Assessing the agricultural reuse of the digestate from microalgae anaerobic digestion and co-digestion with sewage sludge

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Abstract

Microalgae anaerobic digestion produces biogas along with a digestate that may be reused in agriculture. However, the properties of this digestate for agricultural reuse have yet to be determined. The aim of this study was to characterise digestates from different microalgae anaerobic digestion processes (i.e. digestion of untreated microalgae, thermally pretreated microalgae and thermally pretreated microalgae in co-digestion with primary sludge). The main parameters evaluated were organic matter, macronutrients and heavy metals content, hygenisation, potential phytotoxicity and organic matter stabilisation. According to the results, all microalgae digestates presented suitable organic matter and macronutrients, especially organic and ammonium nitrogen, for agricultural soils amendment. However, the thermally pretreated microalgae digestate was the least stabilised digestate in comparison with untreated microalgae and co-digestion digestates. *In vivo* bioassays demonstrated that the digestates did not show residual phytotoxicity when properly diluted, being the co-digestion digestate the one which presented less phytotoxicity. Heavy metals contents resulted far below the threshold established by the European legislation on sludge spreading. Moreover, low presence of *E.coli* was observed in all digestates. Therefore, agricultural reuse of thermally pretreated microalgae and primary sludge co-digestate through irrigation emerges a suitable strategy to recycle nutrients from wastewater.

Keywords:

Anaerobic co-digestion; Biofertiliser; Biogas; Land spreading; Phytotoxicity; Thermal pretreatment

1. Introduction

Microalgae-based wastewater treatment systems represent a cost-effective alternative to conventional activated sludge systems. The major advantage is that mechanical aeration is not required, since oxygen is provided by microalgae photosynthesis. Moreover, microalgae cultures are capable of removing nutrients (N, P) from wastewater by means of different mechanisms, such as assimilation or precipitation (Rawat et al., 2011). Furthermore, these systems can also combine wastewater treatment and bioenergy production if harvested microalgal biomass is downstream processed. In particular, anaerobic digestion is one of the most well-known processes to valorise organic waste generated in a wastewater treatment plant. Over the last decades, several studies on biogas production from microalgae have been carried out (Uggetti et al., 2017). They have demonstrated that some microalgae species have a resistant cell wall, which may hamper their bioconversion into methane. Microalgae cell wall disruption could be enhanced by applying pretreatment methods, being the most suitable those pretreatments with low energy demands (Passos et al., 2014). Besides, in the context of microalgae grown in wastewater, co-digestion of microalgae with sewage sludge is a profitable strategy, since the sludge is generated in the same process chain (Uggetti et al., 2017). This could optimise waste management and increase the organic loading rate of the digester (Mata-Alvarez et al., 2014).

Apart from biogas, microalgae anaerobic digestion also produces a digestate that can be reused in agriculture. Even though several studies have pointed out the necessity of recycling nutrients through digestate reuse to improve the sustainability of biogas production from microalgae (Collet et al., 2011), the properties of microalgae digestate for agricultural reuse have yet to be characterised. In general, anaerobic digestates have proper chemical properties for agricultural reuse (Rowell et al., 2001). For instance, they are rich in ammonia nitrogen, readily available for plant uptake, and other macronutrients such us phosphorus and potassium (Teglia et al., 2011a). However, depending on digestates properties, their reuse could be more addressed to improve or maintain the physico-chemical or biological properties of soils (soil amendment) or to boost the plants growing

(fertilisers). In the first case, digestates with high organic matter, organic carbon and organic nitrogen content are preferred, while digestates with important mineral fractions have a higher potential for application as fertiliser (Nkoa, 2014).

Anaerobic digestion is often designed to achieve the maximum energy production, leading to a low stabilisation of the organic matter of the feedstock. As a consequence, digestates may be characterised by a high labile organic matter content and, thus, their agricultural reuse may face agronomic and environmental issues. In fact, it is known that by adding low-stabilised organic matter the soil microbial activity may be excessively stimulated. Indeed, it can produce high CO_2 fluxes from the soil, soil oxygen consumption with sequential nitrogen losses, and phytotoxicity phenomena (Pezzolla et al., 2013; Abdullahi et al., 2008). In addition, the digestate composition can highly vary depending on the feedstock or anaerobic digestion operating conditions. Even the application of a pretreatment on the feedstock previous to anaerobic digestion can influence the final composition of the digestate (Monlau et al., 2015a). Thus, the characterisation of a digestate before evaluating its potential applications is convenient.

When characterising new digestates, particular attention should be addressed to the macronutrients content, potential phytotoxicity and stabilization of the organic matter. *In vivo* bioassays are useful to assess the potential phytotoxicity (Alburquerque et al., 2012; Zucconi et al., 1985). The quantification of CO_2 emissions and the water extractable organic matter (WEOM) in digestate amended soils are suitable strategies to assess organic matter stabilization (Pezzolla et al., 2013; Said-Pullicino and Gigliotti, 2007). On the other hand, land application of anaerobic digestates may also introduce physical, chemical and biological contaminants into soils which may be up-taken by crops and endanger their long-term agricultural activity (Nkoa, 2014). For instance, European legislation on sewage sludge spreading (EC Directive 86/278/CEC) mainly regulates the heavy metals content in digestates to avoid their accumulation in amended soils. However, a more recent European Directive draft (2003/CEC) also proposes restrictions on the occurrence of bio-accumulative organic compounds and their hygenisation before being spread on soils.

Consequently, the presence of these contaminants in digestates should be assessed if they are going to be reused in agricultural soils.

The aim of this study was to characterise for the first time the quality of microalgae digestates for agricultural reuse. To this end, the effluents from three different anaerobic digesters fed by untreated microalgae, thermally pretreated microalgae and thermally pretreated microalgae in co-digestion with primary sludge were analysed. The main parameters evaluated were organic matter, macronutrients and heavy metals content, hygenisation, potential phytotoxicity and organic matter stabilisation.

2. Material and Methods

2.1 Digestate origin and sampling

The microalgal biomass used in this study consisted of a microalgae-bacteria consortia grown in a pilot raceway pond that treated wastewater from a municipal sewer, as described by (Passos et al., 2015). Microalgal biomass was harvested from secondary settlers and gravity thickened in laboratory Imhoff cones at 4 ºC for 24 hours. The pilot plant was located at the laboratory of the GEMMA research group (Barcelona, Spain). According to optic microscope examinations (Motic BA310E, equipped with a camera NiKon DS-Fi2), predominant microalgae were *Chlorella* sp. and diatoms (Fig. 1).

In order to improve microalgae biodegradability, a part of the harvested and thickened biomass was thermally pretreated at 75 ºC for 10h, as suggested by Passos and Ferrer (2014). The pretreatment of microalgal biomass was carried out in glass bottles with a total volume of 250 mL and a liquid volume of 150 mL, which were placed in an incubator under continuous stirring at 75 ºC for 10h. Untreated (control) and pretreated microalgae were digested in lab-scale reactors under mesophilic conditions. Furthermore, the anaerobic co-digestion of pretreated microalgal biomass with primary sludge (25%-75% VS, respectively) was also evaluated. The thickened primary sludge was collected in a municipal wastewater treatment plant near Barcelona.

Thus, the following effluents from microalgae anaerobic digestion were analysed:

- Digester 1 (D1): Microalgal biomass;
- Digester 2 (D2): Thermally pretreated microalgal biomass;
- Digester 3 (D3): Co-digestion of pretreated microalgal biomass and primary sludge.

Anaerobic reactors (1.5 L) were operated on a daily feeding basis, where same volume was purged from and added to digesters using plastic syringes. Operation conditions of the reactors and feedstock characteristics are shown in Table 1. Digestate samples were analysed weekly over a period of 11 weeks of stable reactors operation. Physico-chemical properties were analysed during 11 weeks (n=11) while macronutrients and pathogens were analysed during the last 6 weeks (n=6) and the heavy metals during the 3 last weeks (n=3).

