Biochar promotes methane production during anaerobic digestion of organic waste

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Abstract

Climate change and energy demand are calling for more sustainable fuels such as biomethane produced by anaerobic diges-tion of organic waste. Biochar addition to waste is presumed to enhance the efficiency of methane production, yet individual reports disclose contradictory results. Therefore, we performed a meta-analysis of 27 selected publications containing 156 paired measurements of control and biochar-amended treatments to assess the impact of biochar on the methanogenic perfor-mance. Results show that biochar promotes biomethane production substantially with a high Hedge's d value of 5.7 ± 1.04, yet sporadic publications report a methane decline. Methanogenic performance is statistically controlled by feedstock type, pyrolysis temperature and biochar concentration, but not controlled by pH, size, surface area and methanogen species. These findings should help to tune the parameters of anaerobic digestion with biochar to optimize biomethane productions. Moreover, our results cast some doubt on the efficiency of adding biochar to soil to sequester carbon in soils because biochar promotes methane generation and, in turn, emissions of methane, a greenhouse gas, to the atmosphere.

Keywords Anaerobic digestion · Biochar · Methane · Meta-analysis · Wastewater treatment

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Introduction

Global warming and the rising energy demand are calling for more circular processes where waste is recycled into materials and energy. Biomethane is a carbon–neutral, sustainable fuel produced by anaerobic fermentation of organic matter in natural and anthropic environments, yet the efficiency of actual processes is limited (Chen et al. 2018; Gao et al. 2020; Garcia-Mancha et al. 2017). Strategies have been recently developed to improve anaerobic fermentation by microbial immobilization, pH buffering and enzymatic induction (Gao et al. 2020; Xiao et al. 2020a). Anaerobic degradation and biomethane production are also promoted by electrometha-nogenesis using electroactive microorganisms and conductive materials such as biochar (Fig. 1; Li et al. 2018; Xiao et al. 2020b; Yuan et al. 2018). Recent research has also focused on the use of nanomaterials to favor methanogenesis (Ma et al. 2020; Xiao et al. 2018, 2019c).

Biochar is carbon negative and comprises a wide variety of complex materials produced by pyrolysis of biomass (Glaser et al. 2009; Gunarathne et al. 2019; Akhil et al. 2021; Fawzy et al. 2021). Biochar has been applied to reduce nutrient leaching from soils, to recover resources from water, to accelerate waste disposal and for biomethane production

Fig. 1 Transformation of organic waste by anaerobic fermentation is promoted by biochar addition
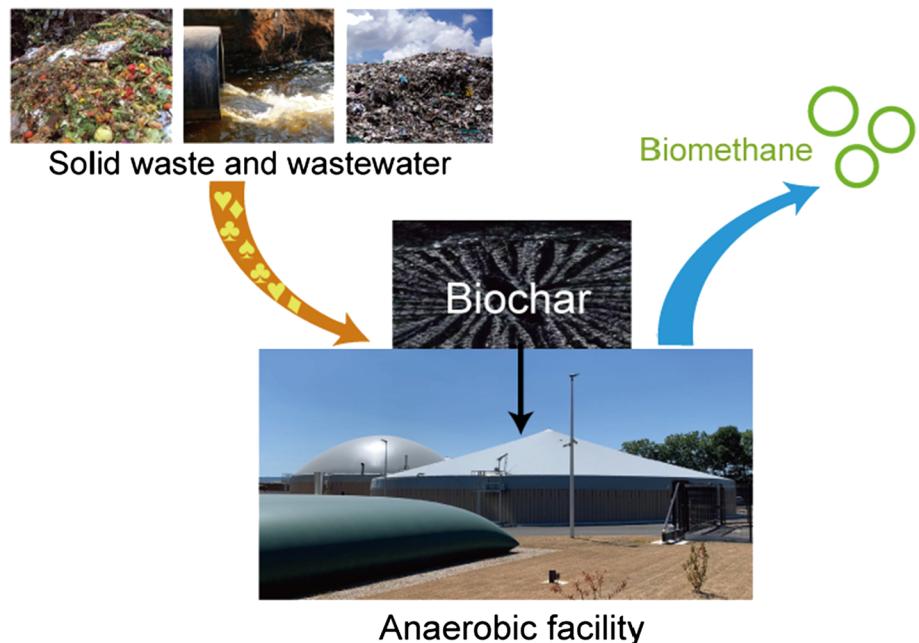
(Lorenz and Lal 2014; Fagbohungbe et al. 2017; Masebinu et al. 2019; Qiu et al. 2019; Yang et al. 2020). Biochar properties and molecular composition vary widely with the nature of the feedstock and pyrolysis conditions (Keiluweit et al. 2010; Gao et al. 2020). Several biochar properties have been proposed to favor biomethane production, e.g., microbial immobilization, pH buffering, and controlling metal ion availability and enzymatic processes (Yuan et al. 2018; Xiao et al. 2019b; Gao et al. 2020; Huang et al. 2020b; He et al. 2020). Overall, mechanisms fostering methanation by biochar are better understood but individual studies report sometimes contradictory results, e.g., rising or declining biomethane production (Cheng et al. 2018; Luo et al. 2015; Shen et al. 2016). Therefore, we report here a meta-analysis to clarify the impact of biochar properties on methanogenesis during anaerobic digestion of environmental waste.

Experimental

Biochar data

We found 105 publications in the Web of Science and Bing search engine for documents on biochar application to methane production during anaerobic digestion (AD) for treating environmental waste and pollution, excluding soil-related research, from January 1 2010 to June 15 2020, using the keywords "biochar" AND "methane" OR "CH$_4$." We extracted the following variables: feedstock, pyrolysis temperature, pH, size, surface area, conductivity, methanogen species and methanogenic performance. Variable means, standard deviations and sample replicate number were extracted from publication tables and text. When data were only reported in image format in graphs, data points were extracted using Plot Digitizer 2.6.8 and Web Plot Digitizer. When relevant data were not present in publications, corresponding authors were contacted to get the data. We first considered the highest rate of methane production, but, if not available, we used the highest yield of methane. When accurate maximum rate of methane production or yield could not be obtained due to too much fluctuation of methane concentrations, publications were excluded from this study.

From this initial pool, we selected only documents reporting three or more replicates for each run, and we found 19 publications containing 105 data pairs of treatment data versus control data (Table S1). Control is defined as runs without biochar. We used the Hedges method, rather than the response ratio, because the Hedges method is adapted to data samples of relatively small size (Jeffery et al. 2016; Larry and Ingram 1985). Therefore, a minimum of two replicates is meeting the analysis standard. Consequently, data on biomethane production were collected from 27 articles containing 156 paired measurements of control and biochar-amended treatments for disposal of environmental waste and pollution.

