Semi-continuous anaerobic digestion of mixed wastewater sludge with biochar addition

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**Abstract:**
This work analysed the effects of biochar (BC) addition to the anaerobic digestion (AD) of wastewater mixed sludge (MS) in semi-continuous mode. A 3 L digester was operated at 37 °C for 100 days, feeding MS collected every three weeks in the same wastewater treatment plant, and 10 g L⁻¹ of BC. The average performance of MS digestion (biogas 188 NmL d⁻¹, 68% methane) improved in presence of BC (biogas 244 NmL d⁻¹, 69% methane). According to the results of the multiple linear regression analysis performed on the experimental data, the 79% variation of the soluble COD in the MS was the driving factor for the 38% increase of biogas and methane yields. Future research investigating the role of BC in the AD of wastewater sludge should explore not only BC properties and dosage, but also the influence of the variability of the substrate’s composition.

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Dear Editor,

We would like to submit the enclosed manuscript, entitled “**Semi-continuous anaerobic digestion of mixed wastewater sludge with biochar addition**” by Marco Chiappero, Franco Berruti, Ondřej Mašek and Silvia Fiore for your consideration for possible publication as original research paper in *Bioresource Technology*. The manuscript is coherent with *Bioresource Technology*’s aims and scopes and specifically with subject classification 20.030 Anaerobic digestion. The manuscript was prepared according to the Instruction for Authors.

This work describes the effects of biochar (BC) addition to the anaerobic digestion (AD) of wastewater mixed sludge (MS) in semi-continuous mode. A 3 L digester was operated at 37 °C for 100 days, feeding MS collected every three weeks in the same wastewater treatment plant, and 10 g L\(^{-1}\) of BC. The average performance of MS digestion (biogas 188 NmL d\(^{-1}\), 68% methane) improved in presence of BC (biogas 244 NmL d\(^{-1}\), 69% methane). According to the results of the multiple linear regression analysis performed on the experimental data, the 79% variation of the soluble COD in the MS was the driving factor for the 38% increase of biogas and methane yields. Future research investigating the role of BC in the AD of wastewater sludge should explore not only BC properties and dosage, but also the influence of the variability of the substrate’s composition.

All authors of this paper directly participated in the planning, execution, and analysis of the research. All authors of this paper approved the final version submitted. All authors mutually agree that the manuscript should be submitted to Bioresource Technology. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not now under consideration for publication elsewhere. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration. If accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher. The authors declare no conflicts of interests.

Thank you very much for your time and consideration.

Sincerely yours,

Silvia Fiore (on behalf of all authors)

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Turin, 10 June 2021
Wastewater treatment

MIXED SLUDGE

SUBSTRATE

Pyrolysis + activation

Mesophilic semi-continuous anaerobic digestion

- BIOGAS PRODUCTION
- METHANE PRODUCTION
- COD REMOVAL
- SOLIDS REMOVAL

multiple linear regression analysis
Highlights

- Biochar slightly improved the anaerobic digestion of wastewater sludge at 37 °C
- Changes in soluble COD of the sludge mostly influenced biogas and methane yields
- Sludge composition was a key process factor, independently of biochar addition
Semi-continuous anaerobic digestion of mixed wastewater sludge with biochar addition

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Abstract

This work analysed the effects of biochar (BC) addition to the anaerobic digestion (AD) of wastewater mixed sludge (MS) in semi-continuous mode. A 3 L digester was operated at 37 °C for 100 days, feeding MS collected every three weeks in the same wastewater treatment plant, and 10 g L\textsuperscript{-1} of BC. The average performance of MS digestion (biogas 188 NmL d\textsuperscript{-1}, 68% methane) improved in presence of BC (biogas 244 NmL d\textsuperscript{-1}, 69% methane). According to the results of the multiple linear regression analysis performed on the experimental data, the 79% variation of the soluble COD in the MS was the driving
factor for the 38% increase of biogas and methane yields. Future research investigating the role of BC in the AD of wastewater sludge should explore not only BC properties and dosage, but also the influence of the variability of the substrate’s composition.

Keywords
biochar; biogas; linear regression; sludge; wastewater

1. Introduction
Wastewater treatment plants (WWTP) in Europe produce currently over 10 Mt/year of wastewater sludge (expressed as dry substance) (Eurostat, 2021). Sludge management is a critical issue for WWTPs, implying significant impacts on their operating costs (Appels et al., 2010) and environmental footprint (Gherghel et al., 2019). In the past decade, many WWTP operators have implemented AD as part of the sludge management processes to recover energy. The optimization of biogas production from wastewater sludge could heavily improve the energy balance of a WWTP (Gu et al., 2017; Jenicek et al., 2013). Even if anaerobic digestion (AD) is commonly applied in full-scale WWTPs, it still has critical issues that need to be addressed to improve its performance. Particularly in the case of waste activated sludge, the complex floc structure and the recalcitrant cell walls limit the hydrolysis and the overall implementation of AD (Zhen et al., 2017), resulting in scarce methane yields and high retention times. Further, the presence of inhibitory substances in the sludge may limit methane production (Yuan and Zhu, 2016). To improve the AD of wastewater sludge, different options have been widely investigated, including physicochemical pre-treatments (Khanh Nguyen et al., 2021; Zhen et al., 2017), and co-digestion with other substrates (Chow et al., 2020; Elalami et al., 2019). At the
same time the financial and environmental impacts of the proposed solutions (i.e., use of chemicals and energy demand) should be contained. A recent perspective in this framework considered the use of additives, mostly carbon-based materials (e.g. biochar, activated carbon, graphene, carbon fibres) (Romero-Güiza et al., 2016), with the aim of improving methane production, AD stability, and digestate quality (Abbas et al., 2021). Among carbon-based additives, biochar (BC) has been receiving increasing attention due to its low cost and environmental sustainability, and the ability to increase methane yield (Chiappero et al., 2020). BC is the solid residue of the thermo-chemical pyrolytic treatment of biomass with limited or no oxygen, and it may derive from many waste biomasses (Li et al., 2019). BC has become highly attractive for many applications (Zhang et al., 2019), due to the wide variety of distinctive properties, including large surface area and porous structure, rich surface functionalities, high ion exchange capacity and adsorption ability. Specifically considering BC as additive in the AD of wastewater sludge, increased methane yields and production rates were observed (Chiappero et al., 2021). The large surface area and porous structure of BC provides a suitable habitat for microbial attachment and colonization during AD (Yin et al., 2019; Zhang et al., 2019). BC was shown to stimulate all phases of AD, from hydrolysis and acidogenesis (Wei et al., 2020; Xu et al., 2020; Yin et al., 2019), to acetogenesis and methanogenesis (Lü et al., 2020; Shen et al., 2021). It has been suggested that BC stimulates the syntrophic metabolisms between fermentative bacteria and methanogens (Lü et al., 2020; Shen et al., 2021) by facilitating interspecies electron transfer on its surface functional groups (Wang et al., 2020; Wang et al., 2018). Further, when supplemented to the AD of wastewater sludge, BC increases the alkalinity of the system, thanks to the content of alkali and alkaline earth metals (Shen et al., 2016; Zhou et al., 2020), and mitigates ammonia
inhibition (Shen et al., 2017; Zhang et al., 2019). Conversely, inhibitory effects on 

methane production from wastewater sludge were observed in case of excessive loads of 