2.2 Digestate characterisation

2.2.1. Physicochemical properties and macronutrients

Total solids (TS), volatile solids (VS), total chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) were analysed according to Standard Methods (APHA, 2005). Ammonium nitrogen (NH_4^+-N) was measured according to the Solorzano method (Solorzano, 1969). Volatile fatty acids (VFA) concentrations were measured by injecting 1 µL of centrifuged (4200 rpm for 8 min) and filtered samples (0.2 µm) into an Agilent 7820A GC after sulphuric acid and diisoprppyl ether addition. The GC was equipped with an auto-sampler, flame ionization detector and a capillary column (DP-FFAB Agilent 30 m x 0.25 mm x 0.25 µm), and operated at injector and detector temperatures of 200 and 300ºC, respectively, with helium as carrier gas. Electric conductivity (EC) was determined with a Crison EC-Meter GLP 31+ and pH with a Crison Portable 506 pH-meter. Total organic carbon (TOC) and total nitrogen (TN) were measured using an automatic analyser (aj-Analyzer multi N/C 2100S). TOC was analysed with an infrared detector (NDIR) according to combustion-infrared method of Standard Methods (APHA, 2005) by means of catalytic oxidation at 800 ºC using CeO_2 as catalyst. Following, a solid-state chemical detector (ChD) was used to

quantify TN as NOx. Phosphorous was determined by means of Olsen-P modified method (Watanabe and Olsen, 1965). Ca^{+2} and Mg^{+2} were analysed by EDTA titrimetric method after ammonium acetate extraction (1N at pH 7), while Na^{+} and K^{+} were determined by flame photometric method after ammonium acetate extraction (1N at pH 7) (MAPA, 1994). Dewaterability was evaluated by means of the capillary suction time (CST) test (Triton Electronics Ltd.).

2.2.2. Heavy metals

In order to determine the heavy metals concentration, samples were dried at 100ºC during 24h. After HCL-HNO_3 (3:1, v/v) digestion (200ºC, 15 min) of dry digestate, Cd, Cr, Cu, Hg, Ni, Pb and Zn were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Elan 6000).

2.2.3. Pathogens

Escherichia coli (*E. coli*) was determined according to Standard Methods (APHA, 2005). The *E. coli* ChromID™ Coli (COLI ID-F) used in this study was supplied by Biomérieux and the culture medium was m-coliBlue24® from Difco.

2.3 Organic matter stabilisation

2.3.1 Soil incubation procedure

Organic matter stabilisation from digestates was evaluated through a microcosm soil experiment. Fresh digestates were used to amend an agricultural soil (soil chemical characterization not shown), using a digestate dose according to the limits prescribed by the European Nitrates Directive (91/676/CEC) for the protection of groundwater against pollution caused by nitrates. Specifically, digestate application doses were calculated to apply 170 kg N ha^{-1}. 200g of soil (dry matter) were amended and placed in an incubation chamber (20 ±2°C) for 30 days at 70% of the water holding capacity.

2.3.2 CO_2 emissions evaluation

176 CO_2 emissions resulting from the organic matter mineralization were measured after 0, 2, 5, 8, 12,
177 20 and 30 days of amending, using an alkaline-trap and subsequent titration. At the same time, 10g
178 (fresh weight) of soil were collected and air-dried for the WEOM determination.
179 *2.3.3 Water extractable organic matter determination*
180 The WEOM was analysed both in the digestates and amended soils. Fresh digestate samples were
181 centrifuged at 4,200 rpm for 6 min and filtered through a 0.45 μm membrane filter (GVS). Soil
182 WEOM was extracted from the dry soil samples with deionised water (solid to water ratio of 1:10
183 w/w) for 24 h. The suspensions were then centrifuged at 4,200 rpm for 6 min and filtered through a
184 0.45 μm membrane filter. Water Extractable Organic Carbon (WEOC) concentration in the filtrates
185 was then measured by an automatic analyser (Analytic Jena-Analyzer multi N/C 2100S) and the
186 WEOM was calculated according the following equation (Pribyl, 2010):
187 $$WEOM = WEOC \cdot 2.0$$
188
189 ***2.4 Potential phytotoxicity***
190 *2.4.1. Seed germination bioassay*
191 To evaluate the germination index (GI), a modified phytotoxicity test employing seed germination
192 was used (Zucconi et al., 1985). Pure digestates together with three dilutions (0.1%, 1% and 10%
193 v/v in deionised water) were used as germination media. A filter paper placed inside a 9 cm
194 diameter Petri dish was wetted with 1 mL of each germination solution and 10 *Lepidium sativum* L.
195 seeds were placed on the paper. 100% deionised water was used as a control. Five replicates were
196 set out for each treatment. The Petri dishes, closed with plastic film to avoid moisture loss, were
197 kept in the dark for 2 days at 20 °C. After the incubation period, the number of germinated seeds
198 and the primary root length were measured. The GI was expressed as a percentage of the control.
199 *2.4.2. Plant growth bioassay*
200 To evaluate the influence of digestate on plant biomass accumulation, a modified phytotoxicity test
201 employing plant growth was used (Alburquerque et al., 2012). Plastic seedbeds made of 12 cells (50

mL/cell with a drainage hole in the bottom) were used for the experiment, after filling them with commercial perlite (2-3 mm diameter). Seedbeds were placed 24 h in a vessel (20x15x5 cm) containing 500 mL of deionised water to reach the saturation of the substrate. Then, 5 seeds of *Lepidium sativum* L. were sown in each cell. After the 3 days needed for the germination and seedlings occurrence, 32 seedlings were left in each seedbed and deionised water was replaced by 500 mL of the digestate dilutions to be tested (0.1 %, 1% and 10% v/v). Pure digestates were not tested in this case, since no germination was observed in the germination test. One seedbed was used as a control, leaving 100% deionised water as growth media. During all the experiment, the vessels were placed in environmental controlled conditions (25±2°C, daily photoperiod of 14 h). At the end of the experiment, after 10 days from the replacement of the growth media, seedlings survived were harvested and their total dry mass (TS) was determined after drying at 105°C. The growth index (GrI) was calculated for each digestates as the percentage of the control (distilled water). The whole experiment was replicated three times.

3. Results and Discussion

3.1 Physico-chemical characterisation

All the digestates analysed presented low dry matter content (~3% TS) (Table 2) and can be considered as liquid products. To ease their management, these digestates could be directly spread on soils in nearby areas. However, if transportation/distribution was required, a dewatering process to reduce the moisture content would be recommended. If we look at the CST measurements, which estimate the ability of each digestate to release water (Gray, 2015), we can see how microalgae digestates presented poor dewaterability (25 and 28 $s \cdot gTS^{-1} \cdot L$ for D1 and D2, respectively), while these results were consistently improved by the co-digestion of primary sludge (8 $s \cdot gTS^{-1} \cdot L$) (Table 2). This is due to the higher dewaterability of primary sludge digestate with respect to microalgae digestate.

On the other hand, the measured pH presented slightly-alkaline values in all digestates

228 (>7.0). Among them, pretreated microalgae digestate (D2) presented the highest pH value, which
229 can be attributed to the higher concentration of NH_4^+-N released from proteins during the thermal
230 pretreatment (Passos and Ferrer, 2014). However, all pH values are compatible with the common
231 pH on soils and therefore, their application should not affect the soil pH.

232 Other factors that may cause an impact on soils after digestate spreading are the EC and
233 VFA's content, since phytotoxicity effects have been correlated to both parameters (Alburquerque et
234 al., 2012; Di Maria et al., 2014). Although EC was moderate in all digestates (5.9-8.2 $dS \cdot m^{-1}$), the
235 digestate from the co-digestion showed the lowest value. Consequently, it would cause less impact
236 on soil. Besides, all digestates showed low VFA's concentrations (Table 2). Again, the lowest value
237 was found in the co-digestion digestate (10 $mgCOD\text{-}eq \cdot L^{-1}$). This indicates that the anaerobic
238 digestion process results in a more stabilised digestate when pretreated microalgae are co-digested
239 with the primary sludge.

