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Research paper

# Optimizing biomethane production and plants growth with biochar-enhanced anaerobic digestion

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A B S T R A C T

Food waste (FW) poses environmental, economic, and ethical challenges, contributing to greenhouse gas emissions. Anaerobic digestion (AD) offers the dual benefit of enhancing FW management while generating biogas as renewable energy source and producing nutrient-rich digestates. This study explored a circular and integrated model where anaerobic co-digestion (AcD) of FW and cow manure is enhanced with biochar-derived digestate applied in two doses (7, 14 g/L) under mesophilic and thermophilic conditions. Residual digestates are tested as fertilizers on *Solanum Lycopersicum* L. growth in greenhouse trials. Digestates, mineral fertilizer, and commercial compost were applied at three different dosages: 85, 170, 340 kgN/ha. Results showed significant improvements in biogas yield, particularly under thermophilic conditions where the AcD with both doses of biochar produced around 890 NL/kgVS of biogas, 25 % v/v more than the AcD without biochar, and 44 % more than mesophilic AcD. Greenhouse trials using digestates as fertilizers for tomato plants revealed differential agronomic impacts. Thermophilic digestates outperformed mesophilic ones as well as mineral fertilizer in promoting biomass accumulation (+20 %) and chlorophyll content (+10 %), while mesophilic digestates favored root development despite no significant differences emerging in their physic-chemical composition. Results support previous research, proving that the addition of biochar affected methane production, nutrient composition, and bioavailability of digestates, with notable benefits for plant root development and nutrient uptake. The novelty of this study is the comprehensive evaluation of both biomethane production and agronomic effects of the residual digestate, that offers a closed circular-bioeconomy model for sustainable FW management, energy production, and crop cultivation.

## 1. Introduction

Food waste (FW) is a huge problem in current society. Around one-third of the food produced is estimated to be lost or wasted along the food production and supply chains. Over 59 million tons of fresh mass FW are generated only in the EU [1], more than half of which come from household consumption [2]. Across the globe, around 20 % of food available to consumers is wasted, with 1.05 billion tons of FW produced in 2022 [3]. This impressive quantity is added to the world's food lost in the supply chain from post-harvest to, and excluding, retail, which is estimated to be 13 % of the total food produced [3]. Since it is difficult to clearly assess whether countries are effectively decreasing food

*Abbreviations: AD*, Anaerobic digestion; AcD, Anaerobic co-digestion; C, Commercial compost; CCI, Chlorophyll content index; d.m.b., Dry matter basis; EC, Electrical conductivity; F, Mineral fertilizer; FW, Food waste; FOS, Flüchtigen organischen säuren (organic volatile fatty acids); GHG, Greenhouse gases; I, Inoculum; OFMSW, Organic Fraction of Municipal Solid Waste; RDI, Root Development Index; S, Substrate; SSA, Specific Surface Area; TAC, Totales Anorganisches Carbonat (inorganic total carbonate); TS, Total Solids; UC, Untreated Control; VS, Volatile Solids.

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wastefulness over the years, it is important to find ways to properly manage it.

FW is an economic and ethical issue, since over 37 million people still cannot afford quality meals, but also a big environmental issue. Food production consumes limited natural resources, and FW only is responsible for 8–10 % of global greenhouse gas (GHG) emissions [4], excluding the huge impact of food production itself. As we became aware of the detrimental effects of GHGs on climate change and their consequence, in 2015 countries from all over the world stated the ambitious goal to hold the global average temperature increase to 1.5 °C above pre-industrial levels in the Paris Agreement [5]. To pursue this goal, the United Nations set the target of reducing GHGs emissions by 45 % by 2030 and reaching net zero by 2050 [6]. The European Union, with the European Green Deal and the "Fit for 55 %", set an even stricter goal of reducing GHG emissions by 55 % by 2030. However, despite most countries having set national climate plans, current commitments would lead to only 2.6 % decrease in GHG emissions by 2030 [7]. Thus, to pursue this goal, many approaches should be explored and developed simultaneously.

Among these, anaerobic digestion (AD) is a mature technology that can contribute to reducing GHG emissions in different productive sectors, by providing clean energy, treating organic waste, and facing resource depletion at the same time [8,9]. AD is the biological process in which biomass is decomposed by anaerobic microbial consortia into a methane-rich gas phase. Four different steps can be identified in the AD process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During the hydrolysis stage, complex polymers are broken down into smaller compounds, that can be more easily accessed and digested by microorganisms. Then, acidogenic microorganisms convert hydrolysis products into intermediate volatile fatty acids, which are used in acetogenesis to produce acetic acid, $H_2$, and $CO_2$. Finally, methanogenic microorganisms use these compounds to produce $CH_4$ [10,11]. Optimal environmental conditions during each phase ensure a balanced coexistence of the various species and so the best performance of the process. Unsuitable content of total solids (TS), imbalance in carbon to nitrogen ratio (C/N), micronutrient deficiency, volatile fatty acids, and ammonia accumulation, can slow down microbial activity and hinder methane production [10,12]. To avoid some of these drawbacks, different substrates are usually coupled in the anaerobic co-digestion (AcD), ensuring optimal values, especially in terms of pH and C/N [13,14]. Another fundamental parameter for AD process is the temperature, which distinguishes psychrophilic (0–25 °C), mesophilic (25–40, commonly 35–37 °C), and thermophilic (40–60, commonly 55–60 °C) operating conditions [11,15]. At each temperature, different microbial consortia are active, affecting AD efficiency and methane production, which are expected to increase with the increasing temperature. Moreover, higher temperatures are useful to sanitize the substrate killing most of the pathogens that may occur in the feedstock materials [11]. On the other side, thermophilic processes require more energy since big volumes must be heated at higher temperatures.

Even though AD is already spread at the industrial level, it still meets some criticalities, mainly related to the possible presence of inhibitors, accumulation of ammonia, generation of volatile fatty acids, and digestate management [16]. The optimal control of the process should limit the production of inhibitors and undesired compounds, while the digestate could be further exploited as organic fertilizer in alternative to mineral ones instead of being disposed of.

With the uninterrupted growth of the world's population, both waste production and food demand are increasing. Consequently, the requirement for fertilizers is expected to grow [17]. In the last few years, the use of inorganic fertilizers in agriculture indeed decreased with respect to previous years [18]. However, the production of mineral fertilizers has huge environmental costs, being very energy-intensive and depleting non-renewable resources [19]. Moreover, land use changes and intensive agricultural practices are affecting soil fertility. Thus, finding alternative sources for crops nutrients has never been so impelling.

The digestate coming from AD not only represents a valid substitute for mineral fertilizers, providing essential nutrients for crop cultivation, but also a good soil conditioner, enhancing plant growth-promoting microbes and water retention, reducing leaching, and improving soil structure [20–22]. These improvements result in increased soil productivity and plant yield [23].

The AD promotes the decomposition of easily degradable organic components, increasing the biological stability of the digestate and its nutrient availability for plants, while reducing its phytotoxicity [24].

However, nutrient composition in digestate is highly variable, depending on the feedstock used for the AD. Both organic and inorganic contaminants may be present in detrimental concentrations to crops or may accumulate over time with repetitive applications [25]. Physical impurities, pathogens, but also viscosity, and odour may hamper digestate application in soil [26]. Organic pollutants are degraded during AD, while heavy metals, coming from anthropogenic activities, can reach stable phases once in the soil [10]. Thus, the direct application of digestate in soil without further treatment is subject to a complete chemical analysis to guarantee its safety. Post-treatment of digestate is mandatory, especially in rural communities of low-income countries, where low-tech digesters are often implemented at the domestic level to treat organic waste and provide clean energy [27]. Here, the uncontrolled AD process may lead to a low stabilized, non-sanitized, and phytotoxic digestate [27]. In this case, post-treatment of digestate would ensure a safe reuse of digestate providing low-cost fertilizing sources in remote rural areas, where the circular-economy model proposed in the present work would represent a valid approach to accomplish many SDGs [27]. The AD of FW in low-tech equipment would avoid the need for waste disposal, and produce clean and affordable energy and fertilizers, allowing self-sustainment of communities.

FW is a suitable feedstock for AD and digestate production thanks to its high organic matter content and its continuous and abundant availability. At the same time, AD represents a valid treatment for FW, ensuring proper management to avoid environmental pollution, GHG emissions, and resource wastefulness [28,29]. However, FW is highly site-specific – being strictly dependent on the local collection rules – and it can be seasonal, very heterogeneous, and made of hard biodegradable components (as 18.6 % cellulose, 9.7 % lignin and 8.6 % hemicellulose), making the use of FW digestate as fertilizer more difficult. Among other types, FW digestate is a good biofertilizer, being usually rich in macro-nutrients and carbon. Ammonium can account for up to 60–80 % of total N in FW digestate, coming from protein degradation, thus contributing to emissions through volatilization ($NH_3$, $N_2O$, $N_2$) and leaching [21]. Moreover, FW digestate usually lacks essential trace elements, requiring the additional application of those compounds or the co-digestion of various feedstocks to improve its composition [20,23]. Little information still exists on the essential trace elements, such as Fe, Ni, Mo, Co, and Se, probably because FW digestates are not often studied and FW is generally co-digested with other substrates, especially manures and sewage sludges [21]. FW digestates are still poorly studied and more research focusing on FW or organic fraction of municipal solid waste (OFMSW) digestates is needed to better understand their short- and long-term effects on soil properties [21]. Additional studies are needed also to compare emissions and pollution from digestates with those from mineral fertilizers [21].

In recent years it has been proposed to use digestate as feedstock for pyrolysis to produce biochar, to overcome the abovementioned complications and face the possible drawbacks of its direct application in agriculture [30]. Given their high concentration of plant nutrients, FW and FW digestate biochar can be used in crop cultivation [20]. At the same time, the addition of biochar during AD can help solve the process criticalities thanks to its physical and chemical properties. The high surface area is suitable for microbial flora colonization and adsorbance of inhibiting compounds; pH can increase solution alkalinity, reducing ammonia inhibition and acid stress to the microbial community; good electrical conductivity (EC) and abundant presence of surface functional groups lead to higher $CH_4$ yield, favoured by the reduction of the lag phase of methanogenesis step related to the addition of biochar itself [16,28]. Additionally, the presence of biochar in the AD process seems to enhance the elemental composition of solid digestate, with positive effects for its subsequent use as fertilizer [16].

