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Comparative life cycle assessment of microalgae cultivation for non-energy purposes using different carbon dioxide sources

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3 **Comparative life cycle assessment of microalgae cultivation**
4 **for non-energy purposes using different carbon dioxide**

5 **sources**

6

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15

16

17 **Abstract**

18

19 The ability of microalgae to sequester carbon and at the same time synthesise valuable compounds
20 with potential applications in nutraceutical, pharmaceutical and cosmetic industries makes them
21 attractive for commercial deployment, especially in view of a blue bioeconomy. Among microalgae,
22 the diatom *Phaeodactylum tricornutum* is considered as an important potential source of omega-3
23 polyunsaturated fatty acids, such as eicosapentanoic acid, an essential polyunsaturated fatty acid with
24 anti-inflammatory and antimicrobial properties. The aim of this study was to perform the Life Cycle

25 Assessment of the cultivation of *P. tricornutum* - at semi-industrial scale in photobioreactor - for the
26 production of high-quality bioactive compounds comparing synthetic carbon dioxide supply to a
27 supply with waste carbon dioxide from a biogas upgrading process hypothesizing industrial symbiosis
28 network. The effect of renewable energy use instead of the European electricity mix was also
29 examined. Primary data on the production process, including the stages of cleaning and sterilisation,
30 cultivation, harvesting and freeze-drying, were used. The midpoint impact categories recommended
31 in the ILCD Handbook were used for performing the impact assessment. A sensitivity analysis was
32 also performed on algal productivity, culture medium recirculation factor and amount of solvents per
33 cleaning cycle. Firstly, results indicate in general cultivation and freeze-drying as the most
34 contributing stages to the impacts. Secondly, they demonstrate in the comparative assessment that the
35 use of carbon dioxide from the biogas upgrading is a feasible and attractive alternative to the synthetic
36 one, as it allows for the improvement of the environmental performance of the production process in
37 all the analysed impact categories. Finally, sensitivity analysis suggests that the environmental
38 performance could be further improved by acting on other key factors, such as electricity source,
39 nutrients culture medium and cleaning solutions.

40

41 **Keywords:** Environmental assessment; Marine microalgae; Bioactive compounds; High-value
42 products; CO₂ fixation; Waste CO₂

43

44

45 **Chemical symbols**

46

47 CH₄ Methane

48 CO₂ Carbon dioxide

49 H₂S Hydrogen sulfide

50

51 **Subscripts**

52

53 DW Dried Weight

54

55 **Acronyms**

56

57 ACP Acidification Potential

58 ADP Abiotic Resources Depletion Potential

59 EPA Eicosapentanoic Acid

60 ETP Terrestrial Eutrophication Potential

61 FAETP Freshwater Aquatic Ecotoxicity Potential

62 FETP Freshwater Eutrophication Potential

63 GHG Greenhouse Gas

64 GWPebc Global Warming Potential, excluding biogenic carbon

65 GWPIbc Global Warming Potential, including biogenic carbon

66 HTPc Human Toxicity Potential with cancer effects

67 HTPnc Human Toxicity Potential with non-cancer effects

68 IRPhh Ionizing Radiation Potential with human health impacts

69 LCA Life Cycle Assessment

70 LCI Life Cycle Inventory

71 LCIA Life Cycle Impact Assessment

72 LUCP Land Use Change Potential

73 METP Marine Eutrophication Potential

74 ODP Ozone Layer Depletion Potential

75 PBR Photobioreactor

76 POFP Photochemical Ozone Formation Potential

77	PUFAs	Polyunsaturated Fatty Acids
78	RIPpm	Respiratory Inorganics Impact Potential with particulate matter
79	TFA	Total Fatty Acids
80	WRDP	Water Resource Depletion Potential

81

82

83 **1. Introduction**

84

85 The importance of algae for the blue bioeconomy has been recognised by the European Commission
86 (2019) both because of their role in the ecosystem and their value for commercial applications that
87 diminish the pressure on land-based products. Microalgae have, as a matter of fact, the ability to
88 convert CO₂, water and sunlight to sugars, from which macromolecules, such as lipids and other many
89 beneficial and valuable compounds, can be obtained (Vonshak, 1993).

90 In the last decades, microalgae have been mostly regarded as a promising bioenergy source - through
91 lipids extraction - due to notable advantages in comparison with other bioenergy feedstocks, such as
92 a higher growth rate than that of terrestrial plants and the possibility to be grown on non-productive
93 land (Pienkos and Darzins, 2009). Therefore, numerous studies have been focused mainly on growing
94 microalgae for energy purposes, as an alternative to current fossil-based sources (Faried et al., 2017).
95 Despite these intense research efforts, at present large-scale microalgae cultivation for commercial
96 production of biofuels has been limited, since current technologies appear to be not sufficient to reach
97 economic viability and sustainability targets (Monari et al., 2016; Quinn and Davis, 2015). The main
98 bottleneck is currently represented by the energy intensity of the operations required for the growing
99 and harvesting of microalgae, as well as of the downstream processes to obtain biofuels (Dasan et al.,
100 2019; Delrue et al., 2012).

101 As a result, microalgae biomass cultivation for the production of added-value chemicals remains at
102 the moment the most attracting application to exploit their potential (Barsanti and Gualtieri, 2018).

103 Microalgae can synthesise a large variety of added-value compounds of particular commercial
104 interest, including pigments, omega-3 fatty acids, proteins, polysaccharides and phenolics (Stengel et
105 al., 2011). Most of these components are considered bioactive compounds, which are essential and
106 non-essential compounds (e.g., vitamins or polyphenols) having a beneficial effect on human health
107 (Biesalski et al., 2009). This explains their applications in various consolidated sectors, including
108 nutraceuticals, pharmaceuticals, cosmetics (Olaizola, 2003), and in emerging sectors such as natural
109 pesticides and plant protectants products (Azmir et al., 2013). Specifically, thanks to their high selling
110 price, algal high-value commercial products can offset the capital and the operating costs of the
111 process (Suganya et al., 2016). As a result, algal compounds are well established in the marketplace
112 and new microalgae products are likely to be developed and commercialised in the next years
113 (Borowitzka, 2013). Attention and expectations have been placed in particular on those co-producing
114 processes typical of integrated biorefineries, which require the valorisation of the entire biomass (Su
115 et al., 2017; Thomassen et al., 2017). Algal residues from biorefinery valorisation have been for first
116 time quantified as residual biomasses by Greggio et al. (2019) with reference to an Italian region.

117 Life Cycle Assessment (LCA) is recognised by the European Commission (2003) as the best
118 framework for assessing the potential environmental impacts of products. However, at present few
119 LCA studies have addressed the environmental aspects of algal production not primarily for energy
120 purposes, despite the increasing economic attractiveness of microalgae exploitation for producing
121 high added-value compounds. Some authors have compared systems providing the same products
122 from traditional sources and microalgae. For instance, Taelman et al. (2015) compare through LCA
123 protein meal from microalgae and from soybean, finding that the algal production system, due to its
124 immature small scale, has high energy consumption and therefore is not competitive with the well-
125 established soy production system; nevertheless, using a sensitivity test, they show the possibility to
126 overcome this gap through scale-up efficiencies and a switch to renewable electricity sources.

127 Smetana et al. (2017) perform the LCA of different cultivation techniques and microalgae species to
128 obtain protein concentrates, eventually comparing their environmental performance with that of

129 traditional protein sources; the study highlights the presence of alternatives which appear to be
130 beneficial with respect to the use of meat sources. A third example of comparison between traditional
131 and innovative source of the same substance is provided by Kyriakopoulou et al. (2015), that perform
132 a comparative analysis between cultivation of *Dunaliella salina* and carrot farming for the production
133 of β -carotene; although microalgae cultivation exhibits a greater environmental impact on biomass
134 basis, the considerably higher content of β -carotene in *D. salina* leads to higher extraction yields and
135 therefore lower impacts.