240

241 ***3.2 Organic matter and fertiliser properties***

242 The three digestates had moderate organic content due to organic matter mineralization during the
243 anaerobic digestion process. While the two microalgae digestates presented a similar VS/TS ratio of
244 53-54%, the percentage of organic matter in the co-digestion digestate was lower (47%) due to the
245 higher mineralization of primary sludge, which is a more readily biodegradable substrate than
246 microalgae. In fact, the percentage of organic matter in digestates is highly dependent on the type of
247 substrate and the operating conditions of anaerobic reactors (Monlau et al., 2015b). For instance,
248 Teglia et al. (2011a) compared digestates from different origins and found that digestates from agri-
249 food industries showed higher organic matter content than digestates from sewage treatment plants.
250 The results obtained in this study are in accordance with those from similar microalgae anaerobic
251 digestion processes (Passos and Ferrer, 2014, 2015).

252 Several studies have shown that anaerobic digestates can be as effective as mineral fertilisers
253 (Nkoa, 2014). To assess the fertiliser properties of the microalgae digestates, the macronutrients

content was here evaluated (Table 3). The main nutrient present in all digestates was nitrogen. Even so, the nitrogen content of microalgae digestates (both untreated and thermally pretreated) was significantly higher than the co-digestion digestate (39-42%), showing values of 80 g·kg TS^{-1} and 56 g·kg TS^{-1}, respectively. Microalgae digestates presented similar nitrogen values compared to those from farm-byproducts that are frequently applied as nitrogen suppliers on soils (Alburquerque et al., 2012; Zucconi et al., 1985). Moreover, the nitrogen content was much higher than the common values found in sewage sludge digestates (36-40 g·kg TS^{-1}) (Di Maria et al., 2014; Gell et al., 2011), even in the co-digestion digestate. The highest concentration of NH_4^+-N was found in the pretreated microalgae digestate. However, the NH_4^+-N/TKN ratio only varied from 30.9 to 33.8% among all digestates, presenting all of them a similar soluble mineral nitrogen fraction. This means that the organic nitrogen fraction is predominating in all digestates, so they should be used as soil amendment rather than fertiliser (Teglia et al., 2011b). As expected, the digestates also showed low C/N ratios around 3 (Table 2). These values are within the typical range for other digestates as sewage sludge, poultry slurry or pig slurry (Alburquerque et al., 2012; Gutser et al., 2005). Unfortunately, with low C/N ratios, N is present in excess and it can be lost by ammonia volatilization or leaching (Bernal et al., 2009). In order to increase the carbon content in microalgae digestates, they could be co-digested with other carbon rich substrates, like waste paper (Yen and Brune, 2007).

Moderate quantities of P and K^+ were also found in all the digestates (Table 3). P content was slightly higher in microalgae digestates (D2 and D3) compared to the digestate obtained by the co-digestion (3.6-3.9 and 3.2 g P·kg TS^{-1}, respectively). On the other hand, the content of K^+ of the microalgae digestates was 2-fold higher compared to the digestate obtained by the co-digestion (4.8-5.2 and 2.2 g K·kg TS^{-1}, respectively). Conversely to nitrogen, no significant differences were found between P and K^+ contents of microalgae and sewage sludge digestates. In particular, literature reported values from 2.2-3.0 g K·kg TS^{-1} and 3.2-3.8 g P·kg TS^{-1} in sewage sludge

279 digestates (Di Maria et al., 2014; Gell et al., 2011; Tambone et al., 2010), which fall within the
280 range of the co-digestion digestate analysed in the present study. Ca^{2+}, Mg^{2+} and Na^{+} presented
281 similar concentrations in all the cases. This can be attributed to the composition of the wastewater
282 treated in both systems where microalgae and primary sludge were obtained, which came from the
283 same water source. The content of salts should be carefully analysed when applying the digestates
284 to the soils to avoid their salinization, especially the presence of Na^{+} (Daliakopoulos et al., 2016).
285 On the whole, microalgae digestates could especially contribute to nitrogen supply on soils.
286 However, with a moderate NH_4^{+}-N/TKN ratio (<35%) their use should be addressed as soil
287 amendment rather than direct biofertiliser. Indeed, the digestates nutrients content was lower than
288 those recommended by the standards of European countries that have regulated the commercial uses
289 of liquid fertilisers (EC 2003/2003). Conversely, their organic matter content and their high mineral
290 and organic nitrogen content make them suitable for land spreading. Nonetheless, the stability of
291 organic matter and potential toxicity of digestates must be taken into account, along with their
292 potential risks on soil contamination. These issues are analyzed and discussed in the following
293 sections.
294
295 ***3.3 Stabilisation of the organic matter***
296 Figure 2a shows the CO_2 emissions measured from the digestate amended soils studied in
297 the microcosm experiment. Whereas the control (un-amended soil) showed moderately constant
298 emission rates throughout the incubation period, the addition of digestates increased the CO_2 fluxes
299 with respect to the control, particularly in the first days after amendment. Similar results were
300 obtained by other authors after amending soils with anaerobic digestate and compost (Alluvione et
301 al., 2010; Pezzolla et al., 2013). The highest emission rates were observed immediately after
302 applying the digestates for the soils treated with pretreated microalgae (D2) and co-digestion (D3)
303 digestates (230 and 245 $mgCO_2\ kg_{dm}^{-1}\ d^{-1}$, respectively). CO_2 emissions decreased steadily over
304 time, reaching constant values similar to the control ones within 13 days. Conversely, the soil

305 treated with unpretreated microalgae (D1) showed a different behaviour, whose highest value was
306 observed after 2 days from the amendment (170 $mgCO_2\ kg_{dm}^{-1}\ d^{-1}$). Besides, cumulative net CO_2
307 emissions at the end of the incubation period increased in the following order: D1 < D3 < D2 (Table
308 4). Considering the amount of organic carbon added to the soil with the microalgae digestates
309 (Table 4), higher fluxes of CO_2 were expected from D1 and D3 amended soils. However, the
310 highest cumulative CO_2 emissions were detected for the soil amended with thermally pretreated
311 microalgae, indicating that the organic matter of this digestate was less stabilised than the organic
312 matter of the other digestates (D1 and D3). This is in accordance with the fact that D1 and D3 also
313 showed lower biodegradability in the soil than D2. It can be deducted from the values of C-
314 mineralization, expressed as the % of the added TOC that was mineralised at the end of the
315 incubation (Table 4). The lower stabilisation of pretreated microalgae digestate with respect to the
316 other digestates could be attributed to the different anaerobic digesters operations. For instance,
317 comparing the anaerobic digestion of unpretreated and thermally pretreated microalgal biomass,
318 higher NH_4^+-N and VFA concentrations were found in the latter (Passos and Ferrer, 2014). As a
319 consequence, the digestate from thermally pretreated microalgae could be less stabilised and could
320 show higher soluble organic matter content that can be quickly mineralized in the soil. On the other
321 hand, the co-digestion with primary sludge could also reduce the NH_4^+-N and VFA concentrations
322 in the reactors. The addition of easily degradable substances to the soil implies the consumption of
323 soil oxygen that, in some circumstances, can lead to anoxic conditions, fermentation processes and
324 to the production of phytotoxic substances (Wu et al., 2000). Stability-dependent respiration rates
325 were reported by various authors for soils amended with organic materials (Sánchez-Monedero et
326 al., 2004). Most of them also observed CO_2 emissions peaks in the first few days after amendment
327 with an intensity related to the contents of WEOM and microbial biomass. In fact, it is well known
328 that organic amendment can change the amount and quality of dissolved organic matter present in
329 the soil solution (Chantigny, 2003). As WEOM is an easily available organic matter fraction for soil
330 microorganisms, it has important implications on microbial activity and soil respiration. Moreover,

331 Said-Pullicino et al., (2007) have shown that the soluble organic matter fraction of organic
332 amendments tends to decrease with organic matter stabilisation.

