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# 1 **Effect of coupling alkaline pretreatment and sewage sludge co-** 2 **digestion on methane production and fertilizer potential of digestate**

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## 12 **Abstract**

13 This study aims at investigating how organic waste co-digestion coupled with alkaline  
14 pretreatment can impact the methane production and fertilizer value of produced digestates. For  
15 this purpose, sludge alone and mixed with olive pomace or macroalgal residues were subjected to  
16 anaerobic digestion with and without alkaline pretreatment. In addition, co-digestion of pretreated  
17 sludge with raw substrates was also carried out and compared to the whole mixture pretreatment.  
18 KOH pretreatment enhanced methane production by 39%, 15% and 49% from sludge, sludge  
19 mixed with olive pomace and sludge mixed with macroalgal residues, respectively. The  
20 digestates were characterized according to their physico-chemical and agronomic properties.  
21 They were then applied as biofertilizers for tomato growth during the first vegetative stage (28 d  
22 of culture). Concentrations in chlorophyll *a* and carotenoids in tomato plants, following sludge

23 digestate addition, rose by 46% and 41% respectively. Sludge digestate enhanced tomato plant  
24 dry weight by 87%, while its nitrogen content rose by 90%. The impact of nitrogen and  
25 phosphorus contents in the digestate was strongest on tomato plant dry weight, thus explaining  
26 the efficiency of sludge digestate relative to other types of digestate. However, when methane  
27 production is considered, the combination of pre-treatment with co-digestion of macroalgal  
28 residues and sludge appears most beneficial for maximizing energy recovery and for biofertilizer  
29 generation.

30 **Keywords:** anaerobic digestion, waste activated sludge, pretreatment, codigestion, biofertilizer,  
31 chlorophyll.

## 32 **Abbreviations**

33 **AcoD** Anaerobic co-digestion

34 **AD** Anaerobic digestion

35 **BMP** biomethane potential test

36 **CEL** Cellulose

37 **FOS** Volatile organic acids

38 **HEM** Hemicelluloses

39 **HRT** hydraulic retention time

40 **LIGN** Lignin

41 **MAR** Macroalgal residues

42 **MonoD** mono-digestion

43 **NDS** Neutral detergent soluble

44 **OP** Olive pomace

45 **PLS** Partial least square

- 46 **TAC** Total alkalinity
- 47 **TKN** Total Kjeldahl nitrogen
- 48 **TWAS** Thickened waste activated sludge
- 49 **TS** Total solids
- 50 **VFAs** Volatile fatty acids
- 51 **VS** Volatile solids

## 52 **1. Introduction**

53 As the world population grows, the consumption of food and water is increasing exponentially.  
54 Wastewater treatment plants, along with the food industry, generate enormous amounts of  
55 organic waste such as sludge, which are, in many developing countries, landfilled or burned  
56 (Babayemi and Dauda, 2010). As a country with fairly significant natural resources, Morocco is  
57 presently experiencing an increasing generation of biomass waste which needs to be dealt with  
58 (Mohamed et al., 2018). Examples of these wastes include olive pomace from oil extraction (OP),  
59 red seaweed or macroalgae residues (MAR). Wet olive pomace is generated at 400 000 tons per  
60 year (Bouknana et al., 2014), MAR at 870 tons per year (Aboulkas et al., 2017) and dry weight  
61 sludge at 255 500 tons per year (Belhadj et al., 2013). The lack in sustainable management of  
62 these wastes would eventually lead to long-term harmful effects on the environment, which might  
63 even be taking place already (Dahhou et al., 2017). Nowadays, effective waste management  
64 strategies are required to ensure the sustainability of the processes and to minimize their impact  
65 on the environment. Indeed, currently applied sludge management techniques include  
66 incineration, composting, anaerobic digestion (AD) and agronomic valorisation (land application)  
67 (Cieřlik et al., 2015).

68 Anaerobic digestion, one of the most widespread solutions for processing organic waste, is a  
69 biological process that transforms organic matter into biogas and into a residue called digestate.  
70 Digestate can have fertilizing or amending properties. The quantity of methane produced and the  
71 quality of the digestate necessarily depend upon the properties of the substrates used. They also  
72 rely on the AD conditions or the application of biomass pretreatments. Among these properties,  
73 substrate composition and its biodegradability are crucial parameters.

74 Parameters such as the C to N ratio, moisture content and pH can be adjusted by adding a co-  
75 substrate prior to AD, known as co-digestion. Other benefits of co-digestion are the dilution of  
76 inhibitory compounds such as fatty acids or phenols but also the improvement of the buffering  
77 capacity (Iacovidou et al., 2012). In addition, co-digestion contributes to the balance, in the  
78 digesters, between macro and micronutrients that are necessary for anaerobic microorganisms.  
79 From a technical point of view, co-digestion allows to take advantage of already existing  
80 digesters to manage additional wastes; furthermore, by increasing their energy production the site  
81 consumption can be covered (Mu et al., 2020). However, although co-digestion can be applied to  
82 improve AD performances, it should be done with caution as there is a risk of modifying the  
83 rheology of the mixture and increasing its total solid content (TS) which in turn may reduce the  
84 digester performance (Miryahyaei et al., 2020).

85 The co-digestion of sludge with other substrates has been investigated intensely during the past  
86 decades. The organic fraction of municipal solid waste and food waste can represent two  
87 potential co-substrates of the AD process of wastewater sludge. Co-substrates such as fatty  
88 wastes and glycerol were also studied, but with sludge/co-substrate ratios generally above 50%  
89 (Volatile solids (VS) basis) (Jensen et al., 2014). On the contrary, food wastes or agricultural

90 residues (manure, fruit and vegetable wastes) can be added at a higher ratio but it generally  
91 remains around 50% (Algapani et al., 2017). For instance, a 19% improvement in methane  
92 production was obtained after co-digestion of sludge with 40% (VS basis) olive pomace (Alagöz  
93 et al., 2015). Biomasses such as macro- and microalgae have also been studied as sludge co-  
94 substrates but not as often as the other aforementioned wastes (Elalami et al., 2019). For instance,  
95 macroalgae (*Ulva spp.*) have been co-digested with mixed sludge at a ratio of 11% (VS basis) but  
96 no synergy was observed (Costa et al., 2012). In parallel, Mahdy et al. (2014) observed a synergy  
97 for methane production during the co-digestion of the microalgae strain *Chlorella vulgaris*, with  
98 primary sludge (+26%), but not with waste activated sludge (Mahdy et al., 2014).

99 Nonetheless, besides the co-digestion benefit, most of these biomasses are relatively difficult to  
100 degrade by AD, as their hydrolysis is the rate limiting step. To overcome this bottleneck, various  
101 pretreatment techniques have been applied, with extensive research in the case of sewage sludge  
102 (Kor-Bicakci and Eskicioglu, 2019). Hydrothermal pretreatment and steam explosion are among  
103 the most effective pretreatments on sludge and lignocellulosic biomasses (Cheah et al., 2020).  
104 However, the costs of these pre-treatments raise questions concerning their efficiency (Cheah et  
105 al., 2020). More recently, researchers have given more attention to low temperature pre-  
106 treatments using a minimum amount of chemicals or by replacing these with organic reagents  
107 that are less harmful for the environment (Kamusoko et al., 2019).

108 Previous works by the authors highlight the potential of alkaline pretreatments for increasing  
109 methane production from both olive pomace (Elalami et al., 2020a) and macroalgal residues  
110 (Elalami et al., 2020b). Although alkaline pretreatments are not yet used at full scale AD, they  
111 favour the enhancement of the sludge methane potential (Heo et al., 2003; Li et al., 2012).

112 Alkaline pretreatments result in the solubilisation of sugars, polyphenols and lipids while the  
113 lignin content in the pretreated OP is reduced (Pellera et al., 2016). In addition, NaOH  
114 pretreatment (pH=12, 90°C for 2 h) has resulted in the solubilisation of 67% of crude proteins in  
115 sludge (Liu et al., 2008). Alkaline pre-treatment also improves the buffering capacity by adjusting  
116 the pH value of the substrate (Kamusoko et al., 2019).

117 Pretreatment can also enhance the effectiveness of co-digestion. It can be applied to a single  
118 substrate as well as to a mixture. However, this approach can generate additional costs, which is  
119 why it has been investigated more seldom than co-digestion alone. Examples of pretreatments  
120 combined with sludge co-digestion include thermo-alkaline techniques for lignocellulosic  
121 residues (Abudi et al., 2016b) and mechanical or thermal treatment for macroalgal and microalgal  
122 biomasses (Mahdy et al., 2014; Tedesco et al., 2013). In fact, Abudi et al. (2017) reported that  
123 thermal pretreatment of sludge synergistically increased the batch methane production of the  
124 mixture containing the organic fraction of municipal wastes, sludge and rice straw at a ratio of  
125 1:1.5:1.5 (Abudi et al., 2016a). In addition, Solé-Bundo et al. (2019) found that thermal  
126 pretreatment of microalgae increased the methane production of its mixture with primary sludge  
127 (Solé-Bundó et al., 2020).

