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Improving acid-stressed anaerobic digestion processes with biochar - towards a combined biomass and carbon management system

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Abstract

Interest in biochar as an additive to enhance anaerobic digestion (AD) has grown in the context of biomass cascading use and the 2050 net-zero goal. However, few studies have investigated the effects of biochar on AD from a biochar production perspective, including biomass feedstocks and pyrolysis temperatures. To valorise biomass and better understand the mechanisms and environmental implications of using biochar in AD, this study investigated the effects of distinct biochar types on AD under acid stress-induced process inhibition using batch tests. The results demonstrated that biochar can mitigate acid stress and enhance the methane production rate. The kinetic rate constant of methane production is positively related to the buffer capacity of the tested biochars ($R^2 = 0.88$). The choice of feedstocks is a crucial factor ($P = 0.003$), particularly the best-performing biochars derived from raw grass silage. In contrast, the pyrolysis temperature effect was less significant ($P = 0.18$). Furthermore, the analysis of biochar indicates that the alkali (K) and alkaline earth (Ca, Mg) metals contained in biochar may be one of the important factors contributing to buffer capacity ($R^2 = 0.82$ to 0.86). Hence, buffer capacity is a crucial quality criteria when evaluating biochar for AD applications. Raw grass silage biochars are promising for acid stress mitigation due to their high buffer capacity, while carbon-rich woody biochars have high CO_2 sequestration potential. A compromise between mitigating acid stress and sequestering carbon is the use of pre-treated grass biochar. Overall, the use of biochar-enriched digestate offers a potential way to close material loops and complete the biomass-to-biochar value chain.

1. Introduction

Biochar (BC) contains recalcitrant carbon derived from the pyrolysis of biomass and, thus, is an emerging tool for managing residual biomass and sequestrating carbon (Lehmann and Joseph 2009, Spokas 2010). The specific properties of BC are influenced by the biomass feedstocks and pyrolysis conditions, in particular the temperature (Lehmann and Joseph 2009, Chiappero *et al* 2020). Generally, as pyrolysis temperature increases, biochar becomes more graphitic and aromatic due to hydrogen (H) and oxygen (O) losses during pyrolysis (Tan *et al* 2015). In addition, the mineral ash content increases with higher pyrolysis temperatures due to greater mass loss of organic compounds, and this mineral content varies significantly depending on the feedstocks (Raveendran *et al* 1995, Li *et al* 2017). As a potential value-added approach for BC, the use of BC as an additive to optimize anaerobic digestion (AD) processes has been increasingly explored in recent years (Masebinu *et al* 2019, Chiappero *et al* 2020). AD processes are well-established as a sustainable method for managing organic waste

and providing renewable energy, particularly biogas. However, AD processes are still facing many challenges including low reactor efficiency and process inhibition caused by organic acid accumulations, which has been reported in previous studies as a main challenge for treating food waste (FW) or organic fraction of municipal solid waste (OFMSW) (Dai *et al* 2015, Luo *et al* 2015, Dang *et al* 2017, Xu *et al* 2018, Ye *et al* 2018). This rapid acid accumulation is related to the high content of easily biodegradable carbohydrates in FW and OFMSW and leads to an inhibition of methanogenesis in AD processes (Anderson and Yang 1992, Dai *et al* 2015). To overcome these challenges, it is important to have a comprehensive understanding of the operation parameters of AD, such as pH, buffer systems and volatile fatty acids (VFAs) (Rosenwinkel *et al* 2015). BC produced from pyrolysis processes exhibits a range of physicochemical properties, such as pH, buffer capacity, which could improve AD processes (Masebinu *et al* 2019, Chiappero *et al* 2020).

Investigations of different biochar types concerning feedstocks or pyrolysis temperatures in AD processes have been reported in previous studies (Fagbohungbe *et al* 2016, Kaur *et al* 2019, Zhang *et al* 2019, Chiappero *et al* 2021, Deng *et al* 2021, Pan *et al* 2022). For example, Deng *et al* (2021) demonstrated distinct effects on digesting whiskey draff in AD processes using biochar produced at pyrolyzing temperatures of 500 °C, 700 °C, and 900 °C. Among these, only the biochar produced at 700 °C exhibited an increase in methane production. The authors proposed that biochar at 700 °C, was characterized by moderate graphitization and abundant active surface functional groups, promoted interspecies electron transfer, consequently enhancing methane production. Similarly, Pan *et al* (2022) investigated biochar produced at different temperatures. Their experiments, conducted under organic substrate overloading conditions, used biochar derived from mushroom bran at 400 °C, 450 °C, 500 °C, 550 °C, and 600 °C. In particular, the addition of biochar produced at 550 °C resulted in a maximum significant increase in methane production and a shortened fermentation period. These results suggest that, for the same feedstock, the positive effects initially increase as the biochar pyrolysis temperature increases, and then decrease once reaching maximum significance. Other studies have tested the biochar derived from different biomass feedstocks. For example, Zhang *et al* (2019) conducted an investigation involving nine distinct biochar types derived from corn straw, coconut shell, and sewage sludge. These biochars were produced at pyrolysis temperatures of 400 °C, 500 °C, and 600 °C, respectively. Remarkably, all the tested biochars demonstrated a significant enhancement in methane production under conditions of organic substrate overloading. The authors attribute these results to the alleviation of VFA accumulations. In another study, Kaur *et al* (2019) explored the effects of biochars obtained from softwood, oilseed rape, and wheat straw, all pyrolyzed at 550 °C and 700 °C. The addition of biochar led to an increase in methane production and the degradation of VFAs when compared to control conditions without biochar.

Previous studies have mainly focused on the investigation of biochars derived from woody and straw biomass. In Europe, however, there is a significant underutilised resource in the form of grasslands, which cover a considerable area and currently account for 17.4% of the EU's total landscape (LUCAS 2021). Thus, there is increasing interest in the use of grassland as a renewable resource in Europe and its conversion to biochar (Heinrich *et al* 2023a). Moreover, previous studies have tested the biochars produced from different biomass feedstocks and pyrolysis temperatures, but how these two parameters affect the characteristics of biochar and subsequently affect the AD process optimization is not well understood. Furthermore, few studies have explored the potential environmental implications of biochar-enriched digestate, regarding closing the AD-based biomass-to-biochar value chain and contributing to a broader biomass and carbon management system.

The earlier study conducted by Hu *et al* (2023) has identified the conditions for positive BC effects on AD, i.e. at a high biochar addition rate and under substrate overloading. The effects of biochar types were however not investigated. Thus, the objectives of the present study were to: (1) determine the biochar properties and their relationships; (2) examine the extent of improvement in acid-stressed AD using biochars from different feedstocks (grass, wood) and pyrolysis temperatures (400 °C, 750 °C); (3) analyse the profile of TVFAs and total alkalinity during AD processes under the influence of biochar addition and acid stress. The potential uses of biochar-enriched digestates were also assessed in terms of their energy potential via incineration and their heavy metal concentrations, comparing the latter to the limit values specified in the European Biochar Certificate (EBC 2023). The current studyuniquely tested distinct biochar types from residual (sawdust) and low-value biomass (grass) at a low and a high pyrolysis temperature, which distinctively shows the properties of biochar over a wide spectrum and increases the validity of the results. Additionally, this study explored the need for pre-treating grass-type biomass. To our knowledge, few researchers have investigated the effects of grass-type biochar on AD processes.

