

Biochar facilitates biofuel production through batch single-stage and two-stage digestion of grass silage and cattle slurry

Xue Ning^{a,b}, Chen Deng^{c,a,b,*}, Xihui Kang^{a,b}, Richard O'Shea^{a,b}, David M. Wall^{a,b},
Richen Lin^{a,b,d}, Jerry D. Murphy^{a,b}

^a SFI MaREI Centre for Energy, Climate and Marine, Environmental Research Institute, University College Cork, Cork, Ireland

^b Civil, Structural and Environmental Engineering, School of Engineering and Architecture, University College Cork, Cork, Ireland

^c School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 210096, China

^d Key Laboratory of Energy Thermal Conversion and Control of Ministry of Education, School of Energy and Environment, Southeast University, Nanjing, 210096, China

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ABSTRACT

Grass silage and cattle slurry are the most abundant feedstocks for biogas in an Irish context. This work describes how to optimize their biomethane yield. Hydrolysis and biomethane yields from co-digestion of grass silage and slurry were enhanced in a biochar-supplemented two-stage digestion process. Biochar addition at the optimal dosage of 10 g/L in batch two-stage digesters led to the highest methane yield of 253 L per kilogram (kg) volatile solid (VS), which was 24% higher than that from two-stage digesters without biochar supplementation. For a batch single-stage digester, the biomethane yields were lower than the two-stage but 10 g/L biochar addition led to an increase to 218 L/kg VS as compared to 198 L/kg VS for the group without biochar supplementation. Volatile fatty acid accumulation increased with the enhancement of biomethane production in the biochar-supplemented two-stage reactors. The addition of biochar in single-stage and two-stage anaerobic digesters presented positive effects on the electrochemical properties of the digester contents, reflected by the increased charge storage capacity, reduced solution resistance and improved digester content conductivity. The methane yields in two-stage anaerobic digestion were shown to be strongly correlated ($R^2 = 0.92$) with the concentration of extracellular polymeric substances. This evidenced that the interlaced conductive network established by extracellular polymeric substances and biochar was essential for direct interspecies electron transfer and biomethane production. This study demonstrated that biochar-supplemented two-stage anaerobic digestion is an optimal route for energy recovery from grass silage and cattle slurry.

1. Introduction

The European Union (EU) has committed to achieve carbon neutrality by 2050 to tackle the challenges of global warming (EU, 2020). The Renewable Energy Directive of the European Commission (EC) sets a target of more than 32% of total energy consumption (electricity, heat and transport) within the EU to be renewable by 2030 (EC, 2021). Significant resources have been applied to develop sustainable fuel alternatives, such as ethanol, hydrogen (H₂) and biomethane (CH₄) (Liu et al., 2021). The literature would suggest that anaerobic digestion (AD) has the potential to provide biomethane as a renewable energy source that can replace significant quantities of natural gas (Bhatnagar et al., 2022), and in doing so alleviate greenhouse gas emissions.

In two-stage anaerobic digestion, the biomass substrates are first

hydrolysed by fermentative bacteria to produce volatile fatty acids (VFAs) and biohydrogen (Ding et al., 2016), and subsequently, the VFAs are fed to a second digester with methanogenic archaea for conversion to carbon dioxide (CO₂) and CH₄. Typically, the two stages are operated at different temperatures and pH, with the optimal growth conditions at 55 °C and pH 6.0 for fermentative bacteria in hydrolysis, acidogenesis and acetogenesis processes in the first stage, and the optimal growth conditions of 37 °C and pH 7.0 for methanogenic archaea in the second stage (Li et al., 2020). The separated decomposition of complex substrates results in a higher production of VFAs which can be further converted to biomethane as an energy vector. However, simplicity and cost-effectiveness need to be considered for applications of two-stage systems.

Grass silage is the most abundant feedstock for biogas production

* Corresponding author. School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 210096, China.

E-mail address: chen.deng@ucc.ie (C. Deng).

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through anaerobic digestion in Ireland (Deng et al., 2019). The biomethane yield for grass silage in mono-digestion varied from 207 to 428 L/kg volatile solids (VS) as reported by Ning et al. (2021). The maximum biomethane potential in single-stage digestion of grass silage was 400 L/kg VS (Wall et al., 2013), which equated to a biodegradability index of 90%. The high biodegradability could be attributed to the lower lignin content in perennial ryegrass and the full degradation of the cellulosic biomass. It must be stated that grass and grass silage are not homogeneous and biomethane values depend on grass species, climatic growing conditions and time of cut but it may be stated that higher biomethane yields have been reported in two-stage digester systems as compared to single-stage reactors (Deng et al., 2019). Although the digestion of cattle slurry typically has lower biomethane yields as compared to energy crops such as grass silage (Wall et al., 2013), the abundance of micro-nutrients such as cobalt, iron and nickel contained in the slurry is beneficial for co-digestion with grass silage. A mix of cattle slurry and grass silage at a ratio of 4:1 on a VS basis was highlighted as the optimal choice when considering process stability and economic feasibility (Wall et al., 2014).

The biomethane yields from co-digestion of cattle slurry and grass silage can be further facilitated by supplementation with conductive materials (Liu et al., 2020; Wang et al., 2021). Biochar is a stable carbon-rich substance that can be recovered from biowastes through the process of pyrolysis. Biochar is electrically conductive due to its high carbon content (70%); it is alkaline due to the enriched carbonate groups on the surface, and has a porous structure with a large surface area formed through pyrolysis (Masebinu et al., 2019). Biochar has shown to be an environmentally sustainable cost-effective conductive material that when added to an anaerobic digester shortens the lag-phase time, stabilizes the microbial communities, boosts VFA production, and increases biomethane yield and production rate (Saif et al., 2022). For example, the peak methane production rate was accelerated by 29% and the biomethane yield increased by 17% with 1.25 g/L of biochar in the digestion of seaweed *Laminaria digitata* (Deng et al., 2020).

The main mechanisms by which biochar enhanced the process of bioenergy recovery via anaerobic digestion were found to be: an increase in buffering capacity to alleviate VFAs accumulation; immobilization of functional microorganisms; and support of direct interspecies electron transfer by passing electrons through conductive structures and the functional redox quinones groups on the biochar surface (Deng et al., 2021; Saif et al., 2022; Yuan et al., 2023). For the latter, biochar acts as a bridge between syntrophic bacteria and methanogenic archaea. Direct interspecies electron transfer can only happen with the presence of conductive pili and cytochromes (Masebinu et al., 2019), whereas biochar supplementation offers an enhanced route to pass electrons between species, hence increasing the electron transfer capability in methanogenesis. Therefore, the authors highlight the potential for biochar supplementation to enhance biomethane production through the facilitation of direct interspecies electron transfer.

To date, the effect of conductive materials (such as biochar) on the two-stage co-digestion of cattle slurry and grass silage has not been reported. Few studies focus on the effects of biochar on the performance of the first-stage hydrogen reactors in a process of two-stage anaerobic digestion. The innovation of this study is the comparative investigation of the degree of bioenergy recovery through different anaerobic digestion systems (including single-stage and two-stage processes) with varying doses of biochar and the exploration of the contributing factors in the stimulation of direct interspecies electron transfer in the process of methanogenesis. The main objectives of this research are as follows:

- (1) compare the effects of varying doses of biochar in single-stage and two-stage anaerobic digestion in terms of biogas production, VFA profiles and energy yields;

- (2) assess the electrochemical properties of the biochar-supplemented digester contents in single-stage and two-stage anaerobic digesters;
- (3) evaluate the secretion of extracellular polymeric substances (EPSs) by microorganisms in single-stage and two-stage anaerobic digesters.