128 Pretreatments can further affect the quality of the digestate (Tampio et al., 2016). For example,  
129 the KOH pretreatment of wheat straw at a dose of 6% (TS basis) resulted in a 138% higher  
130 potassium and 68% higher ammonia content compared to untreated wheat straw digestate (Jaffar  
131 et al., 2016). NaOH pretreatment has been the most widely used alkaline pre-treatment in  
132 literature (Carrère et al., 2010). However, the substitution of NaOH by KOH can reduce the

133 impact of pre-treatment for the agronomic use of the digestate, since sodium can increase the  
134 salinity of the soil (Bolzonella et al., 2018).

135 Very few studies have yet investigated the impact of pretreatment on plant properties after  
136 digestate application. Solé-Bundó et al. (2017) reported that although digestate from thermally  
137 pretreated microalgae improved digestate hygienisation , it did not increase the cress growth  
138 index (Solé-Bundó et al., 2017).

139 In a previous study, a comparison was made between digestates originating from untreated and  
140 KOH-pretreated macroalgal residues. Both types of digestate led to similar results for tomato and  
141 wheat germination and for plant growth. Nevertheless, tomato plant weight increased with the use  
142 of digestate issued from the co-digestion of macroalgal residues and sewage sludge, thus  
143 implying the advantage of sludge addition to the digestion feedstock (Elalami et al., 2020b). Note  
144 that the combination between pretreatment and co-digestion had not yet been investigated.

145 Sludge pre-treatment and co-digestion have been studied extensively in the field of AD processes  
146 and their performances. However, as a novelty, the present study proposes to examine the effect  
147 of pre-treatment, either alone or combined with co-digestion, on the quality of digestate. The  
148 further use of digestate as fertilizer for plant growth is also explored. Here, various pretreatment  
149 and co-digestion strategies were applied to organic wastes in order to achieve both bioenergy  
150 (methane) and biofertilizer (digestate) production. Semi-continuous reactors were thus used both  
151 to confirm the efficiency of alkaline pretreatment on biogas production and to produce the  
152 digestates destined as fertilizers. The various digestates were characterised from a physico-  
153 chemical point of view and then tested for their efficiency on tomato growth during the first

154 vegetative stage. Finally, the correlation between dry weight of tomato plants and digestate  
155 properties was assessed through partial least square regression.

## 156 2. Materials and methods

### 157 2.1. Substrates and soil

158 Macroalgal residues (MAR) from agar extraction (*Gelidium sesquipedale*) were provided by a  
159 company located in Morocco. The MAR had a TS of 89% and a VS content of 79% (TS basis).

160 The olive pomace (OP) originated from a traditional olive oil extraction mill in the region of Beni  
161 Mellal in Morocco. It contained 97% of TS and 88% (TS basis) of VS.

162 The dried substrates were dried and grinded with a knife mill (SM 100, Retsch, Germany) using a  
163 4 mm screen size. The thickened waste activated sludge (TWAS) was collected from a  
164 wastewater treatment plant located in Narbonne-France. This sludge contained 19% TS and 78%  
165 VS (TS basis). A paper mill anaerobic sludge was used as inoculum for running the semi-  
166 continuous assay. It was characterised by a TS and VS content of 64 g/l and 45 g/l respectively.

167 Clay-rich soil used for the experiment was sampled between 10 and 30 cm depth from a farm  
168 located in the South of France. This soil was prepared as described in (Elalami et al., 2020b). It  
169 contained 1.9% organic matter, a C/N ratio of 8.9, a pH of 8.4, 0.13% total nitrogen, 0.04 g/kg  
170 total P<sub>2</sub>O<sub>5</sub>, 0.18 g/kg K<sub>2</sub>O and 0.15 g/kg MgO. The cation exchange capacity (7.8 g/kg) was  
171 measured using the Metson method (NF X 31-130).

### 172 2.2. Alkali pretreatment

173 A potassium hydroxide solution (Merck, 35% w/w) was added at a dose of 5 g KOH/100 g TS at  
174 25°C. Mixture of sludge (6.7 g wet weight) and MAR (1.42 g wet weight) or OP (1.17 g wet  
175 weight) was pretreated by adding 0.36 ml or 0.35 ml of KOH respectively. Water was then added  
176 to achieve a solid to liquid ratio of 2 g VS/100 ml and the mixture was kept under agitation at 100  
177 rpm for 2 d. Similarly, TWAS was pretreated alone to be mono-digested and to be co-digested

178 with untreated MAR or OP. For sludge mono-digestion, 13.4 g of wet sludge was pre-treated  
179 with 0.36 ml KOH at 25°C for 2 d, whereas for the co-digestion, 6.7 g of sludge was pretreated  
180 with 0.18 ml of KOH using 50 ml of water for 2 d. Finally, 1.17 g of OP or 1.42 g of MAR and  
181 50 ml of water were added. For all chemical pretreatments, the temperature, stirring conditions  
182 and the substrate/water ratio were maintained at 25°C, 100 rpm and 2 g of VS/100 ml of water  
183 respectively.

### 184 2.3. Anaerobic digestion assays

185 Continuous stirred tank reactors (CSTR) with a working volume of 2.5 l were used for the semi-  
186 continuous anaerobic digestion of untreated and alkali pretreated mixtures of MAR and OP with  
187 TWAS. The reactors were fed manually once a day, and functioned under mesophilic conditions  
188 (37°C) with a hydraulic retention time (HRT) of 20 d and an organic loading rate of 1 gVS/l.d.  
189 The homogenisation of reactors was ensured by a continuous magnetic stirring system. The  
190 anaerobic digestion lasted for about 4 HRTs (80 d), which is in agreement with the scientific  
191 consensus that requires at least 2-3 HRTs ensure the stability of the system. The various  
192 conditions investigated at CSTR reactor scales are presented in **Table 1**. The monitoring of  
193 reactor performances was carried out according to (Elalami et al., 2020b). Biogas production was  
194 measured online using Ritter milligas counters, and biogas composition was analysed by gas  
195 chromatography (GC CLARUS 480-Perkin Elmer) as described in (Sambusiti et al., 2012).

196 **Table 1** Various feeding conditions applied in CSTR reactors

### 197 2.4. Analysis

198 The APHA method (American Public Health Association) (APHA, 1998) was applied for  
199 measuring total and volatile solids . After KOH pretreatment, the solid and liquid phases of the  
200 mixtures were separated with a centrifuge (5430, Eppendorf, Germany) at 7830 rpm for 15 min.  
201 The solid fraction was then dried overnight at 105°C. The dried solid phase of the mixtures was  
202 subjected to the Van-Soest method in order to determine the contents in Neutral detergent soluble  
203 (NDS), hemicellulose (HEM), cellulose (CEL) and lignin (LIGN) (Van Soest, 1963). The term  
204 "like" was used here to refer to fractions extracted from the Van-Soest method steps, bearing in  
205 mind that sludge and MAR are not lignocellulosic. The C, H, N and S content was measured by  
206 elemental analysis using Thermo Scientific FlashSmart analyser, via flash combustion at 950°C.  
207 Digestate conductivity was measured according to the NF EN 13038. The digestate content in  
208 nutrients and in raw substrates (P, K) was determined with the same experimental protocol as  
209 described in (Elalami et al., 2020b).

## 210 2.5. Tomato growth test

211 A plant growth test was performed to validate the agronomic quality of the digestates. After trial  
212 preparations, the small pots (500 ml) were placed in a growth chamber (Fitotron, Weiss  
213 Gallenkamp, UK), according to the OECD 208 guidelines (2006) under controlled light  
214 conditions (16 hours of light (4670 LUX) and 8 hours of darkness), temperature (25°C in light  
215 period and 18°C in dark period), humidity (60% under light and 80% in the dark).

216 The application of the digestates was not only compared with unfertilized soil but also with  
217 industrial fertilizer. Thus, a dose of 150 kg N/ha was applied for both the digestate and industrial  
218 fertilizer (commercially available ammonium nitrate), while the P concentration in industrial  
219 fertilizer was 50 kg P / ha (by addition of triple superphosphate).

220 Each set of conditions contained 4 pots in which six tomato seeds were sowed. The pots were  
221 placed in the growth chamber. Every 24-48 h, the pots were watered by weighing and adding  
222 distilled water to reach the initial weight. After 70% of the control seeds germinated, three tomato  
223 seeds were left in each pot in order to allow enough space for plant growth for dry weight  
224 measurement. After 28 d, the tomato plants were harvested by cutting the stems at ground level.  
225 A certain amount of fresh plants was kept aside for chlorophyll content determination. The  
226 remaining plants were dried for 48 h at 70°C in a forced-air oven, weighed and analysed. The  
227 germination index and dry weight of the tomato plants are given in (Eq.1 and 2) (Elalami et al.,  
228 2020b).

$$229 \text{ Dry weight (gTS/100 plants)} = \frac{\text{Dry weight of harvested plants (70}^\circ\text{C)}}{\text{Number of plants}} * 100 \text{ (Eq. 1)}$$

$$230 \text{ Germination index (\%)} = \frac{\text{Final number of seeds that sprouted}}{\text{Number of initial seeds}} * 100 \text{ (Eq. 2)}$$

231 Chlorophyll refers to the green pigments present in plant chloroplasts. They have a major role in  
232 photosynthesis, which consists in the absorption of light energy in order to convert carbon  
233 dioxide and water into carbohydrates and oxygen. Chlorophyll *a* has a blue/green colour, while  
234 chlorophyll *b* is a yellow/green pigment. In addition, plants also contain orange/red-coloured  
235 carotenoids that contribute to the photosynthetic system as accessory light energy absorbers and  
236 as photo protectants of the photosynthetic apparatus.

