Evaluation of agronomic properties of digestate from macroalgal residues anaerobic digestion: Impact of pretreatment and co-digestion with waste activated sludge

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Abstract

The aim of this paper is to investigate the impact of pretreating macroalgal residue (MAR) from agar-agar extraction and its co-digestion with sewage sludge on methane production and the agronomic quality of the digestates produced. First different pretreatments were assessed on BMP tests. Among milling technologies used, knife milling with a 4mm-screen improved methane production by 25%. The MAR was then knife milled before alkaline, acid and thermal pretreatment. KOH pretreatment (5% TS basis, 25°C for 2 days) led to the highest methane improvement. It was applied to semi-continuous anaerobic digestion and methane production achieved 237 Nml/gVS which was 20% higher than the control (198 Nml/gVS). In comparison to MAR mono-digestion, co-digestion with thickened activated sludge produced less methane (184 Nml/gVS) but reduced H_2S emission by 91%. None of the digestates was toxic for the

germination or growth of wheat and tomato plants. Particularly, co-digestion had the highest impact on tomato plant dry weight (+94% compared to soil alone) mainly due to the phosphorous brought by sludge. However, the impact of alkaline pretreatment on plants growth was not significant.

Keywords: alkali pretreatment; biogas yield; fertilizer; milling; plant growth; seaweed.

Abbreviations

AD Anaerobic digestion

BMP Biochemical methane potential

CEL Cellulose

CSTR Continuous Stirred Tank Reactor

FOS Volatile organic acids (Flüchtige Organische Saüren)

HEM Hemicelluloses

LCB Lignocellulosic biomass

LIGN Lignin

MAR Macroalgal Residues

NDS Neutral detergent soluble

sCOD Soluble chemical oxygen demand

TAC Total alkalinity concentration

TKN Total Kjeldahl Nitrogen

TS Total solid

TWAS Thickened waste activated sludge

VFAs Volatile fatty acids

VS Volatile solid

1. Introduction

Within the framework of the circular economy, the developed countries are strongly involved in the sustainable management of solid waste (Pires et al., 2011). Thus, to adapt to new regulations concerning environmental protection and waste management, Morocco, being a developing country, is moving towards production and utilization of renewable energies such as solar and wind energies (Nfaoui and Sayigh, 2020). However, the production of renewable energy, such as biogas, from locally generated wastes is not fully explored.

The red macroalgal biomass (*Gelidium* sesquipedale) is produced in large quantities in the Moroccan coasts. They have the greatest gelling capacity among the other red macroalgae species and they are industrially used for agar extraction. After the extraction, the residues are incinerated or landfilled. However, the organic matter contained in the macroalgal residues can be transformed into bioenergy through anaerobic digestion (AD) or co-digestion which could be an interesting valorization route for these residues as it is a mature and cheap technology (Franchetti, 2013).

In fact, anaerobic digestion can be carried out as mono or co-digestion by mixing two or more substrates. Sewage sludge has been extensively used in co-digestion with mainly food wastes and fatty wastes (Siddique and Wahid, 2018). Co-digestion with sludge can enhance AD performance by adjusting C to N balance and moisture and by diluting inhibitors (Mata-Alvarez et al., 2014). According to Ganesh Saratale et al. (2018), macroalgae are generally rich in salts, which may accumulate in digester and thus inhibit the AD process (Ganesh Saratale et al., 2018). In addition,

67 sulfur contained in macroalgae can also lead to unstable AD process and lower methane yields
68 (Tabassum et al., 2017). These problems may be mitigated by co-digestion with the sludge,
69 having the advantage of balancing the nutrients within the digester (Siddique and Wahid, 2018).
70 According to Nghiem et al. (2017), the most financially and environmentally profitable driver of
71 co-digestion is avoiding landfill (Nghiem et al., 2017). In the context of Morocco, the co-
72 digestion of MAR and sludge does not yet exist. However, codigestion seems more feasible than
73 monodigestion since MAR are not available all year round and their generation is quite limited
74 (870 ton/year) (Aboulkas et al., 2017).

75 In the case of hardly degradable materials, hydrolysis may be difficult and slow, which strongly
76 affects the retention time, and then the productivity of the AD plant (Wang et al., 1997).
77 Mechanical pre-treatments have been extensively studied on lignocellulosic matrices, which is
78 not the case for macroalgae biomasses and residues (Thompson et al., 2019). As for alkaline
79 pretreatment, Jard et al. (2013) reported that NaOH pretreatment increases the methane
80 production from macroalgal biomass by 17% under room temperature (Jard et al., 2013), while
81 acid pretreatment was studied by Vanegas et al. (2015) and a negative effect on methane
82 production was obtained (Vanegas et al., 2015). Nevertheless, all studied macroalgae
83 pretreatments were carried out with batch AD. In addition, a comparative study on different
84 pretreatments effect on macroalgal residues has never been investigated. Furthermore,
85 pretreatments are usually optimized considering methane production improvement and more
86 barely energy requirements and chemicals consumption. Nevertheless, the digestate which is rich
87 in nutrients and organic compounds, is generally used as soil biofertilizer substituting the
88 industrial fertilizers (Ronga et al., 2019). It is, thus, important to consider the effect of
89 pretreatments on the digestate agronomic quality and its future reuse (Kor-Bicakci et al., 2019)

but this point has been barely studied (Solé-Bundó et al., 2017; Tampio et al., 2015).

The first aim of this paper is to compare the impact of different milling technologies and thermal (70°C), acid (H_3PO_4) and alkali pretreatments on biomethane potential (BMP) of macroalgal residues (MAR). Moreover, codigestion with thickened activated sludge on methane production was investigated on semi-continuous anaerobic digestion assay. Digestates were then used as fertilizers for wheat and tomatoes growth, two of the most common crops in Morocco.

2. Materials and methods

2.1. Feedstocks, soil and Inoculum

Macroalgal residue (MAR) (*Gelidium sesquipedale*) were collected from a company located in Kenitra-Morocco which extracts agar-agar from red macroalgae. The thickened waste activated sludge (TWAS) used for co-digestion was collected from a wastewater treatment plant located in Narbonne-France. The inoculum used for BMP tests was a paper mill anaerobic sludge with a VS contents of 41 g/l. The properties of MAR and TWAS are presented in **Table 1**.

The soil used for growth plant tests was sampled from the 10 cm to 30 cm layer from a farm located in Rhamna-Benguerir region in Morocco. A sandy loam agricultural soil was used with 30% silt for the incubation experiments. Air-dried soil was sieved (< 4 mm) and stored (15°C) until the beginning of the experiments. The soil contained organic matter (1.8%), C/N ratio (8.4), pH (8.4), total nitrogen (0.13%), P_2O_5 (0.835 g/kg), K_2O (0.41 g/kg) and MgO (0.41 g/kg). The cation exchange capacity (11.8 g/kg) was measured using Metson method (NF X 31-130), which indicated that exchange and fixation of cations in the used soil were quite easy.

2.2. Pretreatments

The MAR was subjected to different techniques of mechanical pretreatment. Knife milling was carried out by using (RETSCH SM 100, Germany) 4 mm and 0.5 mm screens. Vibro and planetary ball milling were operated using (RETSCH MM 400, Germany) and (Pulverisette 7, Fritsch, France) respectively.