2. Material and methods

2.1. Characteristics of inoculum, biomass feedstocks and woody biochar

The inoculum was obtained from a lab-scale continuously stirred tank reactor that processed various types of biomass, including food waste, cattle slurry, grass silage and seaweed at mesophilic temperatures (37 °C). The characteristics of the inoculum were detailed in the Supporting Information Text 1. The grass silage (C₂₄ H₃₆ O₂₃ N) was a perennial ryegrass with a TS content of 28.7% and a VS content of 25.9%. The elemental composition consisted of C (41.3% VS), H (5.1% VS), O (51.6% VS), and N (2.0% VS) yielding a C:N ratio of 20.8. The grass silage was macerated to achieve a particle size of 1 cm and then fed into the anaerobic digesters. The cattle slurry (C₃₁ H₄₅ O₄₃ N) was sourced from a local dairy farm in Cork, Ireland, with average TS and VS contents of 8.3% and 6.5%, respectively. The elemental composition determined by ultimate analysis consisted of C 33.3%, H 4.0%, O 61.4%, and N 1.3% on a VS basis. The grass silage and cattle slurry were kept at −18 °C in a freezer before use.

Biochar was provided by Premier Green Energy. It was originally sourced from an Irish pyrolysis plant pyrolyzing waste wood in a rotary kiln at 700 °C, with a carbon content of 87.8% on a TS basis. Other characteristics of this woody biochar, including pH, electrical conductivity, specific surface area, and crystal structure were detailed in a previous study by Deng et al. (2020). The woody biochar was crushed and sieved to a particle size of less than 150 µm, and then dried in an oven overnight at 105 °C prior to the experiment.

2.2. Batch anaerobic digestion experiments with varying doses of biochar

Grass silage and cattle slurry were co-digested at a ratio of 4:1 on a VS basis in the batch trials as outlined in Fig. 1. The mix ratio (4:1) was picked based on a previous study which highlighted the highest biomethane production at a high organic loading rate of 4.0 g VS/L/d for co-digestion of grass silage and cattle slurry (Wall et al., 2014). The Bioprocess Control Automatic Methane Potential Test System II (AMPTS II) was used to determine the biohydrogen and biomethane potential.

For the single-stage digestion, 4 g VS (235 mL) of biomethane inoculum and 2 g VS of biomass (1.6 g grass silage and 0.4 g cattle slurry on a VS basis) were added to each reactor. Subsequently, varying doses of biochar were added to different groups: group 1 with 5 g/L biochar (S-B5), group 2 with 10 g/L biochar (S-B10), and group 3 with 20 g/L biochar (S-B20). Group 4 was a control group which digested grass silage and cattle slurry with no biochar supplement (S-B0). Each reactor was subsequently made up to a total working volume of 435 mL with deionised water, and adjusted to a pH value of 7.0 ± 0.05 with sodium hydroxide (NaOH, 1 M) solutions and hydrochloric acid (HCl, 1 M). Each group was run in triplicate and operated for 30 days (at which stage all gas production had ceased) under mesophilic conditions (37 °C).

For two-stage digestion, 2 g VS of biomass (1.6 g grass silage and 0.4 g cattle slurry on a VS basis) was fed to each reactor, along with 20 mL of acclimatized biohydrogen inoculum. The biochar was subsequently added to each bottle (in triplicate): group 5 with 5 g/L biochar (T-B5), group 6 with 10 g/L biochar (T-B10), and group 7 with 20 g/L biochar (T-B20). Group 8 was defined as the control group which digested grass silage and cattle slurry without biochar addition (T-B0). Deionised water was added to each reactor to make up the working volume of 200 mL, whereafter the pH was adjusted to 6.0 ± 0.05 using NaOH (1 M) and HCl (1 M). The first stage ran for five days (at which stage all gas production

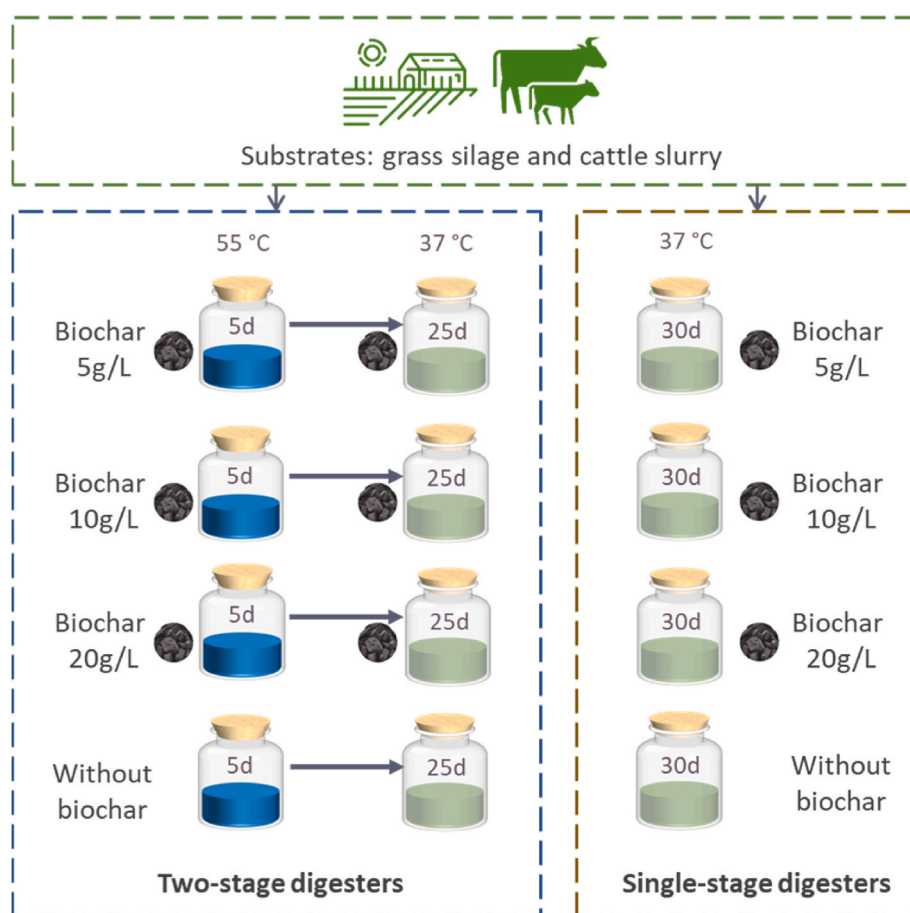


Fig. 1. Schematic of the batch two-stage and single-stage anaerobic digestion systems with varying doses of biochar (5, 10, 20 g/L) or without biochar. Blue represents the first-stage biohydrogen reactors, and green represents the second-stage and single-stage biomethane reactors. Single-stage inoculum control (without biochar) and single-stage cellulose standard (without biochar) are not shown in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

had ceased) under the thermophilic condition (55 °C), and the pH was then adjusted to neutrality (7.0 ± 0.05) for the second-stage biomethane production. As in the single-stage trial, 4 g VS (equivalent to 235 mL, at a substrate-to-inoculum ratio of 1:2 on a VS basis) biomethane inoculum was added at the beginning of the second stage of methanogenesis to make up the total working volume to 435 mL. For group 6, 7, and 8, additional biochar was added to each bottle to maintain the biochar concentration of 5, 10, and 20 g/L, respectively. The second stage of digestion ran for 25 days under the mesophilic condition (37 °C). The overall duration for two-stage digestion was 30 days, the same as the operation period of the single-stage reactors.

The individual reactors were sealed using rubber stoppers and flushed for over 2 min with nitrogen gas to remove oxygen in the headspace. Each digester was stirred and kept in a water bath at the specified temperature throughout the trials. The volume of biohydrogen or biomethane was measured and normalized to standard temperature and pressure (0 °C, 1 atm) by the AMPTS II apparatus. Triplicate measurements were also made for single-stage inoculum control (without biochar) and single-stage cellulose standard (without biochar) groups. The inoculum control was used to correct the volumes of H₂ and CH₄ production from grass silage and cattle slurry.