136 Gong and You (2015) perform a multi-objective optimisation of the co-production of added-value
137 chemicals along with biofuels and confirm its convenience under both economic and environmental
138 criteria; however, it is noteworthy that global warming is the only impact category assessed in this
139 work. Pacheco et al. (2015) consider the co-production of biohydrogen and pigments, concluding
140 that, although it is not possible to disregard the economic benefits of pigments production, the high
141 energy demand for their extraction negatively affects the overall sustainability. Other authors
142 compared production alternatives by identifying possible hotspots and potential improvements before
143 the implementation at larger scale. Among these, Pérez-López et al. (2014a), Pérez-López et al.
144 (2014c) and Pérez-López et al. (2014b) assess the production of eicosapentaenoic acid from *P.*
145 *tricornutum*, a basket of 5 bioactive compounds from *Tetraselmica suecica* and astaxanthin from
146 *Haematococcus pluvialis*, respectively. Similarly, Papadaki et al. (2016) examine different
147 combinations of pre-treatment and extraction procedures to recover a bioactive compound
148 (phycocyanin) from the cyanobacterium *Spirulina platensis*. Very recently, (Espada et al., 2019)
149 compare, from the environmental point of view, two extraction procedures to obtain β -carotene from
150 *D. salina*. Finally, Bussa et al. (2019) evaluate the potentialities of microalgae for the production of
151 polylactid acid and highlight two critical factors: the optimal growing conditions for microalgae and
152 the effect of the properties of the end product.

153 In general, the few existing studies highlight that: i) production of high-value compounds from
154 microalgae is energy-intensive and environmentally improvable; ii) exploiting all algal biomass

155 components increases the economic feasibility (De Bhowmick et al., 2019; Mishra et al., 2019;
156 Vanthoor-Koopmans et al., 2013); iii) biomass production should be combined with bio-remediation
157 and CO₂ mitigation services, when feasible, in order to make the process economically viable and
158 environmentally sustainable (Wang et al., 2008).

159 On this last point, it is to be remarked how the growing concern about global climate change, of which
160 anthropogenic CO₂ emissions are mainly responsible, is leading to focus on CO₂ capture potential of
161 microalgae, ascertained that the overall productivity of algal cultures can benefit from the supply of
162 an external source of CO₂ (Kassim and Meng, 2017; Lam et al., 2012; Rezvani et al., 2016). The
163 amount of additional CO₂ needed in the cultivation process in order to support algal growth depends
164 on several factors, first of all on the algal strain and subsequently on a series of operational
165 parameters, such as temperature, pH, light intensity, O₂ levels, or presence of inhibitory compounds
166 (López et al., 2013). The direct injection of a CO₂-rich gas stream into microalgae cultures can
167 improve the mass transfer of CO₂ and, within certain limits, the rate of photosynthetic CO₂
168 assimilation (Zhao and Su, 2014). This additional CO₂ can conveniently be recycled from different
169 industrial processes, including combustion processes for energy production (Kroumov et al., 2016),
170 cement manufacturing (Cuellar-Bermudez et al., 2015) and biogas upgrading processes (Xia and
171 Murphy, 2016).

172 With reference to the latter, it should be considered that typical biogas contains 55–70% methane
173 (CH₄) and 30–45% carbon dioxide (CO₂). Other components include water, oxygen and other
174 impurities. Reducing CO₂ and impurities content significantly improves the quality of biogas and
175 makes it fit standard requirements. There are several means of reducing the content of CO₂ such as
176 physical, chemical and biological methods (Kao et al., 2012; Seyed Hosseini et al., 2018). Microalgal
177 biomass has also been identified as a means of biogas upgrading, thus achieving the CO₂ removal by
178 biotechnology rather than through conventional physical or chemical removal techniques. However,
179 the potential of biotechnologies for CO₂ removal from biogas has been assessed only at laboratory or
180 pilot scale (Muñoz et al., 2015).

181 In recent years, intensive research efforts have been focused on the investigation of the algal bio-
182 fixation potential of the CO₂ contained in post-combustion flue gases (Huang et al., 2016). These
183 studies are mainly aimed at assessing the algal response in terms of productivity, particularly with
184 regard to possible negative effects of the pollutants typically present in a gas of this type, such as CO,
185 SO_x, NO_x, C_xH_y, particulate matter, halogen acids and heavy metals (Van Den Hende et al., 2012).
186 Moreover, some studies on biotechnological upgrading of biogas have shown that methane does not
187 exert any negative effect on algal growth, at the typical concentrations of a gas produced by anaerobic
188 digestion of biomass (Meier et al., 2015). It should be noted that the use of waste gases from industrial
189 processes is likely to influence not only the productivity but also the quality of the biomass produced,
190 due to the presence of other gases considered as contaminants in the gas. However, the above
191 mentioned reports rarely give information on biomass quality parameters, except for some cases in
192 which the variation induced on the lipid content is mentioned (Chiu et al., 2011; Lizzul et al., 2014),
193 since they are mainly focused on the CO₂ capture function rather than on producing biomass that can
194 be converted into useful products (Ho et al., 2011).

195 Established that it is necessary to ensure that the quality standards of the biomass produced are not
196 compromised when supplying waste gases in the cultivation process, a detailed evaluation of the
197 actual environmental implications arising from this strategy should be performed.

198 Taking this into account, the present study aimed to determine whether the use of waste CO₂
199 recovered from a biogas upgrading process, in place of synthetic commercial CO₂, could bring
200 environmental benefits to the life cycle of the cultivation of microalgae for non-energy purposes. The
201 selected microalga was *Phaeodactylum tricoratum*, employed in the production of bioactive
202 compounds such as Polyunsaturated Fatty Acids (PUFAs). In the study two scenarios, which differ
203 only in terms of typology of carbon dioxide additional source, were compared by means of LCA
204 methodology. In the last part of the paper, a third scenario was analysed in order to test the effect of
205 energy source on the environmental performances of the system. To the best of our knowledge, few
206 papers performed an LCA study to analyse the environmental performances of the cultivation of *P.*

207 *tricornutum* and, in the context of microalgae production for added-value compounds, this is the first
208 paper that uses experimental data when assessing the advantages of using waste CO₂ in the cultivation
209 process.

210

211 **2. Material and methods**

212

213 *2.1 Microalgae description*

214 The microalga selected for the study was the marine diatom *P. tricornutum* (strain PTN0301), isolated
215 from water samples collected in the North Sea in 2003.

216 *P. tricornutum* is a promising source of PUFAs, which are reported to have anti-inflammatory and
217 antimicrobial properties, and to be helpful against cardiovascular diseases (Santos-Sánchez et al.,
218 2016). It is characterised by high growth rates and protein content (40-60% of the dry weight) (Buono
219 et al., 2016; Mirón et al., 2003) as well as by a high amount of Eicosapentaenoic Acid (EPA) that can
220 reach a concentration of up to 30% of Total Fatty Acids (TFAs) in this algal cells (Qiao et al., 2016).
221 Due to its composition and growth performance, this species results suitable for aquaculture feeds,
222 human health supplies, and vegetarian diets (Borowitzka, 2013).

223 Monoclonal cultures were set up using F/2 medium (Guillard and Ryther, 1962) with a salinity of 20.
224 Cultures were maintained at 20 ± 1 °C under a 16 h light:8 h dark photoperiod and irradiance of 100–
225 110 $\mu\text{mol photon/m}^2/\text{s}$.

226

227 *2.2 Life Cycle Assessment methodology*

228 This study applied LCA in order to perform a comparative evaluation of the environmental
229 performance of the different scenarios. LCA is a standardised methodology which allows for the
230 quantification of environmental impacts associated to any product or service considering its entire
231 life cycle, from raw material acquisition through production, transportation, use and ultimately the
232 products' end-of-life. Process LCAs are defined by the ISO 14040 series (ISO, 2006a), stating that

233 four main steps must be followed: (1) goal and scope definition, (2) Life Cycle Inventory (LCI)
234 analysis, (3) Life Cycle Impact Assessment (LCIA) and (4) interpretation.

235

236 *2.3 LCA goal and scope definition*

237 The goal of the present study was to assess the environmental performances of the production of *P.*
238 *tricornutum* for non-energy purposes on a semi-industrial scale, evaluating the use of two different
239 carbon sources. In the first trial the biomass was fed with a stream of commercial CO₂ of high purity
240 grade, while in the second one waste CO₂ from biogas upgrading was used. These trials will be
241 indicated as scenarios from hereafter. A third scenario was implemented in order to compare the
242 improvements that can be obtained by replacing the CO₂ source with those that may derive from the
243 change in the energy source used by the plant.