Biochar variables were grouped to facilitate cross-comparisons, e.g., the nature of biochar feedstock was grouped in 'wood and sawdust,' 'herbaceous and lignocellulosic waste,' 'manure,' and 'sludges' (Table S1). Similarly, pyrolysis temperatures were grouped in 'below 500 °C,' '500–700 °C,' and 'above 700 °C. 'Conductivities were grouped in 'below 450 µS/cm' and 'above 450 µS/cm.' Biochar pH was grouped into 'acidic below 7,' 'weakly alkaline from 7 to 9,' and 'alkaline above 9.' Sizes were grouped in 'below 1 mm'

and 'above 1 mm.' Brunauer, Emmett and Teller (BET) surface areas were grouped in 'below 100 m^2 g^{-1}' and 'above 100 m^2 g^{-1}.' Biochar concentrations were grouped in 'below 10 g dm^{-3},' 'equal to 10 g dm^{-3}' and 'above 10 g dm^{-3}.' Two types of methanogenic archaea were distinguished: acetoclastic methanogens and hydrogenotrophic methanogens.

Meta-analysis

We used the standardized mean difference metric Hedge's d in Eq. 1, which induces less biases that the Hedge's g factor in Eq. 2 (Larry and Ingram 1985):

$$d = \left(1 - \frac{3}{4(n-2)-1}\right)g \tag{1}$$

$$g = \frac{X_1 - X_2}{S_p} \tag{2}$$

$$S_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 - 1) + (n_2 - 1)}} \tag{3}$$

where n denotes the total sample size, and $\overline{x_1}$ and $\overline{x_2}$ depict the means of experimental and control treatments. Experimental data refer to the treatment with biochar, whereas control data refer to the treatment without biochar. A categorical random effect model was applied to d, with means weighted by the inverse of the variance. Here, S_p is the pooled standard deviation in Eq. 3, where n_1 and n_2 are the number of repetitions in the control and experimental groups, and s$_1$ and s$_2$ depict the standard deviations of control and experimental groups.

Contrary to the response ratio commonly used in ecological research, the standardized mean effect sizes are probabilistic (Hedges et al. 1999; Larry and Ingram 1985). That is, the mean effect sizes describe the probability that a sample would fall between the experimental mean and the control mean, assuming a normal distribution (Hedges et al. 1999). Consequently, confidence intervals of 95% were generated based on a normal distribution. When the 95% confidence interval of the parameter does not overlap with Hedge's d of 0, this implies that the variable promotes biomethane production, which suggests the promotion of anaerobic digestion of environmental waste. When the 95% confidence interval of a biochar parameter does not overlap with that of another variable, there is a statistically significant difference.

By convention, for variables that do not overlap with the Hedge's g of 0, a d value higher than 0.8 indicates a large effect, d of 0.2–0.8 shows a moderate effect, and d of 0.0–0.2 displays a small effect (Hedges et al. 1999; Jeffery et al. 2016). A key point is that, using the Hedge's d metric,

an effect size of a variable analysis does not equate to an effect size of others in independent analyses presented in this study. As a consequence, only categories within individual analyses, e.g., feedstocks, as differentiated by the horizontal dotted bars, can be compared. The effect sizes do not mean that the extent of the actual biomethane production increase or decrease. Small effect sizes may indicate significant value in biomethane production in absolute terms. For instance, in small effect sizes, the actual biomethane parameter may be several times larger than that of the large effect sizes.

Results and discussion

Overall effect of biochar addition

We assessed the global effect of biochar addition on anaerobic methanogenesis by calculating the grand mean of the Hedge's d for 156 published data pairs of treatment versus control without biochar (Fig. 2). Results show a d value of 5.70 ± 1.04, which evidences a large effect size and implies that the presence of biochar statistically induces an increase in biomethane in most investigations. Yet sporadic studies have also shown the inhibitory effect of biochar or no effect (Cheng et al. 2018; Shen et al. 2016). This discrepancy is probably due to the high heterogeneous nature of biochar (Diao et al. 2020; Gao and Goldfarb 2019), suggesting that biomethane production may be enhanced by specific biochar properties, as discussed below.

Effect of biochar feedstock

We calculated d values of feedstock including sludges, manure, herbaceous and lignocellulosic waste, and wood and sawdust (Fig. 2). All feedstock types show high d values from 4.71 ± 1.72 to 7.99 ± 1.51, implying that biochar addition improves biomethane generation whatever the type of feedstock. Furthermore, there is no statistical difference within feedstock types, sludges displaying the highest d of 7.99. Manure, plant waste and woody materials appear equally competitive with Hedge's d values around 5.0. High d values for sludges are supported by the fact that sludge biochar provides more nutrients for fermentative bacteria and methanogens (Wang et al. 2020). Moreover, biochar from sludge has also induced better pollutant removal and heavy metal adsorption (Diao et al. 2020; Regkouzas and Diamadopoulos 2019; Singh et al. 2020), which may be explained by a more favorable living environment for microorganisms. Overall, the slight advantage of sludge biochar in terms of methanogenesis is likely due to its ability to adsorb and store nutrients for activating methanogens. We conclude that biochar improves methanogenesis for all biochar feedstocks, but there is no statistical advantage of the feedstock type.