Many studies have been conducted in recent years focusing on AD of various biomasses and the evaluation of agronomic properties of digestates. However, to the best of the authors' knowledge, there are no available studies considering both process optimization for biogas and biomethane production, and agronomic effects of the residual digestate, and analysing how different operative conditions during the AD can affect the properties of the resulting digestate.

In the present study, a virtuous circular economy model is presented, in which digestate from AD of OFMSW is pyrolyzed and used to improve the performance of a subsequent AcD of FW and cow manure. The fertilizing potential of the resulting digestate is then evaluated through greenhouse pot experiments. Specifically, the study focuses on 1) the optimisation of anaerobic co-digestion (AcD) of FW and cow manure in terms of process stability and $CH_4$ production in the biogas by comparing different operative temperatures (35 and 50 °C) and the addition of different doses of biochar (0, 7, 14 g/L), produced through the slow pyrolysis of the dried digestate from OFMSW; 2) the evaluation of the agronomic effects of the produced digestates on plants growth to demonstrate their potential use as fertilizers and understand if the addition of biochar during the AD affects also the agronomic properties of the final digestates. The novel approach used in this study by using the same biomass in different operative conditions allows a better comparison avoiding the variability associated with the different feedstocks usually used for AD research works and aims at supporting the agronomic use of FW digestate as an alternative to mineral fertilizers.

## 2. Materials and methods

### 2.1. Inoculum and feedstock

The mesophilic AcD of FW and cow manure was performed with inoculum from the Speranza farming cooperative in Candiolo, Italy, which runs AD of livestock sewage, manure, agricultural waste, and chopped corn. The thermophilic AcD was done with inoculum from the Acea Pinerolese AD plant in Pinerolo, Italy. FW was taken from the leftovers of the University canteen, which contained pasta, bread, beans, peas, and white meat, and shredded before use for homogenization. Cow manure came from the same farming cooperative "Speranza" where the mesophilic inoculum was taken from. The same feedstock, of both FW and cow manure, was used for both mesophilic and thermophilic AcD to avoid any variability due to different feedstocks. The only different material was the inoculum, since mesophilic and thermophilic inocula were used for mesophilic and thermophilic AcD respectively, to avoid thermal shocks to microbial communities. No pretreatments were performed on feedstock materials, except for the shredding of FW to obtain a homogenized substrate.

The biochar used in the AcD was produced through slow pyrolysis performed in a fixed bed reactor at 500 °C at 5 °C/min with a residence time of 1 h according to [29]. The pyrolysis setup and biochar recovery are described in our previous work [31]. The feedstock for pyrolysis was the dried digestate coming from the anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW) of a real treatment plant performed in batch feeding mode at Substrate to Inoculum ratio (S:I) of 2:1 based on Volatile Solids (VS) and 6 % of Total Solids (TS) under mesophilic conditions (37 °C) according to the optimal operative condition identified in our previous work [32]. The chemical and physical properties of the dried digestate are reported in Table 3. The dried digestate was chosen as a biochar source as part of the closed circular model proposed to explore alternative uses of digestate, as discussed in the previous section.

### 2.2. Experimental set-up: anaerobic digestion

Anaerobic co-digestion (AcD) of FW and cow manure was performed in batch mode feeding. FW and cow manure were mixed in a 0.8: 0.2 w/w ratio to reach the optimal values of pH and C/N ratio for the anaerobic digestion process. AcD was set up with 6 % TS, an S:I equal to 1:1, and a working volume of 80 % of the reactor volume according to [33]. AcD tests were carried out in 500 mL ®Duran glass bottles (Duran, Germany). Each digestor was manually shaken four times per day to avoid the formation of a surface crust and ensure proper contact between substrate and microorganisms.

Agitation is one of the key factors that affect the efficiency of anaerobic digestion but, despite the extensive research, the optimal mixing method is still debated. According to various studies, intermitting mixing is considered even better than continuous and unmixing [34–36]. Moreover, when working with low TS content, anaerobic digestion seems to be unaffected by mixing conditions [35]. Thus, it is reasonable to assume that the mixing condition used in the present study doesn't affect considerably the performance of the anaerobic digestion.

AcD was performed to evaluate the effect of adding biochar under mesophilic and thermophilic conditions. For this purpose, two biochar doses were tested: 7 g/L (B1) [28] and 14 g/L (B2) [16]. Various biochar doses in anaerobic digestion have been tested in the literature, but the optimal dosage is still debated. In this study, two intermediate values were chosen among those reported by other authors, considering also the scaling up of the process, where high biochar doses would be unfeasible. Two experimental runs were carried out. The first run was performed under the mesophilic condition at 35 °C and the second under the thermophilic condition at 50 °C. Temperature control was guaranteed in a 55 L thermostatic water bath (Julabo-Corio-C).

For each experimental run, 4 configurations were tested (Table 1): inoculum alone to measure the net biogas and methane productions, AcD without biochar as a positive control, AcD with the lowest dose of biochar (B1, 7 g/L), and AcD with the highest dose of biochar (B2, 14 g/L). Each configuration was tested in triplicates to assess the uncertainty associated with the experiments. A total of 12 digestors were managed per run. The exact quantities fed into reactors for each configuration are given in Tables S1 and S2 (Supplementary Materials).

Each digestor was connected by 6 mm Teflon tubes (PTFE, Germany) to a 1 L ®Tedlar gas bag acting as a gasholder. The measurement of the biogas volume was performed through the water displacement method. The biogas composition was measured with a SRA Micro-GC, equipped with a Molsieve 5A column (for the analysis of permanent gases like hydrogen, nitrogen, methane, and carbon monoxide) using argon as a carrier (column temperature: 100 °C) and with a TCD detector. The injection temperature was fixed at 90 °C and the pressure at 30 psi.

Daily and cumulative biogas production of each configuration was calculated by subtracting the production of the inoculum (I), to be able

**Table 1**
Abbreviations of AcD configurations.

| ID code | Description |
|---|---|
| **I_M** | AD of Inoculum in Mesophilic conditions |
| **M** | AcD of FW and cow manure without biochar in Mesophilic conditions |
| **M_B1** | AcD of FW and cow manure with the lowest dose of biochar (B1, 7 g/L) in Mesophilic conditions |
| **M_B2** | AcD of FW and cow manure with the highest dose of biochar (B2, 14 g/L) in Mesophilic conditions |
| **I_T** | AD of Inoculum in Thermophilic conditions |
| **T** | AcD of FW and cow manure without biochar in Thermophilic conditions |
| **T_B1** | AcD of FW and cow manure with the lowest dose of biochar (B1, 7 g/L) in Thermophilic conditions |
| **T_B2** | AcD of FW and cow manure with the highest dose of biochar (B2, 14 g/L) in Thermophilic conditions |

AD tests were stopped when the daily biogas rate was below 1 % of the total volume produced up to that time [37].

to evaluate the net production of the substrate (S) alone, according to Eq. (1) that follows.

$$Net\ Biogas\ Production\ of\ Substrate\ \left[NL / kg_{VS\ S}\right] = \frac{Biogas\ Production\ of\ Inoculum\&Substrate\ [NL] - Biogas\ Production\ of\ Inoculum\ \left[NL/kg_{VS\ I}\right]\cdot VS_I}{kg_{VS}} \quad (1)$$

Every week, liquid samples of approximately 5 mL were taken from each digestor to carry out analyses and monitor the progression of the process. The pH was measured through the DIN 38,404 C5 methodology with PC 80+ DHS® (XS Instruments). The FOS (Flüchtigen Organischen Säuren, organic volatile fatty acids) and TAC (Totales Anorganisches Carbonat, inorganic total carbonate) measurements were carried out with a pH340 WTW-pH-meter according to [38]. First, samples were filtered with a 1.2 μm filter. Then, titration was performed with a 1:10 diluted sample by adding sulphuric acid 0.1 N to reach a pH value of 5.0 ($P_0$) and 4.4 ($P_1$). The volumes of acid added to obtain respectively $P_0$ and $P_1$ are $V_0$ and $V_1$. FOS and TAC can then be calculated by Eq. (2) and (3):

$$FOS = (V_1 \cdot 1.66 - 0.15) \cdot 500 \quad (2)$$

$$TAC = V_0 \cdot 250 \quad (3)$$

### 2.3. Kinetic study

To deepen the understanding of dosage effects on methane and biogas production for different AcD conditions tested, kinetic studies were performed. A first-order kinetic model was assumed to evaluate the disintegration rate ($k_d$) of the process through the initial part of the cumulative methane curve, according to Eq. (4) [37].

$$B(t) = B_{exp}(1 - e - k_d t) \quad (4)$$

where *B(t)* is the cumulative methane production at a given time *t*, $B_{exp}$ represents the methane potential yield at the fifth day (NL/$kg_{VS}$), $k_d$ is the disintegration rate ($d^{-1}$), and *t* is time (d). then, the Gompertz-modified model was used to estimate the lag phase, the maximum biogas production rate, and the maximum biogas yield potential, according to Eq. (5) [32,39].

$$y(t) = y_m exp\left\{ - exp\left[\frac{R_m e}{y_m} \cdot (l - t) + 1\right]\right\} \quad (5)$$

where *y(t)* is the predicted biogas yield (NL/kg $_{VS}$) at a given time *t* (d), $y_m$ is the theoretical biogas potential (NL/$kg_{VS}$), Rm is the maximum biogas production daily rate (NL/$kg_{VS}$ d), and λ is the lag phase (d).

The theoretical biogas potential (TBP) is calculated by Buswell and Neave equations (Eq. (6) and (7)) according to [32].

$$C_aH_bO_cN_d + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4}\right)H_2O \rightarrow \left(\frac{4a + b - 2c - 3d}{8}\right)CH_4 + \left(\frac{4a - b + 2c + 3d}{8}\right)CO_2 + dNH_3 \quad (6)$$

$$TBP\left[\frac{Nm^3}{kg_{VS}}\right] = \frac{22,415a}{12a + b + 16c + 14d} \quad (7)$$

### 2.4. Physic-chemical characterization

Humidity, TS, and VS contents of all used biomasses – inoculum, organic food waste, cow manure, and biochar – as well as of produced digestates were assessed according to APHA, 2006. Humidity and TS were measured by drying samples at 105 °C overnight. VS were determined with calcination to ash at 550 °C for 6 h with a heating rate of 5 °C/min. Elemental composition (CHNS) of the dried and liquid samples was also measured with the Elemental Macro Cube system (Vario, Germany), determining percentage composition in terms of C, H, N, S, and O in addition to 100.

