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Abstract

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

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

Keywords: Environmental assessment; Marine microalgae; Bioactive compounds; High-value products; CO_2 fixation; Waste CO_2

Chemical symbols

CH_4	Methane
CO_2	Carbon dioxide
H_2S	Hydrogen sulfide

Subscripts

DW	Dried Weight

Acronyms

ACP	Acidification Potential
ADP	Abiotic Resources Depletion Potential
EPA	Eicosapentanoic Acid
ETP	Terrestrial Eutrophication Potential
FAETP	Freshwater Aquatic Ecotoxicity Potential
FETP	Freshwater Eutrophication Potential
GHG	Greenhouse Gas
GWPebc	Global Warming Potential, excluding biogenic carbon
GWPibc	Global Warming Potential, including biogenic carbon
HTPc	Human Toxicity Potential with cancer effects
HTPnc	Human Toxicity Potential with non-cancer effects
IRPhh	Ionizing Radiation Potential with human health impacts
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LUCP	Land Use Change Potential
METP	Marine Eutrophication Potential
ODP	Ozone Layer Depletion Potential
PBR	Photobioreactor
POFP	Photochemical Ozone Formation Potential

PUFAs	Polyunsaturated Fatty Acids
RIPpm	Respiratory Inorganics Impact Potential with particulate matter
TFAs	Total Fatty Acids
WRDP	Water Resource Depletion Potential

1. Introduction

The importance of algae for the blue bioeconomy has been recognised by the European Commission (2019) both because of their role in the ecosystem and their value for commercial applications that diminish the pressure on land-based products. Microalgae have, as a matter of fact, the ability to convert CO_2, water and sunlight to sugars, from which macromolecules, such as lipids and other many beneficial and valuable compounds, can be obtained (Vonshak, 1993).

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

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

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

Life Cycle Assessment (LCA) is recognised by the European Commission (2003) as the best framework for assessing the potential environmental impacts of products. However, at present few LCA studies have addressed the environmental aspects of algal production not primarily for energy purposes, despite the increasing economic attractiveness of microalgae exploitation for producing high added-value compounds. Some authors have compared systems providing the same products from traditional sources and microalgae. For instance, Taelman et al. (2015) compare through LCA protein meal from microalgae and from soybean, finding that the algal production system, due to its immature small scale, has high energy consumption and therefore is not competitive with the well-established soy production system; nevertheless, using a sensitivity test, they show the possibility to overcome this gap through scale-up efficiencies and a switch to renewable electricity sources. Smetana et al. (2017) perform the LCA of different cultivation techniques and microalgae species to 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

components increases the economic feasibility (De Bhowmick et al., 2019; Mishra et al., 2019; Vanthoor-Koopmans et al., 2013); iii) biomass production should be combined with bio-remediation and CO_2 mitigation services, when feasible, in order to make the process economically viable and environmentally sustainable (Wang et al., 2008).

On this last point, it is to be remarked how the growing concern about global climate change, of which anthropogenic CO_2 emissions are mainly responsible, is leading to focus on CO_2 capture potential of microalgae, ascertained that the overall productivity of algal cultures can benefit from the supply of an external source of CO_2 (Kassim and Meng, 2017; Lam et al., 2012; Rezvani et al., 2016). The amount of additional CO_2 needed in the cultivation process in order to support algal growth depends on several factors, first of all on the algal strain and subsequently on a series of operational parameters, such as temperature, pH, light intensity, O_2 levels, or presence of inhibitory compounds (López et al., 2013). The direct injection of a CO_2-rich gas stream into microalgae cultures can improve the mass transfer of CO_2 and, within certain limits, the rate of photosynthetic CO_2 assimilation (Zhao and Su, 2014). This additional CO_2 can conveniently be recycled from different industrial processes, including combustion processes for energy production (Kroumov et al., 2016), cement manufacturing (Cuellar-Bermudez et al., 2015) and biogas upgrading processes (Xia and Murphy, 2016).

With reference to the latter, it should be considered that typical biogas contains 55–70% methane (CH_4) and 30–45% carbon dioxide (CO_2). Other components include water, oxygen and other impurities. Reducing CO_2 and impurities content significantly improves the quality of biogas and makes it fit standard requirements. There are several means of reducing the content of CO_2 such as physical, chemical and biological methods (Kao et al., 2012; Seyed Hosseini et al., 2018). Microalgal biomass has also been identified as a means of biogas upgrading, thus achieving the CO_2 removal by biotechnology rather than through conventional physical or chemical removal techniques. However, the potential of biotechnologies for CO_2 removal from biogas has been assessed only at laboratory or pilot scale (Muñoz et al., 2015).