Fig. 2 Forest plot of Hedge's *d* calculated from published literature (Table S1). Top: grouping by feedstock, pyrolysis temperature and conductivity of biochar. Bottom: grouping by biochar concentration, size, BET surface area and pH. Points show means, bars show 95% confidence intervals. The numbers in parentheses indicate the number of pairwise comparisons of treatment with biochar versus control without biochar. BET: Brunauer, Emmett and Teller
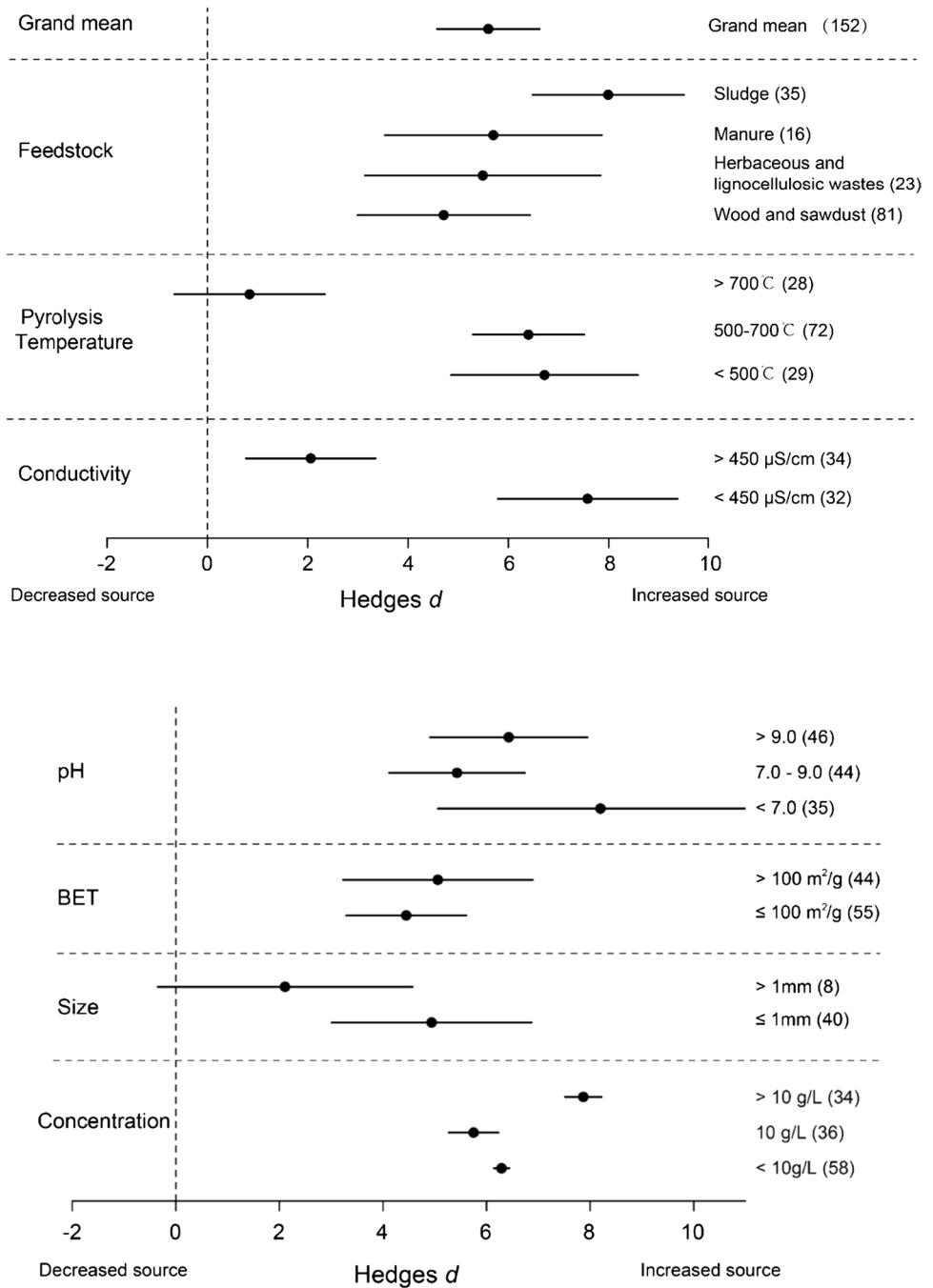
Effect of pyrolysis temperature

We calculated *d* values of biochar produced by pyrolysis below 500 °C, of 6.72 ± 1.86, between 500 and 700 °C, of 6.40 ± 1.11, and above 700 °C, of 0.840 ± 1.50 (Fig. 2). Results imply that biochar favors biomethane generation below 700 °C. There is no significant difference between 500 and 700 °C-produced biochar and biochar produced below 500 °C. On the other hand, pyrolysis above 700 °C induces a drastic decline of biomethane promotion. These findings may be explained by changes of the biochar molecular

structure with temperature (Hao et al. 2018). Indeed, Keiluweit et al. (2010) observed a gradual change in the molecular structure of plant biomass-derived biochar with temperature.

High-temperature biochar is characterized by fewer labile compounds at the surface of biochar particles, and therefore less microbial substrates for fermentative bacteria and methanogenic archaea (Bruun et al. 2011). This explanation is strengthened by the declining CH_4 and N_2O emissions from soils amended with high-temperature biochar, which are thus better suited for mitigation of greenhouse gas emissions (Cayuela et al. 2015). This scenario is also supported

by the biochar release of less degradable organic compounds when the pyrolysis temperature increases (Ji et al. 2020). By contrast, slow pyrolysis at low temperature yields more biochar with diverse chemical groups (Chen et al. 2019; Sohi et al. 2010), which are likely to promote methane production in anaerobic digesters, or methane emissions from soils (Jeffery et al. 2016). Overall, our findings show that biochar produced below 700 °C improves methanogenesis. Pyrolysis at lower temperature is also saving energy.

Effect of biochar conductivity

Biochars having low conductivity, below 450 µS/cm, show a much higher d value, of 7.58 ± 1.79, than high-conductivity biochar, displaying a d value of 2.06 ± 1.29 (Fig. 2). Low conductivity biochar is therefore statistically more effective at accelerating biomethane production. This finding is unexpected because recent research suggests that biochar acts as an electron shuttle, which should favor microbial activity (Viggi et al. 2017; Xiao et al. 2019b; Yuan et al. 2018). Nonetheless, a recent report explains that electrical conductivity of biochar is controlling only the rate of anaerobic degradation, not the yield of biogas (Rasapoor et al. 2020). Moreover, conductivity does not appear as a relevant factor for choosing which biochar should be used for degrading environmental waste (Lu et al. 2020a), and some studies suggest that attributing rising biomethane production to high material conductivity requires caution (Martins et al. 2018; Van Steendam et al. 2019; Wang et al. 2021). Overall, our findings show that low conductivity biochar favors methanogenesis, yet underlying mechanisms are unclear.

Effect of biochar pH

Figure 2 displays the effect of biochar of different pH on biomethane production. Results show that varying the biochar pH induces no statistical difference in biomethane production, despite the fact that pH is known to modify fermentation rates (Begum et al. 2018; Feng et al. 2020; Mao et al. 2017). Yet, most investigations included in this meta-analysis did not report the pH of the system before and after biochar application, though pH is expected to vary widely because some biochar contains oxygen-containing organic anions and carbonates that increase alkalinity (Fidel et al. 2017; Yuan et al. 2011; Meng et al. 2020). Overall, varying the pH of biochar does not statistically improve methanogenesis.

Effect of surface area and biochar size

Values of d for biochar with BET surface area above 100 m²/g, of 5.06 ± 1.83, are not statistically different from those of biochar with surface area below 100 m²/g,

of 4.45 ± 1.06 (Fig. 2). Similarly, the size to biochar particles does not appear to modify biomethane generation, yet a trend for higher d value is observed for particle size below 1 mm. This implies that smaller particles of biochar may be beneficial to the degradation of environmental waste. For instance, the addition of powdered biochar to a pig manure/ wheat straw aerobic compost increased biomethane emissions by 57%, whereas granular biochar decreased biomethane emissions by 22% (He et al. 2018). On the contrary, other investigations have shown that large biochar particles promote methanogenesis (Cheng et al. 2018; Viggi et al. 2017). Overall, there is no clear global effect of surface area and size on anaerobic degradation of waste and pollutant and on biomethane production.