Specific surface area (SSA), pore size, and pore volume of biochar were measured with $N_2$ adsorption method on a BET analyzer (Micromeritics TriStar II 3020), after being degassed under vacuum for 2 h at 300 °C and then cooled in a liquid nitrogen bath at −196 °C for analysis. The main functional groups were assessed with FT-IR Spectrophotometer Tensor 27 (Bruker) within the wavenumber range of 4000 to 400 $cm^{-1}$.

Additionally, Electrical Conductivity (EC), and the main nutrient content of digestates were evaluated. For EC measurement, performed with PC 80+ DHS® (XS Instruments), digestates were extracted with distilled water (1:10 w/v) and then centrifuged at 5000 rpm for 5 min (SL 16R, Thermo Scientific), since suspended solid matter hindered the stabilization of the measure and could damage the instrument. Total nitrogen was measured through elemental analysis, while nitrate, ammonium, orthophosphate, and potassium were detected with HACH LANGE GHB vial tests, respectively LCK340, LCK503, LCK350, LCK228, and quantified by a DR5000 spectrophotometer (HACH), and finally, organic nitrogen was estimated by subtraction, according to Eq. (8).

$$N_{org} = N_{tot} - N \cdot NH_4^+ - N \cdot NO_3^- \quad (8)$$

Again, only the liquid fraction of digestates obtained after centrifugation was used for the assessment of these substances because the presence of suspended solid particles alters the spectrophotometric measure.

### 2.5. Experimental set-up: greenhouse pot trial

A greenhouse experiment was performed to evaluate the agronomic effects of the different produced digestates. For this purpose, triplicates of each configuration were mixed, obtaining a total of six different digestates, as reported in Table 2.

The experimental campaign was carried out over three months during the summer season, from the beginning of June to the end of August 2023. Peat substrate mixed with perlite in 50:50 % v/v was used for cultivation in pots of tomato plants (*Solanum lycopersicum* L., oxheart variety, ESASEM S.p.A.). Digestates were spread on the pot surface and applied in three different doses equal to 85 (N1), 170 (N2), and 340 (N3)

**Table 2**
Abbreviations of treatments used for greenhouse pot trial.

| ID code | Description |
|---|---|
| **D_M** | Digestate from mesophilic AcD without biochar (M) |
| **D_MB1** | Digestate from mesophilic AcD with the lowest biochar dose (M_B1) |
| **D_MB2** | Digestate from mesophilic AcD with the highest biochar dose (M_B2) |
| **D_T** | Digestate from thermophilic AcD without biochar (T) |
| **D_TB1** | Digestate from thermophilic AcD with the lowest biochar dose (T_B1) |
| **D_TB2** | Digestate from thermophilic AcD with the highest biochar dose (T_B2) |
| **C** | Commercial compost |
| **F** | Mineral fertilizer |
| **UC** | Untreated Control |

kg N/ha at the time of transplanting of seedlings in pots (commercial plastic pots, 12 × 12 × 13 cm, 1.25 L), ten days after the germination. One plant was transplanted in each pot. Commercial compost (C) (ACSR S.p.A.) and mineral fertilizer (F) (urea, carbamide, $NH_2CONH_2$, 46 % N, Alfenatura, Italy) were also applied in the same dosages, mixed with cultivation substrate. Non-treated plants were used as control (UC). No other fertilizer has been applied for the whole duration of the trial. 10 replications were used for each thesis.

The intermediate nitrogen dosage of 170 kg N/ha (N2) was selected according to the Council Directive 91/676/CEE, the so-called Nitrates Directive (European Council Directive, 1991b), and the lowest (85 kg N/ha, N1) and highest (340 kg N/ha, N3) ones were chosen as half and double dosages of the intermediate one.

For the trial, designed in completely randomized blocks, automatic sprinkler irrigation was set three times a day and coupled with manual irrigation to avoid water shortages on the hottest days.

### 2.6. Agronomic and physiological parameters

Every week essential parameters of all plants were assessed. Height was measured and leaves were counted. Then, the Chlorophyll Content Index (CCI) was evaluated with a SPAD 502 chlorophyll meter (CCM-200 plus, Opti-Sciences, Inc., Hudson, NH, USA). Specifically, CCI was measured on the first three different fully formed leaves of the same composite leaf from the top of each plant, since they are usually the best developed and exposed to light. SPAD measurement allows instant real-time, rapid, and non-destructive assessment of chlorophyll content and crop nitrogen status, which is essential for efficient crop production and N management [40].

At the end of the experiment, all plants were cut and immediately weighed to measure the fresh biomass. Dry biomass was determined by weighing the samples after oven drying at 105 °C for 72 h. Finally, Root Development Index (RDI) was assigned to evaluate the development of the root apparatus. This index is assigned as a score between 0 (not developed) and 4 (very well developed) based on the visual inspection of the root development in each pot according to [41]. With an RDI equal to 0, the soil slab smashes itself and soil is dispersed once out of the pot. With an RDI equal to 4, roots are grown all over the pot and the soil remains compact and pot-shaped, as shown in Fig. 1.

### 2.7. Statistical analysis

The experimental data of biogas production and greenhouse pot experiments were subjected to one-way ANOVA to compare the mean results of different treatments on AD performances and plant measurements. After the ANOVA, Duncan's post-hoc test ($p < 0.05$) was performed. The statistical software IBM SPSS Statistics was used for all statistical analyses.

## 3. Results and discussion

### 3.1. Characterization of inoculum, substrates and biochar for anaerobic digestion

Physic-chemical properties of mesophilic (M) and thermophilic (T) inoculum, food waste and cow manure used for the AcD, as well as digestate from OFMSW then pyrolyzed and biochar used for the AcD are reported in the following Table 3. Substrates and inoculum are site-specific, and their properties strictly depend on the composition, location, and feedstock (for the inoculum). However, TS and VS values reported in this work align with other studies, for both inoculum [42] and FW [43]. The elemental composition (% of C, H, N, S, and C/N) of FW is also in line with other studies where similar FW substrate was used [32,43,44]. Moreover, C/N ratio and pH values are in the optimal range for the anaerobic digestion process [45]. This confirms that the used FW is a good representative sample of average FW and allows to generalize the presented results to analogous feedstocks.

Concerning biochar properties, specific surface area (SSA), pore size, and pore volume are among the most important factors that affect the adsorption capacity of biochar and its role during the anaerobic digestion, adsorbing potential inhibitors and promoting microbial colonization [16]. The average pore diameter is in line with values found in literature [46] and falls into the mesopores (2–50 nm). The SSA of the biochar used in this study (17.59 $m^2/g$) is quite low compared to biochars obtained from other biomasses but it is aligned with biochars produced in other studies from similar feedstocks, such as sludge [16]. Biochar with similar SSA have been used in AD processes also in other works with good results, proving that it is difficult to assess the role of biochar in AD and to directly correlate its SSA with the effect on AD performances [47]. Finally, FT-IR analysis was performed to assess the main functional groups of biochar. The complete spectrum is given in Figure S1 in Supplementary Materials. The absorption band between 3560 and 2992 $cm^{-1}$ can be attributed to O—H stretching vibrations and suggests the presence of phenols, alcohols, or carboxylic acids [48]. The peaks at 2925 and 2855 $cm^{-1}$ indicate asymmetric and symmetric $CH_2$ aliphatic bonds, while the absorption band between 1800 and 1500 $cm^{-1}$, with peaks in 1730 and 1600 $cm^{-1}$, signals the presence of $C = O$ of either carboxylic acid/ester or aldehyde/ketone groups [48]. The peak at 1025 $cm^{-1}$ indicates C–O, $C = C$, and/or C–C stretching [48,49]. Previous studies suggested that surface redox properties of biochar improve methane production during the AD process [50]. Redox properties are supposed to depend on the type and quantity of surface functional groups, resulting in different effects on AD performances [50]. However, the exact mechanisms of this synergic process are still poorly known and further research is needed to deepen their understanding.


**Fig. 1.** Examples of assigned Root Development Indexes.

**Table 3**
Substrates physic-chemical characterization.

| | Inoculum_M | Inoculum_T | FW | Manure | OFMSW digestate | Biochar |
|---|---|---|---|---|---|---|
| **TS [%]** | 5.922 ± 0.068 | 3.200 ± 0.083 | 27.432 ± 0.078 | 16.776 ± 1.073 | 5.7 ± 0.99 | 96.91 ± 1.004 |
| **VS/TS [%]** | 60.000 ± 2.003 | 60.000 ± 1.993 | 85.000 ± 3.043 | 70.000 ± 1.932 | 52 ± 2.76 | 62.474 ± 2.099 |
| **pH**[1] | 6.3 | 6.7 | 5.8 | 7.2 | 6.9 ± 0.15 | 8.1 |
| **C [%]** | 41.065 ± 0.969 | 43.042 ± 1.008 | 51.815 ± 0.969 | 42.240 ± 1.754 | 39.34 ± 1.34 | 54.260 ± 3.640 |
| **H [%]** | 4.665 ± 0.011 | 5.092 ± 0.002 | 6.811 ± 0.018 | 5.053 ± 0.199 | 6.08 ± 2.1 | 1.490 ± 0.094 |
| **N [%]** | 3.105 ± 0.106 | 3.020 ± 0.138 | 4.255 ± 0.205 | 2.085 ± 0.035 | 3.4 ± 0.45 | 2.030 ± 0.186 |
| **S [%]** | 0.275 ± 0.026 | 0.103 ± 0.001 | 0.221 ± 0.000 | 0.291 ± 0.046 | 1.45 ± 0.01 | 1.217 ± 0.200 |
| **C/N** | 13.225 ± 0.085 | 14.252 ± 0.147 | 12.177 ± 0.360 | 20.259 ± 0.498 | 11.57 ± 0.97 | 26.729 ± 1.226 |
| **Specific surface area [$m^2$/g]** | – | – | – | – | – | 17.59 ± 1.71 |
| **Pore volume [$m^3$/g]** | – | – | – | – | – | 0.056 ± 0.001 |
| **Average pore diameter (nm)** | – | – | – | – | – | 15.458 ± 1.920 |

[1] standard deviation is not present because a single measure has been taken.

