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# Analysis of the influence of activated biochar properties on methane production from anaerobic digestion of waste activated sludge

Marco Chiappero[a], Franco Berruti[b], Ondřej Mašek[c], Silvia Fiore[a] *

[a] DIATI (Department of Engineering for Environment, Land and Infrastructures), Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy

[b] Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Department of Chemical and Biochemical Engineering, Faculty of Engineering, Western University, London, Ontario, N6A 5B9, Canada

[c] UK Biochar Research Centre (UKBRC), School of GeoSciences, University of Edinburgh, King's Buildings, Edinburgh EH9 3JN, United Kingdom

***corresponding author**: S. Fiore (silvia.fiore@polito.it)

## Highlights

- Biochar affected the anaerobic digestion of waste activated sludge at 37 °C
- Activated biochar increased methane yield while not influencing biogas yield
- $CO_2$ adsorption and the sequestration of organic acids were estimated

## Abstract

This work describes the impacts of biochar (BC) addition to the anaerobic digestion (AD) of waste activated sludge (WAS). Three BCs, produced by pyrolysis at 550 °C of different waste biomasses (soft wood, sewage sludge and rice husk), then physically activated at 900 °C with $CO_2$, were investigated as additives. AD tests were performed in batch mode at 37 °C, feeding 2% total solids and 10 g $L^{-1}$ BC. While none of the considered BCs increased biogas yield compared to control digesters, the activated BCs with higher surface area, porosity and hydrophobicity (RH550a from rice husk and SS550a from sewage sludge) boosted methane yield (up to 105% for RH550a). The experimental methane production were: 0.037 $Nm^3$ $kgVS^{-1}$ for SWP550a, 0.081 $Nm^3$ $kgVS^{-1}$ for SS550a, 0.142 $Nm^3$ $kgVS^{-1}$ for RH550a and 0.069 $Nm^3$ $kgVS^{-1}$ for control reactors. $CO_2$ adsorption (3.14 mmol $g^{-1}$ for RH550a, 0.97 mmol $g^{-1}$ SS550a) calculated from experimental data was consistent with literature (0.4-2.3 mmol $g^{-1}$ BC). The fitting of experimental methane productions through the modified Gompertz equation showed an acceleration of methane production for all BCs, with a reduction of the lag phase compared to control reactors (0.5 days vs 2.6 days). This work, although confirming literature data about $CO_2$ adsorption, brings new insights on the influence of specific physico-chemical properties of BC as additive in AD of WAS. Surface area, porosity, hydrophobicity and alkali and alkaline metals content in ashes were the most important BC properties affecting AD of activated sewage sludge.

## Keywords

additive; biogas; fermentation; sewer sludge; wastewater

## 1. Introduction

Wastewater sludge management is challenging both inside and outside wastewater treatment plants (WWTPs). Inside WWTP facilities, sludge management could represent up to 50% of operating costs [1] and 40% of total greenhouse gas emissions [2]. Anaerobic digestion (AD) of wastewater sludge produces biogas (55-75% v/v methane and 25-45% v/v $CO_2$), which when converted into heat and/or electricity can partially cover the energy requirements of a WWTP [3,4]. Therefore, the optimization of sludge AD is crucial for the energy self-sufficiency of a WWTP [5]. Outside WWTP facilities, sludge disposal in EU-27 mostly involves agricultural reuse (44%), incineration (22%), composting (15%), landfilling (11%) and others (8%) [6,7].

Primary sludge (PS) mainly consists of settleable solids, whereas, waste activated sludge (WAS) (i.e. secondary sludge) is made of flocs of bacterial cells and extra-polymeric substances (EPS), more difficult to hydrolyse and biodegrade than PS [8]. PS and WAS, depending on the size of the WWTP and on the outline of the sludge line, are generally mixed in AD (up to medium-size plants) or kept separated (usually in large plants) [9]. To improve AD performances, specifically to accelerate the initial hydrolysis and consequently enhance methane production, various physical, chemical and biological pre-treatment technologies have been proposed to disintegrate WAS cell walls and solubilize lighter organic compounds [10,11]. However, most technologies require energy and/or chemicals or are mainly implemented at lab or pilot scale [8].

Biochar (BC, i.e., the solid residue derived from biomass through pyrolysis or gasification) was studied as additive in the AD of large variety of substrates [12,13], as a result of its large surface area and porous structure, ion exchange capacity and presence of functional groups. Considering that the AD of WAS, compared to PS and mixed PS and WAS, is more challenging for the above-mentioned reasons, the investigation of the effect of BC as additive could be interesting to try to overcome such limitations. Biogas and methane productions observed in WWTP facilities are around 0.07-0.23 $Nm^3/kg_{VS}$ (65% methane) for WAS [14,15] and 0.14-0.91 $Nm^3/kg_{VS}$ (67% methane) for mixed PS+WAS [16]; comparing the average values of the above mentioned ranges, an increase of + 206 % can be estimated for PS+WAS compared to WAS, with almost analogous contents of methane. Moreover, BC doesn't require chemicals nor energy if its production is sustained by the other pyrolysis products [17]. Added to digestate, BC may enhance its quality as soil amendment [18]; this is relevant knowing that, as before mentioned, nearly half of the sludge produced in EU is destined to agricultural reuse and composting [19]. Several studies (Table 1) confirmed the improvement of methane yields and production rates from the AD of wastewater sludge due to BC addition. The most frequently observed benefits of BC supplementation in AD are increased alkalinity/buffering capacity and mitigation of ammonia inhibition [20–26], faster degradation of intermediate organic acids [26–28], enhanced interspecies electron transfer (IET) [20,24,26,28], and $CO_2$ sequestration [21,22]. Conversely, also negative impacts on AD due to excessive doses of BC were observed (i.e. inhibition of methanogenesis and change of sludge rheology) [20,26]. Different and sometimes contrasting effects of BC supplementation on AD could be ascribable to the differences in BC properties, resulting from a range of factors such as feedstocks characteristics and operating conditions employed in BC production; some studies focused on varying the pyrolysis temperature [26,28] or the feedstocks [21,22], or both [27] and investigated different doses of BC [20,25]. However, considering literature (Table 1), most studies investigated a single BC, while only few studied two [27] or three BCs [21], and analysed the improvement of AD performances due to BC addition without a deep comparison of the behaviours of different BCs in the same experimental conditions. Moreover, despite the good number of studies concerning the addition of BC to the AD of raw PS, or mixtures of PS and WAS, limited number of studies focused specifically on BC addition to the AD of WAS [25,26]. To the best of our knowledge, no study investigated the effect of the addition of different BCs to the AD of WAS focusing on the influence of the specific physico-chemical properties of BC on the performance of the AD process, which is the aim of this work. This study investigated three BC samples, deriving from the pyrolysis of different waste biomasses, then physically activated with $CO_2$. Our approach was driven by the following issues. Firstly, a specific focus on waste biomasses, as feedstock to produce BC and as substrate for AD. Secondly, the choice of different waste biomasses as feedstock was due to the interest in obtaining a wide array of physico-chemical features of the BCs. Thirdly, $CO_2$ activation is known to improve some of the BCs' physical characteristics (specific surface and porosity), and it was selected instead of chemical activation to avoid large volumes of washing water needed to remove residues of activation agents from biochar and to bring BC pH values down. A two-step procedure (pyrolysis and activation) was performed because the different steps happened in different times and places (see Section 2.1).

