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Effect of Biochar on Anaerobic Co-Digestion of Untreated Sewage Sludge with Municipal Organic Waste under Mesophilic Conditions

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Abstract: Anaerobic digestion (AD) is a biological process that occurs in the limited presence of oxygen. This process involves various difficulties during the operation, such as acidification and increased concentration of volatile fatty acids, which can inhibit methane production. Therefore, in this work, the impact of biochar on the co-digestion of untreated sludge and residual biomass under mesophilic conditions was studied. For the production of biochar, the gasification process was used at different temperatures: 759 °C (BL), 798 °C (BM), and 888 °C (BH). This biochar was added in concentrations of 0 g/L, 3.33 g/L, and 6.67 g/L at the beginning of the co-digestion process. The results showed that a concentration of 6.67 g/L with BH biochar increased the PBM by 18% compared to the control sample and reduced the chemical oxygen demand (COD) by 88%. In addition, there was a reduction of volatile fatty acids (VFA) of 42.75%. Furthermore, FTIR analysis demonstrated that biochar has appropriate functional groups for this process. These data suggest a good interaction of biochar with the mixture of sludge and municipal waste, indicating that biochar can improve the anaerobic co-digestion of untreated sludge and municipal waste.

Keywords: biochar; sewage sludge; anaerobic digestion; mesophilic conditions; methane; chemical oxygen demand

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1. Introduction

The anaerobic digestion (AD) process could generate a mixture of gases known as biogas. This gas is mainly composed of methane (CH_4), carbon dioxide (CO_2) [1], and traces of hydrogen sulfide (H_2S) [2]. The biodegradation of organic matter is conducted by microorganisms present in the sludge and the aqueous media [1–3]. Processing biomass residues through AD can reduce greenhouse gas emissions and promote a circular economy. Furthermore, the waste produced during AD can be used for the production of nutrient-rich substrates or the generation of fuel, such as biogas [4]. Degrading organic materials obtain biogas under oxygen-free conditions [5,6]. These wastes include livestock manure, food waste, and sewage sludge [5]. Currently, most agricultural biogas plants are located in Asia and Europe. For example, China has more than 100,000 biogas plants. In addition, China has domestic biogas generation programs, so the number of plants is approximately 40 million. Meanwhile, Germany has more than 10,000 biogas plants [7].

The increase in population leads to more food production, which increases the generation of municipal organic waste (ROM). According to the World Bank, in growing and low-income countries, 93% of municipal solid waste is incinerated or deposited in open fields, roads, thoroughfares, and bodies of water. Large-scale waste management continues to be a problem for developing countries [8]. However, only 2% of municipal solid waste in high-income countries is mismanaged [9].

The co-digestion process has been presented as an option for treating organic municipal solid waste to produce biofuels. In this process, sewage sludge provides the microorganisms needed for biomass biological degradation in an oxygen-free environment. For better biogas production results, it is well understood that sewage sludge needs to be stabilized and, in some cases, pretreated [10]. Pretreatment processes help improve codigestion by promoting microbial growth and limiting the inhibition of processing. However, alternatives are required to digest biomass without using pretreatments. Reducing pretreatments could simplify the operation of the digestion systems and reduce operating costs. However, untreated sewage sludge presents specific difficulties due to the possible acidification and contamination of the digested biomass.

Moreover, acidification could decrease biogas production in the AD process since a neutral or almost neutral pH is needed to promote the growth of methanogenic bacteria [11]. While some authors point out that a pH between 6.7 and 8.4 can ensure optimal biogas production [6], others have presented a narrower range between 6.7 and 7.2 [12]. Furthermore, other factors can impact anaerobic digestion performance, such as the accumulation of volatile fatty acids (VFAs), ammonium contamination, or unstable temperature during digestion [13–17]. Several alternatives have been studied to improve biogas production in the co-digestion process, such as using biochar as an additive, applying pretreatments to biomass, and adding stabilizers such as calcium carbonate. Therefore, biochar has been proposed as an alternative to improve the anaerobic digestion of municipal solid waste. Also, applying pretreatments to the biomass should be avoided [18]. In this sense, it has been determined that, in most studies, the sewage sludge used in the AD process has been pretreated, which could generate higher processing costs for biogas production.

Biochar is a high-carbon-based material produced from the thermal conversion of biomass in an oxygen-free atmosphere at temperatures above 250 °C [18]. The implementation of biochar has been proposed in different studies [15,17–19]. Several of these studies have shown that biochar helps stabilize pH and can help adjust the pH level to an optimal range [19–24]. In addition, biochar has been shown to reduce the lag phase of the system and improve methane production [14,25,26].

Biochar production processes such as pyrolysis and hydrothermal conversion have been reported in the literature to improve the digestion of organic waste [27,28]. However, biochar from top-lit updraft gasification has been little used to improve the co-digestion process.

Therefore, this study aimed to evaluate the effect of biochar generated by gasification on the anaerobic co-digestion of untreated sewage sludge. The untreated sewage sludge with municipal organic waste was evaluated under mesophilic conditions.

2. Materials and Methods

2.1. Organic Sludge and Leachate from Municipal Organic Waste

The untreated sewage sludge was collected from the Waste Water Treatment Plant of Juan Díaz (WWTP) (Panama City, Panamá). This sludge was stored at low temperatures (4 °C) to prevent the unexpected growth of microorganisms [29]. The used sludge was untreated in all experiments. Municipal organic waste leachate was prepared for the experiments. The mixture was prepared with 40% fruits, 40% vegetables, and 20% tubers. Residues of cucumber, cabbage, potato peels, orange, pear, and banana, which were crushed in a blender (NINJA, Model BL641, Katy, TX, USA) for subsequent leaching, were used. Three parallel tests were conducted to determine the liquid volume using 600 g of residues. During these tests, it was determined that approximately 125 mL of percolation is generated for every 600 g of crushed biomass. Leachate can provide water, nutrients, and microorganisms, which can influence the productivity of the anaerobic process. It also contributes to the dilution of some products of the biological process, such as volatile fatty acids (VFAs), ammonium, and H_2 [30].