Chemical pretreatments are reported to be efficient in degrading hardly degradable matter especially lignocellulosic biomasses (Pellera and Gidarakos, 2018). In particular acids are efficient to solubilize polysaccharides whereas alkali degrade lignin (Monlau et al., 2012). Thus, alkaline and acid pretreatments were compared for their effectiveness on MAR. Acid pretreatment is generally applied at high temperature (de Jong and Gosselink, 2014) whereas alkali can be operated at room temperature. The choice of acid and alkaline reagents was made with regard to a further agronomic use of the digestate, as KOH and H_3PO_4 bring potassium and phosphorous respectively. Therefore, acid pretreatment was achieved by adding phosphoric acid with a dose of 5% (TS basis), and then the mixture was heated at 70°C under stirring at 100 rpm for 4 h using an incubator shaker (New Brunswick Scientific Innova 43, France). Alkaline pretreatment was carried out by the addition of KOH with a dose of 5% (TS basis). The mixture was stirred at 100 rpm and 25°C for 48 h. Thermal pretreatment (70°C) was carried out to decouple the effect of temperature and acid reagent, observed in thermal acid pretreatment under stirring at 100 rpm for 4 h. For thermal and chemical pretreatments, the same MAR to water ratio (1:40 (wTS/w)) was used.

130 2.3.Biochemical and Physicochemical Analysis

131 The measurement of TS and VS contents was done using the APHA (American Public Health
132 Association) method (APHA, 1998). The neutral detergent soluble matter (NDS), hemicelluloses
133 (HEM), cellulose (CEL) and lignin (LIGN) contents were determined using Van-Soest method
134 (Van Soest, 1963). The term "like" indicates that the quantified material is extracted from the
135 same stage of the Van-soest method as cellulose, hemicellulose and lignin. CHNS content was
136 measured by elemental analysis using Thermo Scientific FlashSmart analyzer, via flash
137 combustion at 950°C. The particle size distribution of the mechanically pretreated samples was
138 determined by laser diffraction using a Mastersizer 2000 in combination with the Scirocco 2000
139 (Malvern Instruments, Worcestershire, UK). Results are expressed in the term of D_{50} that
140 represents the diameter at which 50% of the sample is composed of particles with a diameter
141 smaller and bigger than this value. Samples solubilization was determined by the soluble
142 chemical oxygen demand (sCOD) measurement. The sCOD of chemically and thermally
143 pretreated MAR was measured in the liquid phase. However, the sCOD of raw and mechanically
144 pretreated MAR were determined after maceration of 2 g of MAR for 4 h, in 100 ml of
145 ultrapurified water under stirring at 100 rpm and ambient temperature. Then, the mixture was
146 diluted with ultrapurified water to get the COD in the range of 0-1500 mgO_2/l. The solution was
147 then transferred to the Spectroquant test kits and the sCOD value was read by a HACH DR/2000
148 spectrophotometer at 620 nm.

149 Digestates conductivity was measured according to the NF EN 13038. Total nitrogen (TKN) was
150 determined according to the Kjeldahl method (Kjeldahl, 1883), by using a mineralizator (BUCHI
151 digestion unit K 438) and a BUCHI 370-K distillator/titrator. For practical reasons two methods
152 were used to determine nutrient content (P, K, Mg, Ca and Na). In the substrates (sludge and

153 MAR), microwave-assisted mineralization was carried out after the addition of nitric acid (65%)
154 and hydrogen peroxide (30%). The reaction was conducted for 30 min at room temperature.
155 Then, the mixtures were placed in the microwave reactor (Flexiwave, milestone) and heated for
156 20 min to achieve 210°C which was maintained for 20 min and then cooled for 25 min. The
157 obtained solution was analyzed using the Inductively Coupled Plasma Mass Spectrometry
158 (ThermoFisher Scientific, XSeries 2 ICP-MS) equipped with a cooled spray chamber, a
159 quadruple mass spectrometer and a collision cell. The ICP-MS settings were: Nebulizer flow 0.82
160 $l.min^{-1}$, auxiliary flow 0.80 $l.min^{-1}$, cool flow 13 l/min, forward power 1400 Watts, cell gas flow
161 He/H 4.5 $ml.min^{-1}$. In the digestates, nutrients content (P, K, Mg, S, Ca, Na) were determined
162 according to the NF EN 17053.

163 2.4.Anaerobic digestion (AD)

164 Biochemical methane potential (BMP) tests were carried out to assess macroalgal residues and
165 sewage sludge biodegradability and the impact of pretreatment on MAR biodegradability. After
166 mechanical, chemical and thermal pretreatments, the substrates were subjected to BMP tests
167 carried out in triplicate in 500 ml flasks with 300 ml working volume. Sludge, the mixture of
168 sludge and MAR, untreated and pretreated MAR were added to the inoculum at a ratio of 1
169 $gVS/gVS_{inoclum}$. Oligoelement, macroelement and buffer solutions, whose concentrations are
170 given in (Monlau et al., 2013) were added. Flasks were set at mesophilic conditions (35°C) and
171 were continuously stirred at 100 rpm on an agitated table. The calculation of produced methane
172 volume was based on pressure measurements, on ideal gas law and on the biogas composition,
173 obtained using gas chromatography (GC CLARUS 480, Perkin Elmer). Then, the production of
174 the inoculum alone was subtracted from the total flask methane production.

175 Three continuous stirred tank reactors (CSTR) of 2.5 l were used for the semi-continuous AD of
176 untreated MAR, KOH pretreated MAR and for the co-digestion of MAR and TWAS. The
177 reactors worked under mesophilic conditions (37°C) with a hydraulic retention time of 20 days
178 and an organic loading rate of 1 gVS/l.d. Before being pretreated or fed to the reactors, the
179 macroalgal residue was ground using knife milling with a screen of 4 mm. R1, which was the
180 control reactor, was fed with untreated MAR, whereas; R2 and R3 were fed with KOH treated
181 MAR, and a mixture of MAR and TWAS at a ratio of 1:1 (VS basis) respectively. Monitoring of
182 the reactor performance was carried out by the measure of biogas volume using gas counters
183 (Ritter, Germany) and its analysis by gas chromatography (GC CLARUS 480, Perkin Elmer).
184 The volatile fatty acid content (VFAs) was determined using gas chromatograph as described in
185 (Thomas et al., 2018). The determination of NH_4^+ concentration in digestate was carried out by
186 the titrimetric method after distillation using a BUCHI 370-K distillator (Rodier, 1975). The
187 determination of FOS (volatile organic acids) and TAC (Total Alkalinity Concentration) was
188 achieved by titration of HCl (0.1M) to a pH equal to 5.3 and 4.3 respectively using the following
189 equation (**Eq 1**):

190 $$\frac{FOS}{TAC} = \frac{\mathrm{V}(\mathbf{pH=4.3}) - \mathrm{V}(\mathbf{pH=5.3})}{\mathrm{V}(\mathbf{pH=4.3})} \qquad \text{(Eq 1)}$$

191 2.5.Kinetic parameters

192 The modified Gompertz model (**Eq 2**) was used to determine kinetic parameters of anaerobic
193 digestion in the batch assays. This model is widely used on the assumption that there is a
194 relationship between methane production and bacterial growth in digesters.