237 The extraction of chlorophyll *a*, chlorophyll *b* and carotenoids was carried out by soaking 0.5 g  
238 of fresh biomass in 10 ml methanol (98%) for 5 min. The solution was then centrifuged at 10000  
239 rpm for 15 min (Hettich Zentrifugen Rotanta 460). 0.5 ml of the clarified solution was sampled  
240 and diluted in 4.5 ml methanol (98%). The solution was then analysed with a UV–VIS

241 spectrophotometer (Jenway 7315) to determine the absorbance at 470, 652 and 665 nm. These  
242 pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were quantified using the following  
243 equations (**Eq.3, 4 and 5**) (Lichtenthaler, 1987):

$$244 \quad \text{Chlorophyll a } (\mu\text{g/gTS}) = \frac{16.7A_{665} - 9.16A_{652}}{m_{\text{biomass}}} \times 10 \quad (\text{Eq. 3})$$

$$245 \quad \text{Chlorophyll b } (\mu\text{g/gTS}) = \frac{30.09A_{652} - 15.3A_{665}}{m_{\text{biomass}}} \times 10 \quad (\text{Eq. 4})$$

$$246 \quad \text{Carotenoids } (\mu\text{g/gTS}) = \frac{(1000A_{470} - 1.63Ca - 104.9Cb)}{221m_{\text{biomass}}} \times 10 \quad (\text{Eq. 5})$$

## 247 2.6. Statistical analysis

248 A t-test was applied to evaluate the significance of the results obtained ( $p < 0.05$ ). Partial least  
249 squares (PLS) regression is a statistical method for determining the linear relationship between  
250 two matrices X (digestate properties) and Y (dry weight of tomato plants). The PLS of  
251 experimental data was performed using SIMCA from UMETRICS. A correlation coefficient  $R^2$   
252 was computed to assess the statistical relationship between the two variables. In addition, the root  
253 mean square error of estimation (RMSEE) represents the distance between the observed Y  
254 variable and the predicted Y variable. The cross-validated coefficient ( $Q^2$ ) and root mean square  
255 error of cross validation ( $RMSE_{CV}$ ) generated from an internal method used by SIMCA to define  
256 the accuracy of the prediction were used for validating the model.

## 257 **3. Results and discussion**

### 258 3.1. Elemental analysis of feedstocks

259 First, the composition of the substrates was assessed in order to estimate the benefits in their pre-  
260 treatment and anaerobic digestion. The composition of the substrates used in this study is shown

261 in **Table 2**. The phosphorous content was highest in sludge relative to the other substrates, while  
262 more potassium was found in olive pomace. The C to N ratio was highest in olive pomace,  
263 mainly due to its high lipid and lignin content (Elalami et al., 2018). Conversely, sludge  
264 presented the lowest C/N ratio which explains why its co-digestion with other substrates that  
265 have a better C/N is more favourable. The optimal C to N ratio for anaerobic digestion is within  
266 the 25-30 range (Appels et al., 2008). However, in this study, all mixtures had lower C to N  
267 ratios. Anaerobic digestion also depends on the biodegradability of the substrates. Therefore, pre-  
268 treatment was applied to increase the biodegradability of the mixtures.

269 **Table 2** Composition of the substrates (sludge, olive pomace and macroalgal residues).

### 270 3.2. Impact of pretreatment on Van-Soest fractions

271 Van-Soest fibres in the raw and pretreated substrates as well as in their mixtures are presented in  
272 **Fig.1**. The KOH pretreatment (5% TS basis, at 25°C for 2 d) significantly reduced the  
273 hemicellulose-like and cellulose-like fractions contained in TWAS by 70% and 86% respectively,  
274 while increasing the easily accessible fractions such as NDS (+163%). This observation concurs  
275 with previous studies (Chen et al., 2020; Heo et al., 2003). For example, NaOH pretreatment (10  
276 M added to reach a pH of 12) was found to significantly increase soluble COD, soluble proteins,  
277 sugars and volatile fatty acids in waste activated sludge (Chen et al., 2020).

278 Moreover, the NDS, lignin-, cellulose- and hemicellulose-like composition of the mixtures did  
279 not significantly differ from the calculated composition which was based on the sum of the  
280 different fractions from the two separate substrates forming each mixture. In the mixture of  
281 pretreated sludge and OP, the hemicellulose content fell by 32%, while lignin-like fractions  
282 dropped by 28% and NDS rose by 45%. Similarly, a pretreatment of the whole mixture obviously

283 seemed to be more efficient in degrading the most recalcitrant materials contained in both TWAS  
284 and OP. The lignin-like content decreased by 53% relative to the untreated TWAS and OP  
285 mixture, while the hemicellulose-like fraction fell by 63% and zero effect was observed on the  
286 cellulose-like fraction. Conversely, the NDS increased strongly after KOH pretreatments (+91%).  
287 The pretreatment of sludge alone before MAR addition did not have any significant effect on  
288 lignin-like and cellulose-like fractions relative to an untreated mixture. However, the strongest  
289 effect of alkaline pretreatment could be observed on the whole sludge and MAR mixture, where  
290 lignin- and hemicellulose-like fractions dropped by 73% and 64% respectively, while the NDS  
291 increased by 71%.

292 **Fig.1** Van-Soest fractions in the substrates, their mixtures, pretreated substrates and mixtures. OP (Olive  
293 pomace), MAR (Macroalgae residues), TWAS (Thickened waste activated sludge).

294 The alkaline pretreatment effect on lignocellulosic biomass such as olive pomace has already  
295 been reported in literature. Alkaline pretreatment aims at reducing the lignin content through the  
296 cleavage of ester bonds in lignin/phenolic-carbohydrate complexes (Taylor et al., 2011), thus  
297 explaining the significant impact of pretreatment of a whole sludge and olive pomace mixture on  
298 the solubilisation and lignocellulosic matrix degradation.

299 It is also noteworthy that neither sludge nor MAR are lignocellulosic matrices. These analysed  
300 fractions are only lignocellulose-like fractions that acted as indicators to quantify the impact of  
301 pretreatment on organic matter. However, it was obvious that the pre-treatment of sludge alone  
302 reduced the hemicellulose-like fraction and enhanced NDS, while the pretreatment of whole  
303 mixtures also affected lignin- and cellulose-like fractions. This may be related to the amount of  
304 KOH added.

### 3.3. Anaerobic digesters performance

#### **3.3.1. Methane production**

The reactor performances for the different scenarios are presented in **Table 3**. Over a total operating time of 80 d, all reactors were stable by the 50<sup>th</sup> d (data not shown). According to reactor performance, co-digestion of sludge with OP (R1) and MAR (R4) resulted in increased methane specific production by 75% and 72% respectively relative to R1S, while for co-substrates, co-digestion improved ammonium concentrations and FOS/TAC (Volatile organic acids to total alkalinity ratio). Furthermore, the TS and VS removal increased due to co-digestion of sludge with OP and MAR. This was related to a higher methane production in the co-digesters.

The KOH pretreated sludge (R2S) produced 39% more methane compared to untreated sludge (R1S). The pretreatment of sludge alone and of a whole sludge and OP mixture both showed a similar methane production. Indeed, R2 and R3 produced 13 % and 15 % higher methane volumes compared to R1. This finding might result from an inhibition effect occurring within the R3, probably due to the release of phenolic compounds after delignification of OP by KOH pretreatment, while the R2 functioned normally. For this reason, in similar studies, it is recommended to dose polyphenols in digesters fed with a chemically pre-treated substrate, such as OP, that is rich in lignin. Pellerá et al. (2016) observed that the application of a NaOH pretreatment to OP, led to a linear increase in total polyphenol concentrations along with the dose of NaOH, regardless of temperature. they reached 5 mg gallic acid equivalent/gVS at a dose of 1 mmole NaOH/gVS at 25°C for 16 h (Pellerá et al., 2016). Note that a phenol concentration of 1.5 g/l should not be exceeded in order to maintain a proper methanogen activity (Monlau et al., 2014).

327 Hence, alkali pretreatment, applied to sludge only seems to be a more effective and  
328 environmentally friendly strategy rather than pretreating a whole mixture of TWAS and OP  
329 which requires more KOH addition to the system. Previously, Alagöz et al. (2015) reported that  
330 ultrasonic pretreatment applied to waste activated sludge increased the methane potential by 24%  
331 during co-digestion with olive pomace, although this was not compared with ultrasonic  
332 pretreatment of the whole mixture (TWAS and OP ) (Alagöz et al., 2015). The highest methane  
333 production was achieved in R6 (fed with alkali pretreated mixture of TWAS and MAR). With a  
334 methane yield of 281 Nml CH<sub>4</sub>/gVS, the KOH pretreatment enhanced the methane production by  
335 49% relative to the raw mixture (R4). On the contrary, the R5 (fed with the mixture of pretreated  
336 sludge and macroalgal residues) did not result in any significant enhancement when compared  
337 with the raw mixture.

338 **Table 3** Semi-continuous reactors performance; R1S (untreated sludge), R2S (pretreated sludge), R1  
339 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and  
340 olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6  
341 (pretreated mixture of sludge and macroalgal residue).

