E. Thermally enhanced adsorption and persulfate oxidation-driven regeneration on FeCl₃-activated biochar for removal of microcystin-LR in water. *Bioresour. Technol.* 2021, 286, 131950. [CrossRef] [PubMed]

36. Martínez, E.J.; Rosas, J.G.; Sotres, A.; Moran, A.; Cara, J.; Sánchez, M.E.; Gómez, X. Codigestion of sludge and citrus peel wastes: Evaluating the effect of biochar addition on microbial communities. *Biochem. Eng. J.* 2018, 137, 314–325. [CrossRef]
37. Wei, W.; Guo, W.; Ngo, H.H.; Mannina, G.; Wang, D.; Chen, X.; Liu, Y.; Peng, L.; Ni, B.J. Enhanced high-quality biomethane production from anaerobic digestion of primary sludge by corn stover biochar. *Bioresour. Technol.* **2020**, *306*, 123159. [CrossRef]

38. Shen, Y.; Linville, J.L.; Ignacio-de Leon, F.A.A.; Schoene, R.P.; Urgun-Demirtas, M. Towards a sustainable paradigm of waste-to-energy process: Enhanced anaerobic digestion of sludge with woody biochar. *J. Clean. Prod.* **2016**, *135*, 1054–1064. [CrossRef]

39. Sunyoto, N.M.S.; Zhu, M.; Zhang, Z.; Zhang, D. Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresour. Technol.* **2016**, *219*, 29–36. [CrossRef]

40. Cai, J.; He, P.; Wang, Y.; Shao, L.; Lu, F. Effects and optimization of the use of biochar in anaerobic digestion of food wastes. *Waste Manag. Res.* **2016**, *34*, 409–416. [CrossRef]

41. Fagbohungbe, M.O.; Herbert, B.M.; Hurst, L.; Li, H.; Usmani, S.Q.; Semple, K.T. Impact of biochar on the anaerobic digestion of citrus peel waste. *Bioresour. Technol.* **2016**, *216*, 142–149. [CrossRef]

42. Wang, P.; Peng, H.; Adhikari, S.; Higgins, B.; Roy, P.; Dai, W.; Shi, X. Enhancement of biogas production from wastewater sludge via anaerobic digestion assisted with biochar amendment. *Bioresour. Technol.* **2020**, *309*, 123368. [CrossRef]

43. Shanmugam, S.R.; Adhikari, S.; Nam, H.; Sajib, S.K. Effect of bio-char on methane generation from glucose and aqueous phase of algae liquefaction using mixed anaerobic cultures. *Biomass Bioenergy* **2018**, *108*, 479–486. [CrossRef]

44. 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]

45. Lyu, H.; He, Y.; Tang, J.; Heater, M.; Liu, Q.; Jones, P.D.; Codling, G.; Giesy, J.P. Effect of pyrolysis temperature on potential toxicity of biochar if applied to the environment. *Environ. Pollut.* **2016**, *218*, 1–7. [CrossRef] [PubMed]

46. Smith, C.R.; Hatcher, P.G.; Kumar, S.; Lee, J.W. Investigation into the sources of biochar water-soluble organic compounds and their potential toxicity on aquatic microorganisms. *ACS Sustain. Chem. Eng.* **2016**, *4*, 2550–2558. [CrossRef]

47. Baltrėnas, P.; Paliulis, D.; Kolodynskij, V. The experimental study of biogas production when digesting chicken manure with a biochar additive. *Greenh. Gases Sci. Technol.* **2019**, *9*, 837–847. [CrossRef]

48. Sethupathi, S.; Zhang, M.; Rajapaksha, A.; Lee, S.; Mohamed Nor, N.; Mohamed, A.; Al-Wabel, M.; Lee, S.; Ok, Y. Biochars as potential adsorbers of CH₄, CO₂ and H₂S. *Sustainability* **2017**, *9*, 121. [CrossRef]

49. Creamer, A.E.; Gao, B.; Zhang, M. Carbon dioxide capture using biochar produced from sugarcane bagasse and hickory wood. *Chem. Eng. J.* **2014**, *249*, 174–179. [CrossRef]

50. Wang, J.; Zhao, Z.; Zhang, Y. Enhancing anaerobic digestion of kitchen wastes with biochar: Link between different properties and critical mechanisms of promoting interspecies electron transfer. *Renew. Energy* **2021**, *167*, 791–799. [CrossRef]

51. Ren, S.; Usman, M.; Tsang, D.C.M.; 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]

52. Qin, Y.; Wang, H.; Li, X.; Cheng, J.J.; Wu, W. Improving methane yield from organic fraction of municipal solid waste (OFMSW) with magnetic rice-straw biochar. *Bioresour. Technol.* **2017**, *245*, 1058–1066. [CrossRef]

53. Wu, B.; Yang, Q.; Yao, F.; Chen, S.; He, L.; Hou, K.; Pi, Z.; Yin, H.; Fu, J.; Wang, D.; Li, X. Evaluating the effect of biochar on mesophilic anaerobic digestion of waste activated sludge and microbial diversity. *Bioresour. Technol.* **2019**, *294*, 122235. [CrossRef]