2. Materials and methods

2.1. Biomass preparation and biochar production

2.1.1. Biomass feedstocks

Three biomass feedstocks were selected for this study, i.e. (1) grass silage (**s**); (2) a pre-treated grass silage (**g**) and (3) oak wood sawdust (**w**). The oak wood sawdust was obtained from a commercial sawmill in Hedemünden, Germany. The sawdust is available in large quantities and with less handling demand, hence ideal for biochar production (Hamelin *et al* 2019, Thiffault *et al* 2023). The grass biomass feedstock was obtained from roadside verges along a rural road in the District of Lake Constance, Germany, which need to be valorised (Larsson *et al* 1998, Piepenschneider *et al* 2016). The grass samples were taken from the second cut in September 2020 by mowing with a commercial rotary mower at a stubble height of 5 cm and directly ensiled in 250 kg round bales.

2.1.2. Biomass pretreatment

The pre-treated grass silage was processed after a minimum ensiling duration of six weeks according to the IFBB concept (Bühle *et al* 2014). The IFBB (Integrated Generation of Solid Fuel and Biogas from Biomass) procedure was developed by the University of Kassel to valorise biomass (Wachendorf *et al* 2009, Hensgen *et al* 2011). Briefly, the ensiled biomass was chopped to a length of 10 cm and subsequently processed by hydrothermal conditioning at 40 °C with silage-to-water ratio of 1:4 based on fresh matter for 15 min. Afterwards, mechanical dehydration was carried out with a conical screw press (type AV, Anhydro Ltd., Kassel, Germany) to produce a press cake and a press liquid from the ensiled biomass. The press liquid can be easily degraded for methane production (Richter *et al* 2009). The press cake with reduced ash content, the raw silage and the oak wood sawdust were further pelletized to a size of 6 mm diameter and 10 mm length using a pilot-scale pellet press (PP230UG, qteck GmbH, Bergen, Germany) and the obtained pellets had a water content of 10 ± 2%.

2.1.3. Biochar pyrolysis

Six different types of biochar were produced from the obtained three pellet types in a continuously operated auger reactor (PYREKA, Pyreg GmbH, Dörth, Germany), flushed with nitrogen to avoid uncontrolled oxidation. Since the process parameters during pyrolysis have an important role in characteristics of biochar (Lehmann and Joseph 2009, Ippolito *et al* 2020), the production parameters were set with two temperatures 400 °C (low) and 750 °C (high) and a moderate residence time of approximately 30 min, to ensure the resulting biochar types had a large spectrum of characteristics. The used parameters allow a high transferability of the results in a large-scale reactor, for example, Pyreg P500 (Pyreg GmbH, Dörth, Germany) (Joseph *et al* 2020). Biochars were labelled using abbreviations representing the biomass feedstock (s, g, w) and their corresponding pyrolysis temperatures (400, 750), resulting in a total of six biochar types: s400, s750, g400, g750, w400, and w750. Before being used in batch experiments, the biochars were milled to less than 1 mm and dried overnight at 105 °C. The three biomass feedstocks (table 2) and the six biochar types (table 3) were characterised.

2.2. Batch fermentation tests

2.2.1. Source and characteristics of inoculum and substrate

Commercial dog food pellets (Orlando Gourmet, Germany) were used as a substrate to simulate representative food wastes (Nakasaki *et al* 2004, Dang *et al* 2017, Koch *et al* 2017), which can be obtained worldwide with comparable quality, thus, enables the experiments to be replicated in international laboratories. According to the producer, the dog food was composed of 25.0% crude protein, 16.0% crude lipid, 2.5% crude fibre, 6.5% ash, and the remains including carbohydrates accounted for 50.0% on a dry matter basis. The dog food had a total solid (TS) content of 91.7% based on fresh matter (FM) and a volatile solid (VS) of 93.3%TS prior to digestion tests. Digested sewage sludge was used as inoculum obtained from an anaerobic mesophilic sewage sludge digester that processed sewage sludge from a municipal wastewater treatment plant (Kassel, Germany, population equivalent: 340,000). The inoculum had a TS of 1.83%FM and a VS of 59.5%TS prior to digestion tests. The electrical conductivity (EC) and pH value of inoculum prior to digestion tests were 5.11 mS/cm and 7.37, respectively.

2.2.2. Experimental setup

The experiments were conducted using two Gas Endeavour systems (Bioprocess Control Sweden AB), involving a total of 30 bottles (DURAN Boro 3.3, 500 ml). Each bottle was filled to 400 g_{FM}. The experiments included seven treatments, consisting of a control with only inoculum and dog food, as well as six treatments, each containing inoculum, dog food, and a specific biochar from the six biochar types intended for testing (table 1). Each treatment was conducted in four replicates. A blank test with inoculum only and a positive test with cellulose as substrate were also conducted but without replicates in the remaining two bottles. To simulate a

Table 1. Experimental conditions for different biochar types under substrate overloading conditions with a biochar addition rate of 25% and for control without biochar addition. Positive test for checking inoculum was conducted with cellulose as substrate.

Group	Inoculum		Substrate		Biochar	Deionized water
	[g_{FM}]	[g_{VS}]	[g_{FM}]	[g_{VS}]	[g_{TS}]	[g]
Control	300	3.26	7.62	6.52	0	92.38
s400	300	3.26	7.62	6.52	1.63	90.75
s750	300	3.26	7.62	6.52	1.63	90.75
g400	300	3.26	7.62	6.52	1.63	90.75
g750	300	3.26	7.62	6.52	1.63	90.75
w400	300	3.26	7.62	6.52	1.63	90.75
w750	300	3.26	7.62	6.52	1.63	90.75
blank	400	4.34	—	—	—	—
positive	300	3.26	1.71	1.63	—	98.29

FM fresh matter; *TS* total solids, also dry matter.

process condition of organic overloading, the inoculum-to-substrate ratio (ISR) based on VS was chosen to be 0.5, while a non-stressed condition is usually with ISR 2 (Holliger *et al* 2016, Hu *et al* 2023). The biochar was added at a ratio of 25% according to the previous study (Hu *et al* 2023). This ratio represents the relationship between biochar total solids and substrate volatile solids and was equal to 4.1 g_{TS}/L. All tests were conducted under a mesophilic condition (36.5 ± 0.5 °C) until the daily biogas production during three consecutive days was less than 0.5% of the total biogas production (VDI 2016). With the embedded real-time data acquisition system of the Gas Endeavour, both the biogas and methane volume were recorded and normalised to the standard temperature (0 °C) and pressure (1 atm) automatically. A more detailed description of the experimental setup and procedure can be found in the previous study (Hu *et al* 2023).