333 Figure 2b shows the time course of the WEOM in the digestate amended soils. Digestate
334 application enhanced significantly ($P < 0.05$) the concentration of WEOM in the treated soils with
335 respect to control during the first days after amendment. Following, the WEOM concentration
336 showed a clear decreasing trend during the incubation period due to the soil microbial respiration.
337 While D1 and D3 amended soils showed a decrease of WEOM content to the control level, in the
338 D2 amended soils the WEOM mineralisation appears to be stronger and lead to a final content
339 significantly lower ($P < 0.05$) than the control soils. The WEOM behaviour observed in the D2
340 amended soils and the low biodegradability showed by D1 and D3 appear to be in contrast with the
341 WEOM concentrations in the microalgae-derived digestates (Table 4). In fact, D1 showed a higher
342 content of WEOM with respect to D2 and D3. Therefore, it can be assumed that the labile organic
343 matter of D2 was characterized by a low stability due to the thermal pretreatment of the microalgae
344 biomass that was responsible for the solubilisation of labile and reactive organic compounds. As a
345 consequence, the application of the thermal pretreated microalgae digestate to the soil can lead to
346 the *priming effect*, with strong short-term changes in the turn-over of soil organic matter after the
347 application of low stabilized organic amendments (Kuzyakov et al., 2000).

348 In all the amended soils, the strongest WEOM mineralization appeared to be concluded after
349 13 days from the application, similarly to what was observed for the CO_2 emissions. As already
350 demonstrated by Pezzolla et al. (2013), when an organic amendment is applied to soil, WEOM is
351 strictly related to the soil CO_2 emission rates. In the present work, this fact was confirmed by the
352 correlation between the soil respiration rates of all the soil samples and their WEOM contents.
353 Indeed, a high positive correlation was found ($y = 1.5313x - 2655.5$) to be significant ($r = 0.7750$) at
354 $P < 0.05$ ($n = 28$). In the last two weeks of incubation a constant trend was observed for the WEOM
355 content in the amended soils. This behaviour can be explained considering the dynamic equilibrium

356 that occurs between the consumption of WEOM due to the mineralization and the release of
357 WEOM by the soil microorganism during their hydrolytic activity (Rochette and Gregorich, 1998).
358 In the light of the results obtained, it appears clear that pretreated microalgae digestate is
359 less recommendable for soil application than the other digestates due to the low stabilisation of its
360 soluble organic matter. Indeed, untreated microalgae and co-digestion digestates spreading lead to a
361 lower impact on soil system and higher benefits for the environment and the agriculture.
362
363 ***3.4 Evaluation of the potential phytotoxicity of digestates***
364 Phytotoxicity effects are often found in anaerobic digestates due to the high contents in soluble
365 salts, NH_4^+-N and low weight organic compounds (i.e. volatile fatty acids, phenols) (Alburquerque
366 et al., 2012). In this study, the GI was used to evaluate the digestates phytotoxicity by applying
367 different concentrations of digestate (100%, 10%, 1% and 0.1%) and comparing the germination of
368 cress seeds (*Lepidium sativum* L.) to a control (100% of deionised water) (Fig. 3).
369 The results showed that no germination was detected for any pure digestate. Thus, the GI of
370 pure digestates (0%) indicates that they cannot be spread on agricultural soils without dilution or a
371 stabilisation post-treatment process. For instance, a composting post-treatment would produce a
372 compost where phytotoxic compounds, still abundant in anaerobic digestates and responsible of the
373 absence of germination (Abdullahi et al., 2008), can be reduced. Conversely, positive results in the
374 germination assays were found for digestate dilutions. Untreated and pretreated microalgae
375 digestates (D1 and D2, respectively) gave a similar GI trend, showing the highest GI for the 0.1%
376 dilution (109.9% and 97.3%, respectively). At this dilution (0.1%), the highest GI was observed for
377 D1, probably due to the lower content of ammonia nitrogen with respect to D2 (Table 3). In both
378 cases, the lowest GI value was observed at 10% dilution. On the contrary, no significant differences
379 were observed between 1% and 0.1% dilutions, when values close to the control were achieved. It
380 means that the largest phytotoxic potential was removed at 1% dilution. Concerning D3, there were
381 no significant ($P < 0.05$) differences for the GI between dilutions of 10%, 1% and 0.1% (GI of

97.8%, 109.5% and 101.9% respectively), meaning that the phytotoxicity effect of the microalgae digestate was reduced through the co-digestion. Indeed, co-digestion processes are known to be more advantageous than mono-digestion ones due to a dilution effect of inhibitory compounds, among other factors (Tritt, 1992).

Moreover, the effect of digestates dilutions (10%, 1% and 0.1%) on the biomass production of cress (*Lepidium sativum* L.), expressed as GrI, were evaluated (Fig. 4). Concerning D1, no significant ($P < 0.05$) phytotoxic effect was detected on the production of biomass. Conversely, D2 showed a strong reduction of GrI at the highest concentration tested (10%), which is probably due to the high content of ammonium nitrogen of D2 (Table 3). At lower concentrations (1%, 0.1%), the GrI of D2 increased due to the dilution of the phytotoxic compounds. For both D1 and D2, the 1% dilution which showed a significantly higher ($P < 0.05$) GrI than the 0.1% dilution. As shown for other plants, low level of phytotoxicity can lead to a normal growth, or even higher than the un-stressed control, due to the genetic adaptability of the plants (Wang et al., 2015). This phenomena may be responsible of the GrI behaviours in D1 and D2. Nevertheless, the best performance in the plant growth bioassay was obtained from D3. Thus, co-digestion process appears to be the most suitable process for the reduction of phytotoxicity as already showed by the results obtained from the GI bioassay. Concerning the GrI determination, 10% and 1% dilutions of D3 did not show significant differences with respect to the control, showing the absence of residual phytotoxicity. When diluted at 0.1%, D3 showed plant nutrient, growth stimulant or even phytohormone-like effects (Alburquerque et al., 2012) that lead to a significant increase of the GrI ($P < 0.05$) with respect to the control (128.1%).

In the present work, NH_4^+-N, VFA and EC of the digestates were found to be significantly ($P < 0.05$) and negatively correlated both to GI and GrI, as expected from what described in literature (Alburquerque et al., 2012; Zucconi et al., 1985). Statistical models used in this evaluation are described in Table 5.

In light of what was found in the germination and growth bioassays, agricultural application

of the microalgae-derived digestates through dilution in the irrigation water would be the most suitable option, as the digestate would be diluted before coming in contact with seeds and plants. Moreover, dilution could also avoid salts and heavy metal concentration in the soil (Moral et al., 2005). Co-digestion digestate appeared to be the most suitable for agricultural reuse. In fact, it would require less water for dilution and, thus, it would be a more concentrated organic fertiliser. Moreover, the co-digestion digestate was the only one that did not show residual phytotoxicity; conversely it showed stimulating properties in the *in vivo* assays.

3.5 Potential risks of digestates: heavy metals and pathogens

In order to assess the potential risks of soil contamination after digestate spreading, the occurrence of heavy metals and the presence of pathogens (*E. Coli*) were evaluated.

Concerning heavy metals, their concentrations in the three digestates were lower than the threshold established by the sludge European Directive (EC directive 86/278/CEC), and also by the even more restrictive EU Directive draft (2003/CEC) (Table 6). Although all digestates presented appropriate heavy metal contents for soil application, special attention should be paid to the co-digestion digestate because of its high Zn content that is originated from the primary sludge. This is a particularity of the wastewater treatment plant where the primary sludge was collected, since they receive wastewater from industries generating high Zn concentration in their effluents. With regards to the microalgae digestate, despite microalgae ability for assimilating metals (Suresh Kumar et al., 2015), no significant heavy metal concentrations increase was found in microalgae digestates (D1 and D2) compared to the mixture with the primary sludge (D3) (Table 6).

Regarding the digestate hygenisation, low *E.coli* presence was found in all digestates (Table 7), below the threshold values proposed by the EU Directive draft on spreading sludge on land (less than $5 \cdot 10^5$ colony forming units per gram of wet weight of treated sludge) (2003/CEC). Moreover, it is noteworthy that thermal pretreatment improved the hygenisation leading to absence of *E.coli* in the digestate. In fact, according to the EU draft, the combination of thermal pretreatment and

anaerobic digestion can be considered as an advanced sludge treatment.