In recent years, intensive research efforts have been focused on the investigation of the algal bio-fixation potential of the CO_2 contained in post-combustion flue gases (Huang et al., 2016). These studies are mainly aimed at assessing the algal response in terms of productivity, particularly with regard to possible negative effects of the pollutants typically present in a gas of this type, such as CO, SO_x, NO_x, C_xH_y, particulate matter, halogen acids and heavy metals (Van Den Hende et al., 2012). Moreover, some studies on biotechnological upgrading of biogas have shown that methane does not exert any negative effect on algal growth, at the typical concentrations of a gas produced by anaerobic digestion of biomass (Meier et al., 2015). It should be noted that the use of waste gases from industrial processes is likely to influence not only the productivity but also the quality of the biomass produced, due to the presence of other gases considered as contaminants in the gas. However, the above mentioned reports rarely give information on biomass quality parameters, except for some cases in which the variation induced on the lipid content is mentioned (Chiu et al., 2011; Lizzul et al., 2014), since they are mainly focused on the CO_2 capture function rather than on producing biomass that can be converted into useful products (Ho et al., 2011).

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

Taking this into account, the present study aimed to determine whether the use of waste CO_2 recovered from a biogas upgrading process, in place of synthetic commercial CO_2, could bring environmental benefits to the life cycle of the cultivation of microalgae for non-energy purposes. The selected microalga was *Phaeodactylum tricornutum*, employed in the production of bioactive compounds such as Polyunsaturated Fatty Acids (PUFAs). In the study two scenarios, which differ only in terms of typology of carbon dioxide additional source, were compared by means of LCA methodology. In the last part of the paper, a third scenario was analysed in order to test the effect of energy source on the environmental performances of the system. To the best of our knowledge, few papers performed an LCA study to analyse the environmental performances of the cultivation of *P.*

tricornutum and, in the context of microalgae production for added-value compounds, this is the first paper that uses experimental data when assessing the advantages of using waste CO_2 in the cultivation process.

2. Material and methods

2.1 Microalgae description

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

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

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

2.2 Life Cycle Assessment methodology

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

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

2.3 LCA goal and scope definition

The goal of the present study was to assess the environmental performances of the production of *P. tricornutum* for non-energy purposes on a semi-industrial scale, evaluating the use of two different carbon sources. In the first trial the biomass was fed with a stream of commercial CO_2 of high purity grade, while in the second one waste CO_2 from biogas upgrading was used. These trials will be indicated as scenarios from hereafter. A third scenario was implemented in order to compare the improvements that can be obtained by replacing the CO_2 source with those that may derive from the change in the energy source used by the plant.

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

The final product of the system is the dried algal biomass, with a 5% w/w of water content, which resulted to have a very comparable biochemical composition (Simonazzi et al., 2019) between the two tested conditions (i.e. supply of commercial or waste CO_2). Consequently, the extraction process was not included in the analysis and the functional unit chosen was 1 kg Dried Weight (DW) of algal biomass.

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

waste CO_2 for microalgal cultivation is at an experimental stage not yet applied at semi-industrial scale.