342 Some previous studies on sludge, OP and macroalgal residue pretreatments, are presented in  
343 **Table 4**. In general, alkali pre-treatments are more effective on lignocellulosic residues (Pellera  
344 et al., 2016; Peng et al., 2019; Thomas et al., 2019), since they alter the lignin structure (Cheah et  
345 al., 2020).

346 As reported in (Ruiz-Hernando et al., 2014; Wang et al., 2018), alkaline reagent can increase  
347 methane production from sludge up to 34%. Results from the present study agree with this  
348 observation. In addition, coupling alkaline pre-treatment with heat has further improved sludge  
349 biodegradability and consequently the amount of methane produced. Thermal pre-treatment for 6

350 hours at 80°C prior to NaOH addition resulted in a 172% improvement in methane production  
351 (Zou et al., 2020). For OP, thermal pre-treatment with steam explosion led to a 49% reduction in  
352 hemicelluloses, resulting in higher solubilisation and, in turn, increased methane production in  
353 the liquid fraction of the pre-treated OP. Nevertheless, alkaline or thermo-alkaline pre-treatment  
354 effectively reduce the lignin content of the OP, thus leading to a 17 % and 23% increase in  
355 produced methane, as observed by (Elalami et al., 2020a) and (Pellera et al., 2016), respectively.  
356 Alagöz et al. (2015) also reported that microwave or US pre-treatment of sludge prior to co-  
357 digestion with OP increased methane production by 38% and 44%, respectively (Alagöz et al.,  
358 2015). Results from the present study do not achieve this same level of improvement, therefore  
359 implying that sludge solubilisation can be enhanced with heating.

360 As for macro-algae, studies have been carried out on different species of seaweed but never on  
361 residues of already industrially exploited macroalgae. In the present study, 49% more methane  
362 resulted from KOH pre-treatment of the sludge and MAR mixture. However, to the authors'  
363 knowledge, no studies yet discuss pre-treatment of sludge mixtures and macroalgal residues.  
364 Generally, thermal or chemical pre-treatments of macroalgae biomass have previously been  
365 reported with varying improvement rates ranging between 4 and 26% (Ding et al., 2020; Elalami  
366 et al., 2020b; Jard et al., 2013; Vanegas et al., 2015).

367 The impact of pre-treatment on methane production thus essentially depends on the nature of the  
368 substrate used and on its composition in addition to pre-treatment and AD conditions. For this  
369 reason, the concept of exergy, has been proposed for comparing different pretreatments  
370 (Soltanian et al., 2020). However, future works on exergy and the economic aspects of alkaline  
371 pre-treatment still need to be considered.

372 **Table 4** Comparison between the results from the present study and other sludge, olive pomace and  
373 macroalgal biomass pretreatment assessments.

374

### 375 **3.3.2. Other parameters**

376 The buffer capacity of reactors containing KOH-pretreated substrate was found to be  
377 significantly higher than the buffer capacity of reactors containing untreated substrate. Sambusiti  
378 et al. (2013) reported that the FOS/TAC ratio should ideally remain lower than 0.3 to avoid  
379 inhibition and further acidification of the system (Sambusiti et al., 2013). Here, no inhibition was  
380 observed and the reactors exhibited FOS/TAC ratio values ranging between 0.19 and 0.39. The  
381 maximum FOS/TAC value (0.39) was achieved in R2. The FOS/TAC ratio fell during the AD  
382 experiment, reaching values between 0.13 and 0.24 at steady state. The highest VFA  
383 concentrations were found in R2S (0.37 g eq acetic acid/l which is equivalent to 6.2 mol/m<sup>3</sup>).  
384 This is lower than the inhibitory concentration of 9 mole/m<sup>3</sup> reported in the literature (Appels et  
385 al., 2008).

386 Furthermore, ammonium concentrations ranged between 217 mg/l and 600 mg/l, which lies  
387 within the recommended range for anaerobic microorganisms (200-1500 mg/l) (Rajagopal et al.,  
388 2013). Maximal ammonium concentrations were obtained in KOH pretreated reactors. These  
389 were at 600, 510 and 568 mg/l in R2S, R3 and R6 respectively. Although such increases in NH<sub>4</sub><sup>+</sup>  
390 concentrations may result from the degradation of proteins during KOH pretreatment, ammonium  
391 concentrations decreased towards the end of the AD process, tending to stabilize at 411, 237 and  
392 278 mg/l for R2S, R3 and R6 respectively. High ammonium concentrations contributed to an  
393 increase in alkalinity, which in turn ensures an optimal level of pH between 6.5 and 7.8. Despite  
394 the fact that the pH at the inlet was high (up to 12.8) for pretreated substrates which had not been

395 neutralized, the reactors still worked correctly and digestate pH fell within 6.9 and 7.4 at steady  
396 state. In addition, KOH pretreatment resulted in the enhancement of VS removal, ranging  
397 between 4% and 39%. This result concurs with the study by Monlau et al. (2015) who achieved a  
398 VS removal enhancement of 20% following NaOH pretreatment of sunflowers (1 mmole/gTS,  
399 55°C for 24 h).

400 Although pretreatment conditions were not identical, the KOH concentration (0.9 mmol/gTS)  
401 was similar to that of NaOH used in (Monlau et al., 2015). However, for a weaker lime  
402 concentration (2.8 g/100 gTS of organic fraction of municipal solid waste, room temperature and  
403 6 h), the VS removal increased by 21% (Torres and Lloréns, 2008). The effectiveness of a  
404 pretreatment therefore also depends on the reagent used, on the liquid-solid ratio and on the  
405 studied substrate.

406 To conclude, co-digestion of sludge with OP and MAR led to in an improvement in methane  
407 production relative to sludge alone. This is related to the methane potential of the co-substrates  
408 which is higher than that of sludge. In addition, alkaline pretreatment had the strongest impact on  
409 reactor performance. In particular, co-digestion coupled with pretreatment clearly improved  
410 methane production, digestate ammonium concentrations and VS removal. On the whole, an  
411 economical assessment and optimisation remains necessary, involving both costs and benefits of  
412 biogas production and use of digestate. As examples, (Aghbashlo et al., 2019; Tabatabaei et al.,  
413 2020) have reported exergy based economic analyses.

#### 414 3.4. Digestates properties

415 Digestate properties are summarized in **Table 5**. Comparison between digestates produced from  
416 sludge mono and co-digestion pointed out that co-digestion residues are richer in organic matter  
417 (and carbon) which, in turn, should improve the amending value of sludge digestate.

418 KOH pretreatment reduced the TS and VS content in digestates. This is mainly due to methane  
419 improvement and to the consequent decrease in C and H concentrations in digestates.  
420 Nonetheless, regarding the fibre content, lignin, hemicelluloses and cellulose-like fractions  
421 seemed to decrease after AD. Fibre removal efficiency was strongly related to the nature of the  
422 co-substrate as well as its initial fibre composition. Indeed, the lignin-like fraction decreased in  
423 the R2S digestate, while the hemicellulose-like fraction dropped in the R3. The cellulose-like  
424 fraction in the R6 digestate decreased in comparison with the R4 digestate.

425 Regarding the nutrient profile, KOH pretreatment increased the conductivity level in R2S, R2,  
426 R3, R5 and R6 up to 1370  $\mu\text{S}/\text{cm}$ , probably because of the high potassium content in all the  
427 digestates following KOH pretreatment. This result agrees with (Elalami et al., 2020b), who  
428 observed how the KOH pre-treatment of MAR (5% at 25°C for 2 d) also improved conductivity  
429 and reduced the digestate organic matter content. Jaffar et al. (2015) reported that a KOH  
430 pretreatment (6% TS, room temperature for 3 d) enhanced the total nitrogen, phosphorus and  
431 potassium contents of digestate by 9% ,7% and 138% respectively, in comparison with untreated  
432 wheat straw digestate (Jaffar et al., 2016). Nevertheless, in the current study, the total  
433 phosphorous concentration did not appear to be significantly affected by KOH pretreatment, with  
434 values ranging between 61.6 and 80.8 mg  $\text{P}_2\text{O}_5/\text{kg}$  TS for sludge mono-digestion, 43.5-60 mg  
435  $\text{P}_2\text{O}_5/\text{kg}$  TS and 42.1-54 mg  $\text{P}_2\text{O}_5/\text{kg}$  TS for sludge co-digestion with OP and MAR respectively.  
436 While the potassium content in digestate from untreated substrates varied between 9.4 and 24.6 g

437 K<sub>2</sub>O/kg TS, potassium concentrations varied between 65.2 and 194 g K<sub>2</sub>O /kg in digestate issued  
438 from sludge or pretreated mixtures.

439 The ammonium content increased due to higher protein degradation following KOH  
440 pretreatment. This finding agrees with the study by Zou et al. (2020) in which a thermoalkaline  
441 pretreatment (80°C for 6 h then mixture of NaOH and Ca(OH)<sub>2</sub> at pH= 12 and 25°C for 24 h)  
442 increased ammonia levels by 140% at the end of the AD process, when compared with sludge  
443 alone (Zou et al., 2020). Similarly, thermoalkaline pretreatment (140°C, 60 meq NaOH/l for 1 h)  
444 tripled the ammonium concentrations in a sludge and food-waste mixture (Lee et al., 2019).