Characterization of Organic Sludge and Leachate

COD (chemical oxygen demand), VFA (volatile fatty acids), alkalinity, ammonium, total nitrogen, and TOC (total organic carbon) analyses were performed with HACH methods 10212 [31], 10240 [32], 10239 [33], 10205 [34], 10208 [35], and 10267 [36], respectively. The pH was determined using the ISO 10390 method [37]. A spectrophotometer was used to measure the vials (DR6000 UV-VIS Spectrophotometer, Hach, Düsseldorf, Germany). Before the analysis, the samples were centrifuged (Universal 320 Hettich®, Tuttlingen, Germany) at 6000 rpm. For the initial characterization of the organic waste leachate, a sample of 100 mL of leachate was taken. This sample was taken 24 h after the leaching process began. The HACH method was used for this characterization, and the following parameters were analyzed: COD, VFA, alkalinity, total nitrogen, and TOC. All parameters were represented in mg/L. The samples were centrifuged at 6000 rpm (Hettich® Universal 320®, Tuttlingen, Germany) at 6000 rpm.

2.2. Biochar

Rice husk biochar (Doferra, Panama City, Panama) was used as feedstock for biochar production. The rice husk used in the carbonization process was collected from Molino Doferra S.A. (24 December, Panama City, Panama). Rice husk was selected as a raw material because approximately 67,851 tons of rice waste are generated and distributed in the districts of Tonosí, Pedasí, and Pocrí [38]. Furthermore, rice husk is a low-density porous organic substrate with an organic matter percentage of 85% [39]. It has a surface area of 183 m^2/g at an air flow rate of 20 L min^{-1} [40]. Top-lit updraft (TLUD) gasification was used to produce the biochar for this work. Some studies have reported using rice husks for biochar production in a TLUD [41–43], but little use of TLUD biochar has been reported for co-digestion of municipal waste. The gasification process was carried out using the methodology presented by Bethancourt et al. [44]. Carbonization was performed with three airflows to evaluate biochar's physical and chemical characteristics. The selected airflows were 12 L/min, 16 L/min, and 20 L/min. The gasification of rice husks was performed three times, and the temperatures were recorded. Average maximum temperatures of 759 °C ± 47.52 (12 L/min) (BL), 798 °C ± 36.03 (16 L/min) (BM), and 888 °C ± 10.29 (20 L/min) (BH) were obtained.

Chemical Characterization of Biochar

The rice husk biochar was stored to be used in the anaerobic co-digestion process. Subsequently, characterizations of the biochar were carried out, where nitrogen was determined with the UNE-EN-15407 method, total oxidizable organic carbon (Oxidant/Titulometry/NTC 5167), and total calcium (MAR/A.A/NTC 5167), among other analyses (see Table 1). Fourier transform infrared spectroscopy (FTIR) analysis was performed for the rice husk and biochar feedstock to determine the representative functional groups. The Agilent Cary 660 FTIR (Australia) was used for these tests.

Table 1. Characterization of biochar at different temperatures, where DB means dry basis. BL, BM, and BH are biochar produced at 759 °C, 798 °C, and 888 °C, respectively.

Analysis	Method	Units	BL Results	BM Results	BH Results
Carbon	UNE-EN-15407	% DB	50.90 ± 5.09	50.42 ± 5.04	13.77 ± 1.38
Hydrogen	UNE-EN-15407	% DB	1.57 ± 0.16	1.95 ± 0.20	0.41 ± 0.04
Oxygen	ASTM-D5622-95	% DB	7.66 ± 0.77	13.62 ± 1.36	0.6 ± 0.06
Nitrogen	UNE-EN-15407	% DB	>0.10 ± 0.01	>0.10 ± 0.01	>0.10 ± 0.01
Sulfur	APPLICATION NOTE 42151 THERMO SCIENTIFIC	% DB	>0.10 ± 0.01	>0.10 ± 0.01	>0.10 ± 0.01
Fixed Total Solids	SM-2540-G	% DB	39.87 ± 0.68	34.01 ± 0.68	85.22 ± 0.68
Total oxidizable organic carbon	Oxidant/Titulometry/NTC 5167	%	4.01	11.3	6.07
Total organic nitrogen	Kjeldahl/Titulometry/NTC 370	%	0.427	0.092	0.203
Total phosphorus	MAR/Spectrophotometry/NTC 234	%	0.028	0.068	0.36
Total sulfur	MAR/Gravimetry/NTC 1154	%	0.135	0.075	0.156
Total sodium	MAR/A.A/NTC 5167	%	0.0766	0.0579	0.0998
Total potassium	MAR/A.A/NTC 5167	%	1.12	1.0138	1.453
Total calcium	MAR/A.A/NTC 5167	%	0.615	0.278	0.325

2.3. Digestion Tests Setup and Biogas

Anaerobic codigestion was performed with the OxiTop-C® system (WTW, Troistedt, Germany), with a total volume of 250 mL. The tests were performed in triplicate. For biochar and control samples, 140 mL of untreated sewage sludge and 10 mL of inoculum (municipal organic waste leachate) were added, resulting in an S/I ratio 0.10. For the control sample of organic sludge (LD), 140 mL of sludge plus 10 mL of distilled water was added. Therefore, a useful volume of 150 mL was obtained. Biochar (BL, BM, BH) was added at the beginning of the co-digestion process at three different rates: 0 g/L (CBL0, CBM0, CBH0), 3.33 g/L (CBL3, CBM3, CBH3), and 6.67 g/L (CBL6, CBM6, CBH6). The initial pH data were taken from the biomasses and digestion samples. In addition, the final pH of the digestion process was measured to verify the change in pH.

Once the bottles were loaded, the cylinders were purged with nitrogen for 60 s to displace the oxygen in the cylinders. The reactors were sealed with the measurement head, which had a pressure transducer with a limit of 330 hPa. The pressure transducer can measure the pressure inside the reactor and calculate the samples' biochemical methane potential (BWP). The reactors were stirred continuously with a magnetic stirrer during the test time (15 days). Devlin et al. used a similar methodology, evaluating the effect of acid pretreatment on the digestion of activated sewage sludge [45]. The digestion time was 15 days, and the pressure was measured at intervals of 30 min. This was released once the pressure of 300 hPa was reached. The biogas release was carried out in 1 L TEDLAR bags. Once the digestion time had elapsed, the biogas composition was analyzed; for this, a Gasboard-3200Plus gas meter (Cubic-Ruiyi, China) was used. Analyses were performed on the codigestion samples before and after treatment. COD, VFA, ammonium, TOC, and total nitrogen were analyzed using the HACH method described in the section "Characterization of organic sludge and leachate."

