# Sustainable production of microalgae in raceways: nutrients and water management as key factors influencing environmental impacts

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#### ABSTRACT

Microalgae production has taken on importance for its ability to be more energy efficient than land crops, with low input requirements and a wide number of possible applications.

This work aimed to evaluate the environmental impacts of the production of microalgae for use as bio-stimulants and aquaculture feeds. Inventory data from a real production facility of 1 ha located in Almería (Spain) were acquired, and LCA was applied to compare nine scenarios with alternative water bases (fresh, sea and waste), and nutrient sources (fertilizers, manure and wastewater), and the alternatives were also compared using a CO<sub>2</sub> supply (commercial liquid) versus a default scenario (recovered flue gas). The LCA results outlined that the main inputs affecting environmental performance were electricity use, chemical fertilizer demand (N and P) and transport. Scenarios using recovered nutrients from slurry and wastewater showed reductions in the climate change category (kg CO<sub>2</sub> eq.) of 80% and 20% respectively, compared to standard fertilizer use. The threshold of distance for manure transport was 40 km, beyond that value the scenarios using recovered nutrients performed worse than scenarios using chemical fertilizers. The multifunctionality of the process which included wastewater depuration, permitted compensation in most of the impact categories, yielding negative values in some (all of the toxicity categories).

**Keywords:** CO<sub>2</sub> source; Environmental impact; Life Cycle Assessment (LCA); Microalgae production; Raceway reactors; Wastewater.

## 1. Introduction

Population growth and the increase in the standard of living and consumption, place the search for new sustainable sources for the production of food, feed and feedstock at the centre of the development focus. To respond to these needs, issues such as the sustainable intensification of agriculture and the increase of high-performance forms of production, such as aquaculture, are becoming the focus of attention. Sustainable intensification of agriculture for food, feed and feedstock is the recurring mantra in the 2050 forecast for economic and social scenarios. In the same search for environmental sustainability, it has become mandatory to develop production models that minimise waste and that exploit the waste from other processes as raw materials, in a logic of industrial symbiosis and the circular economy. Within this framework, production of microalgae has been notable for traits like high photosynthetic efficiency reflected in productivity, its capacity to produce a wide range of active compounds, and the possibility of using alternative resources such as land not classed as fertile soil, sea water, and recovered streams (e.g. wastewater) (Cardozo et al., 2007; Clarens et al., 2010; Quinn and Davis, 2015).

At present, microalgae are actively being investigated for their ability to produce active substances (bio-stimulants) which applied in small quantities, can stimulate the growth of several crops, enhance nutrition efficiency and provide protection against abiotic and biotic stresses (Michalak and Chojnacka, 2015; Plaza et al., 2018; Stirk et al., 2013). Amino acids already contained in proteins from microalgae must be adequately hydrolysed to obtain valuable bio-stimulants (Romero García et al., 2012). However, microalgae biomass also provides valuable phytohormones such as auxin-like and cytokinin-like molecules, stimulating the growth and root development of plants (Tarakhovskaya et al., 2007). Microalgae biomass has also been reported as a source of valuable biopesticides, in this case the nature of the molecules involved being less known (Macías et al., 2008).

Microalgae also have interesting applications in animal nutrition and aquaculture as highly nutritional dietary supplements, for their high content of proteins, high quality essential amino acids (methionine, threonine, and tryptophane, scarce and valuable in animal diets), vitamins, carotenoids, antioxidants, and other substances beneficial to animal health (Muller-Feuga, 2000; Vizcaíno et al., 2014;(Koller et al., 2014; Yaakob et al., 2014). This is a relevant issue, as the demand for animal protein will almost double by 2050 and marine based-proteins can contribute significantly to the global food supply (FAO, 2016, 2018).

Although microalgae production does not require arable land, it demands high water use and fertilizers. Microalgae can exploit slurry and wastewater nutrients, turning a problem into resources. In fact, nutrients in slurry are often mismanaged, and may be provided to crops in excess (due to the need to discharge them) and not effectively taken up by plants (Webb et al., 2009) causing problems related to pollution of surface waters and air. Various studies show that microalgae have a high capacity for the efficient removal of nutrients from wastewater and slurry, so that microalgae production could be an appropriate way for nutrient removal and recovery from a liquid stream, producing valuable biomass at the same time (Franchino et al., 2013; Skorupskaite et al., 2015; Ledda et al., 2015; Christenson et al., 2011). The possibility of using wastewater to satisfy nutrient demands could be beneficial for water treatment and reduce costs in the chain of production (Leite et al., 2019). When producing microalgae biomass using wastewater, a consortia of microalgae and biomass is established, quality of the biomass being a function of operation conditions: thus, if adequately managed, more than 95% of the produced biomass will be microalgae (Acién et al., 2016). In this consortium the microalgae provide oxygen (O<sub>2</sub>) for aerobic bacteria to biodegrade organic pollutants and in turn take up the CO<sub>2</sub> that is released by the bacteria via respiration (Muñoz and Guieysse, 2006). Organic compounds are thus mineralized, the released inorganic nutrients, such as N and P, being consumed by microalgae to produce microalgae biomass. Complete treatment, if possible in these systems, releases water which fulfils EU regulations, and the cost of wastewater treatment becomes lower than using conventional technologies (Acién et al., 2016).

In domestic wastewaters, most of the nitrogen is present as ammonium (NH<sub>4</sub><sup>+</sup>), with low concentrations of nitrite and nitrate. This feature favours nitrogen consumption by microalgae since NH<sub>4</sub><sup>+</sup> assimilation requires less energy than NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> conversion into structural nitrogen (Cai et al., 2013). Slurry, on the other hand, holds higher concentrations of organic matter, nitrogen, and phosphorus in comparison with domestic wastewaters, the amounts present depending on animal nutrition and farming practices (Godos et al., 2009; Gupta and Bux, 2019). Although pig slurry can be rich in ammonium that is the favoured form of nitrogen for microalgae growth, NH<sub>4</sub><sup>+</sup> concentrations exceeding 100 mg L<sup>-1</sup> could decrease microalgae growth in some species because of free ammonia toxicity (Posadas et al., 2014). Therefore slurry must be supplied at low loading rates to microalgae (Posadas et al., 2017), while wastewater can be used directly (Acién et al., 2016).