**Table 1.** Literature review concerning the effects of the addition of biochar to the anaerobic digestion of wastewater sludge (abbreviations: BC: biochar; DWAS: dewatered WAS; DIET: direct interspecies electron transfer; FW: food waste; GS: gasification; HRT: hydraulic retention time; HTC: hydrothermal carbonization; IET: interspecies electron transfer; PS: primary sludge; PY: pyrolysis; T: temperature; TPAD: temperature phased anaerobic digester; TS: total solids; VFAs: volatile fatty acids; VS: volatile solids; WAS: waste activated sludge; WWS: unspecified wastewater sludge).

## 2. Materials and methods

### *2.1. Substrate, inoculum and biochars*

WAS and inoculum (digestate from a mesophilic digester operating at 37 °C) were obtained from a WWTP in Piedmont, Italy. Before the experiments, WAS was thickened by settling and stored at 4 °C. The inoculum was then degassed at 37 °C in a water bath for 7 days [29].

The considered BC samples were derived from the pyrolysis of soft wood pellets (SWP550a), sewage sludge (SS550a) and rice husk (RH550a), were produced at the UK Biochar Research Centre (UKBRC) in Edinburgh, UK. The BC samples are “standard” biochar materials produced at the UKBRC, and this work adopts their reference nomenclature. The raw biomasses were in form of Ø6 mm pellets and 4-6 mm long rice husk. BC samples were produced 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 [30]. The BCs then underwent physical activation with 60 mL $min^{-1}$ $CO_2$ at 900°C for 2 hours at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR) at Western University, Canada. The activation conditions were chosen according to [31], selecting physical activation, instead of chemical activation, because of its many advantages, as the high efficiency in increasing the specific surface area and porosity of the BC [32], without producing washing water in need of further treatment. 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. All BCs were characterized (see section 2.3) and manually powdered in an agate mortar prior to AD tests.

### *2.2. Anaerobic digestion tests*

AD tests were performed in batch mode and mesophilic conditions at DIATI, Politecnico di Torino, Italy. The tests investigated the impact of the addition of 10 g BC $L^{-1}$ [26,28] of the 3 BCs (SWP550a, SS550a and RH550a) on the AD of WAS. 6 reactors (3 for biogas and 3 for methane measurement, see below) were operated for each BC, plus 6 reactors as control (CTRL in the following, e.g. substrate and inoculum without BC) and 6 reactors as blanks (only inoculum), reaching 30 reactors in total. Each reactor consisted of a Duran glass bottle (0.25 L working volume, WV), closed by a GL45 PP screw cap and connected to a biogas/bio-methane sampling apparatus (see below and Figure 1). WAS and inoculum underwent the AD tests adopting a substrate to inoculum (S/I) ratio equal to 1:1 on VS basis. All reactors were kept in a temperature-controlled water bath at 37±1 °C and manually mixed 3 times per day. Biogas/methane sampling happened as follows: biogas was collected in 2 L gas bags (30238-U Supelco) and measured daily by water displacement with a Drechsel washing bottle. Each reactor for methane measurement was connected to a 0.1 L glass bottle containing an alkaline washing solution (3 N NaOH) and the outgoing gas flow was measured by water displacement. Biogas and methane volumes were monitored daily until their marginal production was below 1%. Their respective values were normalized to standard temperature and pressure (0 °C, 1 atm).

**Figure 1.** Outline of (a) the experimental setup and of (b) the sampling apparatus

### *2.3. Analytical procedures*

Samples were collected from the digesters at the beginning and at the end of each AD test and stored at 4 °C until characterization. Total solids (TS) and volatile solids (VS) were measured according to standardized methods [33]. pH was measured with a benchtop pH80+DHS meter (XS Instruments). Ammonia nitrogen, chemical oxygen demand (COD) and organic acids were measured using Nanocolor test kits (Macherey-Nagel, Germany) and a PF-12$^{\text{Plus}}$ photometer (Macherey-Nagel, Germany). The samples were centrifuged at 6,000 rpm for 10 min and the supernatant was analyzed for ammonia nitrogen and, after filtration on 0.45 µm, for soluble COD (sCOD) and organic acids (i.e. precursors, and inhibitors if in excess, of methanogenesis). All the analyses were conducted in duplicate.

The surface area, total pore volume and average pore diameter of the BC samples were measured at ICFAR using a Brunauer-Emmett-Teller (BET) analysis through a Nova 2000e Surface Area & Pore Size Analyzer (Quantachome Instruments, Anton Paar QuantaTec Inc., Florida, USA). Liquid nitrogen at -196 °C was used for the adsorption studies. Prior the analyses, the samples were degassed at 105 °C for 1 h and then at 300 °C for 3 h. The inorganic elements (excluding Na, K, Mg and P) were analyzed at DIATI, Politecnico di Torino through a NEX DE VS Rigaku XRF spectrometer. Proximate analysis was carried out at UKBRC using a Mettler-Toledo TGA/DSC1 instrument (Mettler-Toledo Ltd, Leicester, UK); a Thermo-Fisher FlashSmart elemental analyser with MAS Plus Auto-sampler was used to determine carbon, hydrogen and nitrogen contents. The pH and electrical conductivity (EC) values of biochar were analyzed in deionized water at a ratio of 1:10 (w/v).

### *2.4. Kinetics analysis*

In this study, the cumulative methane production curves were fitted by the modified Gompertz equation (eq. 1):

$$B(t) = P \exp\left\{-\exp\left[\frac{R_{max}\, e}{P}\,(\lambda - t) + 1\right]\right\} \qquad (1)$$

where, $B(t)$ is the cumulative methane production ($Nm^3$ $kgVS^{-1}$) at time $t$ (day); $P$ is the methane potential of the substrate ($Nm^3$ $kgVS^{-1}$); $R_{max}$ is the maximum methane production rate ($Nm^3$ $kgVS^{-1}$ $d^{-1}$); $\lambda$ is the lag phase (day); $e$ is the Euler's number. The kinetic parameters were estimated using non-linear regression analysis. This function fits experimental data with the least squares method. The fit of the functions was estimated through the coefficient of determination ($R^2$).

## 3. Results and discussion

### *3.1. Characterization of substrate, inoculum and biochars*

The results of the characterization of the substrate and inoculum are shown in Table 2, where the standard error is referred to the replicates.