2.4. Biochemical Potential of Methane

The biochemical potential of methane (PBM) analysis was performed to determine the ability of a waste to be degraded and produce CH_4 [46]. Methane generation was calculated using the monometric methodology [47,48].

$$\mathrm{PBM}\ (\mathrm{mL/gSV}) = \mathrm{VT}_{\mathrm{CH4}}/\mathrm{gSV}, \tag{1}$$

where,

gSV = grams of volatile solids in the samples;

VT_{CH4} = total volume of methane.

3. Results and Discussion

3.1. Characterizations of Biomass

3.1.1. Untreated Sewage Sludge

Table 2 presents the characterization of the untreated sewage sludge before the co-digestion process. The sludge was observed to have a low C/N ratio (0.49), below the recommended limit of 9–30 for anaerobic digestion [49,50]. Chemical oxygen demand (COD) is the oxygen that oxidizing agents consume. However, in anaerobic conditions, this definition is different. COD is the amount of organic compounds in a material, such as untreated sewage sludge [51]. The analyzed sludge presented a COD of 3179.17 mg/L. The literature mentions that the sludge has a COD of approximately 56 g/L [52,53]. It is inferred that it is due to the type of sludge used and the process applied to the biomass in the wastewater treatment plant.

Table 2. Sewage sludge characterization. COD is the chemical oxygen demand; VFAs are the volatile fatty acids; TOC is the total organic carbon; and C/N ratio is the carbon–nitrogen ratio.

COD	VFA	Alkalinity	Ammonium	TOC	NT	C/N Ratio	pH
mg/L							
3179.17	687.83	5212.5	954.75	463.5	949.5	0.49	7.86

3.1.2. Leachate

The characterization of the leachate is shown in Table 3. The alkalinity of the leachate was 4025 mg/L, which indicates the ability of the percolate to neutralize acids [54]. A similar value was obtained by Moradi et al., who characterized the sludge of a WWTP, obtaining 4500 mg/L of alkalinity. They also evaluated the potential of biochar in the anaerobic digestion process. Moreover, the accumulation of VFAs generates an acidic medium [55]. The higher the concentration of VFAs, the greater the system's instability [56]. In this study, the presence of VFA in percolation reached a value of 5337.5 mg/L. However, the literature mentions that this value should be less than 4000 mg/L. The concentration of VFAs can inhibit methane production [57]. Therefore, the use of biochar is proposed as a preventive measure against the accumulation of this parameter. Chemical oxygen demand (COD) was 800 mg/L. One study reported a value of 13,073.30 mg/L for municipal organic waste [55]. This study reported a ratio of 26.24, which indicates that it is within the 9–30 ratio recommended in the literature [49,50]. In their study, Ihoeghian et al. reported a C/N ratio of 15.70 [49].

Table 3. Characterization of percolation of municipal organic waste (MOW). COD is the chemical oxygen demand; VFAs are the volatile fatty acids; TOC is the total organic carbon; and C/N ratio is the carbon–nitrogen ratio.

COD	VFA	Alkalinity	NT	TOC	C/N Ratio
mg/L					
800	5337.5	4025	250	6560	26.24

3.1.3. Biochar

According to Bethancourt et al., the rice husk biochar made under the gasification process has the presence of microelements such as Mn, Fe, Zn, and Cu [44]. Cubero-Cardoso et al. (2023) evaluated the addition of metals in the anaerobic digestion of strawberry extrusion. They evaluated various metal aggregates in different combinations. However, they noted that regardless of the metal added, an increase in methane production was observed from day one. However, up to day five, the lag phase was observed. Then, an exponential growth in biogas production was observed from day five until system stabilization (day 26) [58]. In addition, rice husk biochar presents CaO and MgO. These two compounds act as alkalizes and are used to neutralize or correct pH [59]. Carbon is present in biochar in a proportion of 13.77% (Table 1) on a dry basis for BH biochar, 50.90% on a dry basis for BL biochar, and 50.42% for BM. However, the carbon content does not intervene in methane generation [60]. Biochar contains less than 0.10% nitrogen. The total oxidizable organic carbon for BH was 6.07%, while for the BL, it was 4.01%. Biochar BM had the highest percentage, (11.30%). Likewise, it had different percentages of phosphorus, sulfur, sodium, potassium, and calcium below 1.5%.

Figure 1 shows the results obtained by the FTIR analysis. Functional groups, such as O-H, C-H, and C=C, are present in rice husks, which could increase the transfer of electrons between microorganisms and promote their metabolic activity. In addition, they could stimulate the degradation of pollutants by producing hydroxyl radicals (OH) [61]. This analysis found a broad band at 3400–3200 cm^{-1} in the biochars and the uncarbonized husk. This band is attributed to the moisture content of the biomass by the intermolecular H-bonds. It is associated with the hydrogen vibration of the hydroxyl groups of alcohols, phenols, organic acids, or by amides (present in proteins and peptides) [62,63]. The intensity of this peak was maintained in the uncarbonized and carbonized biomass, which could indicate that the hydroxyl groups did not decompose after the thermochemical process at high temperatures [64] pikes 2950–2800 cm^{-1} correspond to aliphatic stretching (CH groups). This stretch was found in biochar and feedstock. However, the literature suggests that the gasification process can undo the aliphatic structure of the biomass [62,64–66].