2.2.3. Sampling and determination of AD process parameters

To determine AD process parameters, including pH, electrical conductivity (EC), total volatile fatty acid (TVFA) and total alkalinity (TA), additional 21 glass bottles were prepared. The same seven treatments as described above were conducted but in triplicate (table 1). Measurements were taken periodically until the batch trials for gas production were completed. To avoid sedimentation and to achieve a representative sample, the slurry was stirred before measurement and sampling. The bottles were opened to measure pH and EC at 36.5 ± 0.5 °C, and a 5 g_{FM} sample was collected from each bottle at the same time. Subsequently, samples were centrifuged at 4350 g for 10 min, then the supernatants were frozen at −18 °C for determination of TVFA and TA. After each sampling and measurement, the headspace of bottles was flushed with nitrogen gas at a flow rate of 2.5 l min^{-1}. for one minute, thus, maintaining the anaerobic condition.

2.3. Analytical methods

2.3.1. Characterization of biochar and biomass

TS (105 °C) and ash content (650 °C) of biochar were measured following DIN EN 12902 (2005). TS (105 °C) and ash content (550 °C) of biomass were measured following DIN EN ISO 18134-3 (2015) and DIN EN ISO 18122 (2016), respectively. Volatile matters of both biomass and biochar were determined in the absence of oxygen at 900 °C for 7 min (DIN 51720 2001, ISO 18123 2016). Fixed carbon was calculated by subtracting the ash content and volatile matter from the total dry mass. For both biomass and biochar, the weight percentage of element CHNS based on TS were measured using an elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Oxygen (O) content was calculated based on the CHNS and ash content. The concentration of ash minerals (K, Ca, Mg) and heavy metals (Pb, Cd, Cr, Cu, Ni, Zn) were determined by an ICP-OES analysis (SPECTRO Analytical Instruments GmbH, Kleve, Germany) after microwave digestion with nitric acid. A biochar suspension diluted with deionized water by 10:1 (v/w) was prepared to measure EC and pH of biochar according to Singh *et al* (2017). The buffer capacity was determined by titrating with NaOH in a suspension of HCl and biochar with 20:1 (v/w) (Singh *et al* 2017). For biomass fibre analysis, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the method of van Soest and Wine (1967) using an ANKOM 200 fibre analyser (ANKOM Technologies, USA) (table 2). To assess the energy potential, the higher heating values (HHV) of biochar and digestate were measured using an IKA C200 bomb calorimeter (IKA Werke GmbH & Co, Germany). Replicate samples of digestates were pooled and dried at 105 °C prior to the measurement of the heating value.

Table 2. Characteristics of raw grass silage (s), IFBB pre-treated grass silage (g) and oak sawdust (w) used as biomass feedstocks for biochar production.

Parameters	s	g	w
NDF [%TS]	41.06 ± 0.54	59.68 ± 0.45	85.88 ± 0.31
ADF[%TS]	23.31 ± 0.46	35.93 ± 0.57	63.01 ± 0.11
Lignin (ADL) [%TS]	9.48 ± 0.36	14.08 ± 0.19	14.84 ± 0.33
Hemicellulose [%TS]	17.75	23.75	22.87
Cellulose [%TS]	13.83	21.85	48.17
C [%TS]	44.87 ± 0.05	44.99 ± 0.08	49.91 ± 0.10
H [%TS]	4.68 ± 0.06	4.96 ± 0.12	5.08 ± 0.04
N [%TS]	2.59 ± 0.01	1.99 ± 0.11	0.40 ± 0.07
S [%TS]	0.421 ± 0.127	0.189 ± 0.017	0.060 ± 0.014
O [%TS]	30.22 ± 0.24	33.43 ± 0.07	43.98 ± 0.22
K [mg/g][a]	27.45 ± 0.09	7.80 ± 0.07	1.14 ± 0.02
Ca [mg/g][a]	12.89 ± 0.08	9.62 ± 0.10	1.48 ± 0.03
Mg [mg/g][a]	3.03 ± 0.02	1.58 ± 0.00	0.22 ± 0.01
Ash content [%TS]	17.21 ± 0.10	14.44 ± 0.16	0.57 ± 0.01
Volatile matter [%TS]	66.74 ± 0.02	68.88 ± 0.04	82.91 ± 0.08
Fixed carbon [%TS]	16.05 ± 0.02	16.69 ± 0.04	16.52 ± 0.08

Data shown are mean (± standard deviation, n = 2);
[a] based on TS, *TS* total solids, also dry matter; *ADL* acid detergent lignin; *ADF* acid detergent fibre; *NDF* neutral detergent fibre; *Hemicellulose* = NDF—ADF; *Cellulose* = ADF—ADL.

2.3.2. Analysing the AD samples

After digestion, electrical conductivity (EC) and pH of all the digestate (both for gas production and for process parameters) were immediately measured at 36.5 ± 0.5 °C with the WTW multiparameter meter 3420 (Xylem Analytics, Germany; **Supplementary**). VS and TS of digestate were determined according to the German Standard Methods for the Examination of Water, Wastewater and Sludge (DEV 2020). For total volatile fatty acid (TVFA) and total alkalinity (TA), the digestates were centrifuged at 4350 g for 10 min, the supernatants were stored at −18 °C and later analysed by titrating with sulfuric acid following the Nordmann method (Nordmann 1977).

2.4. Kinetic estimation

First order kinetic model is often used to describe an anaerobic digestion process (equation (1)):

$$\frac{dS}{dt} = -kS \tag{1}$$

where, S is the biodegradable substrate, t is the time and k is a first order kinetic rate constant.

Considering the relation between the degraded substrate and the generated methane (Guwy 2004, Angelidaki *et al* 2009, Li *et al* 2018), separating variables and then integrating, the equation (1) is possible to write as follows (equation (2)):

$$\ln \frac{B_\infty - B}{B_\infty} = -kt \tag{2}$$

where, t is the time and k is the first order methane production rate constant. B_∞ is the ultimate methane yield and B is the cumulative methane yield at a given time t.

2.5. Biochar mass and carbon yield

Biochar mass yield was calculated following equation (3), it equals to the biomass mass conversion rate.

$$\begin{aligned} Biochar \quad mass \quad yield\,[\%] = Output \quad biochar\,[kg \quad TS] \\ \div Input \quad biomass\,[kg \quad TS] \times 100 \end{aligned} \tag{3}$$

The biochar carbon yield was defined as the carbon flow quote from the input biomass to the yield biochar, calculated following equation (4),

$$Biochar \quad carbon \quad yield \quad [\%] = \frac{C_{BC} \quad [\%TS] \times Output \quad biochar\,[kg \quad TS]}{C_{BM} \quad [\%TS] \times Input \quad biomass\,[kg \quad TS]} \times 100 \tag{4}$$

where C_{BC} is the carbon content of biochar, and C_{BM} is the carbon content of biomass.

Table 3. Characteristics of biochars produced from raw grass silage, IFBB pre-treated grass silage and oak sawdust either at a pyrolysis temperature of 400 °C or 750 °C and with a retention time of 30 min.