**Table 2**. Characterization of the substrate and the inoculum (standard error between parentheses; ND: not detected)

**Table 3**. Characterization of SWP550a, SS550a, RH550a biochars (standard deviation between parentheses; ND: not detected)

All BCs were derived from the same type of pyrolysis processes and activation conditions. The differences in their physico-chemical features (Table 3, standard deviation is referred to the replicates) are due to the characteristics of the raw feedstocks; all BCs considered in this work had favourable properties for their use as additive in AD process [31] based on the characteristics of their precursor biomasses. The characteristics of the considered BCs are consistent with literature, as detailed in the following. SWP550a, derived from soft wood pellets, is mainly composed of cellulose, hemicellulose, lignin and some inorganic compounds [34]. RH550a, derived from rice husk, presents significant contents of ash and silica (20-25% wt), together with cellulose, hemicellulose and lignin [35]. SS550a derived from sewage sludge (mostly made of proteins, grease and fats, and cellulose); contents of ash and nutrients, including N, P and K, are higher in SS550a than in plant-derived BCs as SWP550a , RH550a [36]. Surface area and porosity are key parameters affecting BC's role in AD, e.g. high surface area and large porous structure can favour microbial immobilization [37] and/or support physical adsorption of ammonia and carbon dioxide [38]. As a result of the activation, for all BCs the BET surface area ($S_{BET}$) and the total pore volume ($V_T$) were noteworthy, while the average pore diameter was in the meso and macro pore range. SS550a exhibited lower $S_{BET}$ and $V_T$ (109 m$^2$ g$^{-1}$, 0.17 cm$^3$ g$^{-1}$ respectively), compared to RH550a (263 m$^2$ g$^{-1}$, 0.21 cm$^3$ g$^{-1}$) and SWP550a (605 m$^2$ g$^{-1}$, 0.42 cm$^3$ g$^{-1}$). The lower $S_{BET}$ of SS550a is due partly to the structure of the feedstock, which typically contracts under pyrolysis [26], and to the high ash content, which could fill and block micropores during pyrolysis [27]. SS550a and RH550a present higher ash content (58.89% and 47.93%, respectively), compared to SWP550a (1.25%), because the higher amount of inorganic matter in their feedstocks concentrated in BC during pyrolysis. In general, silica (Si), aluminium (Al), calcium (Ca) and potassium (K) were the most abundant elements in the ash fraction of all BCs, being Si and K predominant in RH550a, and Al and Ca in SS550a. The presence of alkali and alkaline earth metals (AAEMs), such as Na, K, Mg, Ca, in the ash fraction could contribute to the buffering capacity of BCs during AD [39]. In addition, large concentrations of AAEMs, in particular K, were shown to promote in-situ $CO_2$ sequestration during AD [20,23]. The content of carbon was rather low in SS550a compared to SWP550a; the C and N contents of BCs deriving from plant-based feedstocks (SWP550a, RH550a) are reported to increase under pyrolysis, and decrease in case of BCs from mineral rich precursors (SS550a) [34]. SS550a had the highest content of nutrients (N, P, K), due to their richness in sewage sludge precursor. As confirmed herein, BCs derived from agricultural residues (as RH550a) generally contain more nutrients than BCs from woody biomass (as SWP550a) [22].

The EC of SS550a was high (280.80 dS m$^{-1}$) (Table 3) due to the significant mineral content. RH550a and SWP550a had lower EC (respectively, 0.48 and 0.09 dS m$^{-1}$). Conductive materials are able to promote direct interspecies electron transfer (DIET) during AD [40]. The capability of stimulating DIET seemed related to the redox characteristics of BCs [41,42], i.e. the electron accepting capacity

(EAC) and the electron donating capacity (EDC) electrons. In general, the electron transfer by BC may be due to different organic structures, being quinone/hydroquinone moieties mainly responsible for EAC and EDC, while conjugated π-electron systems of the condensed aromatic structures of BC are responsible for its conductivity [43]. High H/C and O/C ratios would suggest that DIET is mainly mediated by quinone groups, while lower ratios (<0.35 and 0.09, respectively) would indicate that electron transfer is mostly conducted by the graphitic carbon structures of BC [44]. Therefore, the low molar ratios of RH550a could suggest the possibility of DIET mediated by graphitic structures during AD.

The pH of all BCs was alkaline (7.9-9.7) (Table 2). RH550a presented higher pH (9.71) than SS550a (8.17) and SWP550a (7.91), because of the larger amounts of alkali and alkaline elements in rice husk than in wood [45]. High ash content and lack of acidic functional groups are usually correlated with high pH values of the BC [46]. Thereby, the higher pH of RH550a compared to SWP550a may be related to the lower presence of acidic functional groups, as suggested by the lower H/C and O/C ratios of RH550a compared to SWP550a and SS550a. The H/C ratio is an indication of BC degree of aromatization; a high O/C ratio implies more oxygenated functional groups, contributing to high cation exchange capacity (CEC) values and hydrophilicity of BC [47,48].

### *3.2. Effect of biochar addition on methane production*

The cumulative biogas productions from SS550a, RH550a and CTRL reactors (Figure 2a) were similar during the entire experiment, while biogas production from SWP550a was lower from day 1. The final biogas yields were: SWP550a (0.160 ± 0.007 $Nm^3$ $kgVS^{-1}$), SS550a (0.219 ± 0.005 $Nm^3$ $kgVS^{-1}$), RH550a (0.210 ± 0.006 $Nm^3$ $kgVS^{-1}$), and CTRL (0.238 ± 0.017 $Nm^3$ $kgVS^{-1}$). Apparently, SS550a and RH550a did not increase biogas production, and SWP550a led to a reduction. Shen et al. (2015) [20] observed decreased biogas production during thermophilic AD of wastewater sludge by increasing the dose of BC from corn stover, together with an enhanced methane production. They suggested the key role of BC in $CO_2$ adsorption and mineralization. Overall, our results show that, in the same experimental conditions, BCs having different features had diverse impacts on methane production (Figure 2b). During the first 6 days, methane production from BC supplemented digestors (particularly RH550a) was higher than from CTRL reactors. From day 7, methane production from SSP550a and CTRL digestors increased, exceeding SWP550a. Thus, the final methane yields were: 0.037±0.004 $Nm^3$ $kgVS^{-1}$ from SWP550a, 0.081±0.006 $Nm^3$ $kgVS^{-1}$ from SS550a, 0.142±0.024 $Nm^3$ $kgVS^{-1}$ from RH550a, and 0.069 $Nm^3$ $kgVS^{-1}$ from CTRL. RH550a showed a significantly enhanced methane production compared to CTRL reactors and SS550a. An adverse effect was observed for SWP550a supplemented digestors, consistent with the previous observations of biogas yield. These results are in agreement with those of other studies; for instance, different enhancements of methane yields, ranging 0.07-0.15 $Nm^3$ $kgVS^{-1}$ were observed after 15 days of mesophilic AD of wastewater sludge with 8 g $L^{-1}$ of BCs from corn straw, coconut shell and sewage sludge, produced at different temperatures [27]. Conversely, Wu et al. (2019) [26] reported that BC from pyrolysis of wastewater sludge at varying temperatures had slight positive or even adverse impacts on methane yield (0.10-0.12 $m^3$ $kgVS^{-1}$) during the mesophilic AD of WAS.