2.4 Overview of the production system

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

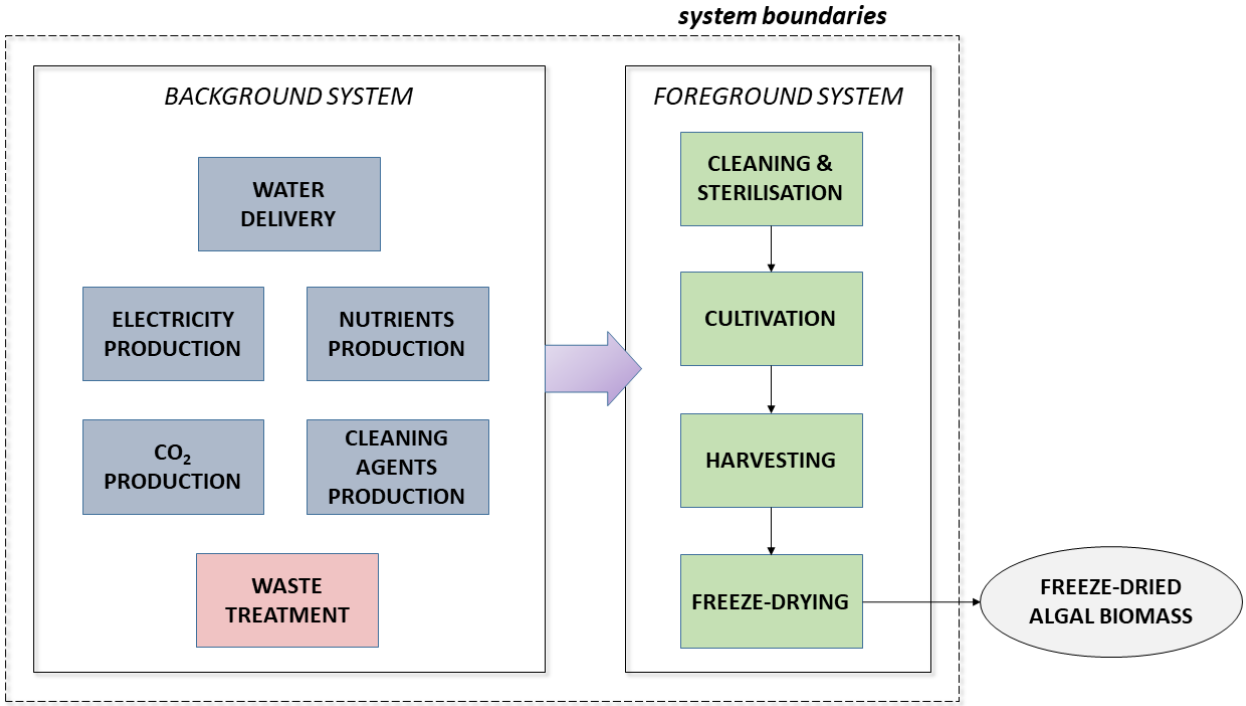

Fig. 1 - *Process chain and system boundaries overview.*

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

Biomass cultivation can take place in many technological configurations, which can be basically divided into open and closed systems (Tredici, 2004). Technologies based on open systems are generally cheaper and much simpler than closed PBR systems. However, PBRs allow for a better control over the process variables, a much lower contamination risk and a higher CO_2 fixation efficiency (Ho et al., 2011; López et al., 2013). For this reason, closed systems are the most effective

method to grow microalgae for the production of bioactive compounds with high-quality standards and the supply of CO_2 at high concentrations (Pulz, 2001).

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

2.4.1 Cleaning and sterilisation

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

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

2.4.2 Cultivation

The 250 l column PBR (column height 220 cm, diameter 55 cm) is inoculated and filled with modified F/2 culture medium (Guillard and Ryther, 1962), and the culture initial concentration is of 0.3 g/l (biomass DW). Temperature in the reactor is maintained at 22 °C. The reactor presents a photoperiod with 16 h light and 8 h dark periods and light intensity of 150 µmol photon/m^2/s. A constant air flow is provided in the culture, essentially for its mixing (approximate bubble size 0.5 cm). In order to support microalgal growth, a CO_2 flow is supplied for 24 minutes per day (6.25 l/min). While in the first scenario this flow consists of synthetic commercial CO_2 of high purity grade (>99.5% v/v), in

the case of waste CO_2 scenario the flow has an average CO_2 content of 75%, also containing residual CH_4 (approximately 16%) and other compounds from biogas upgrading (i.e. H_2S 24 ppm).

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

2.4.3 Harvesting

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

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

2.4.4 Freeze-drying

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

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

2.5 Life Cycle Inventory

Except for microalgae growth rates, any other inventory data for the foreground system were based on primary data from a semi-industrial plant set in Italy producing a different species of microalgae (*Arthrospira platensis*) and adjusted to a hypothetical scenario which consider *P. tricornutum* 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 CO_2" and "Waste CO_2" scenarios*

334

Flow	**Background Process**	**Database**	**Amount ("Synthetic CO_2" scenario)**	**Amount ("Waste CO_2" scenario)**	**unit**
Input flows:					
Tap water	EU-28 Tap water	GaBi	2475	2412	l
Deionised water	EU-28 Water (deionised)	GaBi	1134	1087	l
Synthetic CO_2	DE Carbon Dioxide	GaBi	26747	0	g
Waste CO_2	*(elementary flow)*	-	0	25617	g
Electricity	EU-28 Electricity Grid Mix	GaBi	611	597	kWh
KNO_3	RER potassium nitrate, as N, at regional scale	Ecoinvent	99.8	99.8	g
NaH_2PO_4	RER sodium phosphate, at plant	Ecoinvent	37.3	37.3	g
Na_2SiO_3	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
$FeCl_3$	CH iron(III) chloride, 40% in H2O, at plant	Ecoinvent	4.73	4.73	g
$ZnSO_4 \cdot 7H_2O$	RER zinc monosulphate, $ZnSO_4$.H2O, at plant	Ecoinvent	0.0137	0.0137	g
NaClO	RER sodium hypochlorite, 15% in H2O, at plant	Ecoinvent	1384	1326	g
HCl	DE Hydrochloric acid (32%), production mix, at plant	GaBi	526	504	g

Output flows:					
Algal biomass (dried weight)	*(product)*	-	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
CO_2	*(elementary flow)*	-	24897	23767	g

335

A detailed description of the equipment and its consumption is given in Table 2. The electricity consumption was calculated on the basis of the power of the equipment and the duration of operation. It can immediately be noted that electricity consumption related to harvesting and freeze-drying stages is not dependent on the type of CO_2 used; it is not influenced by productivity indeed, being constant per unit of treated biomass.

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

	Amount (kWh)	
	"Synthetic CO_2"	"Waste CO_2"
Cultivation		
Lamps	191.7	183.6
Compressor	95.9	91.8
Control unit	42.7	40.9
Harvesting		
Centrifuge	8.9	8.9
Freeze-Drying		
Chiller	32.0	32.0
Freeze-Dryer	240.0	240.0

2.5.2 Description of laboratory experimental tests

Information concerning algae growth rates refers to a specific study performed in indoor 70 l column PBRs (column height 110 cm, diameter 40 cm, M2M Engineering, Italy). The PBRs were internally illuminated with cool white neon at continuous irradiance of 300 µmol/m^2/s and a photoperiod of 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 CO_2 (Simonazzi et al., 2019). The same specific inputs of CO_2
354 and nutrients of the hypothetical semi-industrial scale system were used. The waste CO_2 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 CO_2 was equal to 0.046 g/l/d,
359 compared to the one with synthetic CO_2 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 CO_2 was derived by difference between CO_2 input and CO_2 fixation rate. The latter
365 was estimated from the biomass productivity by applying the stoichiometric CO_2 requirement factor
366 for microalgae growth of 1.85 g CO_2/ 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 CO_2 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

hypochlorite 15% and hydrochloric acid 32%); 500 km for synthetic commercial CO_2; 100 km for waste CO_2, assuming a relative proximity to the biogas plant.