Effect of biochar concentration

Biochar concentration caused a strong and statistically significant difference in the strength of biomethane production, with a maximal impact for concentrations exceeding 10 g/L and a d value of 7.87 ± 0.35 (Fig. 2). Increasing biochar concentration is therefore an efficient means to improve methanogenesis, which may further result in a promotion of waste degradation. This finding is supported by biochar properties that are likely to stabilize anaerobic digestion and rise biomethane yield (Gao et al. 2020; Lim et al. 2020; Ma et al. 2021). For instance, providing immobilization sites for microorganisms could explain the higher anaerobic degradation and methanogenic performance (Zhang et al. 2018).

Moreover, even though biochar itself is not a substantial source of labile carbon, biochar is a sponge-like material able to adsorb and store organo-mineral nutrients for further microbial feeding (Cross and Sohi 2011; Demisie et al. 2014). In this line, elevated biochar concentrations have been shown to increase the availability of organic carbon for fermentation bacteria and methanogenic archaea (Lu et al. 2020b; Jiang et al. 2020; Zhang et al. 2020). Based on this, environmental waste and pollution can be degraded more easily, which in turn is more conducive to biological activities (Xiao et al. 2021a; b). Overall, high biochar concentrations foster methanogenesis, yet underlying mechanisms remain undeciphered.

Methanogenic species

Values of d for acetoclastic methanogens, of 5.19 ± 2.06, and hydrogenotrophic methanogens, of 3.08 ± 1.4, are not statistically different, implying a similar contribution of these species to biomethane production (Fig. 3). These high d values also reveal that both acetoclastic and hydrogenotrophic methanogens produce more biomethane following biochar addition. This finding is strengthened by an investigation revealing that *Methanosarcina*, *Methanosaeta* and

Fig. 3 Forest plot of Hedge's *d* calculated from published data grouped by 'Methanogens with the highest abundance' and 'Methanogens with the highest increase in abundance.' Points show means, bars show 95% confidence intervals. The numbers in parentheses indicate the number of pairwise comparisons on which the statistic is based. 'Methanogens with the highest abundance' means the most abundant methanogens in samples. "Methanogens with the highest increase in abundance' means methanogens showing the highest changes in abundance
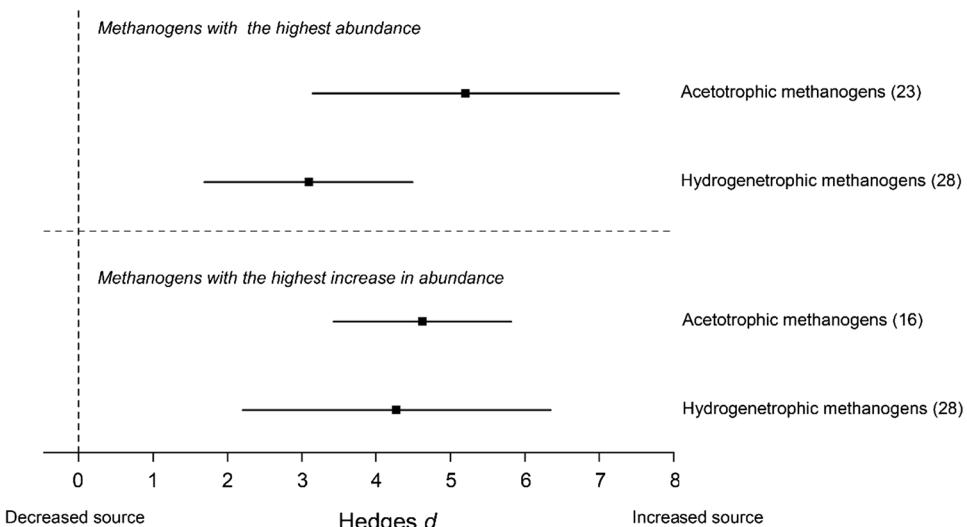
Methanobacterium methanogens predominate in paddy soil-amended biochar during the anaerobic decomposition of rice straw (Huang et al. 2020a). Trophic methanogens, hydrogenotrophic and acetoclastic methanogens may actively participate in the methane production process. Indeed, reports have shown that methanogens that use acetate and hydrogen as substrates coexist in the anaerobic fermentation system (Madigou et al. 2019; Zhang et al. 2019). Compared to hydrogenotrophic methanogens, acetoclastic methanogens should contribute more to methane production with sufficient organic substrates (Garcia-Mancha et al. 2017; Lim et al 2020; Xiao et al. 2019a). Overall, biochar addition improves biomethane production by methanogens, yet acetoclastic and hydrogenotrophic methanogens display similar performances.

Conclusion

Our findings show that, on the average, biochar addition is favoring biomethane generation, whereas this was not clear in previous individual reports. Our identification of biochar properties that favor or do not favor methanogenesis will be helpful for basic research to decipher underlying mechanisms, and for applied research to improve biomethane production as a sustainable fuel and benefit perfection of environmental waste and pollution control measures. Last, the fact that biochar globally promotes biomethane generation in anaerobic media is casting some doubt on the use of biochar to sequester carbon in soils. Indeed, our findings suggest that soils amended with biochar may accelerate methane emissions in the atmosphere, notably in anaerobic soils where fermentation of organic matter and pollution takes place, thus counteracting the sequestering effect of biochar.

Declarations

Conflict of interest Authors declare no competing financial interest.

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Supplementary Material

Biochar promotes methane production during anaerobic digestion of organic waste

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Table SI 1 Literature data and references of the effect of biochar addition on biomethane production during anaerobic disposal of solid waste and wastewater.

Table S1 Literature data and references of the effect of biochar addition on biomethane production during anaerobic disposal of solid waste and wastewater.