**Table 4**
. Cumulative biogas, biomethane production and FOS/TAC values of different AcD configurations: **M**, mesophilic AcD without biochar; **M_B1**, mesophilic AcD with the lowest biochar dose (7 g/L); **M_B2**, mesophilic AcD with the highest biochar dose (14 g/L); **T**, thermophilic AcD without biochar; **T_B1**, thermophilic AcD with the lowest biochar dose (7 g/L); **T_B2**, thermophilic AcD with the highest biochar dose (14 g/L). Different letters indicate differences between treatments and dosages that were significant at $P < 0.05$ (Duncan's).

| | Total biogas production [NL/$kg_{VS}$] | Total $CH_4$ production [NL/$kg_{VS}$] | FOS/TAC [-] |
|---|---|---|---|
| **M** | 553.309 ± 15.308 (bc) | 229.109 | 0.246 ± 0.032 |
| **M_B1** | 527.066 ± 136.147 (bc) | 192.526 | 0.314 ± 0.017 |
| **M_B2** | 485.531 ± 24.302 (c) | 182.889 | 0.347 ± 0.026 |
| **T** | 663.415 ± 63.129 (b) | 368.972 | 0.321 ± 0.007 |
| **T_B1** | 892.869 ± 4.924 (a) | 525.390 | 0.307 ± 0.014 |
| **T_B2** | 886.413 ± 16.988 (a) | 527.961 | 0.338 ± 0.006 |

### 3.2. Anaerobic digestion

The effect of biochar addition on the cumulative biogas and biomethane production process in both mesophilic and thermophilic conditions was investigated. 1-way ANOVA evidenced statistically significant differences between cumulative biogas production of the various tested configurations (Table 4). Thermophilic AcD with both doses of biochar (T_B1 and T_B2) turned out to be the best performing, followed by thermophilic AcD without biochar (T), mesophilic AcD without biochar (M) and with the lowest dose of biochar (7 g/L, M_B1), and finally mesophilic AcD with the highest dose of biochar (14 g/L, M_B2).

Cumulative biogas production of M (Fig. 2a) was 14 % higher than M_B2 and 5 % higher than M_B1, even though the latter was not statistically significant, with 553.31 ± 15.31 NL/$kg_{VS}$. Instead, AcD without biochar was the least productive in thermophilic conditions (T), 25 % less than T_B1 and T_B2 (Fig. 2b), which produced almost the same quantity of biogas in the end, 892.9 ± 4.9 and 886.4 ± 17.0 NL/$kg_{VS}$ respectively.

Total biogas production is significantly higher under thermophilic conditions, where the process is accelerated. T_B1 reached 892.9 ± 4.9 NL/$kg_{VS}$ in <30 d, while M_B1 production was 45 % lower over the same time and it reached 527.1 ± 136.1 NL/$kg_{VS}$ after 35 d when the process ended. Final biogas and biomethane production of each configuration is reported in Table 4, along with FOS/TAC values which are discussed later in this section, while cumulative curves of both biogas and biomethane during the whole experiment are shown in Fig. 2.

Instead, the highest percentage of $CH_4$ in biogas was observed in the AcD without biochar and the lowest one with B2, in both mesophilic and thermophilic conditions, even though the difference was smaller in the latter (Fig. 3). A maximum of 70 % v/v of $CH_4$ was reached in the mesophilic AcD M, almost halfway through the process, while 66 % of $CH_4$ in the biogas was obtained at the end of the thermophilic AcD T_B2. This led to the final highest $CH_4$ production in T_B2 (527.9 $NL_{CH4}/kg_{VS}$) and the lowest one in M_B2 (182.9 $NL_{CH4}/kg_{VS}$). Moreover, mesophilic and thermophilic conditions showed differences in the biogas composition during the evolution of the process. In mesophilic conditions, $CH_4$ in the biogas increased at the beginning, reached the maximum almost halfway through the process, and then steadily decreased until the end, while in thermophilic conditions $CH_4$ percentage in the biogas kept growing day by day and so the highest quantity of $CH_4$ reported in Fig. 3b was reached at the end of the process.

Even though the role of biochar during AD is still debated, results suggest that it has an effect. In mesophilic conditions, it seems to reduce the process efficiency, probably because it adsorbs the microorganisms, reducing their contact with the substrate, or it adsorbs the biogas, hindering its release. In thermophilic conditions, instead, it enhanced the process efficiency.

A possible explanation is that the higher temperature favored the release of undetermined compounds that promoted microbial activity and biogas production, or it favored the adsorption of inhibiting compounds and microbial colonization [16]. Another possible explanation resides in the inoculum used. Mesophilic and thermophilic AcD were performed under the same operative conditions (TS, S:I ratio) and with the same feedstock materials, but the pivoting role has been played by the inoculum, which had different origin for mesophilic and thermophilic AcD. Mesophilic inoculum is generally more stable and less sensitive to temperature and environmental changes than thermophilic inoculum [51]. As a result, thermophilic processes typically require more control. Biochar can serve as an effective biomaterial to help regulate these processes and enhance stability. Even though microbial community was not analysed, this hypothesis is supported by the increased biogas and methane productions in the presence of biochar in thermophilic conditions (Table 5).

Many factors affect the performance of the AD process. Initial conditions of the test, inoculum and substrate used, substrate to inoculum ratio, biochar dose, but also biochar type and its properties [52,53], which are related to the operative conditions and temperature of biochar production, as it has been evidenced by Wambugu et al. [28]. For this reason, it is difficult to generalize the results and make sound comparisons with other works and both total inhibition and huge process improvement are reported in literature because of the biochar addition. Literature treating similar substrates for mono- or co-digestion with various doses of biochar was considered to compare results and even if experimental conditions are not always equal, results remain comparable.

$CH_4$ total production in mesophilic conditions of the present work is like that obtained in other studies. Swiechowski et al. (2022) reported

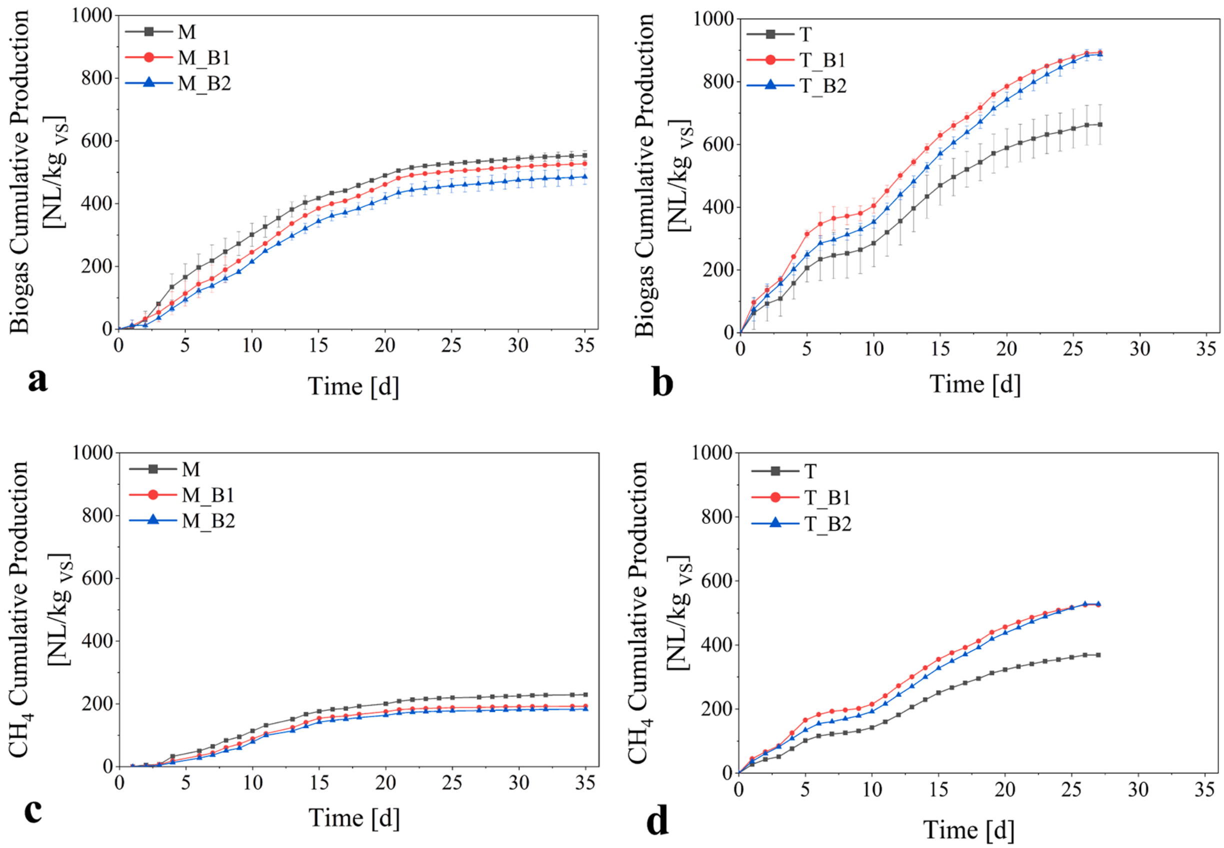


**Fig. 2.** Cumulative biogas production of mesophilic AcD (**2a**) and thermophilic AcD (**2b**) and cumulative methane production of mesophilic AcD (**2c**) and thermophilic AcD (**2d**). Each error bar represents one standard deviation. **M**, mesophilic AcD without biochar; **M_B1**, mesophilic AcD with the lowest biochar dose (7 g/L); **M_B2**, mesophilic AcD with the highest biochar dose (14 g/L); **T**, thermophilic AcD without biochar; **T_B1**, thermophilic AcD with the lowest biochar dose (7 g/L); **T_B2**, thermophilic AcD with the highest biochar dose (14 g/L).