2.3. Analytical methods

Cyclic voltammetry and electrochemical impedance spectroscopy were adopted to characterize the electrochemical performance of the digester content based on a method established previously (Wu et al.,

2020). Briefly, the cyclic voltammetry curve was scanned at a rate of 5 mV/s from −1.0 V to +1.0 V vs. Ag/AgCl using a Biologic VSP three-electrode electrochemical workstation. Electrochemical impedance spectroscopy was assessed at a frequency range of 1–10 kHz. The electric equivalent circuit model, including solution resistance R_1 (Ω), charge transfer resistance R_2 (Ω), and double layer capacitance C_2 (F), was fit using the ZFit module in Bio-Logic software (EC-Lab). The electrical conductivity of the digester content was measured in a linear sweep voltammetry test (at a rate of 25 mV/s from +0.4 V to −0.4 V vs. Ag/AgCl) using a two-gold electrode test platform (Zhao et al., 2016).

Extracellular polymeric substances are the mixture of organic compounds secreted by microorganisms into their surrounding environment, and mainly include polysaccharides and proteins. Extracellular polymeric substances were extracted by a modified heat extraction method (Wu et al., 2022), and then a Thermo Scientific Pierce Bicinchoninic Acid protein assay was performed to measure protein concentrations; the concentration of polysaccharides was determined by the anthrone-sulfuric acid method (Somani et al., 1987). Other analytical methods are detailed in the Supporting Information Text 2.

2.4. Gas production kinetics

Understanding biomethane production kinetics is essential in assessing digester performance. However, the selection of the model is highly dependent on the specific biomass since there is no standardized model for all batch anaerobic digestion processes. Karki et al. (2022) evaluated nine different kinetic models in co-digestion of different

organic wastes, and concluded that advanced models derived from the modified Gompertz model were quite similar in fitting with the biomethane production data. A study by Lin et al. (2018) describing the addition of graphene to a glycine digester, suggested that the modified Gompertz was a better fit to the experimental data when compared to the first-order kinetic model and the Cone model. Therefore, the modified Gompertz model was employed in this study to evaluate the process of biomethane production and simulate the maximum biomethane yield, maximum biomethane production rate and lag-phase time as per Equation (1). The predicted peak time of the process of anaerobic digestion is calculated by Equation (2):

$$P = P_m \exp \left\{ - \exp \left[\frac{R_m \times e}{P_m} (\lambda - t) + 1 \right] \right\} \quad (\text{Equation 1})$$

$$T = \frac{P_m}{R_m \times e} + \lambda \quad (\text{Equation 2})$$

where P is the CH_4 yield (L/kg VS), R_m is the predicted maximum CH_4 production rate (L/kg VS/d), P_m is the predicted maximum CH_4 yield (L/kg VS), e is Euler's number which is 2.71828, λ is the lag-phase time (d), t is the digestion duration (d), and T is the predicted peak time of the digestion process (d).

2.5. Calculations of biodegradability index and energy yields

Biodegradability index is a measure of the efficiency of feedstock conversion to biogas in the digestion process. The biodegradability index is defined as the ratio of the specific CH_4 yield obtained from the batch experiments to the theoretical CH_4 potential as per Equation (3) (Ding et al., 2018). The theoretical CH_4 potential was calculated by the Buswell equation as detailed by Deng et al. (2019). The energy yields in the produced hydrogen and methane were evaluated using the lower heating value (LHV) as per Equations (4) and (5). The energy values in biomass feedstocks were evaluated by converting higher heating value (HHV) to LHV as per Equations (6) and (7). Specifically, the HHV in biomass feedstocks was estimated by the modified Dulong Formula based on the elemental composition of the feedstock (C, H, O, N) on a VS basis (Nizami et al., 2009). The energy consumption for operating the process was mainly considered as thermal energy consumption as per Equation (8) and electrical energy consumption as 10 kWh/t feedstock (Murphy et al., 2004), which equals 222.2 kJ/kg VS in this case. Energy conversion efficiency is defined as the ratio of energy yields in the produced hydrogen and methane to the total energy inputs (including the energy contained in the biomass feedstocks, and electrical and thermal energy consumption).

$$\text{Biodegradability index (\%)} = \frac{\text{Specific } \text{CH}_4 \text{ yield (L/kg VS)}}{\text{Theoretical } \text{CH}_4 \text{ potential (L/kg VS)}} \times 100 \quad (\text{Equation 3})$$

$$\text{H}_2 \text{ energy yield} = \text{SHY} \times \text{LHV}_{\text{H}_2, \text{v}} \quad (\text{Equation 4})$$

$$\text{CH}_4 \text{ energy yield} = \text{SMY} \times \text{LHV}_{\text{CH}_4, \text{v}} \quad (\text{Equation 5})$$

$$\text{HHV} = 337 \times \text{C} + 1419 \times \left(\text{H} - \frac{\text{O}}{8} \right) + 93 \times \text{S} + 23.26 \times \text{N} \quad (\text{Equation 6})$$

$$\text{LHV} = \text{HHV} - 212 \times \text{H} - 8 \times \text{O} \quad (\text{Equation 7})$$

$$\text{Thermal energy consumption} = C_{\text{pw}} \times m \times \Delta T \quad (\text{Equation 8})$$

where SHY is the specific hydrogen yield (L/kg VS), SMY is the specific methane yield (L/kg VS), $\text{LHV}_{\text{H}_2, \text{v}}$ is 10.7 kJ/L, $\text{LHV}_{\text{CH}_4, \text{v}}$ is 35.5 kJ/L, HHV is the higher heating value (kJ/kg VS) of the biomass feedstocks, LHV is the lower heating value (kJ/kg VS) of the biomass feedstocks, C_{pw} is the specific heat of liquid water 4.18 kJ/(kg $\cdot$ °C); m is the mass of the

liquid in each reactor per kilogram VS (kg/kg VS); ΔT is the temperature difference (°C) between the temperature in digesters (55 °C in the first stage and 37 °C in the second stage) and ambient temperature (25 °C).

2.6. Statistical significance analysis

The statistical significance analysis was conducted by the one-way analysis of variance (ANOVA) with a significance level (α) of 0.05. Results with a p-value less than 0.05 were deemed to be statistically significant.

3. Results and discussion

3.1. Effects of biochar on biogas production

3.1.1. Biohydrogen yield from the first stage

In the first stage of the two-stage digestion process, recalcitrant lignocellulosic components in grass silage and cattle slurry were broken down into simple sugars through hydrolysis, and these reducing sugars were then converted to metabolic products such as VFAs and biohydrogen through acidogenesis. Varying doses of biochar were added to the first-stage digesters as defined by groups T-B0, T-B5, T-B10 and T-B20. No biomethane was detected during the first stage, indicating the favourable acclimatized environment for anaerobic fermentative bacteria.

The specific hydrogen yields and hydrogen production rates in the first stage are shown in Fig. 2 (a) and (b). In the first-stage digestion of group T-B0 without biochar supplement, the cumulative hydrogen yield was 19.8 L/kg VS. The hydrogen yield was similar to the yield of 17.5 L/kg VS in a previous study on two-stage digestion of perennial ryegrass silage (Deng et al., 2019). The first-stage digestion processes with 5 g/L (T-B5) biochar addition achieved a hydrogen yield of 21.1 L/kg VS, which is not significantly different from the control group T-B0 ($p > 0.05$). The hydrogen yield of group T-B10 with 10 g/L biochar addition was 29.6 L/kg VS, presenting a significant ($p < 0.05$) rise of 49% as compared to the control group without biochar addition. Similar results were obtained by Sunyoto et al. (2016) where a hydrogen yield in a two-stage digester increased by 31% with 8.3 g/L biochar supplementation. The enhancement in hydrogen production due to biochar supplementation could possibly be attributed to the pH buffer capacity of biochar and the enhanced biofilm formation on the porous structure of biochar (Masebinu et al., 2019). The overdose of biochar (20 g/L in the T-B20 group) led to a reduction in biohydrogen production to 22.6 L/kg VS as compared to the biochar concentration at 10 g/L (T-B10). It is likely that the overdosage of biochar (20 g/L in the T-B20 group) blocked the contact between microbes and substrates (Le et al., 2020), and negatively affected the methanogenesis process through damaging the structure and function of the cell membrane (Dong et al., 2019).