445 In addition, total nitrogen and sulfur concentrations slightly fell in digestates issued from KOH  
446 pretreated substrates. However, as a CHNS analysis was performed on dried digestate, the  
447 volatile forms of sulfur did not contribute to the measured value. This was also the case for  
448 nitrogen; indeed, the N and total Kjeldahl nitrogen (TKN) differences could be essentially  
449 attributed to the volatilization of NH<sub>3</sub> due to digestate drying prior to elemental analysis.

450 To conclude, when compared to mono-digestion of sludge, co-digestion significantly improved  
451 methane production and increased the organic matter content of the digestate. Concurrently,  
452 when compared to untreated substrate digestate, alkaline pretreatment also improved methane  
453 production and enriched the digestate with ammonium.

454 **Table 5** Properties of tomato plants grown on unfertilized soil (control) and with industrial fertilizer or  
455 different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2  
456 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4  
457 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of  
458 sludge and macroalgal residue).

459 3.5. Efficiency of the various digestates on tomato plant growth

460 **3.5.1. Effect on germination and dry weight of tomato plants**

461 The results of tomato growth tests on soil alone, with industrial fertilizer and after digestate  
462 application are summarized in **Fig.2**. Note that the germination index was not significantly  
463 impacted by digestate quality.

464 This finding concurs with Solé-Bundo et al. (2017) who reported that diluted digestates from  
465 untreated, pretreated microalgae and microalgae co-digested with sludge did not present any  
466 significant effect on the germination index of cress seeds. However, at high digestate  
467 concentrations (10%), co-digestion proved to be more effective in maintaining a maximal  
468 germination index while mono-digestion residues reduced germination by 40% (Solé-Bundó et  
469 al., 2017). Similarly, Albuquerque et al. (2012) observed that the germination index of both  
470 cress and lettuce improved when pig slurry digestate was applied at a concentration of 1%  
471 (Albuquerque et al., 2012). Such a dilution avoids phytotoxicity issues. However, in the present  
472 study, digestate dilution was not required, probably due to their low TS, which could be related to  
473 the low OLR applied to the digester. This implies that the content in phytotoxic compounds was  
474 low.

475 **Fig.2** Germination index (a) and dry weight (b) of tomato plants grown on unfertilized soil (control) and  
476 soil fertilized with industrial fertilizer and different digestates. R1S (untreated sludge), R2S (pretreated  
477 sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of  
478 sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal  
479 residues), R6 (pretreated mixture of sludge and macroalgal residues).

480 On the contrary, the dry weight of tomato plants, after digestate addition, increased under all  
481 conditions. Regarding R1S, it rose by 87% compared to unfertilized soil, quite similarly to the  
482 effect of industrial fertilizer addition. However, the addition of R2S did not result in a higher dry

483 weight result compared to R1S. Similarly, R2, R3 and R5, R6 digestates did not show a  
484 significant difference in dry weight compared to R1 and R3 digestates, suggesting that KOH  
485 pretreatment effect was not significant. By comparing co-digestion with sludge mono-digestion  
486 residues, sludge digestate was found to be more beneficial for plant growth, probably thanks to  
487 its high phosphorous content, especially in the case of co-digestion with OP. Previously, Solé-  
488 Bundo et al. (2017) reported that sludge and microalgae co-digestion residues were less  
489 phytotoxic than microalgae digestate, thus suggesting that the addition of sludge enhanced the  
490 agronomic value of the digestate (Solé-Bundó et al., 2017). In addition, thermally pretreated  
491 microalgae (75°C for 10 h) digestate did not show any impact on cress growth. However, in the  
492 case of OP digestates, the presence of phenolic compounds may explain the decrease in dry  
493 weight of tomato plants, in comparison with MAR co-digestion residues. Besides, orange waste  
494 digestate was previously found to strongly reduce the germination rate of ryegrass (-92%) as well  
495 as its dry weight (-50%) (Kaparaju et al., 2012). Similarly, the addition of digestate from the co-  
496 digestion of olive waste and citrus pulp resulted in a significant decrease in the germination (-  
497 90%) of cucumber growth (Panuccio et al., 2019).

### 498 **3.5.2. Effect on tomato plant properties**

499 The properties of tomato plants, cultivated on the various digestates produced, are provided in  
500 **Table 6.** Co-digestion of sludge and OP residues enhanced the C, H, N and pigment contents in  
501 comparison with unfertilized soil. Nevertheless, chlorophyll *b* was unaffected neither by digestate  
502 nor by industrial fertilizer addition, while the carotenoid content increased following the addition  
503 of industrial fertilizer or of each type of digestate (up to +72%). In addition, the R1 digestate  
504 increased the chlorophyll *a* concentration by 8% compared to industrial fertilizer, while the other

505 digestates presented similar or even lower pigment contents, as was the case for the R5 and R6  
506 digestates. This is probably related to a decrease in the absorption of nutrients required for  
507 chlorophyll *a* production within the plant. This effect can occur if metal concentrations in the  
508 digestate are too high. It is therefore recommended to carry out metal analysis of the digestate  
509 before its application, especially for digestate containing macroalgae. Indeed, macroalgae are  
510 known to absorb and concentrate metals from a contaminated environment (Wang and Dei,  
511 1999). Furthermore, raw sludge digestate proved to be more profitable for enhancing tomato  
512 plant properties in comparison with pretreated sludge digestate. Indeed, the pretreated sludge  
513 digestate contained less organic matter than untreated sludge digestate.

514 Reports on the impact of digestate properties on plant composition are scarce in the literature  
515 (Albuquerque et al., 2012; Ronga et al., 2018; Y. Wang et al., 2018). Cow manure digestate is  
516 known to enrich soil with nutrients, mainly N, P and K, which in turn improves the nutrient  
517 content in watermelon fruit (Albuquerque et al., 2012). Similarly, liquid digestate from pig  
518 manure can enhance the sugar and protein content in maize plants by 10 % and 12% respectively  
519 (Y. Wang et al., 2018) while another digestate from agricultural wastes has been observed to  
520 enhance the nitrogen, potassium and phosphorous content in alfalfa leaves by 18%, 17% and 7%  
521 relative to industrial fertilizer (Koszel and Lorencowicz, 2015). In contrast, Sortino et al. (2014)  
522 did not observe any effect of urban bio-waste digestate on the C and N levels of harvested tomato  
523 plants (Sortino et al., 2014). Moreover, Ronga et al. (2018) found that co-digestion residues from  
524 maize silage, triticale silage, cow slurry and grape stalks led to a decrease in the aromatic  
525 compounds of peppermint and basil plants (Ronga et al., 2018). The effect of a digestate  
526 therefore depends on the substrate composition, on AD conditions and on the type of pre-  
527 treatment or co-digestion, in addition to plant and soil properties.



552 on the dry mass of the plants. These results agree with previous studies where the nitrogen and  
553 phosphorus contents were found to be growth-limiting elements, especially for the very early  
554 stages of growth (Cooke et al., 2005; Malhotra et al., 2018).

555 In addition, Razaq et al. (2017) reported that both nitrogen and phosphorus affected plant height,  
556 root morphology and chlorophyll content (Razaq et al., 2017). In contrast, Iocoli et al. (2019)  
557 found that lettuce dry weight was highly related to ammonium nitrogen. They also reported that  
558 organic nitrogen is associated with recalcitrant organic structures which are not readily available  
559 for plants (Iocoli et al., 2019). However, these results still need to be confirmed on long term  
560 assays in field conditions, since results can be strongly affected by the availability of elements,  
561 the soil texture, the climate conditions and the type of plant tested.

562 **Fig.3** PLS results linking dry weight of tomato plants to digestate characteristics, a) centred and scaled  
563 coefficients, b) predicted versus observed dry weight of tomato plants.

#### 564 **4. Conclusions and future prospects**

565 Co-digestion improves methane production relative to sludge mono-digestion and both types of  
566 digestate virtually share the same degree of impact on plant dry weight. This suggests that co-  
567 digestion can improve methane production and ensure a P and N supply, since these nutrients  
568 correlate strongly with plant dry weight. Alkaline pre-treatment has shown to improve methane  
569 production, although it does not seem to affect the growth of tomato plants. Digestate application  
570 increased chlorophyll *a* and carotenoid concentrations in tomato plants. However, the alkaline  
571 pretreatment of MAR mixtures led to a fall in concentrations of these pigments in tomato plants.  
572 In future studies, the impact of digestate application on heavy metals in soil and plants should be  
573 investigated over long-term field trials. In addition, the sustainability of integrating pretreatment

574 and co-digestion strategies will have to be addressed for pilot scales including the analysis of  
575 economic, environmental and societal aspects.