4. Conclusions

Agricultural reuse of the digestate from microalgae anaerobic digestion and co-digestion with primary sludge appears to be a promising solution towards zero waste generation in microalgae-based wastewater treatment systems. All microalgae digestates considered in this study presented organic matter and macronutrients content, especially organic and ammonium nitrogen, suitable for agricultural soils amendment. However, the thermal pretreated digestate presented a higher concentration of easily consumable organic carbon that can be mineralized on soil producing environmental impacts. Conversely, untreated microalgae and co-digestion digestates appeared to be more stabilised. *In vivo* bioassays demonstrated that the digestates did not show residual phytotoxicity when properly diluted, being the co-digestion digestate the one which presented less phytotoxicity. Furthermore, it showed interesting stimulant properties for plants. Heavy metals contents resulted far below the threshold established by the European legislation on sludge spreading. Low presence of *E.coli* was observed in all digestates. In addition, the thermal pretreatment improved the hygenisation obtaining absence of *E.coli* in the digestate. In this context, agricultural reuse of thermally pretreated microalgae and primary sludge co-digestate through irrigation emerges as a suitable strategy to recycle the nutrients and organic matter in agriculture.

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461

462 **References**

463 Abdullahi, Y.A., Akunna, J.C., White, N.A., Hallett, P.D., Wheatley, R., 2008. Investigating the
464 effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of
465 municipal solid waste as soil amendment. Bioresour. Technol. 99, 8631–8636.
466 doi:10.1016/j.biortech.2008.04.027

467 Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal,
468 M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial
469 residues. Biomass and Bioenergy 40, 181–189. doi:10.1016/j.biombioe.2012.02.018

470 Alluvione, F., Bertora, C., Zavattaro, L., Grignani, C., 2010. Nitrous Oxide and Carbon Dioxide
471 Emissions Following Green Manure and Compost Fertilization in Corn All rights reserved. No
472 part of this periodical may be reproduced or transmitted in any form or by any means,
473 electronic or mechanical, including photocopy. Soil Sci. Soc. Am. J. 74, 384–395.
474 doi:10.2136/sssaj2009.0092

475 Association., A.P.H., Eaton, A.D., Association., A.W.W., Federation., W.E., 2005. Standard methods
476 for the examination of water and wastewater. APHA-AWWA-WEF, Washington, D.C.

477 Bernal, M.P., Alburquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical
478 criteria for compost maturity assessment. A review. Bioresour. Technol. 100, 5444–5453.
479 doi:10.1016/j.biortech.2008.11.027

480 CEC. Council Directive 86/278/EEC on the protection of the environment, and in particular of the
481 soil, when sewage sludge is used in agriculture. Brussels, 12 June 1986.

482 CEC. Council Directive 91/676/EEC of concerning the protection of waters against pollution
483 caused by nitrates from agricultural sources. Brussels, 12 December 1991.

484 CEC. Regulation (EC) No 2003/2003 relating to fertilisers. Brussels, 13 October 2003.

485 CEC. Proposal for a Directive of the European Parliament and the Council on spreading of sludge

on land. Brussels, 30 April 2003

Chantigny, M.H., 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113, 357–380. doi:10.1016/S0016-7061(02)00370-1

Collet, P., Hélias, A., Lardon, L., Ras, M., Goy, R.-A., Steyer, J.-P., 2011. Life-cycle assessment of microalgae culture coupled to biogas production. Bioresour. Technol. 102, 207–214. doi:10.1016/j.biortech.2010.06.154

Daliakopoulos, I.N., Tsanis, I.K., Koutroulis, A., Kourgialas, N.N., Varouchakis, A.E., Karatzas, G.P., Ritsema, C.J., 2016. The threat of soil salinity: A European scale review. Sci. Total Environ. 573, 727–739. doi:10.1016/j.scitotenv.2016.08.177

Di Maria, F., Sordi, A., Cirulli, G., Gigliotti, G., Massaccesi, L., Cucina, M., 2014. Co-treatment of fruit and vegetable waste in sludge digesters. An analysis of the relationship among bio-methane generation, process stability and digestate phytotoxicity. Waste Manag. 34, 1603–1608. doi:10.1016/j.wasman.2014.05.017

Gell, K., van Groenigen, J., Cayuela, M.L., 2011. Residues of bioenergy production chains as soil amendments: Immediate and temporal phytotoxicity. J. Hazard. Mater. 186, 2017–2025. doi:10.1016/j.jhazmat.2010.12.105

Gray, N.F., 2015. Chapter Seventeen – Capillary Suction Time (CST), in: Progress in Filtration and Separation. pp. 659–670. doi:10.1016/B978-0-12-384746-1.00017-3

Gutser, R., Ebertseder, T., Weber, A., Schraml, M., Schmidhalter, U., 2005. Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. J. Plant Nutr. Soil Sci. 168, 439–446. doi:10.1002/jpln.200520510

Kuzyakov, Y., Friedel, J.., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498. doi:10.1016/S0038-0717(00)00084-5

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S., 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sustain.

512 Energy Rev. 36, 412–427. doi:10.1016/j.rser.2014.04.039
513 Ministerio de agricultura, pesca y alimentación (MAPA). Métodos oficiales de análisis. Tomo III,
514 Madrid, 1994. ISBN: 84-491-0003-8
515 Monlau, F., Kaparaju, P., Trably, E., Steyer, J.P., Carrere, H., 2015a. Alkaline pretreatment to
516 enhance one-stage CH4 and two-stage H2/CH4 production from sunflower stalks: Mass,
517 energy and economical balances. Chem. Eng. J. 260, 377–385. doi:10.1016/j.cej.2014.08.108
518 Monlau, F., Sambusiti, C., Ficara, E., Aboulkas, A., Barakat, A., Carrere, H., 2015b. New
519 opportunities for agricultural digestate valorization: current situation and perspectives. Energy
520 Environ. Sci. 8, 2600–2621. doi:10.1039/C5EE01633A
521 Moral, R., Perez-Murcia, M.D., Perez-Espinosa, A., Moreno-Caselles, J., Paredes, C., 2005.
522 Estimation of nutrient values of pig slurries in Southeast Spain using easily determined
523 properties. Waste Manag. 25, 719–725. doi:10.1016/j.wasman.2004.09.010
524 Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic
525 digestates: a review. Agron. Sustain. Dev. 34, 473–492. doi:10.1007/s13593-013-0196-z
526 Passos, F., Ferrer, I., 2015. Influence of hydrothermal pretreatment on microalgal biomass anaerobic
527 digestion and bioenergy production. Water Res. 68, 364–73. doi:10.1016/j.watres.2014.10.015
528 Passos, F., Ferrer, I., 2014. Microalgae Conversion to Biogas: Thermal Pretreatment Contribution
529 on Net Energy Production. Environ. Sci. Technol. 48, 7171–7178. doi:10.1021/es500982v
530 Passos, F., Gutiérrez, R., Brockmann, D., Steyer, J.-P., García, J., Ferrer, I., 2015. Microalgae
531 production in wastewater treatment systems, anaerobic digestion and modelling using ADM1.
532 Algal Res. 10, 55–63. doi:10.1016/j.algal.2015.04.008
533 Passos, F., Uggetti, E., Carrère, H., Ferrer, I., 2014. Pretreatment of microalgae to improve biogas
534 production: A review. Bioresour. Technol. 172, 403–412. doi:10.1016/j.biortech.2014.08.114
535 Pezzolla, D., Said-Pullicino, D., Raggi, L., Albertini, E., Gigliotti, G., 2013. Short-term Variations
536 in Labile Organic C and Microbial Biomass Activity and Structure After Organic Amendment
537 of Arable Soils. Soil Sci. 178.