A maximum hydrogen production rate of 11.1 L/kg VS/d was achieved on day 1 in the control group (T-B0), indicating the rapid solubilisation of the biodegradable components such as cellulose and hemicellulose in silage and slurry during the first stage. A significant difference ($p < 0.05$) in hydrogen production rate was evident when comparing Group T-B10 with T-B0: T-B10 displayed a peak of 15.2 L/kg VS/d with 10 g/L biochar addition on day 1, 37% ($p < 0.05$) higher than the peak in the control group. The promotion of hydrogen production rates corresponded to the increasing cumulative hydrogen yields due to biochar supplementation. However, no statistically significant difference ($p > 0.05$) in hydrogen production rates was observed in groups T-B5 and T-B20 when compared to group T-B0.

3.1.2. Biomethane yield in the second and single stage

Biomethane was produced in the second-stage reactor of the two-stage system as demonstrated in Fig. 2 (c) and (d). The standard cellulose digestion achieved 328 L/kg VS which is 79% of the theoretical potential. The theoretical methane potential of the grass silage and

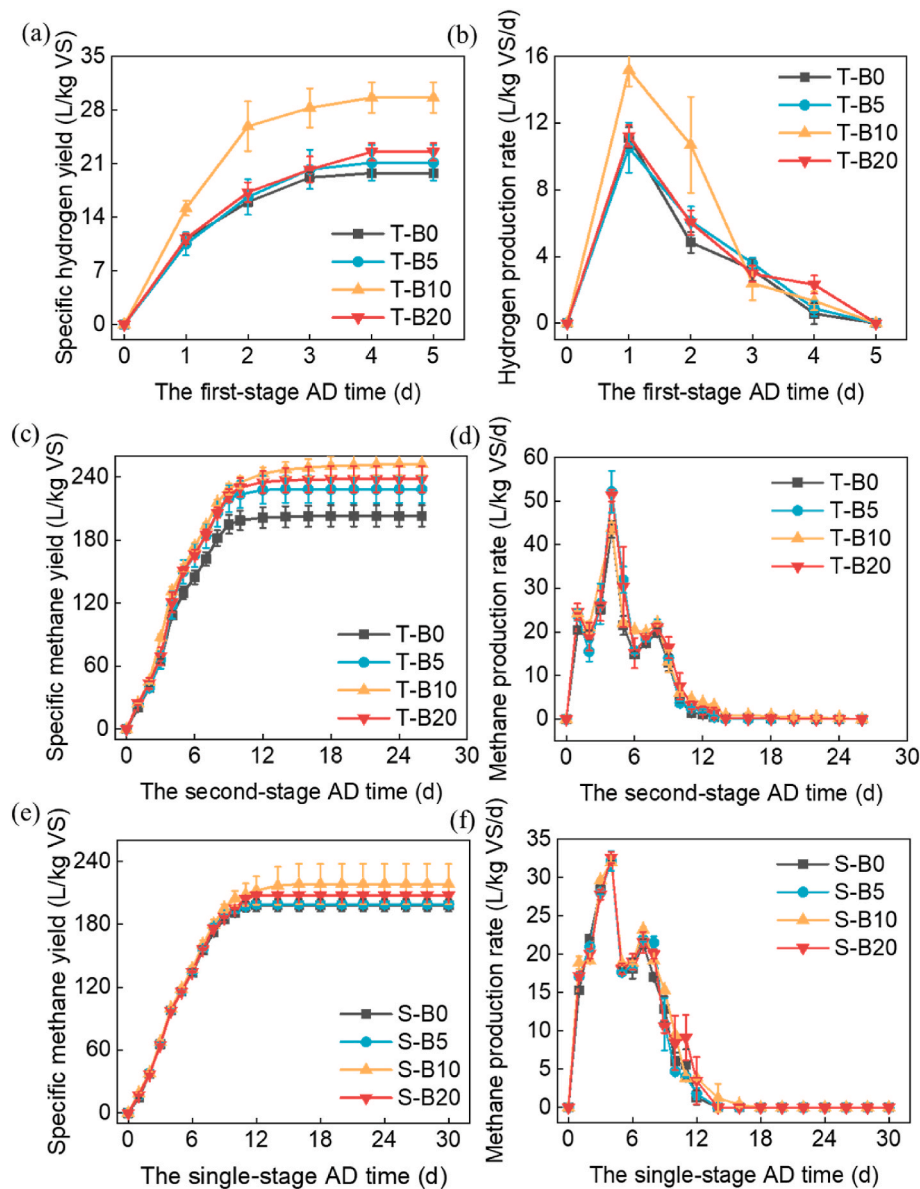


Fig. 2. Biochar-supplemented biogas production through two-stage and single-stage anaerobic digestion (AD). (a) the specific hydrogen yield and (b) hydrogen production rate in the first-stage reactors with biochar dosage of 0, 5, 10, 20 g/L; (c) the specific methane yield and (d) methane production rate in the second-stage reactors of the two-stage anaerobic digestion; (e) the specific methane yield and (f) methane production rate in the single-stage reactors with varying doses of biochar. Data are presented as means $\pm$ standard deviation (SD).

cattle slurry mixture was calculated as 309 L/kg VS by the Buswell equation. The low theoretical methane potential (309 L/kg VS) obtained through the Buswell Equation applied to the co-digested feedstocks, may be ascribed to the abundant lignocellulosic compounds contained in the grass silage as compared to the grass silage used in previous studies of Irish grass silage by Wall et al. (2013). In the control group (T-B0), 203 L/kg VS of biomethane was produced through the second-stage co-digestion of cattle slurry and grass silage. With 10 g/L biochar supplementation (T-B10), the methane yield increased by 24% ($p < 0.05$) at 253 L/kg VS as compared to the control group (T-B0). In two-stage reactors, surplus biochar supplementation at a concentration of 20 g/L (T-B20) did not increase the biomethane yields beyond those with supplementation of 10 g/L, but still had a positive effect on biomethane production, as the methane yield increased by 17% ($p < 0.05$) as compared to the control group (T-B0).

In the absence of biochar, the biomethane production rate in the second-stage digester (T-B0) initially peaked at 20.5 L/kg VS/d after 24

h, and then reached its highest peak at 43.6 L/kg VS/d on day 4. The third peak of 19.9 L/kg VS/d was achieved on day 8. The first two peaks within 4 days can potentially be ascribed to the degradation of available VFAs generated in the first stage, and the third peak resulted from the further breakdown of solid remnants in silage and slurry (Xing et al., 2021). With biochar addition at different dosages in the second-stage digesters, the three peaks of biomethane production rates also occurred on day 1, day 4, and day 8, but the peak values increased. The maximum increase of 19%, 12% and 11% on day 1, day 4 and day 8, respectively, was obtained at a biochar concentration of 10 g/L (T-B10) as compared to the control group (T-B0). It is concluded that biochar accelerated the process of methanogenesis, most likely due to its function as a biofilm supporter (Sunyoto et al., 2016) and the facilitation of interspecies electron transfer between the syntrophic bacteria and archaea (Deng et al., 2021).