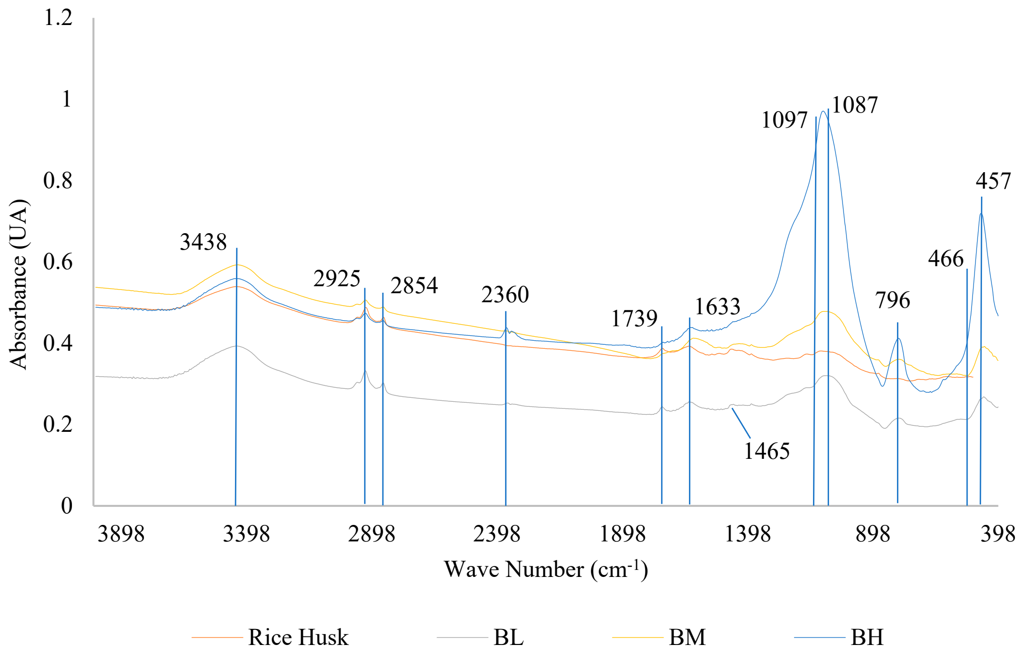

Figure 1. Fourier transform infrared spectroscopy for rice husks and rice husk biochar at temperatures of 759 °C, 798 °C, and 888 °C.

In the BH sample, a 2360 cm^{-1} band corresponds to group C≡C, representing organic waste [66]. The four samples have a band between 1780 and 1700 cm^{-1}, corresponding to aldehyde, ketone, ester, and carboxylic acid. In the band 1633 cm^{-1}, there is a decrease in vibration of the stretch region of the C=C ring [66]. The absorption peak of 1633 cm^{-1} could correspond to areas of proteins associated with nitrogenous compounds. It is also associated with water from the hydrophilicity of the hemicellulose of the biomass [63]. The 1098 cm^{-1} band is the symmetrical stretch of CO for cellulose, hemicellulose, and lignin for the BH, BM, and BL samples. Meanwhile, the band of 1087 cm^{-1} is proper to the sp3 hybridization bond of the carbon atoms [67].

The absorption band 860–680 cm^{-1} belongs to the aromatic flexure C-H, corresponding to monosubstituted phenyl-type benzenes [68]. The BH sample has a steeper peak in this band. In addition, this region coincides with peaks associated with cellulose, hemicellulose, and lignin. The BH sample had a more pronounced peak in this region, which could be related to a greater presence of lignocellulosic compounds in the biochar [63]. Moreover, it has been shown that adding biochar to the co-digestion process could accelerate the degradation of lignocellulosic compounds [63]. Rice husk biochar has functional groups that could facilitate the process of anaerobic digestion. In their study, Ihoeghian et al. reported similar functional groups when using biochar from food waste [49].

3.2. Influence of Biochar on COD Removal after the Co-Digestion Process

In Figure 2, a comparison of the COD removal percentage is made for the samples 0 g/L (CBL0, CBM0, CBH0), 3.33 g/L (CBL3, CBM3, CBH3), and 6.67 g/L (CBL6, CBM6, CBH6). COD is the amount of organic compounds in a material, such as untreated sewage sludge [51]. The control sample is located in this figure; that is, it contains 0 g/L of biochar. This is to make a comparison with the samples containing biochar. It can be seen that the codigestion and added biochar sample groups present a higher percentage of COD removal (Figure 2).

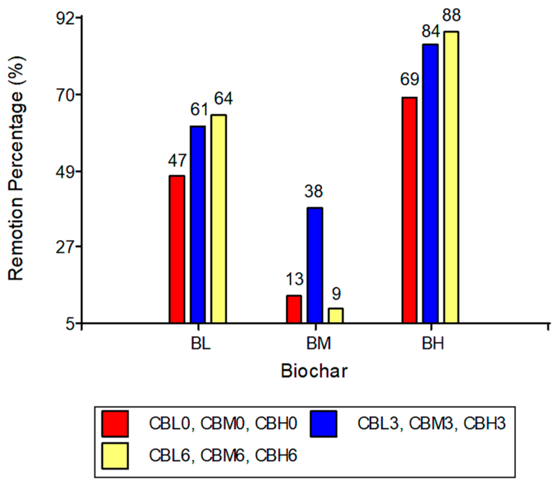

Figure 2. Percentage of COD removal at 0 g/L, 3.33 g/L, and 6.67 g/L of biochar at different temperatures (759 °C, 798 °C, and 888 °C).

On the other hand, the sludge used for each sample group was different, so a different removal rate was observed for the control samples with 0 g/L biochar. When biochar is added to the codigestion samples, it can be observed that there is a tendency to increase the removal percentage with a higher rate of biochar in the mixture. However, with the BM sample group, there is a different behavior. The CBM0 removal percentage was 13%, and sample CBM6 reported 9% COD removal. Meanwhile, with the CBM3 sample, a higher removal percentage was obtained compared to CBM0 and CBM6, which was 38%. On the other hand, sample CBH6 reported the highest removal percentage of 88%. This behavior could show that the greater the addition of biochar, the greater the COD removal [55,69].

Furthermore, it could be observed that the temperature of the biochar intervenes in the behavior of the COD elimination process; that is, at a higher biochar production temperature (888 °C), it presented greater COD removal. Meanwhile, the biochar with a temperature of 759 °C removed 64% of COD at 6.67 g/L (CBL6). The chemical oxygen demand indicates the decomposition of organic matter in biomasses [55]. In their study, Pant and Rai reported that biochar at 12.5 g/L helped remove 54.8% of COD. Biochar enhances microbial activities, leading to faster metabolization of organic waste, helping to decrease COD and increase methane production [69]. The reduction of COD is also limited by the presence of ammonium in the anaerobic process. The greater the presence of ammonium, the lower the COD reduction since the activity of microorganisms that can degrade biomass is inhibited [70]. It is estimated that the improvement of the addition of biochar is due to the preservation of the microbial communities attached to the biochar. This can minimize system lag time. In addition, it maintains the generation of methane [71].