Methane content gradually increased in all reactors until the end of the test (Figure 2c) up to: 23±3% for SWP550a, 37±3% for SS550a, 67 ± 12 % for RH550a, and 29±2% for CTRL. RH550a and SS550a increased methane content compared to control reactors. A possible explanation may be the capture of $CO_2$ from biogas; in details, the possibility of sequestering $CO_2$ with BC during AD, thus achieving an in-situ biogas upgrading, was explored [20], reporting methane contents equal to 88-97% in presence of BC and to 68% in CTRL reactors, reaching $CO_2$ removal equal to 55-86%. $CO_2$ adsorption capacity of the investigated BCs was calculated as the difference between methane content in CTRL reactors and in the ones supplemented with BC, obtaining 3.14 ± 1.49 mmol $g^{-1}$ for RH550a and 0.97 ± 0.87 mmol $g^{-1}$ for SS550a, in agreement with literature (0.4 - 2.3 mmol $g^{-1}$ BC) [31]. The main mechanism for $CO_2$ sequestration by BC has been reported to be physical adsorption through van der Waals and electrostatic forces, suggesting the importance of high surface area and pore volume, along with reduced pore size [38]. RH550a and SWP550a presented high surface area (263 and 605 $m^2$ $g^{-1}$) and porosity (0.21 and 0.42 $cm^3$ $g^{-1}$), which could favour $CO_2$ adsorption. The presence of alkali and alkaline earth metals in the ash fraction of RH550a may promote chemical sorption of $CO_2$ via mineralogical reactions [49]. In addition, RH550a showed the lowest H/C and O/C atomic ratios compared to SWP550a and SS550a, suggesting significant hydrophobicity and aromatization. Non-polarity and high hydrophobicity of BC can effectively improve $CO_2$ adsorption in presence of water [21]. Another option for the enhanced methane yield and the reduced biogas yield may be the major conversion of $CO_2$ to $CH_4$ by aceticlastic methanogens through DIET [25,27]. In this work, a clear positive impact of the high EC of SS550a linked to its mineral constituents on the methane production from WAS, was not observed, similarly to other studies [41].

The fitting of the cumulative methane productions by the modified Gompertz (Table 3), was reasonably good for SS550a ($R^2$ 0.951), RH550a ($R^2$ 0.848) and CTRL reactors ($R^2$ 0.953). In case of all BCs supplemented digestors, the lag phase ($\lambda$) was shorter (0.5 days in SS550a and 0 days in SWP550a and RH550a) than in CTRL reactors (2.6 days) thereby, methane production from WAS seemed to be significantly accelerated by BC supplementation. The maximum methane potential ($R_{max}$) was enhanced by the addition of RH550a (0.016 $Nm^3$ kg $VS^{-1}$ $d^{-1}$), compared to other reactors (0.005-0.006 $Nm^3$ kg $VS^{-1}$ $d^{-1}$). Consistently, Wang et al. (2020) [28] observed a reduction of $\lambda$ and an enhancement of P and $R_{max}$ during the AD of wastewater sludge with BC from Douglas fir, resulting in the faster degradation of organic acids and methane production. It could be assumed that RH550a, and to a lesser extent SS550a, provided favourable conditions for microbial immobilization and growth, given the high surface area, proper porous structure, and hydrophobicity [37]. The reduced distance between syntrophic microbial partners could promote IET and exchanges of volatile fatty acids or other metabolites [31], potentially enhancing the different steps of the AD process.

**Figure 2**. Cumulative biogas and methane production (mean ± standard error): (a) Cumulative biogas production for SWP550a, SS550a, RH550a, and CTRL reactors; (b) Cumulative methane production for SWP550a, SS550a, RH550a, and CTRL; (c) Methane content for SWP550a, SS550a, RH550a, and CTRL.

**Table 3**. Kinetic parameters of methane productions from different biochars with modified Gompertz model (P: methane potential of the substrate; $R_{max}$: maximum methane production rate; λ: lag phase)

### *3.3. Digestate characterization*

Sludge and digestate have been characterised for all the tested conditions (Figures 3 and 4). When BCs were supplemented, the initial (i.e. before AD) pH was not enhanced by any type of BC (Figure 3a), while other studies reported significant increase of the initial pH values after the addition of BCs due to their alkaline nature [20,25]. The different behaviour observed can be ascribed to the different origin of BCs and the higher doses adopted in those studies, from 16 to 66 g $L^{-1}$ compared with the 10 g $l^{-1}$ used in the present work. The final pH remained close to neutral for all conditions tested. In all cases, the pH of the substrate was within the optimal range for methanogens [10]. The initial (i.e. before AD) total solids (TS) (Figure 3b) increased by 10 g $l^{-1}$ resulting from BC addition. The final (i.e. after AD) TS of BCs supplemented digesters were not significantly different, reduced by AD between 20% and 30%, as in CTRL reactors. The volatile solids (VS) (Figure 3c) increased after AD to different extents due to the BCs supplements (SWP550a > RH550a > SS550a > CTRL), depending on their ash contents.

BC Addition enhanced the total COD (SWP550a 49 g $L^{-1}$, SS550a 54 g $L^{-1}$, RH550a 50 g $L^{-1}$) with respect to CTRL reactors (34 g $L^{1}$), probably due to the volatile matter of the BC that is mostly recalcitrant for AD [20,50]. At the end of AD, the total COD decreased by 27% in RH550a, 21% in SS550a and CTRL, 14% in SWP550a reactors, in line with methane yields. For all tested conditions, the final sCOD values were below 1 g $L^{-1}$, corresponding to a removal exceeding 54% (Figure 4a). BC addition resulted in a slight increase, compared to CTRL reactors, of the organic acids removal (Figure 4b), equal to 3%, 10 % and 22% for SWP550a, SS550a, and RH550a, respectively, corresponding to estimated net removals equal to 4, 20 and 12 mg of total organic acids $g^{-1}$ BC, respectively. These results are consistent with the removal of sCOD, and they provide a quantitative estimate of organic acids removal, which has not been covered yet by literature. BCs supplementation did not affect the initial $NH_3$-N of the sludge, in the range of 400-450 mg $L^{-1}$ as in CTRL reactors. $NH_3$-N increased during AD (Figure 4c), due to ammonification, by 46% in CTRL reactors. In BCs amended reactors the increment was lower: 20%, 34%, and 21%, for SWP550a, SS550a, and RH550a, respectively. BC addition resulted in an estimated removal of $NH_3$-N equal to 38 mg $g^{-1}$ BC, 16 mg $g^{-1}$ BC, and 50 mg $g^{-1}$ BC for SWP550a, SS550a, and RH550a, respectively, consistently with literature (25-137 mg $NH_3$-N $g^{-1}$ BC) [31]. Ammonia decrease by BC can result from its adsorption, which is favoured by high surface area, large porous structure, high CEC and acidic functional groups, or from struvite precipitation, thanks to Mg, K and Ca present in the mineral fraction of BCs [22,31]. These results suggested that BCs could effectively mitigate ammonia inhibition as confirmed by other studies [20,27].