Parameters	Raw grass silage		Pre-treated grass silage		Oak sawdust	
	400 °C	750 °C	400 °C	750 °C	400 °C	750 °C
EC [μS/cm][a]	10413.0 ± 130.2	10323.0 ± 28.7	1037.7 ± 7.1	1461.0 ± 15.0	82.0 ± 2.8	587.0 ± 3.6
pH [-][a]	10.50 ± 0.00	10.59 ± 0.00	7.75 ± 0.03	10.21 ± 0.09	7.12 ± 0.02	10.00 ± 0.04
Buffer [%TS][a,b]	12.02 ± 0.06	18.59 ± 0.76	5.86 ± 0.19	10.94 ± 0.75	4.26 ± 0.41	5.98 ± 0.66
Buffer Class[c]	2	2	1	2	1	1
K [mg/g_{TS}]	58.95 ± 1.04	71.44 ± 0.56	14.16 ± 1.55	18.14 ± 0.44	2.64 ± 0.00	3.80 ± 0.06
Ca [mg/g_{TS}]	31.76 ± 0.34	40.61 ± 0.34	23.77 ± 3.02	30.46 ± 0.27	7.07 ± 0.20	10.39 ± 0.06
Mg [mg/g_{TS}]	8.16 ± 0.11	10.27 ± 0.03	4.11 ± 0.51	5.44 ± 0.01	0.24 ± 0.03	0.43 ± 0.03
Ash content [% TS][a]	41.52 ± 0.46	53.59 ± 0.22	32.29 ± 0.27	43.98 ± 0.11	2.23 ± 0.07	3.37 ± 0.06
Volatile matter [%TS]	22.01 ± 0.05	10.80 ± 0.31	24.84 ± 0.07	8.33 ± 0.02	28.54 ± 0.17	6.03 ± 0.08
Fixed carbon [%TS]	36.47 ± 0.05	35.61 ± 0.31	42.87 ± 0.07	47.69 ± 0.02	69.23 ± 0.17	90.60 ± 0.08
C [% TS]	41.47 ± 0.16	38.71 ± 0.10	49.85 ± 0.03	48.46 ± 0.08	75.97 ± 0.55	90.10 ± 0.51
H [% TS]	2.04 ± 0.02	0.59 ± 0.01	2.53 ± 0.03	0.69 ± 0.01	2.62 ± 0.03	1.11 ± 0.02
N [% TS]	2.48 ± 0.01	1.35 ± 0.00	2.39 ± 0.01	1.30 ± 0.01	0.38 ± 0.01	0.32 ± 0.00
O [% TS]	12.28 ± 0.11	5.56 ± 0.09	12.90 ± 0.06	5.53± 0.08	18.78 ± 0.58	5.10 ± 0.49
S [% TS]	0.203 ± 0.014	0.200 ± 0.005	0.043 ± 0.001	0.046 ± 0.009	0.005 ± 0.000	0.003 ± 0.002
Molar O/C ratio	0.222	0.108	0.194	0.086	0.185	0.042
Molar H/C ratio	0.591	0.184	0.608	0.170	0.415	0.148
BC mass yield [%]	39.8	32.5	41.2	31.6	31.3	20.1
BC carbon yield [%]	36.8	28.0	45.6	34.0	47.7	36.3

Data shown are mean (± standard deviation, n = 2);

[a] data shown are mean (± standard deviation, n = 3);

[b] buffer capacity shown as $CaCO_3$ eq.;

[c] Class of buffer capacity from 0 to 3: Class 0 (<1% $CaCO_3$ eq.), Class 1 (1%–10% $CaCO_3$ eq.), Class 2 (10%–20% $CaCO_3$ eq.) and Class 3 (>20% $CaCO_3$ eq.) (Camps-Arbestain *et al* 2015); *TS* total solids, also dry matter; *EC* electrical conductivity indicating salt contents in the leachate of biochar; *BC* biochar.

2.6. Statistical analysis

The normality of data was checked with the Shapiro-Wilk test. The homogeneity of data variance was tested using Levene's test. Significant differences of means were assessed using analysis of variance (ANOVA), considering the experimental factors biochar addition, biomass feedstocks, pyrolysis temperatures and interactions between feedstocks and temperatures. Kruskal–Wallis test was used for checking the significant differences of results with non-normal distributed data and Dunn's test was for further pairwise comparison to identify the significance level of treatments. Linear regression analyses were performed for kinetic rate constants, total volatile fatty acids and digestate heating values, to determine their relationships with biochar addition. Relationships between biochar properties were also determined with linear regression analyses. The threshold for significance was set as $P \leqslant 0.05$. Statistical analysis was carried out using R software (Version 4.1.2) (R Core Team 2021) with additional functions provided by R packages car (Fox and Weisberg 2019) and FSA (Ogle *et al* 2022).

3. Results

3.1. Biochar and biomass characterization

3.1.1. Biomass pre-treatment, biomass feedstocks and pyrolysis temperatures effects on biochar characteristics

The IFBB treatment successfully reduced ash content in raw grass silage by 16% (table 2). The ash minerals, including K, Ca, and Mg, showed a reduction in the range of 25% to 70% after the IFBB treatment but were still much higher than that in oak wood by up to 10 times. However, increasing pyrolysis temperature decreased the biochar mass yield and increased the ash content (table 3). In addition to the pyrolysis temperature, biomass feedstocks had a much stronger influence on biochar's ash content. The grass-type biochars had an almost 15 times higher ash content than that of woody type biochars, which is also reflected in the significantly higher content of ash minerals in biomass feedstocks (table 2). Furthermore, the high biochar mass yield of up to 40% for grass-type biochar is positively related to the substantial retention of ash minerals after pyrolysis (ash content up to 50%). Thus, the biochar carbon content of grass-type biochar was only less than 50% (table 3). However, the pre-treatment of grass silage enhanced the carbon content in biochar by around 10%.

In addition, only the carbon content of woody biochars was affected by the pyrolysis temperatures, with a 15% difference, which can be related to the significantly higher cellulose content of oak wood with 48%

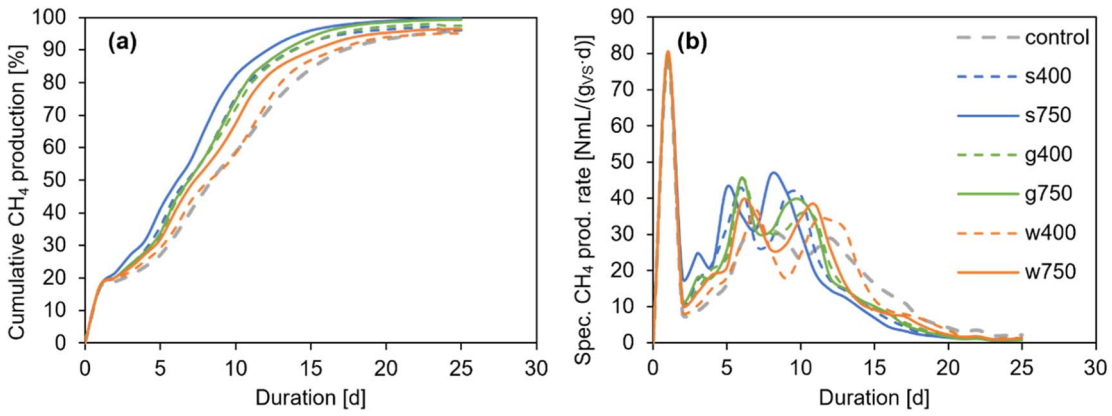

Figure 1. (a) Cumulative methane production curves with the addition of different biochar types and (b) specific methane production rates with different biochar types. The data shown are average values of 4 replicates.