Unlike synthetic CO_2, waste CO_2 was assumed to enter the system with "zero burden", meaning that the impacts linked to the processes of anaerobic digestion and subsequent upgrading of biogas were entirely allocated to the main product (i.e. the biomethane). On the contrary, the by-product containing CO_2 (i.e. the off-gas) was considered a waste flow, hence it is not responsible of the impacts related to its generation; nevertheless, impacts related to its recovery and delivery to the algal production plant were taken into account.

In addition, since the recovered CO_2 from biogas upgrading would otherwise be directly emitted into the atmosphere, in the second scenario direct emissions from the cultivation process, which are related to the CO_2 previously imported as a material input, were not accounted. Diversely, in the first scenario CO_2 direct emissions from the cultivation process are also generated within the system boundaries, precisely in the Haber-Bosch production process, through steam reforming of natural gas, and thus included in the inventory. These considerations follow the Greenhouse Gas (GHG) accounting approach proposed in Supekar and Skerlos (2014), which distinguishes between the generation of GHGs and the emission of GHGs into the atmosphere, suggesting to account for CO_2 emissions only when they are generated, in order to avoid double counting or leakage of recovered CO_2.

At the same time, a storage of CO_2 embodied in the bio-product was considered for both scenarios.

2.6 Life Cycle Impact Assessment

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

403 including biogenic carbon (GWPibc); 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 (HTPc); Human
406 Toxicity Potential with non-cancer effects (HTPnc); 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 (RIPpm); 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 CO_2" 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

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

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

3. Results and discussion

Table 3 presents the results of the LCIA for all impact categories and for both CO_2 source scenarios and Figure 2 provides a comparative environmental profile of these two scenarios. The results indicate that the use of waste CO_2 in place of synthetic CO_2 allows for a reduction of the impact scores of a few percentage points. The reasons for the better performance of the "Waste CO_2" scenario are the absence of the synthetic CO_2 production process and a slightly higher productivity in the cultivation stage, although not statistically significant. The higher productivity in "Waste CO_2" scenario could be due to the presence of impurity in the off-gas that acts as micro-nutrient in the algal culture. It is clear that this result requires confirmation through further tests on a larger scale. It can be noted that for the climate change impact categories (GWPebc and GWPibc) the impact reduction is higher (approximately 14%). In fact, CO_2 direct emissions from the cultivation process in the second case were not accounted, since the microalgae production system is considered as not being responsible of their generation (as explained in paragraph 2.5.3). However, it is noteworthy that the algal productivity can vary depending on the composition of the off-gas, which in turn depends on the process and the plant that generates it. Moreover, the use of waste CO_2 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 CO_2 is related to the technical realisation of a cost-effective system to reuse the
458 effluent in the microalgae cultivation system and the CO_2 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 PM2.5 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