3.3. Influence of Biochar on VFA Variation before and after the Co-Digestion Process

The acidification of anaerobic digestion and the accumulation of VFAs is due to a high organic load rate, which inhibits methane production. Alkaline treatments, such as calcium hydroxide and baking soda, remedy these conditions [72]. In addition, amendments such as biochar help reduce VFAs and maintain a pH close to neutrality, facilitating methane production [25,49]. The reduction or removal of VFAs and ammonia, among other components, is due to the biochar's high surface area, which improves biogas generation [49]. It should be remembered that rice husk biochar has a surface area of 183 m^2/g for an airflow of 20 L/min [40]. The surface area of the biochar helps microbial immobilization, which improves the system's digestibility and shortens the process's lag phase [49]. The increase or decrease in volatile fatty acids in the system indicates possible alterations in the process. For example, an accumulation of acetate suggests a good development of the acetogenesis stage. Another indicator is that a high accumulation of VFA could indicate an acidification of the system [73].

Table 4 shows the initial and final values of volatile fatty acids (VFA) from the co-digestion process. Sample CBL3 presented an increase in VFA after the co-digestion process. Meanwhile, in CBM3 and CBH3, this variable decreased after the process. A 1.72% and 40.83% removal rate was obtained for CBM3 and CBH3 biochar, respectively. The samples CBL6 and CBM6 showed an increase in VFA. However, for the CBH6 sample, a VFA decrease of 42.75% (from 1017.5 mg/L to 582.5 mg/L) was reported. Therefore, it is observed that the CBH6 sample presented a greater removal of VFA, which could indicate that the higher the biochar concentration, the smaller the delay phase of the process [73].

Table 4. Volatile fatty acids before and after the co-digestion process. VFA i are the initial volatile fatty acids; VFA f are the final fatty acids; and Std. Dev is the standard deviation of the samples.

Samples	VFA i (mg/L)	Std. Dev	Tukey HSD	VFA f (mg/L)	Std. Dev	Tukey HSD
CBL0	1157.5	3.54	A	1755	7.07	A
CBM0	937.5	2.12	B	1200	0	B
CBH0	941.25	12.37	B	560.5	2.12	C
CBL3	1170	0	A	1610	0	A
CBM3	962	2.83	B	945.5	7.78	B
CBH3	962.5	14.1	B	569.5	2.12	C
CBL6	1180	14.1	A	1690	0	A
CBM6	953	2.83	C	1480	0	B
CBH6	1017.5	3.54	B	582.5	6.36	C

In their study, Zhu et al. reported that the concentration of fatty acids tends to accumulate in the first days of the codigestion process. The VFA was assimilated and transformed into methane [74]. However, a lag phase can vary depending on the biomass used and the process conditions. This is why an increase in VFA can occur during the co-digestion process. The samples were subjected to codigestion treatment for 15 days so the process could remain in the VFA accumulation stage.

The accumulation of VFAs could indicate a slow hydrolysis of proteins and polysaccharides. This slows down the system, and methane generation tends to be slower. A rapid degradation of VFA occurs due to the enzymatic activity and the microbial variety developed during the system [75]. Therefore, the accumulation of VFA could be interpreted as a slow hydrolysis of the system, meaning that its digestion process had not yet been completed.

3.4. Influence of Biochar on Ammonium Variation before and after the Co-Digestion Process

Several components cause toxic environments for bacteria. However, due to an acclimatization process, bacteria can tolerate the toxic environment at low concentrations. One component that limits biogas generation is ammonia nitrogen, which can be found in two distinct forms: ammonium ion (NH_4^+) and ammonia (NH_3) [76]. Because of this, various treatments have been used to prevent ammonium concentration within anaerobic co-digestion, such as heat pretreatment, biological prehydrolysis, and biochar addition [77,78].

Table 5 shows the variation in ammonium concentration before and after the anaerobic co-digestion process. The CBL3 sample absorbed 21% ammonium, and the CBH3 test absorbed 34.94%. For its part, CBL6 absorbed 33.09% and CBH6 59.15% of the contaminant. Therefore, it can be observed that sample CBH6 removed a higher percentage of ammonium than sample CBH3. Therefore, the higher the concentration of the same biochar, the greater the absorption of this inhibitor. This is because biochar can absorb inhibitors such as ammonium and VFA [78]. The control sample, CBL0, had an absorption of 45.09%, and the sample, CBH0, had an absorption of 34.29%. In their study, Hou et al. (2016) showed that samples should have a pH between 7 and 9 for better ammonium absorption using biochar. Furthermore, it is mentioned that a high surface area and the presence of compounds such as MgO, CaO, and KCl improve the ammonium absorption process [79].

Table 5. Ammonium before and after the co-digestion process. Ammonium i and ammonium f are the initial and final ammonium present in the samples, respectively. Std. Dev is the standard deviation of the samples.

Samples	Ammonium i (mg/L)	Std. Dev	Tukey HSD	Ammonium f (mg/L)	Std. Dev	Tukey HSD	Removal Percentage (%)
CBL0	1143.75	1.77	C	628	0	C	45.09
CBM0	2970	0	A	6315	7.07	A	-
CBH0	1357.5	0	B	892	0	B	34.29
CBL3	1098.75	1.77	C	868	0	C	21.00
CBM3	3290	0	A	5320	0	A	-
CBH3	1362.5	0	B	886.5	0.707	B	34.94
CBL6	1157.5	0	C	774.5	0.707	B	33.09
CBM6	2975	7.07	A	7535	7.07	A	-
CBH6	1356.25	1.77	B	554	0	C	59.15

Another behavior that was recorded in the analysis was that in samples CBM0, CBM3, and CBM6, an increase in the ammonium concentration was reported. The CBM3 test showed an increase in ammonium from 3290 mg/L to 5320 mg/L at the end of the anaerobic co-digestion process, that is, an increase of 38.16%. For sample CBM6, ammonium increased from 2975 mg/L to 7535 mg/L (60.52% ammonium). It should be noted that the control sample (CBM0) also recorded an increase in the presence of ammonium of 52.97%. In their study, Gnaoui et al. also reported an increase in the presence of ammonium [77]. Regarding the behavior of biochar, Choudhury et al. obtained a similar behavior by increasing the ammonium concentration during the anaerobic co-digestion process using biochar. They mentioned that the ammonium absorption capacity could be affected by the presence of microbial biomass on the surface of the biochar [80]. Furthermore, Kizito et al. demonstrated that cations such as K, Ca, Mg, Fe, and Zn inhibit biochar's adsorption capacity through competition for active binding sites [81]. Therefore, samples where ammonium does not decrease may be due to these behaviors, where the surface is affected by microbial biomass or by competition for active binding sites.