compared to the two grass-type biomass with less than 20% (table 2). The carbon derived from cellulose remained above 400 °C, while the H and O in biomass were further lost at pyrolysis temperature from 400 °C to 750 °C, resulting in a 90% carbon content of woody biochar produced at 750 °C. However, the H and O content were reduced significantly at 750 °C compared to 400 °C, which resulted in lower molar O/C and H/C ratios at higher pyrolysis. All biochars exhibit molar O/C ratios lower than 0.2, except for s400 BC. Volatile matter of biochar also decreased with increasing pyrolysis temperatures (table 3) and showed a positive correlation with molar O/C ratios ($R^2 = 0.84$, $P = 0.01$).

3.1.2. Correlations between AAEMs, buffer capacity and ash content

The biochar's ash content was positively related to its buffer capacity ($R^2 = 0.72$, $P = 0.03$) (table 3). The contents of alkali (K) and alkaline earth (Ca, Mg) metals (AAEMs) in biochars were also positively linked to both their ash content ($R^2 = 0.63$ to 0.98, $P < 0.06$) and buffer capacity ($R^2 = 0.82$ to 0.86, $P < 0.01$). With increasing content of AAEMs, the ash content and buffer capacity also increased to different extents. Marginal enhancements of Mg related to a pronounced increase of buffer capacity ($LRS = 0.7$, $R^2 = 0.86$, $P = 0.007$; linear regression slope = *LRS*) and ash content ($LRS = 0.18$, $R^2 = 0.90$, $P = 0.004$), followed by Ca and K. However, the linear regression between ash content and K was weaker ($R^2 = 0.63$, $P = 0.06$) than that of Ca ($R^2 = 0.98$, $P < 0.001$) and Mg ($R^2 = 0.90$, $P = 0.004$). Generally, biochar produced with high pyrolysis temperatures and grass-type feedstocks had a higher pH-value and buffer capacity (table 3). Raw grass silage-derived biochar exhibits higher values in terms of buffer capacity ($t_{(12)} = 15.1$, $P < 0.001$) and ash content ($t_{(12)} = 46.6$, $P < 0.001$) compared to biochar derived from pre-treated grass silage. However, biochar produced from both grass-type feedstocks as well as at high pyrolysis temperature had a higher buffer capacity and pH value, greater content in alkaline ash minerals and lower molar O/C and H/C ratios, which may favour AD processes.

3.2. Anaerobic digestion process

3.2.1. Methane production

The addition of biochar accelerated methane production under acid stress, with the effect varying depending on the biochar (BC) feedstock and pyrolysis temperatures (figure 1(a)). In general, higher ash content in feedstocks and higher pyrolysis temperatures of BC enhanced the acceleration of methane production. This was particularly pronounced for grass silage biochar produced at 750 °C (s750 BC), while the least effect was observed for oak wood biochar produced at 400 °C (w400 BC). For instance, the time to reach half of the actual achievable BMP decreased from 216 h (control) to 151 h (s750 BC). Furthermore, the maximum cumulative methane yield was weakly affected by the biochar addition (figure 1(a); *one-way ANOVA*, $P = 0.08$) with a maximum increase by the addition of s750 BC of 4% compared to control. Additionally, biochar had also no significant effect on the methane content (figure S3; *one-way ANOVA*, $P = 0.54$).

The specific methane production rate, however, was affected by different biochar types under acid stress and showed the same dependence on biochar types as in methane production process (figure 1(b)). Additionally, multiple peaks were observed indicating the inhibitions during the methane production process. After the decline of the first peak due to the high organic loading, the methane production rate was able to increase again with different recovery speeds. The group with s750 BC recovered significantly faster than other treatments, and the slowest recovery was obtained with w400 BC and then control.

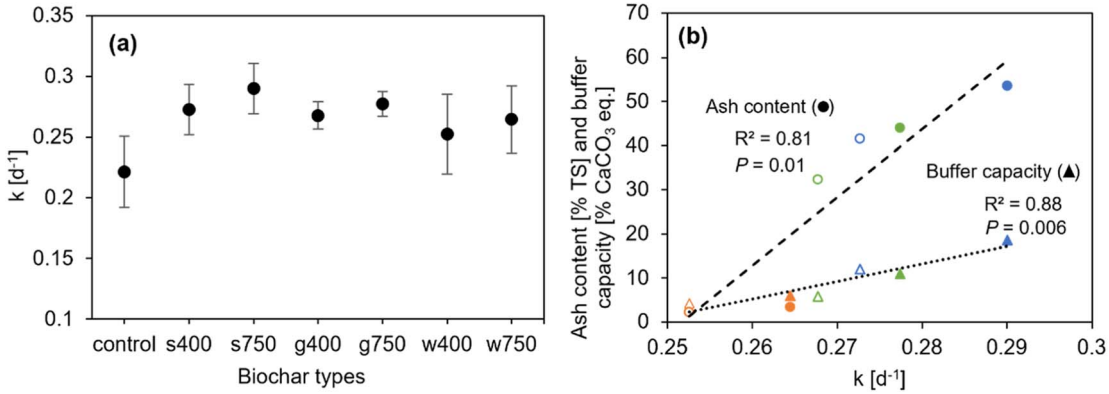

Figure 2 (a) Mean effect (± standard deviation, n = 4) of biochar produced from grass silage (s), IFBB pre-treated grass silage (g) and oak sawdust (w) either at 400 °C or 750 °C on the methane production kinetic rate constant (k) in comparison to the control treatment (0% biochar); (b) mean effects of contribution of biochar's buffer capacity (n = 3) and ash content (n = 3) on the first order kinetic rate constant (n = 4) of the methane production process. Biochar produced from grass silage (s) shown in blue colour (▬), from IFBB pre-treated grass silage (g) in green colour (▬) and from oak sawdust (w) in orange colour (▬), with 400 °C shown in unfilled symbols (○ △) and 750 °C in solid filled symbols (● ▲).