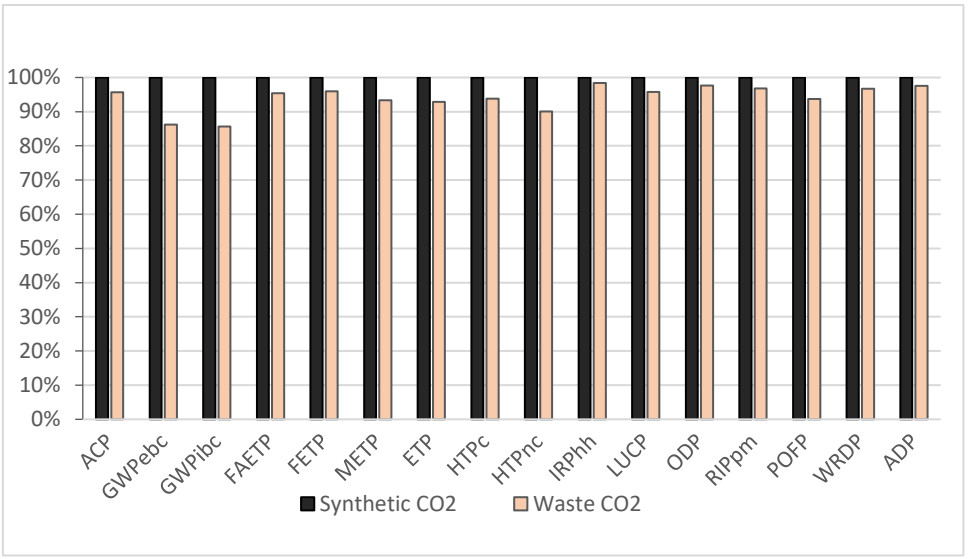

Fig. 2 - *Comparative environmental profile of both scenarios.*

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

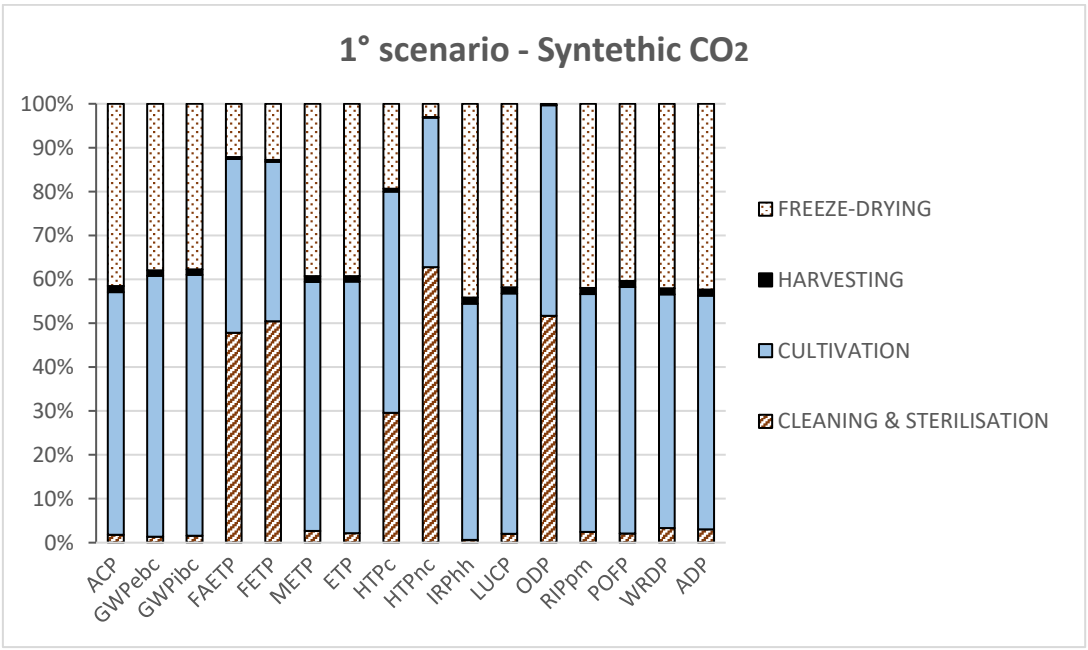

Fig. 3 - *Relative contribution of each process step in the first scenario ("Synthetic CO_2").*

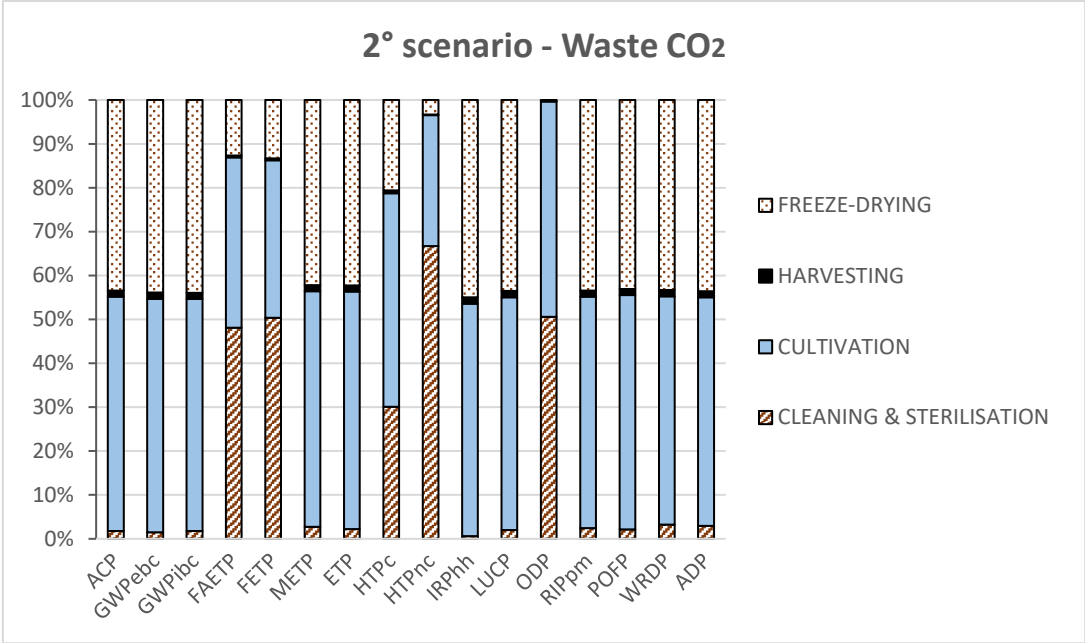

Fig. 4 - *Relative contributions of each process step in the second scenario ("Waste CO_2").*