Feedstock	Pyrolysis temperature (°C)	pH	Conductivity (mS/m)	Size (mm)	BET (m²/g)	Doze	Methanogens [3]	Methanogens [4]	Control group Max rate or yield	SD	Number	Experimental group Max rate or yield	SD	Number	Hedge's d	References
Pine wood	980	NA[1]	2.2 ± 0.46	2.0-2.4	451 ± 11	NU[2]	NA	NA	0.87	0.018	2	1.06	0.0792	2	1.89	(Cheng et al., 2018)
Pine wood	980	NA	2.2 ± 0.46	2.0-2.4	451 ± 11	NU	NA	NA	0.935	0.015	2	1.3	0.0532	2	5.33	(Cheng et al., 2018)
Pine wood	980	NA	2.2 ± 0.46	2.0-2.4	451 ± 11	NU	NA	NA	1.16	0.145	2	1.11	0.0166	2	-0.277	(Cheng et al., 2018)
Pine wood	980	NA	2.2 ± 0.46	0.21-0.25	531 ± 31	NU	NA	NA	1.08	0.037	2	0.741	0.0748	2	-3.28	(Cheng et al., 2018)
Pine wood	980	NA	2.2 ± 0.46	0.21-0.25	531 ± 31	NU	NA	NA	1.11	0.014	2	0.748	0.184	2	-1.59	(Cheng et al., 2018)
Pine wood	980	NA	2.2 ± 0.46	0.21-0.25	531 ± 31	NU	NA	NA	1.08	0.014	2	0.413	0.0317	2	-15.54	(Cheng et al., 2018)
Fruitwoods	800	8.63 ± 0.13	NA	NA	NA	2	NA	NA	2.81	0.624	3	2.25	0.32909	3	-0.899	(Luo et al., 2015)
Fruitwoods	800	8.63 ± 0.13	NA	NA	NA	4	NA	NA	1.12	0.087	3	2.09	0.121244	3	7.37	(Luo et al., 2015)
Fruitwoods	800	8.63 ± 0.13	NA	0.5-1	NA	6	*Methanobacterium*	*Methanobacterium*	1.26	0.104	3	1.53	0.121244	3	1.91	(Luo et al., 2015)
Fruitwoods	800	8.63 ± 0.13	NA	NA	NA	8	*Methanobacterium*	*Methanobacterium*	0.97	0.104	3	1.02	0.103923	3	0.385	(Luo et al., 2015)
Sawdust	500	9.15 ± 0.12	NA	0.25-1	248 ± 34	10	NA	NA	1.349	0.095	2	1.351	0.0843	2	0.0127	(Li et al., 2018)

Substrate															Reference
Sawdust	500	9.15 ± 0.12	NA	0.25-1	248 ± 34	10	NA	NA	2.606	0.058	2	2.619	0.0635	2	0.123	(Li et al., 2018)
Sawdust	500	9.15 ± 0.12	NA	0.25-1	248 ± 34	10	NA	NA	3.502	0.163	2	5.482	0.192	2	6.36	(Li et al., 2018)
Sawdust	500	9.15 ± 0.12	NA	0.25-1	248 ± 34	10	NA	NA	4.675	0.075	2	8.473	0.114	2	22.5	(Li et al., 2018)
Sawdust	500	9.15 ± 0.12	NA	0.25-1	248 ± 34	10	*Methanosaeta*	*Methanosaeta*	5.696	0.2	2	10.21	0.534	2	6.40	(Li et al., 2018)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	5	NA	NA	1.813	0.142	3	2.872	0.245	3	4.23	(Paritosh and Vivekanand, 2019)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	10	NA	NA	1.813	0.142	3	3.662	0.254	3	7.19	(Paritosh and Vivekanand, 2019)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	15	NA	NA	1.813	0.142	3	2.993	0.238	3	4.82	(Paritosh and Vivekanand, 2019)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	20	NA	NA	1.813	0.142	3	2.611	0.12	3	4.86	(Paritosh and Vivekanand, 2019)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	25	NA	NA	1.813	0.142	3	2.463	0.214	3	2.86	(Paritosh and Vivekanand, 2019)
Hardwood	NA	7.9 ± 0.3	14 ± 10	NA	NA	30	NA	NA	1.813	0.142	3	2.49	0.228	3	2.85	(Paritosh and Vivekanand, 2019)
Rice straw	600	10.40 ± 0.02	2.853 ± 0.086	<2	38.8 ± 0.99	20	*Methanosarcina*	*Methanosarcina*	0.0257	0.031	3	0.347	0.217	3	1.66	(Yuan et al., 2018)
Wood chips	600	9.60 ± 0.03	0.225 ± 0.006	<2	14.75 ± 0.95	20	*Methanosarcina*	*Methanosarcina*	0.0257	0.031	3	0.0019	0.0014	3	-0.868	(Yuan et al., 2018)
cow Manure biomasses	600	10.24 ± 0.06	0.277 ± 0.007	<2	16.91 ± 0.73	20	*Methanobacterium*	*Methanobacterium*	0.0257	0.031	3	0.825	0.238	3	3.77	(Yuan et al., 2018)
Pine sawdust	650	9.6	NA	0.0035–	130	8.3	NA	NA	113	8.66	3	156	12.12436	3	3.27	(Sunyoto et al., 2016)

Pine sawdust	650	9.6	NA	0.0259 0.0035– 0.0259	130	16.6	NA	NA	113	8.66	3	160	5.196152	3	5.27	(Sunyoto et al., 2016)
Pine sawdust	650	9.6	NA	0.0035– 0.0259	130	25.1	NA	NA	113	8.66	3	145	5.196152	3	3.58	(Sunyoto et al., 2016)
Pine sawdust	650	9.6	NA	0.0035– 0.0259	130	33.3	NA	NA	113	8.66	3	138	13.85641	3	1.73	(Sunyoto et al., 2016)
Switchgrass	500	7.3 ± 0.3	33.8 ± 1.2	0.669 ± 0.453	5.7 ± 2.0	NA	NA	NA	190.8	20.61	3	328.7	4.458	3	7.40	(Shanmugam et al., 2018)
Ashe juniper	400	7.0 ± 0.2	35 ± 14	1.068 ± 0.838	8.0 ± 3.0	NA	NA	NA	190.8	20.61	3	327.2	14.7	3	6.10	(Shanmugam et al., 2018)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	4	NA	NA	490	14.5	3	480.5	5.6	3	-0.691	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	10	NA	NA	490	14.5	3	493.1	4.8	3	0.230	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	20	NA	NA	490	14.5	3	507.5	4.6	3	1.30	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	2	NA	NA	440	39.1	3	460.3	3.5	3	0.586	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	5	NA	NA	440	39.1	3	530.5	4.2	3	2.60	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	10	NA	NA	440	39.1	3	476.6	4	3	1.05	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	1.6	NA	NA	340	31.7	3	490.2	7.1	3	5.23	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	4	NA	NA	340	31.7	3	478.1	3.7	3	4.90	(Cai et al., 2016)
Fruitwoods	800–900	8.63 ± 0.13	NA	NA	NA	8	NA	NA	340	31.7	3	471.9	3	3	4.69	(Cai et al., 2016)

