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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. In conclusion, in the considered experimental conditions, the variability of the substrate's composition was the key factor driving the performances of the AD of MS, independently of the addition of BC.

**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 (Alenzi et al., 2021; Mohammad Mirsoleimani Azizi et al., 2021). 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) (Lu et al., 2020), 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; S. 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 (e.g., mixing primary and secondary sludge and accounting the variability of the characteristics of the sludge sampled in a certain WWTP), 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 over 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 as discussed in Section 2.2 (Chiappero et al., 2021, Chiappero et al., *under review*). 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 in a continuously fed rotary kiln pyrolyzer (inner diameter 0.244 m, heated length 2.8 m) with mean residence time of 30 min. Subsequently, the BC underwent physical activation with $CO_2$ at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR) at Western University, Canada. Physical activation was performed in a horizontal 316 stainless steel tubular reactor 19 mm in diameter and 0.9 m long. The biochar was placed between two stainless-steel woven mesh pads and the activation was carried out in a furnace at 900°C with a constant $CO_2$ flow rate of 200 mL/min and a holding time of 60 minutes. 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).

*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 after vigorous mixing, 0.150 L of MS at 3 % of TS load 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 and an OLR in the range 0.9-1.4 $g_{VS}$ $L^{-1}$ $d^{-1}$. 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. This BC dose, recently used by other studies on the AD of wastewater sludge (Kaur et al., 2020; Liang et al., 2021; Wang et al., 2020) was selected based on the results of previous mesophilic AD batch tests performed on sludge sampled in the same WWTP and supplementing the same SS550s

BC, where it was found to enhance the methane yield from MS by 22 % (Chiappero et al., *under review*) and from waste activated sludge by 17 % (Chiappero et al., 2021), in order to investigate its effects on AD in semi-continuous feeding mode.

**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$^{Plus}$ 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 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.

*2.5. Preliminary economic analysis*

A simplified economic analysis was carried out to estimate the maximum unit cost of BC (USD $kg^{-1}$) that equals the higher revenues deriving from BC supplementation to the AD process. The input data of the AD process (Appendix) correspond to real tested conditions from the present study and recent literature, comparing five AD scenarios (PS, MS, food waste, and co-digestion of sewage sludge), where BC was supplemented to the continuous or semi-continuous digesters, at lab- or pilot-scale. Considering the costs, the simplified analysis was based exclusively on the cost of the BC; the capital costs and operational costs associated with the eventual improvement of the mixing inside the digester (due to the TS increase caused by BC) were not involved. Considering the revenues, it was assumed that they derive from the "extra" (compared to the performances of AD without any supplement) energy obtained with BC addition, with production of electrical energy in a combined heat and power (CHP) unit and supplying the thermal energy to the plant's energy needs. In details, the extra methane production was calculated as difference between the yield of BC amended reactors minus that of control reactors. The net economic profit was estimated given the methane lower heating value, the electrical energy efficiency of the CHP, and the average EU-27 electricity price for non-household consumers (Appendix).

## 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 (Souza et al., 2021). 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 (Kaur et al., 2020). 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., 2020).

**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) $CH_4$, $CO_2$, and $H_2S$ 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 $g_{VS}^{-1}$ $d^{-1}$, then increased to 191 ± 9 NmL $g_{VS}^{-1}$ $d^{-1}$ (phase 3), remaining almost stable during phase 4, and finally jumped up to 286 ± 8 NmL $g_{VS}^{-1}$ $d^{-1}$ 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 $g_{VS}^{-1}$ $d^{-1}$), followed by a slow rise (up to 145 ± 7 NmL $g_{VS}^{-1}$ $d^{-1}$) in phase 4 and a marked increase to 198 ± 6 NmL $g_{VS}^{-1}$ $d^{-1}$ in phase 5.