In general, it can be stated that for most of the impact categories (ACP, GWPebc, GWPibc, METP, ETP, IRPhh, LUCP, RIPpm, POFP, WRDP, ADP) the main contribution derives from Cultivation (about 50%) and Freeze-drying (about 40%) stages, while the contribution of Cleaning and sterilisation and Harvesting step are very low (< 5%). Cleaning and sterilisation stage turns out to be relevant only in 5 impact categories: FAETP, FETP and ODP, for which a contribution equal to about half of the total impact can be observed, and HTPc and HTPnc with a contribution of 30% and 65% respectively. These results can be found in both scenarios, although in the "Waste CO_2" scenario the relative contribution of the cultivation stage compared to drying is slightly reduced. In order to better interpret the results, the relative contribution per process typology (Figure 5 and Figure 6) was analysed. All processes have been included in one of the following user-defined groups: (1) electricity consumption, (2) nutrients production, (3) solvents production, (4) water consumption, (5) water or waste treatment, (6) carbon dioxide production and usage and (7) transport. The main finding is that electricity consumption is distinctly the most remarkable contributor to the majority of the impact categories, specifically those in which the cultivation and drying stages were found to be the most important. On the other hand, the contribution of solvents production emerges only in the impact

categories in which the cleaning and sterilisation step appears to be significant (FAETP, FETP, ODP, HTPc, HTPnc). In the same categories the contribution of nutrients production, for which the cultivation step is responsible, is noticeable, in particular in ODP, where it contributes for about 50% of the total impact. Water production and water and waste treatment show a small influence on the impacts, which can be spotted, in order of importance, in the categories FETP, HTPnc and HTPc. The transport phase also shows a negligible contribution, except for one category (HTPnc) in the "Synthetic CO_2" scenario, where the share of impact exceeds 10%. From the comparison between the two scenarios (Figure 5 and Figure 6), it can be seen how the contribution to the impact profile deriving from the use of CO_2 can be almost completely eliminated through the use of waste CO_2.

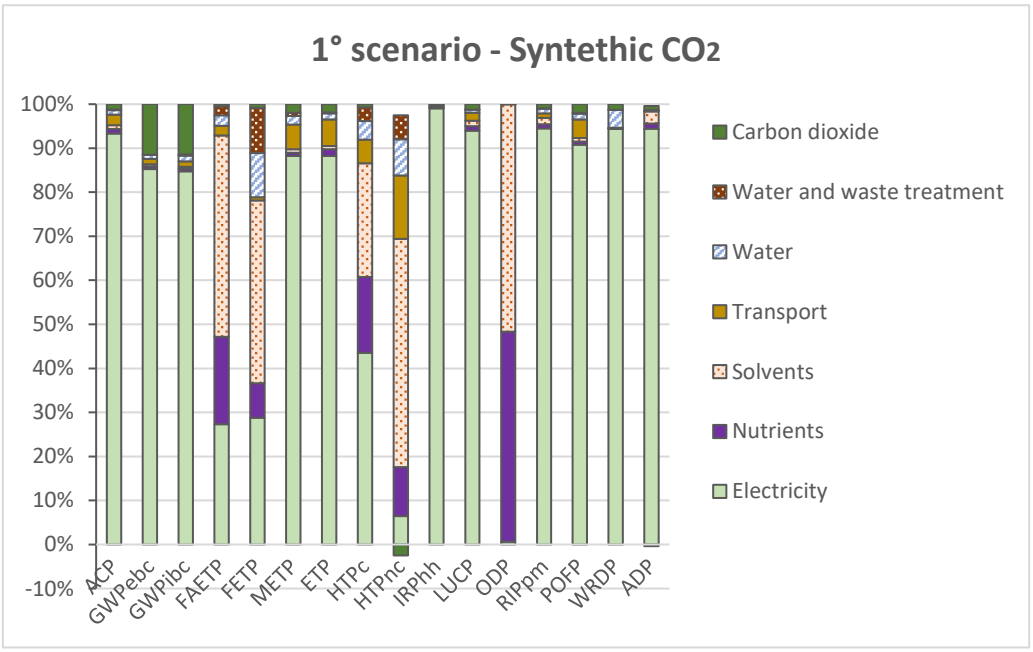

Fig. 5 - *Relative contribution of each process typology to each impact category in the first scenario ("Synthetic CO_2").*