538 Pribyl, D. W., 2010. A critical review of the conventional SOC to SOM conversion factor.
539 Geoderma. 156(3), 75-83. doi: 10.1016/j.geoderma.2010.02.003.
540 Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2011. Dual role of microalgae:
541 Phycoremediation of domestic wastewater and biomass production for sustainable biofuels
542 production. Appl. Energy 88, 3411–3424. doi:10.1016/j.apenergy.2010.11.025
543 Rochette, P., Gregorich, E.G., 1998. Dynamics of soil microbial biomass C, soluble organic C and
544 CO2 evolution after three years of manure application. Can. J. Soil Sci. 78, 283–290.
545 doi:10.4141/S97-066
546 Rowell, D.M., Prescott, C.E., Preston, C.M., 2001. Decomposition and nitrogen mineralization
547 from biosolids and other organic materials: relationship with initial chemistry. J. Environ.
548 Qual. 30, 1401–1410.
549 Said-Pullicino, D., Erriquens, F.G., Gigliotti, G., 2007. Changes in the chemical characteristics of
550 water-extractable organic matter during composting and their influence on compost stability
551 and maturity. Bioresour. Technol. 98, 1822–1831. doi:10.1016/j.biortech.2006.06.018
552 Said-Pullicino, D., Gigliotti, G., 2007. Oxidative biodegradation of dissolved organic matter during
553 composting. Chemosphere 68, 1030–1040. doi:10.1016/j.chemosphere.2007.02.012
554 Sánchez-Monedero, M.A., Mondini, C., de Nobili, M., Leita, L., Roig, A., 2004. Land application
555 of biosolids. Soil response to different stabilization degree of the treated organic matter. Waste
556 Manag. 24, 325–332. doi:10.1016/j.wasman.2003.08.006
557 Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite
558 method. Limnol. Ocean. 14, 799.
559 Suresh Kumar, K., Dahms, H.-U., Won, E.-J., Lee, J.-S., Shin, K.-H., 2015. Microalgae – A
560 promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. 113, 329–352.
561 doi:10.1016/j.ecoenv.2014.12.019
562 Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010.
563 Assessing amendment and fertilizing properties of digestates from anaerobic digestion through

564 a comparative study with digested sludge and compost. Chemosphere 81, 577–583.
565 doi:10.1016/j.chemosphere.2010.08.034
566 Teglia, C., Tremier, A., Martel, J.-L., 2011a. Characterization of Solid Digestates: Part 2,
567 Assessment of the Quality and Suitability for Composting of Six Digested Products. Waste and
568 Biomass Valorization 2, 113–126. doi:10.1007/s12649-010-9059-x
569 Teglia, C., Tremier, A., Martel, J.-L., 2011b. Characterization of Solid Digestates: Part 1, Review of
570 Existing Indicators to Assess Solid Digestates Agricultural Use. Waste and Biomass
571 Valorization 2, 43–58. doi:10.1007/s12649-010-9051-5
572 Tritt, W.P., 1992. The anaerobic treatment of slaughterhouse wastewater in fixed-bed reactors.
573 Bioresour. Technol. 41, 201–207. doi:10.1016/0960-8524(92)90002-F
574 Uggetti, E., Passos, F., Solé, M., Garfí, M., Ferrer, I., 2017. Recent Achievements in the Production
575 of Biogas from Microalgae. Waste and Biomass Valorization 8, 129–139. doi:10.1007/s12649-
576 016-9604-3
577 Wang, Q., Que, X., Zheng, R., Pang, Z., Li, C., Xiao, B., 2015. Phytotoxicity assessment of atrazine
578 on growth and physiology of three emergent plants. Environ. Sci. Pollut. Res. 22, 9646–9657.
579 doi:10.1007/s11356-015-4104-8
580 Watanabe, F.S., Olsen, S.R., 1965. Test of an Ascorbic Acid Method for Determining Phosphorus in
581 Water and NaHCO3 Extracts from Soil1. Soil Sci. Soc. Am. J. 29, 677–678.
582 doi:10.2136/sssaj1965.03615995002900060025x
583 Wu L.; Ma Q.; Martinez G.A. Comparison of methods for evaluating stability and maturity of
584 biosolids compost. J Environ Qual. 2000, 29: 424-429. DOI
585 10.2134/jeq2000.00472425002900020008x.
586 Yen, H.-W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce
587 methane. Bioresour. Technol. 98, 130–4. doi:10.1016/j.biortech.2005.11.010
588 Zucconi, F., Monaco, A., Forte, M., Bertoldi, M. de., 1985. Phytotoxins during the stabilization of
589 organic matter. Compost. Agric. other wastes / Ed. by J.K.R. Gasser.

590 **Table 1.** Main parameters of the anaerobic digestion and feedstock properties.

	Digester 1 (D1): Microalgae	Digester 2 (D2): Pretreated microalgae	Digester 3 (D3): Co-digestion
Operation conditions			
Temperature (ºC)	36.2 ± 1.1	36.6 ± 1.8	35.7 ± 1.8
OLR (gVS/L.day)	0.83 ± 0.04	0.82 ± 0.02	0.83 ± 0.01
HRT (days)	30	30	30
Feedstock			
Composition (% VS)	100 % MB	100 % P-MB	25 % P-MB + 75% PS
TS (%)	3.9 ± 0.4	3.7 ± 0.3	3.7 ± 0.4
VS (%)	2.5 ± 0.2	2.4 ± 0.1	2.4 ± 0.1
VS/TS (%)	66 ± 5	66 ± 6	66 ± 8
COD (g/L)	43.4 ± 8.1	44.0 ± 7.0	48.1 ± 8.0

591 Note: OLR= organic loading rate, HRT= hydraulic retention time, TS= total solids, VS= volatile solids, COD= chemical
592 oxygen demand, MB= microalgal biomass; P-MB= pretreated microalgal biomass, PS= primary sludge.
593 Pretreatment conditions: 75ºC, 10h.

Table 2. Main physico-chemical properties and organic matter of the three microalgae digestates analysed (mean ± SD; n=11, except for TOC and TN (n=3)).

Parameter	Units	Digestate D1: Microalgae	Digestate D2: Pretreated microalgae	Digestate D3: Co-digestion
pH	-	$7.35^{a} \pm 0.11$	$7.55^{b} \pm 0.08$	$7.30^{a} \pm 0.15$
EC	$dS \cdot m^{-1}$	$7.0^{b} \pm 0.7$	$8.2^{a} \pm 0.3$	$5.9^{c} \pm 0.4$
TS	$g \cdot g^{-1}$,%	$3.0^{a} \pm 0.1$	$2.9^{a} \pm 0.2$	$3.0^{a} \pm 0.2$
VS	$g \cdot g^{-1}$,%	$1.6^{b} \pm 0.1$	$1.5^{b} \pm 0.1$	$1.4^{a} \pm 0.1$
VS/TS	%	$54^{b} \pm 2$	$53^{b} \pm 1$	$47^{a} \pm 2$
COD	$g \cdot L^{-1}$	$26^{a} \pm 2$	$25^{a} \pm 2$	$24^{a} \pm 1$
TOC	$g \cdot L^{-1}$	7.6 ± 0.1	6.4 ± 0.0	6.1 ± 0.1
TN	$g \cdot L^{-1}$	2.4 ± 0.0	2.2 ± 0.1	1.9 ±0.1
C/N	-	3.17	2.98	3.27
VFA	$mgCOD\text{-}eq \cdot L^{-1}$	$100^{a} \pm 138$	$270^{a} \pm 365$	$10^{a} \pm 25$
CST	s	$795^{b} \pm 71$	$919^{b} \pm 122$	$272^{a} \pm 21$
	$s \cdot gTS^{-1} \cdot L$	$25^{b} \pm 3$	$28^{b} \pm 4$	$8^{a} \pm 1$

Note: TS= total solids, VS= volatile solids, COD= chemical oxygen demand, TOC= total organic carbon, TN= total nitrogen, C/N= Carbon-Nitrogen ratio, VFA= volatile fatty acids, CST= capillary suction time

[a,b,c] letters indicate a significant difference between digestates at a level of $p < 0.05$ after Tuckey's test.

599 **Table 3.** Macronutrients characterisation of the three digestates analysed (mean ± SD, n=6).