In single-stage digesters, as shown in Fig. 2 (e) and (f), however, no significant difference ($p > 0.05$) in the biomethane yield was observed

between each group of S-B0, S-B5, S-B10, and S-B20. The biochar addition of 10 g/L (S-B10) led to the highest CH₄ yield of 218 L/kg VS, which is slightly higher but without significant difference ($p > 0.05$) than that of 198 L/kg VS encountered in the control group (S-B0). In the absence of biochar (S-B0), the biomethane production rate achieved the first peak value of 32.1 L/kg VS/d on day 4, corresponding to the rapid utilization of accessible organic compounds in the feedstock by methanogens. A second peak value of 21.4 L/kg VS/d was obtained on day 7, which could be attributed to the degradation of rigid lignocellulosic components in the silage (Kang et al., 2022). Similar trends were observed in the single digesters with biochar supplementation. The first peak of the biomethane production rate on the fourth day showed no significant difference ($p > 0.05$) when adding biochar. The second peak values were 21.9 L/kg VS/d, 23.2 L/kg VS/d, 21.6 L/kg VS/d with 5 g/L, 10 g/L and 20 g/L biochar supplement. The biochar addition in the single-stage digesters in groups S-B05, S-B10 and S-B20 showed no significant increase in the methane production rates as compared to the single-stage anaerobic digestion without biochar supplementation.

The second-stage digesters performed better than the single-stage digesters with respect to the specific methane yields and methane production rates with the same dosage of biochar addition. For example, the maximum biomethane yield in the second stage was significantly ($p < 0.05$) higher (up to 16%) than the single-stage digester at the optimal biochar dosage of 10 g/L. This could be attributed to the enhanced decomposition of recalcitrant compounds in a separated hydrolysis process. Interestingly, the reactor type had an effect on biochar facilitation of the anaerobic digestion process: significant increases in biomethane yields and biomethane production rates with biochar supplementation were only observed in two-stage reactors (T-B10 and T-B20 group) as compared to the two-stage control group (T-B0), whereas the increases detected when adding biochar in the single-stage digesters as compared to the single-stage control group (S-B0) were insignificant. The separation of fermentative bacteria and methanogenic archaea into optimal growth conditions in two-stage digesters may have a mutually beneficial effect leading to the promotion of biomethane as a whole and enhanced biomethane production with the addition of biochar. This may be explained as follows. Hydrolysis is the rate-limiting step in the digestion process of lignocellulosic components such as grass silage (Deng et al., 2019). A previous study observed that biochar could significantly accelerate the acidogenesis, acetogenesis, and methanogenesis in the digestion of lignin monomer but it was less effective in facilitating the digestion of whole lignocellulosic biomass (Deng et al., 2023). In two-stage digesters, the hydrolysis process has been significantly improved in the first stage as exemplified by the increase in hydrogen production, facilitating more degradable components to be fed to the second stage. Therefore, the effect of biochar on biomethane yield in the second-stage digester is enhanced through increased production of readily degradable components in the first-stage digester. Quantitative analysis on increased biomethane production (as compared to the control group without biochar supplement) per gram biochar

when adding different dosages of biochar is further discussed in the Supporting Information Text 3.

The kinetic parameters obtained from the modified Gompertz model, including the predicted maximum biomethane yield, production rate, lag-phase time, predicted peak time and T_{80} , are exhibited in Table 1. The modelling outcomes closely matched the experimental results with an adjusted R^2 (a measure of model accuracy) of over 0.996. The predicted lag-phase time was the shortest at 0.7 d when adding 10 g/L biochar into the second-stage digester (T-B10) as compared to other second-stage digesters including the control group, which further confirmed the optimal biochar concentration for improving biomethane production. No differences in the lag-phase time ($\lambda = 0.7$) were noticed among the single-stage digesters with varying doses of biochar addition. Interestingly, T_{80} , which is the predicted time required to produce 80% of the total CH₄ yield, would be longer with a higher dosage of biochar supplementation both in two-stage and single-stage anaerobic digestion. The prolongation of methane production time induced by biochar were also observed in similar studies (Deng et al., 2020, 2021).

3.2. Effects of biochar on volatile fatty acids profiles

For two-stage digesters, the complex biomass feedstocks were decomposed into simpler molecules such as VFAs through the processes of hydrolysis and acidogenesis in the first-stage hydrogen digesters. Such soluble VFAs provided the main substrates for methanogenic archaea in the second stage. The profiles of VFAs generated in the T-B10 group with the optimal biochar dosage of 10 g/L in the first stage were compared with the control group (T-B0) as shown in Fig. 3 (a). The total VFAs produced in groups T-B0 and T-B10 were measured in an increasing trend during the five-day fermentation in the first stage, and reached 3050 and 3752 mg/L on the fifth day, respectively. Compared to the control group, VFAs generated in 10 g/L biochar-supplemented first-stage reactor were 23% higher at the end of the five days and in accordance with the enhanced biohydrogen production. Acetic acid was the main VFA produced in the first stage, which accounted for approximately 73% in T-B0 and 69% in T-B10 at the end of the first-stage trials. This result verified the positive effects of biochar on the hydrolysis and acidification processes of anaerobic digestion, most likely due to the acclimatization and immobilisation of fermentative bacteria on the large surface area of biochar (Zhang et al., 2020).

The VFAs in group T-B0 and T-B10 were transferred from the first-stage to the second-stage reactor for biomethane production; the VFA concentrations at the start of the second stage were measured as 922 mg/L and 1500 mg/L, respectively (Fig. 3 (b)), as they were diluted by adding biomethane inoculum for the second stage. At the start of the second-stage trials group T-B10 contained 63% more VFAs that were accessible for methanogenic archaea to produce biomethane compared with the control group T-B0. Biochar addition did not significantly change the composition of the VFAs in the second-stage digesters as acetic acid made up approximately 75% of the total VFAs both in group

Table 1

The kinetic analysis of on-farm feedstock digestion with varying doses of biochar (0, 5, 10, 20 g/L) using the modified Gompertz model. P_{measured} is the specific methane yield (L CH₄/kg VS) in the trial, P_m is the predicted maximum biomethane yield (L CH₄/kg VS), Deviation is the difference (%) between P_{measured} and P_m , R_m is the predicted maximum biomethane production rate (L CH₄/kg VS/d), λ is the lag-phase time (d), T is the predicted peak time (d) of the anaerobic digestion process, T_{80} is the predicted time (d) required to produce 80% of the total CH₄ yield, and adjusted R^2 is a corrected goodness-of-fit measure. Data are presented as means $\pm$ standard deviation (SD).

Parameters	T-B0	T-B5	T-B10	T-B20	S-B0	S-B5	S-B10	S-B20
P_{measured} (L CH ₄ /kg VS)	203.2 $\pm$ 10.3	228.4 $\pm$ 12.9	252.6 $\pm$ 10.7	238.1 $\pm$ 12.5	198.2 $\pm$ 5.6	199.3 $\pm$ 1.3	218.4 $\pm$ 19.3	207.9 $\pm$ 0.8
P_m (L CH ₄ /kg VS)	205.7 $\pm$ 1.8	233.5 $\pm$ 3.1	250.7 $\pm$ 1.7	242.0 $\pm$ 2.9	208.5 $\pm$ 3.8	211 $\pm$ 4.3	223.5 $\pm$ 2.2	219.8 $\pm$ 4.4
Deviation (%)	1.2	2.2	0.7	1.6	5.2	5.9	2.3	5.8
R_m (L CH ₄ /kg VS/d)	31.8 $\pm$ 0.1	37 $\pm$ 0.1	36.3 $\pm$ 0.1	35.7 $\pm$ 0.1	28 $\pm$ 0.1	28.2 $\pm$ 0.1	28.6 $\pm$ 0.1	27.5 $\pm$ 0.1
λ (d)	0.8 $\pm$ 0.1	1.0 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1
T (d)	3.2 $\pm$ 0.1	3.3 $\pm$ 0.1	3.2 $\pm$ 0.1	3.3 $\pm$ 0.1	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1	3.6 $\pm$ 0.1	3.7 $\pm$ 0.1
T_{80} (d)	6.8 $\pm$ 0.1	6.8 $\pm$ 0.1	7.0 $\pm$ 0.1	7.1 $\pm$ 0.1	7.6 $\pm$ 0.1	7.6 $\pm$ 0.1	7.9 $\pm$ 0.1	8.1 $\pm$ 0.1
Adjusted R^2	0.996	0.996	0.998	0.996	0.997	0.996	0.998	0.997