195 $$B = Pm.exp\left(-\exp\left[\left[\frac{Rm.e.(\lambda - t)}{Pm}\right] + 1\right]\right) \qquad \text{(Eq 2)}$$

196 Where B (Nml/gVS) is the cumulative methane production, P_m (Nml/gVS) is the maximal
197 methane production, R_m (Nml/gVS.d) is the maximal methane production rate and λ (d) is the lag
198 phase time.

199 Kinetic parameters were then calculated using the minimization of the sum of least square
200 between the observed and predicted values. The coefficient of determination R^2 (**Eq 3**) was
201 calculated to indicate the variation in estimated methane potential (Yi) that is explained by the
202 measured methane potential (Xi).

203 $$R^2 = 1 - \frac{\sum_{i=1}^{N}(Xi-Yi)^2}{\sum_{i=1}^{N}\left[Xi-\frac{(\sum_{i=1}^{N}Xi)}{N}\right]^2} \qquad \text{(Eq 3)}$$

204 2.6.Phytotoxicity growth tests of wheat and tomatoes plants

205 The agronomic quality of digestates generated in the three continuous stirred tank reactors was
206 investigated through phytotoxicity growth plant tests. Plants trials with seeds of wheat and
207 tomatoes were performed in small pots with a volume of 0.5 l, placed in a growth chamber
208 (Fitotron, Weiss Gallenkamp, UK) according to the OECD 208 guidelines (2006) under
209 controlled conditions. During the trial, the environmental conditions were the following ones: 16
210 h of light and 8 h of darkness, temperature at 25 °C for the periods of light and 18 °C during
211 periods of darkness and 60 % relative humidity for the periods of light and 80 % during periods
212 of darkness. Three conditions were tested as: soil alone, soil + industrial fertilizers and soil +
213 digestate. A dose of 150 kg N / ha was applied for both digestate and industrial fertilizer
214 (commercially available ammonium nitrate). For P dosage of industrial fertilizers, 50 kg P / ha
215 from triple superphosphate were applied. Such doses are within the range of N and P application
216 rates for different crops systems (Gell et al., 2011). The mixtures of soil and digestates or

industrial fertilizer were prepared into 10 cm diameter plastic pots and the mixture was brought to 70% of the water holding gravimetric capacity. Ten wheat seeds and six tomato seeds were planted in each pot using four replicates for each condition. Each pot was manually watered every 48h by weighing and adding water to achieve the initial weight. After that 70% of control seeds were germinated, five and three seeds of wheat and tomatoes respectively, were let in each pot, to make space for the plants to growth for dry weight measurement. After 21 days and 28 days respectively for wheat and tomatoes, plants were harvested by cutting them off at the soil level and then dried for 48 h at 70°C in a forced air oven and weighed. For each condition, germination index expressed in % of initial seeds and biomass dry matter were measured and calculated as follows **(Eq 4 and 5):**

$$Germination\ index\ (\%) = \frac{Final\ number\ of\ seeds\ that\ germinated}{\text{Number of initial seeds}} * 100 \text{ (Eq 4)}$$

$$Dry\ weight\ (\text{gTS/100 plants}) = \frac{Dry\ weight\ of\ harvested\ plants\ (70°C)}{Number\ of\ plants} * 100 \text{ (Eq 5)}$$

The results were compared two by two using the t-test under a Student law, assuming the variance equality, normality and independence of repetitions.

3. Results and discussion

3.1. Optimization of pretreatment on BMP

3.1.1. Substrates composition

Table 1 presents the composition of macroalgal residue (MAR) and the activated sludge (TWAS) used in this study. Overall, TWAS and MAR had similar VS (%TS), carbon (%TS) and hydrogen (%TS) content. However, the waste activated sludge was richer in nutrients compared to macroalgal residues which suggests that the sludge may provide nutrients for co-digestion which could remain in the digestate.

239 *3.1.2. Effect of Milling on BMP*

240 **Table 2** presents some properties of MAR after different milling technology pretreatments. The
241 smallest granulometry of milled samples was obtained after vibro-milling with a mean particle
242 size of 91 µm, while planetary ball milling gave average particle size of 173 µm. However, a
243 medium size of about 1mm was obtained using knife milling with a screen size of 4 mm. The
244 sCOD of all milled samples was enhanced compared to the raw. In fact, after vibro-ball milling,
245 the sCOD was enhanced by 83%. Soluble COD increased when particle size decreased, which
246 favors the cell walls disruption and the ease of organic matter release. Moreover, all mechanical
247 pretreatments of MAR enhanced the methane production rate (R_m) compared to raw MAR,
248 especially knife milling; while the lag phase time (λ) remained stable. All pretreatments gave
249 between 10% and 25% higher methane than the raw material.

250 In brief, knife milling using screen size of 4 mm was sufficiently effective in homogenizing the
251 raw MAR and increasing its accessibility for anaerobic digestion. Thus, the milled macroalgal
252 residue (screen of 4 mm) was subjected to thermal and chemical pretreatments, used for reactors
253 feed and was appointed as (Untreated MAR).

254 *3.1.3. Effects of Thermal and Chemical Pretreatments on BMP*

255 **Table 3** presents the biochemical composition of solid and liquid fractions after pretreatments as
256 well as the methane potential of untreated, pretreated MAR and mixture of MAR and sludge. In
257 fact, the solubilization was expressed in terms of soluble COD which increased after acid
258 pretreatment by 225%. Thermal pretreatment at 70°C resulted in increasing the sCOD by 216%,
259 while alkaline pretreatment achieved 132% of sCOD compared to control.

Moreover, all pretreatments seem to impact the least accessible organic compounds (lignin-like) in MAR. In fact, lignin-like reductions of 26%, 43% and 38% were obtained after acid, alkali and thermal pretreatments respectively. Similarly, cellulose-like content was highly reduced by all studied pretreatments and was decreased by 29-37%. Acid pretreatment had the highest impact on hemicelluloses-like content which decreased by 47%. Indeed, acid pretreatment at low temperatures was reported to cause the hydrolysis of hemicelluloses which increased the accessibility to cellulose. However, the acid pretreatment at high temperature can generate furfural and 5-(hydroxymethyl)furfural which inhibits methanogenesis at high concentrations (2-4g/l)(Barakat et al., 2012). The acid pretreatment was, thus, not efficient in enhancing methane production which may be due to the low temperature used, the low pretreatment time or the low solid to liquid ratio which probably weakened the action of the acid. In fact, acid pre-treatments are generally carried out at temperatures above 170°C (Monlau et al., 2013), or if the temperature is lower, they are carried out for several days (e.g. 7 days at 25°C (Song et al., 2014).

Contrarily, alkali pretreatment causes lignin degradation and the weakening of the bonds with other lignocellulosic components. The alkaline pretreatments are generally effective in methane production. However, the inhibitors formation such as phenols can be obtained depending on reagents strengths and their applied doses (Chen et al., 2008).Besides, the fiber reduction in thermally pretreated MAR may be due to the transfer of thermally extractable matter to the liquid phase. In fact, during thermal and acid pretreatments, gel phases were formed which may be due to residual agar. Thus, thermal and acid pretreatments can be used to extract more value-added products and the remained solid fractions can be valorized through AD.