3.5. Influence of Total Organic Carbon (TOC)

In terms of total organic carbon (TOC), in Table 6, it can be seen that there is an increase in the presence of TOC after the anaerobic digestion process. However, this increase is not related to the temperature of the biochar. For example, for sample CBM3, the initial TOC (TOC i) was 387.5 mg/L, while the final TOC (TOC f) was 690 mg/L, with a percentage increase of 43.84%. The CBL3 test showed a greater percentage increase in this variable (63.23%). Meanwhile, the CBH3 sample presented an increase of 57.81%. Furthermore, the comparative analysis (Tukey HSD) shows that the samples differ significantly.

Table 6. Total organic carbon (TOC) before and after the co-digestion process. TOC i and TOC f are the tests' initial and final total organic carbon. Std. Dev is the standard deviation.

Samples	TOC i (mg/L)	Std. Dev.	Tukey HSD	TOC f (mg/L)	Std. Dev.	Tukey HSD	Percent Increase (%)
CBL0	380	0	C	1365	7.07	B	72.161
CBM0	570	7.07	A	920	0	A	38.043
CBH0	475	0	B	1145	0	C	58.515
CBL3	570	0	B	1550	0	A	63.226
CBM3	387.5	0	A	690	0.707	B	43.841
CBH3	472.5	17.7	C	1120	0	C	57.813
CBL6	450	0	C	1920	0	A	76.563
CBM6	590	0	A	1450	0	C	59.310
CBH6	520	0	B	1685	0	B	69.139

For the CBM6 variable, a percentage increase of 59.31% and 76.53% for the CBL6 sample is observed. Furthermore, CBH6 presented a percentage of 69.14%. However, it should be noted that in the controls, there was also an increase in OCD of 72.16%, 38.043%, and 58.52% for the CBL0, CBM0, and CBH0 controls, respectively. This behavior was reported by García and attributed to an error in the procedure or in the equipment used [82]. Furthermore, a characteristic behavior of biochar is that it can store recalcitrant organic carbon, indicating that bacteria cannot degrade it [83].

The decrease in TOC is attributed to the transformation of organic carbon into CO_2 [84]. However, in this study, an increase in TOC is identified, which could indicate that organic carbon is not transformed into CO_2. Therefore, the digestate could be used for soil improvement [85].

3.6. Influence of Total Nitrogen (NT)

Important parameters such as total nitrogen (TN) must be regulated in anaerobic digestion. This parameter was affected by the addition of biochar in the DA process. In Table 7, you can see the change in TN at the beginning and the end of the AD process. Initial data of 250 mg/L, 1040 mg/L, and 1911.25 mg/L were obtained for samples CBL3, CBM3, and CBH3, respectively. At the end of the process, values such as 4110 mg/L, 1015 mg/L, and 1280 mg/L were obtained for samples CBL3, CBM3, and CBH3, respectively. In addition, initial data of 242.75 mg/L, 965.5 mg/L, and 1160 mg/L were obtained for the CBL6, CBM6, and CBH6 tests, respectively. At the end of the process, values such as 2250 mg/L, 1120 mg/L, and 1310 mg/L were obtained for samples CBL6, CBM6, and CBH6, respectively. It should be noted that the mixture of raw and leached sludge from sample CBL0 presented a value of 243.75 mg/L of TN. This sample presented a low TN value compared to CBM0 and CBH0, with a value of 952 mg/L and 1033.75 mg/L, respectively. Studies have reported that adding biochar to the AD process does not cause significant changes in this parameter due to the low concentrations of biochar used [83,86].

Table 7. Nitrogen before and after the co-digestion process. Nitrogen i and Nitrogen f are the initial and final nitrogen levels for the tests, respectively. Std. Dev. is the standard deviation of the samples.

Samples	Nitrogen i (mg/L)	Std. Dev.	Tukey HSD	Nitrogen f (mg/L)	Std. Dev.	Tukey HSD
CBL0	243.75	0.707	C	1590	0	A
CBM0	952	0	B	1140	0	C
CBH0	1033.75	1.77	A	1250	0	B
CBL3	250	0	C	4110	0	A
CBM3	1040	0	B	1015	7.07	C
CBH3	1911.25	1.77	A	1280	0	B
CBL6	242.75	0	B	2250	0	A
CBM6	965.5	0.707	A	1120	0	C
CBH6	1160	177	A	1310	0	B

3.7. Influence of Biochar on SV Ratio Change before and after Co-Digestion

Volatile solids (VS) analyses were performed before and after the co-digestion process to analyze the PBM and evaluate the reduction of volatile solids (see Table 8). Volatile solids decreased between 12% and 34% after the co-digestion process. However, this reduction does not correlate with the addition of biochar. Furthermore, the initial volatile solids are unrelated to adding biochar. The highest value obtained was initial volatile solids between 23 mg/L and 49 mg/L. Meanwhile, the final VS ranged between 16 mg/L and 42 mg/L. In their study, Moradi et al. obtained a similar behavior, reporting a reduction of up to 43%. This could indicate a high potential for biodegradability and potential to produce biogas [55]. The higher the SV concentration, the higher the organic matter and bacterial communities. Furthermore, a VS of 20 g/L to 60 g/L is recommended. If the biomass has a value greater than the established range, dilutions must be made, and these dilutions must not be less than 10 g/L for the inoculum [87].

Table 8. Volatile solids before and after the co-digestion process.