**Figure 2**. Sludge characteristics before and after anaerobic digestion (mean ± standard error): (a) pH; (b) total solids; (c) volatile solids

**Figure 3**. Sludge characteristics before and after anaerobic digestion (mean ± standard error): (a) soluble COD; (b) Organic acids; (c) $NH_3$-N.

## 4. Conclusions

The physico-chemical features of the three investigated biochars, even if diverse, were all favourable for their use as additives in AD. The results of the AD tests did not show positive effects of the selected BC addition on biogas production, while methane production was accelerated and enhanced (up to 105% for RH 550a) in samples supplemented with activated BCs exhibiting the highest specific surface area and pore volume, lower pore diameter and electrical conductivity, higher hydrophobicity and amounts of alkali and alkaline metals in the ashes. The performance improvement in methane production due to BC addition (in the case of RH550a) was enough to obtain from WAS a methane production that is equivalent to the one usually obtained from PS+WAS according to literature [16]. The influence of each BC property was correlated with the results of this work, achieving consistency with literature and bringing new quantitative insights on organic acids sequestration. This study shed light on the role of key biochar properties and their relevant contributions to improved performances of the AD of WAS under the considered experimental conditions. Further research is needed to better understand the specific mechanisms involved and particularly to elucidate BC influence on complex microbial communities.

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**Table 1**.

| Biochar production | | | Anaerobic digestion | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Feedstock | Production | BC dose | Substrate | Temperature [°C] | Mode | Working volume [L] | HRT [days] | Increase methane yield, +/- % respect to control (Nm$^3$ kgVS$_{fed}^{-1}$) | Potential effects of BC addition | Reference |
| Corn stover | GS | 1.82-3.64 g BC gTS$^{-1}$ | WWS | 55 | Batch | 0.55 | 26 | 0.30-0.31 m$^3$ kg COD$_{deg}^{-1}$ (0.29 control) | - increased alkalinity<br>- mitigated $NH_3$ inhibition<br>- enhanced IET | [20] |
| Corn straw | PY, 400-600 °C | 8 g l$^{-1}$ | WWS | 35 | Batch | 0.3 | 35 | + 57-86 % (0.18-0.22) | - immobilization of microbes<br>- increased buffering capacity<br>- enhanced degradation of VFAs<br>- mitigated $NH_3$ inhibition | [27] |
| Coconut shell | PY, 400-600 °C | 8 g l$^{-1}$ | WWS | 35 | Batch | 0.3 | 35 | + 8-49 % (0.13-0.17) | | |
| Sewage sludge | PY, 400-600 °C | 8 g l$^{-1}$ | WWS | 35 | Batch | 0.3 | 35 | + 33-77 % (0.16-0.21) | | |
| Douglas fir | PY, 400-730 °C | 10 g l$^{-1}$ | WWS | 25 | Batch | 0.06 | > 30 | + $CH_4$ yield (0.08-0.17 m$^3$ kgCOD$^{-1}$) | - faster organic acids degradation<br>- higher abundance of methanogens<br>- BC as electron shuttles by redox active moieties<br>- BC as electron conduits from high electrical conductivity | [28] |
| Douglas fir | PY, 400-730 °C | 10 g l$^{-1}$ | WWS | 37 | Batch | 0.06 | 13 | + $CH_4$ yield (0.17-0.21 m$^3$ kgCOD$^{-1}$) | | |
| Douglas fir | PY, 400-730 °C | 10 g l$^{-1}$ | WWS | 55 | Batch | 0.06 | 21 | + $CH_4$ yield (0.15-0.22 m$^3$ kgCOD$^{-1}$) | | |
| Pine wood | GS | 2.49, 4.97 g BC g$^{-1}$ TS | PS | 37 | Batch | 0.55 | 25 | + 2-3 % | - increased alkalinity<br>- mitigated $NH_3$ inhibition<br>- $CO_2$ sequestration<br>- inhibition at high BC doses | [21] |
| Pine wood | GS | 2.49, 4.97 g BC g$^{-1}$ TS | PS | 55 | Batch | 0.55 | 25 | + 2-6 % | | |
| White oak wood | GS | 2.20, 4.40 g BC g$^{-1}$ TS | PS | 37 | Batch | 0.55 | 25 | + 4-8 % | | |
| White oak wood | GS | 2.20, 4.40 g BC g$^{-1}$ TS | PS | 55 | Batch | 0.55 | 25 | + 2-4 % | | |
| Corn stover | PY, 600 °C | 1.82-3.06 g BC g TS$^{-1}$ | PS | 55 | Batch | 0.1 | 40 | + 4-14 % (0.34-0.37) | - enhanced buffering capacity<br>- mitigated $NH_3$ inhibition<br>- increased sludge conductivity (DIET)<br>- inhibition at high BC doses | [23] |
| Corn stover | PY, 600 °C | 1.82 g BC g TS$^{-1}$ | PS | 55 | Continuous | 1.5 | 15 | + 14 % | | |
| Corn stover | GS<br>850 °C | 0.25-1.0 g BC d$^{-1}$ | PS + WAS | 55 | Semi-continuous TPAD | 0.4 | 1st stage: 5-15<br>2nd stage: 13-30 | 0.28-0.34 (0.23-0.28 control) | - increased alkalinity<br>- mitigated $NH_3$ inhibition<br>- shift in bacterial community<br>- $CO_2$ sequestration<br>- inhibition at high BC doses | [22] |
| Pine wood | GS<br>850 °C | 0.25-1.0 g BC d$^{-1}$ | PS + WAS | 55 | Semi-continuous | 0.4 | 1st stage: 5-15 | 0.26-0.32 (0.23-0.28 control) | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TPAD | | 2nd stage:<br>13-30 | | | |
| Sawdust | PY, 500 °C | 2-15 g $l^{-1}$ | FW + DWAS (4:1 w/w) | 35 | Batch | 0.09 | | - 28–64 % lag-phase<br>+22–40% $CH_4$ max prod. rate | - enhanced buffer capacity<br>- enhanced DIET | [24] |
| Dewatered WAS | PY, 300-700 °C | 10 g $l^{-1}$ | WAS | 37 | Batch | 0.3 | 32 | + 4 % (0.116) or negative effect | - mitigated $NH_3$ inhibition<br>- promotion VFAs degradation<br>- immobilization of microbes<br>- enhanced DIET<br>- enhanced acidification activity | [26] |
| Dewatered WAS | HTC, 180-240 °C | 10 g $l^{-1}$ | WAS | 37 | Batch | 0.3 | 32 | + 3-18 % (0.115-0.132) | | |
| Corn stover | PY, 500 °C | 16 g $l^{-1}$ | WAS | 37 | Batch | 0.08 | 32-34 | + 6 % (0.29) | - increased buffering capacity<br>- high organic compounds in BC | [25] |
| Corn stover | PY, 500 °C | 32 g $l^{-1}$ | WAS | 37 | Batch | 0.08 | 32-34 | + 14 % (0.31) | | |
| Corn stover | PY, 500 °C | 66.6 g $l^{-1}$ | WAS | 37 | Batch | 0.08 | 32-34 | + 26 % (0.34) | | |