281 Regarding methane potential, it was found that the alkaline pretreatment was the most efficient
282 pretreatment with 11% more methane produced than control. In addition, alkaline and acid
283 pretreatments decreased the methane production rate (R_m) by 17% and 12% compared to
284 untreated MAR, which may be due to the release of inhibitors such as phenolic compounds and
285 furans, in the liquid phases (Jönsson and Martín, 2016). Taking into account MAR and TWAS
286 methane potential, the specific methane potential of the mixture MAR and TWAS should be
287 equal to 190 Nml/g$VS_{mixture}$ which was approximately achieved (182 Nml/g $VS_{mixture}$). The co-
288 digestion with sludge resulted in lag phase time decrease because sludge contained a higher NDF
289 fraction and less lignin-like materials, and thus it is more rapidly degraded than MAR. In
290 addition, it decreased the specific methane produced because of the low methane potential of
291 sludge. This finding highlights the interest of using MAR as a feedstock for anaerobic co-
292 digestion with sludge.

293 According to Thompson et al. (2019), the most efficient pretreatments for brown macroalgae are
294 thermochemical ones. In fact, when acid pretreated macroalgae was subjected to low temperature
295 pretreatment (80°C for 2 h), methane potential was increased by 130% compared to untreated
296 algal biomass, while single thermal pretreatment (80°C for 2 h) reduced methane production by
297 9% (Barbot et al., 2015). The biological pretreatment with white rot fungi was also used to
298 improve methane production from brown macroalgae and an increase of 20% was reported
299 besides lignin removal (Ben Yahmed et al., 2017). Thompson et al. (2019) suggested that
300 hydrothermal pretreatment can be more attractive at industrial scale (Thompson et al., 2019). In
301 the case of this study, KOH addition had a positive impact on macroalgal residues without the
302 need for heating. Apart from the cost of the chemical reagent, KOH seems to be the most suitable
303 for pretreating these residues and for improving the agronomic quality of its digestate. Thus, the

304 production of digestate and methane from semi-continuous anaerobic digestion of raw and KOH-
305 pretreated MAR was investigated in the next section.

306 3.2. Effect of alkaline pretreatment and co-digestion with sludge on methane production in
307 semi-continuous assays and digestate properties

308 **Fig.1** presents reactors performance parameters such as specific methane volume, FOS/TAC and
309 ammonium concentration. Considering the steady state phase from day 50 to day 116, the
310 methane yields from untreated and pretreated MAR were 197.6 Nml/gVS and 236.7 Nml/gVS
311 respectively. Therefore, methane production was enhanced by 20% after alkaline pretreatment of
312 MAR. Regarding the co-digestion reactor, the methane yield was around 184 Nml/gVS.

313 The maximal VFAs concentrations were 0.42, 0.27 and 0.37 g/l at 40th day in R1, R2 and R3
314 respectively. The VFAs from R1 (untreated MAR) and R2 (alkali pretreated MAR) were
315 composed of acetic (C2), propionic (C3), butyric (C4), iso-butyric (IC4) and iso-valeric (IC5)
316 acids, but their concentrations were highly reduced after the 60th day. Contrarily, VFAs from R3
317 (co-digestion reactor) were only composed of acetic (C2) and propionic (C3) acids. Appels et al.
318 (2008) reported that the VFA accumulation can be toxic to methanogens if it is beyond 2-2.7 g eq
319 acetic acid/l (Appels et al., 2008). In this study, the maximal VFA accumulation occurred in R1
320 in which the VFA concentration reached 0.32 g eq acetic acid/l in the 39th day (data not shown).
321 However, in R3 and R2 the maximal concentrations were 0.23 and 0.21 g eq acetic acid/l
322 respectively. For the three reactors, VFAs were degraded as they were not detected over the
323 stabilization period (after the 77th day). In all cases, pH ranged from 6.8 to 7.4. It thus always
324 remained within the range for optimal methanogenic activity (6.5-7.5), even if the pretreated

MAR pH (pH=12) was not adjusted. The reactor (R2) successfully withstood the high pH of the feed.

Moreover, FOS/TAC ratio was steady after the 60th day for the three reactors **(Fig.1).** In all cases, the FOS/TAC ratio was between 0.1 and 0.35. It reached its peak for all 3 reactors between the 40th and 60th day and then remained constant at 0.1-0.2, which is lower than the threshold for a stable AD (0.3) (Sambusiti et al., 2013). The alkalinity of R2 was higher than that of R1. Thus, the alkaline pretreatment increased the alkalinity which is in accordance with Sambusiti et al. (2013) (Sambusiti et al., 2013), but the FOS/TAC of both reactors remained similar as FOS also increased in R2. In addition, the maximal ammonium concentrations in the three reactors were around 200 mg/l. Ammonium concentrations between 50 and 200 mg/l are recommended for anaerobic microorganisms' growth (Chen et al., 2008). During all phases, ammonium concentrations remained very low compared to the threshold reported in literature (2 g/l) (Chen et al., 2016). The total N contained in MAR was 40 mg/gTS, in which the concentration of ammonium was 1.5 mg NH_4^+/gTS. In the beginning of the AD, ammonium in digestate was originated from inoculum. Then, its concentration decreased in both R1 and R2 from 0.16 g/l and 0.11 g/l respectively, to stabilize at 0.05 g/l in the last 40 days which can be linked to some ammonium deficiency. However, co-digester (R3) did not seem to have this issue, a relatively stable ammonium concentration was maintained (0.2 g/l). As sludge is richer in ammonium and bicarbonates (Fonoll et al., 2015), it increases the buffer capacity of R3.

Table 4 presents the methane yields and digestate properties of the three reactors. In fact, the biogas originating from MAR was composed of 59% of methane and 0.5% of H_2S due to the

sulfur contained in macroalgal residues. These proportions were not affected by the alkaline pretreatment; the biogas was composed of 60% of methane and 0.32% of H_2S. The co-digestion with sludge reduced the H_2S yield to only 0.05%, while CH_4 proportion attained 56% of the biogas. Indeed, the total S content in the sludge was higher compared to MAR (**Table 1**). However, co-digestion with sludge reduced the production of H_2S which may be explained by the possible precipitation of metal sulfides, such as FeS or FeS_2, in the presence of metals contained in sludge (e.g. iron) (Möller and Müller, 2012). In addition, it should be pointed out that the competitiveness between methanogens and sulfate-reducing bacteria depends on the COD/SO_4^{2-} ratio within the digester which was not measured in this study (Dar et al., 2008). Hydrogen sulfide emitted from anaerobic digesters is typically around 2000 ppmv (Zhuo et al., 2019). In this study, a concentration of 5000 ppmv of H_2S was obtained from R1, while in R2 this concentration decreased to 3200 ppmv. However, co-digestion was found to effectively reduce the hydrogen sulfide concentration (500 ppmv). In all cases, if the anaerobic digestion of MAR is designed on an industrial scale, an H_2S elimination step is essential before biogas use.