244 The product system under investigation was assessed “from cradle to gate”, omitting the use and end-
245 of-life phases. As the importance of the infrastructure of a chemical production plant is commonly
246 assumed to be low (Geisler et al., 2004) or insignificant (Hischier et al., 2005), in this study the
247 impacts from construction and maintenance of production plant and equipment were neglected.
248 Transportation phase was assessed for all products delivered to the plant, following recommendations
249 by Frischknecht et al. (2005).

250 The final product of the system is the dried algal biomass, with a 5% w/w of water content, which
251 resulted to have a very comparable biochemical composition (Simonazzi et al., 2019) between the
252 two tested conditions (i.e. supply of commercial or waste CO₂). Consequently, the extraction process
253 was not included in the analysis and the functional unit chosen was 1 kg Dried Weight (DW) of algal
254 biomass.

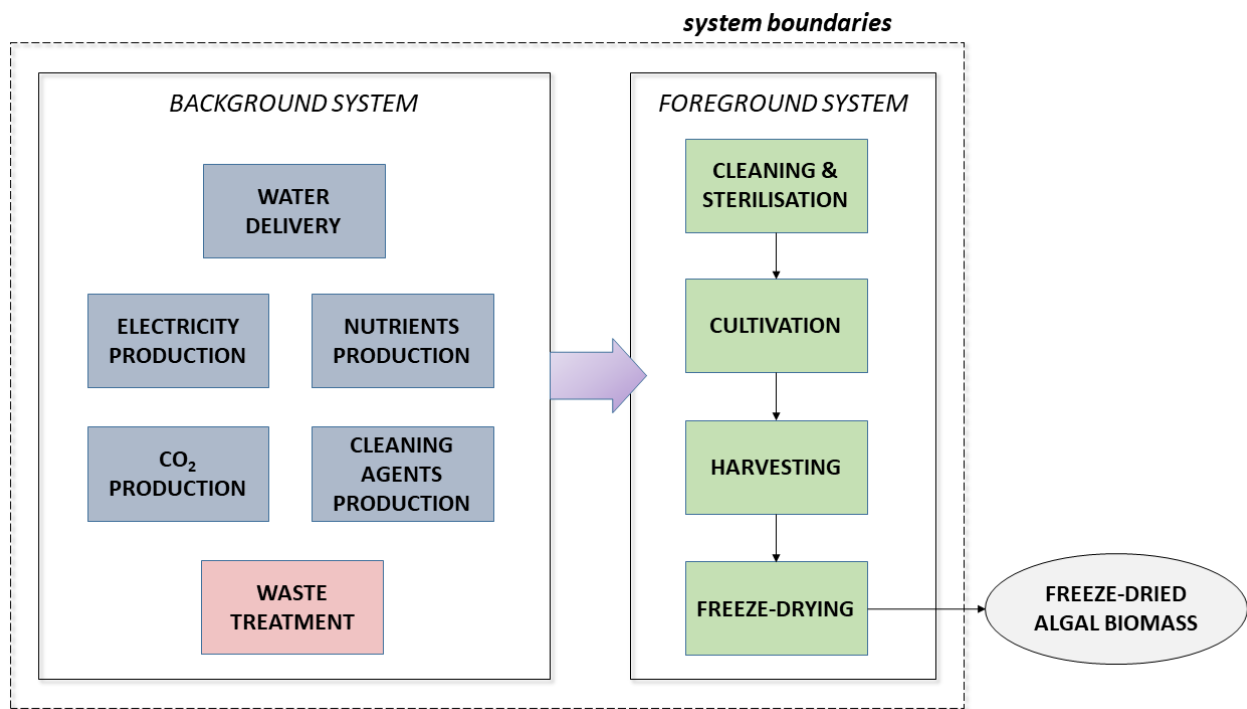
255 This study was based mainly on primary data from a semi-industrial plant (250 l column
256 Photobioreactor (PBR)) for all that concerns the equipment and its consumption and modelled
257 according to the system described in paragraph 2.4. Microalgae growth rates were derived from
258 laboratory tests at a smaller scale (70 l column PBR, described in paragraph 2.5.2) since the use of

259 waste CO₂ for microalgal cultivation is at an experimental stage not yet applied at semi-industrial
260 scale.

261

262 2.4 Overview of the production system

263 The production system is outlined in Figure 1, giving an overview of the process units and system
264 boundaries.



265

266 **Fig. 1** - *Process chain and system boundaries overview.*

267 The process chain was analysed considering four main steps: (1) Cleaning and sterilisation, (2)
268 Cultivation, (3) Harvesting and (4) Freeze-drying.

269 Biomass cultivation can take place in many technological configurations, which can be basically
270 divided into open and closed systems (Tredici, 2004). Technologies based on open systems are
271 generally cheaper and much simpler than closed PBR systems. However, PBRs allow for a better
272 control over the process variables, a much lower contamination risk and a higher CO₂ fixation
273 efficiency (Ho et al., 2011; López et al., 2013). For this reason, closed systems are the most effective

274 method to grow microalgae for the production of bioactive compounds with high-quality standards
275 and the supply of CO₂ at high concentrations (Pulz, 2001).

276 Accordingly, in the present study microalgae cultivation in PBRs was considered.

277

278 *2.4.1 Cleaning and sterilisation*

279 Initially, the PBR is cleaned with a solution of hydrochloric acid, in order to remove saline concretions
280 deriving from previous usage; thereafter it is sterilised with a solution of sodium hypochlorite and
281 washed twice with tap water and deionised water. These operations can take place between one
282 production cycle and the following one, that is to say whenever the PBR is emptied for biomass
283 collection. Although the frequency with which this occurs depends on the biomass growth rate, since
284 harvesting is carried out at a fixed biomass concentration, it was assumed that cleaning and
285 sterilisation procedures take place, on average, every 20 days in any case (hypothesis based on
286 experimental data).

287 At the end of each cleaning cycle, the solution containing hydrochloric acid is sent to a wastewater
288 treatment facility, while sodium hypochlorite is collected in solid state by evaporating the solution
289 and sent to a hazardous waste disposal process.

290

291 *2.4.2 Cultivation*

292 The 250 l column PBR (column height 220 cm, diameter 55 cm) is inoculated and filled with modified
293 F/2 culture medium (Guillard and Ryther, 1962), and the culture initial concentration is of 0.3 g/l
294 (biomass DW). Temperature in the reactor is maintained at 22 °C. The reactor presents a photoperiod
295 with 16 h light and 8 h dark periods and light intensity of 150 μmol photon/m²/s. A constant air flow
296 is provided in the culture, essentially for its mixing (approximate bubble size 0.5 cm). In order to
297 support microalgal growth, a CO₂ flow is supplied for 24 minutes per day (6.25 l/min). While in the
298 first scenario this flow consists of synthetic commercial CO₂ of high purity grade (>99.5% v/v), in

299 the case of waste CO₂ scenario the flow has an average CO₂ content of 75%, also containing residual
300 CH₄ (approximately 16%) and other compounds from biogas upgrading (i.e. H₂S 24 ppm).

301 In this phase, electricity consumption was considered for artificial lightning of the PBR (internal and
302 external), air blowing and monitoring with electronic equipment.

303

304 *2.4.3 Harvesting*

305 Biomass concentration is constantly monitored, and harvesting takes place when the concentration of
306 1 g/l is reached. Only 70% of total biomass in the PBR is recovered for each harvesting step, since a
307 certain amount of culture is needed for the following productive cycle. Wet algal biomass is collected
308 by centrifugation. The water content of algal biomass after centrifugation was assumed to be of about
309 85%_w. Waste culture medium separated from algal biomass is discharged and sent to wastewater
310 treatment (no recirculation).

311 The power consumption resulting from this phase is attributable solely to the continuous flow
312 centrifuge that performs the separation of the biomass from the cultivation medium.

313

314 *2.4.4 Freeze-drying*

315 After centrifugation, algal biomass is stored at -20 °C in a chiller and subsequently lyophilised. The
316 final water content of the freeze-dried algal biomass was assumed to be less than 5% w/w.

317 The inputs considered for this phase were solely the electrical consumption of the two devices used
318 (chiller and freeze-dryer).

319

320 *2.5 Life Cycle Inventory*

321 Except for microalgae growth rates, any other inventory data for the foreground system were based
322 on primary data from a semi-industrial plant set in Italy producing a different species of microalgae
323 (*Arthrospira platensis*) and adjusted to a hypothetical scenario which consider *P. tricornutum*
324 production.