	BL			BM			BH		
Samples	**SV i (g/L)**	**SV f (g/L)**	**% Reduction**	**SV i (g/L)**	**SV f (g/L)**	**% Reduction**	**SV i (g/L)**	**SV f (g/L)**	**% Reduction**
LD	32.88	24.95	24.12	24.62	16.875	31.45	27.075	22.85	15.60
COD	30.95	25.8	16.64	23.1	16.25	29.65	38.78	33.75	12.97
3.33 g/L	28.45	23.725	16.61	28.22	18.725	33.66	49.02	42.2	13.91
6.67 g/L	28.38	34.4	-	28.05	18.35	34.58	36.89	30.35	17.73

3.8. Influence of Biochar on Biogas Generation

The biochemical methane potential (PBM) is conditioned by different parameters, such as the type of biomass, the environmental or process conditions (temperature), and the retention time. In the same way, the materials used as inoculum play an essential role in the generation of biogas. This study evaluated the influence of biochar on the improvement of anaerobic co-digestion of the organic sludge and leachate of organic waste. For this purpose, different samplings were carried out in the Juan Díaz WWTP to evaluate the varying conditions of the untreated sewage sludge. Two biochar aggregates (3.33 g/L and 6.67 g/L) were used at a co-digestion temperature of 28 °C.

The results show that the highest PBM was obtained for sample CBM6, with 212.75 mL/SVg (Figure 3b). Sample CBL3 generated 188.27 mL/SVg, the second-highest value of the biochar-containing samples (Figure 3a). On the other hand, sample CBH6 produced 107.16 mL/SVg of methane (Figure 3c). Therefore, it can be seen that the temperature of the biochar can influence the generation of biogas. However, it should be noted that the initial conditions of the sludge influence this parameter.

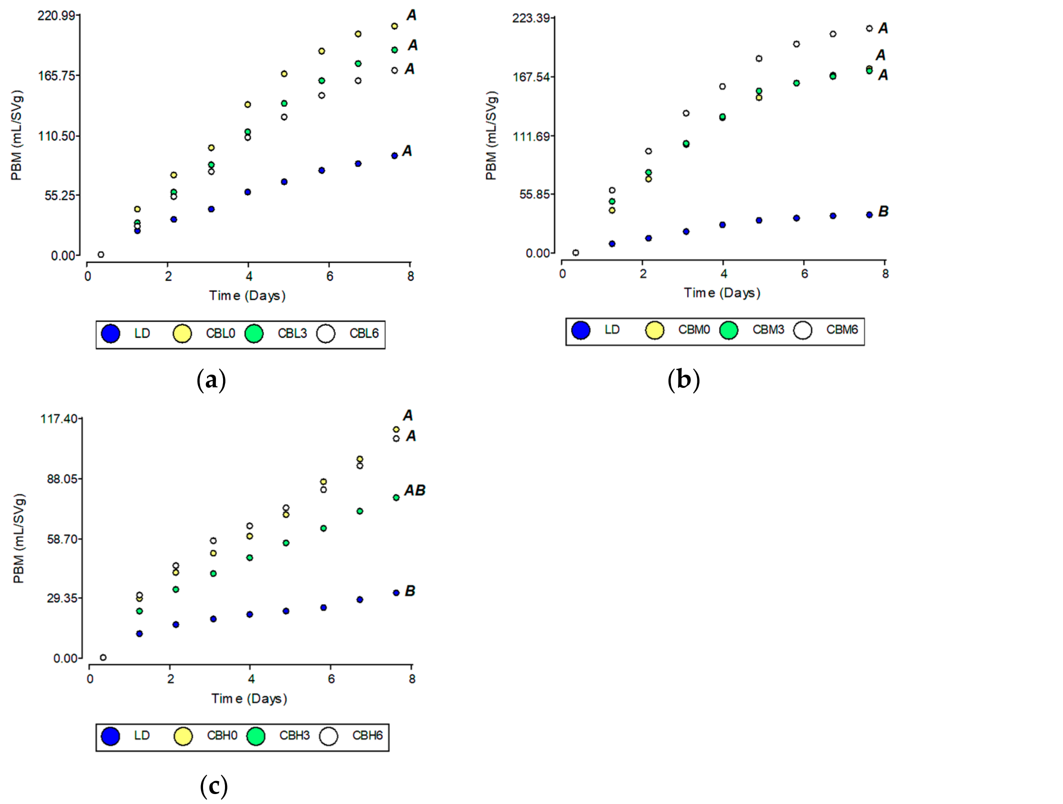

Figure 3. Effect of the PBM of biochar at different temperatures and three different concentrations (0 g/L, 3.33 g/L, and 6.67 g/L) on the anaerobic co-digestion of untreated sludge and leachate from municipal organic waste: (**a**) effect of BL biochar; (**b**) effect of BM biochar; (**c**) effect of BH biochar.

Figure 3b shows a significant difference between the organic sludge (LD) sample and the rest of the samples. It was observed that the co-digestion of raw sludge and organic waste leachate improved the PBM compared to the LD samples; for example, the CBL0 sample generated 175.02 mL/SVg, while the LD test (Figure 3b) produced 36.37 mL/SVg. Similar behavior was obtained in all test groups (Figure 3a,c). Ambaye [88] evaluated the addition of biochar from sewage sludge in the digestion of fruit waste; the control sample generated 150 mL/SVg, a value close to that obtained in this study for the control sample of sludge and leachate [88]. Furthermore, Figure 3a,b show that the PBM curves tend towards growth; that is, they do not approach the system's stabilization. The evaluation curves of LD, CBM0, CBM3, and CBM6 approached stabilization after eight days of data collection.

Adding 3.33 g/L of biochar does not significantly change the biogas generation in the sample groups. However, doubling this dose can cause an increase in methane generation. Cimon et al., in their study, mention that increasing the addition of biochar increases methane generation [19]. There was no significant effect (according to Tukey HSD) on methane generation in the samples with BL and BH biochar additions. According to Quintana-Nájera, this behavior could be due to substrate sequestration and changes in the variety of microorganisms [89]. Retention time is an important parameter that could influence the methane generation for this assay [90].

3.9. Influence of Biochar on Biogas Composition

The composition of the biogas is observed in graphs Figure 4a–c. The cumulative percentage of methane in Figure 4c was 2.81 for untreated sewage sludge digestion (LD), and for CBH0, it was 8.38%, while for CBH3, it was 10.54%, and for CBH6, it was 13.22%. An increase in the percentage of methane in the biogas can be observed by adding biochar. The sample with biochar at 3.33 g/L (CBH3) increased methane concentration by 25.78%, and 6.67 g/L (CBH6) increased methane in the biogas by 57.76%. Tukey HSD analysis showed that the samples are not significantly different.