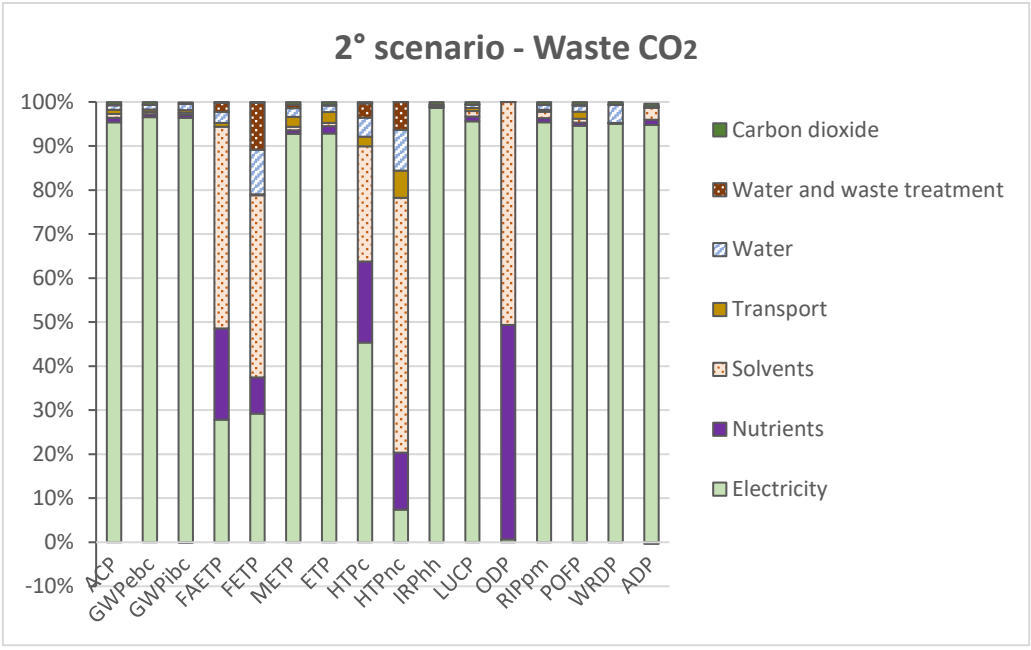

Fig. 6 - *Relative contribution of each process typology to each impact category in the second scenario ("Waste CO_2").*

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

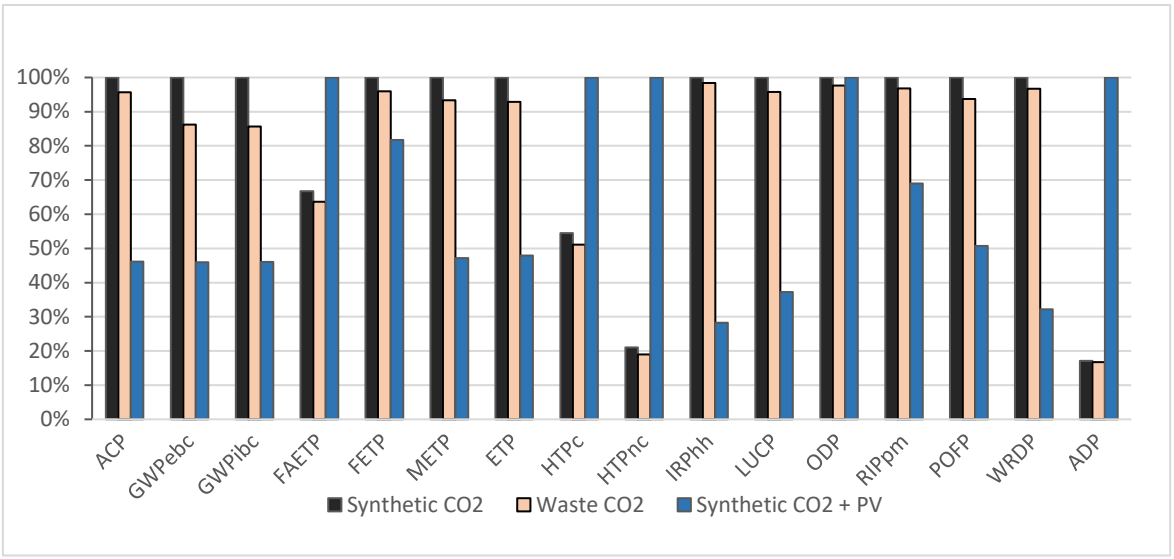

Fig. 7 - *Comparative environmental profile of the three scenarios analysed*

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

by Pérez-López et al. (2014a), which in analogy with this work performs the LCA of the cultivation of *P. tricornutum* for the production of bioactive compounds in an indoor vertical bubble column PBR. However, even in this case important differences can be found: the productivity reported is about eight times higher, the extraction phase is included in the system boundaries and the comparison is not immediate because a different functional unit is considered (i.e. kg PUFAs) and a different impact method (i.e. CML 2001) is adopted. As an example, with regard to the GWP impact, assessed in terms of $kgCO2_{eq}$ in both studies, reporting the results to 1 kg_{DW} of biomass, in the present study values were found between 257 for the scenario with waste CO_2 and of 298 for the "Synthetic CO_2" scenario, while in Pérez-López et al. (2014a) a value of 47.3 was found. This gap is quite wide but can be easily justified considering the disparity in terms of productivity. On the other hand, for the 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 than that found in the previous article ($5.20 \cdot 10^{-6}$ kg CFC-11_{eq}); in this case it must be considered that the main contribution for this impact found in Pérez-López et al. (2014a) originates from the transport phase, which in the present study resulted in general as having a minor relevance.