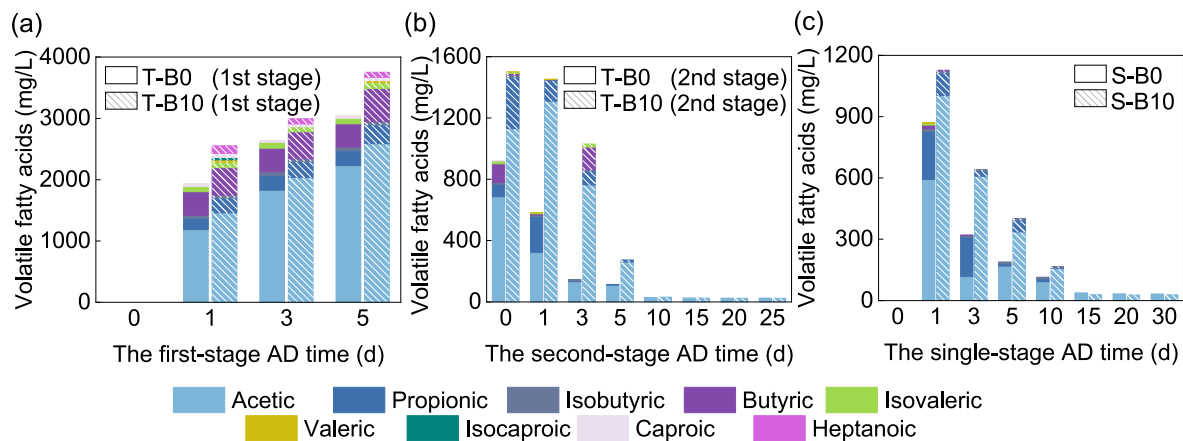


Fig. 3. Volatile fatty acids (VFAs) variation through two-stage and single-stage anaerobic digestion (AD): biochar (10 g/L) effects on the VFAs concentration and composition in the (a) first-stage, (b) second-stage and (c) single-stage reactors.

T-B0 and T-B10. The predominance of acetic acid in the VFA composition demonstrated the system efficiency and stability as acetic acid is the most easily accessible VFA for methanogenic archaea (Valentino et al., 2021). A significant reduction of 50% in total VFAs in group T-B10 on day 5 compared with day 3, and a reduction of 75% on day 10 compared with day 5 during the second-stage trial, were evident as shown in Fig. 3 (b). The two sharp decreases in total VFAs corresponded to the peaks of methane production rates.

The VFA profiles were also compared between the 10 g/L biochar-supplemented single-stage digester (S-B10) and the control group (S-B0) in Fig. 3 (c). The VFAs peaked after one day of digestion at values of 871 and 1127 mg/L in the groups S-B0 and S-B10, respectively. A 29% increase was evident in the maximum VFAs concentration in group S-B10 with 10 g/L of biochar addition as compared to the single-stage control group S-B0. Among the total VFAs in S-B0 and S-B10, acetic acid was predominant, followed by propionic acid as shown in Fig. 3 (c). The acetic acid accounted for 68% and 89% of the total VFAs on day 1 in the group S-B0 and S-B10, respectively, indicating biochar supplement could adjust and optimize the VFAs distribution, and enhance acidogenesis in single-stage digesters. The potential reason for this may be the acclimatization of electron-donating bacteria attached to the surface of biochar (Saif et al., 2022). The degradation of VFAs in groups S-B0 and S-B10 showed a similar trend: VFAs concentration fell below 200 mg/L on day 8, and further reduced below 50 mg/L after 15 days of operation, indicating the efficient conversion from VFAs to biomethane.

Overall, the total VFAs released from the biomass substrates were lower in the single-stage digesters than the first-stage digesters. The pH was measured in the optimum range from 5.25 to 5.36 (see the Supporting Information Table S1) at the end of the first-stage digestion process; this is ideal for the growth of acidogenic bacteria (Qin et al., 2019). In the first acidogenic stage, the most commonly reported bacterium phyla include *Firmicutes*, *Chloroflexi*, and *Proteobacteria*. *Clostridium* sp. was recognised to be the typical acetogen with tolerance under high H_2 partial pressure (D' Silva et al., 2021; Zahedi et al., 2016). In the second methanogenic stage, accumulation of propionic acid was observed in T-B0 on the first day without biochar supplementation in Fig. 3 (b). Excessive accumulation of propionic acid may inhibit methanogenesis. However, the concentration of propionic acid was significantly reduced by 60% (without accumulation) on the first day when adding 10 g/L biochar. Thermodynamically, propionate oxidation is favourable in the direct electron transfer pathway when adding conductive materials (Deng et al., 2023). Electroactive bacteria such as *Geobacter* were reported to be enriched with biochar addition in anaerobic digesters (Tyagi et al., 2021). In this circumstance, the accelerated propionate consumption in T-B10 was postulated to be due to improved direct electron transfer between enriched methanogens and

electroactive bacteria with the supplementation of biochar.

In accordance with VFA consumption and conversion to biomethane, the overall COD removal rates were compared in two-stage and single-stage reactors. Specifically, the highest COD removal of 84% was achieved with 10 g/L of biochar addition in the second-stage digester, which was 20% higher ($p < 0.05$) than the two-stage control group (T-B0). Similar effects of biochar on COD removal were also reported, where the maximum COD removal efficiency of 86% was obtained when digesting sulfamethoxazole pharmaceutical wastewater with the addition of 15 g/L of iron-modified biochar (Ni et al., 2023). The highest COD removal efficiency in the single-stage digesters was 76% with 10 g/L of biochar addition (S-B10), and this removal rate was 20% higher ($p < 0.05$) than the COD removal achieved in the single-stage control group (S-B0). Overall, the COD removal efficiency in the single-stage reactors was lower than in the second-stage reactors. This demonstrated the enhanced hydrolysis in the first stage of two-stage anaerobic digesters, and the facilitated conversion of recalcitrant components to soluble organic matter (Ding et al., 2018).

3.3. Biochar-supplemented reactor properties

3.3.1. Characterization of electrochemical properties

The electrochemical properties of the biochar-supplemented digester content at varying biochar dosages were investigated with respect to the cyclic voltammetry curves, electrochemical impedance spectroscopy and electrical conductivity, as shown in Fig. 4 (a)–(e). The integral areas of the cyclic voltammetry curves were positively correlated to the biochar dosage both from the two-stage digesters ($R^2 = 0.87$) and single-stage digesters ($R^2 = 0.81$). Specifically, the integral area of the biochar-supplemented digester content in group T-B20 with the highest biochar dosage of 20 g/L increased 1.8-fold as compared with the group T-B0 in the absence of biochar, revealing the enhanced charge storage capacity of biochar-supplemented anaerobic digestion systems. The increase in charge storage capacity would allow frequent electron exchange between the species (Deng et al., 2021).