BC by several authors (Wei et al., 2020; Wu et al., 2019; Zhang et al., 2019; Zhang and 

Wang, 2020). Despite the variable effects on AD due to the differences in the features of 

the BC, the above cited studies proved the enhancement of methane production during 

batch AD experiments, suggesting different potential mechanisms. Taking into account 

the experimental conditions involving continuously-fed digesters, the only available 

studies reporting positive effects of BC on methane production concerned primary sludge 

in thermophilic conditions (Wei et al., 2020) and temperature-phased semi-continuous 

AD of mixed sludge (Shen et al., 2017).

To our knowledge, the effects of BC supplementation on the AD of wastewater sludge 

under “realistic” AD conditions, have not been explored yet. Full-scale WWTPs mostly 

feed AD with mixed sludge (primary and waste activated sludge), in continuous mode. 

The intrinsic high variability of the physicochemical features of the sludge in the same 

facility (Arhoun et al., 2019; Panepinto et al., 2016), which influences the performance of 

the AD, is well-known and should be considered as a key factor. The present study aims 

to investigate the effects of BC addition on the AD of wastewater sludge under operating 

conditions simulating common full-scale installations, e.g. mesophilic AD of mixed 

sludge sampled multiple times in the same WWTP during a period of three months. A 3 

L reactor was operated at 37 °C in semi-continuous mode over 100 days, feeding mixed 

sludge sampled from the same WWTP every three weeks. The specific objectives of the 

study were: 1. investigating the effect of BC addition on raw mixed sludge (no pre- 

treatments), also accounting for the variability of the substrate, on biogas yield and
composition; 2. exploring the relationship among BC addition, sludge biodegradability and AD performance through a linear regression approach.

2. Material and Methods

2.1. Substrate, inoculum, and biochar

Mixed sludge (MS) was sampled from a WWTP in northern Piedmont, Italy every three weeks (4 samplings in total). The MS was collected at the outflow of the dynamic settling tank receiving primary sludge and waste activated sludge before the AD, obtaining representative samples (30 L). At each sampling, the MS was characterized (see section 2.3) and stored at 4 °C. The inoculum employed for the start-up of the AD process was sampled from the digester operating at 37 °C in the same WWTP.

The biochar (Sewage Sludge - SS550a) considered in this work was selected among other BCs according to the results of previous studies (Chiappero et al., 2021, Chiappero et al., in preparation). SS550 is a “standard” BC produced at the UK Biochar Research Centre (UKBRC) at the University of Edinburgh, UK (Mašek et al., 2018), through the slow pyrolysis of sewage sludge pellets at 550 °C. Subsequently, the BC underwent physical activation with CO₂ (60 mL min⁻¹ at 900 °C for 2 hours) at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR) at Western University, Canada. Therefore, the BC considered in this work was defined as “SS550a” (i.e. “SS550” according to the reference ID adopted at UKBRC, and “a” to refer to its activation). Full details about SS550a production and activation are given in the Appendix.

2.2. Semi-continuous anaerobic digestion test
A lab-scale (3 L working volume) continuously stirred stainless-steel reactor (Methan Tube®, Biological Care, Italy) (Figure 1) was operated under a semi-continuous feeding mode for 100 days. The temperature was set to 37 °C (± 0.4 °C) and controlled through a built-in heater. Continuous mechanical mixing (75 rpm, reversed every 5 min) was provided by a brushless DC motor. For the start-up of the AD process, the digester was filled with digestate (see section 2.1), previously stored at 37 °C for 5 days. Once a day, at the same time, 0.150 L of MS was manually fed to the reactor and 0.150 L of digestate automatically discharged, resulting in a hydraulic retention time (HRT) equal to 20 days. The AD tests involved two consequent phases. Phase 1 (control phase, CTRL): from day 0 to day 49, the digester was fed with MS. At day 31, a slight decrease of pH and total alkalinity was observed, thus the initial HRT equal to 15 days was adjusted to 20 days. Phase 2: from day 50 to 100, 10 g L⁻¹ of BC was supplemented to the digester, feeding 1.5 g of BC and 0.150 mL of MS daily. The selected BC dosage was based on the literature (Chiappero et al., 2020) and on the results of previous AD tests (Chiappero et al., 2021, Chiappero et al., in preparation).

**Figure 1.** Configuration of the 3 L stainless-steel reactor: (1) motor and mixer; (2) gas outlet; (3) closing screws; (4) discharging port; (5) silicone stopper; (6) inflow; (7) heater connection; (8) outflow.

**2.3. Analytical procedures**

MS was characterized at every sampling (see section 2.1) and digestate was analysed every 3-4 days to monitor the key operating key parameters. pH and electrical conductivity (EC) were measured using a pH80+DHS (XS Instruments) multi-meter.
Total solids (TS) and volatile solids (VS) were determined according to Standard Methods (APHA-AWWA-WEF, 2005). The total alkalinity (TA) was measured using the 2320B volumetric/potentiometric method (APHA-AWWA-WEF, 2005), on 40 mL of 1:10 diluted digestate titrated to pH 4.5 with 0.02 N hydrochloric acid. Total and soluble chemical oxygen demand (tCOD and sCOD), organic acids, and ammonia nitrogen were analyzed using Nanocolor test kits (Macherey-Nagel, Germany) and a PF-12\textsuperscript{Plus} photometer (Macherey-Nagel, Germany). The samples were centrifuged (6,000 rpm for 10 min) and the supernatant used for ammonia nitrogen determination. The supernatant was filtered on 0.45 μm cellulose acetate membranes and analyzed for sCOD and organic acids. All analyses were carried out at least in duplicate.

The parameters and analytical procedures involved in the characterization of the BC, obtained in a previous study (Chiappero et al., 2021), are detailed in the Appendix.