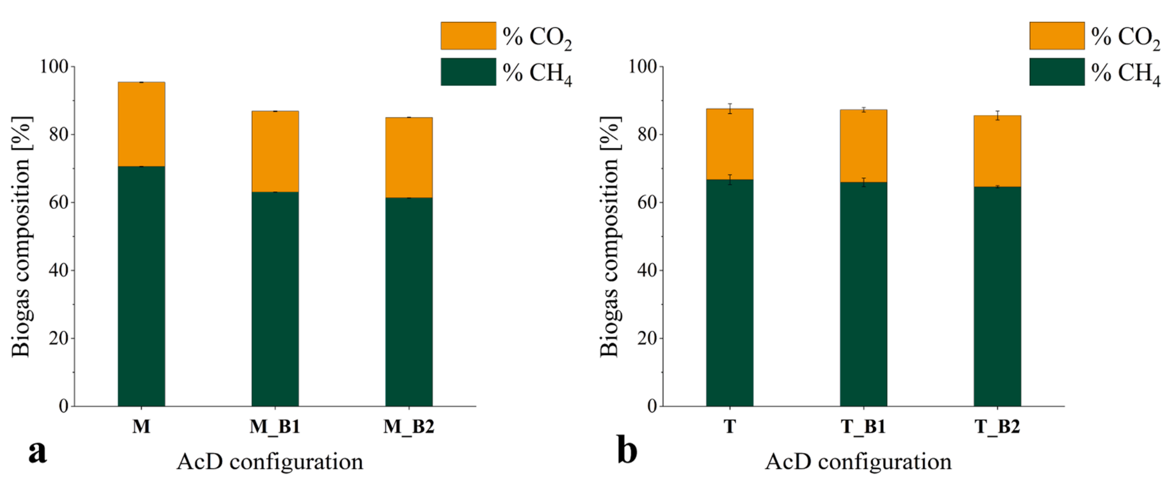


**Fig. 3.** Percentage biogas composition in terms of $CH_4$ and $CO_2$ of AcD in mesophilic (**2a**) and thermophilic (**2b**) conditions, at the maximum of $CH_4$ production. The remaining 10 % or less, consisting of air and humidity, has been excluded from the representation. Each error bar represents one standard deviation. **M**, mesophilic AcD without biochar; **M_B1**, mesophilic AcD with the lowest biochar dose (7 g/L); **M_B2**, mesophilic AcD with the highest biochar dose (14 g/L); **T**, thermophilic AcD without biochar; **T_B1**, thermophilic AcD with the lowest biochar dose (7 g/L); **T_B2**, thermophilic AcD with the highest biochar dose (14 g/L).

higher $CH_4$ production than this study, between 347.9 and 365.2 $mL_{CH4}/g_{VS}$, and reached higher methane production with the addition of only 0.65 g/L of FW biochar [53]. Jiang et al. (2020) reported a huge increase in $CH_4$ production with the addition of biochar compared to the control, for every tested dose (0.5, 1, 1.5 $g_{BC}/g_{VS\ of\ co\text{-}substrate}$, corresponding to 8.4, 16.8, 25.2 g/L), and the $CH_4$ production was close to that obtained in this study, between 188 and 250 $mL_{CH4}/g_{VS}$ with the increasing dose of biochar [54]. Analogously, Kaur et al. (2020) reached the highest cumulative $CH_4$ production (381.92 $L_{CH4}/kg_{VS}$) with the addition of 10 $g_{BC}/L$ [47].

Concerning the thermophilic AcD, Zhang et al. (2020) obtained the highest cumulative $CH_4$ production (786 $mL_{CH4}/g_{VS}$) from the AD of FW with 7.5 $g_{BC}/L$, significantly higher than that obtained in the present study with a similar biochar dose (525.39 NL/kgVS with 7 g/L of

**Table 5**
Comparison of the results from other works in literature on the effects of biochar addition on anaerobic digestion of food waste.

| Reference | Feedstock | Operative Temperature (°C) | Biochar (type and concentration)[1] | | Biochar effect[2] | Total $CH_4$ production [$NL/kg_{VS}$] |
|---|---|---|---|---|---|---|
| Present study | University canteen food waste and cow manure (80 % FW) | 35 | Dried digestate | 7 g/L | - 20 % | 192.5 |
| | | | | 14 g/L | - 16 % | 182.9 |
| | | 50 | | 7 g/L | + 42.4 % | 525.39 |
| | | | | 14 g/L | + 43 % | 527.96 |
| [53] | Artificial food waste[3] | n.d. | Food waste | 0.65 g/L | + 3.6 % | 331 – 365.2 |
| [54] | University canteen food waste and sewage sludge (VS SS/VS FW 1:1) | Mesophilic (not defined) | Citrus peel | 0.5 $g_{BC}/g_{VS\ of\ co\text{-}substrate}$ | + 86.4 % | 188.6 |
| | | | | 1 $g_{BC}/g_{VS\ of\ co\text{-}substrate}$ | + 105 % | 208.0 |
| | | | | 1.5 $g_{BC}/g_{VS\ of\ co\text{-}substrate}$ | + 147 % | 250.8 |
| [47] | Artificial food waste[3] and sewage sludge | 35 | Wheat straw pellet | 10 g/L | + 24 % | 381.9 |
| [43] | Canteen food waste | 55 | Sawmill waste wood pellets gasification | 7.5 g/L | + 31 % | 786 |
| [56] | Artificial food waste[3] and sewage sludge (4:1) | 35 | Sawdust | 10 g/L | Reduction of lag phase | ~350 |
| [55] | Artificial food waste[3] and sewage sludge (75: 25) | 37, 55 | – | – | | 460 |

[1] When not differently specified, the biochar has been produced through slow pyrolysis.
[2] Where not specified, biochar effect is referred to increase of methane yield with respect to AD without biochar, of each paper.
[3] Food waste was simulated mixing different fresh foods to bring carbohydrates, proteins, and fibres.

biochar) [43]. Gu et al. (2020) compared mesophilic and thermophilic AD of different ratios of FW and sewage sludge, without the addition of biochar [55]. The highest $CH_4$ production was obtained with FW only and it was similar in both cases, of approximately 460 $mL_{CH4}/g_{VS}$, higher than the mesophilic one of this study, but in line with the thermophilic one.

In Table 5 the main data and results of other works dealing with anaerobic digestion of food waste with different co-substrates and doses of biochar are summarized.

Concerning physic-chemical properties of the digestates at the end of AD, mesophilic ones have similar properties among themselves as well as thermophilic ones, but some differences emerged between them (Table 7). Despite a similar content of TS, VS of mesophilic digestates is significantly lower than that of thermophilic digestates, around 10 % on average, but still TS and VS are in line with other studies [57].

Final pH results slightly lower for mesophilic digestates, suggesting that the different temperature at which AcD has been performed may affect the properties of the digestate. In any case, during the whole process pH always remained in the optimal range values without hindering the microbial activity. For all digestates, final pH is in line with common values of AD and in accordance with the literature [58,59]. Lower content of C and N emerged from the elemental analysis for thermophilic digestates compared to the mesophilic ones, resulting however in a similar C/N ratio, in line with other studies also in this case [58,60].

Another parameter used to assess the stability of the AD process is the FOS/TAC ratio. FOS/TAC values below 0.2 are usually associated with a too low biomass input, while above 0.5 excessive biomass is present [61]. For nearly all the configurations, FOS/TAC value was between 0.3

**Table 6**
Results of kinetic study of AcD configurations. **M**, mesophilic AcD without biochar; **M_B1**, mesophilic AcD with the lowest biochar dose (7 g/L); **M_B2**, mesophilic AcD with the highest biochar dose (14 g/L); **T**, thermophilic AcD without biochar; **T_B1**, thermophilic AcD with the lowest biochar dose (7 g/L); **T_B2**, thermophilic AcD with the highest biochar dose (14 g/L).

| | 1st order kinetic | | Gompertz modified model | | |
|---|---|---|---|---|---|
| | $k_d$ ($d^{-1}$) | $R^2$ | λ (d) | Biogas theoretical ($NL/kg_{VS}$) | Biogas experimental ($NL/kg_{VS}$) |
| **M** | 0.0091 | 0.922 | 2 | 922.615 | 553.309 |
| **M_B1** | 0.0071 | 0.9003 | 2 | 830.666 | 527.066 |
| **M_B2** | 0.0056 | 0.9234 | 3 | 851.320 | 485.531 |
| **T** | 0.0248 | 0.9777 | 1 | 911.716 | 663.415 |
| **T_B1** | 0.042 | 0.9807 | 0.5 | 932.622 | 892.869 |
| **T_B2** | 0.0314 | 0.9978 | 0.5 | 925.518 | 886.413 |

**Table 7**
Physic-chemical characterization and nutrient composition of digestates for plants growth trial: **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L).