Parameter	Units	Digestate D1: Microalgae	Digestate D2: Pretreated microalgae	Digestate D3: Co-digestion
TKN	$gN \cdot L^{-1}$	$2.4^{a} \pm 0.1$	$2.3^{a} \pm 0.1$	$1.7^{b} \pm 0.0$
	$gN \cdot kg\ TS^{-1}$	$79.8^{a} \pm 4.0$	$80.6^{a} \pm 2.2$	$56.0^{b} \pm 1.1$
NH_4^+-N	$gN \cdot L^{-1}$	$0.7^{b} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.5^{a} \pm 0.1$
NH_4^+-N/TKN	%	30.9	33.8	32.5
P	$gP_2O_5 \cdot L^{-1}$	$0.25^{b} \pm 0.02$	$0.27^{b} \pm 0.02$	$0.21^{a} \pm 0.03$
	$gP \cdot kg\ TS^{-1}$	$3.6^{b} \pm 0.3$	$3.9^{b} \pm 0.2$	$3.2^{a} \pm 0.5$
K	$gK_2O \cdot L^{-1}$	$0.17^{b} \pm 0.03$	$0.19^{b} \pm 0.02$	$0.08^{a} \pm 0.03$
	$gK \cdot kg\ TS^{-1}$	$4.8^{b} \pm 0.8$	$5.2^{b} \pm 0.7$	$2.2^{a} \pm 1.0$
Ca	$gCaO \cdot L^{-1}$	$0.43^{a} \pm 0.13$	$0.37^{a} \pm 0.10$	$0.54^{b} \pm 0.07$
	$gCa \cdot kg\ TS^{-1}$	$10.2^{a} \pm 3.1$	$8.9^{a} \pm 2.4$	$13.4^{b} \pm 1.7$
Mg	$gMgO \cdot L^{-1}$	$0.18^{a} \pm 0.09$	$0.21^{a} \pm 0.09$	$0.17^{a} \pm 0.10$
	$gMg \cdot kg\ TS^{-1}$	$3.6^{a} \pm 1.8$	$4.2^{a} \pm 1.8$	$3.6^{a} \pm 2.0$
Na	$gNa_2O \cdot L^{-1}$	$0.40^{b} \pm 0.05$	$0.38^{b} \pm 0.06$	$0.32^{a} \pm 0.03$
	$gNa \cdot kg\ TS^{-1}$	$10.0^{b} \pm 1.3$	$9.4^{b} \pm 1.4$	$8.1^{a} \pm 0.8$

600 Note: TKN= total Kjeldahl nitrogen

601 [a,b] letters indicate a significant difference between digestates at the level of $p < 0.05$ after Tuckey's test.

602 **Table 4.** Carbon mineralization rate from digestate amended soils after 30 days of incubation (mean
603 ± SD, n=3).

Parameter	Units	Digestate D1: Microalgae	Digestate D2: Pretreated microalgae	Digestate D3: Co-digestion
*Total N**	$mg \cdot L^{-1}$	2.4± 0.1	2.2± 0.1	1.9± 0.2
Application dose	mL	13.0	14.3	16.6
TOC_{added}	mg	98.1	92.0	101.1
WEOM	$mg \cdot L^{-1}$	1335.9	892.3	790.5
WEOM added	mg	17.4	12.8	13.1
Net CO_2 emission	mg-C	21.2± 1.9	47.1± 2.1	30.7± 2.6
TOC_{added} *mineralized*	%	21.6 ± 1.7	51.2 ± 6.7	30.4 ± 5.2

604 Note: TOC= total organic carbon, WEOM= water extractable organic matter
605 *: total N values used for the dosage calculation
606

607 **Table 5.** Linear regression equations (y = mx + q) calculated for selected parameters of the
608 digestates (n=11).

Y	x	m	q	r
$N\text{-}NH_4^+$	*GI*	-0.0073	0.7254	0.9054*
VFA		-0.6728	67.351	0.9301*
EC		-0.0067	6.7041	0.9572*
$N\text{-}NH_4^+$	*GrI*	-0.0068	0.6826	0.8691*
VFA		-0.6270	63.0660	0.8862*
EC		-0.0628	6.2935	0.9156*

609 Note: GI= Germination Index, GrI= Growth Index, VFA= volatile fatty acids, EC= electric conductivity
610 *: significant at $P < 0.05$

611 **Table 6.** Concentration of heavy metals in microalgae digestates (mean+SD, n=3).

Parameter	Units	Digestate D1: Microalgae	Digestate D2: Pretreated microalgae	Digestate D3: Co-digestion	Limit values*	Limit values**
Cd	mg·kg TS^{-1}	2.2 ^{a} ± 1.9	2.7 ^{a} ± 0.3	8.6 ^{a} ± 5.4	20-40	10
Cu	mg·kg TS^{-1}	584 ^{a} ± 108	593 ^{a} ± 100	491 ^{a} ± 23	1000-1750	1000
Pb	mg·kg TS^{-1}	47 ^{a} ± 3	49 ^{a} ± 1	221 ^{b} ± 112	750-1200	750
Zn	mg·kg TS^{-1}	637 ^{a} ± 53	592 ^{a} ± 9	2202 ^{b} ± 135	2500-4000	2500
Ni	mg·kg TS^{-1}	104 ^{a} ± 9	127 ^{a} ± 9	101 ^{a} ± 5	300-400	300
Cr	mg·kg TS^{-1}	69 ^{a} ± 2	75 ^{a} ± 14	127 ^{b} ± 9	-	1000
Hg	mg·kg TS^{-1}	2.0 ^{a} ± 0.5	1.7 ^{a} ± 0.6	<1.1 ^{a} ± 0.2	16-25	10

612 *: Limit values according to current European legislation (EC directive 86/278/CEC)
613 **: Limit values according to the European draft (2003/CEC)
614 ^{a,b} letters indicate a significant difference between digestates at the level of $p < 0.05$ after Tuckey's test.

615 **Table 7.** *Escherichia coli* content (CFU/ml) in microalgae digestates (mean ± sd; n=6).

Digestate	**Mean**	**Maximum value**
D1 (microalgae)	39.8	316.2
D2 (pretreated microalgae)	0.0	Absence
D3 (co-digestion)	25.1	199.5