Besides the energetic interest of AD process, the quality of the digestate generated was also investigated and results are provided in **Table 4**. Overall, nutrient concentrations in D2 (digestate from R2) were lower than those from D1 (digestate from R1) except for potassium concentration which was obviously brought by the KOH, while D3 (co-digestate) contained high concentrations of NH_4^+, P and K compared to D1. In fact, N, P and K are essential for plant growth and, in the case of the co-digestion, were in higher concentration due to the addition of TWAS (**Table 1**). Moreover, less cellulose-like and more lignin-like were found in the D2 compared to D1 which is due to the degradation during AD process. In fact, the ratio (CEL+HEM)/LIGN was reported to be an indicator of humification degree (Teglia et al., 2011). As humic substances are essential for

370 soil fertility and health due to their stability, D2 was more stabilized and can be more beneficial
371 for soil at the long-term showing the interest of applying pretreatment on digestate stabilization.
372 In both France and Morocco, there are no special guidelines on dose limits for Ca, Mg and Na for
373 land application. Only regulations around metallic traces and micro-pollutants are available.
374 However, these nutrients can increase the salinity of soil, especially Na can present a risk to plant
375 growth if a threshold is exceeded. The salinity can reduce the nutrients adsorption, limit the
376 photosynthesis and thus reduce the chlorophyll production resulting in plants with nutritional
377 deficiencies (Daliakopoulos et al., 2016). However, the tolerance to salinity depends on the
378 plants, wheat is highly tolerant to soil salinity while tomato is moderately tolerant (conductivity
379 should not exceed 3000 µS/cm) (Daliakopoulos et al., 2016). The conductivity of the present
380 digestates (**Table 4**) shows that their application is not likely to affect soil salinity.

381 3.3.Agronomic Valorization of the digestates

382 **Fig.2** presents germination and biomass growth (g TS / 100 plants) of wheat (Fig.2a) and tomato
383 (Fig.2b) plants. The analysis of variance of the results showed that germination index was not
384 affected by any of the trials conditions **(Fig.2)** suggesting that the germination was not inhibited
385 by digestate addition. This finding is in agreement with Solé-Bundo et al. (2017) who found that
386 germination index of cress was not significantly changed after applying three digestates diluted at
387 0.1 % and 1% (Solé-Bundó et al., 2017). However, Opatokun et al. (2017) reported a negative
388 effect of food waste digestate on tomato germination (Opatokun et al., 2017).

389 Regarding wheat biomass growth, the three digestates were as beneficial as industrial fertilizer
390 which suggests that digestates contained nutrients that can offset N and P requirements. The
391 excess of potassium in D2 also had no noticeable effect on wheat growth. Unlike wheat, tomatoes

392 growth was significantly improved by D3 addition, followed by the industrial fertilizer and D2
393 and D1. This finding showed clearly the positive effect of MAR and TWAS co-digestion over the
394 mono-digestion due to probably its high phosphorous content (**Table 4**). The latter had many
395 advantages such as enzymes activation, sugars transport and stomatal activity regulation for
396 optimized water absorption (Hasanuzzaman et al., 2018). However, potassium is absorbed in
397 earlier growth stage compared to nitrogen and phosphorous that can explain why the low amount
398 of D3 was not prejudicial (Prajapati, 2012). An excessive potassium uptake may reduce
399 absorption of other nutrients like magnesium (Farhat et al., 2016). Investigating the interaction
400 between nutrients and micronutrients is required to optimize their concentrations in added
401 fertilizers/digestate and to maximize their uptake.

402

403 **Table 5** reports agronomic tests of digestates in literature and their main results. In general rules,
404 digestates were reported as a good fertilizer, improving soil properties, plant growth and health
405 (Panuccio et al., 2016; Westphal et al., 2016). Nevertheless, despite its nutrients, digestate can be
406 toxic for plant germination at too high concentration (Opatokun et al., 2017). Its impact on seeds
407 and soil depends on its composition and concentration. Dilution of digestate is sometimes
408 needed. Alburquerque et al. (2012) reported the impact of two digestate dilutions on lettuce and
409 cress germination. In fact, at a 1% dilution in water of both digestates increased crops
410 germination, while lower (0.1%) and higher (10%) dilutions were found ineffective
411 (Alburquerque et al., 2012).
412 Moreover, Tampio et al. (2016) reported positive effects of applying food waste and organic
413 fraction of municipal solid waste digestates on ryegrass growth which was enhanced by 167%
414 and 213% respectively. These results were related to the high nitrogen concentration and the

soluble fraction (50-70%) of phosphorus contained in the digestates (Tampio et al., 2016). Similarly, Gell et al. (2011) investigated the application of three digestates having different impacts on lettuce plant growth. At a dose of 150 g N/kg, the human excreta digestate decreased the growth yield by 10% while cow manure digestates resulted in a 20% increase. This finding was explained by the fact that human excreta digestate slowly released organic matter and nutrients in soil compared to the other digestates (Gell et al., 2011).

Depending on digestate composition, sometimes pure digestates cannot be used directly in the soil fertilization, a dilution or post-treatment may be necessary to avoid germination inhibition. This was the case of Solé-Bundo et al. (2017) study which showed that a dilution of mono-digestate at 1% was needed to avoid phytotoxicity issues caused by microalgae digestate application (Solé-Bundo et al., 2017). In addition, microalgae digestate increased the growth index of cress by 10% which was lower than the growth index after co-digestion residue application (75% VS of sludge and 25% VS of microalgae). Despite its lower nutrient content compared to mono-digestate, the co-digestion residue was found to present less phytotoxicity compared to untreated microalgae digestate, while the digestate of thermally pretreated microalgae had no impact on cress growth (Solé-Bundó et al., 2017).

In the case of the current study, plant growth tests were successful, without any previous dilution. In addition, digestate from pretreated MAR presented similar benefits for plant growth as digestate of untreated MAR showing that digestate from pretreated biomass did not exhibit phytotoxicity effect.

Nonetheless such conclusions should be moderated and compared with caution as the impact of digestate application in soil depends not only on digestate properties but also on the soil

properties and structure as well as on experimental protocols used and operational conditions applied (temperature, luminosity, humidity…) (Nkoa, 2014).

4. Conclusion

Milling was necessary to increase the accessibility and methane potential of macroalgae residues. Moreover, alkali pretreatment enhanced methane production of MAR by 20% and the generated digestate had the same effect on plant growth compared to untreated MAR digestate. In contrast, co-digestion with sludge led to lower methane production than mono-digestion of macroalgal residues, but lower H_2S emission and higher digestate agronomic value due to nutrients brought by sludge. However, this study should be completed by further work before any extrapolation of these results. In particular, experiments with higher OLR will be of high interest.