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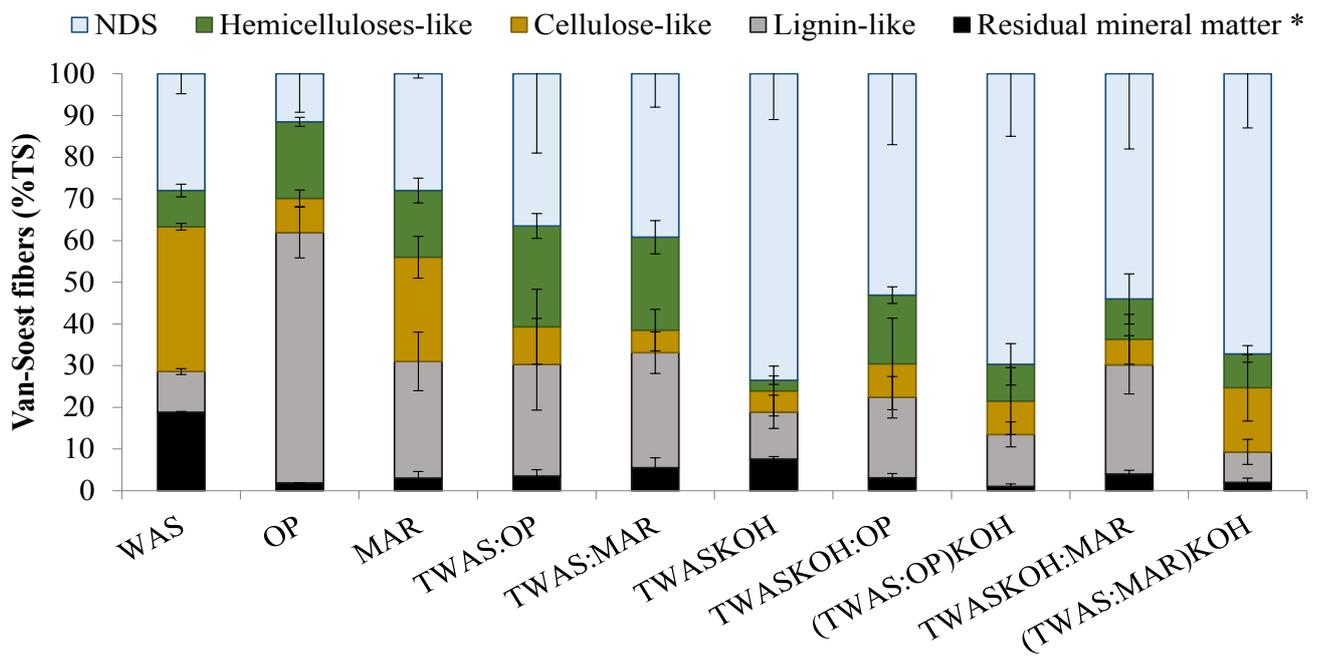
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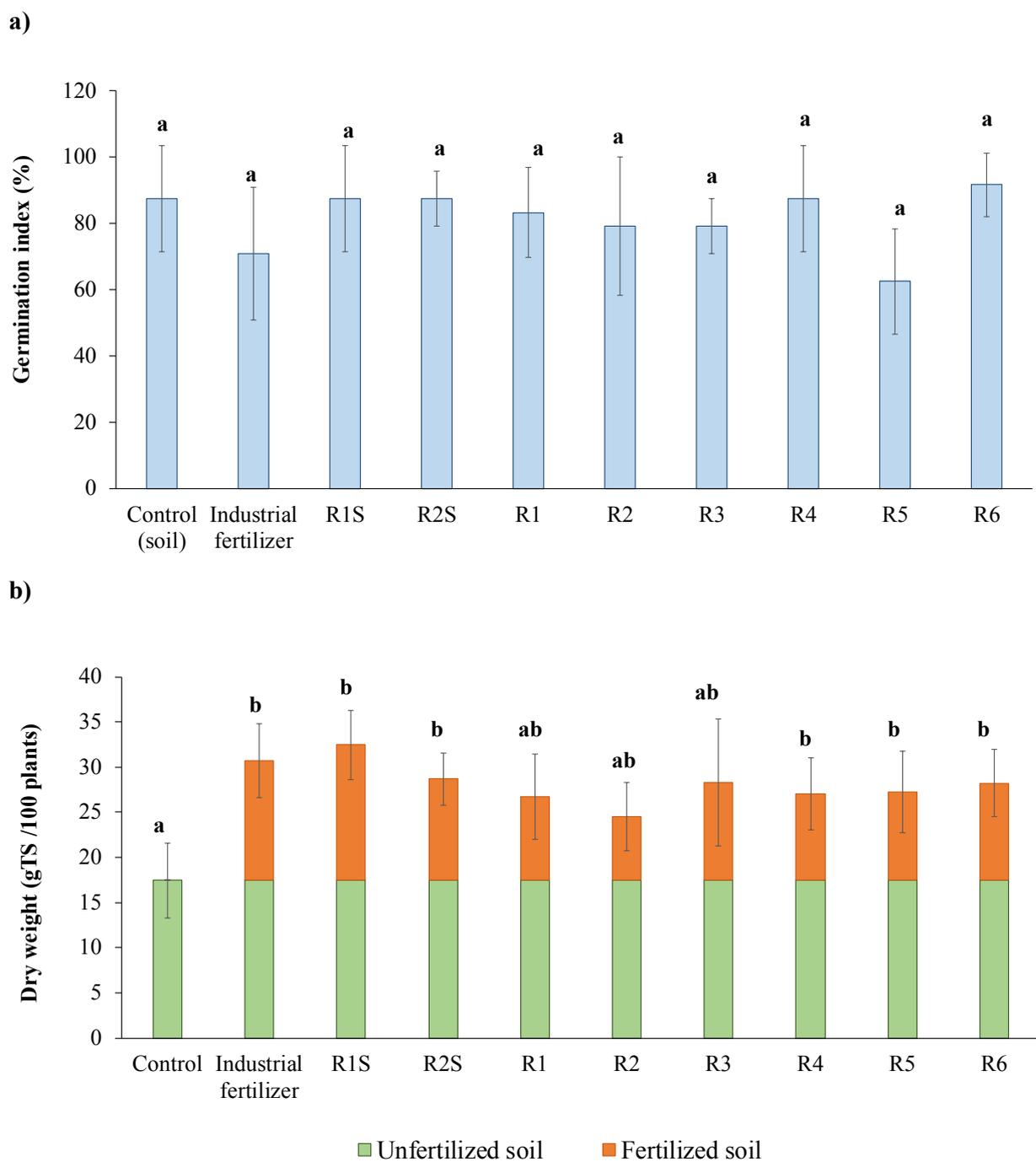
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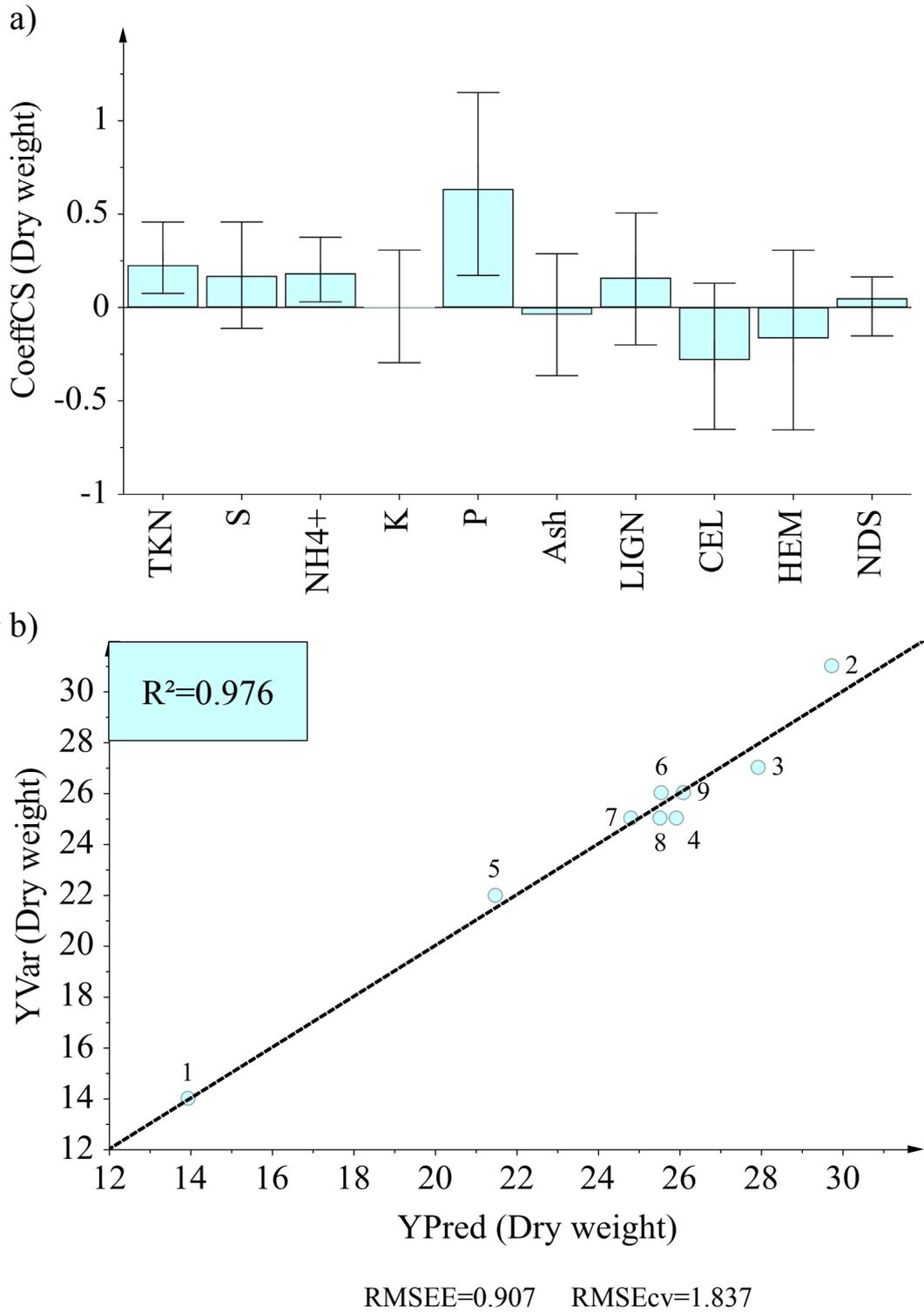


\*After burning the "lignin-like" fraction.