Similarly, specific biogas and methane yields expressed as NmL g $_{tCOD}^{-1}$ $d^{-1}$ (Figure 2C) confirmed the trends shown in Figure 2B, with minimum average values in phase 2 (92 ± 5 and 64 ± 4 NmL g $_{tCOD}^{-1}$ $d^{-1}$ respectively), and maximum values (157 ± 4 and 109 ± 3 NmL g $_{tCOD}^{-1}$ $d^{-1}$) 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 $CH_4$ and $CO_2$ 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 $H_2S$ 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 asVS 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 $g_{VS}^{-1}d^{-1}$, 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 $y = b_0 + b_1 x_1 + b_2 x_2$, where y is the estimated parameter, $x_1$ is the OLR, $x_2$ 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 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 $kg_{VS}^{-1}d^{-1}$) 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 $R^2$ of 0.60, and between the biogas yield and the sCOD/tCOD (%) ($F(1.63) = 93.87$, $p = 4.2$ e-14) with an $R^2$ of 0.62. As for biogas, significant linear relationships between the specific methane yield expressed as NmL $kg_{VS}^{-1}d^{-1}$ 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 $R^2$ 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 ($F(1.63) = 79.17$, $p = 1.0$ e-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 sCOD $L^{-1}$ $d^{-1}$ and BC concentration (g $L^{-1}$) 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 (NmL $d^{-1}$) were significant, with $R^2$ equal to 0.69 and 0.73, respectively. While significant positive relationships ($b_1>0$, $p_1<0.05$) for biogas or methane yields and OLR were confirmed, there were significant negative relationships ($b_2< 0$, $p_2<0.05$) with BC concentration. Further, positive linear regressions were found between the specific biogas and methane yields (NmL $gVS^{-1}$ $d^{-1}$) and the OLR. However, there was 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 $CH_4$ and $CO_2$ 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 $CO_2$ 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 $CO_2$ sequestration were suggested as main mechanisms. Compared to the BCs used in the two mentioned studies, SS550a presents comparable specific surface area (109.2 $m^2$ $g^{-1}$), micro-porous structure (total pore volume 0.169 $cm^3$ $g^{-1}$, 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 $BC/g_{VS}$ added, and 0.32 ± 0.01 g $BC/g_{TS}$), being 3.5-7 times lower than those of the other studies, with an optimum of 1.75-3.5 g $BC/g_{VS}$ (Shen et al., 2017) and 1.82 g $BC/g_{TS}$ (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. The obtained tCOD removal values in phase 5 (Table 2) are consistent with literature, where tCOD removals are in the range 34-55 % (Astals et al., 2012; Choi et al., 2018; Hidaka et al., 2013). 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. 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 (Xu et al., 2020) 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 $l^{-1}$ $CaCO_3$ during CTRL phase, and from 3000 to 3300 mg $l^{-1}$ $CaCO_3$ with BC addition. Multiple linear regression analysis found a significant positive relationship between BC concentration and TA in the digestate ($b_2>0$, $p_2<0.05$), 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 (Ahmed et al., 2021; 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 (Jiang et al., 2019). 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.

*3.4. Preliminary economic analysis*

The key question is whether the economic benefits deriving from BC addition exceed its cost. The main benefit is the extra (compared to AD without any supplement) methane yield, resulting in higher incomes from the sale of electricity. However, extra costs derive from the supplementation of BC. A simplified economic analysis (section 2.5) estimated the maximum unit cost of BC that equals the profits from the enhanced methane yield in different scenarios. In this study (semi-continuous AD of MS at 37 °C), an enhanced methane yield of 22 % due to a dose of 10 g $L^{-1}$ could be cost-effective with a unit cost of BC below 0.043 USD $kg^{-1}$. This value is borderline with respect of a typical range cost for BC of 0.05 - 0.5 USD $kg^{-1}$ (Chiappero et al., 2020; Chiappero et al., under review). Other studies presented different results (see Appendix) depending on OLR, enhancement of methane yield, and BC dose. For instance, values ranging 0.017-0.022 USD $kg^{-1}$ were found for the AD of MS, due to similar enhancements of methane yield (+ 21-27 %) but larger doses of BC (18.75 g $L^{-1}$). Conversely, the AD of PS resulted in a higher maximum unit cost of 0.184 USD $kg^{-1}$, due to the larger OLR and the lower dose of BC. Further, promising results were obtained for other scenarios regarding the AD of food waste and co-digestion of sewage sludge and orange peel with maximum BC unit costs of 0.329 and 0.524 USD $kg^{-1}$, respectively, where the authors found larger improvements of $CH_4$ yields (38 % and 61 %) by adding similar doses of BC (15 and 10 g $L^{-1}$). Overall, the optimization of BC dosage may be a key step towards the economic feasibility of BC supplementation in AD.