325 The databases used for obtaining background data are Gabi Professional Database and Ecoinvent v.2
326 Database.

327

328 *2.5.1 Primary data of the semi-industrial production system*

329 Total amounts of the input and output flows for the analysed foreground system in both scenarios are
330 shown in Table 1, including the reference to the databases used for the corresponding background
331 processes.

332

333 **Tab. 1** - *Input and output flows of the analysed foreground system in “Synthetic CO₂” and “Waste CO₂” scenarios*

334

Flow	Background Process	Database	Amount ("Synthetic CO ₂ " scenario)	Amount ("Waste CO ₂ " scenario)	unit
<i>Input flows:</i>					
Tap water	EU-28 Tap water	GaBi	2475	2412	l
Deionised water	EU-28 Water (deionised)	GaBi	1134	1087	l
Synthetic CO₂	DE Carbon Dioxide	GaBi	26747	0	g
Waste CO₂	<i>(elementary flow)</i>	-	0	25617	g
Electricity	EU-28 Electricity Grid Mix	GaBi	611	597	kWh
KNO₃	RER potassium nitrate, as N, at regional scale	Ecoinvent	99.8	99.8	g
NaH₂PO₄	RER sodium phosphate, at plant	Ecoinvent	37.3	37.3	g
Na₂SiO₃	RER sodium silicate, Furnace Process, pieces	Ecoinvent	13.0	13.0	g
EDTA	RER, EDTA, ethylenediaminetetraacetic acid, at plant	Ecoinvent	3.59	3.59	g
FeCl₃	CH iron(III) chloride, 40% in H ₂ O, at plant	Ecoinvent	4.73	4.73	g
ZnSO₄ · 7H₂O	RER zinc monosulphate, ZnSO ₄ .H ₂ O, at plant	Ecoinvent	0.0137	0.0137	g
NaClO	RER sodium hypochlorite, 15% in H ₂ O, at plant	Ecoinvent	1384	1326	g
HCl	DE Hydrochloric acid (32%), production mix, at plant	GaBi	526	504	g

<i>Output flows:</i>					
Algal biomass (dried weight)	<i>(product)</i>	-	1000	1000	g
Wastewater	EU-28 Municipal wastewater treatment (mix)	GaBi	2468	2406	l
NaClO	EU-28 Glass/inert waste on landfill	GaBi	1384	1326	g
CO₂	<i>(elementary flow)</i>	-	24897	23767	g

335

336

337 A detailed description of the equipment and its consumption is given in Table 2. The electricity
338 consumption was calculated on the basis of the power of the equipment and the duration of operation.
339 It can immediately be noted that electricity consumption related to harvesting and freeze-drying
340 stages is not dependent on the type of CO₂ used; it is not influenced by productivity indeed, being
341 constant per unit of treated biomass.

342

343 **Tab. 2** - *Summary of the equipment and its electricity consumption for the production of 1 kg_{DW} of*
344 *P. tricornutum in the base scenario*

345

	Amount (kWh)	
	“Synthetic CO ₂ ”	“Waste CO ₂ ”
<i>Cultivation</i>		
Lamps	191.7	183.6
Compressor	95.9	91.8
Control unit	42.7	40.9
<i>Harvesting</i>		
Centrifuge	8.9	8.9
<i>Freeze-Drying</i>		
Chiller	32.0	32.0
Freeze-Dryer	240.0	240.0

346

347 2.5.2 Description of laboratory experimental tests

348 Information concerning algae growth rates refers to a specific study performed in indoor 70 l column
349 PBRs (column height 110 cm, diameter 40 cm, M2M Engineering, Italy). The PBRs were internally
350 illuminated with cool white neon at continuous irradiance of 300 μmol/m²/s and a photoperiod of
351 16:8 h, maintained at a constant room temperature of 20 ± 1 °C.

352 The experimental tests consisted in the simultaneous cultivation of *P. tricornutum* in two PBRs
353 differing only for the type of feeding CO₂ (Simonazzi et al., 2019). The same specific inputs of CO₂
354 and nutrients of the hypothetical semi-industrial scale system were used. The waste CO₂ consisted of
355 the off-gas produced by a biogas upgrading process with membranes, implemented in the GoBioM
356 project (European Regional Fund 2014-2020 programme). Biomass growth was monitored until the
357 stationary growth phase (day 12), retrieving information about algal growth rates. Experiments
358 showed a similar average productivity, which in the case of waste CO₂ was equal to 0.046 g/l/d,
359 compared to the one with synthetic CO₂ equal to 0.044 g/l/d. Final biomass composition was analysed
360 in terms of main compounds (i.e. proteins, polysaccharides, lipids) and cell elemental composition,
361 revealing the absence of significant differences between the two cultures. The possible assimilation
362 by the alga of other off-gas compounds has not been evaluated, considering it negligible for the
363 purposes of the environmental assessment.

364 The output of CO₂ was derived by difference between CO₂ input and CO₂ fixation rate. The latter
365 was estimated from the biomass productivity by applying the stoichiometric CO₂ requirement factor
366 for microalgae growth of 1.85 g CO₂/ g biomass (Posten, 2009).

367

368 2.5.3 Secondary data

369 Concerning the background system, inventory data for the production and delivery of water,
370 electricity production, synthetic CO₂ production and waste treatment and management were taken
371 from the Gabi Professional Database. Inventory data for the production of nutrients and washing
372 agents were taken mostly from the Ecoinvent Database, since they were not available in the Gabi
373 Professional Database.

374 The transport of the different inputs to the plant was modelled considering a small diesel truck with
375 a 9.3 t payload. Following the recommendations by Frischknecht et al. (2005) for transport distances
376 in absence of real market information, the following distances were assumed: 600 km for chemicals
377 used in the preparing of the culture medium, delivered as salts; 100 km for washing agents (sodium

378 hypochlorite 15% and hydrochloric acid 32%); 500 km for synthetic commercial CO₂; 100 km for
379 waste CO₂, assuming a relative proximity to the biogas plant.

380 Unlike synthetic CO₂, waste CO₂ was assumed to enter the system with “zero burden”, meaning that
381 the impacts linked to the processes of anaerobic digestion and subsequent upgrading of biogas were
382 entirely allocated to the main product (i.e. the biomethane). On the contrary, the by-product
383 containing CO₂ (i.e. the off-gas) was considered a waste flow, hence it is not responsible of the
384 impacts related to its generation; nevertheless, impacts related to its recovery and delivery to the algal
385 production plant were taken into account.

386 In addition, since the recovered CO₂ from biogas upgrading would otherwise be directly emitted into
387 the atmosphere, in the second scenario direct emissions from the cultivation process, which are related
388 to the CO₂ previously imported as a material input, were not accounted. Diversely, in the first scenario
389 CO₂ direct emissions from the cultivation process are also generated within the system boundaries,
390 precisely in the Haber-Bosch production process, through steam reforming of natural gas, and thus
391 included in the inventory. These considerations follow the Greenhouse Gas (GHG) accounting
392 approach proposed in Supekar and Skerlos (2014), which distinguishes between the generation of
393 GHGs and the emission of GHGs into the atmosphere, suggesting to account for CO₂ emissions only
394 when they are generated, in order to avoid double counting or leakage of recovered CO₂.

395 At the same time, a storage of CO₂ embodied in the bio-product was considered for both scenarios.

396

397 *2.6 Life Cycle Impact Assessment*

398 The software GaBi 8.0 was used for the computational implementation of the inventories. Due to
399 robustness and completeness, the midpoint impact categories recommended in the ILCD Handbook
400 (ILCD/PEF recommendations v1.09) (JRC European Commission, 2011) were used for performing
401 the LCIA step. Accordingly, 16 midpoint impact categories were considered: Acidification Potential
402 (ACP); Global Warming Potential, excluding biogenic carbon (GWPebc); Global Warming Potential,

403 including biogenic carbon (GWP_{ibc}); Freshwater Aquatic Ecotoxicity Potential (FAETP);
404 Freshwater Eutrophication Potential (FETP); Marine Eutrophication Potential (METP); Terrestrial
405 Eutrophication Potential (ETP); Human Toxicity Potential with cancer effects (HTP_c); Human
406 Toxicity Potential with non-cancer effects (HTP_{nc}); Ionizing Radiation Potential with human health
407 impacts (IRPhh); Land Use Change Potential (LUCP); Ozone Layer Depletion Potential (ODP);
408 Respiratory Inorganics Impact Potential with particulate matter (RIP_{pm}); Photochemical Ozone
409 Formation Potential (POFP); Water Resource Depletion Potential (WRDP); Abiotic Resources
410 Depletion Potential (ADP).