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

3.1 Sensitivity Analysis

The results of the sensitivity analysis are presented in Figure 8. The algal productivity affects all impact categories to the same extent, determining variations of 3-4% in the worst case scenario and 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 CO_2 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 GWPibc, 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.
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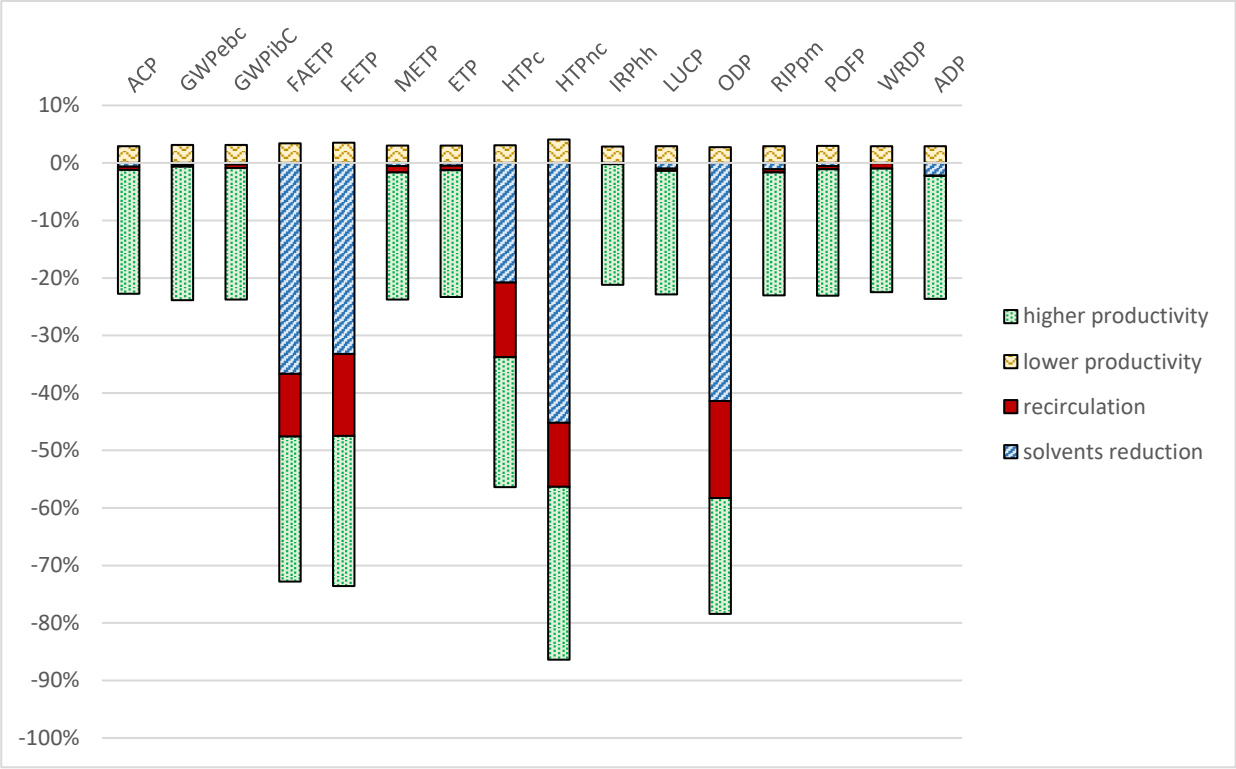

Fig. 8 – *Results of the sensitivity analysis, considering higher (+63%) algal productivity, lower (+5%) algal productivity, culture medium recirculation and reduced amount (-80%) of solvents per cleaning cycle.*

4. Conclusions

The main goal of this study was to perform the comparative life cycle assessment of the production of *P. tricornutum* through the process using synthetic commercial CO_2 ("Synthetic CO_2" scenario) and the process using the off-gas from the upgrading process of biogas to biomethane ("Waste CO_2" scenario). It was found that the semi-industrial production of *P. tricornutum* using waste gas containing CO_2 in place of synthetic CO_2 allows for an overall improvement in the environmental profile of the process in all the analysed impact categories and in particular in terms of GHG emissions reduction, benefiting from the absence of the synthetic CO_2 production process and the slightly higher productivity in the cultivation stage. Both scenarios indicated Cultivation and Freeze-

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

The third scenario – which assumed the use of photovoltaic electricity – showed that moving towards renewable energy sources could notably decrease many environmental impacts. Obviously, further environmental advantages could be obtained from the combined use of waste CO_2 and renewable energy.

Finally, it is noteworthy that the results are mainly influenced by data about algal productivity, which is a particularly high-sensitive parameter. Indeed, sensitivity analysis confirmed that a slight worsening of algal productivity may be sufficient to offset the positive effects of replacing the CO_2 source; At the opposite, a higher - but observed in laboratory experiments - algal productivity could improve the environmental performances by up to 20-25%.

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

In conclusion, it can be stated that the use of CO_2 from the biogas upgrading is a feasible and attractive alternative to the synthetic one, as it allows for the improvement of the environmental performance of the production process without reducing its productivity. The possibility of using waste CO_2 in the added-value compounds production through microalgae exploitation is along the path to sustainability. It complies with the principles of circular economy and industrial symbiosis and can facilitate the move towards the blue bioeconomy. The study also highlighted that the environmental performance of microalgae cultivation for producing valuable substances could be further improved by acting on other key factors, such as the electricity source and nutrient substances. Anyway, it must be considered that technical and economic challenges have to be overcome before CO_2 obtained from biogas upgrading could be used for microalgae cultivation.

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