**Table 2**

| | Substrate | Inoculum |
|---|---|---|
| TS (g/L) | 18.75 (0.01) | 21 (0.1) |
| VS/TS (-) | 77.8 (0.06) | 12.71 (0.02) |
| pH (-) | 6.40 (0.02) | 6.72 (0.02) |
| COD (g $O_2$/L) | 27 (1) | 40 (5) |

**Table 3**.

| | SWP550a | SS550a | RH550a |
|---|---|---|---|
| $S_{BET}$ (m$^2$ g$^{-1}$) | 605.1 | 109.2 | 263.4 |
| $V_T$ - Total pore volume (cm$^3$ g$^{-1}$) | 0.416 | 0.169 | 0.209 |
| D - Average pore diameter (nm) | 2.75 | 6.19 | 3.18 |
| pH (-) | 7.91 (0.30) | 8.17 (0.64) | 9.71 (0.26) |
| EC (dS m$^{-1}$) | 0.09 (0.03) | 280.80 (15.3) | 0.48 (0.14) |
| Moisture (% wt) | 1.52 (0.16) | 2.48 (0.08) | 1.54 (0.30) |
| Ash (% wt) | 1.25 (0.42) | 58.89 (0.45) | 47.93 (5.43) |
| Volatile matter (% wt) | 14.20 (0.81) | 21.37 (0.03) | 7.48 (1.22) |
| Fixed C (% wt) | 83.03 (0.93) | 17.26 (0.46) | 43.05 (5.57) |
| Total C (% wt) | 85.52 (1.22) | 29.53 (0.42) | 48.69 (2.37) |
| H (% wt) | 2.77 (0.09) | 1.33 (0.07) | 1.24 (0.12) |
| N (% wt) | < 0.10 | 3.75 (0.08) | 1.04 (0.09) |
| O (% wt) | 10.36 (1.19) | 6.50 (0.47) | 2.47 (0.37) |
| P (% wt) | 0.06 (0.04) | 2.29 (0.05) | 0.10 (0.07) |
| K (% wt) | 0.25 (0.07) | 0.34 (0.01) | 0.39 (0.17) |
| H:C (molar ratio) | 0.39 (0.01) | 0.54 (0.03) | 0.28 (0.06) |
| O:C (molar ratio) | 0.09 (0.01) | 0.17 (0.01) | 0.04 (0.01) |
| Elements (mg kg$^{-1}$) | | | |
| Al | 1520 (-) | 28967 (153) | ND |
| As | ND | 13 (2) | 0.2 (-) |
| Ca | 2630 (20) | 16533 (58) | 1737 (84) |
| Cd | ND | ND | ND |
| Cl | 60 (1) | 633 (5) | 744 (1) |
| Co | ND | 201 (8) | 8.4 (0.8) |
| Cr | ND | 299 (6) | 6.2 (0.6) |
| Cu | 3.0 (0.1) | 92.4 (0.6) | 11.3 (0.3) |
| Hg | ND | ND | ND |
| Mg | ND | ND | ND |
| Na | ND | ND | ND |
| Ni | 1.2 (0.2) | 13 (1) | 5.8 (0.5) |
| Pb | ND | 99 | ND |
| S | 32 (8) | 2457 (23) | 114 (8) |
| Se | ND | ND | ND |
| Si | 204 (3) | 48833 (115) | 125333 (577) |
| Zn | 11.07 (0.06) | 296 (2) | 35.4 (0.2) |

**Table 4**

| | P<br>($Nm^3$ $kgVS^{-1}$) | $R_{max}$<br>($Nm^3$ $kgVS^{-1}d^{-1}$) | λ<br>(days) | $R^2$<br>(-) |
|---|---|---|---|---|
| SWP550a | 0.037 | 0.005 | 0.00 | 0.517 |
| SS550a | 0.081 | 0.006 | 0.53 | 0.951 |
| RH550a | 0.142 | 0.016 | 0.00 | 0.848 |
| CTRL | 0.069 | 0.006 | 2.59 | 0.953 |