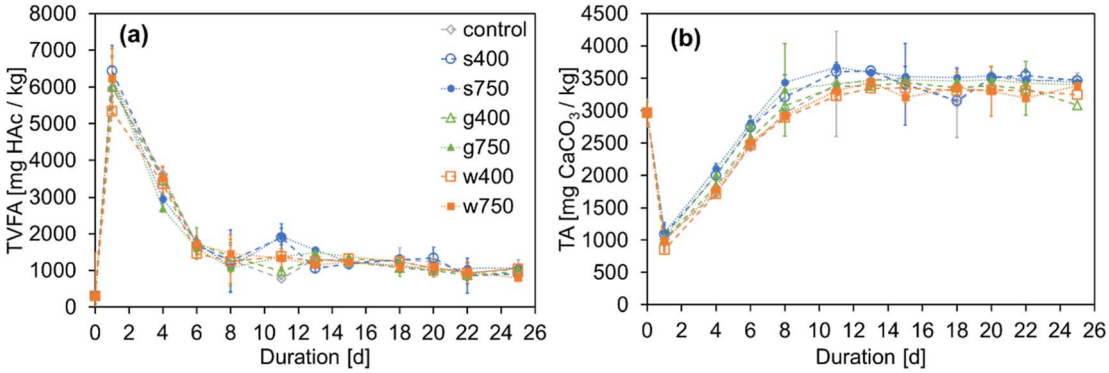

Figure 3. Mean effect (± standard deviation, n = 3) of biochar produced from grass silage (s), IFBB pre-treated grass silage (g) and oak sawdust (w) at either 400 °C or 750 °C on the development of (a) total volatile fatty acid (TVFA), (b) total alkalinity (TA), during the whole incubation time in comparison to the control treatment (0% biochar).

3.2.2. Pseudo-first-order kinetic of methane production and its correlation with biochar properties

All the used biochar elevated the pseudo-first-order kinetic rate constants of methane production compared to the control treatment (figure 2(a)), the degree of fit (R^2) ranged from 0.90 to 0.95. The feedstocks of biochar had a significant impact on the kinetic rate constants (*two-way ANOVA*, $F_{(3,21)} = 6.49$, $P = 0.003$). However, no significant effects were observed for pyrolysis temperatures (*two-way ANOVA*, $F_{(1,21)} = 1.88$, $P = 0.18$) and its interaction with feedstocks (*two-way ANOVA*, $F_{(2,21)} = 0.06$, $P = 0.94$) on the methane production kinetic rate constants. Thus, the grass biochar s750 achieved the maximal improvement in kinetics ($t_{(21)} = 4.18$, $P < 0.001$) of ca. 31%, whereas the oak wood biochar w400 obtained the minimal improvement in kinetics ($t_{(21)} = 1.90$, $P = 0.07$) of ca. 14% compared to the control (figure 2(a)). In addition, increasing the pyrolysis temperature of biochar from 400 °C to 750 °C resulted in slightly improved kinetics (figure 2(a)).

The pseudo-first-order kinetic rate constant of the methane production process was positively related to the buffer capacity and ash content of biochar under the high organic loading condition (figure 2(b)). While marginal increases of the buffer capacity caused a conceivable improvement of the methane production kinetic (*LRS* = 398), a greater extent of ash content was needed to achieve the same kinetic improvement (*LRS* = 1544).

3.2.3. VFAs and alkalinity

During the anaerobic digestion (AD) process, the total volatile fatty acids (TVFAs) were generally not affected by the biochar addition (*Kruskal–Wallis*, $P = 0.98$) (figure 3(a)). However, in the TVFAs degradation phase at the beginning of the experiment, biochar addition exhibited some effects. Especially on day 4, the high TVFAs were reduced rapidly with the addition of g750 BC ($t_{(14)} = -2.05$, $P = 0.06$) by 26% compared to control, whereby

Table 4. Heavy metal concentrations (mg/kg_{TS}) in biomass feedstocks, including grass silage (s), IFBB pre-treated grass silage (g), and oak sawdust (w), as well as in the resulting biochars produced at either 400 °C or 750 °C with a retention time of 30 min.

	Pb	Cd	Cr	Cu	Ni	Zn
Biomass feedstock						
s	3.53 ± 0.08	<0.5[a]	33.93 ± 0.31	16.90 ± 0.60	36.05 ± 1.76	62.72 ± 0.34
g	5.22 ± 0.25	<0.5[a]	55.77 ± 2.76	25.26 ± 0.53	72.98 ± 11.74	54.02 ± 4.79
w	0.18 ± 0.00	<0.5[a]	2.86 ± 0.49	1.87 ± 0.31	11.28 ± 6.30	3.20 ± 0.72
Biochar						
s400	7.48 ± 1.92	<0.5[a]	55.46 ± 8.18	32.54 ± 6.84	93.67 ± 12.03	122.35 ± 20.96
s750	6.13 ± 0.50	<0.5[a]	67.98 ± 1.64	41.41 ± 1.11	62.30 ± 7.52	131.63 ± 3.34
g400	8.28 ± 2.06	<0.5[a]	95.14 ± 12.22	43.72 ± 7.57	35.41 ± 5.37	103.21 ± 7.62
g750	13.43 ± 2.75	<0.5[a]	123.58 ± 4.44	49.20 ± 2.72	37.98 ± 7.99	132.23 ± 8.05
w400	<0.5[a]	<0.5[a]	2.76 ± 0.32	5.80 ± 0.15	32.77 ± 6.33	22.78 ± 3.14
w750	<0.5[a]	<0.5[a]	3.66 ± 0.74	7.74 ± 1.49	21.82 ± 2.56	14.99 ± 1.05
EBC-Agro limit values						
	120	1.5	90	100	50	400

Data shown are mean (± standard deviation, n = 3);
[a] below detection limit; *EBC-Agro* European biochar certificate for agricultural applications.

Table 5. Heating values of biochars and digestates in MJ/kg_{TS}, inoculum had HHV with 11.87 ± 0.05 MJ/kg_{TS}.

Biochar addition	Biochar HHV	Digestate HHV
None	—	12.24 ± 0.09
s400	15.63 ± 0.08	13.31 ± 0.08
s750	13.06 ± 0.05	12.06 ± 0.21
g400	18.58 ± 0.04	13.39 ± 0.14
g750	16.94 ± 0.04	12.92 ± 0.00
w400	28.04 ± 0.03	15.26 ± 0.32
w750	32.23 ± 0.01	17.70 ± 0.36

Data shown are means (± standard deviation, n = 2); *HHV* higher heating value; HHV for digestates were measured with pooled samples.

with the addition of s750 BC obtained also a considerable reduction of TVFAs ($t_{(14)} = -1.46$, $P = 0.17$) by 18% compared to the control. Data for TVFAs on day 11 are not representative due to an error in measurement process (figure 3(a)). In contrast to TVFAs, the total alkalinity (TA) was significantly affected by biochar addition (*Kruskal–Wallis*, $P = 0.005$) (figure 3(b)). Treatments with s750 BC addition showed a greater TA than both oak wood biochars (comparing to w400 BC: *Dunn-Test*, $P = 0.009$ and comparing to w750 BC: *Dunn-Test*, $P = 0.05$) (figure 3(b)).

3.3. Heavy metal concentrations and energy potential of biochars and digestates

Comparison of the heavy metal concentrations in biochars with EBC-Agro limits (EBC 2023) indicates the potential use of these biochar in agricultural applications (table 4). All woody biochars meet the EBC-Agro limits. In contrast, both pre-treated grass biochars (g400 and g750) have higher chromium (Cr) concentrations than the limits, while both raw grass silage biochars (s400 and s750) exceed the nickel (Ni) concentration limits. This is strongly coupled to the high Cr and Ni concentrations in the biomass. Except for zinc (Zn), the IFBB pre-treatment concentrated the heavy metals from raw grass silage (s) in the resulting press cake (g).