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**Fig.3** PLS results linking dry weight of tomato plants to digestate characteristics, a) centred and scaled coefficients, b) predicted versus observed dry weight of tomato plants.

**Table 1** Various feeding conditions applied in CSTR reactors.

	Reactors	Feedstock		
		OP (% VS)	TWAS (% VS)	MAR (% VS)
Sludge mono-digestion	R1S-TWAS	0	100	0
	R2S-TWASKOH	0	100 + KOH*	0
Co-digestion	R1-TWAS:OP	50	50	0
	R2-TWASKOH:OP	50	50 + KOH*	0
	R3-(TWAS:OP)KOH	50+ KOH*	50+ KOH*	0
	R4- TWAS:MAR	0	50	50
	R5-TWASKOH:MAR	0	50 + KOH*	50
	R6-(TWAS:MAR)KOH	0	50+ KOH*	50+ KOH*

\*Pretreatment conditions: 5% TS, 25°C for 2 d. OP (Olive pomace), MAR (Macroalgae residues), TWAS (thickened waste activated sludge).

**Table 2** Composition of the substrates (sludge, olive pomace and macroalgal residues).

<b>Substrates</b>	<b>OP</b>	<b>TWAS:OP</b>	<b>TWAS</b>	<b>TWAS:MAR</b>	<b>MAR</b>
C (% TS)	52±0.2	-	38.3±0.6	-	38.8±0.2
H (% TS)	7.2±0.3	-	5.68±0.05	-	6.1±0.3
N (% TS)	1.0±0.2	-	6.13±0.06	-	4.0±0.2
S (% TS)	0.10±0.01	-	1.12±0.03	-	0.65±0.01
C/N	52.0	12.0	6.7	7.6	9.7
P (g /kg TS)	10.1±0.4	-	39.2±0.4	-	11.5±0.3
K (g /kg TS)	65±4	-	37.3±0.1	-	3.6±0.4
Lipids (%)	16.4±0.8	-	-	-	-

OP (Olive pomace), MAR (macroalgal residues), TWAS (thickened waste activated sludge).

**Table 3** Semi-continuous reactors performance; R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

Reactors	pH <sub>in</sub>	pH at steady state	Methane (Nml /gV <sub>Sin</sub> )	Methane enhancement (% untreated)	TS removal (%TS <sub>in</sub> )	VS removal (%VS <sub>in</sub> )	Max VFA (g eq acetic acid/l)	FOS/TAC		NH <sub>4</sub> <sup>+</sup> (mg/l)		
								Max	Steady state	Max	Steady state	
<b>MonoD</b>	<b>R1S</b>	7	6.9	109±5	-	20	28	0.02	0.19	0.13	254	254
	<b>R2S</b>	9	7.5	152±14	39	29	39	0.37	0.29	0.21	600	411
<b>AcoD</b>	<b>R1</b>	6.4	7.2	191±10	-	30	37	0.05	0.23	0.21	217	178
	<b>R2</b>	7.2	7.1	215±24	13	32	42	0.18	0.39	0.24	428	217
	<b>R3</b>	12.6	7.2	220±16	15	34	43	0.31	0.31	0.16	510	237
	<b>R4</b>	7.1	7.2	188±19	-	40	46	0.15	0.19	0.14	239	201
	<b>R5</b>	7.3	7.3	194±28	3	38	48	0.3	0.33	0.22	468	257
	<b>R6</b>	12.8	7.4	281±25	49	45	58	0.04	0.32	0.16	568	278

MonoD (mono-digestion), AcoD (anaerobic co-digestion), TS (total solids), VS (volatile solids), VFA (volatile fatty acids), FOS (volatile organic acids), TAC (total alkalinity).

**Table 4** Comparison between the results from the present study and other sludge, olive pomace and macroalgal biomass pretreatment assessments.

Substrates	Pretreatment	Conditions	Methane enhancement	Ref
Waste activated sludge	Thermoalkaline	80°C for 6h, then a mixture of NaOH and Ca(OH) <sub>2</sub> was added (at a ratio of NaOH: Ca(OH) <sub>2</sub> =4:1)	+172%	(Zou et al., 2020)
Waste activated sludge	Alkaline	157 g NaOH/kg TS at 25°C for 24 h. Biomethane potential test (BMP) at 37°C for 35 d.	+34%	(Ruiz-Hernando et al., 2014)
Waste activated sludge	Alkaline	NaOH (pH=8) for 6 d at 35°C. BMP at 35°C for 56 d.	+30%	(Wang et al., 2018)
Olive pomace	Steam explosion	200°C for 5 min and 1.57 MPa. BMP at 35°C for 23 d.	<p><b>Untreated OP:</b> 366 ml/g VS.</p> <p><b>Liquid fraction of OP:</b> 589 ml/g VS.</p> <p><b>Solid fraction of OP:</b> 263 ml/g VS.</p>	(Rincón et al., 2016)
Olive pomace	Thermoalkaline	NaOH (1 mmol/gVS) for 4h at 90°C Biomethane potential test (BMP) at 35°C for 50 d.	+23%	(Pellera et al., 2016)
Olive pomace	Alkaline	NaOH (4%) for 2 d and 25°C BMP at 35°C for 30 d.	+17%	(Elalami et al., 2020a)
Waste activated sludge and olive pomace (1:1 (g/g))	Ultrasonic	200W for 30 min <b>on sludge alone.</b> batch-fed anaerobic reactors at 37 °C for 30 days.	+23%	(Alagöz et al., 2015)
Waste activated sludge and olive pomace (1:1 (g/g))	Microwaves	For 30 min at 175 °C and 2000 kPa <b>on sludge alone.</b> batch-fed anaerobic reactors at 37 °C for 30 days.	+44%	(Alagöz et al., 2015)
Brown macroalga “ <i>Laminaria digitate</i> ”	Thermal	140 °C for 20 min Two-stage anaerobic digestion (batch dark fermentation at 35°C for 3 d, then batch AD at 35°C for 21 d).	+26%	(Ding et al., 2020)
Red macroalga “ <i>Palmaria palmata</i> ”	Alkaline	NaOH (4% TS) at 20°C for 24 h. BMP for 35°C.	+18%	(Jard et al., 2013)
Brown macroalga “ <i>Laminaria digitate</i> ”	Acid	2.5% citric acid 120 °C; 1 h; 1 atm BMP at 35°C for 32 d.	+4%	(Vanegas et al., 2015)
Red macroalgal residues	Alkaline	KOH (5%) for 2 d and 25°C AD CSTR: HRT of 20 d at 37°C.	+20%	(Elalami et al., 2020b)
Sewage sludge	Alkaline	5% of KOH for 2 d and 25°C AD CSTR: HRT of 20 d at 37°C.	+39%	
Waste activated sludge and olive pomace	Alkaline	5% of KOH for 2 d and 25°C <b>on sludge alone.</b> Codigestion ratio 1:1 (VS) AD CSTR: HRT of 20 d at 37°C.	+13%	In this study
Waste activated sludge and olive pomace	Alkaline	5% of KOH for 2 d and 25°C Codigestion ratio 1:1 (VS)	+15%	
Waste activated sludge and red macroalgal residues	Alkaline	AD CSTR : HRT of 20 d at 37°C.	+49%	

**Table 5** Properties of tomato plants grown on unfertilized soil (control) and with industrial fertilizer or different digestates. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