411 Normalization and weighting were not performed because they are optional analysis required in the
412 ISO standard and are not necessary to realise the objectives of this study.

413

414 *2.7 Sensitivity Analysis*

415 A sensitivity analysis was performed in order to evaluate the influence of some input parameters on
416 the model outcome (ISO, 2006b, 2006a). Three parameters were selected: (1) algal productivity, (2)
417 culture medium recirculation factor and (3) amount of solvents per cleaning cycle.

418 Regarding the algal productivity, the values obtained from a single experimental test were used, as
419 described in paragraph 2.5.2. However, this parameter can vary considerably, since even small
420 changes in environmental conditions can significantly affect algal growth (Pérez-López et al., 2017).
421 In order to take account of these possible variations, different productivity values were considered,
422 obtained from other experiments carried out with the same type of system but with different
423 conditions of salinity, light and temperature, thus defining a range of variation, from -5% to 63% with
424 respect to the “Synthetic CO₂” scenario (Casciaro, 2016).

425 Concerning the culture medium recirculation factor, in the base situation no recycling of the culture
426 medium is expected, being completely discharged after separation with the centrifuge in the
427 harvesting step, as stated in paragraph 2.4.3. However, waste culture medium separated from algal
428 biomass can be recycled for the following productive cycle, which therefore would not require fresh

429 culture medium, but only an appropriate reintegration of macronutrients (nitrogen and phosphorus).
430 For this reason, the parameter linked to recirculation was made to vary between the base scenario and
431 a new scenario with the maximum allowed recirculation. In order to do so, a recirculation was
432 modelled, estimating the amount of macronutrients for reintegration from experimental data about
433 nutrient uptake.

434 Lastly, the third parameter considered was the amount of solvents per cleaning cycle. Although it is
435 not a procedure currently implemented in the plant, there is the possibility of reusing the same
436 washing solution for more PBRs. Accordingly, in the sensitivity test the quantity of solvents needed
437 was reduced by up to 80% in the hypothesis of reuse of the same solution for 5 PBRs (hypothesis
438 based on experts' judgment).

439

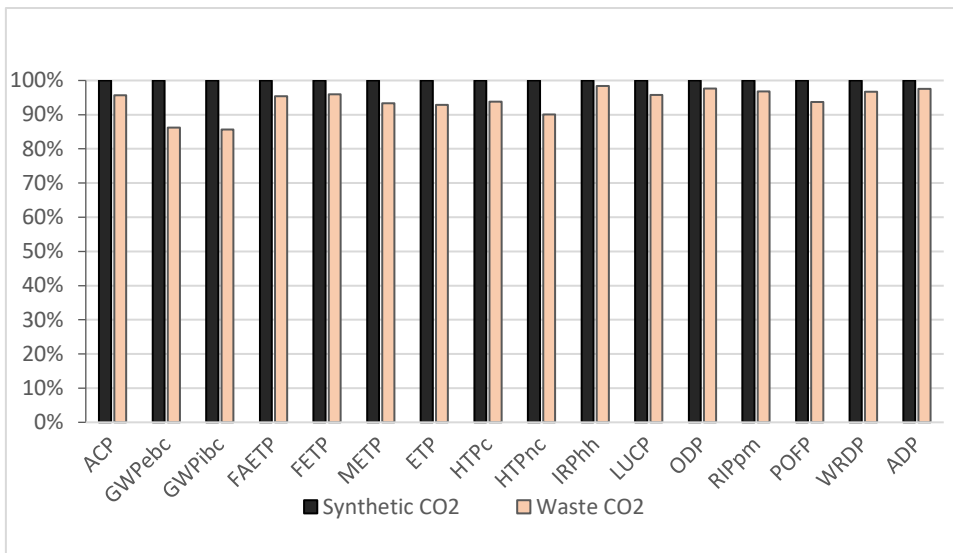
440 **3. Results and discussion**

441 Table 3 presents the results of the LCIA for all impact categories and for both CO₂ source scenarios
442 and Figure 2 provides a comparative environmental profile of these two scenarios. The results indicate
443 that the use of waste CO₂ in place of synthetic CO₂ allows for a reduction of the impact scores of a
444 few percentage points. The reasons for the better performance of the “Waste CO₂” scenario are the
445 absence of the synthetic CO₂ production process and a slightly higher productivity in the cultivation
446 stage, although not statistically significant. The higher productivity in “Waste CO₂” scenario could
447 be due to the presence of impurity in the off-gas that acts as micro-nutrient in the algal culture. It is
448 clear that this result requires confirmation through further tests on a larger scale. It can be noted that
449 for the climate change impact categories (GWPebc and GWPibc) the impact reduction is higher
450 (approximately 14%). In fact, CO₂ direct emissions from the cultivation process in the second case
451 were not accounted, since the microalgae production system is considered as not being responsible
452 of their generation (as explained in paragraph 2.5.3). However, it is noteworthy that the algal
453 productivity can vary depending on the composition of the off-gas, which in turn depends on the
454 process and the plant that generates it. Moreover, the use of waste CO₂ in microalgal cultivation, even

455 if it could improve productivity, must be supported by qualitative analyses of the produced biomass
 456 that demonstrate the compliance to quality standards. Finally, it should not be forgotten that the
 457 exploitation of waste CO₂ is related to the technical realisation of a cost-effective system to reuse the
 458 effluent in the microalgae cultivation system and the CO₂ utilisation technologies are in early stage
 459 of development and their cost-effectiveness is not well-known (Hendriks et al., 2013).

460 **Tab. 3** - *Life Cycle Impact Assessment results for both scenarios associated to the production of 1*
 461 *kg_{DW} of algal biomass.*

Impact category	Unit	Acronym	“Synthetic CO ₂ ”	“Waste CO ₂ ”
Acidification Potential	Mole of H ⁺ eq	ACP	8.52E-01	8.15E-01
Global Warming Potential, excl. biogenic carbon	kg CO ₂ eq	GWPebc	2.98E+02	2.57E+02
Global Warming Potential, incl. biogenic carbon	kg CO ₂ eq	GWPIbc	3.00E+02	2.57E+02
Freshwater Aquatic Ecotoxicity Potential	CTUe	FAETP	2.83E+01	2.70E+01
Freshwater Eutrophication Potential	kg P eq	FETP	2.46E-03	2.36E-03
Marine Eutrophication Potential	kg N eq	METP	1.81E-01	1.69E-01
Terrestrial Eutrophication Potential	Mole of N eq	ETP	1.83E+00	1.70E+00
Human Toxicity Potential with cancer effects	CTUh	HTPc	4.84E-07	4.54E-07
Human Toxicity Potential with non-cancer effects	CTUh	HTPnc	1.71E-06	1.54E-06
Ionizing Radiation Potential with human health impacts	kBq U ²³⁵ eq	IRPhh	1.26E+02	1.24E+02
Land Use Change Potential	kg C deficit eq	LUCP	1.41E+02	1.35E+02
Ozone Layer Depletion Potential	kg CFC-11 eq	ODP	1.73E-07	1.69E-07
Respiratory Inorganics Impact Potential with particulate matter	kg PM _{2.5} eq	RIPpm	4.10E-02	3.97E-02
Photochemical Ozone Formation Potential	kg NMVOC	POFP	4.63E-01	4.34E-01
Water Resource Depletion Potential	m ³ eq	WRDP	1.81E+01	1.75E+01
Abiotic Resources Depletion Potential	kg Sb eq	ADP	1.23E-03	1.20E-03