Biogas production was monitored daily through a gas flow meter (μFlow, Bioprocess Control, Sweden), automatically normalized to standard temperature and pressure (0 °C, 1 atm). Biogas was collected in a 10 L gas sampling bag (30229-U, Supelco) and characterized (methane, carbon dioxide, hydrogen sulfide and oxygen) three times per week through an Optima 7 Biogas analyser (Mru GmbH, Germany).

2.4. Data analysis

During the AD test, mean and standard error of the different parameters were determined for the time periods in which a constant organic loading rate (OLR) was fed to the digester. The relationship among BC addition, sludge characteristics and AD performances was assessed through single and multiple linear regression analyses.
3. Results and Discussion

3.1. Characterization of substrate, inoculum, and biochar

The average characteristics of the MS and of the inoculum are reported in Table 1. MS showed the typical characteristics of a mixed sludge (in average, pH 6.0 and TS 3.1%-wt). BC supplementation increased TS correspondingly to the dose of 10 g L\(^{-1}\), and reduced VS/TS due to the high ash content of the BC.

Table 1. Chemical characteristics of the mixed sludge, mixed sludge with 10 g L\(^{-1}\) of SS550a biochar, and the digestate. Data are expressed as average ± standard error (number of values).

Considering the characteristics of the BC (see Appendix), the most significant are as follows. The specific surface area (109.2 m\(^2\) g\(^{-1}\)) and total pore volume (0.169 cm\(^3\) g\(^{-1}\)) are the result of the physical activation. The relevant ash content (58.9%-wt) and low total carbon (29.5%-wt) are expected based on the composition of the parent wastewater sludge (Metcalf & Eddy, 2013) from which the BC derives. The significant content of micro-elements contributed to the electrical conductivity (28 S m\(^{-1}\)) and to the alkaline pH (8.17). The main mineral constituents were Si, Al, Ca, S, P, K, present in the parent biomass and concentrated during the pyrolysis (Zielińska et al., 2015). Nutrients and alkali and alkaline earth metals are present in significant concentrations, compared to plant-based BCs (Qambrani et al., 2017). An adequate amount of alkali and alkaline earth metals in BC can contribute to the buffering capacity (Shen et al., 2015). The H/C and O/C atomic ratios (respectively 0.54 and 0.17), indicate an intermediate hydrophobicity of the BC, in agreement with the literature (Zhang et al., 2015).
Figure 2. Biogas, methane, and carbon dioxide production during the semi-continuous AD of mixed sludge with and without BC: (A) Gas production as NmL d⁻¹; (B) Gas production as NmL g tCOD⁻¹ d⁻¹; (C) Gas production as NmL g VS⁻¹ d⁻¹; (D) CH₄, CO₂, and H₂S concentration; (E) Organic loading rate as g VS L⁻¹ d⁻¹ and g tCOD L⁻¹ d⁻¹.

3.2. Biogas and methane production

As detailed in section 2.2, the digester was fed for the first 50 days with MS (CTRL phase), and from day 51 to 100 supplementing 10 g l⁻¹ of BC. Figure 2 shows the production of biogas, methane and carbon dioxide, biogas composition and OLR during the different stages of the AD test. The MS was sampled four times during the test, and different physico-chemical features were observed (Table 1), thus the change of the substrate composition (specifically, VS/TS, tCOD and sCOD) and, consequently, of the organic loading rate (OLR) determined five constant sub-phases (Figure 2E): 1 to 3 during CTRL phase, 4 and 5 during BC addition.

Table 2. Summary of the experimental results of the semi-continuous AD test, in each phase, reported as average (standard error).

The results of the AD test are detailed in Table 2. Daily biogas and methane yields (Figure 1A) reached stability around day 10, implying the conclusion of the start-up phase. Overall, during the experimental phase, biogas yield was in the range of 408-1120 NmL d⁻¹ and methane yield in the range of 315-724 NmL d⁻¹. During the CTRL stage, biogas yield slightly decreased from phase 1 to 3 (from 791 ± 45 NmL d⁻¹ to 637 ± 28 NmL d⁻¹),
and during phase 4 in the presence of BC (527 ± 18 NmL d⁻¹) until day 74, when a sharp increase occurred at the beginning of phase 5 (917 ± 26 NmL d⁻¹). Methane yield showed a similar trend, with a decline from a 525 ± 28 NmL d⁻¹ in phase 1 during CTRL to 379 ± 19 NmL d⁻¹ in phase 4, followed by an increase to 636 ± 19 NmL d⁻¹ in phase 5. It should be noticed that BC supplementation from day 50 did not seem to correspond to any variation of the decreasing trends observed for biogas and methane yields from phase 1 to 4. Conversely, the sharp increase at day 74 during the BC addition corresponded to a change in MS composition: sCOD of the MS was 1555 ± 5 mg L⁻¹ in phase 1, 1123 ± 11 mg L⁻¹ in phase 2 and 3, 1870 ± 90 mg L⁻¹ in phase 4, and 3340 ± 30 mg L⁻¹ in phase 5.

The daily specific biogas and methane yields were also determined. The specific biogas yield (Figure 2B) decreased between phase 1 and 2 from 211 ± 12 to 163 ± 9 NmL g⁻¹ VSd⁻¹, then increased to 191 ± 9 NmL g⁻¹ VSd⁻¹ (phase 3), remaining almost stable during phase 4, and finally jumped up to 286 ± 8 NmL g⁻¹ VSd⁻¹ in phase 5. The specific methane yield followed a specular trend with a decrease between phases 1 and 2 (from 140 ± 7 to 113 ± 7 NmL g⁻¹ VSd⁻¹), followed by a slow rise (up to 145 ± 7 NmL g⁻¹ VSd⁻¹) in phase 4 and a marked increase to 198 ± 6 NmL g⁻¹ VSd⁻¹ in phase 5.

Similarly, specific biogas and methane yields expressed as NmL g⁻¹ tCODd⁻¹ (Figure 2C) confirmed the trends shown in Figure 2B, with minimum average values in phase 2 (92 ± 5 and 64 ± 4 NmL g⁻¹ tCODd⁻¹ respectively), and maximum values (157 ± 4 and 109 ± 3 NmL g⁻¹ tCODd⁻¹) in phase 5. The addition of BC from day 50 did not correspond to any clear variation in specific biogas and methane yields between phases 3 and 4.