| Parameter | D_M | D_MB1 | D_MB2 | D_T | D_TB1 | D_TB2 |
|---|---|---|---|---|---|---|
| **TS [%]** | 2.76 ± 0.231 | 2.65 ± 0.727 | 3.28 ± 0.304 | 3.33 ± 0.294 | 3.29 ± 0.334 | 4.14 ± 0.097 |
| **VS [% d.m.b.[1]]** | 50.57 ± 0.373 | 49.80 ± 1.582 | 52.08 ± 1.351 | 60.63 ± 1.362 | 60.24 ± 0.466 | 64.22 ± 0.447 |
| **Ashes [% d.m.b.[1]]** | 49.43 | 50.20 | 47.92 | 39.37 | 39.76 | 35.78 |
| **pH [-][2]** | 7.41 | 7.40 | 7.37 | 8.74 | 8.23 | 9.03 |
| **EC [mS/cm][2]** | 9.06 | 12.72 | 10.24 | 11.32 | 10.63 | 14.45 |
| **C [%]** | 1.12 ± 0.098 | 1.41 ± 0.014 | 1.24 ± 0.212 | 0.7 ± 0.085 | 1.05 ± 0.0424 | 0.98 ± 0.099 |
| C [%][1] | 37.90 ± 1.138 | 40.94 ± 4.971 | 40.44 ± 0.332 | 25.25 ± 0.071 | 25.78 ± 6.300 | 24.50 ± 2.956 |
| **N [%]** | 0.27 ± 0.043 | 0.32 ± 0.057 | 0.315 ± 0.007 | 0.34 ± 0.0424 | 0.345 ± 0.0353 | 0.32 ± 0.000 |
| N [%][1] | 3.42 ± 0.134 | 3.27 ± 0.283 | 3.23 ± 0.092 | 2.34 ± 0.141 | 2.16 ± 0.431 | 1.83 ± 0.113 |
| **C/N [-]** | 4.25 ± 1.057 | 4.499 ± 0.712 | 3.939 ± 0.547 | 2.061 ± 0.332 | 3.046 ± 0.172 | 3.050 ± 0.265 |
| C/N [-][1] | 11.10 ± 0.103 | 12.63 ± 2.613 | 12.54 ± 0.461 | 10.81 ± 0.623 | 11.85 ± 0.548 | 13.46 ± 2.448 |
| **S [%]** [1] | 0.59 ± 0.003 | 0.45 ± 0.052 | 0.38 ± 0.009 | 0.46 ± 0.023 | 0.42 ± 0.067 | 0.34 ± 0.058 |
| **$N\text{-}NO_3^-$ [mg/L][2]** | 37.5 | 62.5 | 54.5 | 36.5 | 31.55 | 40.6 |
| **$N\text{—}NH_4^+$ [mg/L][2]** | 1170 | 1390 | 1080 | 1470 | 1840 | 1620 |
| **N-org [mg/L][2]** | 1492.5 | 1747.5 | 2015.5 | 1893.5 | 1578.5 | 1539.4 |
| **K [mg/L][2]** | 1760 | 2130 | 1870 | 1480 | 1740 | 1900 |
| **ortho-P [mg/L][2]** | 15.82 | 18.47 | 7.78 | 18.1 | 29.45 | 18.7 |

[1] dm.b.: dry matter basis.
[2] standard deviation is not present because a single measure has been taken.

and 0.4 (Table 4), which is the optimal range value for maximum biogas production. According to the FOS/TAC value measured at the beginning of the process, only AcD without biochar in mesophilic conditions (M) resulted in a low biomass input, however no more biomass was added to observe the initial design parameters and the batch mode feeding, which does not provide any feeding once the process has started.

### 3.3. Kinetic study

First-order kinetic and Gompertz modified model were used to verify and confirm experimental data through the kinetic study. The main results are reported in Table 6, while graphics of Gompertz modified model for mesophilic and thermophilic configurations are shown in Figures S2, S3 in Supplementary Material, respectively. From the first-order kinetic, disintegration rate ($k_d$) was obtained for each configuration, representing the velocity at which substrate is degraded. In the mesophilic AcD, $k_d$ confirms the inhibiting effect of biochar with the increase of the applied dose. The Gompertz modified model shows that there are not significant differences between M and M_B1, that have the same lag phase (λ), while MB2 is slowed down, while in thermophilic AcD the addition of biochar reduced λ without relevant differences between the two doses (T_B1, T_B2). Additionally, thermophilic AcD with both doses of biochar were the best performing, getting close to the theoretical biogas production values modeled.

### 3.4. Characterization of digestates for plant growth experiments

In addition to proximate analysis and elemental analysis, the main nutrient composition for plants growth was measured in both digestates and commercial compost used for comparison.

The commercial compost (C) had 70.41 % of TS, pH equal to 5.69, and EC to 4.85 mS/cm. Elemental composition revealed 25.64 % ± 1.9 of C, 1.99 % ± 0.0 of N, 0.275 % ± 0.03 of S, and a C/N ratio equal to 12.89 ± 0.96, on d.m.b. (dry matter basis). Finally, the main nutrients were measured on d.m.b. and resulted in 147.47 mg/Kg N—$NO_3^-$, 3084.11 mg/Kg N—$NH_4^+$, 7657.11 mg/Kg K, 163.07 mg/Kg ortho-P. Physic-chemical and nutritious characterization of digestates is reported in Table 6 and most of the values are in line with the literature, proving that FW digestate has similar properties on average, despite the variability usually attributed to it depending on the season and locality.

pH of mesophilic digestates is 1 point lower than thermophilic ones, meaning that also nutrients availability for plants is different. Nitrogen availability is reduced with pH higher than 8. Moreover, total and ammonium nitrogen are lower in mesophilic digestates than in

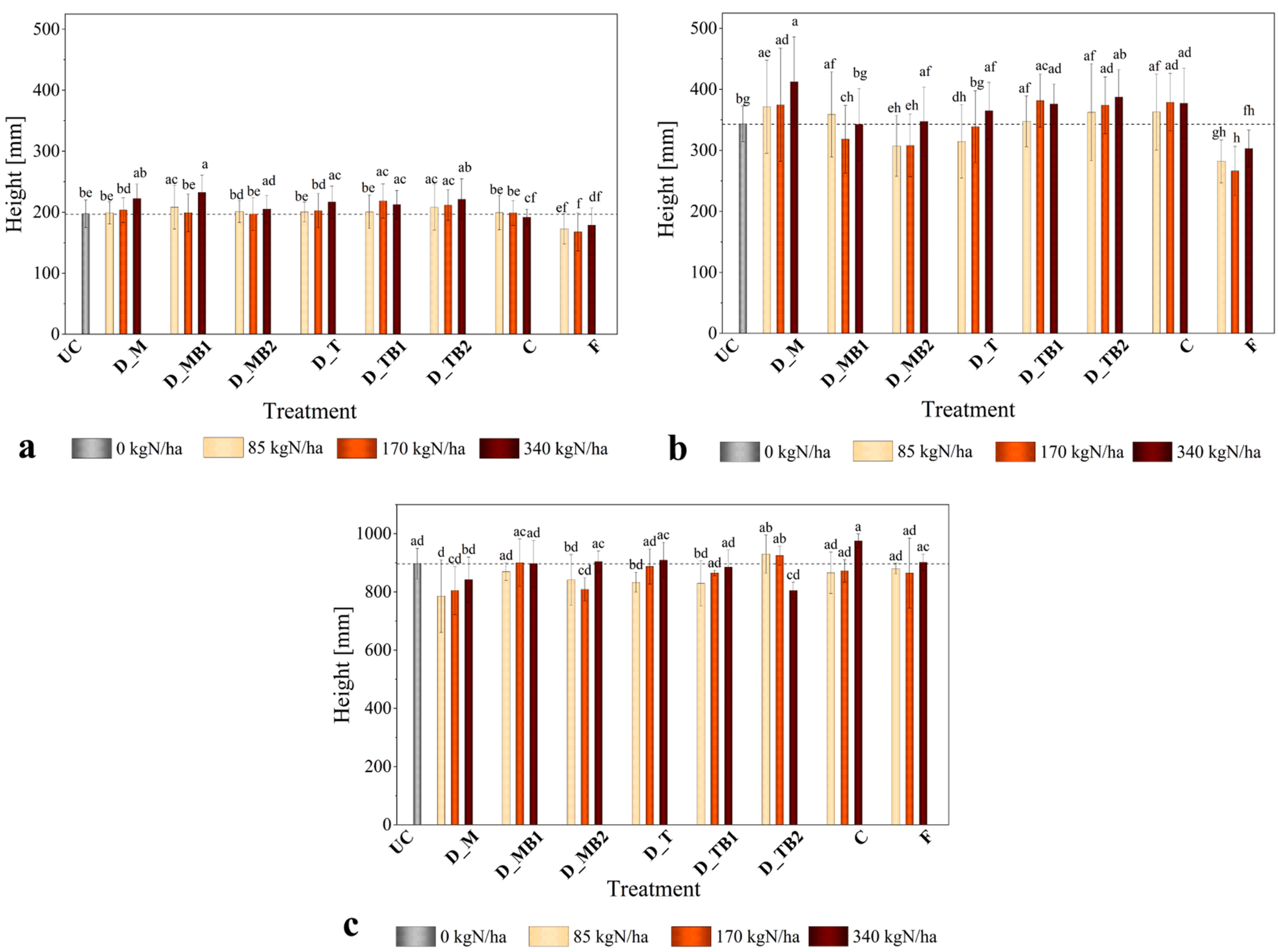


**Fig. 4.** Mean height of plants under each treatment and dosage (a) 3 weeks, (b) 6 weeks, and (c) 11 weeks after transplanting and treatment. Different letters indicate differences between treatments and dosages that were significant at $P < 0.05$ (Duncan's). The horizontal dashed lines indicate the value of the untreated control (UC) to ease the comparison. Each error bar represents one standard deviation. **UC**, untreated control; **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L); **C**, commercial compost; **F**, mineral fertilizer.

thermophilic ones. Other studies reported similar values for total and ammonium nitrogen, also in digestates coming from different types of wastes [21,57,59,62,63]. Digestates produced in this study resulted in higher quantities of nitrates compared to other works where nitrates were almost absent, probably because of the different feedstock. Phosphorus is instead quite lower in the present study, but it must be noted that only orthophosphate has been measured and not total phosphorus. Potassium concentration is again in accordance with literature, slightly but not significantly higher than other studies in which food waste digestate was produced [59,64].

During AD, most of the organic matter is removed and the residual digestate results in high concentrations of ammonia nitrogen, phosphorus, potassium, and other elements and metals. In feedstock, nitrogen is mainly present as ammonium or as organic N, the latter being converted to ammonium from protein degradation during AD process. Ammonium and potassium are not consumed by anaerobic microorganisms, as well as phosphorus, which is converted to the soluble form at the most [65]. According to literature, high concentrations of P affect microbial metabolism during AD, and thus methane production, and inhibiting effects become relevant when ortho-P is higher than 250 mg/L [66]. Digestates produced in the present study have low concentration of ortho-P, while ammonium is much more abundant. The higher concentration of ammonia nitrogen in thermophilic digestates could be due to a higher degradation of the substrate during the AD, confirmed also by the higher biogas production in that case.

### 3.5. Greenhouse pot trial

Significant differences were revealed in the growing trend among the different treatments and dosages (Fig. 4). The results of three relevant times of the trial will be discussed in this section, that are three weeks after transplanting, when differences among treatments started to be visible, six weeks after transplanting, corresponding to the middle of the trial, and the end of the trial, eleven weeks after transplanting.