To increase the productivity of microalgae related systems,  $CO_2$ , which is an essential macronutrient and maybe limiting in ambient air, should be provided. When only  $CO_2$  from the atmosphere is available the biomass productivity is limited, whereas by providing additional  $CO_2$  the biomass productivity has been reported to increase significantly (Acién et al., 2016). The total amount of  $CO_2$ required is a function of overall production capacity, theoretically up to 1.8 kg of  $CO_2$  are required per kg of biomass to be produced. Its supply from a concentrated source has proved to effectively increase the availability of carbon for the growth of microalgae, and also to improve the recovery of nutrients by assimilation in their biomass (Assis et al., 2019; Cai et al., 2013). As compressed  $CO_2$  is costly, both from the economic and environmental points of view, it may be supplied from a recovered source in place of compressed  $CO_2$  gas, by using flue gas from power plants fired with fossil fuels (Acien et al., 2012; Benemann et al., 2003; Wang et al., 2008).

In order to assess the sustainability of a product in a new production chain, it is essential to rely on a standardised approach, by proceeding with completely validated evaluations. One of the tools used is the life cycle assessment (LCA). This procedure includes the calculation of all the inputs (energy and resources) and outputs (emissions) for each production step of the life cycle of the study. Using the Life Cycle Assessment (LCA) methodology has become increasingly widespread for the

evaluation of products and services, with several studies evaluating the production of microalgae as food and energy outcomes. The LCA tool allows to precisely quantify emissions to the environment (physical quantities), highlight critical hot spots in the production process, compare production processes, and finally evaluate the opportunity to adopt an innovative production process in comparison to the already existing options.

Many LCA studies have modelled virtual microalgae facilities with downstream processing, arranging different available technologies and reporting widely available data on microalgae productivity (Holma et al., 2013; Hou et al., 2011; Medeiros et al., 2015; Bauer et al., 2016a). Some references focus on the synergy of different production chain elements such as energy and feedstock or depuration and energy (Murthy, 2011), while others have an in-depth look at the emissions related to the microalgae growth steps such as ammonia and N<sub>2</sub>O (Campbell et al., 2010). Some of the LCA studies are quite optimistic about the future applicability and convenience of microalgae cultivation for commodities purposes, e.g. energy (Hossain et al., 2019; Pegallapati and Frank, 2016) and feedstock (Bussa et al., 2020), while others are more cautious in delineating and delimiting the role that microalgae production may have in the production systems of the future (Branco-Vieira et al., 2020; Pérez-López et al., 2017; Schneider et al., 2018a; Yadav et al., 2020).

However, many of the evaluations, however accurate, are made on virtual production plants, extrapolated from small pilots and laboratory data. The contribution this article intends to provide is the evaluation of sets of different working conditions, by including recovery practices that can highlight the environmental outcomes, all based on solid data from a full-scale facility.

# 2. Materials and Methods

#### 2.1 Goal and Scope definition

The aim of this work is to provide a reliable attributional LCA of the production of microalgae for agriculture and aquaculture related applications, using primary data monitored from a full-scale production facility for microalgae based on raceway ponds and using different recovered inputs for water and nutrient sources, through the evaluation of different alternative scenarios. Some inquiries

that this study will try to answer are: what is the most effective production model (including recovery of waste streams) for microalgae production? What are the differences between a production model using primary sources compared with a production model using recovered streams? What are the shifts in environmental impacts due to the use of recovered resources?

# 2.2 System description

Data to perform the LCA were collected from a demonstration facility at IFAPA (Investigación y Formación Agraria y Pesquera de Andalucia) Research Centre in Almería, Spain. On this location different reactors were available. The specific data included in this work were obtained from a raceway reactor of 1,000 m<sup>2</sup>, which operated in a continuous mode for over one year. These data provided the basis for modelling a 5 ha model plant composed of nine large open raceway ponds for producing microalgae biomass (5,000 m<sup>2</sup> reactor<sup>-1</sup>), 3 photobioreactors used for producing inoculum (1,500 m<sup>2</sup> reactor<sup>-1</sup>, made of PVC linear with a length to width ratio value of 10), and a 1,000 m<sup>2</sup> surface area used for the auxiliary equipment (biomass harvesting and processing). The real unit has a biomass productivity of 20 g m<sup>-2</sup>·day<sup>-1</sup>, the reactors operated in continuous mode at 0.2 day<sup>-1</sup>, during an 8-hour day<sup>-1</sup> for 300 days year<sup>-1</sup>, and ten years was the lifespan assumed for the structure and equipment. The location has an average annual solar radiation in a daylight period of 815  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> and of 1630  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> at noon, with a temperature range from 9°C to 29°C, and an average value of 18°C. System substitution was included for considering the service provided by the management and depuration of wastewaters, which is described in the inventory analysis.

## 2.2.1 Production process

The production process started with producing a strain of microalgae in dedicated reactors to prepare inoculum. The inoculum was used as a seed culture for the ponds. Open ponds comprise a lined, shallow raceway in which water containing microalgae is circulated by paddle wheels. The culture medium was prepared from three different sources of water: freshwater, seawater and wastewater (Table 1). The fertilizers added as N and P sources were calcium nitrate and triple superphosphate respectively, quantities of water demanded and its partial recovering with wastewater, are summarised in Table 2, as is  $CO_2$ . Slurry was supplied to provide the same quantity of N as that supplied by the commercial fertilizer, an average value of 1.5 g kg<sup>-1</sup> content of N was used for manure (average data from measurements). The energy demand was mainly due to the electricity supply for water pumping, mixing devices and gas injection (ambient air and  $CO_2$  or flue gas, see scenarios); the input considered was the Spanish energy mix at the grid, medium voltage. An index of  $CO_2$  absorption equal to 2 has been taken into account for each functional unit (FU) of produced microalgae biomass, based on measurements made at the demoplant and consistent with previous references (Posten and Schaub, 2009; Putt et al., 2011).

# 2.2.2 Harvesting

The harvesting was performed in a two-step process, including pre-concentration by Dissolved Air Flotation (DAF) and a dewatering step using a nozzle separator (GEA Westfalia). At the end of the dewatering steps, the biomass sludge achieved a final biomass concentration of 100 g L<sup>-1</sup> dry matter (dw), ready to be processed, and FeSO<sub>4</sub> was applied as a flocculant. We wish to underline that this two-stage harvesting is an optimised design, capable of stable operation and with a total energy demand of 0.2 kWh kg<sub>biomass</sub><sup>-1</sup>, i.e. the lowest value within the range of 0.2-5 kWh kg<sub>biomass</sub><sup>-1</sup>, reported in recent literature (Fasaei et al., 2018) for dilute solutions from open production systems. As for the growth, the input data of this phase are not from lab scale or theoretical consumption using equipment adapted for microalgae functioning, but data from equipment working at full-scale to harvest microalgae.

# 2.2.3 Processing

After centrifugation, the paste biomass underwent cell disruption by a High Pressure Homogeniser (HPH) (Niro-GEA Westfalia) to be finally processed by enzymatic hydrolysis (commercial Alcalase and Flavourizyme). Base and acid supply in addition to heating was applied in this phase to control reactor pH and temperature to the optimum values imposed by the enzymes used.