Considering the biogas composition (Figure 2D), the stability of CH₄ and CO₂ contents over time was clear, in the range 67.1-69.0% and 28.6-30.8% during the CTRL phase, and 68.9-69.1 % and 29.6-30.1 % during BC addition, respectively. In all phases, the H₂S
concentration in biogas was almost negligible (below 16 ppm). Methane content of almost 70% confirmed a good stability of the AD process (Duan et al., 2012). However, under the specific experimental conditions, BC did not positively affect methane content, in contrast with literature. For instance, Shen et al. (2017) found an increase of 14-25% of the average methane content with the addition of BCs derived from corn stover and pine, compared to a control reactor, during the temperature-phased semi-continuous AD of sludge at 55 °C. Wei et al. (2020) showed that supplementation of corn stover BC enhanced methane content up to 21% compared to control reactors during the continuous AD of primary sludge at 55 °C.

Figure 2E shows the trend of the OLR, expressed as VS and tCOD (as commonly found in literature), during the AD test. In general, OLR did not vary significantly over the duration of the test, ranging between 0.9-1.4 g VS L⁻¹ d⁻¹ and 1.6-2.1 g tCOD L⁻¹ d⁻¹, which are typical values adopted in full-scale mesophilic digesters in WWTPs (Bolzonella et al., 2005). However, OLR did not seem to positively affect the specific biogas and methane yields observed in this work. In phase 2, the highest OLR values (1.4 g VS L⁻¹ d⁻¹, 2.1 g tCOD L⁻¹ d⁻¹) corresponded to the minimum specific biogas and methane yields, equal to 163 ± 9 and 113 ± 7 NmL gVS⁻¹ d⁻¹, respectively. More importantly, the marked increase in specific biogas (+42%) and methane yields (+37%) between phases 4 and 5 did not match a pronounced variation of the OLR.

Table 3. Results of multiple linear regression to predict biogas and anaerobic digestate parameters based on OLR as sCOD (g sCOD L⁻¹ d⁻¹) and BC concentration (g L⁻¹). The linear model is expressed in the form y = b₀ + b₁ x₁ + b₂ x₂, where y is the estimated parameter, x₁ is the OLR, x₂ is BC concentration.
In parallel to the usual notations applied to OLR (expressed as VS and tCOD), we decided to investigate the influence of sCOD on the OLR and included it in the further evaluation of our experimental results. The soluble COD represents the fraction of COD immediately accessible by microorganisms for degradation. The ratio between sCOD and tCOD is used to quantify the degree of solubilization of the sludge, usually considered as a performance indicator to investigate the efficiency of pre-treatments in improving the methane yield (Nguyen et al., 2021). In this study, despite the stability of TS content (around 3.1%), the MS presented a variable sCOD/tCOD, in the range 2.9 – 8.6% (Figure 2E).

Since the increment in the methane yields during the BC addition occurred concurrently with a change in MS composition, a single linear regression was used to investigate the relationships between the specific biogas and methane yields (NmL kgVS⁻¹d⁻¹) and the key characteristics of the MS, namely the OLR (expressed as VS, tCOD and sCOD), and the sCOD/tCOD (%). A significant linear relation was not identified between the specific biogas yield and the OLR expressed as g VS L⁻¹ d⁻¹ (F(1.63) = 3.48, p > 0.05) or as g tCOD L⁻¹ d⁻¹ (F(1.63) = 2.56, p > 0.05). Conversely, a significant positive linear relation was found between biogas yield and the OLR expressed as g sCOD L⁻¹ d⁻¹ (F(1.63) = 102.38, p = 7.9 e-15) with an R² of 0.60, and between the biogas yield and the sCOD/tCOD (%) (F(1.63) = 93.87, p = 4.2 e-14) with an R² of 0.62. As for biogas, significant linear relationships between the specific methane yield expressed as NmL kgVS⁻¹d⁻¹ and the OLR expressed as VS (F(1.37) = 3.56, p > 0.05) or as tCOD (F(1.37) = 2.47, p > 0.05) were not found. In contrast, a significant positive linear relationship was identified for methane yield and OLR expressed as sCOD with an R² of 0.67 (F(1.37) = 75.41, p = 1.9 e-10) and
methane yield and sCOD/tCOD (%) with an $R^2$ of 0.68 ($F(1.63) = 79.17$, $p = 1.0 \times 10^{-10}$).

Therefore, the marked increase of the specific biogas and methane yields in phase 5 may be ascribable to the corresponding increase of sCOD/tCOD (%) in the MS from 5.8% of phase 4 to 8.6% of phase 5 (Figure 2E).

Given the significant role of MS composition (specifically, sCOD content) on biogas and methane productions, the relative effect of BC addition and MS features on biogas production and composition was further investigated through a multiple linear regression analysis. From the results of the simple linear regression analysis, OLR expressed as sCOD was identified as the most appropriate parameter to describe the MS composition.

Two independent variables, namely OLR expressed as $g \text{sCOD L}^{-1} \text{d}^{-1}$ and BC concentration ($g \text{L}^{-1}$) were chosen to predict biogas and methane yields, and biogas composition. The linear model was expressed in the form $y = b_0 + b_1 x_1 + b_2 x_2$, where $y$ is the estimated parameter, $x_1$ is the OLR, $x_2$ is the BC concentration. Considering the results of the multiple linear regression analysis (Table 3), in general, the linear regressions for daily biogas and methane yields ($\text{NmL d}^{-1}$) were significant, with $R^2$ equal to 0.69 and 0.73, respectively. While noteworthy positive relationships ($b_1 > 0$, $p_1 < 0.05$) for biogas or methane yields and OLR were confirmed, there were important negative relationships ($b_2 < 0$, $p_2 < 0.05$) between OLR and BC concentration. Further, positive linear regressions were found between the specific biogas and methane yields ($\text{NmL gVS}^{-1} \text{d}^{-1}$) and the OLR. However, there was in general insufficient evidence ($p_2 > 0.05$) to conclude that specific biogas and methane yields were positively ($b_2 > 0$) affected by BC supplementation. As expected, the linear regressions of $\text{CH}_4$ and $\text{CO}_2$ contents based on OLR expressed as sCOD and BC concentration did not show any relationship. Conversely, other studies proved that BC supplementation can enhance methane
production from sludge during continuous AD, adopting different operating conditions or BC dosage and characteristics, compared to this work. As mentioned earlier, Shen et al. (2017) demonstrated the enhancement of methane production from two-stage AD of mixed sludge at 55 °C with the addition of corn stover BC. In that study, the positive effects of BC were attributed to CO₂ removal, mitigation of ammonia inhibition, increased alkalinity, shifts of microbial community, and linked to peculiar BC features as the large specific surface area and micro-porous structure, high hydrophobicity and content of aromatics and alkali and alkaline earth metals. Also Wei et al. (2020), testing a BC having similar characteristics than the one considered by Shen et al. (2017), found that BC increased methane production during thermophilic AD of primary sludge; enhanced buffering capacity, alleviated ammonia inhibition, and CO₂ sequestration were suggested as main mechanisms. Compared to the BCs used in the two mentioned studies, SS550a presents comparable specific surface area (109.2 m² g⁻¹), micro-porous structure (total pore volume 0.169 cm³ g⁻¹, 6.19 average pore diameter), and contents of alkali and alkaline earth metals, but also a less alkaline pH, lower hydrophobicity, and higher H/C and O/C ratios. However, the main difference in the BC, compared with the two cited studies, may consist in the lower dosage of BC adopted in the present work, equal to 10 g L⁻¹ of BC (corresponding to 0.50 ± 0.03 g BC/gVS added, and 0.32 ± 0.01 g BC/gTS), being 3.5-7 times lower than those of the other studies, with an optimum of 1.75-3.5 g BC/gVS (Shen et al., 2017) and 1.82 g BC/gTS (Wei et al., 2020). Moreover, the cited studies fed the same substrate during the whole duration of their AD tests, therefore the influence of the variability of sludge composition on the performance of the AD in the presence of BC, which was highlighted as a key issue by the results of this work, was not specifically explored in the literature.
**Figure 3.** Characteristics of the digestate during the AD test: (A) total and soluble COD; (B) Total Solids and Volatile Solids; (C) Removals of total COD and soluble COD; (D) Removals of Total Solids and Volatile Solids; (E) pH and Electrical Conductivity; (F) Total Alkalinity, Organic Acids, Ammonia Nitrogen.