**Figure 1**. Outline of (a) the experimental setup and of (b) the sampling apparatus

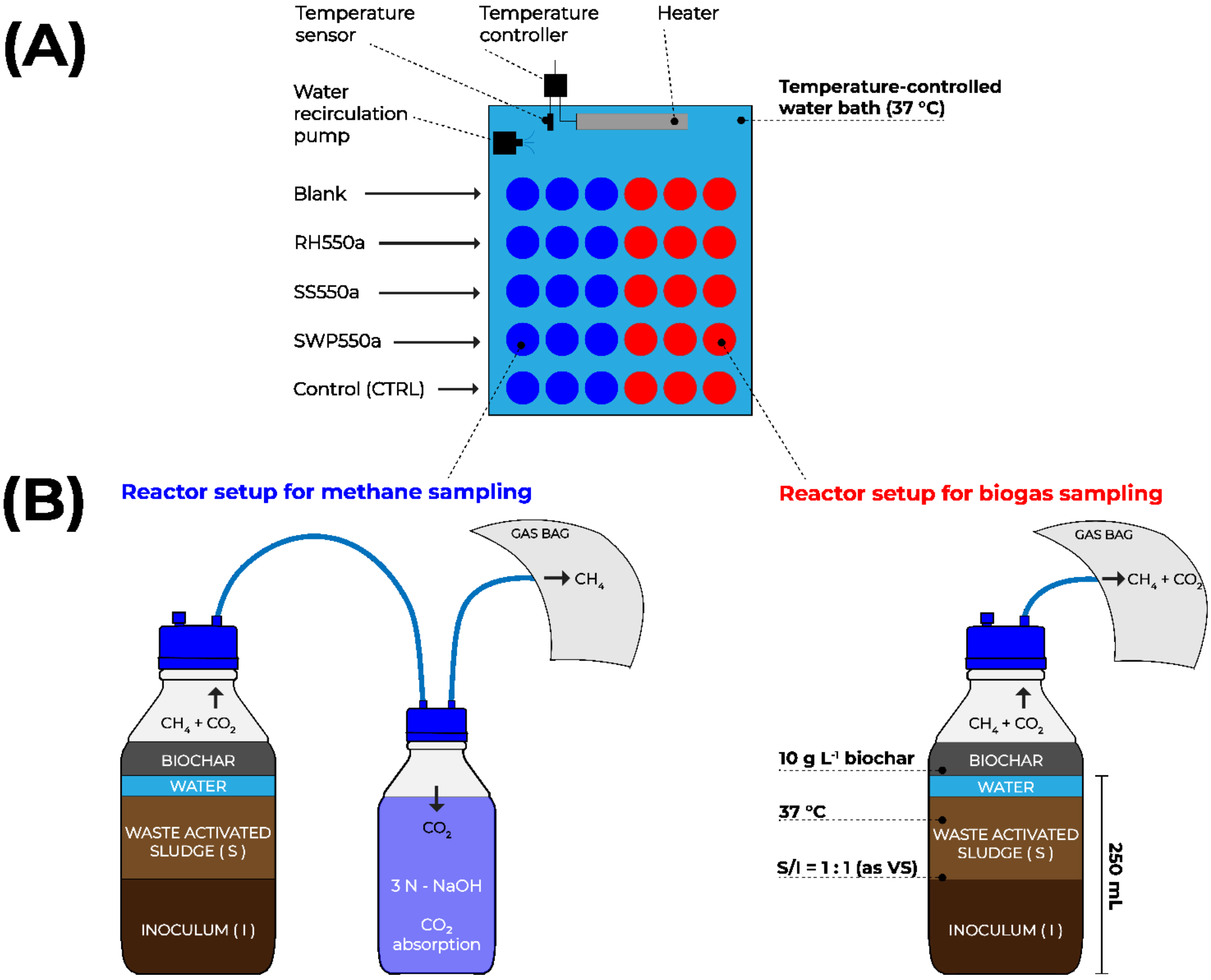


**Figure 2**

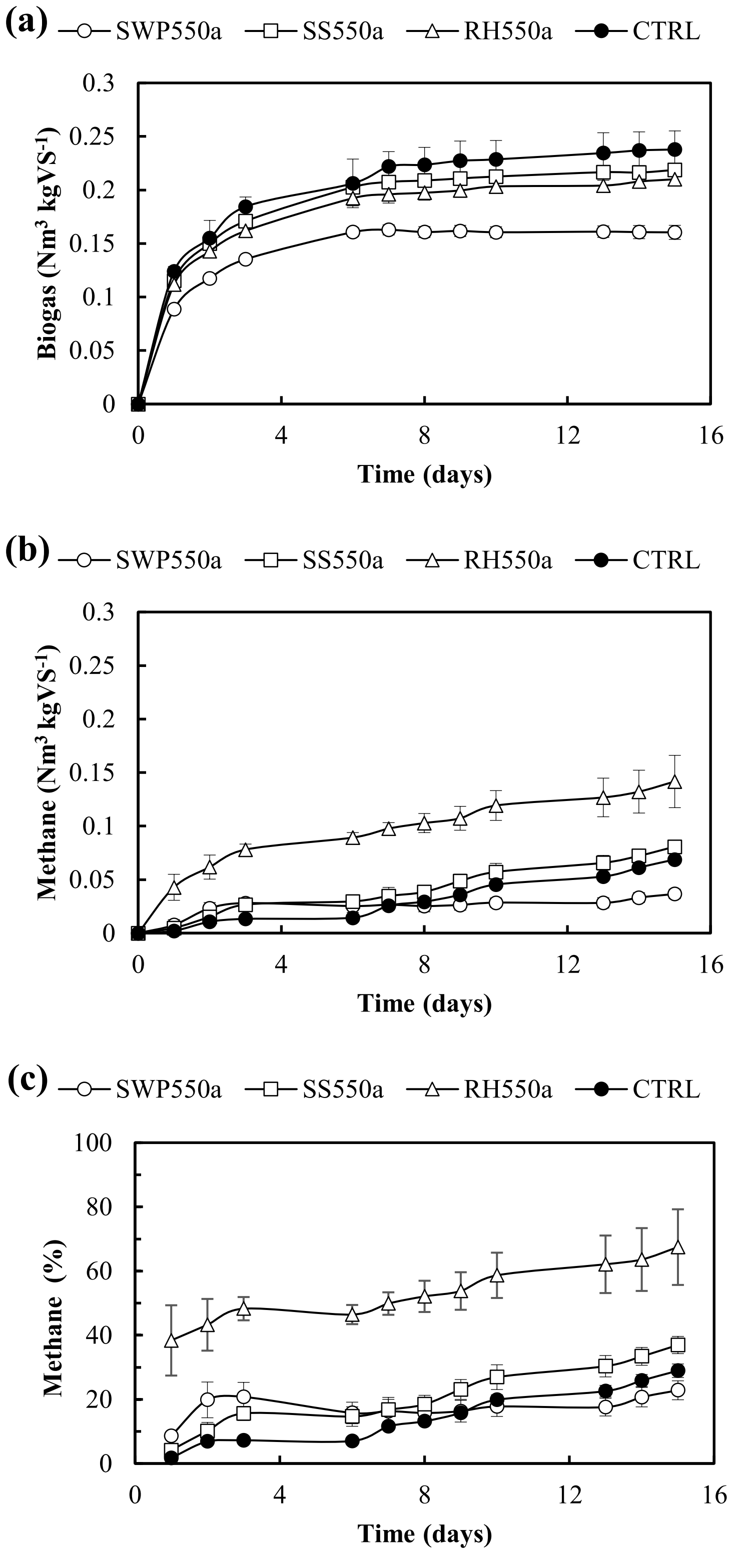

(a)
SWP550a
SS550a
RH550a
CTRL
Biogas (Nm$^3$ kgVS$^{-1}$)
0
0.05
0.1
0.15
0.2
0.25
0.3
0
4
8
12
16
Time (days)
(b)
SWP550a
SS550a
RH550a
CTRL
Methane (Nm$^3$ kgVS$^{-1}$)
0
0.05
0.1
0.15
0.2
0.25
0.3
0
4
8
12
16
Time (days)
(c)
SWP550a
SS550a
RH550a
CTRL
Methane (%)
0
20
40
60
80
100
0
4
8
12
16
Time (days)