An oxidation peak at approximately +0.1 V vs. Ag/AgCl was observed with the 20 g/L biochar supplement. The same oxidation peak at +0.12 V vs. Ag/AgCl was detected in a previous study (Yin et al., 2019), which could be the result of exoelectrogens transferring electrons to other electron-accepting microorganisms at this potential (Kumar et al., 2016). The enhanced oxidation peak suggested that electron transfer was facilitated by biochar. The reduction peak at −0.2 V vs. Ag/AgCl could be an indication of the presence of electrosynthetic microbes as electron acceptors, which are capable of accepting electrons to reduce carbon dioxide or VFAs to biomethane (Di et al., 2022). Biochar might act as a bridge for electron transfer between these electrosynthetic

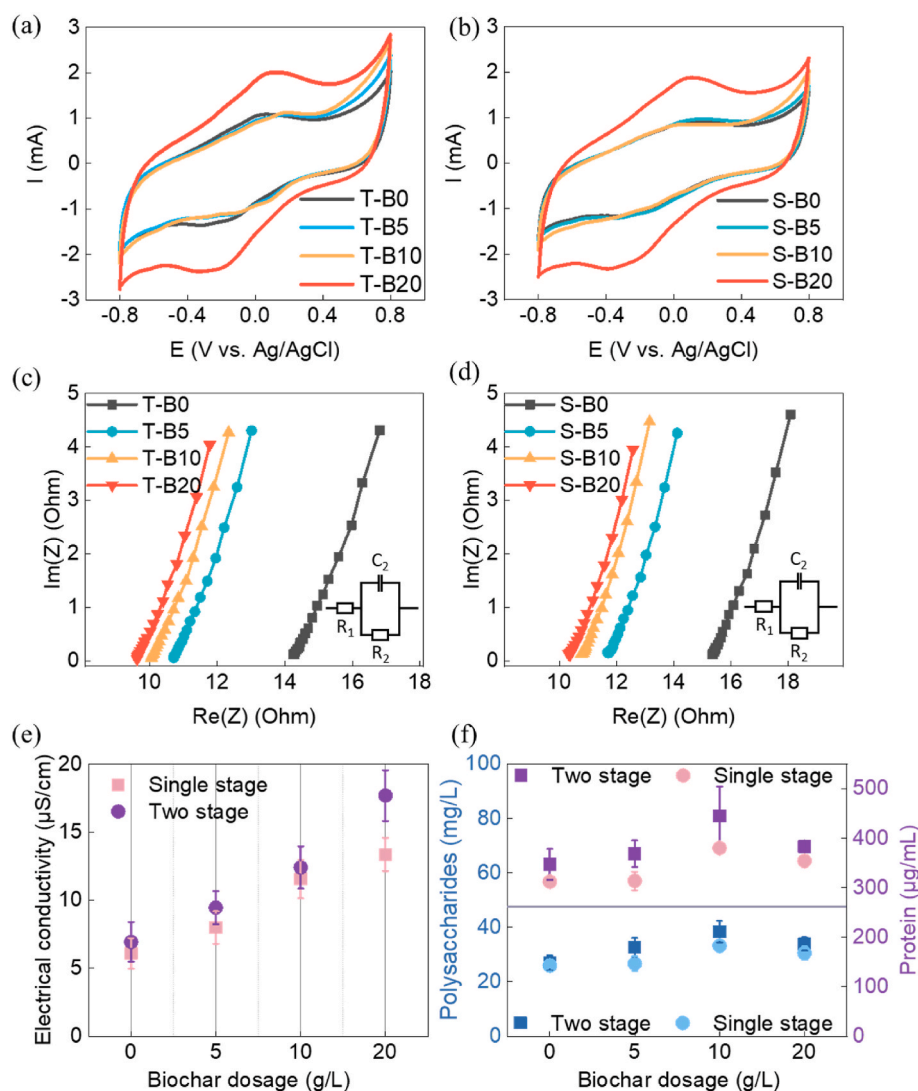


Fig. 4. The electrochemical performance of the biochar-supplemented digester content at the end of the anaerobic digestion trials. Cyclic voltammograms of the digester content from (a) two-stage reactors and (b) single-stage reactors with biochar dosage of 0, 5, 10, 20 g/L; Nyquist plots for the digester content from (c) two-stage reactors and (d) single-stage reactors with varying doses of biochar; the inserted figures in (c) and (d) are equivalent electrical circuit model of the anaerobic digesters, R_1 – solution resistance (Ω), R_2 – charge transfer resistance (Ω), C_2 – double-layer capacitance (F); (e) the electrical conductivity of the digester content in two-stage and single-stage reactors with varying doses of biochar; (f) the concentrations of proteins and polysaccharides in extracellular polymeric substance from the digester content in two-stage and single-stage reactors with varying doses of biochar. Data are presented as means $\pm$ standard deviation (SD).

microbes (He et al., 2023).

The Nyquist plots were drawn based on the results from electrochemical impedance spectroscopy as shown in Fig. 4 (c) and (d), and were subsequently fitted to a typical equivalent circuit model consisting of solution resistance R_1 (Ω), charge transfer resistance R_2 (Ω), and double layer capacitance C_2 (F) (Ségalen et al., 2015). The solution resistance R_1 primarily represents the resistance of suspended biochar-supplemented digester content in the anaerobic digestion systems. The suspended digester content with 20 g/L biochar in the second-stage digester (T-B20) attained the lowest solution resistance of 9.8 Ω , which was 28% lower ($p < 0.05$) than the control group (T-B0). There was no significant difference ($p > 0.05$) in the solution resistance between second-stage digesters and single-stage digesters when the same dosage of biochar was added (5, 10 and 20 g/L). In particular, the biochar proportion and the solution resistance R_1 exhibit strong negative correlation at the values of -0.81 and -0.83 in two-stage and single-stage digestion systems, respectively. This result further illustrates that the mix of biochar and digester content lowered the electron exchange barriers and improved electron transfer rates.

It was also observed that biochar addition increased the electrical conductivity of the digester content as shown in Fig. 4 (e). The bulk conductivity of biochar particles is up to 10^4 S/cm (Chen et al., 2023), which could accelerate the interspecies electron transfer. The maximum electrical conductivity of the digester content was 18 μ S/cm achieved with the addition of 20 g/L biochar in two-stage digestion (T-B20), which was 2.6-fold higher than the digester content from the control group (T-B0). There are strong positive correlations between digester content conductivity and the biochar dosage with coefficients of 0.99 and 0.92 for second-stage digesters and single-stage digesters (see Fig. S1 and Fig. S2 in the Supporting Information), respectively.

Overall, biochar exhibited positive effects on the electrochemical properties of the digester content in the digestion systems, demonstrated by the increased charge storage capacity, reduced solution resistance and improved conductivity. The maximum biochar dosage of 20 g/L improved the electrochemical properties to the greatest extent. However, the maximum biomethane production was achieved at the biochar dosage of 10 g/L. This finding suggests that the electrochemical properties of the digester content might not be the single factor influencing

biomethane production.

3.3.2. Secretion of extracellular polymeric substances

Extracellular polymeric substances were measured to determine the factors affecting biomethane production. In brief, one of the main functions of extracellular polymeric substances secreted by microorganisms is to facilitate the attachment of microbes to the surface of biochar and the formation of biofilms as adhesives between cells (Wen et al., 2020). Polysaccharides and proteins are the main components in extracellular polymeric substances with a total percentage of more than 70% of the substance (Costa et al., 2018), hence, they were measured as the representative compounds in this study as shown in Fig. 4 (f). When 10 g/L of biochar was added into the second-stage digester (T-B10), the concentrations of polysaccharides and proteins achieved the highest values, which were 41% and 28% higher ($p < 0.05$) than the control group of the two-stage digester without biochar (T-B0). The amounts of extracellular polymeric substances (the total amounts of polysaccharides and proteins) are strongly correlated with the biomethane yields obtained in the second-stage digesters with a coefficient of 0.92 (Fig. S3 in the Supporting Information), regardless of the biochar dosage. Therefore, extracellular polymeric substance secretion is argued to be an important factor affecting biomethane yields.

Similarly, in single-stage digesters with the presence of 10 g/L biochar (S-B10), the concentrations of polysaccharides and proteins increased by 27% and 22% (both $p < 0.05$), as compared to the control group (S-B0). A strong positive correlation between extracellular polymeric substances and methane yields was validated by a high coefficient of 0.95 (Fig. S4 in the Supporting Information). Sufficient extracellular polymeric substances allowed the microorganisms to adhere tightly to the surface of biochar to develop electroactive biofilms, and also facilitated the electron transfer within biofilms (Chen et al., 2023; Li et al., 2016). The interlaced conductive network established by extracellular polymeric substances and biochar is essential for direct interspecies electron transfer and biomethane production enhancement.