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#### 2.3 Functional unit and boundaries

The Functional Unit (FU) provides the reference to which all data in the assessment were normalised. In this study, the FU is 10 kg of produced microalgae paste after hydrolysis, containing 1 kg of dry weight biomass. The system boundaries included "cradle to gate", starting from producing a strain of microalgae in a dedicated reactor to prepare inoculum, and the correlated processes for producing at large scale the biomass at the farm gate. Details of the main processes (Figure 1) considered in the LCA include the inputs and outputs of material and energy such as the construction of facilities, production of inoculum and biomass, harvesting, cell disruption and hydrolysis of microalgae, the supply of  $CO_2$  and nutrients, transport of all the materials to the facility, and emissions to soil, water and air due to the managing of the microalgae production structure.

#### 2.4 Scenarios' inventories

The managing of the facility relies on three inputs (water, nutrients and CO<sub>2</sub>) supplied as follows. The water can come from three different sources: fresh, sea and wastewater (sewage). The management of water is also considered as a factor, i.e. with a recirculation or non-recirculation mode, where recirculation has the advantage of being more efficient for nutrient uptake and of requiring both lower water and energy consumption because of water pumped from the network. Nutrients are supplied either by chemical fertilizers or slurry, and with the wastewater scenario, its counted nutrients are provided by the stream itself. Nutrients in both sources are dosed according to microalgae growth, thus the release of N and P in discharged water is minimal for the non-recirculation mode and equal to zero for water within recirculation. For the case of CO<sub>2</sub> supply, two sources for the set of scenarios are considered: (i) recovery (used by default), i.e. CO<sub>2</sub> was provided as compressed purified gas from external providers while heat for the hydrolysis step was provided by methane burning, and (ii) external supply (C), i.e. CO<sub>2</sub> was provided by methane combustion. For CO<sub>2</sub> scenarios, it was assumed that the productivity yield should not be sensitive to the source of supply, but the environmental burdens change. CO<sub>2</sub>, apart from when supplied as a purified compressed gas, was assumed to be recovered from the burning of gas used for

other purposes. The CO<sub>2</sub> produced in the burning of natural gas in a boiler for the quota of heat needed in the microalgae processing (hydrolysis) was accounted as "recovered". In fact, more CO<sub>2</sub> than this supply is needed, and thus it was assumed that combustion was performed for some other purposes (industrial processing, heating, production of electricity), and flue gas was used in the microalgae facility with no burden accounted for it. Table 1 lists the total scenarios (nine) evaluated in this study. A system substitution is used to solve the multifunctionality in relation to wastewater treatment, as the production of microalgae also delivered depurated water as a product. In this case, the system boundaries of the wastewater scenario are subtracted from the inventory of wastewater treatment: energy, chemicals, structure for delivering depurated water. The process used in the Ecoinvent database for the wastewater treatment was *treatment, sewage, unpolluted, class 3*. Transport of goods, handcraft and commodities to the plant (chemicals, fertilizers, equipment) was assumed to be performed by a 32 Mg transport lorry, Euro 5. For an average 100 km each transport distance is expected to be with empty return. Slurry was assumed to have 20 km transport, while water and wastewater were presumed to be on site (water and wastewater networks).

About ammonia emission, a conservative approach was used, and an average of 30% of the total N supplied was assumed to be lost via ammonia stripping when nitrogen is provided to microalgae into the medium via slurry (Rockne and Brezonik, 2006; Woertz et al., 2009) and 20% when wastewater is used, according to correlation with initial ammonia concentrations (Zimmo et al., 2003). When commercial fertilizer was applied (nitrate salts) no ammonia emission was considered. As in each agricultural activity which involves the use of nitrogen and the availability of carbon, N<sub>2</sub>O emission may occur. The proper mixing allowed high oxygen content during the entire cycle, that should prevent N<sub>2</sub>O formation and emission (Fagerstone et al., 2011). The time of emission remains at night, when oxygen formation from photosynthesis stops and concentration of oxygen in the ponds decreases. Even if the N<sub>2</sub>O emission is contained by adequate conditions, up-to-date literature stresses the importance of considering the N<sub>2</sub>O metrics in LCA calculations (Bauer et al., 2016a) so as not to underestimate the real potential of CO<sub>2</sub> equivalent emissions. In the facility described, mixing was

optimised and continuously monitored, thus the best conditions to reduce N<sub>2</sub>O emission were guaranteed. The N<sub>2</sub>O emission was assumed to be of 0.002% N input (for well-mixed ponds) (Fagerstone et al., 2011). Lastly, methane emissions were considered to be of 0.01592 g CH<sub>4</sub> kg<sup>-1</sup> microalgae, when biomass was calculated according to Ferrón et al., (2012) on the basis of water-air interface in the Almería facility. The basic concept recently discovered is that CH<sub>4</sub> may be produced aerobically through bacterial uptake or degradation of algal products such as methyl-phosphonate (Ferrón et al., 2012). The primary data compiled for the inventory denoted to the FU are presented in Table 2. All the inputs from trials were inserted including uncertainty and type of distribution.

#### 2.5 Impact assessment

In the Life Cycle Impact Assessment (LCIA) phase, emissions and resource data identified during the LCI (Life Cycle Inventory) are translated into indicators that reflect environmental pressures and resource scarcity. The software SimaPro® Analyst 9.0.0.41 was used for the computational implementation of the inventories (Goedkoop et al., 2008), and the set of libraries covered by Ecoinvent databases v3.5, 2018 to analyse the environmental impacts. Because of its representativeness at a global scale, the ReCiPe 2016 (Huijbregts et al., 2017a) mid-point method (hierarchist approach) (version 1.13) was used to assess the environmental performance of microalgae production. Robustness of the LCA results was assessed by Montecarlo analysis, setting 10,000 runs (Burmaster and Anderson, 1994).

#### 2.6 Sensitivity analysis

To evaluate the influence of relevant parameters involved in using of recovered nutrients (slurry), i.e. the transport and the productivity, a sensitivity analysis was performed for scenario W3 considering the slurry transportation distance (10 and 30 km as a minimum and maximum value, i.e. 20 km as default value), and the productivity loss for the use of recovered nutrients (72 and 48 ton ha<sup>-1</sup> as a minimum and maximum, i.e. 60 ton ha<sup>-1</sup> as default). The sensitivity coefficient is calculated using the Equation 1.

$$S = \frac{(\text{IC high-IC low})}{\frac{\text{IC default}}{(\text{I high-I low})}}{\text{I default}}$$

(Eq.1)

Where IC is the value of the environmental Impact Category (max, min and default) and I is the value of the input considered for the analysis. Later, simulation provided threshold values for a maximum distance of transport and acceptable production losses due to the use of recovered nutrients.