Heating values indicate energy potential via incineration. After adding biochar in AD process, the digestates show increased heating values than without biochar, which is positively linked to the biochar heating values ($R^2 = 0.93$, $P < 0.001$; table 5). Woody biochars, as well as their corresponding biochar-enriched digestates demonstrated the highest heating values because of the high carbon content in the woody biochar (table 3). Remarkably, even for grass, an atypical fuel source, their biochars significantly increased the higher heating values of digestates. Both pre-treated grass-type biochar significantly increased the higher heating value (HHV) of digestates by up to 10% compared to control without biochar addition (g400 BC, $t_{(7)} = 5.42$, $P < 0.001$; g750 BC, $t_{(7)} = 3.21$, $P = 0.01$). In contrast, raw grass silage derived biochar enhanced the HHV of digestates only at 400 °C by 9% ($t_{(7)} = 5.01$, $P = 0.002$). Adding 750 °C raw grass silage biochar did not affect the HHV of digestate ($t_{(7)} = -0.85$, $P = 0.43$), probably due to its higher ash content. However, the IFBB pre-treatment process

reduced the ash content of raw grass silage and increased the heating value of the resulting biochar, thereby enhancing the energy potential of the digestates enriched with them.

4. Discussion

4.1. Role of biochar in acid-stressed AD processes

Biochar-assisted anaerobic digestion systems demonstrated generally a stable process and rapid methane production. As acid stress is the main challenge for treating food waste or organic fraction of municipal solid waste (OFMSW) usually resulting from substrate overloading (Luo *et al* 2015, VDI 2016, Dang *et al* 2017), this study used the inoculum to substrate ratio (ISR) 0.5 to simulate a substrate overloading, which led to the conditions of acid stress. At the beginning of AD processes, the volatile fatty acids (VFAs) were accumulated intensively due to the high loading of food waste containing high content of carbohydrates. Carbohydrates are easily anaerobically biodegraded and quickly converted to VFAs (Pavlostathis and Giraldo-Gomez 1991). However, methanogens could not convert VFAs as quickly as they were produced due to the lower growth rate of methanogens than acidogens (Hill and Holmberg 1988, Pavlostathis and Giraldo-Gomez 1991). Therefore, the specific methane production rates decreased rapidly in the early days indicating a strong inhibition. In contrast, the total alkalinity (TA) was consumed strongly at the beginning, presumably due to the rapid VFA production. Subsequently, the TA recovered slowly until the VFAs were nearly completely degraded. This study showed that TA concentration in the system with s750 BC was significantly higher than that of w400 BC or control during the AD processes. This could be a result of the high buffer capacity of s750 BC (table 3), which increased the TA concentration in the AD system, and consequently mitigated the VFA inhibition. The present study also revealed that under the acid stress, the most rapid recovery of specific methane production rate was obtained with the addition of s750 BC, and the slowest recovery with w400 BC compared to control, which are in consistent with their TA concentrations in AD systems. This is similar to a previous study, in which a sour digester was successfully treated with the addition of biochar and alkalis (Aramrueang *et al* 2022). Hence, the results suggest that the methane production processes can be inhibited by VFA accumulation, and the TA concentration in AD systems is a crucial parameter to mitigate this inhibition which could be facilitated by adding biochars.

4.1.1. The mechanism: alkaline properties of biochar

Consistent with previous studies, all of the used biochar exhibited an alkaline characteristic. For example, Luo *et al* (2015) conducted experiments using easily degradable glucose under high substrate loading rates (ISR 1/4, 1/6, 1/8), resulting in acid stress. In their study, the utilized biochar exhibited an alkaline pH of 8.63 and increased the maximum methane production rate. Similarly, Wang *et al* (2017) also increased methane production rate through the addition of biochar to AD systems under high loading rate (ISR 0.3), using chicken manure and kitchen waste. The biochar used in their study also exhibited an alkaline pH of 8.96 and a substantial ash content of 75%. Therefore, it implies that BC's alkaline properties are primarily responsible for reducing acid stress in AD processes. Indeed, the addition of biochar kept AD processes in the pH range between 6 and 8, which is favourable for methanogens (figure S5) (Williams and Crawford 1984, Garcia *et al* 2000, Megonigal *et al* 2004).

4.1.2. Effects of biochar feedstocks

The results demonstrated that biochar addition improved the first-order methane production rate constants in acid-stressed AD processes. The extent of this improvement varied depending on the type of biochar feedstock used. For example, s750 BC showed the most significant increase in kinetics compared to the control, mainly due to its high buffer capacity, highest ash content, and the highest pH value among the tested biochar types (table 3). These parameters correlated with the concentration of alkali (K) and alkaline earth (Ca, Mg) metals (AAEMs) in the biomass feedstocks.As suggested in previous studies (Shen *et al* 2015, Wang *et al* 2017), the results revealed that the AAEMs are closely linked to buffer capacity and ash content. However, the absence of a linear correlation between K and ash content can be attributed to the initially high K concentration in grass silage and its substantial reduction through the IFBB pre-treatment process (table 2). This suggests that the role of K in relation to ash content may not be as significant as its role in determining buffer capacity. The finding is consistent with prior research indicating that K, as an alkali metal, can increase the pH value (Shen *et al* 2015). Furthermore, the comparison revealed that g400 BC and w750 BC exhibited similar buffer capacities, despite a tenfold difference in their ash contents. This significant variation in ash content may be attributed to disparities in their respective feedstock sources. However, the comparable buffer capacities of g400 BC and w750 BC are influenced not only by their AAEM concentrations but also potentially by specific functional groups, such as amines, which can adsorb H^+ (Zhang *et al* 2018, Chiappero *et al* 2020).

4.1.3. Effect of pyrolysis temperature

Biochar from the same feedstocks, produced at 750 °C, showed higher buffer capacity, ash content, and pH than produced at 400 °C. This observation may explain the slightly higher kinetic rate constants associated with using 750 °C biochar compared to 400 °C biochar. Indeed, the results demonstrated that the pyrolysis temperature has a significant impact on the elemental ratios, while the feedstocks seem to play a minor role (table 3). Some researchers proposed that biochar promotes the AD process as an electron conductor by facilitating direct interspecies electron transfer (DIET) (Liu *et al* 2012, Chen *et al* 2014). The molar H/C ratio below 0.35 and molar O/C ratio below 0.09 are indicative of a more graphitic structure of biochar (Sun *et al* 2017). This suggests a decreased obstacle to the transfer of electrons and thus facilitates the DIET (Johnravindar *et al* 2021). Both the molar H/C and O/C ratio of the used biochars pyrolyzed at 750 °C meet the suggested ratios (table 3), which indicates that 750 °C biochars may improve the methane production rate via facilitating DIET due to their carbon matrices (Sun *et al* 2018). Alternatively, other factors may also facilitate the process of DIET. For example, some redox-active functional groups of biochar, *e.g.* phenolic and quinoid, were also suggested as electron shuttles, which could promote methanogenesis *via* facilitating the DIET (Kappler *et al* 2014, Klüpfel *et al* 2014, Saquing *et al* 2016), and consequently, improving the methane production rate. However, further analysis, such as Fourier-transform infrared spectroscopy (FTIR), is necessary to identify these functional groups.