3.4. Energy yields and biodegradability index of the anaerobic digestion systems with varying biochar dosages

The energy yields and biodegradability index are shown in Fig. 5. In the absence of biochar, the energy yield from the control group of two stage T-B0 was 7.4 MJ/kg VS (7.2 MJ/kg VS from biomethane and 0.2 MJ/kg VS from biohydrogen), equivalent to a biodegradability index of 66%. This energy gain is quite similar to the study conducted by Yuan et al. (2023), revealing that H_2 and CH_4 made up approximately 2.8–3.1% and 96.9–97.2% of the total energy yields, respectively. The energy yield increased to 9.3 MJ/kg VS (9.0 MJ/kg VS from biomethane and 0.3 MJ/kg VS from biohydrogen) with the addition of 10 g/L biochar in the two-stage group T-B10, corresponding to a biodegradability index of 82%. The biodegradability index exceeded 80% in the

two-stage co-digestion of silage and slurry at the same VS ratio of 4:1 (Wall et al., 2013), but without biochar supplement. The high energy yield obtained in two-stage digesters may have been due to the improvement of degradation of grass silage in the rate-limiting hydrolysis process (Deng et al., 2019).

In single-stage digesters, an energy yield of 7.0 MJ/kg VS was reached in the control group S-B0. Different dosages of biochar (5, 10, 20 g/L in group S-B5, S-B10 and S-B20, respectively) enhanced the energy yields to 7.1, 7.8 and 7.4 MJ/kg VS respectively, as compared to the control group S-B0. The energy yields in single-stage digestion were lower than those in two-stage digestion when adding the same dosage of biochar. This can be explained by the degradability of the VFAs produced in the first stage alongside the hydrogen production and the resultant lower methane yields in single-stage digestion.

In an overview of the energy balance, the total energy inputs included the energy contained in the biomass feedstocks (9.5 MJ/kg VS), electrical energy consumption (0.2 MJ/kg VS) and thermal energy consumption (11.1 MJ/kg VS for two-stage digestion process and 10.9 MJ/kg VS for single-stage digestion process). In two-stage digestion processes, the highest energy conversion efficiency was 44.8% when adding 10 g/L biochar, while the energy conversion efficiency was only 35.9% without biochar supplementation. An energy conversion efficiency of 37.8% was achieved in the single-stage digestion process with 10 g/L of biochar addition.

From a practical perspective, biochar was produced through the energy consuming pyrolysis process, which needs to be taken into consideration in an energy analysis. Based on a previous study by Deng et al. (2020), the energy consumption within the pyrolysis process to produce biochar was 6.5 MJ/kg TS of woody waste treated (0.5 MJ of electricity input for pretreatment, 4.0 MJ of heat for drying the woody waste, and 2.0 MJ of heat for heating the pyrolysis reactor). This equals an energy consumption of 29.5 kJ/g biochar production considering 220 g biochar could be obtained from 1 kg TS of woody waste. On a per kilogram of feedstock VS digested basis, the addition of 1 g biochar led to an extra 11.6 L CH_4 production (which equals an extra energy yield of 412 kJ) in the two-stage digestion with 10 g/L biochar supplement, whilst the addition of 1 g biochar led to an extra 4.6 L CH_4 production (which equals an extra energy yield of 163 kJ) in the single-stage digestion with 10 g/L biochar supplement (see the Supporting Information Text 3). The extra energy yield in the biochar-supplemented digestion system exceeded the energy consumption for biochar production. Therefore, biochar addition would be an energy-efficient approach to promote biomethane production through anaerobic digestion.

This study demonstrated the biochar-supplemented two-stage anaerobic digestion process as a potentially advantageous route for biomethane production from grass silage and cattle slurry. However, it would be necessary to conduct continuous trials and a life cycle assessment prior to the implementation of the biochar-supplemented

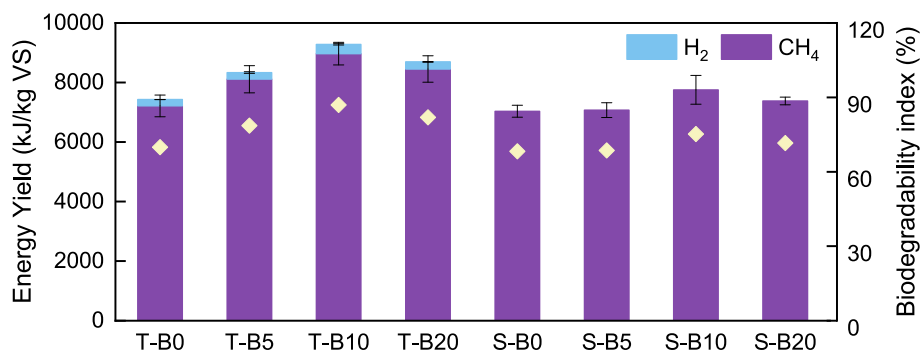


Fig. 5. The energy yields (columns) and biodegradability index (yellow diamond-shape points) of different scenarios in two-stage and single-stage anaerobic digestion (AD). Data are presented as means $\pm$ standard deviation (SD). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

technology in large-scale anaerobic digestion plants. Understanding the mechanism of the effects of biochar in two-stage digestion is also vital to facilitate full-scale application. Firstly, biochar addition facilitated the hydrolysis of rigid lignocellulosic components, and promoted biohydrogen and VFAs production in the first-stage digester, possibly due to the pH buffering capacity and the provision of large surface area to support the growth of fermentative bacteria (Yuan et al., 2023). The extra VFAs produced in the enhanced first-stage digestion led to enhanced conversion to biomethane in the second-stage digester. Secondly, biochar addition stimulated the direct interspecies electron transfer in the second-stage digesters enhancing biomethane production (An et al., 2022). This is evidenced by the enhanced electrochemical properties of the suspended biochar-supplemented digester content. With the addition of 10 g/L biochar, the increasing secretion of extracellular polymeric substances by microorganisms further supports direct interspecies electron transfer within the process of methanogenesis.

4. Conclusions

The overall efficiency of co-digestion of cattle slurry and grass silage can be enhanced by biochar addition in two-stage anaerobic digestion. The highest methane yield of 253 L/kg VS, equivalent to an energy yield of 9.3 MJ/kg VS and a biodegradability index of 82%, was achieved with 10 g/L biochar supplement in two-stage digesters. The methane yield was 24% higher than that from two-stage digesters without biochar supplementation. Biochar has a positive effect on the hydrolysis and acidification processes of anaerobic digestion, as volatile fatty acid accumulation was seen in the first stage. With the same biochar dosage of 10 g/L, the biomethane yield was 218 L/kg VS in single-stage digestion. The maximum biochar dosage of 20 g/L improved the electrochemical properties of the digester content to the greatest extent whilst the biomethane yield was not the highest, suggesting that electrochemical properties of the digester content might not be the single factor influencing biomethane production. The amounts of extracellular polymeric substances (the total amount of polysaccharides and proteins) were strongly correlated with the biomethane yields ($R^2 = 0.92$) in the two-stage anaerobic digestion. The interlaced conductive network established by extracellular polymeric substances and biochar, promoted microbial electron transfer within the methanogenesis process.

CRedit authorship contribution statement

Xue Ning: Writing – original draft, Investigation, Data curation, Formal analysis, Visualization. **Chen Deng:** Conceptualization, Validation, Supervision, Writing – review & editing. **Xihui Kang:** Investigation, Data curation. **Richard O'Shea:** Validation, Supervision, Visualization, Funding acquisition, Writing – review & editing. **David M. Wall:** Funding acquisition, Writing – review & editing. **Richen Lin:** Validation, Supervision, Funding acquisition, Writing – review & editing. **Jerry D. Murphy:** Validation, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Abbreviations

AD	Anaerobic digestion
VFAs	volatile fatty acids
TS	total solid
VS	volatile solid
COD	chemical oxygen demand
EPSs	extracellular polymeric substances
LHV	lower heating value
HHV	higher heating value

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2023.139155>.

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