# **3** Results and discussion

#### 3.1 Environmental Impact assessment

The potential environmental impact associated with the nine scenarios at mid-point level is indicated in Table 3 and represented in Figure 2, results are reported as a relative value (%) achieved, assuming that the highest values for each impact would be equal to 100%. Results show that the scenarios with nutrient recovery (from both slurry and wastewater) are the most environmentally friendly alternatives with noticeable differences regarding the others, in areas that concern climate change, freshwater eutrophication, water depletion, terrestrial acidification and human toxicity. The other scenarios studied showed similar pattern-response in the considered categories.

Robustness of the LCA results was assessed by Montecarlo analysis. When comparing scenarios using recovered resources vs not using them, for 11 categories, the scenarios including the use of fertilizers displayed higher results than the scenarios with recovered nutrients for more than 90% of the runs. For three categories (Human carcinogenic, Ozone depletion and Land use) results of scenarios including fertilizer use were higher in 60% of the runs, while for ozone formation (terrestrial and human) was the opposite: the scenario with fertilizers displayed lower results than the scenario with recovered nutrients in 62% of the runs. For the last two categories (Particulate matter and Terrestrial acidification) the results of scenarios including recovered nutrients were higher than that of fertilizer use in 100% of the runs. The obtained results are consistent with other studies (Arashiro et al., 2018; Tasca et al., 2019). In particular, from those studies it was clear that the inputs of interchangeable factors such as the nutrient source, water type, and recirculation have a sharp effect

in how they can impact the environment, thus supporting the re-use of resources (recovered nutrients) and the full exhaustion (water recirculation to exploit slurry nutrients). The negative bars reported in Figure 2 represent the avoided impacts in the related categories such as ecotoxicity (in the entire compartment, i.e. terrestrial, marine and freshwater), human carcinogenic toxicity, and mineral scarcity. These prevented impacts are attributed to Scenario W9, justified for the depuration of wastewater and for the avoided impacts linked to these processes (i.e. the saving of energy for depuration performed in a standard wastewater depuration plant). The removal of nutrients from wastewater has been previously reported with positive effects (Collotta et al., 2018) in reducing the impact of eutrophication, and terrestrial - freshwater ecotoxicity. The contribution of the process for the different impact categories is reported in the following paragraphs.

# 3.1.1 Climate change.

Global Warming Potential (GWP), which represents the amount of additional emission pressure combined over 100 years because of an emission of 1 kg of CO<sub>2</sub> (expressed as CO<sub>2</sub> eq.) (Huijbregts et al., 2017a), is the Impact Category broadly used for climate change. For the scenarios considered its value was mainly dependent (Figure 3) on the use of energy and the production of fertilizers used to produce microalgae (still linked to the energy use), according to previous works (Acién et al., 2012; Imporzano et al., 2018; Molina et al., 2001). For Scenario W1, for instance, Ca(NO<sub>3</sub>)<sub>2</sub> addition caused 24% of the GWP, and electricity use, 38%. Recirculation of growth medium, in the corresponding Scenarios W1, W3, W5 and W7, allowed pumping less water, causing a slightly lower impact in this category (Table 3). It should be noted that transport caused higher impacts in the scenarios in which manure was used, because of manure transport: i.e. for each unit of fertilizer a large amount of water was also moved. The use of recovered fertilizer saved 0.39 kg CO<sub>2</sub> eq. in comparison with the chemical fertilizers' use, giving a better result (13%) for the GWP category (Table 3). The transport of materials for the facility construction was equal for all scenarios studied, so that it did not affect environmental impacts (Figure 3, GWP category).

In the production model under study, the energy input was optimised for both microalgae production (0.84-0.94 kWh kg<sub>biomass</sub><sup>-1</sup>) and harvesting (0.19 kWh kg<sub>biomass</sub><sup>-1</sup>). In the first case, optimisation was due to optimised reactor design and to the optimised fluid movement and the slow speed of the liquid adopted; in the second case, it was obtained via two-stage harvesting. Thus the large gain that can be considered within scenarios is completely due to the inputs of recovered nutrients and the corresponding emissions for production and use. The lowest impact measured was registered for Scenario W9 (Figure 2, Table 3), where the service provided by wastewater depuration (system substitution accounting for the function of wastewater depuration) compensated for part of the CO<sub>2</sub> emissions. In Figure 2 are reported both positive and negative (credits) impacts. The values reported are similar to those presented by Collotta et al. (2018), in which the use of wastewater, in addition to the injection of CO<sub>2</sub> recovered from a cement plant flue gas, gave the lowest impact emission, i.e. 0.306 kg CO<sub>2</sub> eq. for each kg of biomass, that is quite comparable with that reported for Scenario W9 scenarios, i.e. 0.47 kg CO<sub>2</sub> eq.

## 3.1.2 Stratospheric ozone depletion.

Emissions of ozone-depleting substances (ODSs) (expressed in kg CFC 11 eq.) which leads to increases in UVB radiation (Huijbregts et al., 2017a; WMO, 2014), are relatively small, with the lowest value reported for Scenario W9. As in GWP, the impact category was mainly due to the use of electricity, Ca(NO<sub>3</sub>)<sub>2</sub> and fuel combustion (transport and building of infrastructure).

# 3.1.3 Ionising radiation.

The ionising radiation potential (IRP) reported as a Cobalt-60 eq. to air, quantifies radionucleotides emitted not only during nuclear activity but also in ordinary activities such as fuel burning and phosphate rock extraction. The process contribution that mainly explains its appearance was electricity, with 87% of contribution in Scenarios W1-W2-W5-W6 (in which fertilizers were used), followed by  $Ca(NO_3)_2$  with 6.5%. In the other scenarios, the contribution of electricity was higher than 90%.

#### 3.1.4 Photochemical Ozone Formation.

The category quantifies, as NOx equivalent, the potential molecules leading to the formation of ozone, i.e. the photochemical reactions of NOx and Non-Methane Volatile Organic Compounds (NMVOCs) (Huijbregts et al., 2017a). Many of the same processes mentioned in the previous categories explained the intensification of EOFP, where the impact was low and similar for Scenario W1-W8, and smaller for Scenario W9. The impact categories: ozone formation, human health, ozone formation and terrestrial ecosystem, despite low differences in absolute values, displayed the same pattern.