Among the mesophilic digestates, three weeks after transplanting and treatment administration (Fig. 4a), D_MB1 favored the highest growth at the highest dosage (340 kgN/ha, N3), with a height of +13 % higher than the untreated control (UC). Opposite results were obtained with thermophilic digestates since the best one resulted in D_TB2, followed by D_TB1 and D_T, but the differences were not significant in this case. Mineral fertilizer (F) turned out to be the worst treatment after three weeks, with a lower growth also compared to the untreated control (–15 % N2). This could be because the mineral fertilizer used was urea and hence only mineral nitrogen was given to plants, missing the other nutrients fundamental to plants' growth. Furthermore, the application of urea mineral fertilizers can cause adverse effects on early plant growth in soil [67].

The same trend was still present six weeks after treatment (Fig. 4b), when differences between treatments became more accentuated. D_M at the highest dosage (N3, 340 kgN/ha) gave +20 % higher plants than UC and +32 % higher than F at the same dosage.

However, at the end of the experimental trial, eleven weeks after treatment (Fig. 4c), plants were all similarly tall, even though some statistically significant differences are still present. Nearly all treated plants were shorter than the untreated control and unexpectedly the shortest ones resulted in those treated with D_M in the end, while the best treatment turned out to be compost applied in the highest dose. A possible explanation is that plants passed the phytotoxic phase and aligned, reaching the maximum possible height based on the pot size.

Among digestates, thermophilic ones performed better than mesophilic ones, especially after eleven weeks. This could be due the fact that thermophilic digestates, especially D_TB1 and D_TB2, had similar content of ammonium nitrogen to mineral fertilizer, which also had similar effects. The better performance is clear when mean height of plants is analyzed considering only the treatment, independently on the dosage (Fig. 5), that is, that statistical analysis was performed joining the data of all the dosages for each treatment. In this way, it is possible to better evaluate the effect of each treatment without considering the applied dosage, and some statistical differences emerge. Six weeks after transplanting, mineral fertilizer was notably worse than all other treatments and even than UC (–18 %). At the end of the experiment (week 11), D_MB1 resulted the worst treatment, –15.4 % than C, –13.5 % than F,

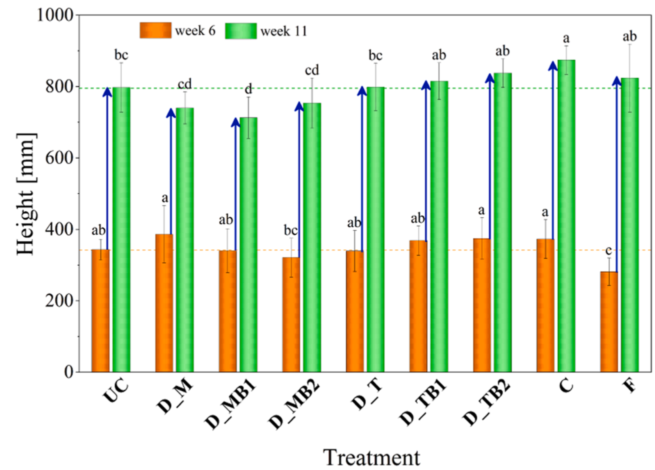


**Fig. 5.** Mean height of plants under each treatment 6 weeks and 11 weeks after transplanting. Different letters indicate differences between treatments that were significant at $P < 0.05$ (Duncan's). The horizontal dashed lines indicate the value of the untreated control (UC) to ease the comparison. Each error bar represents one standard deviation. **UC**, untreated control; **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L); **C**, commercial compost; **F**, mineral fertilizer.

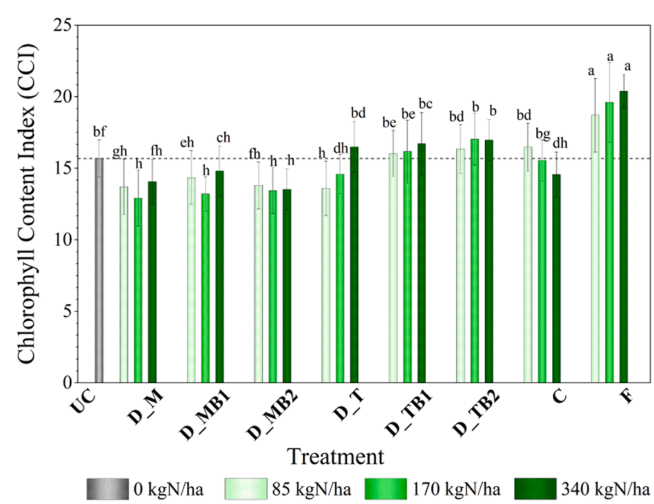


**Fig. 6.** Mean Chlorophyll Content Index (CCI) of leaves 3 weeks after transplanting. Different letters indicate differences between the treatments with the different concentrations of N that are significant at $P < 0.05$ (Duncan's). The horizontal dashed line indicates the value of the untreated control (UC) to ease the comparison. Each error bar represents one standard deviation. **UC**, untreated control; **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L); **C**, commercial compost; **F**, mineral fertilizer.

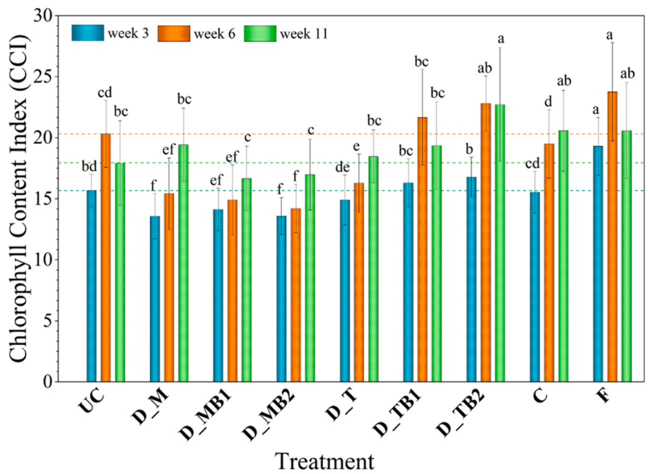


**Fig. 7.** Mean Chlorophyll Content Index (CCI) of leaves 3 weeks, 6 weeks, 11 weeks after treatments. Different letters indicate differences between the treatments that are significant at $P < 0.05$ (Duncan's). The horizontal dashed lines indicate the value of the untreated control (UC) to ease the comparison. Each error bar represents one standard deviation. **UC**, untreated control; **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L); **C**, commercial compost; **F**, mineral fertilizer.

and –10.6 % than UC.

Concerning the Chlorophyll Content Index (CCI) of leaves, the only significant difference among treatments and dosages is detected three weeks after transplanting (Fig. 6). CCI of plants treated with mesophilic digestates is significantly lower than that of untreated plants, –13 % with D_M and D_MB2. The opposite trend to other treatments is observed with commercial compost (C), where CCI decreased with the increase of applied dosage. The highest CCI was obtained with F at every dosage, +19 % to +30 % higher than UC with increasing dose. No relevant differences emerged between different dosages of the same treatment, except for D_T, for which CCI increased with the increasing dose.

More significant differences are evidenced between every treatment, independently on the dosage, 3, 6, and 11 weeks after treatment administration (Fig. 7). Mesophilic digestates always gave the worst results, up to –30 % than UC and –40 % than F after six weeks. The lower content of total and ammonium nitrogen could have led to a lower nitrogen uptake by plants and thus to a lower CCI. Plants treated with D_TB2 always resulted in CCI higher than untreated control (+27 % after eleven weeks), and slightly higher than CCI of plants treated with mineral fertilizer and commercial compost.

Interesting results emerged by the measurement of dry biomass and root development index (RDI) performed at the end of the experimental trial (Fig. 8b,c). As expected, dry biomass of plants grown with mesophilic digestates was lower than that of plants grown with other treatments and untreated control, coherently with the lower final height reached. Unexpectedly, dry biomass of plants treated with D_T was also significantly smaller than that of untreated control (–31 %), even though they were almost equally tall on average. Fresh biomass (Fig. 8a) with D_T was much lower than UC as well, suggesting that D_T produced thinner and lighter plants, with less tissues, maybe because of the absence of essential nutrients or the presence of inhibiting substances that have not been detected. Dry biomasses of plants that received D_TB1, D_TB2, and C were instead higher than both UC and F, with D_TB2 +34 % than UC and +20 % higher than F. Moreover, different trends emerged in fresh and dry biomasses, suggesting that D_TB2, maybe because of the higher content of biochar, promoted tissues growth and so thicker, even if shorter, plants.

The opposite trend was revealed in root development since RDI resulted higher for plants treated with D_MB2, D_T, D_MB1, and D_MB2, the same treatments that produced less aboveground biomass. However, all the digestates are produced from the same feedstock, thus they are expected to have the same, or at least similar, nutrient composition. This suggests that different operative conditions of AcD and different biodegradation efficiencies – reflected by the biogas and biomethane production – may have led to different concentrations of nutrients that favor aboveground or belowground biomass development. Moreover, the addition of biochar during the thermophilic AcD seems to improve the bioavailability of shoot-stimulating nutrients, since D_T gave very different results compared to D_TB1 and D_TB2 in aboveground biomass and roots growth.

Finally, plants treated with D_TB1, D_TB2, C, and F have a higher number of leaves than untread control (Fig. 8d), as expected from the higher dry biomass. D_M, D_MB1, D_MB2, and D_T had a similar number of leaves, but a significantly lower biomass than UC. This could suggest that these digestates produced lighter plants. A similar trend was observed in the number of leaves both 6 and 11 weeks after treatment.

An accurate comparison with other studies is difficult because many variables must be considered. First, substrates used and conditions in which AD is performed, that deeply affect digestate properties; secondly, conditions in which agronomic effects are evaluated, on field or in greenhouse, and the plant species used; finally, the specific goal of the studies, that may be different from those of the present work. Nevertheless, some studies have been found with similarities to the present one and the results of this study on digestates effects on plants growth are in line with literature.