3.3. Digestate characterization

The trends of the characteristics of the digestate (Figure 3A) show that tCOD grew during phase 1, then stabilized during the subsequent phases at 30-32 g L\(^{-1}\) (Table 2). The stabilization of tCOD from the start-up phase was slower than that of the biogas and methane yields. The sCOD remained relatively stable, below 860 mg L\(^{-1}\), with average values ranged between 540 and 730 mg L\(^{-1}\) during the different phases of the test (Figure 3A). The linear relationships between output tCOD or sCOD and BC concentration and OLR (expressed as sCOD) were not significant (Table 3).

The TS concentration (Figure 3B) was relatively stable during CTRL phase, with average values in the range 2.92-3.25%, and slowly increased from day 50. Conversely, the VS concentration remained relatively stable at 1.64-1.91% during the whole test. The VS/TS ratio decreased from 56.3-57.3 % in the CTRL phase to 52.8-53.2 % with the BC addition (Table 2). The results of the multiple linear regression analysis showed a significant positive relationship between BC concentration and TS content, along with a significant negative relationship between BC and VS/TS (Table 3). Obviously, OLR expressed as sCOD did not significantly influence the TS and VS/TS ratio.

Despite the variability of sCOD in the input, the AD system reached a stable concentration in the output. Overall, the resulting removals of tCOD and sCOD over time were
consistent with the biogas and methane yields (Figure 3C). The trend of tCOD removal decreased from the initial values of phase 1 in the CTRL phase to minimum values in phase 4, followed by an increase from day 70 up to an average of 32 ± 3 % (Table 2) in phase 5. Consistently with the trend of specific methane yield, sCOD removal (Figure 3C) showed a decline from a mean of 65 ± 8 % of phase 1 to 35 ± 4 % of phase 3, rising to 80 ± 2 % in phase 5. Therefore, as for biogas and methane yields, the multiple linear regression analysis found significant positive relationships (b1>0, p1<0.05) for the removals of tCOD or sCOD and OLR as sCOD, due to the relative stability of the output concentrations and the variability of sCOD in the feed. Conversely, there was no evidence of positive effects of the BC supplementation on COD removal.

The removal of TS and of VS (Figure 3D) were in the range 3.8-10.4% and 9.6-22.1% in the CTRL phase, respectively, and in the range 4.5-32.5 % and 3.9-31.3 % with BC supplementation. The multiple linear regression analysis did not find significant linear relationships between solids removals and predictor variables (Table 3). Consistently with biogas and methane productions, BC was not found to enhance the removal of organic matter during the mesophilic semi-continuous AD of mixed sludge. These results differ from those of Wei et al. (2020) who found an increase of 14.9% of VS removal in presence of BC, compared to the control reactor, during the continuous AD of primary sludge.

The pH was stable during the whole AD tests around 6.9-7.2 (Figure 3E), in the optimal range for methanogens (Appels et al., 2008) and indicating a good process stability. The Electrical Conductivity (EC) of the digestate ranged between 4.1 and 5.7 mS cm⁻¹, showing the highest values in phase 5 (5.1 ± 0.1 mS cm⁻¹). However, there was no
evidence of a significant positive effect of the BC concentration on the EC of the digestate (Table 3), despite the relevant contents of cations in the BC (Table 2).

Figure 3F shows the trends of total alkalinity (TA), organic acids and ammonia nitrogen concentrations in the digestate. Organic acids are important intermediates in the AD processes, converted to carbon dioxide and methane by syntrophic acetogens and methanogens, can accumulate with potential inhibitory effects on methanogens. The total alkalinity is an indicator of the buffering capacity of the system, e.g. of the ability of neutralizing organic acids. This is the reason why the control of total alkalinity and organic acids concentrations is crucial for the stability of any AD system. TA ranged from 2700 to 2900 mg l⁻¹ CaCO₃ during CTRL phase, and from 3000 to 3300 mg l⁻¹ CaCO₃ with BC addition. Multiple linear regression analysis found a significant positive relationship between BC concentration and TA in the digestate (b₂>0, p₂<0.05), possibly related to a BC contribution to the buffering capacity of the system. The TA increase is related to the alkalinity of SS550a BC, due to the presence of high contents of K, Ca, Mg, Al in the ashes, as reported by other studies (Shen et al., 2015; Wang et al., 2017; Zhou et al., 2020). The organic acids in the digestate were relatively stable below concentrations of concern, with average values ranging 117-233 mg l⁻¹ of acetic acid.

Despite the variability of sCOD in input, there was not a significant effect of OLR expressed as sCOD on the concentration of organic acids in output (Table 3), consistently with previous results related to sCOD in the digestate. Further, the ratio between organic acids and TA was relatively stable between 0.03 and 0.09. A ratio below 0.4 is generally recommended for stable AD operations (Sri Bala Kameswari et al., 2012; Zhou et al., 2020). Ammonia nitrogen concentration in the digestate (Figure 3F) remained relatively stable around 400-600 mg l⁻¹, below inhibitory concentrations for the AD process (Chen...
et al., 2008). There was no evidence of a significant relationship between BC concentration and ammonia nitrogen in the digestate from the multiple linear regression analysis. Instead, other studies found a reduction of ammonia nitrogen in presence of BC (Shen et al., 2015; Zhang et al., 2019). Ammonia nitrogen, produced during AD through ammonification, could further contribute to the buffering capacity of AD system. A significant positive relationship \((F(1.20) = 30.70, p = 2e^{-5}, R^2 0.61)\) between Ammonia nitrogen and TA was found by the multiple linear regression of the experimental data of this work.