## 4. Conclusions

This research proved that, under the considered experimental conditions, the sCOD of the substrate was the driving factor for the performances of the AD of MS, independently of BC addition. This happens particularly in the AD of MS (because of the high variability of the quality of PS), compared to the digestion of WAS, whose composition is less variable. The same BC, tested in batch AD of sludge from the same WWTP, was able to improve of 17% the methane yields obtained from WAS (Chiappero et al., 2021) and of 22% from MS (Chiappero et al. under review).

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**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).

| | Mixed sludge | Mixed sludge + SS550a (10 g $l^{-1}$) | Digestate |
|---|---|---|---|
| pH [-] | 6.1 ± 0.1 (4) | 6.0 ± 0.3 (2) | 7.33 ± 0.003 (1) |
| TS [%-wt] | 3.1 ± 0.1 (4) | 3.9 ± 0.1 (2) | 2.51 ± 0.04 (1) |
| VS [%-wt] | 2.0 ± 0.1 (4) | 2.2 ± 0.2 (2) | 1.36 ± 0.03 (1) |
| VS/TS [%-wt] | 64 ± 2.0 (4) | 55 ± 2.4 (2) | 54.3 ± 0.1 (1) |
| tCOD [g $L^{-1}$ $O_2$] | 35 ± 2 (4) | 38 ± 6 (2) | 21.0 ± 0.7 (1) |
| sCOD [g $L^{-1}$ $O_2$] | 2.0 ± 0.5 (4) | 2.6 ± 0.7 (2) | 0.34 ± 0.04 (1) |

**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 $H_2S$ concentration; (E) Organic loading rate as gVS $L^{-1}$ $d^{-1}$ and g tCOD $L^{-1}$ $d^{-1}$