616 Note: CFU= colony forming units

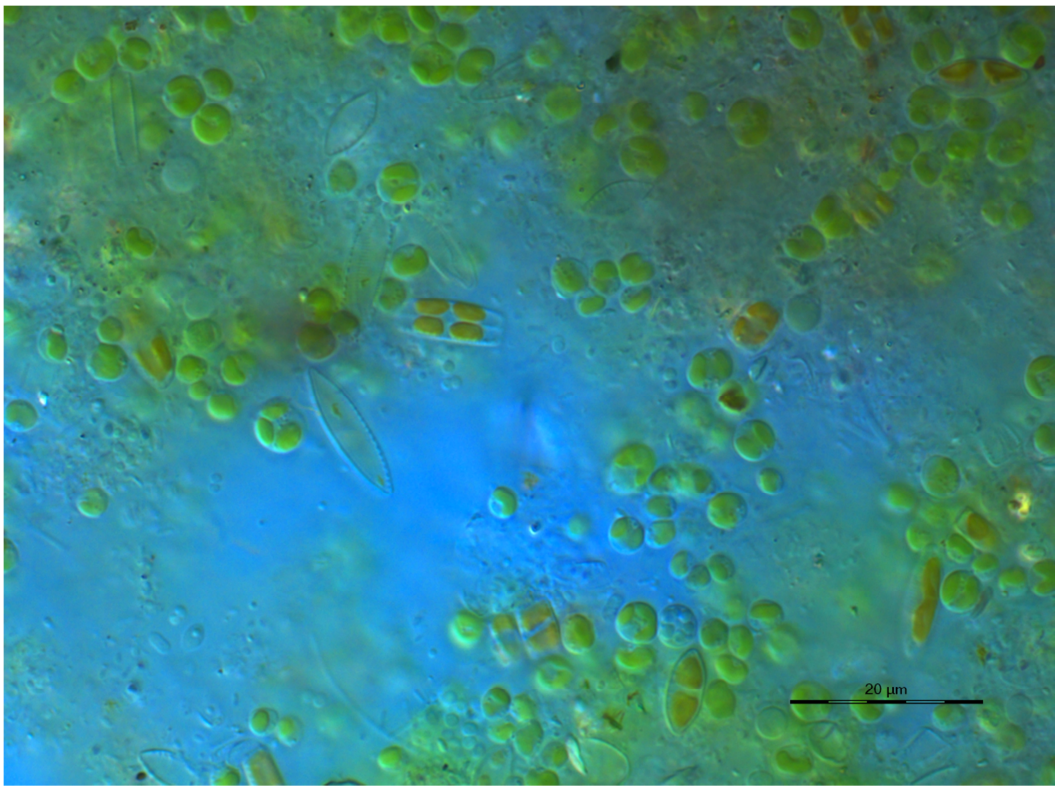
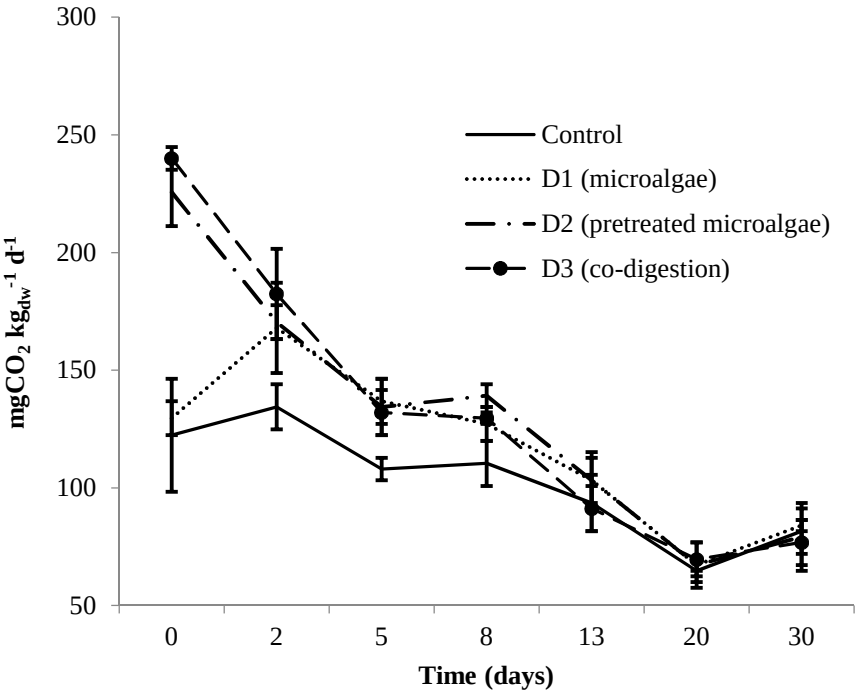
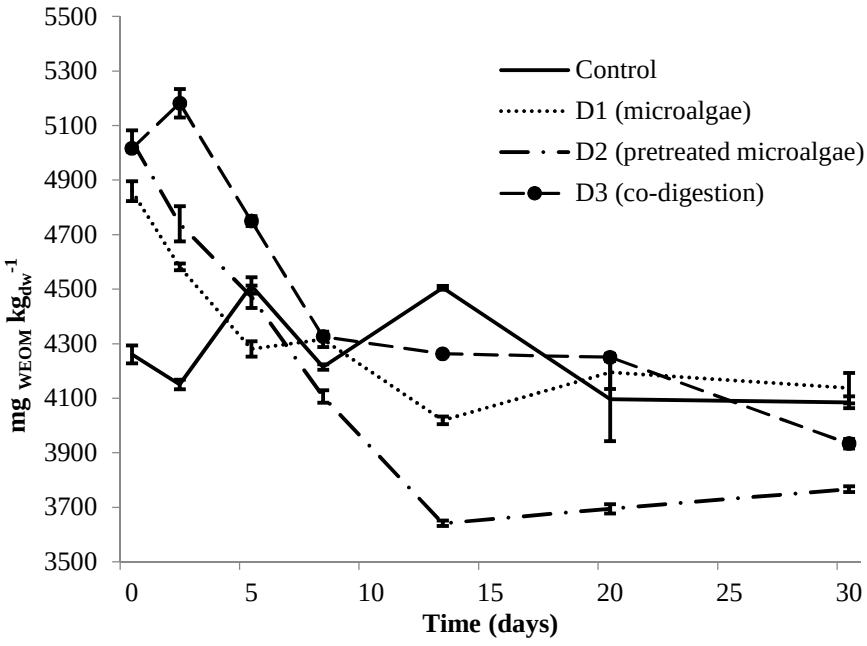


Figure 1. Microscopic image of microalgal biomass mainly composed by *Chlorella* sp. and diatoms.



620 **a)**

621



622 **b)**

623

624

625 **Figure 2.** (a) CO_2 emissions from microalgae-derived digestates amended soil (mean+SD, n=3); (b)

626 Water extractable organic matter content in microalgae-derived digestates amended soil during the

627 incubation period (mean±SD, n=3). Results are expressed on soil dry matter basis.

628

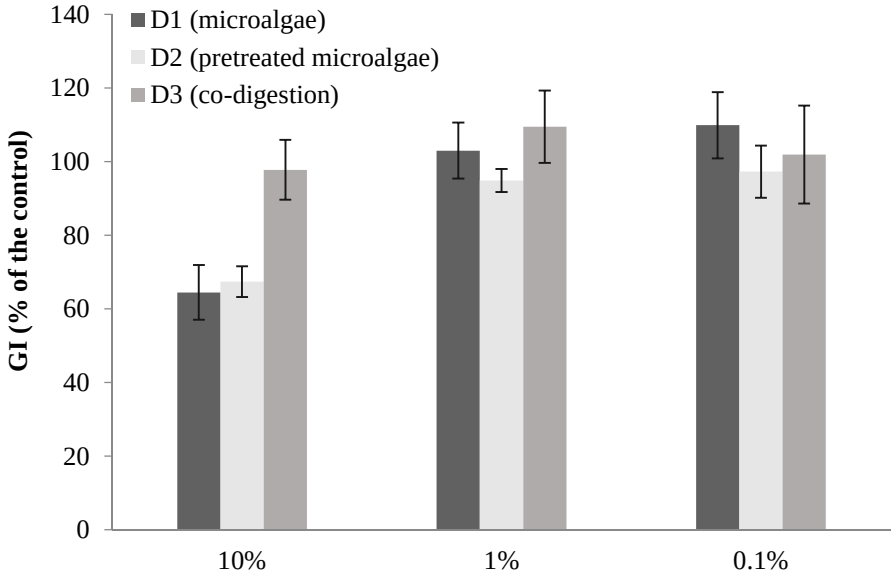


Figure 3. Effects of microalgae digestates and their dilutions on the germination index (GI) of cress (*Lepidium sativum* L.) (mean+SD, n=5). GI was 0% for all the pure (100%) digestates.

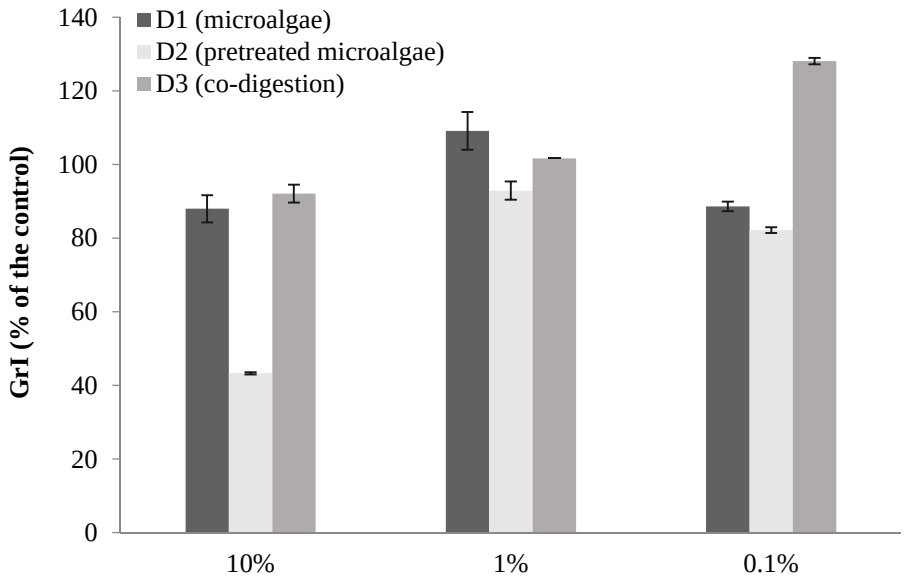


Figure 4. Effects of microalgae digestates and their dilutions on the growth index (GrI) of cress (*Lepidium sativum* L.) (mean+SD, n=5).