4. Conclusions

Under the considered experimental conditions, the selected biochar didn’t clearly improve the AD of mixed wastewater sludge. The results of the multiple linear regression analysis showed that the average 38\% increase in the specific biogas and methane yields in the presence of BC in phase 5, compared to phase 4, was related to the corresponding 79\% increase in the sludge soluble COD. In conclusion, future research should explore not only the role of BC properties and dosage, but also the influence of the variability of the substrate’s composition, which plays a key role, as shown by this study.

Acknowledgements

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References


7. Chiappero, M., Berruti, F., Mašek, O., Fiore, S., 2021. Analysis of the influence of activated biochar properties on methane production from anaerobic digestion of...


https://doi.org/10.2166/wst.2013.423


https://doi.org/10.1016/j.fuel.2020.119105


https://doi.org/10.1016/j.jclepro.2019.01.106


https://doi.org/10.1016/j.biortech.2019.03.146


https://doi.org/10.1021/acssuschemeng.0c00571

https://doi.org/10.1016/j.jaap.2015.01.025
Figure 1. Configuration of the 3 L stainless-steel reactor: (1) motor and mixer; (2) gas outlet; (3) closing screws; (4) discharging port; (5) silicone stopper; (6) inflow; (7) heater connection; (8) outflow.
Table 1. Chemical characteristics of the mixed sludge, mixed sludge with 10 g L\(^{-1}\) of SS550a BC, and of the digestate. Data expressed as mean ± standard error (number of values).

<table>
<thead>
<tr>
<th></th>
<th>Mixed sludge</th>
<th>Mixed sludge + SS550a (10 g L(^{-1}))</th>
<th>Digestate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH [-]</td>
<td>6.1 ± 0.1 (4)</td>
<td>6.0 ± 0.3 (2)</td>
<td>2.51 ± 0.04 (1)</td>
</tr>
<tr>
<td>TS [%-wt]</td>
<td>3.1 ± 0.1 (4)</td>
<td>3.9 ± 0.1 (2)</td>
<td>1.36 ± 0.03 (1)</td>
</tr>
<tr>
<td>VS [%-wt]</td>
<td>2.0 ± 0.1 (4)</td>
<td>2.2 ± 0.2 (2)</td>
<td>54.3 ± 0.1 (1)</td>
</tr>
<tr>
<td>VS/TS [%-wt]</td>
<td>64 ± 2.0 (4)</td>
<td>55 ± 2.4 (2)</td>
<td>7.33 ± 0.003 (1)</td>
</tr>
<tr>
<td>tCOD [g L(^{-1}) O(_2)]</td>
<td>35 ± 2 (4)</td>
<td>38 ± 6 (2)</td>
<td>21.0 ± 0.7 (1)</td>
</tr>
<tr>
<td>sCOD [g L(^{-1}) O(_2)]</td>
<td>2.0 ± 0.5 (4)</td>
<td>2.6 ± 0.7 (2)</td>
<td>0.34 ± 0.04 (1)</td>
</tr>
</tbody>
</table>
Figure 2. Biogas, methane and carbon dioxide productions during the semi-continuous AD of mixed sludge with and without SS550a biochar: (A) Gas production as NmL d\(^{-1}\); (B) Gas production as NmL g tCOD\(^{-1}\) d\(^{-1}\); (C) Gas production as NmL g VS\(^{-1}\) d\(^{-1}\); (D) CH\(_4\), CO\(_2\), and H\(_2\)S concentration; (E) Organic loading rate as gVS L\(^{-1}\) d\(^{-1}\) and g tCOD L\(^{-1}\) d\(^{-1}\).
Table 2. Summary of the experimental results of the semi-continuous AD test, in each phase, reported as mean (standard error).