Feedstock																	
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	0.5	NA	NA	156.5	3.226	3	187.9	4.839	3	6.11	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1	NA	NA	156.5	3.226	3	208.9	4.032	3	11.5	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1.5	NA	NA	156.5	3.226	3	190.3	5.645	3	5.88	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	2	NA	NA	156.5	3.226	3	182.3	4.839	3	5.02	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	0.5	NA	NA	156.5	4.839	3	166.9	2.42	3	2.17	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1	NA	NA	156.5	4.839	3	188.7	3.225	3	6.26	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1.5	NA	NA	156.5	4.839	3	208.9	4.032	3	9.41	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	2	NA	NA	156.5	4.839	3	196.8	4.032	3	7.25	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	0.5	NA	NA	156.5	6.14	3	233.3	4.386	3	11.5	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1	NA	NA	156.5	6.14	3	227.2	4.386	3	10.6	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1.5	NA	NA	156.5	6.14	3	214.9	3.509	3	9.34	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	2	NA	NA	156.5	6.14	3	207	4.384	3	7.57	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	0.5	NA	NA	146.5	4.386	3	166.7	4.386	3	3.68	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1	NA	NA	146.5	4.386	3	194.7	3.509	3	9.71	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1.5	NA	NA	146.5	4.386	3	211.4	4.385	3	11.8	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	2	NA	NA	146.5	4.386	3	185.1	5.263	3	6.37	(Ambaye et al., 2020)

Feedstock	Temp	pH					Genus 1	Genus 2			n			n		Reference
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	0.5	NA	NA	162.2	4.196	3	274.5	3.148	3	24.2	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1	NA	NA	162.2	4.196	3	227.3	4.196	3	12.4	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	1.5	NA	NA	162.2	4.196	3	210.49	3.146	3	10.4	(Ambaye et al., 2020)
Sewage sludge	350	6.4 ± 0.1	44.22 ± 0.02	NA	NA	2	NA	NA	162.2	4.196	3	202.1	3.147	3	8.61	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	0.5	NA	NA	155.9	3.147	3	172.7	3.147	3	4.27	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1	NA	NA	155.9	3.147	3	222	5.245	3	12.2	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	1.5	NA	NA	155.9	3.147	3	204.2	2.098	3	14.4	(Ambaye et al., 2020)
Sewage sludge	550	9.5 ± 0.1	21.07 ± 0.02	NA	NA	2	NA	NA	155.9	3.147	3	189.5	2.098	3	10.1	9
NA	NA	NA	NA	NA	NA	20	Methanosarcina	Methanosarcina	0.0054	2E-04	3	0.0068	0.00024	3	5.60	(Huang et al., 2020)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	10	Methanosaeta	Methanobacterium	10.89	0.21	3	14.27	0.28	3	10.9	(Martinez et al., 2018)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	30	Methanosaeta	Methanofollis	10.89	0.21	3	14.15	0.28	3	10.5	(Martinez et al., 2018)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	10	Methanosaeta	Methanosphaerula	18.73	0.37	3	23.13	0.46	3	8.43	(Martinez et al., 2018)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	30	Methanosaeta	Mathanolinea	18.73	0.37	3	33.39	0.66	3	21.9	(Martinez et al., 2018)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	10	NA	NA	14.35	0.28	3	66.34	1.15	3	49.7	(Martinez et al., 2018)
Vineyard prunings	NA	1.59 ± 0.60	NA	NA	NA	30	NA	NA	14.35	0.28	3	75.53	3.2	3	21.5	(Martinez et al., 2018)
Wood pellet	NA	9.4 ± 0.2	NA	NA	NA	NA	Methanosaetaceae	NA	1.4	0.1	2	1.6	0.04	2	1.50	(Indren et al., 2020)

Wheat straw	NA	10.2 ± 0.03	NA	NA	NA	NA	Methanosaetaceae	NA	1.4	0.1	2	1.2	0.1	2	-1.14	(Indren et al., 2020)
Sheep manure	NA	11 ± 0.1	NA	NA	NA	NA	Methanosaetaceae	NA	1.4	0.1	2	1.2	0.1	2	-1.14	(Indren et al., 2020)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	1	NA	NA	20.19	0.96	2	21.4	1.09	2	0.673	(Jang et al., 2018)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	10	NA	NA	20.19	0.96	2	24.32	1.12	2	2.26	(Jang et al., 2018)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	1	NA	NA	28.23	0.64	2	29.85	0.62	2	1.47	(Jang et al., 2018)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	10	NA	NA	28.23	0.64	2	37.35	0.67	2	7.96	(Jang et al., 2018)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	1	NA	NA	25.58	0.87	2	31.07	1.01	2	3.33	(Jang et al., 2018)
Dry dairy manure	350	NA	NA	0.42–0.6	6.3	10	NA	NA	25.58	0.87	2	38.49	1.44	2	6.20	(Jang et al., 2018)
Corn stover	500	NA	NA	841 to <74	NA	NA	NA	NA	620	10.39	3	611	46.76537	3	-0.152	(Achi et al., 2020)
Corn stover	500	NA	NA	841 to <74	NA	NA	NA	NA	620	10.39	3	611	27.71281	3	-0.246	(Achi et al., 2020)
Douglas fir wood	400	5.62 ± 0.01	46.8±1.9	NA	16.99 ± 0.58	10	*Methanothermobacter*	*Methanosaeta*	19.3	1.4	2	31	1.9	2	4.01	(Wang et al., 2020b)
Douglas fir wood	500	5.77 ± 0.00	49.9±1.2	NA	13.17 ± 4.05	10	*Methanothermobacter*	*Methanosarcina*	19.3	1.4	2	38	2.2	2	5.80	(Wang et al., 2020b)
Douglas fir wood	600	6.08 ± 0.04	51.2±5.7	NA	18.36 ± 2.61	10	*Methanothermobacter*	*Methanosarcina*	19.3	1.4	2	33.5	1.6	2	5.40	(Wang et al., 2020b)
Douglas fir wood	730	6.13 ± 0.08	63.6±3.4	NA	18.39 ± 0.29	10	*Methanothermobacter*	*Methanosarcina*	19.3	1.4	2	25.6	1.8	2	2.23	(Wang et al., 2020b)
Douglas fir wood	400	5.62 ± 0.01	46.8±1.9	NA	16.99 ± 0.58	10	*Methanothermobacter*	*Methanothermobacter*	3.9	1.8	2	7.4	0.5	2	1.51	(Wang et al., 2020b)
Douglas fir wood	500	5.77 ± 0.00	49.9±1.2	NA	13.17 ± 4.05	10	*Methanothermobacter*	*Methanobacterium*	3.9	1.8	2	6.7	0.2	2	1.25	(Wang et al., 2020b)
Douglas fir wood	600	6.08 ± 0.04	51.2±5.7	NA	18.36 ± 2.61	10	*Methanothermobacter*	*Methanobacterium*	3.9	1.8	2	6.8	1	2	1.14	(Wang et al., 2020b)
Douglas fir wood	730	6.13 ± 0.08	63.6±3.4	NA	18.39 ± 0.29	10	*Methanothermobacter*	*Methanothermobacter*	3.9	1.8	2	4	0.7	2	0.0418	(Wang et al., 2020b)
Douglas fir wood	400	5.62 ± 0.01	46.8±1.9	NA	16.99 ± 0.58	10	*Methanobrevibacter*	*Methanobrevibacter*	30.7	1.4	2	35.8	1.9	2	1.75	(Wang et al., 2020b)