#### 3.1.5 Terrestrial Acidification.

The category is linked to the atmospheric deposition of sulphates, nitrates and phosphates that cause a change in the acidity of soils. The highest values were linked to the scenarios using recovered nutrients, slurry and wastewater, that contain nitrogen in the form of ammonia which undergoes volatilisation during the production of microalgae. Once having considered direct ammonia emission, the other source of acidifying substances, far less relevant, is the use of electricity, i.e. the burning of fuel and corresponding NOx production. Electricity accounts for 5% of this category in the scenarios in which ammonia volatilisation occurred, while it was 70% for the others (Figure 2). For Scenario W9 the profile of impacts was analogous to the scenarios with recovered nutrients (Scenarios W3-4-7-8) due to less volatilisation of ammonia and the lowest electricity demand, the latter because there is no burden of transport of nutrients (i.e. manure) and there was a small avoided impact for wastewater depuration.

# 3.1.6 Eutrophication.

Eutrophication is due to the release of nutrients in water bodies; with freshwater eutrophication potentials (FEP) the impact is quantified as kg P eq. In the scenarios that considered recirculation, the main contribution to this category was the release of phosphorus (P) in the discharged water, i.e. 80% of the contribution, even if moderate (see Table 2). Other minor contributions are because of the use of electricity, production of  $Ca(NO_3)_2$  and P discharged as waste during the production steps of

triple phosphate typical of the scenarios using chemical fertilizers. When recirculation was performed, P discharge disappeared (Table 2) reducing the impact, and instead electricity became the main contributor to the footprint. In all the scenarios the impact encompassed both local (P released on site) and global emissions (P released globally in the process of tailing management). For Marine Eutrophication, expressed as N equivalent, the impact is completely due to the release of water in the non-recirculation scenarios (Figure 3), other contributions are negligible. Collotta et al., (2018), showed a significant favourable effect (negative value of impact categories) in the eutrophication impact when wastewater is used, because of the credit of avoided emissions for nutrients uptake and removal from wastewater. In this work, the system substitution took into consideration the avoided wastewater treatment, i.e. the credit was not relative to N and P, but it was relative to the energy demand for the wastewater treatment that was avoided, thus the eutrophication category for Scenario W9 was analogous to all the scenarios with a non-recirculation mode.

# 3.1.7 Ecotoxicity and human toxicity.

The emissions of 1,4-dichlorobenzene-equivalents (1,4DCB-eq) expressed in kg is used as the characterisation factor of ecotoxicity in freshwater, marine and terrestrial ecosystems (Huijbregts et al., 2017a). Ecotoxicity showed the same pattern described above for the other categories, i.e. a remarkable decrease of emission in the scenarios using recovered nutrients (-27%). The main factors affecting ecotoxicity were the use of both electricity and synthetic fertilizers (when supplied), and the processes related to transport all along the lifecycle (i.e. the use and the disposal of vehicles). As outlined in the discussion for other categories, the impacts of transport rose in the scenarios using slurry, with a very marked difference compared to scenarios not including slurries, this being particularly shown in the categories of freshwater and marine ecotoxicity.

In Scenario W9 the use of recovered nutrients within wastewater and the added service of water depuration, resulted in this scenario having a negative impact, i.e. the impact of the energy used for the production was "counterbalanced" by the service of waste depuration. This is easily understood if we consider that the direct electricity used for 1 m<sup>3</sup> of wastewater depurated by the microalgae

system is 0.49 kWh m<sup>-3</sup>, encompassing the energy for biomass production, while the depuration of wastewater by a conventional system costs on average 0.3-2.1 kWh m<sup>-3</sup> of wastewater<sup>-1</sup> (Gandiglio et al., 2017).

For terrestrial ecotoxicity (Figure 3) the role of transport was higher than that played in freshwater and marine ecotoxicity, and it was comparable to the share attributable to electricity and fertilizer use. Similarly, human non-carcinogenic toxicity was mainly due to electricity and fertilizers, and presented a significant reduction in the scenarios with recovered nutrients (Figure 2), while the human carcinogenic category was explained by electricity use as first contributor, and fertilizers and transport related process (building of vehicles and roads) as a second one. The decrease in impact due to the non-use of synthetic fertilizers was eliminated by the greater impact related to the transport of slurry. This led to an equality of this category in the various scenarios considered, excluding, as before, Scenario 9, where the crediting for wastewater treatment brings a big decrease in the impact.

# 3.1.8 Use of resources: Land use, Fossil, Mineral and Water depletion

Land use expressed as the area occupied by the facility was almost equal in all scenarios. Fossil exploitation, quantified as kg oil eq., was explained by electricity use,  $Ca(NO_3)_2$  (30% in the scenarios using fertilizer) and heating (natural gas). Due to the high contribution of  $Ca(NO_3)_2$ , the scenarios with recovered nutrients displayed a 20% decrease in this category, the remaining "credit" being compensated by the transport of slurry. Outside this array is Scenario W9, in which electricity and heat processes mainly provide the contribution, presenting a reduction of 61% in comparison with the scenarios using fertilizer.

Considering the category of mineral resource scarcity, Scenario W9, again, was the only one that displayed negative values, in the sense of preventing environmental impacts in the long term. By contrast, scenarios (Scenario W1-2-5-6) using an external artificial nutrient source presented the higher impact, this was mostly due to Ca(NO<sub>3</sub>)<sub>2</sub> and triple superphosphate, respectively 42% and 24% of the category value. Water depletion level, expressed as m<sup>3</sup> of water consumed over water extracted, depended mostly on the upstream process of energy production. Thus, it was influenced

by the use of electricity which is higher when there is constant pumping of water (recirculation off), and where in fact water consumption increases. The wastewater scenario displaced the lowest impact due to the release of depurated freshwater (2 m<sup>3</sup> kg<sup>-1</sup> microalgae). When seawater and wastewater were used the impact on this process was lower (Figure 2).

## 3.2 Recovered fertilisers and sustainable transport: threshold distance

Markedly, using wastewater or recovered fertilizer from slurry, carried substantial environmental benefits compared with the external supply of macronutrients, this finding being backed up by recent literature (Collotta et al., 2018; Schneider et al., 2018b). Although the management of recovered nutrients showed a net environmental gain, it is appropriate to dwell on an item often cited as a significant component of some impact categories: the transport of recovered fertilizers. The concentrations of nutrients in slurry was not comparable with the concentration of nutrients in synthetic fertilizers; in addition, much water was involved in transport. In the illustrated scenarios, the slurry used had a concentration in N and P of 1.5 g kg<sup>-1</sup> w/w and 0.16 g kg<sup>-1</sup> w/w, from data in Table 2. Therefore, it becomes essential for proper programming, to understand the distances for sustainable transport of slurry, or the distance at which the emissions balance is still acceptable compared to the use of synthetic fertilizers. Moreover, LCA studies using wastewater as the alternative culture medium in growing microalgae (Schneider et al., 2018) showed higher growth resulted by using NPK synthetic fertilizers, since the growth medium supplied was less turbid, allowing higher radiation infiltration compared to wastewater or slurry, both of them rich in suspended organic matter.