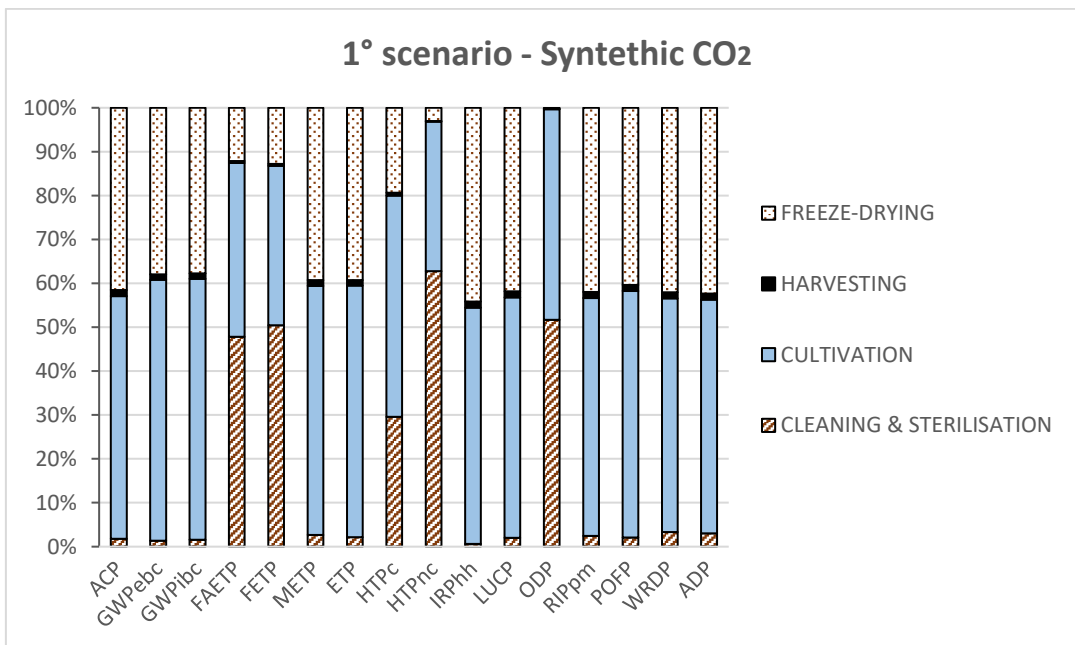


462

463 **Fig. 2 - Comparative environmental profile of both scenarios.**

464 Detailed results for each scenario are presented in Figure 3 and Fig. , showing the relative
 465 contributions of the different process steps (Cleaning and sterilisation, Cultivation, Harvesting and
 466 Freeze-drying) to each impact category score.

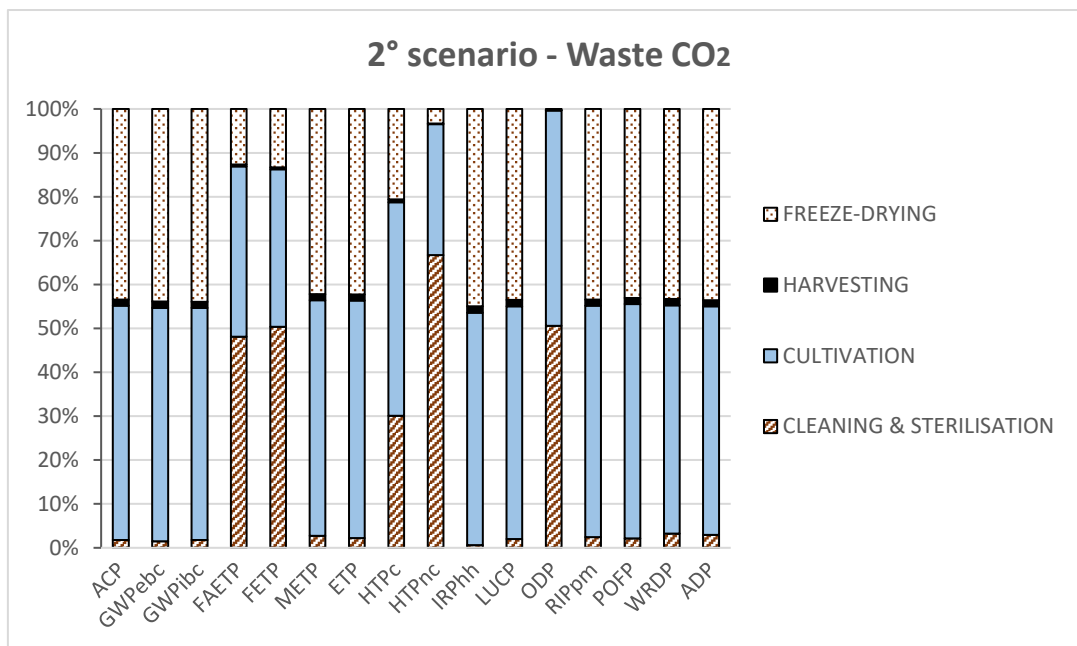
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468

469 **Fig. 3 - Relative contribution of each process step in the first scenario ("Synthetic CO₂").**

470

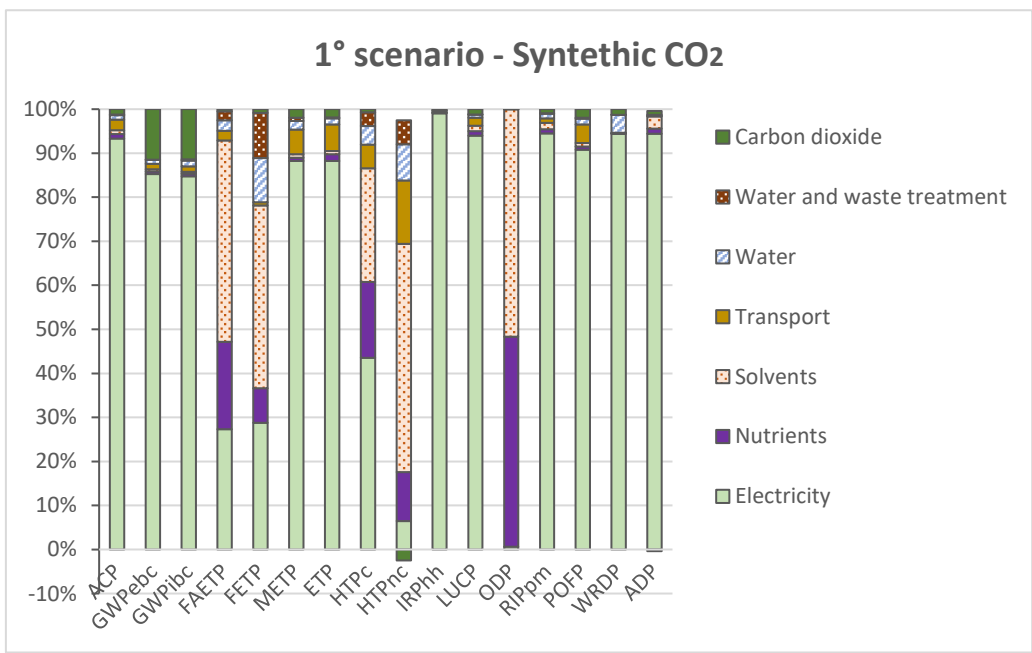


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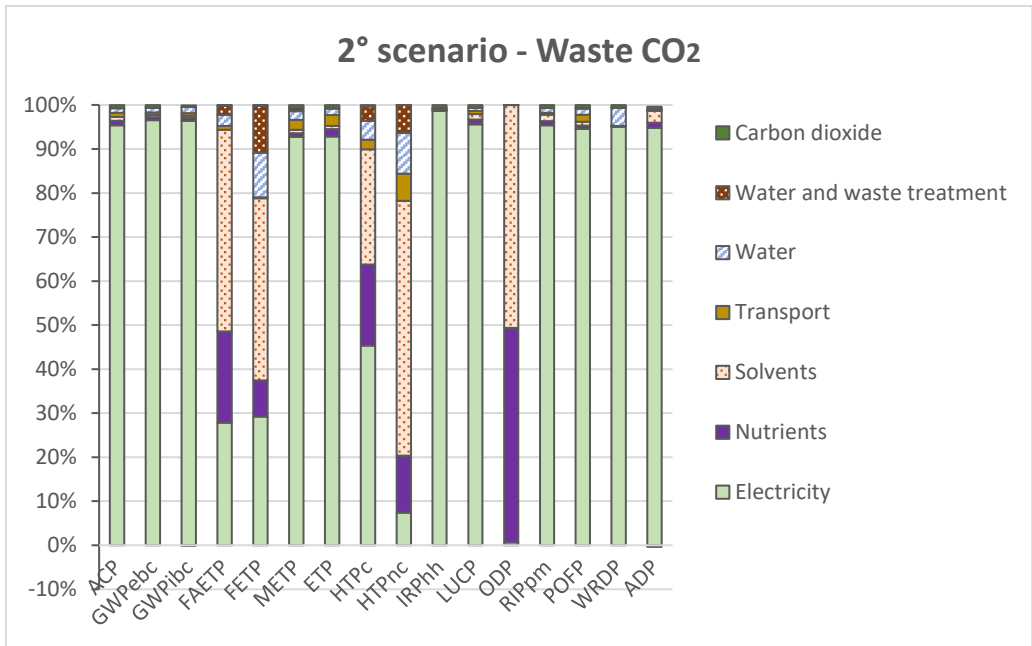
472 **Fig. 4** - Relative contributions of each process step in the second scenario (“Waste CO₂”).