Feedstock																Reference
Douglas fir wood	500	5.77 ± 0.00	49.9±1.2	NA	13.17 ± 4.05	10	*Methanothermobacter*	*Methanomassiliicoccus*	30.7	1.4	2	35.5	2.2	2	1.49	(Wang et al., 2020b)
Douglas fir wood	600	6.08 ± 0.04	51.2±5.7	NA	18.36 ± 2.61	10	*Methanothermobacter*	*Methanomassiliicoccus*	30.7	1.4	2	36.2	2.2	2	1.70	(Wang et al., 2020b)
Douglas fir wood	730	6.13 ± 0.08	63.6±3.4	NA	18.39 ± 0.29	10	*Methanothermobacter*	*Methanomassiliicoccus*	30.7	1.4	2	33.8	2.1	2	0.992	(Wang et al., 2020b)
Douglas fir wood	400	5.62 ± 0.01	46.8±1.9	NA	16.99 ± 0.58	10	*Methanothermobacter*	*Methanothermobacter*	17.6	1.6	2	25	2.8	2	4.44	(Wang et al., 2020b)
Douglas fir wood	500	5.77 ± 0.00	49.9±1.2	NA	13.17 ± 4.05	10	*Methanothermobacter*	*Methanothermobacter*	17.6	1.6	2	24.6	1	2	2.0487	(Wang et al., 2020b)
Douglas fir wood	600	6.08 ± 0.04	51.2±5.7	NA	18.36 ± 2.61	10	*Methanothermobacter*	*Methanothermobacter*	17.6	1.6	2	24.2	1.7	2	1.28	(Wang et al., 2020b)
Douglas fir wood	730	6.13 ± 0.08	63.6±3.4	NA	18.39 ± 0.29	10	*Methanothermobacter*	*Methanothermobacter*	17.6	1.6	2	22.6	1.9	2	0.723	(Wang et al., 2020b)
Sewage sludge	300	6.78	64.1	NA	21.3196	10	*Methanosaeta*	NA	111.4	0.87	3	132.1	4.775	3	4.83	(Wu et al., 2019)
Sewage sludge	500	7.5	27.4	NA	39.7573	10	*Methanosaeta*	NA	111.4	0.874	3	123.4	2.581	3	4.98	(Wu et al., 2019)
Sewage sludge	700	7.92	24.85	NA	31.7746	10	*Methanosaeta*	NA	111.4	0.874	3	114.7	0.735	3	3.27	(Wu et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	2	*Methanospirillum*	*Methanospirillum*	220.1	7.7	2	263.6	8.9	2	2.99	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	6	*Methanospirillum*	*Methanospirillum*	220.1	7.7	2	341.5	3.6	2	11.5	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	10	*Methanospirillum*	*Methanospirillum*	220.1	7.7	2	401.8	7.7	2	13.5	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	14	*Methanospirillum*	*Methanospirillum*	220.1	7.7	2	358.1	4.2	2	12.7	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	2	*Methanosaeta*	*Methanosaeta*	310.4	9.2	2	350	3.9	2	3.20	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	6	*Methanosaeta*	*Methanosaeta*	310.4	9.2	2	391.8	5.5	2	6.14	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	10	*Methanosaeta*	*Methanosaeta*	310.4	9.2	2	456.8	7.7	2	9.86	(Sun et al., 2019)
Cow manure	500	8.5	NA	0.5–1.0	112.6	14	*Methanosaeta*	*Methanosaeta*	310.4	9.2	2	416.9	8.9	2	6.72	(Sun et al., 2019)
Corn straw	400	8.2	NA	NA	29.8	8	NA	NA	117.2	8.244	3	184.2	9.178	3	6.14	(Zhang et al., 2019)
Corn straw	500	8.3	NA	NA	32.8	8	NA	NA	117.2	8.244	3	196.1	6.859	3	8.32	(Zhang et al., 2019)

Feedstock																Reference
Corn straw	600	8.3	NA	NA	56.6	8	NA	NA	117.2	8.244	3	218.4	9.352	3	9.18	(Zhang et al., 2019)
Sewage sludge	400	9.3	NA	NA	16.1	8	NA	NA	117.2	8.244	3	155.9	8.986	3	3.59	(Zhang et al., 2019)
Sewage sludge	500	9.5	NA	NA	18.9	8	NA	NA	117.2	8.244	3	207.6	8.092	3	8.85	(Zhang et al., 2019)
Sewage sludge	600	9.7	NA	NA	26.3	8	NA	NA	117.2	8.244	3	165.8	7.662	3	4.89	(Zhang et al., 2019)
Coconut shell	400	8.7	NA	NA	2.32	8	NA	NA	117.2	8.244	3	143.8	8.923	3	2.48	(Zhang et al., 2019)
Coconut shell	500	9.5	NA	NA	1.92	8	NA	NA	117.2	8.244	3	125.4	9.005	3	0.760	(Zhang et al., 2019)
Coconut shell	600	11.1	NA	NA	12.7	8	NA	NA	117.2	8.244	3	173.7	5.964	3	6.28	(Zhang et al., 2019)
Corn straw	600	8.3	NA	NA	56.6	6.2	NA	NA	126.9	1.997	3	149	3.472	3	6.24	(Zhang et al., 2019)
Corn straw	600	8.3	NA	NA	56.6	15.9	NA	NA	126.9	1.997	3	185.3	5.015	3	12.2	(Zhang et al., 2019)
Corn straw	600	8.3	NA	NA	56.6	26.1	NA	NA	126.9	1.997	3	199.2	5.016	3	15.2	(Zhang et al., 2019)
Corn straw	600	8.3	NA	NA	56.6	34.2	NA	NA	126.9	1.997	3	137.5	3.858	3	2.76	(Zhang et al., 2019)
Sewage sludge	600	9.7	NA	NA	26.3	6.2	NA	NA	126.9	1.997	3	140.2	4.659	3	2.97	(Zhang et al., 2019)
Sewage sludge	600	9.7	NA	NA	26.3	15.9	NA	NA	126.9	1.997	3	155.5	1.997	3	11.5	(Zhang et al., 2019)
Sewage sludge	600	9.7	NA	NA	26.3	26.1	NA	NA	126.9	1.997	3	168.5	3.661	3	11.3	(Zhang et al., 2019)
Sewage sludge	600	9.7	NA	NA	26.3	34.2	NA	NA	126.9	1.997	3	124.9	2.663	3	-0.680	(Zhang et al., 2019)
Coconut shell	600	11.1	NA	NA	12.7	6.2	NA	NA	126.9	1.997	3	142.7	3.802	3	4.16	(Zhang et al., 2019)
Coconut shell	600	11.1	NA	NA	12.7	15.9	NA	NA	126.9	1.997	3	154.1	2.662	3	9.25	(Zhang et al., 2019)
Coconut shell	600	11.1	NA	NA	12.7	26.1	NA	NA	126.9	1.997	3	169.7	2.661	3	14.6	(Zhang et al., 2019)
Coconut shell	600	11.1	NA	NA	12.7	34.2	NA	NA	126.9	1.997	3	184.9	3.042	3	18.0	(Zhang et al., 2019)
Bamboo biochar	NA	8.42 ± 0.02	0.024 ± 0.002	2-3	54.5	NA	NA	NA	9.01	0.96	3	4.11	0.678	3	-4.72	(Mao et al., 2018)
Pine	800	NA	NA	0.5–1	5.21	NA	*Methanothermobacter*	*Methanothermobacter*	9.11	0.53	3	7.06	0.66	3	-2.74	(Lu et al., 2019)
Pine	800	NA	NA	< 0.005	210.78	NA	*Methanolinea*	*Methanolinea*	9.11	0.53	3	9.55	0.53	3	0.664	(Lu et al., 2019)
Pine	800	NA	NA	0.5–1	5.21	NA	*Methanomicrobia*	*Methanomicrobia*	9.08	0.69	3	10.56	0.53	3	1.92	(Lu et al., 2019)