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>Phase 2 (day 21-31)</th>
<th>Phase 3 (day 32-49)</th>
<th>Phase 4 (day 50-73)</th>
<th>Phase 5 (day 74-101)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biogas properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas [NmL d^{-1}]</td>
<td>791 (45)</td>
<td>707 (37)</td>
<td>637 (28)</td>
<td>527 (18)</td>
<td>917 (26)</td>
</tr>
<tr>
<td>Biogas [NmL g VS^{-1} d^{-1}]</td>
<td>211 (12)</td>
<td>163 (9)</td>
<td>191 (9)</td>
<td>202 (7)</td>
<td>286 (8)</td>
</tr>
<tr>
<td>Biogas [NmL g COD^{-1} d^{-1}]</td>
<td>125 (7)</td>
<td>92 (5)</td>
<td>108 (5)</td>
<td>109 (4)</td>
<td>157 (4)</td>
</tr>
<tr>
<td>Methane [NmL d^{-1}]</td>
<td>525 (28)</td>
<td>491 (30)</td>
<td>445 (18)</td>
<td>379 (19)</td>
<td>636 (19)</td>
</tr>
<tr>
<td>Methane [NmL g VS^{-1} d^{-1}]</td>
<td>140 (7)</td>
<td>113 (7)</td>
<td>132 (6)</td>
<td>145 (7)</td>
<td>198 (6)</td>
</tr>
<tr>
<td>Methane [NmL g COD^{-1} d^{-1}]</td>
<td>83 (4)</td>
<td>64 (4)</td>
<td>75 (3)</td>
<td>78 (4)</td>
<td>109 (3)</td>
</tr>
<tr>
<td>CO_{2} [NmL d^{-1}]</td>
<td>224 (12)</td>
<td>214 (12)</td>
<td>201 (8)</td>
<td>163 (8)</td>
<td>277 (9)</td>
</tr>
<tr>
<td>CO_{2} [NmL g VS^{-1} d^{-1}]</td>
<td>60 (3)</td>
<td>49 (3)</td>
<td>60 (3)</td>
<td>62 (3)</td>
<td>86 (3)</td>
</tr>
<tr>
<td>CO_{2} [NmL g COD^{-1} d^{-1}]</td>
<td>35 (2)</td>
<td>28 (2)</td>
<td>34 (2)</td>
<td>34 (2)</td>
<td>47 (1)</td>
</tr>
<tr>
<td>CH_{4} [% vv]</td>
<td>67.1 (2.3)</td>
<td>69.0 (0.3)</td>
<td>68.2 (0.2)</td>
<td>68.9 (0.2)</td>
<td>69.1 (0.1)</td>
</tr>
<tr>
<td>CO_{2} [% vv]</td>
<td>28.6 (0.9)</td>
<td>30.1 (0.2)</td>
<td>30.8 (0.1)</td>
<td>29.6 (0.2)</td>
<td>30.1 (0.1)</td>
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<tr>
<td>H_{2}S [ppm vv]</td>
<td>0.8 (0.3)</td>
<td>1.4 (0.2)</td>
<td>9.5 (1.4)</td>
<td>2.7 (0.6)</td>
<td>6.4 (0.9)</td>
</tr>
<tr>
<td><strong>Digestate properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS [%]</td>
<td>2.9 (0.1)</td>
<td>3.2 (0.2)</td>
<td>3.3 (0.1)</td>
<td>3.5 (0.1)</td>
<td>3.6 (0.1)</td>
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<tr>
<td>VS [%]</td>
<td>1.6 (0.04)</td>
<td>1.8 (0.1)</td>
<td>1.9 (0.1)</td>
<td>1.9 (0.02)</td>
<td>1.9 (0.1)</td>
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<td>VS/TS [%]</td>
<td>56.3 (0.1)</td>
<td>57.0 (0.8)</td>
<td>57.3 (0.2)</td>
<td>53.2 (1.0)</td>
<td>52.8 (0.5)</td>
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<td>pH [-]</td>
<td>7.2 (0.1)</td>
<td>7.1 (0.1)</td>
<td>6.9 (0.1)</td>
<td>7.1 (0.1)</td>
<td>7.1 (0.03)</td>
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<tr>
<td>tCOD [g L^{-1}]</td>
<td>22 (4)</td>
<td>30 (3)</td>
<td>32 (1)</td>
<td>30.5 (0.4)</td>
<td>32 (1)</td>
</tr>
<tr>
<td>sCOD [mg L^{-1}]</td>
<td>541 (128)</td>
<td>637 (51)</td>
<td>730 (43)</td>
<td>645 (43)</td>
<td>675 (53)</td>
</tr>
<tr>
<td>Ammonia Nitrogen [mg L^{-1}]</td>
<td>505 (3)</td>
<td>442 (29)</td>
<td>463 (6)</td>
<td>505 (19)</td>
<td>534 (17)</td>
</tr>
<tr>
<td>Organic Acids [mg L^{-1} CH_{3}COOH]</td>
<td>117 (13)</td>
<td>204 (23)</td>
<td>233 (16)</td>
<td>200 (15)</td>
<td>208 (21)</td>
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<tr>
<td>Electrical conductivity [mS cm^{-1}]</td>
<td>4.5 (0.1)</td>
<td>4.1 (0.1)</td>
<td>4.4 (0.2)</td>
<td>4.6 (0.1)</td>
<td>5.1 (0.1)</td>
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<td>Total Alkalinity [mg L^{-1} CaCO_{3}]</td>
<td>2900 (25)</td>
<td>2764 (145)</td>
<td>2721 (51)</td>
<td>2997 (108)</td>
<td>3293 (47)</td>
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<tr>
<td>TS removal [%]</td>
<td>5 (2)</td>
<td>9 (2)</td>
<td>5 (1)</td>
<td>12 (4)</td>
<td>16 (4)</td>
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<tr>
<td>VS removal [%]</td>
<td>12 (2)</td>
<td>21 (1)</td>
<td>17 (1)</td>
<td>11 (3)</td>
<td>21 (3)</td>
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<tr>
<td>tCOD removal [%]</td>
<td>29 (11)</td>
<td>28 (1)</td>
<td>20 (1)</td>
<td>10 (6)</td>
<td>32 (3)</td>
</tr>
<tr>
<td>sCOD removal [%]</td>
<td>65 (8)</td>
<td>43 (5)</td>
<td>35 (4)</td>
<td>67 (4)</td>
<td>80 (2)</td>
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Table 3. Results of multiple linear regression to predict biogas and anaerobic digestate parameters based on OLR as sCOD (g sCOD L\(^{-1}\) d\(^{-1}\)) and biochar concentration (g L\(^{-1}\)). The linear model is expressed in the form \(y = b_0 + b_1 x_1 + b_2 x_2\), where \(y\) is the estimated parameter, \(x_1\) is the OLR, \(x_2\) is the BC concentration.