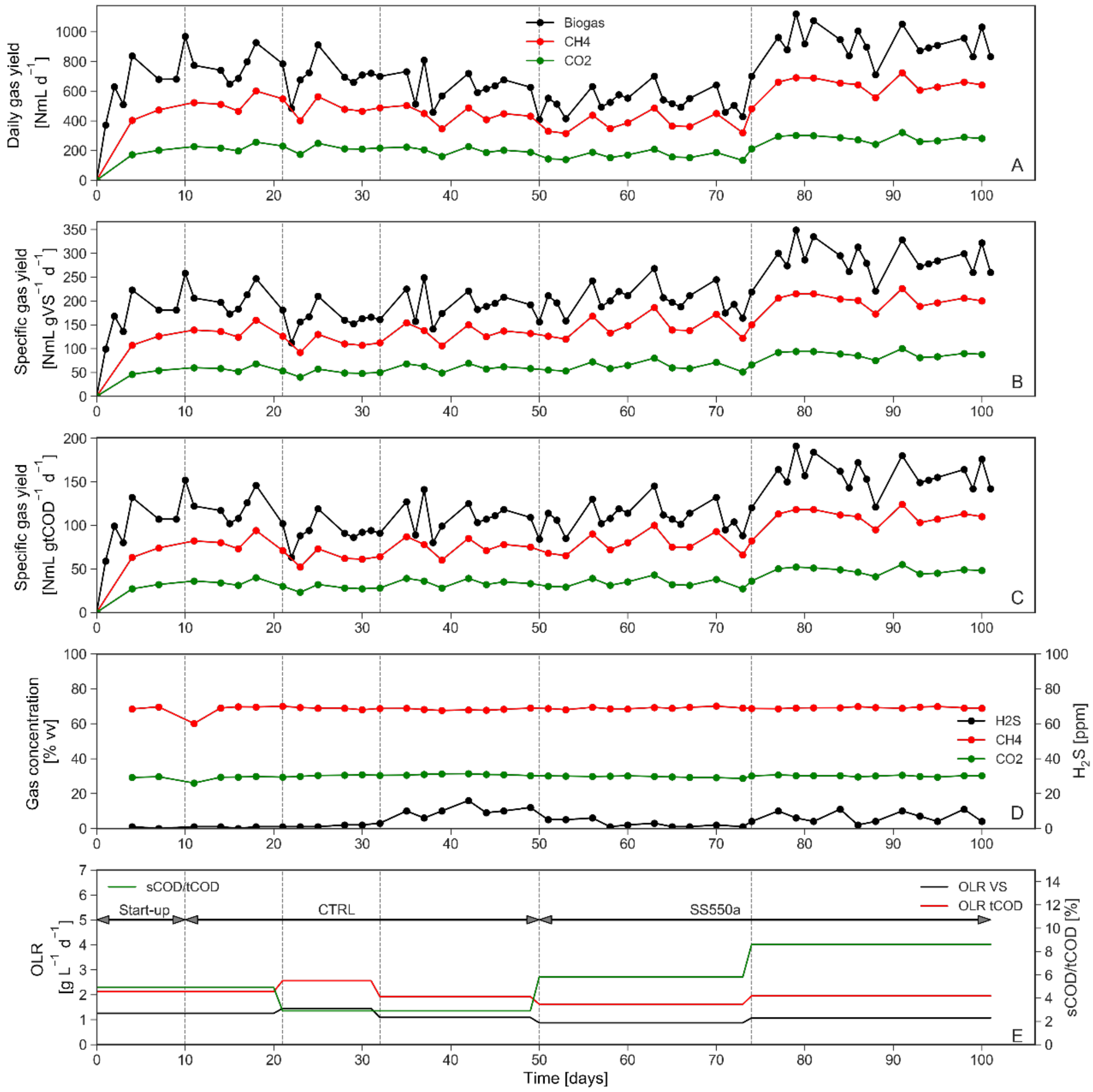

**Table 2.** Summary of the experimental results of the semi-continuous AD test, in each phase, reported as mean (standard error).