Feedstock																Reference
Pine	800	NA	NA	< 0.005	210.78	NA	*Methanomicrobia*	*Methanomicrobia*	9.08	0.69	3	9.44	0.75	3	0.400	(Lu et al., 2019)
Sawdust	300	7.3 ± 0.1	NA	0.25–1	53.2 ± 2.8	15	NA	NA	115.9	2.362	3	159.7	2.362	3	14.8	(Wang et al., 2020a)
Sawdust	500	9.2 ± 0.1	NA	0.25–1	248.6 ± 3.5	15	NA	NA	115.9	2.362	3	168.7	5.196	3	10.5	(Wang et al., 2020a)
Sawdust	700	10.0 ± 0.2	NA	0.25–1	511.3 ± 3.8	15	NA	NA	115.9	2.362	3	150.7	1.89	3	13.0	(Wang et al., 2020a)
Straw	350–550	NA	143	NA	NA	1	*Methanosaetaceae*	*Methanobacteriaceae*	0.756	0.092	3	0.811	0.094	3	0.0744	(Xiao et al., 2019)
Sludge	500	8.01 ± 0.05	46.6 ± 2.8	NA	NA	NA	*Methanothermobacter*	NA	20.14	1.49	3	30.06	2.87	3	3.47	(Yin et al., 2019)
Sawdust	500	9.2	NA	0.25-1	248.6	2	NA	NA	6.7	0.1	2	8.7	0.2	2	7.23	(Wang et al., 2018)
Sawdust	500	9.2	NA	0.25-1	248.6	6	NA	NA	6.7	0.1	2	9.4	0.2	2	9.76	(Wang et al., 2018)
Sawdust	500	9.2	NA	0.25-1	248.6	10	NA	NA	6.7	0.1	2	8.2	0.2	2	5.42	(Wang et al., 2018)
Sawdust	500	9.2	NA	0.25-1	248.6	15	*Methanosaeta*	*Methanosaeta*	6.7	0.1	2	7.8	0.2	2	3.98	(Wang et al., 2018)
Red spruce woodchips	NA	8.4	NA	NA	327	NA	NA	NA	486.9	13.12	3	640.6	16.88	3	8.13	(Mainardis et al., 2019)
Red spruce woodchips	NA	8.4	NA	NA	327	NA	NA	NA	291	7.693	3	402.6	8.974	3	10.7	(Mainardis et al., 2019)
Pine biochar	NA	NA	NA	NA	310.19	NU	NA	NA	72.45	1.11	3	82.9	0.28	3	10.3	(Mainardis et al., 2019)
Pine biochar	NA	NA	NA	NA	310.19	NU	NA	NA	72.45	1.11	3	71.35	1.19	3	-0.765	(Shen et al., 2016)
White oak biochar	NA	NA	NA	NA	296.81	NU	NA	NA	72.45	1.11	3	83.22	0.28	3	10.6	(Shen et al., 2016)
White oak biochar	NA	NA	NA	NA	296.81	NU	NA	NA	72.45	1.11	3	79.41	1.27	3	4.67	(Shen et al., 2016)
Pine biochar	NA	NA	NA	NA	310.19	NU	NA	NA	101.8	6.46	3	107.1	3.35	3	0.824	(Shen et al., 2016)
Pine biochar	NA	NA	NA	NA	310.19	NU	NA	NA	101.8	6.46	3	103.9	2.81	3	0.337	(Shen et al., 2016)
White oak biochar	NA	NA	NA	NA	296.81	NU	NA	NA	101.8	6.46	3	106.1	3.59	3	0.658	(Shen et al., 2016)
White oak biochar	NA	NA	NA	NA	296.81	NU	NA	NA	101.8	6.46	3	101.5	3.04	3	-0.0475	(Shen et al., 2016)
Wheat bran pellets	800	NA	49900	1.7–2	55 ± 1	25	*Methanosarcina*	*Methanosarcina*	14.154	4.001	2	58.308	2.308	2	4.17	(Viggi et al., 2017)

Coppiced woodlands	500	NA	1600	1.7–2	61 ± 1	25	Methanosarcina	Methanosarcina	14.154	4.001	2	66.923	2.461	2	4.28	(Viggi et al., 2017)
Orchard pruning	500	NA	500	1.7–2	13.7 ± 0.5	25	Methanosarcina	Methanosarcina	14.154	4.001	2	72.154	1.539	2	4.12	(Viggi et al., 2017)

Notes: 1, NA, not available; 2, Not used; 3, The predominant methanogens; 4, Methanogens with the highest increase in abundance. These paired measurements of control and biochar-amended treatments was in duplicate with a gray background.

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