For these reasons it is clearly important to carefully evaluate nutrient source and slurry, not only to outline the benefits of slurry as an alternative nutrient source, but also because of the high variability that it could carry in real contexts (productivity), then it becomes reasonable to determine the effect that they could have in a production system. Table 4 shows the results of the sensitivity analysis for transport and productivity in scenario W3. For transport, the category mainly affected was mineral resource scarcity, since it was directly linked to the use of resources for the road infrastructure and

maintenance. The other categories involved were all those related to toxicity (human and ecosystem), the effect on ozone (both stratospheric depletion and atmospheric formation) and the GWP. The simulation of different transport scenarios showed that 40 km was the limit because up to that distance, the solution with nutrient recovery was still sustainable and comparable to the scenario with synthetic fertilizers (W1) for the categories most affected. Beyond this threshold, the solution with recovered fertilizer was not advantageous. Concerning a possible decrease in production due to any issue related to recovered nutrients, the production system with recovered fertilizers was advantageous up to a productivity loss of 20%, i.e. at almost 48 Mg ha<sup>-1</sup> of slurry the environmental benefits due to the recovery of nutrients were cancelled out by the drop in production.

# 3.3 CO<sub>2</sub> recovery

An additional scenario was taken into consideration for compressed and transported CO<sub>2</sub>, instead of recovered CO<sub>2</sub> produced by the combustion of fuel. This approach is important because of the role that commodities such as the recovered CO<sub>2</sub> will play in the immediate future (Naims, 2016), and to show how on site recovery, without the need for storage and compression, is an essential option for the sustainability of microalgae-related production chains. Using compressed CO<sub>2</sub> caused an impact increase of about 2-3 times, of the most relevant impact categories, i.e. global warming, eutrophication and toxicity. Terrestrial ecotoxicity, which is heavily influenced by the use of fuels (CO<sub>2</sub> transport, compression and purification) reported an increase of impact of as much as six times. Indeed, literature outlined that CO<sub>2</sub> injection was the primary factor affecting the environmental impact in the entire chain (Tasca et al., 2019), followed by both the nutrient supply and energy consumption (Collotta et al., 2018), indicating the relevance of the use of recovered sources. In the two years of experimentation that provided data for this work, CO<sub>2</sub> from flue gas (methane combustion) was used routinely, demonstrating how productivity is not damaged by any NOx compounds produced during combustion, which actually work as a micronutrient. The topic was already addressed in literature (Porcelli et al., 2020), with the recommendation for more detailed investigations in full-scale systems.

#### 3.4 Evaluation of impacts and perspectives of microalgae cultivation

To compare and discuss LCA results of microalgae cultivation -including the use of recovered resources- with existing literature, we can use the Global Warming Potential (GWP) category, as it is a robust indicator, widely used and common to different LCA assessment methods.

Schneider et al. (2018) carried out cultivation trials in open raceways, with continuous working, using wastewater as one of the culture media, and reported values of GWP equal to 5.34 and 2.69 when using fertilizers and wastewater respectively.

Completely different findings came from Porcelli et al. (2020), who found values of 257 and 298 CO2 eq kgbiomass-1 using fertilizers and recovered CO<sub>2</sub> vs synthetic CO<sub>2</sub>. In this case, the production involved the use of artificial light, sterilization steps and energy-intensive practices for biomass treatment. So even if only 50% of the impacts were due to cultivation (around 120 CO<sub>2</sub> eq kgbiomass-1) nonetheless the use of artificial light completely shifted the orders of magnitude of the impact in comparison to a field-based production based on sunlight. Other studies that exploited recovered resources (Bauer et al., 2016b; Medeiros et al., 2015; Yuan et al., 2015) reported values of 1.4, 1.03, and 1.26 CO<sub>2</sub>eq for each kg of algae biomass, respectively. These studies modelled inputs from the scale-up of lab data, thus some uncertainty is present in the estimation of inputs or in the evaluation of other factors that, in the continuous operation, may reduce productivity, and provide a low and optimistic value of impacts for the GWP category. The values obtained in our study, as regards the scenarios with recovered nutrients (W3 and W4, 1.24. and 1.29 kg CO2 eq kgbiomass-1 respectively), are close to the lowest values reported in literature, even if performed on a full scale, thus considering the actual measured productivity and consumption of inputs.

Various studies (Clarens et al., 2010; Collet et al., 2011; Lardon et al., 2009) outlined how, in microalgae production, fertilizers consumption, harvesting, and downstream processing, risk

nullifying the benefits of the efficient photosynthetic yield of microalgae. As the critical work of Ketzer (Ketzer et al., 2018) highlights, the energy consumption for the cultivation of algae varies from the most optimistic at 4 MJ kg<sup>-1</sup> up to a value of two orders of magnitude higher (800MJkg<sup>-1</sup>). In this study the consumption is lower than 10 MJ kgbiomass-1, thus together with recovered resource valorization, it allows the achieving of low GWP values, although the inputs of energy and the need for equipment are the main drawbacks of microalgae production.

The results obtained in this work depict the state-of-the-art technology, at present, and report, in the best scenarios, the lowest GWP scores, confirming previous studies that scaled up lab production data in an industrial frame. Based on these data it is interesting to understand the effective potential and role of microalgae production, and thus to compare the environmental burden of microalgae grown in this industrial setting (open raceway, optimized equipment) with the production of land-based commodity plants, e.g. maize and soy, on a dry matter basis. Values of GWP for silage maize biomass or soy range from 0.1 to 0.7 CO<sub>2</sub> eq kg<sup>-1</sup> (Bacenetti and Fusi, 2015; Dalgaard et al., 2008), thus consistently below that of microalgae production according to the state of the art technologies and circular recovery concept.

Considering both the inputs necessary for microalgae production and the environmental impact measured by LCA, the peculiarity of microalgae production systems becomes clear in comparison with traditional agricultural production. Microalgae production allows a higher productivity than "traditional agriculture" and it can limit some emissions due to the management of nutrients in the plant-soil system, i.e. N<sub>2</sub>O emission and nutrients leaching in both surface and deep waters. The microalgae production systems are well isolated from the soil, carefully monitored and managed, and they have high technological input. Thus, microalgae systems allow an optimised management of the recovered nutrients, while traditional agro-systems present more critical issues in the open field, as they involve complex natural systems such as soil and water bodies. The main drivers of these advantages are: (i) the possibility of adding nutrients step by step following the microalgae uptake curve, thus ensuring a great efficiency in using both chemical and recovered fertilizers; (ii) the

possibility of water recirculation, that allows the water to be discharged only once all the nutrients have been used; (iii) the closed and waterproof system of tanks used to produce microalgae prevents any leaching of nutrients before the water used for the production is discharged; (iv) the possibility of controlling the pH, together with the correct delivery of nutrients on demand and the high dissolved oxygen saturation of the media (greater than 100% of air saturation), which allows the reduction of both ammonia and N<sub>2</sub>O emissions.