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615

616 **Table 1.** Composition of macroalgal residues and thickened waste activated sludge

Parameters	MAR	TWAS
TS (%)	89±2	19±1
VS (%TS)	79±2	78±1
Elementary analysis (%TS)		
C	38.82±0.2	38.34±0.58
H	6.18±0.3	5.68±0.05
N	4.04±0.2	6.13±0.06
S	0.65±0.01	1.12±0.03
Fibers		
NDS soluble (%TS)	13±1	39±5
Hemicelluloses (%TS)[a]	22.7±0.4	8.7±1.5
Cellulose (%TS)[b]	36.8±0.7	34.7±0.8
Lignin (%TS)[c]	24.4 ±0.3	9.8±0.7
Nutrients		
NH_4^+(mg/gTS)	1.5±0.1	4.6±0.3
Na (mg/gTS)	7.9±0.6	6.6±0.4
Mg (mg/gTS)	13.2±0.2	28.2±0.1
K (mg/gTS)	3.6±0.4	37.3±0.1
Ca (mg/gTS)	62.10±0.6	59.40±1.4
P (mg/gTS)	11.49±0.3	39.18±0.4

617 [a]Hemicelluloses-"Like"; [b]Cellulose-"Like"; [c]Lignin-"Like"

618

619 **Table 2** Effects of mechanical pretreatments on MAR solubilization and methane potential and kinetic
620 parameters results

	D_{50} (µm)	sCOD (mg/gVS)	Methane produced (Nml/gVS)	Methane enhancement (%Raw)	Kinetic parameters			
					P_m (Nml/gVS)	R_m (Nml/gVS.d)	λ (d)	R^2
Raw	-	115±1[a]	203±42[a]	-	197±42	40±9	3.7±0.2	0.999
Knife Milling								
4mm	1115	124±4[b]	253±5[b]	+25	245±5.9	58.1±6.8	3.7±0.1	0.999
0.5 mm	530	142±1[c]	225±4[a]	+11	222±2.7	58.1±4.3	3.6±0.2	0.999
Ball milling								
Planetary	173	173±1[d]	234±3[a]	+15	226±2.6	44.5±2.2	3.4±0.1	0.999
Vibro	91	211±3[e]	224±2[a]	+10	218±1.3	44.1±2.2	3.5±0.0	0.999

621 Values with the same letter correspond to insignificant differences ($p<0.1$).

622

623 **Table 3** Composition and methane potential of substrates

		MAR (milled at 4 mm)				TWAS	MAR+TWAS 50/50 (VS/VS)
		Untreated	**Alkaline KOH-5%**	**Acid H_3PO_4-5%-70°C**	**Heating-70°C**		
Liquid phase	**pH**	6.7	12.2	4.1	6.8	7.2	7.1
	sCOD (mg/gVS$_{un}$)	124±6^{a}	164±2^{b}	404±2^{c}	392±9^{c}	43±13	121±4
Solid phase	**NDS(%TS$_{un}$)**	13±1^{a}	20.3±1.9^{b}	13±3^{a}	13±2^{a}	39±5	-
	HEM (%TS$_{un}$)^{a}	22.7±0.4^{a}	14.2±0.2^{c}	12±2^{c}	17±2^{b}	8.7±1.5	-
	CEL (%TS$_{un}$)^{b}	36.8±0.7^{a}	23.26 ±0.02^{c}	23±2^{c}	26±1^{b}	34.7±0.8	-
	LIGN (%TS$_{un}$)^{c}	24.4 ±0.3^{a}	14 ±2^{c}	18±1^{b}	15± 3^{bc}	9.8±0.7	-
Total	**Methane produced (Nml/gVS)**	253±4^{a}	281±10^{b}	252 ±6^{a}	263 ±2^{a}	127±10	182±2
	Enhancement (%untreated)	-	+11*	0	+4	-	-
	Kinetic parameters						
	P_m (Nml/gVS)	247±6.5	255±13.7	234±4.2	252±7.1	124±14.6	153±34.5
	R_m (Nml/gVS.d)	46±1.3	38.3±3.2	40.5±0.1	57.2±4.0	4.7±0.2	12.9±4.2
	λ (d)	3.4±0.0	3.2±0.2	3.3±0.1	3.5±0.2	0	0
	R^2	0.999	0.998	0.997	0.998	0.999	0.972

624 TS_{un} (Total solids in untreated MAR)

625 Values with the same letter correspond to insignificant differences ($p<0.1$).

626

627 **Table 4.** Biogas production and characteristics of final digestates from CSTR reactors of untreated MAR,
628 alkali treated MAR and codigestion of MAR and TWAS

		R1 (untreated MAR)	***R2 (pretreated MAR)***	***R3 (TWAS+MAR)***
Methane production	Methane production (Nml/gVS)	198±10	237±13	184±12
	Methane production (% BMP)	78	84	100
	Methane (%biogas)	59±5	60±6	56±4
	Hydrogen sulfide (Nml/gVS)	1.7±0.4	1.3±0.9	0.15±0.1
Digestates	Conductivity (µS/cm)	307±30	642±24	482±26
	pH	8.0±0.1	8.3±0	7.6±0
	Matter profile			
	TS (%)	0.7±0.1	1.0±0.1	1.0±0
	Organic matter (%TS)	75.5±1.1	60.0±0.2	74.8±0.1
	Ash (%TS)	24.5±1.1	40.0±0.2	25.2±0.1
	Elemental analysis (%TS)			
	C	39.09±0.07	34.47±0.17	37.22±0.19
	H	5.30±0.26	4.55±0.09	5.24±0.11
	N	7.99±0.13	6.60±0.19	5.63±0.23
	S	1.09±0.02	1.01±0.08	1.50±0.01
	Fiber content			
	NDS (%TS)	19±7	7±2	38±11
	HEM (%TS)[a]	28±1	32±1	20±3
	CEL (%TS)[b]	16±4	6±3	7±4
	LIGN (%TS)[c]	36.0±3.6	51±2	29±3
	(CEL+HEM)/LIGN	1.22	0.74	0.93
	Nutrient profile			
	NH_4^+ (g N/kg TS)	9.1±1.5	4.7±1.0	15.6±0.6
	TKN (gN/kg TS)	40.0±14.1	37.8±4.7	46.5±4.9
	Ca (g CaO/kg TS)	98.5±2.2	79.6±2.6	64.6±1.4
	K (g K_2O/kg TS)	2.1±0.1	110.5±10.6	9.4±0.6
	Mg (g MgO/kg TS)	14.0±1.6	10.3±0.2	14.1±0.6
	Na (g Na_2O/kg TS)	15.4±2.2	10.5±0.2	7.2±0.5
	P (g P_2O_5/kg TS)	17.2±1.1	12.8±1.1	54.0±0.7