473 In general, it can be stated that for most of the impact categories (ACP, GWPebc, GWPIbc, METP,
 474 ETP, IRPhh, LUCP, RIPpm, POFP, WRDP, ADP) the main contribution derives from Cultivation
 475 (about 50%) and Freeze-drying (about 40%) stages, while the contribution of Cleaning and
 476 sterilisation and Harvesting step are very low (< 5%). Cleaning and sterilisation stage turns out to be
 477 relevant only in 5 impact categories: FAETP, FETP and ODP, for which a contribution equal to about
 478 half of the total impact can be observed, and HTPc and HTPnc with a contribution of 30% and 65%
 479 respectively. These results can be found in both scenarios, although in the “Waste CO₂” scenario the
 480 relative contribution of the cultivation stage compared to drying is slightly reduced. In order to better
 481 interpret the results, the relative contribution per process typology (Figure 5 and Figure 6) was
 482 analysed. All processes have been included in one of the following user-defined groups: (1) electricity
 483 consumption, (2) nutrients production, (3) solvents production, (4) water consumption, (5) water or
 484 waste treatment, (6) carbon dioxide production and usage and (7) transport. The main finding is that
 485 electricity consumption is distinctly the most remarkable contributor to the majority of the impact
 486 categories, specifically those in which the cultivation and drying stages were found to be the most
 487 important. On the other hand, the contribution of solvents production emerges only in the impact

488 categories in which the cleaning and sterilisation step appears to be significant (FAETP, FETP, ODP,
 489 HTPc, HTPnc). In the same categories the contribution of nutrients production, for which the
 490 cultivation step is responsible, is noticeable, in particular in ODP, where it contributes for about 50%
 491 of the total impact. Water production and water and waste treatment show a small influence on the
 492 impacts, which can be spotted, in order of importance, in the categories FETP, HTPnc and HTPc.
 493 The transport phase also shows a negligible contribution, except for one category (HTPnc) in the
 494 “Synthetic CO₂” scenario, where the share of impact exceeds 10%. From the comparison between the
 495 two scenarios (Figure 5 and Figure 6), it can be seen how the contribution to the impact profile
 496 deriving from the use of CO₂ can be almost completely eliminated through the use of waste CO₂.
 497



498
 499 **Fig. 5 - Relative contribution of each process typology to each impact category in the first scenario**
 500 **(“Synthetic CO₂”).**

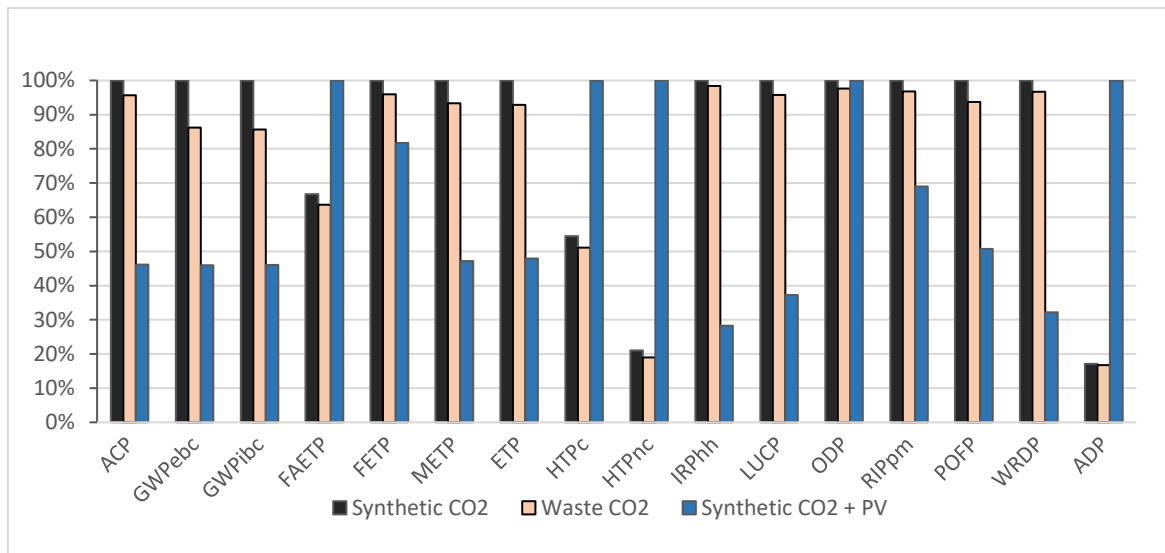


501

502 **Fig. 6** - Relative contribution of each process typology to each impact category in the second
 503 scenario ("Waste CO₂").

504 In the third scenario - built to study the role of the energy source - the European electricity grid mix
 505 was partially replaced with a share of photovoltaics (dataset name: IT Electricity from photovoltaic);
 506 this share was assumed equal to 75% of the energy demand of the production plant, considering the
 507 seasonal availability of an independent photovoltaic system (evaluation based on experts'
 508 judgement). The comparative environmental profile of the three scenarios is presented in Figure 7.
 509 The chart clearly shows that, modifying the energy source, the impact profile can be considerably
 510 altered. Most of the analysed impact categories benefit from the introduction of an important share
 511 of photovoltaic in the electricity mix, with a reduction by 50% (ACP, GWPebc, GWPibc, METP
 512 ETP, POFP) or more (IRPhh, LUCP, WRDP). On the contrary, FAETP, HTPc, HTPnc and ADP
 513 exhibit a marked worsening.

514



515

516 **Fig. 7 - Comparative environmental profile of the three scenarios analysed**

517 A comparison of results reported in this paper with other similar studies is possible but subject to
 518 certain limitations. First of all, almost all available LCA studies on the cultivation of microalgae are
 519 related to the production of biomass for energy purposes. This fact determines important differences,
 520 such as a predilection for open pond systems compared to more sophisticated closed PBRs, given the
 521 relatively low economic value of the product (Collotta et al., 2016; Ketzer et al., 2018). As a result,
 522 the use of open systems leads by itself to lower energy consumption, as demonstrated also in some
 523 comparative studies (Jorquera et al., 2010; Seigné Itoiz et al., 2012). Furthermore, it can be
 524 misleading to compare consumption or impacts related to the cultivation of microalgae species which
 525 exhibit very different productivities. By way of example, the biomass productivity values referring to
 526 different publications reported in Ho et al. (2011) can be considered, obtaining a range of variability
 527 between 0.040 and 1.250 g/l/d. The upper end refers to a cultivation of the same type as that
 528 considered in this study (i.e. bubble column PBR), yet it is found a productivity value that differs by
 529 almost two orders of magnitude from that considered in the present work (i.e. 0.044÷0.046 g/l/d).
 530 From this it follows that, even having comparable consumption per unit of volume and time, such as
 531 those for lighting and air pumping, there could be found very different consumption per unit of
 532 biomass produced. Bearing this in mind, the study most suited for a comparison is seemingly the one

533 by Pérez-López et al. (2014a), which in analogy with this work performs the LCA of the cultivation
534 of *P. tricornutum* for the production of bioactive compounds in an indoor vertical bubble column
535 PBR. However, even in this case important differences can be found: the productivity reported is
536 about eight times higher, the extraction phase is included in the system boundaries and the comparison
537 is not immediate because a different functional unit is considered (i.e. kg PUFAs) and a different
538 impact method (i.e. CML 2001) is adopted. As an example, with regard to the GWP impact, assessed
539 in terms of kgCO_{2eq} in both studies, reporting the results to 1 kg_{DW} of biomass, in the present study
540 values were found between 257 for the scenario with waste CO₂ and of 298 for the “Synthetic CO₂”
541 scenario, while in Pérez-López et al. (2014a) a value of 47.3 was found. This gap is quite wide but
542 can be easily justified considering the disparity in terms of productivity. On the other hand, for the
543 ODP impact the values found in the present work ($1.73 \cdot 10^{-7}$ and $1.69 \cdot 10^{-7}$ kg CFC-11_{eq}) are lower
544 than that found in the previous article ($5.20 \cdot 10^{-6}$ kg CFC-11_{eq}); in this case it must be considered that
545 the main contribution for this impact found in Pérez-López et al. (2014a) originates from the transport
546 phase, which in the present study resulted in general as having a minor relevance.