On the other hand, the production of microalgae requires an amount of energy (in the form of electricity) higher than that for traditional agricultural activities, i.e. the direct electricity demand to produce microalgae biomass is of 8.6 MJ kg biomass<sup>-1</sup>, as primary energy, much higher than that reported for a crop, i.e. 1 MJ kg biomass<sup>-1</sup> (Hege et al., 2004). As outlined by the LCA analysis, the use of electricity is the most significant input in determining almost all the impact categories.

Another important element that differentiates microalgae from crop production is the use of the soil, not only, as previously discussed, in terms of the amount of soil required per unit of biomass produced but considering other soil services. The soil reserved for microalgae production is a sealed soil that cannot offer any eco-systemic services to the environment, such as, for example, draining and filtering of rainwater, habitat for entomofauna, capture of carbon in the soil etc.

Thus, the LCA numbers outline that microalgae production, even when performed on a large scale, and according to a circular approach and using low energy processes, is not, at present for commodity purposes, either energy or food. However, microalgae production has a role for specific functions achieved thanks to its valuable and unique components: e.g. hormone-like molecules with bio-stimulating activity on land plants, shown by assays performed using microalgae grown in the reported trials of this work (Plaza et al., 2018; Roncero-Ramos et al., 2019) and PUFA, for the production of aquafeed (Galafat et al., 2020), which replaces fish oils and decreases the pressure on marine ecosystems (trials performed in the frame of the same project). These kinds of products can provide functions not comparable to those of land-based commodities, and thus justify higher impacts.

Moreover, the evaluation of environmental pros and cons of microalgae production depends on contextual conditions, not entirely captured by all the LCA approaches, such as the possibilities for using seawater and wastewater for production, mainly in the areas where pressure on freshwater resources is high, or the opportunity to use non-arable land, including soils poor and low in quality, or former industrial sites, not suitable for land crop production. For this reason, as often underlined in strategic studies for the location of microalgae plants, it is important to dedicate only industrial or low environmental value soils to a microalgae facility, while valorising high quality soils in proper crop agroecosystems (Koyande et al., 2019; Rosch et al., 2009).

Beyond the added value of microalgae components and the valorisation of low-grade resources in place of scarce resources (by using seawater and non-arable soils), another critical issue in the environmental evaluation of microalgae production is when other services can be achieved. In scenario W9 the depuration of wastewater is performed, and the global sustainability of microalgae production changes completely and becomes better in comparison with the production of land-based commodities. This type of algae-based remediation biotechnology is increasingly being investigated, even in the case of difficult to treat waste streams rich in heavy metals. Salam's work (Salam, 2019) has highlighted how microalgae can effectively remove heavy metals, and depurate waters. As in this work, Salam demonstrated that the sustainability of this technology is hampered by drawbacks due to low biomass concentration (harvesting energy demand). Up to date innovations such as the immobilization of microalgae could be a further step on the road towards greater sustainability of microalgae cultivation systems.

#### 4 Conclusions

Data inventory from an actual operating facility outlined that the main relevant inputs affecting environmental performance were electricity consumption, chemical fertilizer demand (N and P) and transport. Scenarios with recovered nutrients from slurry and the use of wastewater led to the better environmental performances. The threshold of distance for manure transport was 40 km, beyond that value the scenarios using recovered nutrients performed worse than the scenarios using chemical fertilizers. The maximum drop in productivity that the system with recovered nutrients could withstand was 20%, in addition to the environmental performance which was worse than in the scenario with chemical fertilizers. The multifunctionality of the process including wastewater depuration, allowed this scenario to compensate most of the impact categories, yielding negative values in some (e.g. all the toxicity categories). If CO<sub>2</sub> used is not from a recovered source, impacts are 2-3 times higher. Finally, state of the art technology by now justifies the role of microalgae not for commodities production but for specific functions achievable thanks to microalgae metabolism, unless wastewater depuration is included.

Thus microalgae are currently not the panacea for sustainable feedstock production in a world with a growing population, but they can be an excellent tool for the implementation of circular economy processes, such as the depuration of wastewater and livestock wastes combined with the production of aquafeed and the production of biostimulants for a sustainable intensification of agriculture.

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# LIST OF TABLES

Watar true a	Nutrients	Nutrients		Carbon	
water type	supplied by	Recirculation	Code	supply	
Freshwater	Fertilizers	Recirculation	W1	R/C	
Freshwater	Fertilizers	Non-recirculation	W2	R/C	
Freshwater	Manure	Recirculation	W3	R/C	
Freshwater	Manure	Non-recirculation	W4	R/C	
Seawater	Fertilizers	Recirculation	W5	R/C	
Seawater	Fertilizers	Non-recirculation	W6	R/C	
Seawater	Manure	Recirculation	W7	R/C	
Seawater	Manure	Non-recirculation	W8	R/C	
Wastewater	None	Non-recirculation	W9	R/C	

Table 1. List of scenarios related to water and nutrient use.

Parameter	Unit	W1	W2	<i>W</i> 3	W4	W5	W6	W7	W8	W9		
	Natu					ural resources						
Soil occupation	m <sup>2</sup> kg algae <sup>-1</sup>	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17		
Freshwater demand	m <sup>3</sup> kg algae <sup>-1</sup>	0.54	2.333	0.54	2.33	0.00	0.00	0.00	0.00	0.00		
Seawater demand	m <sup>3</sup> kg algae <sup>-1</sup>	0.00	0.00	0.00	0.00	0.54	2.33	0.54	2.33	0.00		
Wastewater demand	m <sup>3</sup> kg algae <sup>-1</sup>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.33		
Water Release in the environment	m <sup>3</sup> kg algae <sup>-1</sup>	0.24	2.00	0.24	2.00	0.24	2.00	0.24	2.00	2.00		
	Nutrients supply											
N input	kg kg <sup>-1</sup> algae	0.1	0.1	0	0	01	0.1	0	0	0		
P input	kg kg <sup>-1</sup> algae	0.016	0.016	0	0	0.016	0.016	0	0	0		
Slurry	m <sup>3</sup> kg algae <sup>-1</sup>	0	0	0.1	0.1	0	0	0.1	0.1	0		
	Other chemicals											
Enzyme	g kg <sup>-1</sup> algae	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03		
NaOH	g kg <sup>-1</sup> algae	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08		
Flocculant	kg kg <sup>-1</sup> algae	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02		
					Energy							
Energy demand for culture medium preparation	kwh kg <sup>-1</sup> algae	0.27	0.39	0.27	0.39	0.27	0.39	0.27	0.39	0.39		
Energy demand for algae growth	kwh kg <sup>-1</sup> algae	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57		
Energy demand for Harvesting (DAF unit)	kwh kg <sup>-1</sup> algae	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11		
Energy demand for Harvesting (Noozle concentrator)	kwh kg <sup>-1</sup> algae	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
Cell disruption (HPH)	kwh kg <sup>-1</sup> algae	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024		
Electricity for hydrolysis	kwh kg <sup>-1</sup> algae	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
Heat for hydrolysis	MJ kg <sup>-1</sup> algae	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8		

**Table 2.** Data inventory used for each scenario for the calculation of impacts. When not indicated primary data measured on the plant, data from literature and assumption are indicated and critically discussed in the text.