4.2. Biochar for environmental management

4.2.1. Improving the efficacy of treating organic waste

The results showed that the kinetic rate constant of methane production increased with biochar addition up to 30% compared to the control. The pseudo-first-order kinetic rate constant is an important parameter to assess the speed of a substrate conversion to methane. With s750 BC, the kinetic rate constant was increased to 0.29 d^{-1} (figure 2(a) and table S16) under acid stress (ISR 0.5). This result is similar to two previous standard batch experiments under ISR 2 with food waste or dog food as substrate, in which the kinetic rate constant was 0.30 d^{-1} (Koch *et al* 2015) and 0.35 d^{-1} (Koch *et al* 2017) respectively. However, the kinetic rate constant is usually fitted using data from batch tests conducted under standard fermentation conditions (ISR 2). This condition avoids inhibitions caused by substrate overload, thereby ensuring an accurate estimation of the maximum methane production rate. The outcomes indicate that biochar could enhance the throughput of a digester by maintaining the kinetic rate constant under non or less-inhibited condition, thus increasing the efficacy of treating organic waste. However, the experiments were conducted using short-term batch trials and do not include the potential effects of a long-term microbiological adaption between biochar and inoculum, which may include biofilm formation on biochar surface or selective colonization of functional microbes (Luo *et al* 2015). This could affect the speed of conversion of substrates to methane as well as the degradation extent of substrates.

4.2.2. Making use of grass-type biomass

The study demonstrated that grass-type biochar has a more significant effect on the elevation of methane production rates than woody biochar. This points out a potential way to valorise grass-type biomasses. For example, in this study, grass biomass from roadside verges was employed as feedstock for biochar production. This type of grass is usually not used, but cut and left in place to decay, i.e. mulching (Piepenschneider *et al* 2016). However, there is a growing concern that the mulching process has negative environmental effects due to NH_3 and N_2O emissions (Larsson *et al* 1998). On the contrary, roadside verges are potential habitats for various species to protect biodiversity (Kaur *et al* 2019). Although, considering the complexity of roadside verges management, using this biomass for biochar production offers an attractive alternative for future biomass utilization and potential profitability in biogas plants, such as AD process enhancement and increased energy potential of digestate. Furthermore, the pre-treatment of grass silage increased the carbon content in biochar while reducing the ash content. This makes pre-treated grass silage biochar a compromise between raw grass silage biochar and woody biochar, thus, offering both good buffer capacity and high carbon sequestration potential. However, the pre-treatment did not assist in reducing the high heavy metal concentrations in roadside verges. The roadside verges derived biochars had higher heavy metal concentrations compared to EBC limits for agricultural applications. Therefore, it is not recommended to use this digestate in the field for soil improvement or as fertilizer. Nonetheless, extensive grasslands in nature conservation areas are expected to have lower heavy metal concentrations (Heinrich *et al* 2023b, Heinrich *et al* 2023a), suggesting their potential as feedstocks for grass-type biochars. Future research should explore grass types with lower heavy metal concentrations and investigate new pre-treatment methods to meet these limits.

4.2.3. Potential implications and future perspectives

The results suggest that biochar could alleviate an acid shock due to its alkaline properties, thus, when the goal is to mitigate acid stress, the grass-type biochar has a significant advantage. Additionally, the carbon fractions in biochars used in this study showed a higher stability due to their lower molar O/C ratios, suggesting a longevity for CO_2 sequestration (Spokas 2010, Ladygina and Rineau 2013). In particular, woody biochar had a significantly higher carbon sequestration potential due to their high carbon content. When the goal is carbon sequestration, woody biochar seems to be more attractive. Furthermore, this application of woody biomass prioritized material use over energy use (Olsson *et al* 2016, Fehrenbach *et al* 2017), which is a value-added process and follows the biomass cascading use principle. A conceptual large-scale discontinuous CSTR as an indicative example was simulated, which received biochar and food waste using the parameters from batch tests obtained in this study (Supplementary). The indicative energy balance showed that the additional biomass preparation and pyrolysis processes are energy self-sufficient due to the surplus energy from pyrolysis gas and energy-rich press liquid from IFBB pre-treatment of grass. Thus, the CO_2 emissions from fossil sources are also reduced due to the renewable energy. Consequently, the amount of biochar added to the digester approximately equals its carbon sequestration potential, preventing the release of carbon back into the atmosphere.

Generally, woody biochar, due to their high carbon content, tends to store more carbon, while grass-type biochar had higher ash content indicating more alkaline properties that can mitigate acid stress in AD processes. IFBB-processing of biomasses might be a good compromise solution, providing both effective acid mitigation in AD and carbon sequestration. However, the roadside verges derived biochar exceeds the EBC-Agro limits, making it unsuitable for field use. Nevertheless, the heating values of all biochar-enriched digestates, except s750 BC, increased significantly compared to the digestates without biochar, indicating improved energy potential via incineration. Grass-type biochars can be employed in AD processes that handle sewage sludge or other substrates, whose digestates are unsuitable for use on arable lands and require eventual incineration. On the other hand, woody biochar is suitable for agricultural biogas plants, where the digestate is typically used as fertilizer or soil improvement. Overall, the use of biochar-enriched digestates from AD points to the potential of closing the material loop. A comprehensive study of these proposed applications is needed.

5. Conclusion

This study reveals the importance of the alkaline buffering mechanism for mitigating acid stress and elevating methane production rate. The effectiveness of acid mitigation level was significantly higher with grass-type biochar compared to woody biochar, highlighting a potential approach for valorising grass-type biomass. However, woody biochars can sequestrate more CO_2 than grass-type biochars due to their high carbon content. Hence, IFBB-processing of grass-type biomasses might be a good compromise solution, providing both effective acid mitigation and carbon sequestration. The present study contributes to the understanding of the broad application potential of biochar and their biomass feedstocks, by demonstrating the environmental implications of using biochar during and after AD. In light of the increasing demand for sustainable CO_2 sequestration and the growing attention to biomass cascading use for enhancing resource efficiency, it is recommend adding biochar in AD processes to treat organic waste. This can lead to the simultaneous mitigation of acid stress and CO_2 sequestration, thereby contributing to a combined biomass and carbon management system. However, a comprehensive life cycle assessment is needed in future work.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

JH: study conception, experiment design, material preparation, data collection and analysis, writing-original draft; MW: funding, supervising, revising, editing; WG: mentoring, revising, editing; BJ: validation-carbon and energy balance; KS: funding, revising, editing; KK: funding, project administration, supervising, revising, editing. All authors read and approved the final manuscript.

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