Cristina et al. (2020) tested various dosages of digestates from sewage sludge anaerobic digestion on tomato plants grown in greenhouse [19]. Similarly to the present study (Fig. 4a), they didn't notice significant differences in plants height after the first month. They reported a much more significant difference in treated plants with respect to the non-treated control. However, the final height of treated plants (around 800 mm) and the number of leaves (between 12 and 18) are comparable, despite dry biomass is 4–8 folds lower. In Cristina et al. (2020), CCI after the second month almost doubled the values of the present study at the sixth week, but in the third month (comparable to the eleventh week) values heavily decreased and are in line with those of the present study [19].

Mickan et al. (2022) tested the effects of food waste digestate at different volumetric concentrations on tomato plants in greenhouse pot tests [68]. A maximum of 2 g of dry biomass was obtained, which is 0.2–0.6 folds those of this study. This could be explained by using smaller pots with more than one plant in each. Moreover, the various volumetric concentrations applied correspond to a maximum of 250 mg of N, which is comparable to N2 (170 kgN/ha) in this study, thus it is reasonable to assume that plants have received low quantities of nutrients. Concerning shoot and root mass instead, the same trend was observed by Mickan et al. (2022), both increasing with the increasing dose of digestate applied, while in this work the opposite trend was found for shoot and root mass [68].

Goat manure digestate was tested in greenhouse pot experiments by Funes-Pinter et al. (2022) [69]. They grow tomato plants for 120 days in a 1:1 peat:perlite substrate and measured biomass and root weight and CCI, like this study. Digestate was diluted at 10 % and applied every week, resulting in a total application of 500 mg of N per pot, slightly higher than N3 of this study (490 mg). Plants growth with digestate was better than that with untreated control but lower than the one with chemical fertilizer. Dry biomass of plants treated with digestate (~ 9 g) is comparable to the best grown plants in this study, even though Funes-Pinter et al. (2022) detected more relevant differences between organic treatments and fertilizer [69].

Despite opposing results found in literature, digestate remains an interesting possible substitute, at least partially, of mineral fertilizers for

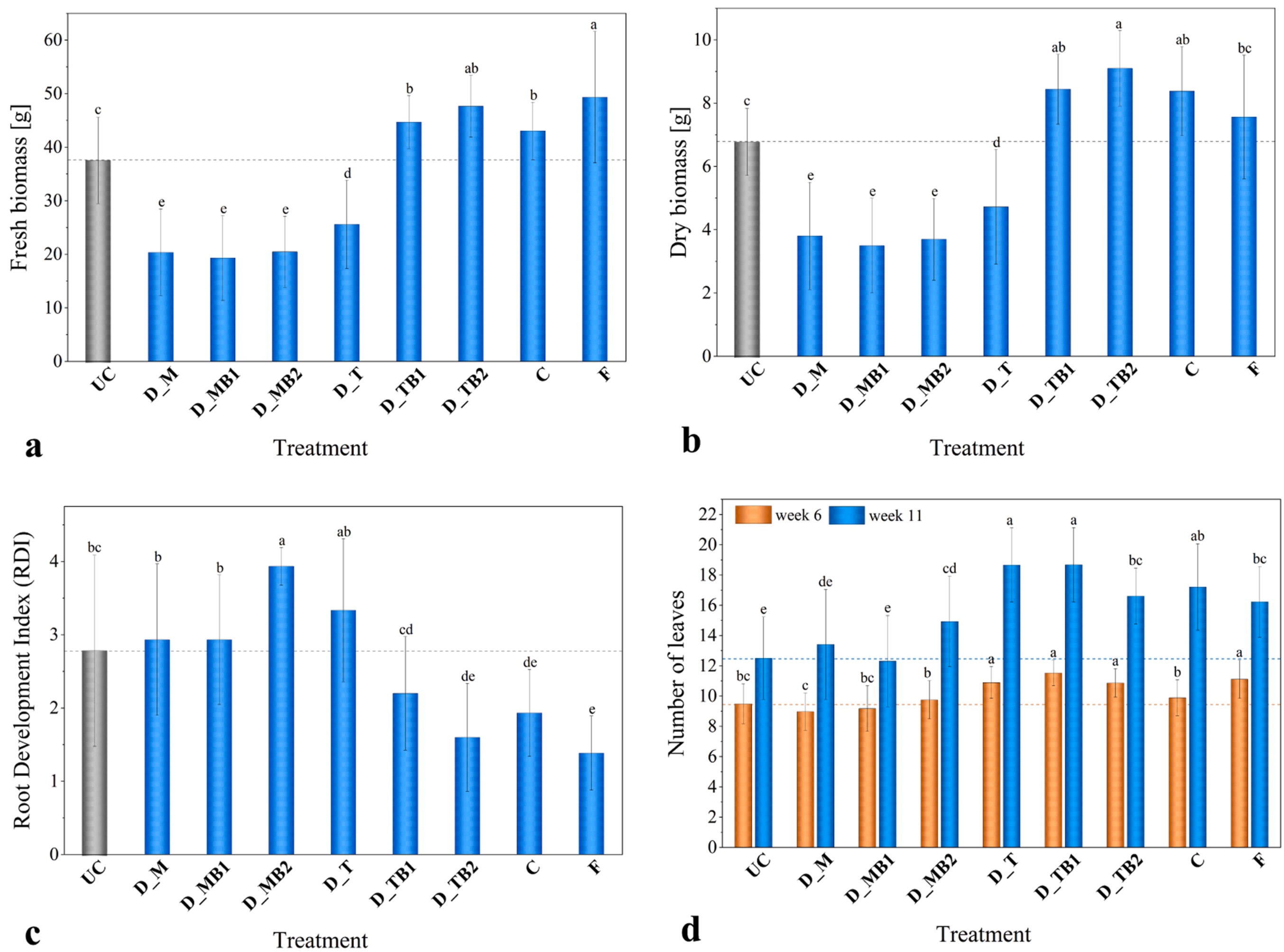


**Fig. 8.** Fresh **(a)** and dry biomass **(b)**, and Root Development Index **(c)** measured 11 weeks after treatment administration, at the end of the trial; number of leaves **(d)** measured after 6 and 11 weeks. Different letters indicate differences between the treatments that are significant at $P < 0.05$ (Duncan's). The horizontal dashed lines indicate the value of the untreated control (UC) to ease the comparison. Each error bar represents one standard deviation. **UC**, untreated control; **D_M**, digestate from mesophilic AcD without biochar; **D_MB1**, digestate from mesophilic AcD with the lowest biochar dose (7 g/L); **D_MB2**, digestate from mesophilic AcD with the highest biochar dose (14 g/L); **D_T**, digestate from thermophilic AcD without biochar; **D_TB1**, digestate from thermophilic AcD with the lowest biochar dose (7 g/L); **D_TB2**, digestate from thermophilic AcD with the highest biochar dose (14 g/L); **C**, commercial compost; **F**, mineral fertilizer.

plants growth. For tomato plants, particularly, Barzee et al. (2019) obtained comparable or even higher quantities of tomatoes compared to mineral fertilizer, applying liquid fraction of different digestates through subsurface drip fertigation system in open field [70]. Li et al. (2023) tested cattle manure digestate in both greenhouse and field tests on tomato plants [60]. They compared digestate to N-P-K synthetic fertilizer and tested also the administration of the two together. In greenhouse tests, the best fertilization turned out to be the digestate coupled with N-P-K, even if not significantly better than the digestate alone. The combined application of digestate and mineral fertilizer in the optimum ratio could be a good strategy to improve soil quality and ensure proper plants growth and production even with digestates that alone aren't optimal.

## 4. Conclusions

In this work, anaerobic co-digestion (AcD) of food waste (FW) and cow manure was performed testing different operative conditions and the addition of biochar. Anaerobic co-digestion was conducted at both mesophilic and thermophilic temperatures with two different doses of biochar, i.e. 7 g/L and 14 g/L, and without biochar. Subsequently, pot experiments under greenhouse conditions were performed to evaluate the agronomic effects of the obtained digestates as potential substitutes of chemical fertilizers.

The addition of biochar during AcD significantly influenced the process performances and the properties of the resulting digestates, under both mesophilic and thermophilic conditions.

The AcD of FW and cow manure with both doses of biochar in thermophilic conditions achieved an increase of 25 % in biogas production compared to digestion without biochar. Moreover, thermophilic conditions performed better than mesophilic conditions, offering higher methane yields and faster biogas production.

The digestates produced under thermophilic conditions showed also better agronomic performance. The one coming from the AcD with 14 g/L of biochar (D_TB2) promoted greater plant growth and chlorophyll content than other digestates as well as mineral fertilizer. Mesophilic digestates promoted root development instead.

AcD of FW and cow manure with the highest dose of biochar (14 g/L, D_TB2) in thermophilic conditions resulted to be the best performing, both for biogas and biomethane production, giving the highest yield, and for agronomic potential, showing similar effects on plants growth compared to mineral fertilizer. However, additional research is needed to understand the role of biochar during AD process and its interactions with microbial communities in the anaerobic digestion consortium as well as in the plant-root-soil system, where complex mechanisms regulate the exchange of substances. Moreover, scale-up studies would be necessary to collect data and evaluate the techno-economic feasibility of the proposed model, since thermophilic AD and pyrolysis are high-energy demanding processes.

In this study, a preliminary proof of concept was performed, and the proposed model was successfully demonstrated on a small laboratory scale. The integration of biochar in the AD process enhances both the energy and nutrient recovery potential of FW improving the fertilizing effects of the resulting digestates. This approach not only supports renewable energy production through increased biogas yields but also provides a valuable alternative to synthetic fertilizers, in a circular bioeconomy model.

## CRediT authorship contribution statement

**Melania Fiore:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Francesca Demichelis:** Writing – original draft, Methodology, Data curation, Conceptualization. **Fabio Alessandro Deorsola:** Supervision, Conceptualization. **Debora Fino:** Methodology, Funding acquisition, Conceptualization. **Guido Saracco:** Validation, Methodology, Conceptualization. **Massimo Pugliese:** Validation, Resources, Methodology, Funding acquisition, Data curation. **Tonia Tommasi:** Supervision, Funding acquisition, Conceptualization.

## Declaration of competing interest

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

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rineng.2025.104883.

## Data availability

Data will be made available on request.

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