					Emission	IS				
Nitrogen released in water	kg kg <sup>-1</sup> algae	0	0.015	0	0.015	0	0.015	0	0.015	0.015
Phosphorus released in water	kg kg <sup>-1</sup> algae	0.00	0.0017	0.00	0.0017	0.00	0.0017	0.00	0.0017	0
Organic carbon released in water (TOC)	kg kg <sup>-1</sup> algae	0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.07	0.07
Flocculant released in water	kg kg <sup>-1</sup> algae	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
$N_2O$ released in atmosphere	mg kg <sup>-1</sup> algae	2.03	2.033	2.85	2.85	2.03	2.03	2.85	2.85	2.85
$NH_3$ released in atmosphere	kg kg <sup>-1</sup> algae	0	0.00	0.045	0.045	0.000	0.000	0.045	0.045	0.030
CH4 in the atmosphere	mg kg <sup>-1</sup> algae	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05

Table 3. Characterization of the 9 scenarios at the midpoint level impact categories according to

Impact category	Unit	W1	W2	W3	W4	W5	W6	W7	W8	W9
Global warming	kg CO <sub>2</sub> eq	1.45	1.50	1.24	1.29	1.45	1.50	1.24	1.29	0.33
Stratospheric ozone depletion	kg CFC11 eq	5.53E -07	5.73E -07	5.44E -07	5.64E -07	5.53E -07	5.73E -07	5.44E -07	5.64E -07	3.15E- 07
Ionizing radiation	kBq Co- 60 eq	0.41	0.43	0.38	0.41	0.41	0.43	0.38	0.41	0.25
Ozone formation, Human health	kg NOx eq	3.67E -03	3.84E -03	3.80E -03	3.97E -03	3.67E -03	3.84E -03	3.80E -03	3.97E -03	1.48E- 03
Fine particulate matter formation	kg PM2.5 eq	2.49E -03	2.63E -03	1.29E -02	1.30E -02	2.49E -03	2.63E -03	1.29E -02	1.30E -02	1.20E- 02
Ozone formation, Terrestrial ecosystems	kg NOx eq	3.72E -03	3.89E -03	3.86E -03	4.03E -03	3.72E -03	3.89E -03	3.86E -03	4.03E -03	1.48E- 03
Terrestrial acidification	kg SO <sub>2</sub> eq	6.93E -03	7.32E -03	9.42E -02	9.46E -02	6.93E -03	7.32E -03	9.42E -02	9.46E -02	9.23E- 02
Freshwater eutrophication	kg P eq	4.29E -04	2.18E -03	3.24E -04	2.08E -03	4.29E -04	2.18E -03	3.24E -04	2.08E -03	1.88E- 03
Marine eutrophication	kg N eq	8.51E -05	4.54E -03	2.38E -05	4.48E -03	8.51E -05	4.54E -03	2.38E -05	4.48E -03	4.47E- 03
Terrestrial ecotoxicity	kg 1,4- DCB	2.77	2.82	1.99	2.05	2.77	2.82	1.99	2.05	-0.97
Freshwater ecotoxicity	kg 1,4- DCB	2.02E -02	2.10E -02	1.46E -02	1.54E -02	2.02E -02	2.10E -02	1.46E -02	1.54E -02	- 1.39E- 02
Marine ecotoxicity	kg 1,4- DCB	3.11E -02	3.21E -02	2.20E -02	2.30E -02	3.11E -02	3.21E -02	2.20E -02	2.30E -02	- 1.90E- 02
Human carcinogenic toxicity	kg 1,4- DCB	4.15E -02	4.33E -02	3.85E -02	4.03E -02	4.15E -02	4.33E -02	3.85E -02	4.03E -02	- 8.42E- 02
Human non-carcinogenic toxicity	kg 1,4- DCB	0.66	0.68	0.47	0.49	0.66	0.68	0.47	0.49	-0.31
Land use	m <sup>2</sup> a crop eq	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.72
Mineral resource scarcity	kg Cu eq	5.97E -03	6.02E -03	2.50E -03	2.56E -03	5.97E -03	6.02E -03	2.50E -03	2.56E -03	- 2.63E- 02
Fossil resource scarcity	kg oil eq	0.44	0.45	0.37	0.39	0.44	0.45	0.37	0.39	0.16
Water consumption	m <sup>3</sup>	6.35	6.78	5.95	6.37	6.05	6.47	5.65	6.06	0.34

# ReCipe 2016 Midpoint (H) V.1.03.

Table 4. Sensitivity indices for transportation distance and productivity in a nutrient recovered source	ce
(Scenario W3: Slurry).	

Impact category	Sensitivity coefficient transport	Sensitivity coefficien Productivity		
Global warming	0.157	-0.48		
Stratospheric ozone depletion	0.189	-0.21		
Ionizing radiation	0.038	-0.23		
Ozone formation, Human health	0.193	-0.22		
Fine particulate matter formation	0.080	-0.22		
Ozone formation, Terrestrial ecosystems	0.197	-0.22		
Terrestrial acidification	0.073	-0.22		
Freshwater eutrophication	0.056	-0.22		
Marine eutrophication	0.050	-0.22		
Terrestrial ecotoxicity	0.298	-0.20		
Freshwater ecotoxicity	0.216	-0.24		
Marine ecotoxicity	0.221	-0.24		
Human carcinogenic toxicity	0.230	-0.23		
Human non-carcinogenic toxicity	0.245	-0.24		
Land use	0.003	-0.40		
Mineral resource scarcity	0.332	-0.20		
Fossil resource scarcity	0.185	-0.19		
Water consumption	0.037	-0.23		

# **Caption Figures**

Figure 1. System boundary considered in the LCA for the production of 1 kg of microalgae biomass.

Figure 2. Comparative environmental results for the nine scenarios considered. Impacts assessment

calculated according to ReCiPe 2016 midpoint (H) V 1.03 method.

**Figure 3.** Contribution of the main inputs to the different impact categories. ReCiPe 2016 midpoint (H) V 1.03 method.



Figure 1



Figure 2