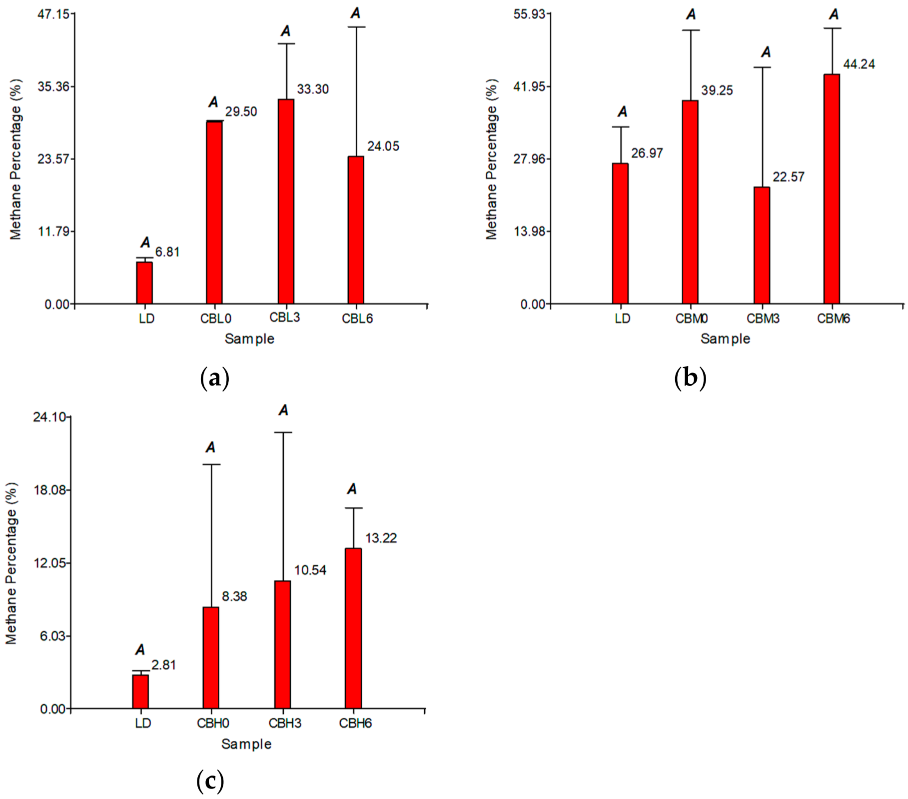

Figure 4. Influence of biochar on the percentage of methane: (**a**) BL effect; (**b**) BM effect; (**c**) BH effect.

See Figure 4a for the samples with biochar at 759 °C (BL). However, there is a difference; sample CBL6 presented a low methane percentage (24.05%) compared to samples CBL0 (29.50%) and CBL3 (33.30%). However, the sample with a biochar concentration of 3.33 g/L presented the highest percentage of methane, and the LD sample had the lowest rate (6.81%), which would indicate that the addition of biochar increased by 4.89 times more than the LD sample for CBL3, and by 3.53 times more for CBL6. In addition, the sludge and leachate co-digestion sample increased by 4.33 times more methane. It should be noted that this co-digestion has an adequate mixture by which to generate methane from raw sludge from a WWTP and leachate from municipal organic waste. Figure 4b shows the sample's particular behavior. CBL6 presented the highest percentage of methane (44.24%), that is, 64.03% more than the LD sample (26.97% methane). On the other hand, the CBL3 sample reported a lower percentage of methane than the sludge (22.57% methane), that is, 16.31% less than the LD. Meanwhile, the CBL0 sample generated 39.25% methane, which is 45.53% higher than the LD.

Shen et al. obtained higher methane percentages than the controls by adding biochar at thermophilic conditions. However, under mesophilic conditions, this percentage of methane decreased during the retention time or the study time. The authors estimated that this behavior was because the biogas production rate could exceed the CO_2 sorption limit, which would cause a decrease in the percentage of methane in the AD [86].

Figure 3c shows the behavior of the PBM of the samples with BH. We can observe a correlation between the PBM and the percentage of methane in the samples. The largest PBM obtained in Figure 3c corresponds to samples CBH6 and CBH0. Figure 4c shows that sample CBH6 generated the highest percentage of methane, and CBH0 produced a rate of 8.38% of methane. Meanwhile, the sludge sample (LD) generated the lowest percentage of methane (2.81%) and the lowest PBM (31.51 mL/SVg).

Similarly, Figures 3a and 4a show similar behavior. In both cases, the LD sample produced the lowest value. CBL6 is the second sample with the lowest PBM (169.90 mL/SVg) and methane percentage (24.05%). However, this behavior was not observed with samples CBL0 and CBL3.

In Figures 3b and 4b, the behavior of samples CBM6 and CBM0 coincide. These generated the highest PBM and percentage of methane in the sample group. Meanwhile, CBM3 and LD did not match behavior because LD generated the lowest PBM of the sample group. However, it produced a higher percentage of methane compared to the CBM3 sample. Furthermore, the samples do not show stabilization behavior in the PBM, so it is recommended that the retention time be extended.

4. Conclusions

It was observed that rice husk biochar could improve the anaerobic co-digestion process of raw sludge and leachate from municipal waste biomass.

1—The improvement in the anaerobic co-digestion could be due to biochar's porosity and a surface area that can lead to retaining contaminants. Sample CBH6 reported an ammonium reduction of 59.15%.

2—Increasing the biochar concentration in the co-digestion process can improve methane generation. It was observed that methane production was increased by 18% for CBH6 compared to the control sample. On the other hand, VFA reduction was enhanced by 42.75% for sample CBH6. Adding biochar did not affect the pH of the samples because the biomass already maintained a pH close to neutral at the beginning of the treatment. TOC exhibited an increase of 76.56% for the CBL6 sample.

3—Untreated sludge biomass is generally not used as a raw material in studies. Raw sludge was used in this study. It was observed that methane can be generated by adding biochar to the media. Although this study did not report values similar to those of the literature, the process's tendency was similar. Therefore, pretreatment processes could be eliminated, reducing the operating costs of co-digestion processes.

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