	R1S untreated TWAS	R2S TWASKOH	R1 TWAS:OP	R2 TWASKOH:OP	R3 (TWAS:OP)KOH	R4 TWAS:MAR	R5 TWASKOH:MAR	R6 (TWAS:MAR)KOH
Conductivity ( $\mu\text{S}/\text{cm}$ )	645 $\pm$ 1	1370 $\pm$ 30	412 $\pm$ 3	870 $\pm$ 22	1070 $\pm$ 35	482 $\pm$ 26	684 $\pm$ 26	921 $\pm$ 28
pH	7.8 $\pm$ 0.1	8.3 $\pm$ 0.5	7.5 $\pm$ 0.1	8.1 $\pm$ 0.3	8.2 $\pm$ 0.4	7.6 $\pm$ 0.0	8.3 $\pm$ 0.2	8.3 $\pm$ 0.2
TS (%)	1.2 $\pm$ 0.0	1.1 $\pm$ 0.02	1.2 $\pm$ 0.4	1.3 $\pm$ 0.03	0.6 $\pm$ 0.01	1.0 $\pm$ 0.0	0.9 $\pm$ 0.01	1.1 $\pm$ 0.02
VS (%TS)	70.7 $\pm$ 0.1	51 $\pm$ 1.2	73.2 $\pm$ 0.4	62 $\pm$ 1.4	44.5 $\pm$ 1.1	74.8 $\pm$ 0.1	56.8 $\pm$ 0.8	53.8 $\pm$ 0.9
Ash (%TS)	29.3	49	26.8	38	55.5	25.2	43.2	46.2
<b>Elemental analysis* (% TS)</b>								
C	34.7 $\pm$ 0.1	29.06 $\pm$ 0.03	38.7 $\pm$ 0.2	34.2 $\pm$ 0.1	35.7 $\pm$ 0.1	37.2 $\pm$ 0.2	33.63 $\pm$ 0.03	34.5 $\pm$ 0.2
H	6.0 $\pm$ 0.2	4.17 $\pm$ 0.03	5.7 $\pm$ 0.3	4.58 $\pm$ 0.01	5.3 $\pm$ 0.2	5.2 $\pm$ 0.1	4.75 $\pm$ 0.08	4.5 $\pm$ 0.1
N	4.7 $\pm$ 0.1	3.0 $\pm$ 0.1	3.9 $\pm$ 0.3	3.19 $\pm$ 0.01	3.4 $\pm$ 0.1	5.6 $\pm$ 0.2	4.79 $\pm$ 0.04	6.6 $\pm$ 0.2
S	1.27 $\pm$ 0.01	1.06 $\pm$ 0.03	0.98 $\pm$ 0.01	0.89 $\pm$ 0.02	0.96 $\pm$ 0.01	1.50 $\pm$ 0.01	1.26 $\pm$ 0.02	1.01 $\pm$ 0.06
<b>Van-Soest fractions (%TS)</b>								
NDS	31 $\pm$ 1	40 $\pm$ 5	19 $\pm$ 4	31 $\pm$ 5	37 $\pm$ 3	38 $\pm$ 11	32 $\pm$ 3	41 $\pm$ 2
HEM	33 $\pm$ 1	36 $\pm$ 4	29 $\pm$ 2	38 $\pm$ 2	16 $\pm$ 2	20 $\pm$ 3	32 $\pm$ 11	26 $\pm$ 5
CEL	2 $\pm$ 1	2 $\pm$ 1	2 $\pm$ 1	4 $\pm$ 1	8 $\pm$ 3	7 $\pm$ 4	3.9 $\pm$ 0.1	0.3 $\pm$ 0.1
LIGN	26 $\pm$ 1	15 $\pm$ 3	41 $\pm$ 2	21 $\pm$ 4	35 $\pm$ 4	29 $\pm$ 3	25 $\pm$ 3	28 $\pm$ 3
<b>Nutrients profile</b>								
NH <sub>4</sub> <sup>+</sup> (g N/kg TS)	21.2 $\pm$ 0.1	37.4 $\pm$ 1.5	10.8 $\pm$ 2.8	16.7 $\pm$ 0.9	39.5 $\pm$ 2.3	15.6 $\pm$ 0.6	28.6 $\pm$ 2.1	25.3 $\pm$ 1.7
TKN (gN/kg TS)	50.9 $\pm$ 1.2	69.1 $\pm$ 0.9	34.7 $\pm$ 7.6	38.5 $\pm$ 0.9	71.7 $\pm$ 1.2	46.5 $\pm$ 4.9	57.8 $\pm$ 0.3	58.2 $\pm$ 0.7
K (g K <sub>2</sub> O/kg TS)	18.0 $\pm$ 0.1	97.8 $\pm$ 1.3	24.6 $\pm$ 2.3	48.4 $\pm$ 2.3	194.0 $\pm$ 4.2	9.4 $\pm$ 0.6	65.2 $\pm$ 0.9	98.2 $\pm$ 1.8
P (g P <sub>2</sub> O <sub>5</sub> /kg TS)	80.8 $\pm$ 1.2	61.6 $\pm$ 0.6	60.0 $\pm$ 5.1	43.4 $\pm$ 2.5	45.4 $\pm$ 1.3	54.0 $\pm$ 0.7	50.3 $\pm$ 3.2	42.1 $\pm$ 2.6

\*Elemental analysis on the solid fraction of the digestate only. TS (total solids), VS (volatile solids), NDS (neutral detergent soluble), CEL (cellulose), HEM (hemicelluloses), LIGN (lignin), TKN (total Kjeldahl nitrogen).

**Table 6** Properties of tomato plants from different seeding conditions. R1S (untreated sludge), R2S (pretreated sludge), R1 (sludge+olive pomace), R2 (pretreated sludge and olive pomace), R3 (pretreated mixture of sludge and olive pomace), R4 (sludge+macroalgal residues), R5 (pretreated sludge and macroalgal residue), R6 (pretreated mixture of sludge and macroalgal residue).

	<b>C</b> (%TS)	<b>H</b> (%TS)	<b>N</b> (%TS)	<b>S</b> (%TS)	<b>Chlorophyll a</b> ( $\mu\text{g/gTS}$ )	<b>Chlorophyll b</b> ( $\mu\text{g/gTS}$ )	<b>Carotenoid</b> ( $\mu\text{g/gTS}$ )
<b>Control (soil)</b>	35.8 $\pm$ 0.2 <sup>a</sup>	5.19 $\pm$ 0.01 <sup>a</sup>	1.14 $\pm$ 0.02 <sup>a</sup>	0.77 $\pm$ 0.02 <sup>a</sup>	48.8 $\pm$ 9.9 <sup>b</sup>	22.2 $\pm$ 13.2 <sup>a</sup>	13.9 $\pm$ 2.1 <sup>a</sup>
<b>Industrial fertilizer (100%)</b>							
<b>R1S</b>	37.6 $\pm$ 0.1 <sup>c</sup>	5.29 $\pm$ 0.01 <sup>b</sup>	1.63 $\pm$ 0.05 <sup>b</sup>	0.485 $\pm$ 0.05 <sup>b</sup>	72.7 $\pm$ 0.0 <sup>c</sup>	22.2 $\pm$ 0.0 <sup>a</sup>	24.3 $\pm$ 1.6 <sup>c</sup>
<b>R2S</b>	37.4 $\pm$ 0.1 <sup>c</sup>	5.3 $\pm$ 0.1 <sup>ab</sup>	2.17 $\pm$ 0.05 <sup>c</sup>	0.74 $\pm$ 0.05 <sup>a</sup>	90.4 $\pm$ 2.2 <sup>d</sup>	27.8 $\pm$ 2.0 <sup>a</sup>	23.6 $\pm$ 3.5 <sup>bc</sup>
<b>R1</b>	36.9 $\pm$ 0.1 <sup>b</sup>	5.2 $\pm$ 0.1 <sup>b</sup>	1.48 $\pm$ 0.03 <sup>c</sup>	0.81 $\pm$ 0.07 <sup>c</sup>	61.5 $\pm$ 3.0 <sup>b</sup>	18.6 $\pm$ 1.2 <sup>a</sup>	19.6 $\pm$ 0.6 <sup>b</sup>
<b>R2</b>	35.2 $\pm$ 0.1 <sup>b</sup>	5.04 $\pm$ 0.03 <sup>ab</sup>	2.2 $\pm$ 0.1 <sup>ab</sup>	1.12 $\pm$ 0.02 <sup>a</sup>	78.7 $\pm$ 3.2 <sup>d</sup>	21.7 $\pm$ 1.5 <sup>a</sup>	23.9 $\pm$ 1.8 <sup>c</sup>
<b>R3</b>	36.5 $\pm$ 0.1 <sup>c</sup>	5.3 $\pm$ 0.1 <sup>c</sup>	2.0 $\pm$ 0.2 <sup>c</sup>	1 $\pm$ 0.1 <sup>a</sup>	65.1 $\pm$ 1.4 <sup>c</sup>	20.0 $\pm$ 1.2 <sup>a</sup>	22.1 $\pm$ 0.5 <sup>c</sup>
<b>R4</b>	37.7 $\pm$ 0.1 <sup>b</sup>	5.4 $\pm$ 0.1 <sup>c</sup>	2.0 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.04 <sup>b</sup>	67.0 $\pm$ 3.0 <sup>c</sup>	20.0 $\pm$ 0.9 <sup>a</sup>	22.3 $\pm$ 0.4 <sup>c</sup>
<b>R5</b>	36.75 $\pm$ 0.04 <sup>b</sup>	5.31 $\pm$ 0.01 <sup>c</sup>	1.6 $\pm$ 0.1 <sup>c</sup>	0.67 $\pm$ 0.07 <sup>c</sup>	69.6 $\pm$ 1.5 <sup>c</sup>	27.8 $\pm$ 2.0 <sup>a</sup>	23.6 $\pm$ 3.5 <sup>c</sup>
<b>R6</b>	35.9 $\pm$ 0.03 <sup>a</sup>	5.03 $\pm$ 0.02 <sup>c</sup>	2.1 $\pm$ 0.2 <sup>a</sup>	0.81 $\pm$ 0.08 <sup>a</sup>	21.1 $\pm$ 0.5 <sup>a</sup>	21.4 $\pm$ 0.7 <sup>a</sup>	23.1 $\pm$ 1.1 <sup>c</sup>
<b>R6</b>	37.2 $\pm$ 0.02 <sup>c</sup>	5.27 $\pm$ 0.02 <sup>b</sup>	1.68 $\pm$ 0.1 <sup>ab</sup>	0.713 $\pm$ 0.03 <sup>ab</sup>	21.5 $\pm$ 0.5 <sup>a</sup>	18.3 $\pm$ 0.7 <sup>a</sup>	18.5 $\pm$ 0.0 <sup>b</sup>

KOH pretreatment:  
↗ CH<sub>4</sub> production

Plant growth after digestate application