| | | CTRL | | | SS550a | |
|---|---|---|---|---|---|---|
| | | Phase 1 (day 10-20) | Phase 2 (day 21-31) | Phase 3 (day 32-49) | Phase 4 (day 50-73) | Phase 5 (day 74-101) |
| Biogas properties | Biogas [NmL $d^{-1}$] | 791 (45) | 707 (37) | 637 (28) | 527 (18) | 917 (26) |
| | Biogas [NmL $g_{VS}^{-1}$ $d^{-1}$] | 211 (12) | 163 (9) | 191 (9) | 202 (7) | 286 (8) |
| | Biogas [NmL $g_{COD}^{-1}$ $d^{-1}$] | 125 (7) | 92 (5) | 108 (5) | 109 (4) | 157 (4) |
| | Methane [NmL $d^{-1}$] | 525 (28) | 491 (30) | 445 (18) | 379 (19) | 636 (19) |
| | Methane [NmL $gVS^{-1}$ $d^{-1}$] | 140 (7) | 113 (7) | 132 (6) | 145 (7) | 198 (6) |
| | Methane [NmL $gCOD^{-1}$ $d^{-1}$] | 83 (4) | 64 (4) | 75 (3) | 78 (4) | 109 (3) |
| | $CO_2$ [NmL $d^{-1}$] | 224 (12) | 214 (12) | 201 (8) | 163 (8) | 277 (9) |
| | $CO_2$ [NmL $g_{VS}^{-1}$ $d^{-1}$] | 60 (3) | 49 (3) | 60 (3) | 62 (3) | 86 (3) |
| | $CO_2$ [NmL $g_{COD}^{-1}$ $d^{-1}$] | 35 (2) | 28 (2) | 34 (2) | 34 (2) | 47 (1) |
| | $CH_4$ [% vv] | 67.1 (2.3) | 69.0 (0.3) | 68.2 (0.2) | 68.9 (0.2) | 69.1 (0.1) |
| | $CO_2$ [% vv] | 28.6 (0.9) | 30.1 (0.2) | 30.8 (0.1) | 29.6 (0.2) | 30.1 (0.1) |
| | $H_2S$ [ppm vv] | 0.8 (0.3) | 1.4 (0.2) | 9.5 (1.4) | 2.7 (0.6) | 6.4 (0.9) |
| Digestate properties | TS [%] | 2.9 (0.1) | 3.2 (0.2) | 3.3 (0.1) | 3.5 (0.1) | 3.6 (0.1) |
| | VS [%] | 1.6 (0.04) | 1.8 (0.1) | 1.9 (0.1) | 1.9 (0.02) | 1.9 (0.1) |
| | VS/TS [%] | 56.3 (0.1) | 57.0 (0.8) | 57.3 (0.2) | 53.2 (1.0) | 52.8 (0.5) |
| | pH [-] | 7.2 (0.1) | 7.1 (0.1) | 6.9 (0.1) | 7.1 (0.1) | 7.1 (0.03) |
| | tCOD [g $L^{-1}$] | 22 (4) | 30 (3) | 32 (1) | 30.5 (0.4) | 32 (1) |
| | sCOD [mg $L^{-1}$] | 541 (128) | 637 (51) | 730 (43) | 645 (43) | 675 (53) |
| | Ammonia Nitrogen [mg $L^{-1}$] | 505 (3) | 442 (29) | 463 (6) | 505 (19) | 534 (17) |
| | Organic Acids [mg $L^{-1}$ $CH_3COOH$] | 117 (13) | 204 (23) | 233 (16) | 200 (15) | 208 (21) |
| | Electrical conductivity [mS $cm^{-1}$] | 4.5 (0.1) | 4.1 (0.1) | 4.4 (0.2) | 4.6 (0.1) | 5.1 (0.1) |
| | Total Alkalinity [mg $L^{-1}$ $CaCO_3$] | 2900 (25) | 2764 (145) | 2721 (51) | 2997 (108) | 3293 (47) |
| | TS removal [%] | 5 (2) | 9 (2) | 5 (1) | 12 (4) | 16 (4) |
| | VS removal [%] | 12 (2) | 21 (1) | 17 (1) | 11 (3) | 21 (3) |
| | tCOD removal [%] | 29 (11) | 28 (1) | 20 (1) | 10 (6) | 32 (3) |
| | sCOD removal [%] | 65 (8) | 43 (5) | 35 (4) | 67 (4) | 80 (2) |