547 Greater consistency with previous findings can be observed about the importance of the relative
548 contributions of processes and inputs to the final impacts: in all studies focused on the production of
549 microalgae for bioactive compounds (Pérez-López et al., 2014c, 2014a, 2014b) the cultivation phase
550 is always the most impacting, whereas the collection phase is generally negligible; in the same studies
551 the remarkable contribution to impacts associated with electricity input is underlined, also confirmed
552 in previous LCA studies concerning biomass crops for energy purposes (Khoo et al., 2011; Lardon et
553 al., 2009).

554

555 *3.1 Sensitivity Analysis*

556 The results of the sensitivity analysis are presented in Figure 8. The algal productivity affects all
557 impact categories to the same extent, determining variations of 3-4% in the worst case scenario and
558 between 20 and 26% in the best case scenario. On the other hand, parameters related to recirculation

559 and solvents reduction are able to significantly reduce 5 impact category scores (FAETP, FETP,
560 HTPc, HTPnc and ODP) but they have a negligible influence on all other impacts.

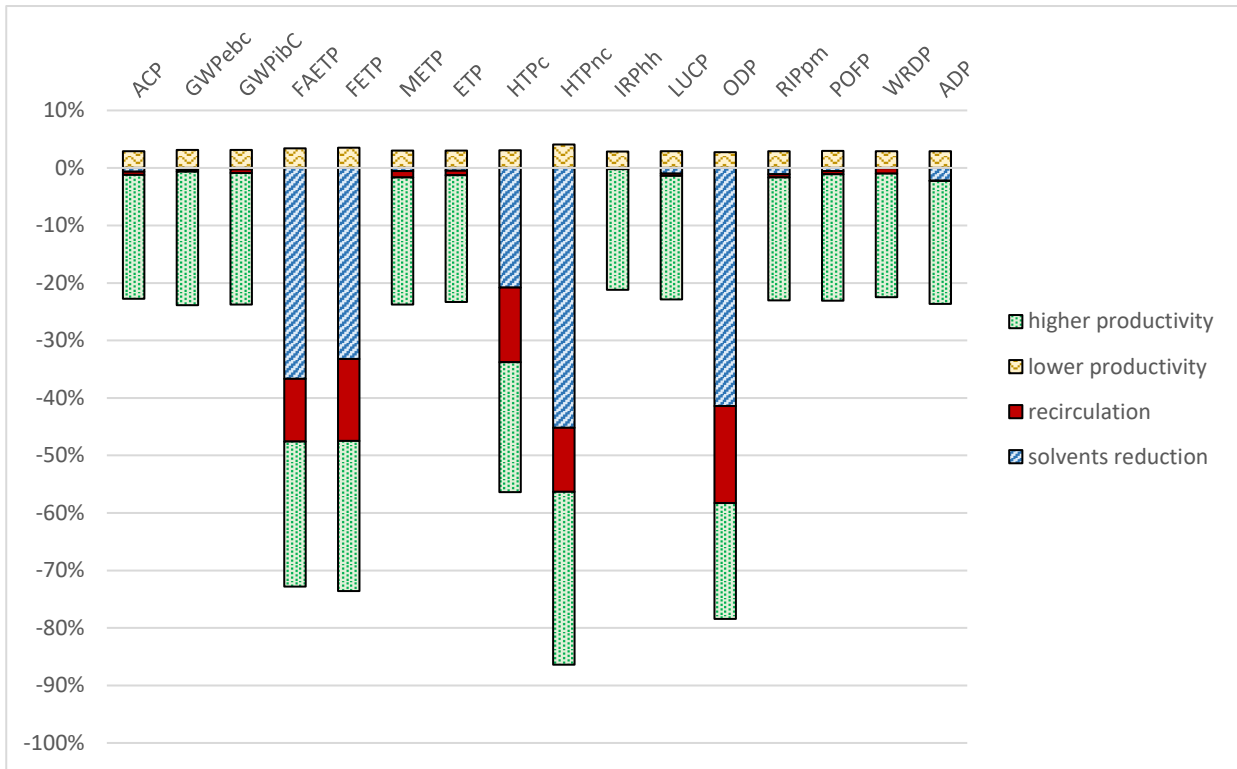
561 From this analysis, it especially appears that even a slight decrease in productivity may be sufficient
562 to counteract, in most of the impact categories, the positive effects due to the change in the type of
563 CO₂ used.

564 Furthermore, the sensitivity analysis results suggest that the objective to minimise all impacts could
565 be pursued through a mixed strategy, namely combining the use of more photovoltaic energy in the
566 electric mix with the reduction of nutrients and solvents usage. Precisely, the categories FAETP,
567 HTPc and HTPnc, whereas exhibiting a marked worsening in the case of an increased use of
568 photovoltaic energy, would particularly benefit from a reduced use of nutrients and solvents.

569 Comparing Figure 7 and Figure 8, it can be stated that, overall, a combined strategy would result in
570 a reduction by 50% or more of the impacts in most of the categories analysed (ACP, GWPebc,
571 GWPi bc, FETP, METP, ETP, IRPhh, LUCP, ODP, POFP, WRDP); in any case, ADP would remain
572 a critical point, since none of the strategies analysed allows for a clear improvement that can
573 counterbalance the great use of abiotic resources necessary for the construction of photovoltaic
574 panels.

575

576



577

578 **Fig. 8** – Results of the sensitivity analysis, considering higher (+63%) algal productivity, lower
 579 (+5%) algal productivity, culture medium recirculation and reduced amount (-80%) of solvents per
 580 cleaning cycle.

581

582 4. Conclusions

583

584 The main goal of this study was to perform the comparative life cycle assessment of the production
 585 of *P. tricornutum* through the process using synthetic commercial CO₂ (“Synthetic CO₂” scenario)
 586 and the process using the off-gas from the upgrading process of biogas to biomethane (“Waste CO₂”
 587 scenario). It was found that the semi-industrial production of *P. tricornutum* using waste gas
 588 containing CO₂ in place of synthetic CO₂ allows for an overall improvement in the environmental
 589 profile of the process in all the analysed impact categories and in particular in terms of GHG
 590 emissions reduction, benefiting from the absence of the synthetic CO₂ production process and the
 591 slightly higher productivity in the cultivation stage. Both scenarios indicated Cultivation and Freeze-

592 drying as the most significant stages and electricity consumption as the main cause of the
593 environmental impacts for the majority of impact categories.

594 The third scenario – which assumed the use of photovoltaic electricity – showed that moving towards
595 renewable energy sources could notably decrease many environmental impacts. Obviously, further
596 environmental advantages could be obtained from the combined use of waste CO₂ and renewable
597 energy.

598 Finally, it is noteworthy that the results are mainly influenced by data about algal productivity, which
599 is a particularly high-sensitive parameter. Indeed, sensitivity analysis confirmed that a slight
600 worsening of algal productivity may be sufficient to offset the positive effects of replacing the CO₂
601 source; At the opposite, a higher - but observed in laboratory experiments - algal productivity could
602 improve the environmental performances by up to 20-25%.

603 At the same time, from the sensitivity analysis, other impact reduction strategies, such as recirculation
604 of the culture medium and recycling of the cleaning solutions, have emerged as very successful,
605 although they appear to be effective only on specific impact categories.

606 In conclusion, it can be stated that the use of CO₂ from the biogas upgrading is a feasible and attractive
607 alternative to the synthetic one, as it allows for the improvement of the environmental performance
608 of the production process without reducing its productivity. The possibility of using waste CO₂ in the
609 added-value compounds production through microalgae exploitation is along the path to
610 sustainability. It complies with the principles of circular economy and industrial symbiosis and can
611 facilitate the move towards the blue bioeconomy. The study also highlighted that the environmental
612 performance of microalgae cultivation for producing valuable substances could be further improved
613 by acting on other key factors, such as the electricity source and nutrient substances. Anyway, it must
614 be considered that technical and economic challenges have to be overcome before CO₂ obtained from
615 biogas upgrading could be used for microalgae cultivation.

616

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618

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625

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627

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