<table>
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<tr>
<th>Dependent variable (y)</th>
<th>df regression</th>
<th>df residuals</th>
<th>F</th>
<th>p</th>
<th>R(^2)</th>
<th>(b_0)</th>
<th>p(_0)</th>
<th>(b_1)</th>
<th>p(_1)</th>
<th>(b_2)</th>
<th>p(_2)</th>
</tr>
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<tbody>
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<td>Biogas properties</td>
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<tr>
<td>Biogas [NmL d(^{-1})]</td>
<td>2</td>
<td>61</td>
<td>68.96</td>
<td>2.2E-16</td>
<td>0.693</td>
<td>330.5</td>
<td>6.6E-13</td>
<td>4898.3</td>
<td>3.8E-17</td>
<td>-24.60</td>
<td>3.4E-09</td>
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<tr>
<td>Biogas [NmL gvs(^{-1}) d(^{-1})]</td>
<td>2</td>
<td>61</td>
<td>50.94</td>
<td>9.8E-14</td>
<td>0.625</td>
<td>111.4</td>
<td>3.4E-13</td>
<td>999.6</td>
<td>1.1E-09</td>
<td>0.24</td>
<td>0.84</td>
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<tr>
<td>Methane [NmL d(^{-1})]</td>
<td>2</td>
<td>36</td>
<td>48.52</td>
<td>6.0E-11</td>
<td>0.729</td>
<td>245.5</td>
<td>2.7E-10</td>
<td>3182.7</td>
<td>1.7E-11</td>
<td>-15.13</td>
<td>5.6E-06</td>
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<tr>
<td>Methane [NmL gvs(^{-1}) d(^{-1})]</td>
<td>2</td>
<td>36</td>
<td>37.76</td>
<td>1.4E-09</td>
<td>0.677</td>
<td>83.0</td>
<td>3.0E-10</td>
<td>621.3</td>
<td>3.0E-06</td>
<td>0.81</td>
<td>0.41</td>
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<td>CO(_2) [NmL d(^{-1})]</td>
<td>2</td>
<td>36</td>
<td>42.47</td>
<td>3.4E-10</td>
<td>0.702</td>
<td>110.3</td>
<td>4.2E-10</td>
<td>1372.6</td>
<td>7.8E-11</td>
<td>-6.86</td>
<td>6.5E-06</td>
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<tr>
<td>CO(_2) [NmL gvs(^{-1}) d(^{-1})]</td>
<td>2</td>
<td>36</td>
<td>31.49</td>
<td>1.2E-08</td>
<td>0.636</td>
<td>37.0</td>
<td>6.3E-10</td>
<td>269.3</td>
<td>7.8E-06</td>
<td>0.24</td>
<td>0.59</td>
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<tr>
<td>CH(_4) [% vv]</td>
<td>2</td>
<td>36</td>
<td>1.63</td>
<td>0.21</td>
<td>0.083</td>
<td>68.3</td>
<td>1.8E-45</td>
<td>-1.8</td>
<td>0.82</td>
<td>0.10</td>
<td>0.16</td>
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<tr>
<td>CO(_2) [% vv]</td>
<td>2</td>
<td>36</td>
<td>0.41</td>
<td>0.67</td>
<td>0.022</td>
<td>30.3</td>
<td>6.3E-41</td>
<td>-2.3</td>
<td>0.64</td>
<td>-0.01</td>
<td>0.84</td>
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<tr>
<td>H(_2)S [ppm vv]</td>
<td>2</td>
<td>36</td>
<td>0.12</td>
<td>0.89</td>
<td>0.006</td>
<td>4.4</td>
<td>0.03</td>
<td>9.3</td>
<td>0.68</td>
<td>-0.09</td>
<td>0.64</td>
</tr>
<tr>
<td>Digestate properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TS [%]</td>
<td>2</td>
<td>20</td>
<td>10.53</td>
<td>7.5E-04</td>
<td>0.513</td>
<td>3.2</td>
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<td>-1.0</td>
<td>0.57</td>
<td>0.05</td>
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<tr>
<td>VS [%]</td>
<td>2</td>
<td>20</td>
<td>1.62</td>
<td>0.22</td>
<td>0.140</td>
<td>1.8</td>
<td>2.1E-15</td>
<td>-0.5</td>
<td>0.60</td>
<td>0.01</td>
<td>0.13</td>
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<tr>
<td>VS/TS [%]</td>
<td>2</td>
<td>20</td>
<td>38.54</td>
<td>1.4E-07</td>
<td>0.794</td>
<td>56.9</td>
<td>1.6E-26</td>
<td>0.4</td>
<td>0.96</td>
<td>-0.43</td>
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</tr>
<tr>
<td>pH [-]</td>
<td>2</td>
<td>20</td>
<td>0.56</td>
<td>0.58</td>
<td>0.053</td>
<td>7.0</td>
<td>3.9E-26</td>
<td>1.0</td>
<td>0.35</td>
<td>-0.004</td>
<td>0.69</td>
</tr>
<tr>
<td>tCOD [g L(^{-1})]</td>
<td>2</td>
<td>20</td>
<td>1.25</td>
<td>0.31</td>
<td>0.111</td>
<td>30.4</td>
<td>1.4E-10</td>
<td>-21.7</td>
<td>0.47</td>
<td>0.38</td>
<td>0.15</td>
</tr>
<tr>
<td>sCOD [mg L(^{-1})]</td>
<td>2</td>
<td>20</td>
<td>0.06</td>
<td>0.94</td>
<td>0.006</td>
<td>678.7</td>
<td>7.4E-08</td>
<td>-333.2</td>
<td>0.73</td>
<td>2.19</td>
<td>0.79</td>
</tr>
<tr>
<td>Ammonia Nitrogen [mg L(^{-1})]</td>
<td>2</td>
<td>20</td>
<td>6.80</td>
<td>0.01</td>
<td>0.405</td>
<td>438.3</td>
<td>7.2E-14</td>
<td>410.3</td>
<td>0.16</td>
<td>3.11</td>
<td>0.21</td>
</tr>
<tr>
<td>Organic Acids [mg L(^{-1})]</td>
<td>2</td>
<td>19</td>
<td>0.50</td>
<td>0.61</td>
<td>0.050</td>
<td>218.8</td>
<td>2.1E-06</td>
<td>-350.2</td>
<td>0.37</td>
<td>3.04</td>
<td>0.37</td>
</tr>
<tr>
<td>CH(_3)COOH]</td>
<td>2</td>
<td>20</td>
<td>18.35</td>
<td>3.0E-05</td>
<td>0.647</td>
<td>3.9</td>
<td>2.4E-16</td>
<td>5.0</td>
<td>0.02</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Electrical conductivity [mS cm(^{-1})]</td>
<td>2</td>
<td>19</td>
<td>26.41</td>
<td>3.3E-06</td>
<td>0.735</td>
<td>2531.9</td>
<td>1.2E-16</td>
<td>3201.1</td>
<td>0.01</td>
<td>23.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Total Alkalinity [mg L(^{-1}) CaCO(_3)]</td>
<td>2</td>
<td>18</td>
<td>1.39</td>
<td>0.28</td>
<td>0.133</td>
<td>4.7</td>
<td>0.37</td>
<td>42.1</td>
<td>0.47</td>
<td>0.28</td>
<td>0.58</td>
</tr>
<tr>
<td>TS removal [%]</td>
<td>2</td>
<td>18</td>
<td>2.00</td>
<td>0.16</td>
<td>0.182</td>
<td>8.3</td>
<td>0.08</td>
<td>98.1</td>
<td>0.06</td>
<td>-0.51</td>
<td>0.25</td>
</tr>
<tr>
<td>VS removal [%]</td>
<td>2</td>
<td>18</td>
<td>5.81</td>
<td>0.01</td>
<td>0.392</td>
<td>1.6</td>
<td>0.82</td>
<td>278.4</td>
<td>0.003</td>
<td>-1.59</td>
<td>0.04</td>
</tr>
<tr>
<td>sCOD removal [%]</td>
<td>2</td>
<td>20</td>
<td>23.23</td>
<td>6.1E-06</td>
<td>0.699</td>
<td>30.8</td>
<td>1.3E-04</td>
<td>229.2</td>
<td>0.01</td>
<td>1.32</td>
<td>0.06</td>
</tr>
</tbody>
</table>
**Figure 3.** Characteristics of the digestate during the AD test: (A) total and soluble COD; (B) Total Solids and Volatile Solids; (C) Removals of total COD and soluble COD; (D) Removals of Total Solids and Volatile Solids; (E) pH and Electrical Conductivity; (F) Total Alkalinity, Organic Acids, Ammonia Nitrogen.
Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: