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## **Industrial symbiosis of anaerobic digestion and pyrolysis: Performances and agricultural interest of coupling biochar and liquid digestate**

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1           **Industrial symbiosis of anaerobic digestion and pyrolysis: performances and**  
2           **agricultural interest of coupling biochar and liquid digestate**

3  
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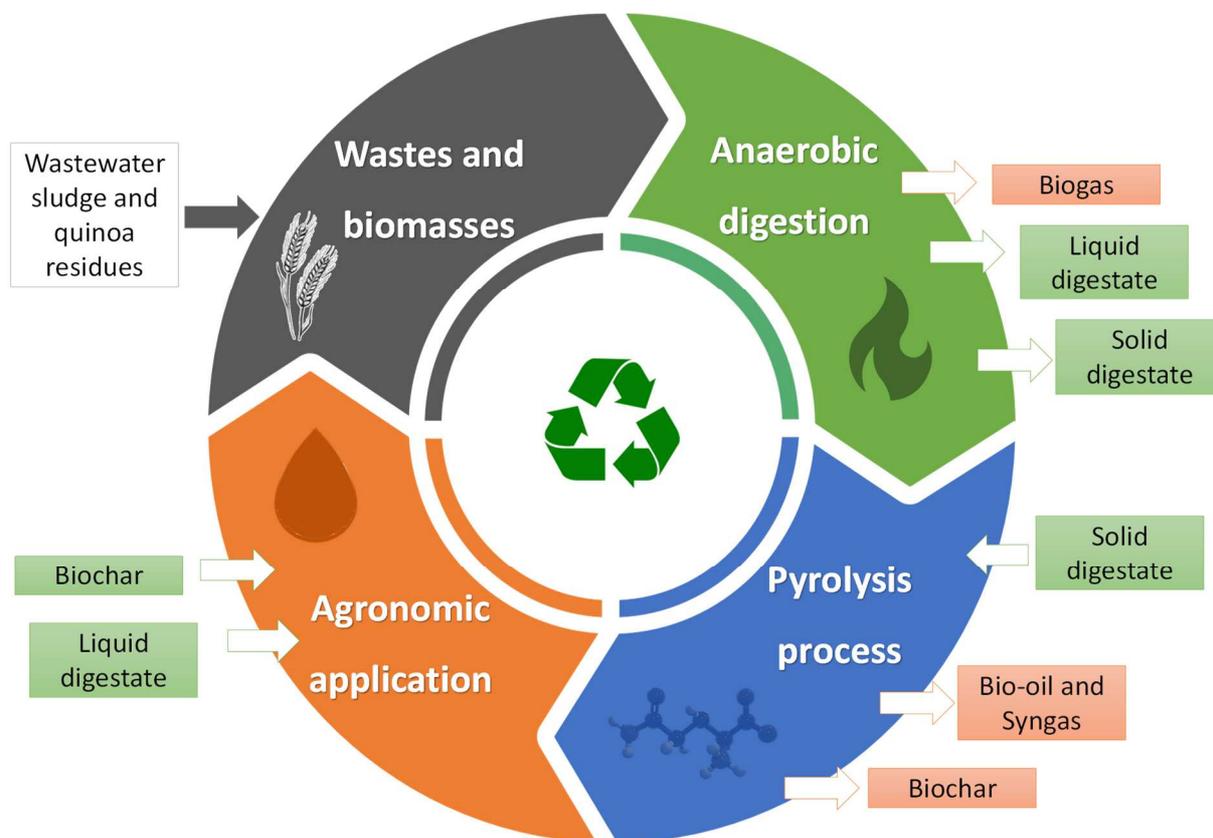
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22   **GRAPHICAL ABSTRACT**



23

24

25 **Keywords:** Anaerobic digestion, biochar, biogas, bio-oil, mineralization, plant growth tests,  
26 syngas

27

28 **Abbreviations:**

29 **AD:** Anaerobic digestion

30 **B25:** Biochar at 25 tons/ha

31 **B50:** Biochar at 50 tons/ha

32 **BMP:** Biochemical Methane Potential

33 **CHP:** Cogeneration Heat and Power

34 **COD:** Chemical Oxygen Demand

35 **CSTR:** Continuous Stirred Tank Reactor

36 **DM:** Dry Matter

37 **EBC:** European Biochar Certificate

38 **IBI:** International Biochar Initiative

39 **IF:** Industrial Fertilizer (DAP and ammonium nitrate)

40 **LD:** Liquid Digestate

41 **VS:** Volatile Solid

42

43 **ABSTRACT**

44 The sustainability of the anaerobic digestion industry is closely related to proper digestate  
45 disposal. In this study, an innovative cascading biorefinery concept coupling anaerobic  
46 digestion and subsequent pyrolysis of the digestate was investigated with the aim of  
47 enhancing the energy recovery and improving the fertilizers from organic wastes. Continuous  
48 anaerobic co-digestion of quinoa residues with wastewater sludge (45/55% VS) exhibited  
49 good stability and a methane production of 219 NL CH<sub>4</sub>/ kg VS. Subsequent pyrolysis of the  
50 solid digestate was carried out (at 500 °C, 1 h, and 10 °C/min), resulting in a products  
51 distribution of 40 wt% biochar, 36 wt% bio-oil, and 24 wt% syngas. The organic phase (OP)  
52 of bio-oil and syngas exhibited higher and lower heating values of 34 MJ/kg and 11.8  
53 MJ/Nm<sup>3</sup>, respectively. The potential synergy of coupling biochar with liquid digestate (LD)

54 for agronomic purposes was investigated. Interestingly, coupling LD (at 170 kgN/ha) with  
55 biochar (at 25 tons/ha) improved the growth of tomato plants up to 25% compared to LD  
56 application alone. In parallel, co-application of biochar with LD significantly increased the  
57 ammonia volatilization (by 64%) compared to LD application alone, although their  
58 simultaneous use did not impact the C and N mineralization rates.

## 59 **1. Introduction**

60 The increase in the world's population is creating environmental issues such as over-  
61 exploitation of fossil fuels and the accumulation of wastes. In recent decades, the scientific  
62 community has increasingly engaged in the development of processes that optimize the  
63 recovery and use of organic wastes (biowastes, agricultural wastes, agro-industrial wastes,  
64 urban wastes, etc.) in a diverse range of products according to the concept of environmental  
65 biorefineries (Demichelis et al., 2020; Monlau et al., 2015a). Morocco has rapidly  
66 transformed into a largely urban society over the past decade, with approximately 60% of its  
67 citizens now living in cities and urban areas due to a trend of rural migration to coastal  
68 centers. The high population growth rate in urban agglomerations in recent years has been  
69 accompanied by environmental problems such as an increase in wastes and effluents  
70 (Alhamed et al., 2018; Belloulid et al., 2017). Morocco has achieved significant improvement  
71 in the wastewater sector in the past ten years, and 123 wastewater treatment plants (WWTP)  
72 have been built, increasing the treatment capacity to 900 million m<sup>3</sup> per year (Alhamed et al.,  
73 2018). In 2015, the potential production of sludge in Morocco at the level of WWTP was  
74 estimated to 155,450 tons of dry matter (Belloulid et al., 2017).

75 Anaerobic digestion (AD) is a well-known process that has been used for many decades to  
76 treat organic wastes, such as wastewater sludges (Sawatdeenarunat et al., 2016). This process  
77 transforms organic matter in the absence of oxygen into biogas (a mixture of CH<sub>4</sub> and CO<sub>2</sub>)  
78 and an undegraded residue called digestate (Monlau et al., 2015b). Biogas is a source of

79 energy that is generally used to generate heat and electricity through cogeneration and a heat  
80 power system or it can be injected into the natural gas grid after an upgrading process  
81 (Roubaud and Favrat, 2005). Nonetheless, mono-AD of organic wastes is limited by the need  
82 to maintain an optimal C/N ratio between 15 and 30 (Chandra et al., 2012), which can lead to  
83 instability and decreased performance (Sawatdeenarunat et al., 2016). Anaerobic co-digestion  
84 with lignocellulosic biomass is a promising technology to improve digester performance with  
85 wastewater sludge by improving the C/N ratio (Giuliano et al., 2013; Zhao et al., 2018).  
86 Despite the potential of co-digestion technology, lignocellulosic biomass as a co-substrate can  
87 lead to a significant increase in the volume of digestate, thus requiring a final  
88 elimination/valorization step (González et al., 2020). Generally, the anaerobic digestate is  
89 separated into a liquid fraction (rich in nutrients, especially N and K) and a solid fraction rich  
90 in P and fibers that are mostly separated by a filter press, centrifuge, or a vibrating sieve  
91 (Akhiar et al., 2017).

92 The cascading biomass valorization approach by coupling two or three processes has become  
93 a new strategy to achieve the “zero waste” goal at the industrial scale. Recently, the coupling  
94 of anaerobic digestion (AD) and pyrolysis processes through the pyrolysis of solid digestate  
95 has been considered as a solution to the challenge of AD digestate management and a way to  
96 increase the sustainability of the entire process by the production of a higher amount of  
97 biofuels (syngas and bio-oil) (Fabbri and Torri, 2016; Ghysels et al., 2020; González et al.,  
98 2020; Pecchi and Baratieri, 2019). Pyrolysis is a thermochemical process in which biomass is  
99 thermally degraded under an inert or a very low stoichiometric oxygen atmosphere (Tripathi  
100 et al., 2016), yielding three products: syngas (mainly CO<sub>2</sub>, H<sub>2</sub>, and CO), bio-oil (composed of  
101 an organic and an aqueous phases), and biochar (Monlau et al., 2015). Syngas can be  
102 converted into heat or heat/electricity (combined heat and power, CHP) alone or mixed with  
103 biogas in boilers and engines (Seyedi et al., 2019). The organic phase of bio-oil can be used as

104 a fuel, or it can be added to petroleum refinery products or upgraded by catalysts to produce  
105 premium-grade refined fuels, or it may have use as building blocks (Pütün et al., 2005)  
106 whereas the aqueous phase can be recirculated as feedstock for the AD process (Torri and  
107 Fabbri, 2014). The energetic interest of coupling AD and pyrolysis compared to stand-alone  
108 AD has been demonstrated previously (Ghysels et al., 2020; Monlau et al., 2015b). In parallel,  
109 González-Arias et al. (2019) demonstrated that the combined approach of pyrolysis and  
110 digestion solves the digestate disposal problem by generating biochar. Indeed, biochar, which  
111 is the carbonaceous material obtained from pyrolysis, has several advantages (stable C,  
112 hygienization, water retention, etc.) and it can be used in several environmental applications  
113 (Abdeljaoued et al., 2020). For instance, biochar has gained much attention in recent decades  
114 due to its potential to enhance soil quality and soil preservation, as well as mitigation of  
115 climate change by carbon sequestration (Fernández et al., 2014; Semida et al., 2019).

116 Nonetheless, although coupling AD and pyrolysis has been well described and discussed in  
117 the literature from an energetic point of view, less information is available regarding the use  
118 of biochar derived from solid digestate for agronomic applications used alone or in  
119 combination with liquid digestate (Glaser et al., 2015; Opatokun et al., 2017; Tayibi et al.,  
120 2021). Ronga et al. (2020) assessed the effect of combining biochar (from pine wood chips)  
121 with LD (from AD of a mixture of maize silage, triticale silage, cow slurry, and grape stalks)  
122 on the fruit yield of tomatoes produced by organic farming. The results demonstrated that  
123 tomato plants fertilized with LD and biochar achieved a maximum yield of 72 tons/ha, while  
124 the lowest production of 47 tons/ha was recorded with unfertilized plants (Ronga et al., 2020).  
125 Similarly, Tayibi et al., (2020a) investigated the coupling of LD with biochar (produced from  
126 the solid digestate fraction) for agronomic applications on nutrient leaching and wheat  
127 growth. Interestingly, the addition of biochar increased the cumulative leaching of all  
128 nutrients, except nitrate, with a significant decrease of 82% at 50 tons/ha, compared to soil

129 treated only with LD alone. In parallel, co-application of biochar and LD improved the aerial  
130 dry biomass production of wheat (up to 27.5%) compared to soil treated only with LD.  
131 Nonetheless, there is still little information available regarding the coupling of LD and  
132 biochar, not only in terms of crop yields but also on changes in the physicochemical  
133 properties of soil. The present study, therefore, evaluated the following:

- 134 • The performance of a continuous stirred reactor (CSTR) at a pilot scale for co-  
135 digestion of quinoa residues and wastewater sludge;
- 136 • The pyrolysis products (biochar, bio-oil, and syngas) from a solid digestate at 500 °C,  
137 1 h, and 10 °C/min;
- 138 • Characterization of the syngas, bio-oil, and biochar by evaluation of the content of the  
139 inorganic elements contained in the biochar with the range suggested by the  
140 International Biochar Initiative (IBI) and the European Biochar Certificate (EBC).
- 141 • The phytotoxicity (germination index) of co-application of biochar (25 tons/ha and 50  
142 tons/ha) and LD (170 kg N /ha) on tomato plants;
- 143 • The effect of co-application of biochar (25 tons/ha) and liquid digestate (170 kg N/ha)  
144 on microbial respiration (CO<sub>2</sub>), the mineralization of nitrogen, and the volatilization of  
145 ammonia.

## 146 **2. Materials and Methods**

147

### 148 **2.1. Feedstocks, inoculum, and soil properties**

149 Two different residues were used for the anaerobic digestion tests: wastewater sludge and  
150 quinoa residues. The wastewater sludge was collected from a WWTP located in Lescar  
151 (France) and stored at -16 °C. Quinoa residues were collected from a private farm located in  
152 Benguerir (Morocco), the residues were dried at 40 °C for three days and crushed twice using  
153 an electric vegetable mill (Ge250, Stihl Viking®, Germany). The inoculum used for the  
154 biochemical methane potential (BMP) tests and for the semi-continuous assays was an

155 internal mesophilic inoculum produced at the APESA center and fed with a mixture of  
156 wastewater sludge and grass. For the agronomic tests, an agricultural soil sampled at the  
157 surface (0-25 cm) was collected at the INRAE site in Mauguio (15.8 km from Montpellier,  
158 France). The soil was air-dried and passed through a 2-mm sieve to remove large fragments.  
159 The main characteristics of the soil were (per thousand parts of raw material): 210 parts clay  
160 ( $< 2\mu\text{m}$ ), 93 parts fine silt (2 to 20  $\mu\text{m}$ ), 208 parts coarse silt (20 to 50  $\mu\text{m}$ ), 196 parts fine  
161 sand (50 to 200  $\mu\text{m}$ ), and 302 parts coarse sands (200 to 2,000  $\mu\text{m}$ ). The soil was classified as  
162 a clay loamy soil. Some of the other characteristics of the soil were a pH of  $7.4 \pm 0.1$  (water),  
163  $1.9 \pm 0.2\%$  organic matter, a C/N ratio of 10.1, a total nitrogen (TN) content of 0.1 wt%,  
164  $0.1 \pm 0.02$  g/kg phosphorus ( $\text{P}_2\text{O}_5$ ), 0.4 g/kg potassium ( $\text{K}_2\text{O}$ ), and a  $9.9 \pm 1.0$  cmol (+)/kg  
165 cation exchange capacity (CEC). Two commercial fertilizers were used: DAP ( $(\text{NH}_4)_2\text{HPO}_4$ ,  
166 characterized by 18 wt% N- $\text{NH}_4$  and 46 wt% P- $\text{P}_2\text{O}_5$ , provided by the Cherifian Office for  
167 Phosphates (OCP) company and complemented with ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) (99%),  
168 from Sigma-Aldrich<sup>®</sup>, to obtain a ratio of 170 kg N/ha.

## 169 **2.2. Anaerobic digestion (AD) process**

170

171 The BMP tests were performed under anaerobic mesophilic conditions ( $35 \pm 2$  °C, pH = 7)  
172 and in duplicate with a working volume of 500 mL at a substrate inoculum (S/I) ratio of 0.35  
173 according the recommendations made in the European Interlaboratory study (Hafner et al.,  
174 2020). A blank control (only the inoculum) was carried out in parallel with the other  
175 biomasses that were tested (wastewater sludge and quinoa residues) to subtract the amount of  
176 biogas generated by just the inoculum. Once the bottle was prepared, it was purged with  
177 nitrogen gas ( $\text{N}_2$ ) to maintain anaerobic conditions. The bottles were then sealed and placed in  
178 an oven at  $35 \pm 2$  °C to maintain mesophilic conditions. The biogas production was monitored  
179 daily by pressure measurements using a manometer with an LC display (Testo 502,  
180 TESTOON, France). The composition of the biogas ( $\text{H}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$ ,  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{S}$ ) was

181 determined using a micro gas chromatograph (490, Agilent Technology, USA). The first  
182 column (Molsieve 5Å PLOT) was used at 110 °C to separate the O<sub>2</sub>, N<sub>2</sub>, and CH<sub>4</sub>, and the  
183 second column (HayeSep A) was used at 70 °C to separate the CO<sub>2</sub> from the other gases. The  
184 injector and the detector temperatures were 110 °C and 55 °C, respectively. Detection of the  
185 gaseous compounds was performed using a thermal conductivity detector. The calibration was  
186 performed with two standard gases composed of either 9.5% CO<sub>2</sub>, 0.5% O<sub>2</sub>, 81% N<sub>2</sub>, and 10%  
187 CH<sub>4</sub> or 35% CO<sub>2</sub>, 5% O<sub>2</sub>, 20% N<sub>2</sub>, and 40% CH<sub>4</sub> (Air Liquide®). All of the results are  
188 presented under standardized conditions of temperature and pressure (P<sub>atm</sub>, 0 °C). After  
189 determination of the BMP of the substrates (quinoa residues and wastewater sludge), a  
190 continuously stirred tank reactor (CSTR) assay was set up with a total working volume of 20  
191 L under mesophilic conditions (35 ± 2 °C) and homogeneity was maintained using a  
192 continuous agitation system. The reactor was initially supplied with mesophilic inoculum as  
193 previously described. The feedstock input and the discharge digestate were performed  
194 manually once a day (5 days per week). The feedstock mixture was composed of 42.5% VS  
195 wastewater sludge and 57.5% VS quinoa residues. The organic load rate and the hydraulic  
196 retention time were set at 2.06 g VS/m<sup>3</sup> day and 41 days, respectively. The CSTR was  
197 monitored by measurement of the pH and the temperature on a daily basis, the FOS/TAC  
198 (Free Organic Acids / Total Inorganic Carbonate) ratio twice a week, and the volatile fatty  
199 acids (VFAs) as well as the ammonium concentration once a week. Details for all the  
200 experimental protocols are provided in the previous section. Measurement of the Dry Matter  
201 (DM) and the Volatile Solid (VS) contents of the digestate was carried out every weekend.  
202 The biogas produced was recorded every day by a PC system connected to a Ritter volumetric  
203 counter cell. The biogas was collected in a gas pocket directly connected to the gas meter; the  
204 analysis of the biogas was carried out 5 days/week using micro gas chromatography (490,  
205 Agilent Technology, USA) as previously described. At the end of the process, a solid-liquid

206 separation was carried out using a wine press. A mass balance of the chemical oxygen  
207 demand (COD) was also carried out at the end of the process. To assess the mass balance, it  
208 was assumed that 350 NL CH<sub>4</sub> can be produced per kg of COD. The COD of the solid  
209 digestate was assessed by a modified protocol using a double acid hydrolysis of biomasses as  
210 described by Cazaudehore et al. (2019). The supernatant liquids from the solid fraction and  
211 the LD were analyzed for COD using commercial kits (Spectroquant 14,155, Merck,  
212 Germany). The concentrations ranged from 500 to 10,000 mg COD/L. The tubes were then  
213 heated to 148 °C in a preheated thermoreactor for 120 min. Finally, the COD was measured  
214 by an automatic spectrophotometer (photoLab® S6, WTW, Germany).

### 215 **2.3 Pyrolysis test of the solid digestate**

216 The dried solid digestate sample (approximately 300 g) was subjected to pyrolysis in a steel  
217 reactor. Before the pyrolysis, the basket of feedstock and the furnace were purged with N<sub>2</sub> for  
218 30 min to ensure an oxygen-free environment. The experiments were then carried out in  
219 duplicate at 500 °C with a heating rate of 10 °C/min, for 1 hour at high temperature. The  
220 collected pyrolysis products were biochar, bio-oil (condensable gas), and syngas (non-  
221 condensable gas). The furnace was then cooled to 25 °C under nitrogen flow and the biochar  
222 and bio-oil were collected and weighed. The syngas (hydrogen, oxygen, nitrogen, carbon  
223 monoxide, and dioxide, methane, ethane, and ethylene) was quantified using a micro-  
224 chromatography device (Varian CP-4900, Agilent, Germany). The first column (CP-Sil CB)  
225 was operated at 37 °C to separate the H<sub>2</sub> and the O<sub>2</sub>, while the second column (Molsieve 5Å  
226 PLO) was operated at 37 °C to separate the N<sub>2</sub>, CO, and CH<sub>4</sub>, and the third column (HayeSep  
227 A) was operated at 35 °C to separate the CO<sub>2</sub>, C<sub>2</sub>H<sub>2</sub>, C<sub>2</sub>H<sub>4</sub>, and C<sub>2</sub>H<sub>6</sub>. The gaseous compounds  
228 were detected by a thermal conductivity detector. The injector and the detector temperatures  
229 were 50 °C and 55 °C, respectively. The analyses of the syngas were carried out every 5  
230 minutes during the pyrolysis reaction using the micro-GC. The mass of the produced syngas

231  $M_{\text{Syngas}}$  (g) was determined by the difference between the output and input gas flow during the  
232 pyrolysis process using an acquisition system linked to two flowmeters measuring the input  
233 (flowmeter BROOKS) and the output (flowmeter M.M.T). Equation (1) was used to calculate  
234 the mass of the syngas, as described below:

$$\begin{aligned} 235 & M_{\text{Syngas}}(g) \\ 236 & = \sum (\text{output flow (g/min)} - \text{input flow (g/min)})/122 \end{aligned} \quad (1)$$

237 where 122 is the number of acquisitions per min.

238 The biochar, bio-oil, and syngas product yield percentages were calculated as follows:

$$239 \quad \text{Biochar (wt\%)} = M_{\text{Biochar}}(g)/M_{\text{Solid digestate}}(g) \times 100 \quad (2)$$

$$240 \quad \text{Bio - oil (wt\%)} = M_{\text{Bio-oil}}(g)/M_{\text{Solid digestate}}(g) \times 100 \quad (3)$$

$$241 \quad \text{Syngas (wt\%)} = M_{\text{Syngas}}(g)/M_{\text{Solid digestate}}(g) \times 100 \quad (4)$$

242 where  $M_{\text{Biochar}}$ ,  $M_{\text{Bio-oil}}$ , and  $M_{\text{Syngas}}$  represent the biochar, bio-oil, and syngas masses produced  
243 during the pyrolysis, and  $M_{\text{Solid digestate}}$  represents the mass of the solid digestate.

244 The losses during pyrolysis (approximately 6%) were considered to be the bio-oil trapped in  
245 the walls of the cooling system, and they were taken into account in the bio-oil calculation.

246 The bio-oil obtained in this work was separated into two different phases: an organic phase  
247 and an aqueous phase obtained by decantation using dichloromethane (99.8%, from Sigma-  
248 Aldrich®) as the organic solvent. The weight and the yield were measured after decantation.

249 Anhydrous  $\text{Na}_2\text{SO}_4$  was added to the solvent mixture/organic phase to remove residual water.

250 The solvent was then evaporated using a rotary evaporator, the organic phase of bio-oil was  
251 weighed, and its yield was measured.

252 The average lower heating value (LHV) of the syngas was calculated based on Equation (5)

253 (Lv et al., 2004), assuming that the  $\text{N}_2$  had been separated from the produced syngas. The

254 predictive higher heating value (HHV) (MJ/kg) of the organic phase of the bio-oil was  
255 calculated using ultimate analysis in Equation (6) (Troy et al., 2013) as follows:

$$256 \quad LHV(MJ / Nm^3) = [30 \times v/v\% CO + 25.7 \times v/v\% H_2 + 85.4 \times v/v\% CH_4 + 151.3 \times$$
$$257 \quad \quad \quad v/v\% (C_2H_4 + C_2H_6)] \times 0.42] \quad (5)$$

$$258 \quad HHV (MJ/ kg) = [3.55 \times C^2 - 232 \times C - 2230 \times H + 51.2 \times (C \times H) + 131 \times N +$$
$$259 \quad 20,600]/1000 \quad (6)$$

## 260 **2.4 Analytical methods**

### 261 **2.4.1. Physicochemical analysis**

262 The Dry Matter (DM) and Volatile Solids (VS) contents in the organic wastes and the co-  
263 products were determined according to the protocol outlined by the American Public Health  
264 Association (APHA, 2005). The pH of the digestate was determined using a WTW 340i  
265 reference pH meter. Ammonium (NH<sub>4</sub><sup>+</sup>) was determined using reagent kits (Spectroquant<sup>®</sup>,  
266 Germany) for NH<sub>4</sub><sup>+</sup> by spectrophotometry (photoLab<sup>®</sup> S6, WTW, Germany). To determine  
267 the concentration of the VFAs, the digestate was centrifuged for 20 minutes at 9,600 rpm  
268 using a centrifuge system (Hettich Zentrifugen, Rotanta 460) to recover the liquid fraction. An  
269 internal standard solution was prepared from 1 g of ethyl-2-butyric acid diluted in 1 L of  
270 water acidified with 2.5% phosphoric acid. A 1 mL aliquot of internal standard solution was  
271 then added to 2 mL of the liquid sample. The mixture was filtered using a 0.2-μm nylon filter  
272 attached to a syringe. The filtered liquid was introduced into a vial for analysis by a gas  
273 chromatography system (GC-7890B) coupled to a flame ionization detector (FID). The  
274 FOS/TAC (Free Organic Acids / Total Inorganic Carbonate) ratio, which corresponds to the  
275 total acid and buffer capacity levels, was determined by acidification of the sample prepared  
276 as 2 g of digestate diluted in 50 mL of deionized water under magnetic agitation with sulfuric  
277 acid (0.1 N) from a graduated burette. A first acidification to a pH of 5.1 determines the  
278 buffer capacity of the medium. A second acidification to a pH of 3.5 can quantify the amount  
279 of total acids in the medium. The Chemical Oxygen Demand (COD) of the LD was analyzed

280 using commercial kits (Spectroquant<sup>®</sup>, Merck, Germany). A 1 mL aliquot of centrifuged LD  
281 was placed in the commercial tubes. The tubes were then heated to 148 °C in a preheated  
282 thermoreactor for 120 min. Finally, the COD was measured by an automatic  
283 spectrophotometer (photoLab<sup>®</sup> S6, WTW, Germany). The theoretical COD of the feedstocks  
284 (wastewater sludge and quinoa residues) and the solid digestate were determined based on  
285 CHNS analysis according to the method reported by Wei et al., (2018). For the COD balances  
286 and biogas equivalence presented in **Fig. 2**, it was assumed that 1g of COD is equivalent to  
287 1NmL of CH<sub>4</sub> (Im et al., 2020). The total Kjeldahl Nitrogen (TKN) content of the soil and the  
288 LD was determined according to the Kjeldahl method (Kjeldahl, 1883) by using a  
289 mineralizator (BUCHI digestion unit K-438) and a distillator/titrator (BUCHI K-370).  
290 Moreover, the N-NH<sub>4</sub><sup>+</sup> content of the LD was determined by the titrimetric method after  
291 distillation using a BUCHI K-370 distillatory (Rodier, 1975).

292 A thermogravimetric analyzer (TGA 2-LF, Mettler Toledo<sup>®</sup>, Switzerland) was used to assess  
293 the thermal degradation of the solid digestate and the stability of its biochar, as well as to  
294 determine the moisture, volatile matter, fixed carbon, and ash contents according to the  
295 protocol reported in Tayibi et al., (2020b). The fibers (cellulose, hemicelluloses) and the  
296 Klason lignin content of the dry solid digestate were determined using the NREL protocol  
297 (Sluiter et al., 2008). Briefly, the Klason lignin content was determined as the weight of the  
298 residues retained on a sintered glass crucible filter (Ø=25mm), and the soluble fractions were  
299 analyzed by high-pressure liquid chromatography (HPLC) to quantify the monosaccharides  
300 content (i.e., glucose, xylose, and arabinose). The HPLC (Alliance<sup>®</sup> HPLC System, Waters,  
301 USA) analysis was performed using a column (Aminex<sup>®</sup> HPX-87H, BioRad, France) at 40 °C  
302 and 0.3 mL/min of 0.005 M H<sub>2</sub>SO<sub>4</sub>. All of the measurements were performed in triplicate.  
303 The cellulose and the hemicellulose percentages were determined by Equations (7) and (8),  
304 respectively.

305  $Cellulose(\%) = [(Glucose(g/L) \times V_{tot})/M_{ini}] \times 100/1.11$  (7)

306  $Hemicelluloses(\%) = [(Xylose(g/L) + Arabinose(g/L) \times V_{tot})/M_{ini}] \times$   
307  $100 / 1.13$  (8)

308 where  $V_{tot}$  and  $M_{ini}$  represent the total volume of the hydrolysis medium (0.025 L) and the  
309 initial mass of the sample in grams, respectively, while 1.11 represents the conversion factor  
310 between glucose and cellulose and 1.13 represents the conversion factor between monomers  
311 (xylose and arabinose) and hemicelluloses (Barakat et al., 2015).

312 The nutrient content (P, K, Mg, S, Ca, and Na) and the minor metallic content (Pb, Cd, Cu,  
313 Ni, Hg, Zn, Cr, and As) were determined by inductively coupled plasma mass spectrometry  
314 (ICP-MS) (X SERIES 2 ICP-MS, Thermo Fisher Scientific, USA) equipped with a cooled  
315 spray chamber, a quadrupole mass spectrometer, and a collision cell. The ICP-MS settings  
316 were as follows: a nebulizer flow of 0.82 L/min, an auxiliary flow of 0.80 L/min, a cool flow  
317 of 13 L/min, a forward power of 1,400 Watts, and a cell gas He/H flow rate of 0.0045 L/min.  
318 For this purpose, microwave-assisted mineralization of the solid digestate and its biochar was  
319 performed after the addition of nitric acid (65%) and hydrogen peroxide (30%). The reaction  
320 was conducted for 30 min at room temperature, and the mixtures were then placed in the  
321 microwave reactor (flexiWAVE, Milestone, USA) and heated for 20 min to reach 210 °C,  
322 which was maintained for 20 min and then cooled for 25 min. The obtained solutions were  
323 filtered using 0.2- $\mu$ m filters and then analyzed by ICP-MS. The ultimate analyses (C, H, N, O,  
324 and S) of the dry solid digestate, its biochar, and the organic phase of bio-oil and dry tomato  
325 plants were determined in duplicate using an elemental analyzer (varioMicro V4.0.2,  
326 Elementar<sup>®</sup>, Germany).

## 327 2.5 Agronomic tests

### 328 2.5.1. Carbon mineralization test

329

330 The effect of biochar (25 tons/ha) on soil CO<sub>2</sub> emission with or without the addition of LD  
 331 (170 kg N/ha) was explored by a 91-day indoor incubation experiment based on the AFNOR  
 332 FD U44-163, (2018) standard. The biochar and the liquid digestate (LD) were added and  
 333 mixed with the soil as follows: soil, soil + biochar (B25), soil + LD + biochar (B25), and soil  
 334 + LD. Each condition was distributed into cups containing the equivalent of 25g of dry soil.  
 335 These cups were then placed in hermetically sealed containers in the presence of sodium  
 336 hydroxide and incubated at 28 °C. The humidity was kept constant throughout the experiment  
 337 (equivalent to the field water retention capacity of pF 2.8). The carbon mineralized by the  
 338 sample was measured after 1, 3, 7, 14, 21, 28, 49, 70, and 91 days of incubation by  
 339 assessment of the C-CO<sub>2</sub> trapped in the sodium hydroxide (NaOH; 0.5 mol/L). This  
 340 measurement was performed on three repeats. A mixture of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and  
 341 sodium hydroxide was assayed. The carbonates were precipitated with an excess of barium  
 342 chloride (BaCl<sub>2</sub>; 20 wt%) solution. The sodium hydroxide that remained free was titrated with  
 343 hydrochloric acid (HCl; 0.1 mol/L). Thymolphthalein (a 0.1% solution in ethanol) was used  
 344 as the color indicator. The microbial respiration was calculated according to the following  
 345 Equation (9):

$$\begin{aligned}
 346 \quad & \text{Microbial respiration} \left( \frac{mg(C - CO_2)}{kg \text{ dry soil}} \right) \\
 347 \quad & = 0.5 \times [(M_{NaOH}(mol/L) \times Vol_{NaOH}(L)) - (M_{HCl}(mol/L) \times Vol_{HCl \text{ used}}(L))] \\
 348 \quad & \times Mm_C \left( \frac{g}{mol} \right) \times 1000 / m_{soil}(kg) \quad (9)
 \end{aligned}$$

349 where,  $M_{NaOH}$  and  $M_{HCl}$  are the concentrations of the NaOH and the HCl solutions used in  
 350 mol/L, respectively,  $Vol_{NaOH}$  is the volume of the NaOH solution,  $Vol_{HCl}$  is the volume of  
 351 HCl used in the titration (L),  $Mm_C$  represents the molar mass of carbon (12.01 g/mol), and  
 352  $m_{soil}$  is the amount of soil in each condition (0.025 kg).

353        **2.5.2.     Nitrogen mineralization test**

354     This test was also based on the AFNOR FD U44-163, (2018) standard. Biochar (25 tons/ha)  
355     and LD (170 tons/ha) were added and mixed with the soil as follows: soil, soil + biochar  
356     (B25), soil + LD + biochar (B25), and soil + LD. The experiment was carried out in soil cups  
357     (25 g of dry soil). Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) ions were extracted from three  
358     repeats after 0, 7, 14, 28, 56, and 90 days of incubation at 28 °C with moisture maintenance  
359     (pF 2.8). For each sampling date, a procedure to extract the mineral nitrogen contained in the  
360     samples by mixing the soil with 100 mL of a KCl solution (1 mol/L) was carried out. The  
361     samples were placed on a rotating agitation device for 1 hour (Heidolph, Reax 20, Germany).  
362     The samples were then filtered using filter paper. The filtrate samples were frozen before  
363     being sent to the laboratory for determination of the ammonia nitrogen levels as  $\text{NH}_4^+$  and  
364     ammonium nitrate. The measurement of  $\text{NH}_4$  was performed according to the modified NF U  
365     42-125, (1985) standard, while the measurement of  $\text{NO}_3$  was performed by colorimetry  
366     (Hood-Nowotny et al., 2010).

367        **2.5.3.     Ammonia nitrogen volatilization ( $\text{NH}_3$ )**

368     The objective of this experiment was to estimate the loss of nitrogen by evaporation of  
369     ammonia nitrogen ( $\text{NH}_3$ ) related to the supply of LD (170 kg N/ha), with or without biochar at  
370     25 tons/ha (B25), using a closed dynamic flow system. The following conditions were  
371     investigated: soil, soil + biochar (B25), soil + LD + biochar (B25), and soil + LD. The altered  
372     soils were incubated for 15 days and added at 183 g. All of the conditions were repeated four  
373     times. To allow microbiological activity in each enclosure, distilled water was added to reach  
374     60% of the soil retention capacity. Volatile ammonia nitrogen was captured in an acid trap.  
375     The traps were changed eight times over the 15-day period to determine the kinetics of the  
376     volatilization. The experimental system comprised three main parts: an incoming air condition  
377     control system (consisting of a bottle of distilled water, a bottle of sulfuric acid, and a second

378 bottle of distilled water); a 500 mL closed volatile chamber containing the modified soil  
379 (biochar with or without LD); and an acidic ammonia trapping system. The airflow was set to  
380 600 L/h (1.25 L/min) at the chamber outlet. For each device, 9 samples were taken during this  
381 test, at 1, 2, 3, 6, 8, 10, 13, and 15 days. The acid trap (0.05 mol/L sulfuric acid) provides the  
382 protons needed to switch from gaseous  $\text{NH}_3$  to  $\text{NH}_4^+$ . The acid traps were assayed by  
383 colorimetry to obtain the concentration of  $\text{NH}_4^+$  in the volume of acid, thereby allowing  
384 determination of the amount of  $\text{NH}_3$  that had evaporated. These amounts were related to the  
385 dry soil mass. The quantities were accumulated to determine the kinetics of the ammonia  
386 nitrogen volatilization.

#### 387 **2.5.4. Plant growth test**

388 The agronomic value of coupling biochar (at doses of 25 and 50 tons/ha) with LD (170 kg  
389 N/ha) was evaluated by determining the growth parameters during the first vegetative stage  
390 (relative seed germination and aerial dry biomass) using tomato as the plant model. Plant  
391 experiments with tomato seeds were performed in small pots with a volume of 0.5 L placed in  
392 a growth chamber (Fitotron®, Weiss Gallenkamp, UK) according to the OECD 208  
393 guidelines (2006) under controlled conditions. The environmental conditions during the  
394 testing were as follows: 16 h of light at 25 °C, 8 h of darkness at 18 °C, with 60% relative  
395 humidity for the periods of light and 80% relative humidity during the periods of darkness.  
396 Seven conditions were tested: soil alone; soil + industrial fertilizers using a mixture of DAP  
397 and ammonium nitrate to reach an application of 170 kg N/ha of mineral nitrogen and 10 kg  
398 P/ha of phosphorus (soil + IF); soil with LD applied at 170 kg N/ha (soil +LD); soil with LD  
399 and biochar application at 25 tons/ha (Soil + LD + B25); soil with LD and biochar application  
400 at 50 tons/ha (Soil + LD + B50); soil with biochar application at 25 tons/ha (soil + B25), and  
401 soil with biochar application at 50 tons/ha (soil + B50). The mixture for each condition was  
402 prepared at 70% of the water retention of soil. Six seeds were planted in each pot, using four

403 replicates for each condition. Each pot was manually sub-irrigated every 48 h by the addition  
404 of water to reach the initial weight. After 70% germination of the control, two germinated  
405 seeds were removed and four were kept for the growing period. After 41 days, the plants were  
406 harvested by cutting them at ground level and they were then dried in an oven at 70 °C for 24  
407 h. For each condition, the relative seed germination expressed as the percentage according to  
408 Equation (9) and the aerial dry biomass expressed in (g DM/100 plants) was determined  
409 according to Equation (10). Generally, a relative seed germination above 70% indicates low  
410 phytotoxicity.

411 Relative Seed Germination (%)

$$412 \quad = (\text{Mean of germinated seed} / \text{Initial number of seeds}) \times 100 \quad (9)$$

$$413 \quad \text{Aerial dry biomass (g DM/ 100 plants)} = (M_{\text{dry}(70\text{ }^{\circ}\text{C})} / 4) \times 100 \quad (10)$$

414 where  $M_{\text{dry}(70\text{ }^{\circ}\text{C})}$  represents the mean of the aerial dry biomass (g DM) for each condition and  
415 4 is the number of plants in each pot at harvesting time.

416 To compare the different conditions tested, the analysis of variance (ANOVA) method was  
417 used to analyze the impact of the various fertilization modes, and the confidence level  
418 considered was 95%.

### 419 **3. Results and Discussion**

#### 420 **3.1. Anaerobic digestion**

421 In the first instance, anaerobic co-digestion of wastewater sludge and quinoa residues was  
422 investigated in mesophilic CSTR anaerobic digesters. The main physicochemical properties of  
423 the quinoa residues and the wastewater sludge are reported in **Table 1**. The quinoa residues  
424 were composed of 24.6%DM of cellulose, 14.1 %DM of hemicelluloses, and 7.0 %DM of  
425 lignin. The quinoa residues had a low nitrogen content of 0.21%DM and a high C/N ratio of  
426 216. All of these values are in agreement with what has previously been reported for  
427 lignocellulosic biomass in the literature (Monlau et al., 2013). By contrast, the wastewater

428 sludge had a higher nitrogen content of 7.0%DM and a lower C/N ratio of 5.8. Interestingly,  
429 co-digestion of quinoa residues and wastewater sludge in the AD process resulted in a C/N  
430 ratio of 13, which is more in agreement with the C/N ratios that have been reported to be  
431 optimal for the AD process, with values ranging from 15 to 30 (Morales-Polo et al., 2018;  
432 Van et al., 2020).

433 First of all, biochemical methane potential tests were carried out, and a value of  $236 \pm 2$  NL  
434  $\text{CH}_4/\text{kg VS}$  was determined for the wastewater sludge and  $237 \pm 2$  NL  $\text{CH}_4/\text{kg VS}$  for the  
435 quinoa residues (data not shown). In this study, quinoa and wastewater sludge exhibited  
436 similar methane potentials and such values are in agreement with previous studies that  
437 investigated methane potential of lignocellulosic biomass (Monlau et al., 2012) and  
438 wastewater sludge (Elbeshbishy et al., 2012). Monlau et al. (2012) have reported methane  
439 potential ranging from 155 NL  $\text{CH}_4/\text{kg TS}$  to 300 NL  $\text{CH}_4/\text{kg TS}$  for various lignocellulosic  
440 biomasses. In parallel, co-digestion of quinoa residues and wastewater sludge (at 57.5/42.5%  
441 VS) resulted in a methane potential of 237 NL  $\text{CH}_4/\text{kg VS}$  demonstrating that the co-digestion  
442 did not exhibit positive synergy at BMP scale which has been previously reported in literature  
443 as the synergy effect depends on the biomass that are co-digested but also on the inoculum  
444 initially used (Elalami et al., 2019; Kim et al., 2019).

445 Then, co-digestion of quinoa residues and wastewater sludge was simulated for 16 weeks  
446 (corresponding to approximately three hydraulic retention times (HRTs) of 38 days) in  
447 mesophilic CSTR digesters, as shown in **Fig. 1A**. These durations are thought to be the  
448 minimum to assess the stability of the AD process (Sambusiti et al., 2013). After an increase  
449 of the organic loading rate during the two first weeks, the OLR was further set at 2 kg  
450  $\text{VS}/\text{m}^3/\text{day}$ . During the overall period of the assay, the pH remained stable around 7.4 and the  
451 temperature was  $38 \pm 2$  °C. All of the values presented below are averages of the values of the  
452 last HRTs (3<sup>rd</sup> HRT).

453 The concentration of ammonium remained below 2.5 gN-NH<sub>4</sub>/L, and no specific inhibition  
454 was observed in terms of methane production. The threshold inhibition level for total  
455 ammonia nitrogen reported in the literature varies from 1.5 to 2.5 g/L (Jiang et al., 2019;  
456 Sambusiti et al., 2013; Yenigün and Demirel, 2013), but after inoculum acclimation  
457 concentrations up to 2.5 g/L can be reach inside the anaerobic digester (Jiang et al., 2019).  
458 The total ammonia nitrogen (TAN), which is generally defined as the sum of free ammonia  
459 nitrogen (FAN, NH<sub>3</sub>-N) and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), is generated during the  
460 hydrolysis of proteins, urea, and nucleic acids (Morozova et al., 2020). The content of VFAs  
461 (data not shown) remained lower than 0.1 g<sub>eq.acetate</sub>/L throughout the assay, thus demonstrating  
462 good stability of the process and no acidification. These results were also reflected by the  
463 FOS/TAC ratio (**Fig. 1B**), which was below the safety threshold value of 0.3 (Sambusiti et al.,  
464 2013). To assess the performances and to identify the absence of inhibition, the average  
465 specific methane yield was computed from the slope of the trend line fitting the data of the  
466 cumulative methane production versus the cumulative VS fed to each reactor. As can be seen  
467 in **Fig. 1C**, the final specific methane production was 238 NL CH<sub>4</sub>/kg VS, corresponding to  
468 100% of the result obtained by the biochemical methane potential (BMP) batch tests. These  
469 observations are in agreement with previous studies that investigated the methane production  
470 of organic wastes on a pilot scale (Sambusiti et al., 2013). In a previous study, Alagöz and  
471 Yenigün, (2015) investigated mesophilic co-digestion of olive residue and waste activated  
472 sludge, and they reported a methane potential of 210 NL CH<sub>4</sub>/kg VS. Similarly, Li et al.  
473 (2017) investigated mesophilic anaerobic co-digestion of waste activated sludge with tobacco  
474 residues, and they reported a methane potential of 181 to 204 NL CH<sub>4</sub> /kg VS depending on  
475 the substrate ratio. Finally, COD (chemical oxygen demand) balances were performed in  
476 parallel on the overall AD system to monitor the absence of losses and closure of the organic  
477 matter cycles. The balances are presented in **Fig. 2**. The COD recovery in the output (sum of

478 the COD of biogas, liquid and solid digestate) was equivalent to 95% of the COD of the input,  
479 thus demonstrating that the matter fluxes are well evaluated and identified. At the end of the  
480 3<sup>rd</sup> HRT, the digestate was separated into a liquid and a solid fraction by a wine press, and the  
481 solid fraction was oven-dried. Pyrolysis of the solid digestate was investigated in the next  
482 section.

### 483       **3.2.       Pyrolysis**

484 Pyrolysis was carried out on the solid fraction of the digestate at 500 °C for 1 h after drying of  
485 the solid digestate. The pyrolysis products distribution was as follows: 40 ± 1.2 wt% biochar,  
486 35.8 ± 2.9 wt% bio-oil, and 23.7 ± 4.9 wt% syngas. The syngas produced during the pyrolysis  
487 process was analyzed and the distribution of the syngas compounds is presented in **Fig.3**. The  
488 LHV of the syngas was 11.8 MJ/Nm<sup>3</sup>, which is in agreement with previously reported values  
489 ranging from 12.9 MJ/Nm<sup>3</sup> to 15.7 MJ /N m<sup>3</sup> (Monlau et al., 2015b; Neumann et al., 2015;  
490 Tayibi et al., 2021). The syngas mainly consisted of CO<sub>2</sub>, CH<sub>4</sub>, CO, and H<sub>2</sub>, with a small  
491 amount of C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>, and C<sub>2</sub>H<sub>2</sub>. The production of CO<sub>2</sub> and CO was mostly due to  
492 decarboxylation and decarbonylation reactions (Jęczmionek and Porzycka-Semczuk, 2014).  
493 The high content of CO<sub>2</sub> can also result from the cracking of remaining fibers such as  
494 cellulose and hemicelluloses in the solid digestate fraction (Liu et al., 2011). In a recent study,  
495 Ghysels et al. (2020) reported a similar composition of syngas from cocoa wastes after  
496 pyrolysis at 500 °C. Indeed, on an N<sub>2</sub>-free basis, the dominant compound in this stream was  
497 carbon dioxide, at a concentration of 79.5 vol% for pyrolysis at 500 °C, and CO, CH<sub>4</sub>, and H<sub>2</sub>  
498 were also present at concentrations of 13.2 vol%, 3.9 vol%, and 2.5 vol%, respectively.

499 In parallel to syngas, bio-oil was also generated during the pyrolysis process. At industrial  
500 level, bio-oil separation by solvent seems to be a promising option with an organic phase that  
501 can be used as a fuel or building blocks (Hossain et al., 2016; Pütün et al., 2005), whereas the  
502 aqueous phase can be recirculated as feedstock for the AD process (Torri and Fabbri, 2014).

503 Hossain et al. (2016) have recently demonstrated the feasibility in an engine combustion of  
504 using organic oil from solid digestate pyrolysis in blend with waste cooking oil and butanol.  
505 In our study, the bio-oil obtained was separated into an organic and aqueous phase, at a ratio  
506 of 23.1 wt%, and 76.9 wt%, respectively. Similarly, Ghysels et al. (2020) reported a ratio of  
507 organic and aqueous phases of 25% and 75%, respectively, after pyrolysis of solid digestate  
508 derived from cocoa wastes at 500 °C, which is in agreement with our study. The main  
509 physicochemical properties of the organic phase of the bio-oil are summarized in **Table 2**.  
510 The organic phase of the bio-oil had a high carbon content (70.5 wt%) and a low oxygen  
511 content (14.9 wt%), which is required for bio-oil fuel applications. The higher heating value  
512 (HHV) of the organic phase of the bio-oil (**Table 2**) was estimated to be 33.9 MJ/kg. This  
513 HHV of bio-oil is higher than that reported by Opatokun et al. (2015) from food digestate  
514 pyrolyzed at 500 °C with a calorific value of 13.5 MJ/kg, but in their case all the bio-oil was  
515 considered and not only the organic phase. Nonetheless, our value was in the same range as  
516 the value of 29.7 MJ/kg reported by Ghysels et al. (2020) after pyrolysis at 500 °C of solid  
517 digestate from the anaerobic digestion of cocoa wastes. Generally, the organic phases from  
518 bio-oil after pyrolysis of solid digestate at 500 °C have been reported to be dominated by  
519 phenolic compounds (Ghysels et al., 2020; Tayibi et al., 2020a; Wei et al., 2018). For  
520 instance, Wei et al. (2018) reported that the content of phenolic compounds was 70.4% in the  
521 OP of the bio-oil produced from digestate (originating from sargassum anaerobic digestion)  
522 pyrolyzed at 450 °C (Wei et al., 2018).

523 Finally, a third product was generated during the pyrolysis process (at 40 wt%), as a  
524 carbonaceous material called biochar. Similarly, Opatokun et al. (2017) reported biochar  
525 yields from 25 to 61%wt after pyrolysis at temperatures ranging from 300 °C to 700 °C of  
526 solid digestate from anaerobic digestion of food wastes. Similarly, Neumann et al. (2015)  
527 reported a biochar yield of 36%wt after pyrolysis at 500 °C of solid anaerobic digestate. The

528 produced biochar was characterized, and their main physicochemical properties are reported  
529 in **Table 3** and compared to the EBC (European Biochar Certificate) and IBI (International  
530 Biochar Initiative). The carbon content of the biochar was 47.2 wt%, indicating that it is a  
531 class 2 biochar according to the IBI and that it is considered a bio carbon mineral (BCM) and  
532 not a biochar according to the EBC (**Table 3**). Compared to the solid digestate, the amount of  
533 O and H decreased in the biochar due to the decarboxylation and dehydration reactions during  
534 the pyrolysis process. The ash content of the biochar was 38.2 wt% compared to only 15.9  
535 wt% in the solid digestate (**Table 3**). The relatively high ash content in the solid digestate can  
536 be attributed to the fact that during the AD process the microorganisms convert the organic  
537 fraction of the organics into CO<sub>2</sub> and CH<sub>4</sub> (Monlau et al., 2015a), resulting in a higher  
538 concentration of inorganics in the digestate than in the original feedstocks. Macronutrients  
539 (i.e., N, P, K, Mg, and Ca) were also analyzed in the solid digestate and the respective  
540 biochar. Except for N, all the macronutrients were enriched in the biochar, which is in  
541 agreement with previous publications that investigated biochar production from solid  
542 digestate (Calamai et al., 2019; Monlau et al., 2016). Minor metals (i.e., Pb, Cd, Cu, Ni, Hg,  
543 Zn, Cr, and As) were also analyzed in the biochar. All of the values obtained were lower than  
544 the maximal threshold levels recommended by the IBI (International Biochar Initiative).  
545 Nonetheless, most of the values were higher than the threshold levels recommended by the  
546 EBC (European Biochar Certificate), except for Hg, as shown in **Table 3**.

### 547 **3.3. Agronomic potential of coupling biochar and LD**

#### 548 **3.3.1. Plant growth tests on tomato plants**

549 The impact of biochar (at 25 and 50 tons/ha) on the growth of tomatoes was investigated  
550 during the first vegetative stage of the plants. The biochar concentration was chosen based on  
551 the data in the literature (Glaser et al., 2015; Greenberg et al., 2019). Biochar has recently  
552 been shown to be of considerable relevance to sustainable agriculture (Mandal et al., 2020;

553 Semida et al., 2019), even though biochar does not have any fertilizing actions and should  
554 generally be added with a fertilizer (Greenberg et al., 2019; Ronga et al., 2020; Tayibi et al.,  
555 2021). Since biochar does not provide an adequate supply of nutrients to serve as the sole  
556 source of fertilization, combined effects with the LD (applied at 170 kg N/ha) were evaluated  
557 on the relative seed germination and the aerial dry biomass of tomatoes. The liquid digestate  
558 was composed of 3.8 wt% dry matter, 2.4 wt% volatile matter, 0.37 wt% total Kjeldahl  
559 nitrogen (TKN), 0.15 wt% ammonium ( $\text{NH}_4^+$ ), 0.27 wt% potassium ( $\text{K}_2\text{O}$ ), and 0.25 wt%  
560 phosphorus ( $\text{P}_2\text{O}_5$ ).

561 Seed germination is a relevant indicator of potential phytotoxicity. LD added alone did not  
562 affect the relative seed germination with 88 ( $\pm 8$ ) % in comparison to soil alone (92 ( $\pm 9$ ) %).  
563 Interestingly, biochar addition at 25 and 50 tons/ha did not exhibit a negative impact on the  
564 germination rate in comparison with soil alone and soil with industrial fertilizers, as shown in  
565 **Fig. 4**. Indeed, relative seed germination of 92 ( $\pm 9$ ) %, 83 ( $\pm 16$ ) %, 79 ( $\pm 8$ ) % were reported  
566 for soil alone, soil with 20 tons and 50 tons of biochar, respectively. Similarly, Bu et al.  
567 (2020) investigated the effect of rice husk and woodchip biochar at various application rates  
568 (1%, 2%, and 5% by weight) on seed germination of *Robinia pseudoacacia* in calcareous soil,  
569 and they did not find that there was a negative impact (Bu et al., 2020). Furthermore, the  
570 combination of LD and biochar did not exhibit a negative impact on germination in  
571 comparison with soil alone and soil with industrial fertilizers. These results are also in  
572 agreement with those of Tayibi et al. (2020a), who similarly did not observe a negative impact  
573 on the germination of wheat when biochar (at 50 tons/ha) was added in combination with LD.

574 In parallel, the growth efficiency of tomato plants when the soil was amended by biochar (at  
575 25 and 50 tons/ha) in combination with LD was also investigated during the first vegetative  
576 growth stage (41 days). As shown in **Fig. 4**, all the conditions tested resulted in higher aerial  
577 dry biomass production than the soil alone. More specifically, the application of LD increased

578 the aerial dry biomass to 18 gDM/100 plants compared to unamended soil (11.6 gDM/100  
579 plants), although it was slightly lower than with industrial fertilizers (20.8 gDM/100 plants).  
580 These results confirm the ability of LD to improve plant growth as previously demonstrated  
581 (Elalami et al., 2020; Nkoa, 2014; Solé-Bundó et al., 2017). A higher aerial dry biomass was  
582 obtained for the condition that combined biochar and LD, with an increase of 33% compared  
583 to soil amended with biochar alone (ANOVA test:  $p < 0.05$ ) and an increase of 88%  
584 compared to soil only (ANOVA test:  $p < 0.05$ ). In parallel, the dry biomass obtained for the  
585 condition combining soil and LD was lower by 13.8% compared to the soil treated with  
586 industrial fertilizers, although the results were not significantly different according to the  
587 ANOVA test ( $p = 0.18$ )

588 Adding biochar with LD increased the dry biomass by 4.5% and 7.9% for 25 tons/ha and 50  
589 tons/ha, respectively, compared to soil fertilized with industrial fertilizers, which shows the  
590 positive effect of coupling biochar and LD.

591 Ronga et al. (2020) also assessed the effect of digestate and biochar fertilizers on the yield and  
592 fruit quality of tomatoes grown in an organic farming system, and they obtained similar  
593 results. Indeed, they demonstrated that plants fertilized with LD and biochar had the  
594 maximum marketable yield (72 tons/ha), followed by BC (67 tons/ha), and LD (59 tons/ha);  
595 while the lowest production (47 tons/ha) was recorded with unfertilized plants. These results  
596 are in agreement with Glaser et al. (2015), who reported a positive effect on maize silage by  
597 combining digestate with biochar. Glaser et al. (2015) demonstrated that the application of  
598 biochar-digestate (at a biochar rate of 1 and 40 tons/ha and digestate at 200 kg N /ha) to maize  
599 increased the yields and plant nutrition compared to pure digestate. Interestingly, at a  
600 concentration of 40 tons/ha of biochar, the co-application of digestate with biochar  
601 significantly increased the maize yield by 42% compared to untreated plants without biochar  
602 addition (Glaser et al., 2015). Greenberg et al. (2019) also investigated the effect of coupling

603 LD with biochar added at 2 and 40 tons/ha on *Zea mays*. For both biochar applications, there  
604 were no significant differences in terms of the rye above-ground biomass. Nonetheless, such  
605 experiments must be extended in the future at a field-scale, and several parameters should be  
606 carefully investigated such as the nature and the quantity of biochar, the digestate origin and  
607 properties, the type of the soil, and climatic conditions.

### 608 **3.3.2. Ammonia volatilization, C and N mineralization**

609 In light of the results obtained with tomato plant growth during the first vegetative stage, the  
610 effect of coupling LD with biochar at a dose of 25 tons/ha on other agronomic parameters  
611 (*e.g.*, ammonia volatilization, C and N mineralization) was investigated. The addition of  
612 biochar on the C mineralization compared with the control (soil alone) was investigated first,  
613 as shown in **Fig. 5A**. A slight but non-significant reduction of C mineralization was observed  
614 in the presence of biochar compared to the control (soil alone). Carbon dioxide is generally  
615 released by microbial decay of residual organic matter (Semida et al., 2019). These results  
616 confirm the capacity of biochar to sequester C in soils and to contribute to carbon  
617 sequestration (Clough et al., 2013; Semida et al., 2019). Bruun and EL-Zehery, (2012)  
618 investigated the effect of biochar addition to soil amended by barley straw. Without biochar,  
619  $48 \pm 0.2\%$  of the straw carbon was mineralized during the 451 days of the experiment. In  
620 comparison,  $45 \pm 1.6\%$  of C was mineralized after biochar addition at  $1.5 \text{ g kg}^{-1}$ . Similarly,  
621 Yoo and Kang, (2012) demonstrated that the addition of biochar (at 2 wt%) in silt loam soils  
622 did not affect the C mineralization compared with the soil alone. Fidel et al. (2019) also  
623 investigated the impact of biochar (derived from wood) added at 0.5 wt%/wt% of silt soil on  
624  $\text{CO}_2$  mineralization at different temperatures and humidity. They did not observe any  
625 significant differences for any of the conditions between the  $\text{CO}_2$  mineralization in the  
626 biochar-amended soil and the unamended soil. The soil sequestration capacity of biochar  
627 appears to depend on the soil typology, the climatic conditions, and the biochar origin

628 (Semida et al., 2019; Yoo and Kang, 2012). The addition of LD to the soil led to an  
629 enhancement of the C mineralization due to the presence of soluble organic carbon that is  
630 mineralized and  $\text{NH}_4^+$ , and the C mineralization rate was determined to be 1,517 mg C-  
631  $\text{CO}_2/\text{kg}$  dry soil. Finally, as shown in **Fig. 5A**, the combination of biochar and LD led to  
632 equivalent C mineralization compared to soil amended with LD only, and a net C  
633 mineralization of 1,539 mg C- $\text{CO}_2/\text{kg}$  dry soil. Aside from potentially sequestering carbon,  
634 the impact of biochar added alone or in combination with LD on the N dynamics by  
635 performing N mineralization experiments was also investigated. The addition of biochar did  
636 not appear to affect the N mineralization dynamics compared to soil alone, as shown in **Fig.**  
637 **5B**. These results confirm that, aside from a high biochar C/N ratio of 17 that can further  
638 increase the C/N of soil, no N immobilization was noted, thus suggesting that biochar is  
639 composed of recalcitrant organic carbon (Semida et al., 2019). Furthermore, the absence of N  
640 mineralization enhancement after biochar application can be explained by the biochar  
641 capacity to absorb  $\text{NH}_4^+$  and  $\text{NO}_3^-$  that masks N mineralization or by the low amount of  
642 hydrolyzable organic N forms in biochar (Ameloot et al., 2015; Clough et al., 2013). The  
643 same tendency was observed in the presence of LD, as shown in **Fig. 5B**, demonstrating that  
644 the biochar did not affect the microbial population involved in N mineralization. The increase  
645 in N mineralization was 129-135 mg  $\text{N}_{\text{mineral}}/\text{kg}$  dry soil.

646 The impact of coupling LD and biochar was also investigated in regard to ammonia  
647 volatilization, as shown in **Fig. 5C**. Interestingly, the addition of biochar alone at 25 tons/ha  
648 did not have any influence on the ammonia volatilization compared to soil alone. The addition  
649 of digestate to the soil led to an increase of the ammonia volatilization of 1.8 mg N/kg dry soil  
650 versus 0.4 mg N/kg dry soil for the non-amended soil. This increase of 1.8 mg N/kg dry soil  
651 corresponded to 3.5% of the total nitrogen provided by the LD. This ammonia volatilization is  
652 due to the presence of ammoniacal nitrogen present in the digestate that became volatilized

653 once applied in the soil (Nkoa, 2014; Plaimart et al., 2021). Similarly, Plaimart et al. (2021)  
654 have reported that approximately 4.8% of the nitrogen was evaporated in the form of  
655 ammonia after the application of digestate on a clay loam soil. Surprisingly, the co-application  
656 of biochar and LD enhanced the ammonia volatilization by as much as 3 mg N/kg dry soil,  
657 which can be explained by the fact that the alkaline nature of biochar added to soil promotes  
658 ammonia volatilization (Sha et al., 2019). Such value of ammonia volatilization corresponded  
659 to 5.8% of the total nitrogen initially provided and consequently biochar addition  
660 simultaneously with LD resulted in 66% more N loss than LD application alone. Similar as  
661 well as contradictory results have been observed in the literature, and the impact of biochar  
662 addition on ammonia volatilization appears to depend on several parameters such as the  
663 nature of the soil, the origin of the biochar, and the experimental conditions (Plaimart et al.,  
664 2021; Sha et al., 2019).

#### 665 **4. Conclusions**

666 In this study, an original cascading biorefinery approach that coupled AD and pyrolysis was  
667 investigated for the valorization of organic wastes. AD led to a methane production of 219  
668 NL CH<sub>4</sub>/kg VS, and the organic phase (OP) of bio-oil and syngas from subsequent pyrolysis  
669 of the solid digestate exhibited higher and lower heating values of 34 MJ/kg and 11.8  
670 MJ/Nm<sup>3</sup>, respectively. Specific attention was paid to combining biochar and anaerobic LD  
671 for agronomic purposes. The characteristics of the biochar were in accordance with the IBI  
672 recommendations for soil amendment. The co-application of biochar with LD significantly  
673 increased the ammonia volatilization by 64% compared to LD application alone. Although  
674 co-application of biochar with LD did not impact the C and N mineralization, their  
675 simultaneous use improved the growth of tomato plants up to 25% compared to LD  
676 application alone.

#### 677 **Acknowledgments**

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679 ATCLASS project.  
680

681 **Table and Figure Captions:**

682 **Table 1.** The main chemical constituents of the feedstocks introduced into the anaerobic lab-  
683 scale CSTR digester.

684 **Table 2.** The properties of the organic phase of bio-oil from the pyrolysis of the solid  
685 digestate.

686 **Table 3.** The physicochemical properties of the solid digestate and the produced biochar  
687 compared to the IBI (International Biochar Initiative) and the EBC (European Biochar  
688 Certificate) recommendations.

689 **Fig.1.** (A) Organic loading rate effect, temperature, and pH changes during the AD process;  
690 (B) Biomethane production and change in the  $\text{NH}_4^+$  concentration during the AD; (C)  
691 Cumulative biogas and methane production during the AD process vs. the cumulative VS  
692 added.

693 **Fig.2.** Chemical oxygen demand (COD) mass balances of the AD CSTR process with quinoa  
694 residues and wastewater sludge co-digestion.

695 **Fig.3.** Syngas distribution during the pyrolysis experimentation according to the temperature  
696 process. Overall syngas composition produced during the pyrolysis process of solid digestate  
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698 **Fig.4.** Relative seed germination (%) and aerial dry biomass (gTS/100 plants) of tomato  
699 plants.

700 **Fig.5.** (A) Microbial respiration, (B) Total nitrogen mineralization over time (from 0 to 91  
701 days), and (C) Ammonia volatilization for the four conditions: soil, soil with biochar (at 25  
702 tons/ha), soil with liquid digestate (LD), and soil with liquid digestate (LD) and biochar at 25  
703 tons/ha (B25).

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705

706 **Table 1.** The main chemical constituents of the feedstocks introduced into the anaerobic lab-  
707 scale CSTR digester.

708

<b>Parameter (units)</b>	<b><i>Quinoa residues</i></b>	<b><i>Wastewater sludge</i></b>
<b>DM (wt% FM)</b>	90.1 ± 0.1	18.7 ± 0.1
<b>VS (wt% DM)</b>	88.9 ± 0.3	79.6 ± 2.2
<b>C (wt%)</b>	43.3 ± 0.2	41.2 ± 0.2
<b>H (wt%)</b>	6.0 ± 0.1	6.2 ± 0.2
<b>N (wt%)</b>	0.2 ± 0.0	7.0 ± 0.2
<b>S (wt%)</b>	0.1 ± 0.0	0.6 ± 0.0
<b>O<sup>a</sup> (wt%)</b>	40.5 ± 0.3	41.2 ± 0.5
<b>Cellulose (wt%)</b>	24.6 ± 0.4	-

<b>Hemicelluloses (wt%)</b>	14.1 ± 0.5	-
<b>Klason lignin (wt%)</b>	7.0 ± 0.3	-
<b>Ash (wt%)</b>	10.0 ± 0.2	3.8 ± 0.4

<sup>a</sup> $O\% = 100\% - C\% - H\% - N\% - S\% - Ash\%$

Parameters (units)	Solid digestate	Biochar	IBI standards V2.0	EBC standards V4.8
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709

710 **Table 2.** The properties of the organic phase of the bio-oil from pyrolysis of the solid  
711 digestate.

712

Parameter (units)	Organic phase of bio-oil	713
<b>C (wt%)</b>	70.6 ± 0.9	714
<b>H (wt%)</b>	8.1 ± 0.7	715
<b>N (wt%)</b>	5.6 ± 0.5	716
<b>S (wt%)</b>	0.8 ± 0.1	716
<b>O (wt%)<sup>a</sup></b>	14.9 ± 1.0	717
<b>HHV (MJ/kg)</b>	33.9	718
<b>Density (kg/L)</b>	1.1	718
<b><sup>a</sup> determined by difference</b>		719

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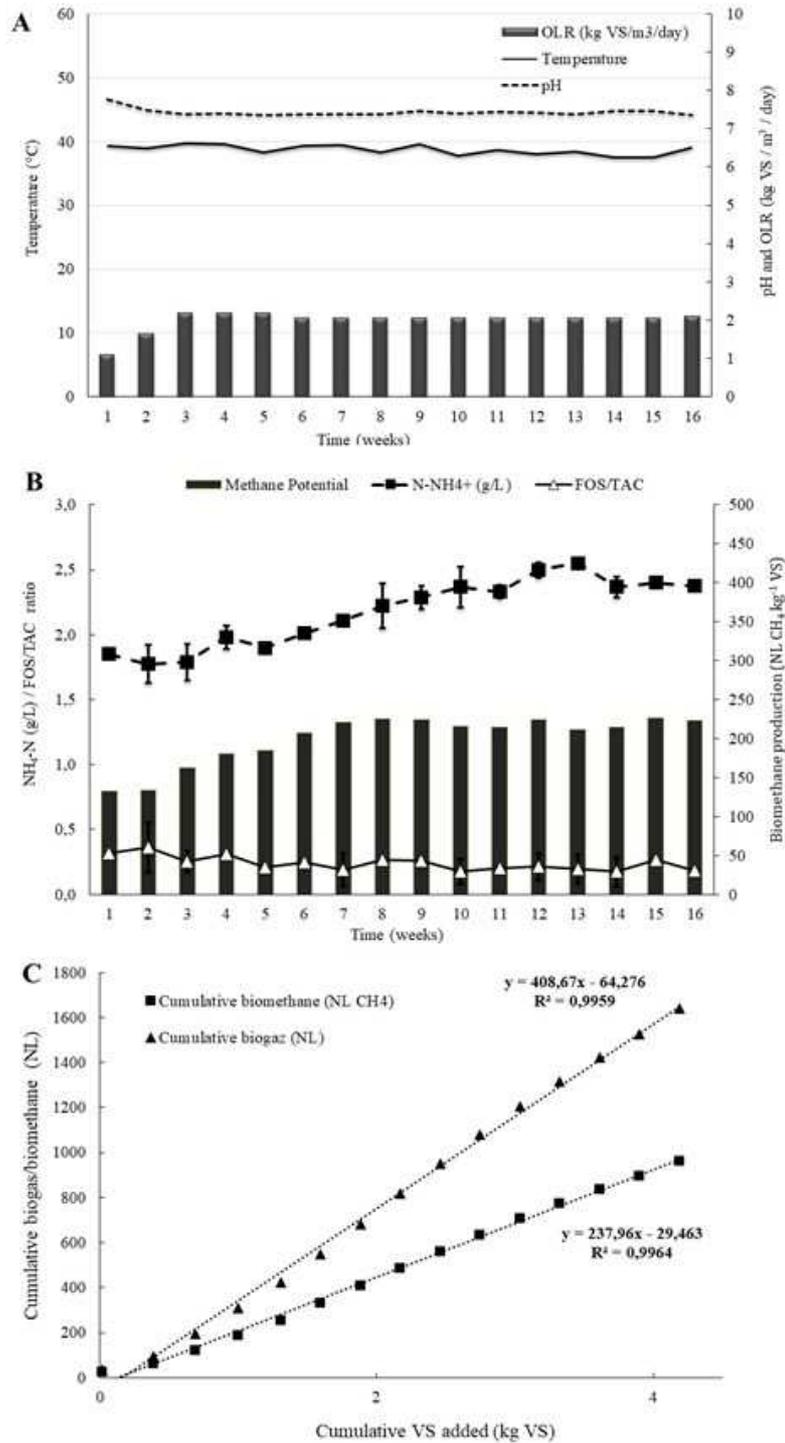
728 **Table 3.** The physicochemical properties of the solid digestate and the produced biochar  
729 compared to the IBI (International Biochar Initiative) and the EBC (European Biochar  
730 Certificate) recommendations.

<b>pH</b>	7.7 ± 0.02	9.8 ± 0.06	-	-
<b>C (wt%)</b>	37.0 ± 2.0	47.2 ± 5.3	10 wt% Minimum •Class 1: ≥ 60wt% •Class 2: ≥ 30wt% and ≤ 60wt%. •Class 3: ≥ 10wt% and ≤ 30wt%.	Biochar ≥ 50wt% Bio Carbon Minerals (BMC) < 50wt%
<b>H (wt%)</b>	4.6 ± 0.4	1.6 ± 0.1	-	-
<b>N (wt%)</b>	2.8 ± 0.6	2.8 ± 0.2	-	-
<b>S (wt%)</b>	0.6 ± 0.1	0.4 ± 0.1	-	-
<b>O<sup>a</sup> (wt%)</b>	39.1 ± 1.7	9.7 ± 5.2	-	-
<b>K (wt%)</b>	1.3	3.1	-	-
<b>P (wt%)</b>	1.7	3.4	-	-
<b>Mg (wt%)</b>	0.4	0.6	-	-
<b>Fe (wt%)</b>	0.5	0.9	-	-
<b>Ca (wt%)</b>	1.7	3.6	-	-
<b>Na (wt%)</b>	0.2	0.4	-	-
<b>H/C</b>	0.13 ± 0.0	0.03 ± 0.0	-	-
<b>O/C</b>	1.06 ± 0.1	0.22 ± 0.13	-	-
<b>Moisture (wt%)</b>	8.3 ± 1.2	4.9 ± 1.3	-	-
<b>Volatile matter (wt%)</b>	60.4 ± 0.6	19.0 ± 0.7	Optional	Required
<b>Fixed carbon (wt%)</b>	15.8 ± 0.7	43.0 ± 0.0	-	-
<b>Ash (wt%)</b>	15.9 ± 1.2	38.2 ± 5.7	Required	Required
<b>Cellulose (wt%)</b>	17.8 ± 1.1	-	-	-
<b>Hemicelluloses (wt%)</b>	5.5 ± 0.0	-	-	-
<b>Lignin (wt%)</b>	18.3 ± 0.6	-	-	-
<b>Minor metallic (mg/kg DM)</b>				
<b>Pb</b>	28	54	70 - 500	< 150
<b>Cd</b>	2.8	5.2	1.4 - 39	< 1.5
<b>Cu</b>	220	389	63 - 1500	< 100
<b>Ni</b>	40	73	47 - 600	< 50
<b>Hg</b>	0.05	< 0.05	1 - 17	< 1
<b>Zn</b>	516	989	200 - 7000	< 400
<b>Cr</b>	120	209	64 - 1200	< 90
<b>As</b>	2.9	4.1	12 - 100	-

<sup>a</sup>O% = 100% - C% - H% - N% - S% - Ash%

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734 **Fig.1.** (A) Organic loading rate effect, temperature, and pH changes during the AD process;

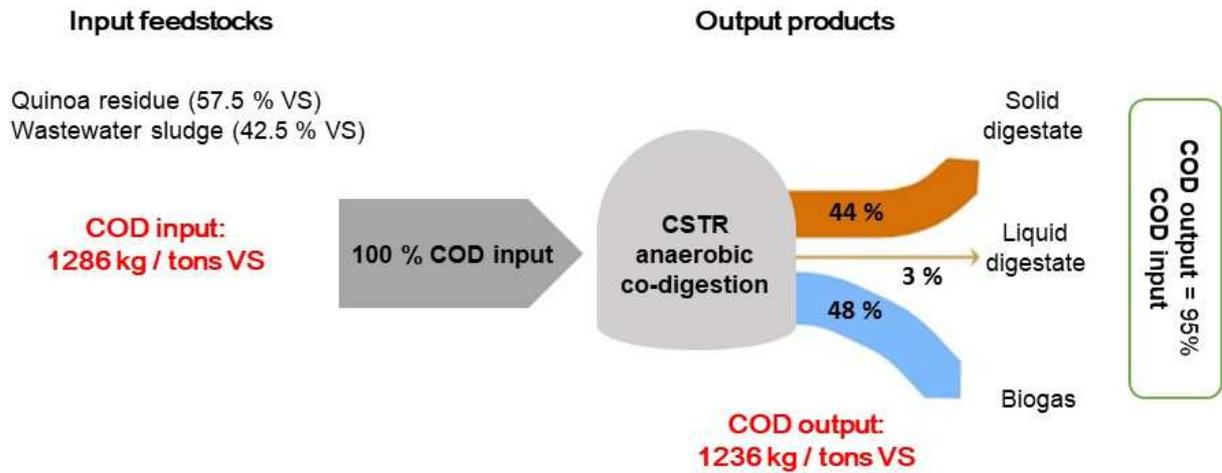
735 (B) Biomethane production and change in the NH<sub>4</sub><sup>+</sup> concentration during the AD; (C)

736 Cumulative biogas and methane production during the AD process vs. the cumulative VS

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added.

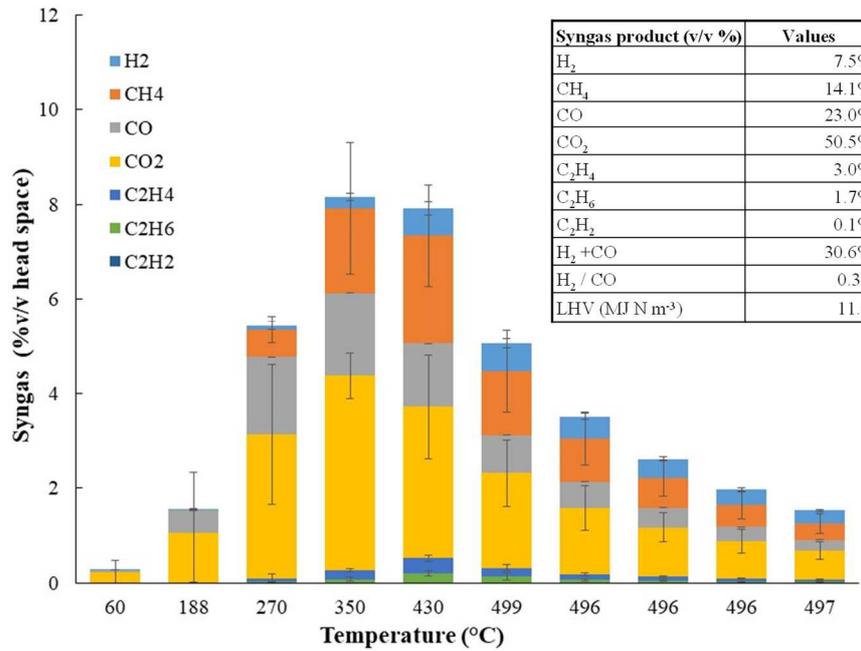
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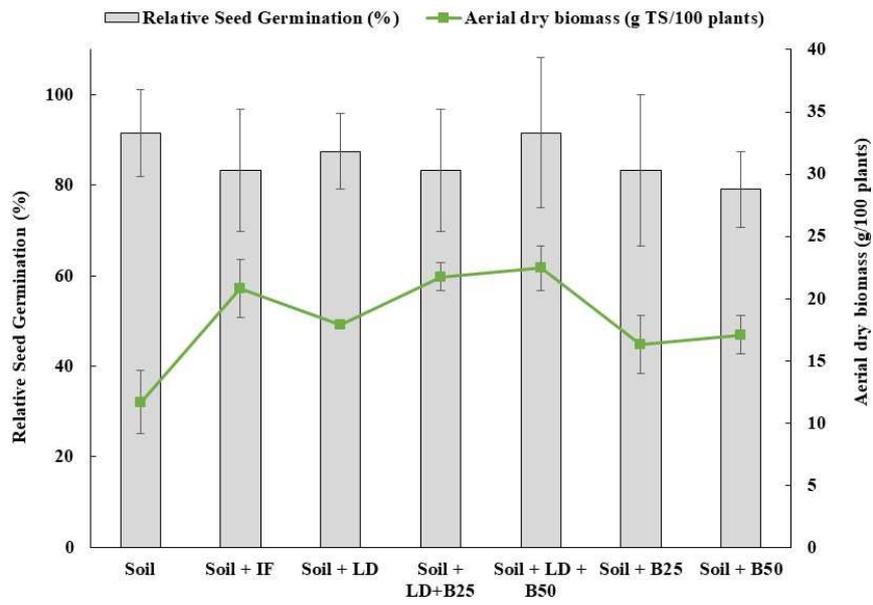


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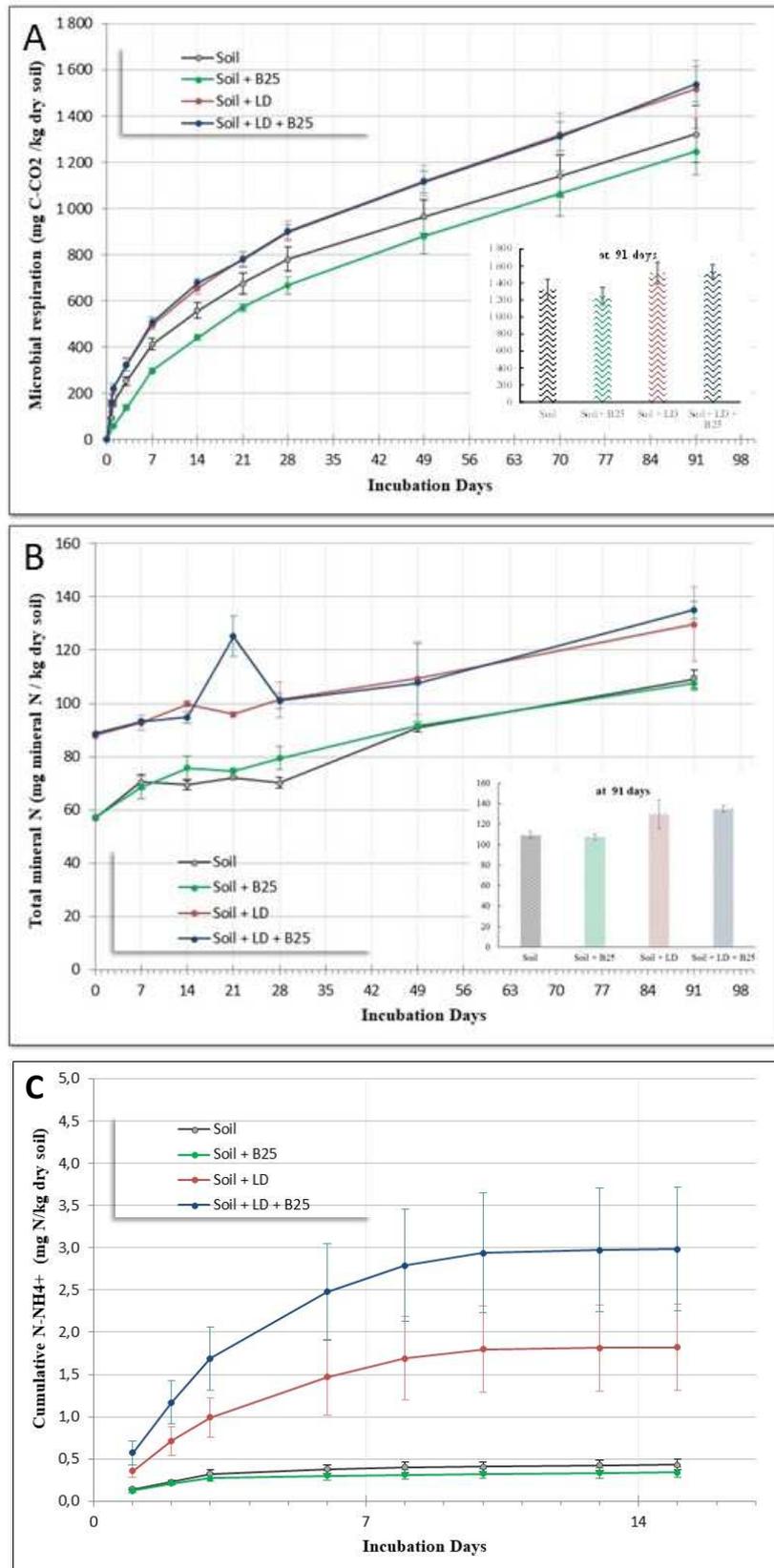


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 755 liquid digestate and biochar application at 25 tons/ha (soil + LD + B25).

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