**Figure 3**

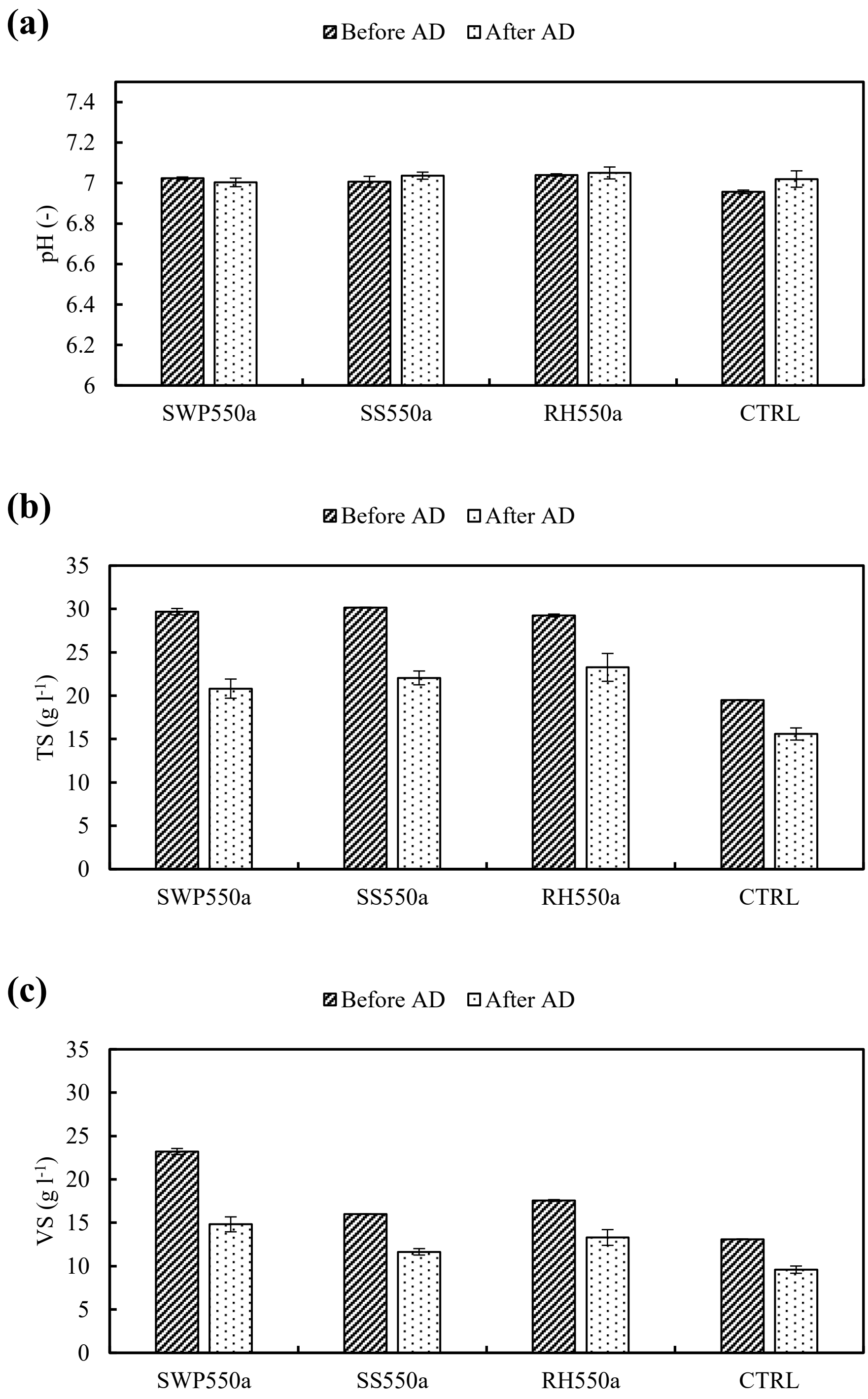
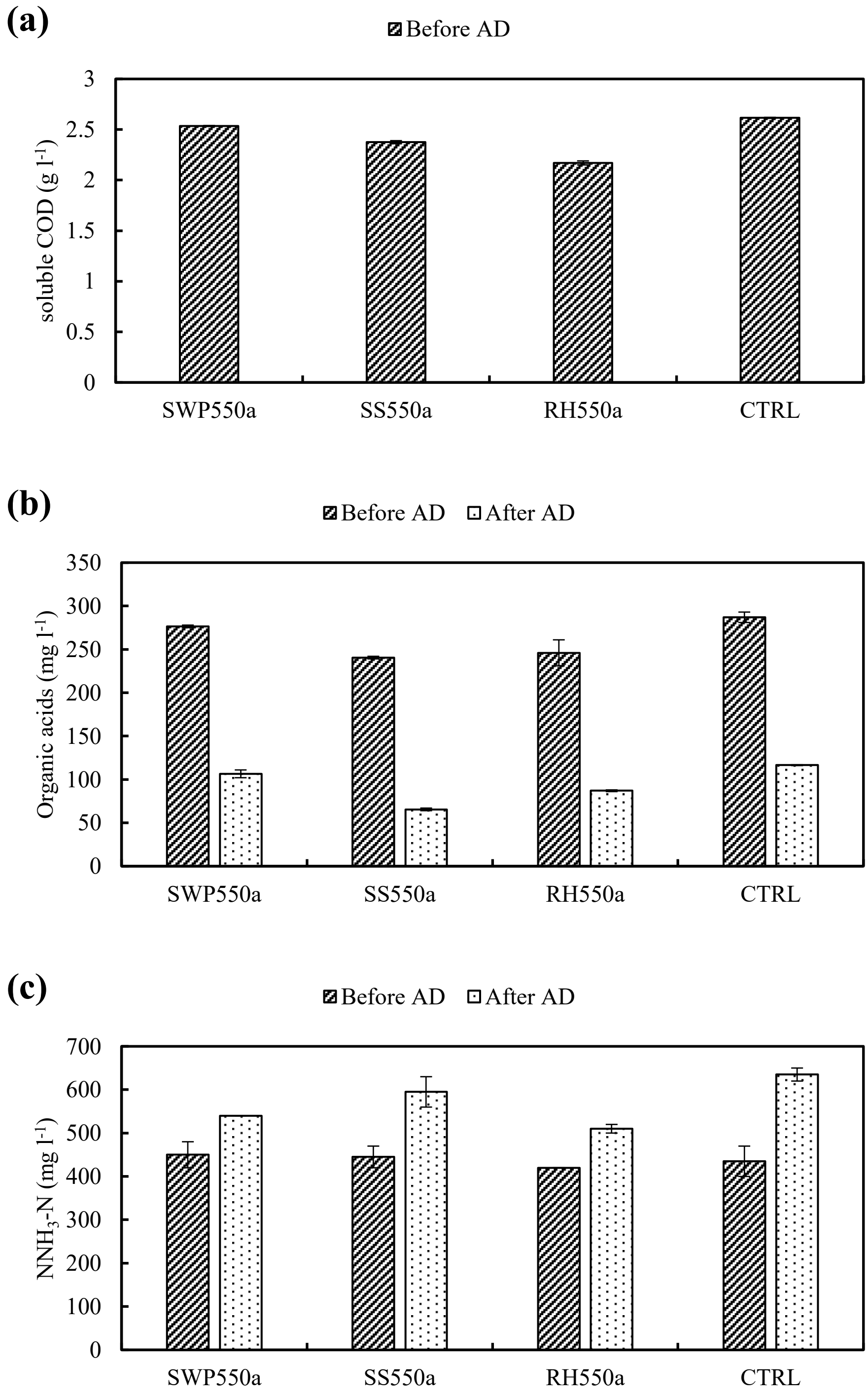

(a)
▨ Before AD ☐ After AD
pH (-)
7.4
7.2
7
6.8
6.6
6.4
6.2
6
SWP550a
SS550a
RH550a
CTRL
(b)
▨ Before AD ☐ After AD
TS (g l$^{-1}$)
35
30
25
20
15
10
5
0
SWP550a
SS550a
RH550a
CTRL
(c)
▨ Before AD ☐ After AD
VS (g l$^{-1}$)
35
30
25
20
15
10
5
0
SWP550a
SS550a
RH550a
CTRL

**Figure 4**


(a)
▨ Before AD
soluble COD (g $l^{-1}$)
0
0.5
1
1.5
2
2.5
3
SWP550a
SS550a
RH550a
CTRL
(b)
▨ Before AD
□ After AD
Organic acids (mg $l^{-1}$)
0
50
100
150
200
250
300
350
SWP550a
SS550a
RH550a
CTRL
(c)
▨ Before AD
□ After AD
$NNH_3$-N (mg $l^{-1}$)
0
100
200
300
400
500
600
700
SWP550a
SS550a
RH550a
CTRL