629 [a]Hemicelluloses-like"; [b]Cellulose-like; [c]Lignin-like

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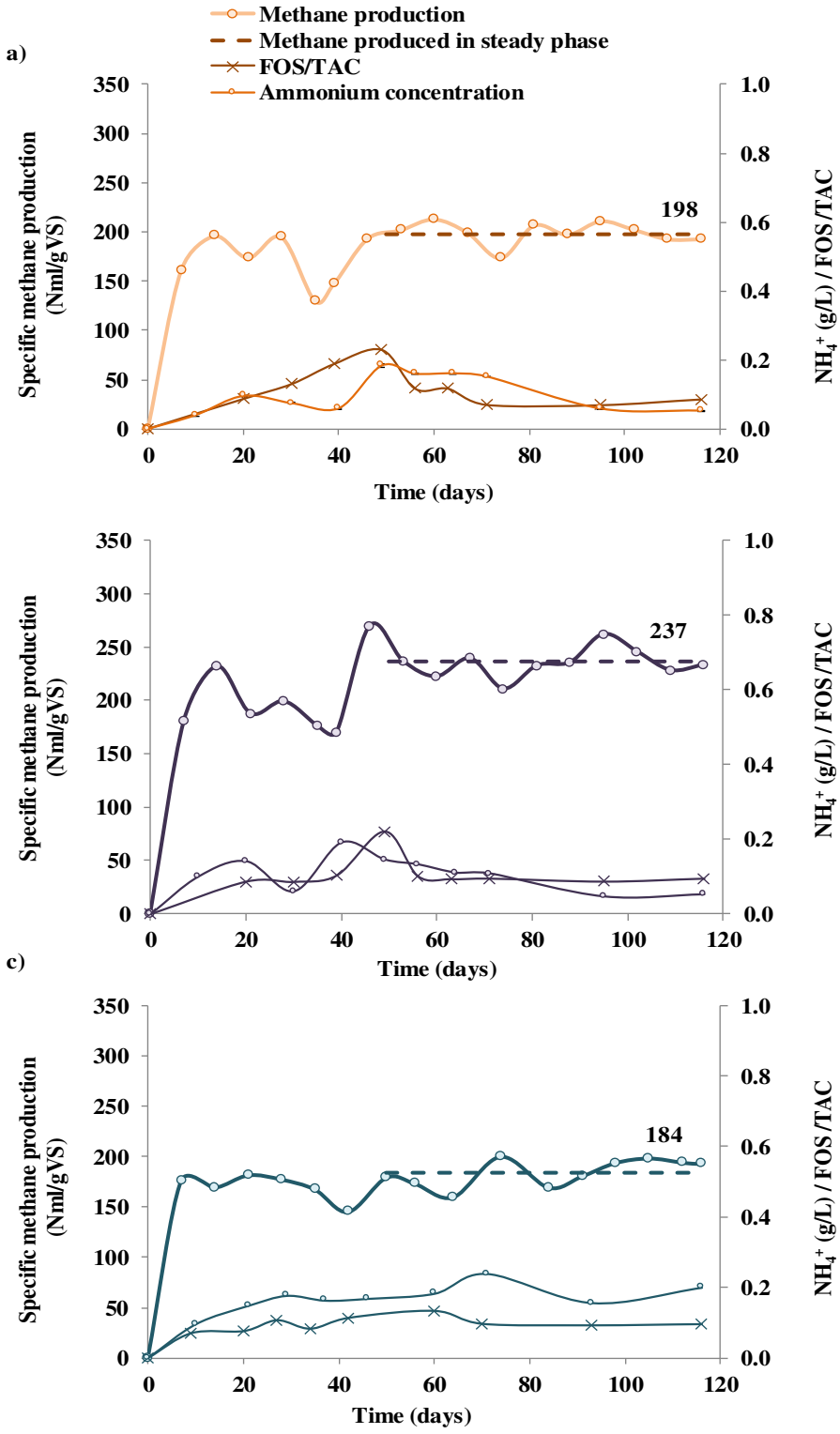
631 **Table 5** Agronomic tests of digestates from organic wastes in literature and in this study

Feedstock	Test	Conditions	N dose	Results of germination and/or plants growth	Ref
Food waste	Plant growth and germination of tomato seeds	Petri plate at room temperature in the dark for 5 days.	7.5g of N added/kg of dry soil	Low germination index (40% only)	(Opatokun et al., 2017)
Food waste	Ryegrass growth	A glass roof outdoors at ambient air temperature for the first 110 days and for days 110–160 in a greenhouse (14 h light in 16 °C and 10 h dark in 14 °C).	1500 mg TKN/5 l	+167% of biomass (DM) compared to control.	(Tampio et al., 2016)
Organic fraction of municipal solid waste				+213% of biomass (DM) compared to control.	
Microalgae	Cress (*Lepidium sativum* L.) growth	Incubation chamber (20 ± 2 °C) for 30 days at 70% of the water holding capacity	170 kg N/ha	Diluted microalgae digestate at 1% results in 10% higher growth index.	(Solé-Bundó et al., 2017)
Thermally pretreated microalgae				Maximal growth index at 1% of dilution, but no improvement compared to control.	
Sewage sludge and microalgae codigestion				+28% of growth index when diluted at 0.1%	
Human excreta	Lettuce shoots growth	Plastic bins at 20 °C and 40% air humidity.	150 kg N/ha	-10% compared to the control	(Gell et al., 2011)
Pig manure				0% compared to the control	
Cow manure				+20% compared to the control	
Mixture of pig slurry and animal by-products +1.0% sludge +6.5% biodiesel wastewater	Lettuce germination	Petri dishes under 17 °C and darkness for 5 days	N.D	At a concentration of 1%: +40% compared to control. At a concentration of 0.1%: -20% compared to control.	(Alburquerque et al., 2012)
Mixture of pig slurry and animal by-products +0.6% pasteurized slaughterhouse residues	Cress germination	Petri dishes under 23 °C and darkness for 3 days		At a concentration of 1%: +50% compared to control. At a concentration of 10%: -60% compared to control.	
Alkali pretreated macroalgal residue	Wheat (*Triticum aestivum.* L) and tomato (*Solanum lycopersicum* .L*)* growth	Small pots or 0.5 L 16 h of light and 8 h of darkness, temperature at 25 °C for the periods of light and 18 °C during periods of darkness and 60 % relative humidity for the periods of light and 80 % during periods of darkness.	150 kg N/ha	Wheat: +27% of biomass (DM) compared to the control Tomato: +30% of biomass (DM) compared to the control	This study
Macroalgal residue				Wheat :+29% of biomass (DM) compared to the control Tomato: +23% of biomass (DM) compared to the control	
MAR and TWAS codigestion				Wheat :+ 24% of biomass (DM) compared to the control Tomato: +94% of biomass (DM) compared to the control.	

632



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634 **Fig.1** Specific methane production, FOS/TAC and NH_4^+ of the reactors, a) Untreated MAR (R1), b) Alkali
635 pretreated MAR (R2), c) Co-digested MAR and TWAS (R3).