**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.

| | Dependent variable (y) | df regression | df residuals | F | p | $R^2$ | $b_o$ | $p_0$ | $b_1$ | $p_1$ | $b_2$ | $p_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biogas properties | Biogas [NmL $d^{-1}$] | 2 | 61 | 68.96 | 2.2E-16 | 0.693 | 330.5 | 6.6E-13 | 4898.3 | 3.8E-17 | -24.60 | 3.4E-09 |
| | Biogas [NmL $g_{VS}^{-1}$ $d^{-1}$] | 2 | 61 | 50.94 | 9.8E-14 | 0.625 | 111.4 | 3.4E-13 | 999.6 | 1.1E-09 | 0.24 | 0.84 |
| | Methane [NmL $d^{-1}$] | 2 | 36 | 48.52 | 6.0E-11 | 0.729 | 245.5 | 2.7E-10 | 3182.7 | 1.7E-11 | -15.13 | 5.6E-06 |
| | Methane [NmL $g_{VS}^{-1}$ $d^{-1}$] | 2 | 36 | 37.76 | 1.4E-09 | 0.677 | 83.0 | 3.0E-10 | 621.3 | 3.0E-06 | 0.81 | 0.41 |
| | $CO_2$ [NmL $d^{-1}$] | 2 | 36 | 42.47 | 3.4E-10 | 0.702 | 110.3 | 4.2E-10 | 1372.6 | 7.8E-11 | -6.86 | 6.5E-06 |
| | $CO_2$ [NmL $g_{VS}^{-1}$ $d^{-1}$] | 2 | 36 | 31.49 | 1.2E-08 | 0.636 | 37.0 | 6.3E-10 | 269.3 | 7.8E-06 | 0.24 | 0.59 |
| | $CH_4$ [% vv] | 2 | 36 | 1.63 | 0.21 | 0.083 | 68.3 | 1.8E-45 | -1.8 | 0.82 | 0.10 | 0.16 |
| | $CO_2$ [% vv] | 2 | 36 | 0.41 | 0.67 | 0.022 | 30.3 | 6.3E-41 | -2.3 | 0.64 | -0.01 | 0.84 |
| | $H_2S$ [ppm vv] | 2 | 36 | 0.12 | 0.89 | 0.006 | 4.4 | 0.03 | 9.3 | 0.68 | -0.09 | 0.64 |
| Digestate properties | TS [%] | 2 | 20 | 10.53 | 7.5E-04 | 0.513 | 3.2 | 1.3E-15 | -1.0 | 0.57 | 0.05 | 0.002 |
| | VS [%] | 2 | 20 | 1.62 | 0.22 | 0.140 | 1.8 | 2.1E-15 | -0.5 | 0.60 | 0.01 | 0.13 |
| | VS/TS [%] | 2 | 20 | 38.54 | 1.4E-07 | 0.794 | 56.9 | 1.6E-26 | 0.4 | 0.96 | -0.43 | 6.1E-06 |
| | pH [-] | 2 | 20 | 0.56 | 0.58 | 0.053 | 7.0 | 3.9E-26 | 1.0 | 0.35 | -0.004 | 0.69 |
| | tCOD [g $L^{-1}$] | 2 | 20 | 1.25 | 0.31 | 0.111 | 30.4 | 1.4E-10 | -21.7 | 0.47 | 0.38 | 0.15 |
| | sCOD [mg $L^{-1}$] | 2 | 20 | 0.06 | 0.94 | 0.006 | 678.7 | 7.4E-08 | -333.2 | 0.73 | 2.19 | 0.79 |
| | Ammonia Nitrogen [mg $L^{-1}$] | 2 | 20 | 6.80 | 0.01 | 0.405 | 438.3 | 7.2E-14 | 410.3 | 0.16 | 3.11 | 0.21 |
| | Organic Acids [mg $L^{-1}$ $CH_3COOH$] | 2 | 19 | 0.50 | 0.61 | 0.050 | 218.8 | 2.1E-06 | -350.2 | 0.37 | 3.04 | 0.37 |
| | Electrical conductivity [mS $cm^{-1}$] | 2 | 20 | 18.35 | 3.0E-05 | 0.647 | 3.9 | 2.4E-16 | 5.0 | 0.02 | 0.03 | 0.08 |
| | Total Alkalinity [mg $L^{-1}$ $CaCO_3$] | 2 | 19 | 26.41 | 3.3E-06 | 0.735 | 2531.9 | 1.2E-16 | 3201.1 | 0.01 | 23.23 | 0.03 |
| | TS removal [%] | 2 | 18 | 1.39 | 0.28 | 0.133 | 4.7 | 0.37 | 42.1 | 0.47 | 0.28 | 0.58 |
| | VS removal [%] | 2 | 18 | 2.00 | 0.16 | 0.182 | 8.3 | 0.08 | 98.1 | 0.06 | -0.51 | 0.25 |
| | tCOD removal [%] | 2 | 18 | 5.81 | 0.01 | 0.392 | 1.6 | 0.82 | 278.4 | 0.003 | -1.59 | 0.04 |
| | sCOD removal [%] | 2 | 20 | 23.23 | 6.1E-06 | 0.699 | 30.8 | 1.3E-04 | 229.2 | 0.01 | 1.32 | 0.06 |

**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.