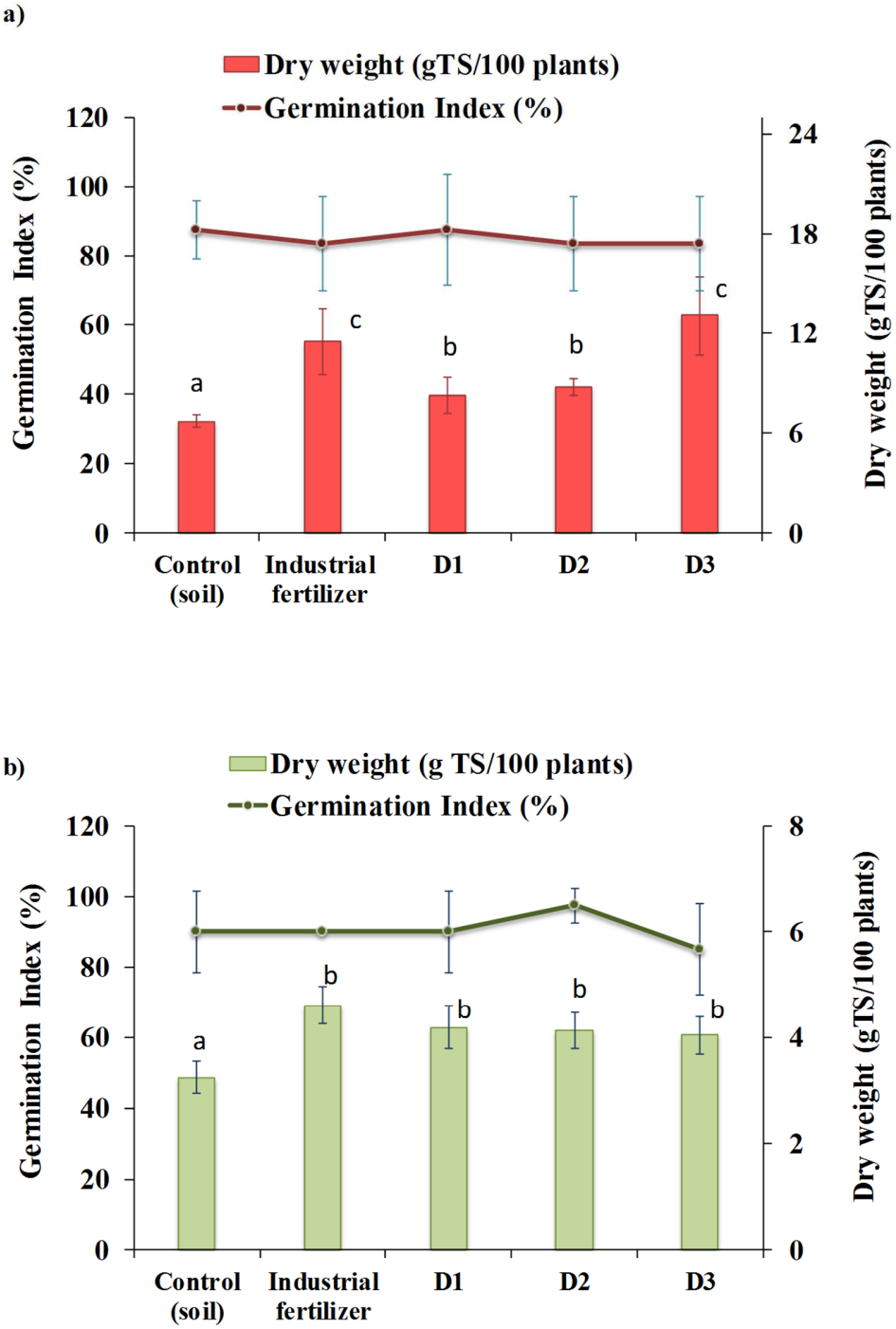
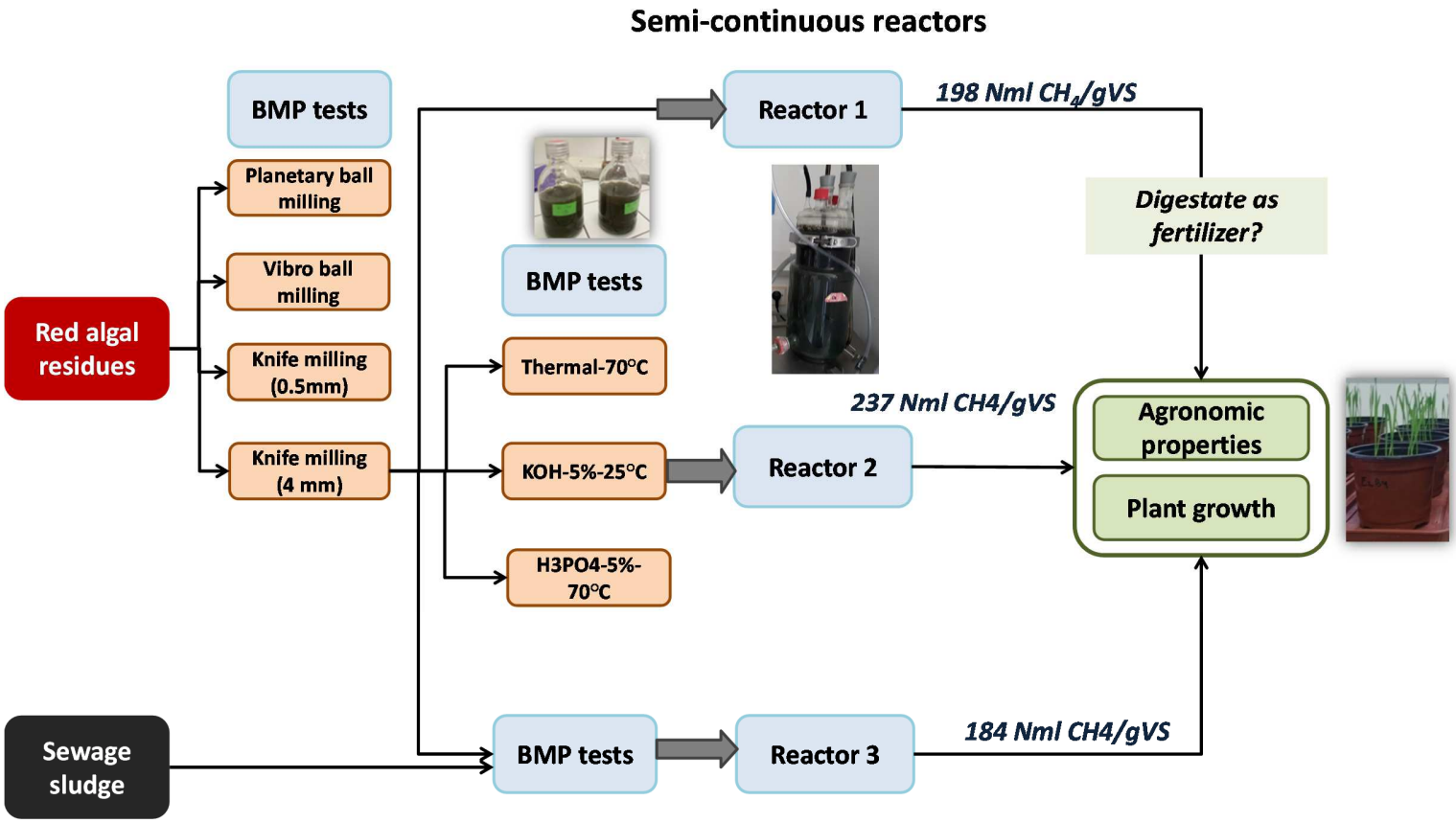


Fig.2 Germination index and dry weight of biomasses for: a) wheat plants, b) tomato plants. Values that are annotated with the same letter correspond to insignificant differences ($p < 0.05$).


Semi-continuous reactors
BMP tests
Planetary ball milling
Vibro ball milling
Knife milling (0.5mm)
Knife milling (4 mm)
Red algal residues
BMP tests
Thermal-70°C
KOH-5%-25°C
H3PO4-5%-70°C
Reactor 1
198 Nml CH4/gVS
Digestate as fertilizer?
237 Nml CH4/gVS
Reactor 2
Agronomic properties
Plant growth
Sewage sludge
BMP tests
Reactor 3
184 Nml CH4/gVS