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Life cycle assessment of an innovative rotating biofilm technology for microalgae production: An Eco-design approach

Diego Penaranda^{a*}, Hubert Bonnefond^a, Freddy Guihéneuf^b, Marjorie Morales^c, Olivier Bernard^a

^a INRIA BIOCORE, BP 93 06902, Sophia Antipolis Cedex, France.

^b Inalve S.A, CEEI Nice Premium 61 Avenue Simone Veil, 06200, Nice, France.

^c Industrial Ecology Programme, Department of Energy and Process Engineering, Norwegian University of Science and Technology (NTNU), Høgskoleringen 5, Trondheim 7034, Norway.

*Corresponding author: diego.penaranda-sandoval@inria.fr

Abstract

Microalgae are recognized as a high-nutritional quality non-conventional feed resource which can contribute to address the increasing food demand. This study focuses on a breakthrough microalgal production system, based on rotating biofilm, with the objective of assessing and reducing its environmental impact for two microalgae-based products: algal biomass and protein concentrate (algae meal). The methodology is based on Life Cycle Assessment (LCA) with an eco-design approach. We provide a methodology to support the LCA of emerging technologies at low technology readiness level. Eco-design parameters were identified from the process modelling and its environmental assessment through an iterative sensitivity analysis. The results for algae meal were compared with soymeal and fishmeal and benchmarked with alternative production technologies. The NH₃ emission factor, fabric support properties (lifespan and composition) and electricity consumption (power rotor and blower) turned out to be the crucial eco-design parameters. Impact reduction ranging from 25% to 88.3% were obtained by eco-designing this new technology. Environmental footprint of algae meal from rotating algal biofilms outperformed the other scenarios. Algae meal from eco-designed rotating biofilm has an environmental impact reduced by at least 70.8% in comparison to conventional aquafeeds (fishmeal and soymeal).

Keywords: *Aquafeed, Biofilms, Emerging technology, LCA, Protein, Technology readiness level (TRL).*

1 Introduction

The world population will reach 9.7 billion by 2050, and food production must double by 2050 to cover the demand (Alexandratos and Bruinsma, 2012; de Medeiros et al., 2021). This may lead to an overexploitation and limitation of conventional protein sources such as livestock and fishes,

which represent 43% of the global protein demand (Yarnold et al., 2019). Sustainable and secure feed resources for animal production becomes now a challenge for the future (Nagarajan et al., 2021). Corn, rice, soybeans, soymeal, fishmeal, are the main conventional feed (de Medeiros et al., 2021). Fish meal is the main feed in the aquaculture industry, due to its high content of protein (Chen, Fufeng et al., 2021). Protection of marine fishery resources limited the fish meal production. Moreover, fish meal safety is threatened by the pollution due to microplastics, chemicals and heavy metals accumulating in fishes (Hanachi et al., 2019).

Microalgae are recognized as a promising non-conventional feed for their high-quality nutritional composition. They are beneficial for animal growth, improve the nutritional and sensory quality of meat and enhance the immunological response and survival rates in farmed animals (de Medeiros et al., 2021; Kibria and Kim, 2019). Protein is the first desirable component in a feed and commercial microalgae species contain from 40 to 69% of proteins (Da Silva et al., 2016; Tarento et al., 2018). These proteins are rich in essential amino acids, similarly to other protein sources such as meat, milk, eggs, and soybean (Nagarajan et al., 2021). In addition, microalgae proteins meet the requirements of digestibility, absorption and quality required as animal feed (Nagarajan et al., 2021). Microalgae also contain beneficial biologically compounds such as essential amino acids, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, minerals, phenolic and other compounds (Morales et al., 2021). Microalgae production does not require the use of pesticides, avoiding finding traces of these contaminants in the feed. Their possible cultivation on infertile lands do not compete with agricultural activities. Finally, microalgae productivity is higher than conventional agricultural crops (Chen, Fufeng et al., 2021; de Medeiros et al., 2021). It results that microalgae are an emerging sustainable, and economically feasible feed resource (de Medeiros et al., 2021). The global market for microalgae is expected to grow from 32.6 billion USD in 2017 to 53.4 billion USD in 2026 (Rahman, 2020). Currently, between 22,000 and 25,000 tons of microalgae biomass are produced worldwide. Around 30% of this is dedicated to the feed industry (Levasseur et al., 2020) in hatchery production of mollusk, crustaceans, shrimps and fish farming (Dineshababu et al., 2019).

Microalgae cultivation is mostly performed in open raceway ponds (ORP), due to their reduced building and operational costs. However, ORP have limitations such as water loss due to evaporation, susceptibility to contamination, low-density cell cultures and low photosynthetic efficiency (Nagarajan et al., 2021; Suparmaniam et al., 2019). Photobioreactors (PBRs) are alternative technologies with a higher photosynthetic efficiency, lower contamination risks and

more accurate control (Barsanti and Gualtieri, 2018). PBRs reach higher productivities, but at higher building and operational costs than ORP (Suparmaniam et al., 2019). Nevertheless, both ORP and PBRs need energy-intensive and expensive biomass harvesting methods. Harvesting can reach up to 20-30% of the total biomass production cost (Mata et al., 2010). Microalgae production technologies must still progress to meet the expected biomass productivity and reduce economic and environmental costs (Dineshbabu et al., 2019). Intense research efforts in the last decades have led to new cultivation processes with enhanced performances (Li et al., 2021; Lu et al., 2021).

In this study, we focus on an alternative innovative process where biomass grows under biofilm form, attached to a mobile support. Biofilm cultivation drastically reduces the harvesting cost, offers a better light penetration, and higher areal productivity (Wang et al., 2015). The environmental benefits of biofilm processes were studied by Morales et al. (2020), revealing decisive advantages compared to standard technologies.

Life Cycle Assessment (LCA) is a useful methodology for assessing the environmental impact of products and services over their life cycle. Several works have attempted to evaluate the potential environmental assessment of microalgae-based products using LCA (see Morales et al. (2019) for a review of 30 studies). Microalgae cultivation systems can be optimized using LCA results, and many studies have used the potential of this tool for eco-designing a process. According to Tu et al. (2018), the existing LCA studies on microalgae can be divided into three groups. The first group of studies report overall impact results, where the global warming potential, cumulative energy demand and water consumption are the most common environmental indicators. The second group of studies extend the overall impacts results to the identification of the “hotspots” of the processes. The third group extend the analysis to technological options analysis. For example, by comparing different microalgae cultivation systems.

Traditionally, LCA has been used to evaluate well established technologies (Moni et al., 2020). The maturity of technology can be assessed by the concept of technology readiness level (TRL). TRL is a systematic scaling method to analyze the state of work on technologies and their readiness for commercial implementation (Moni et al., 2020). It sets nine levels of technology maturity, from the conceptualization phase (TRL 1) to the maturity stage, when the technology can be implemented in practice (TRL 9) (Dębowski et al., 2020). Most of current technologies (photobioreactors and raceways) have been estimated at high technology readiness level (TRL 7) (Tu et al., 2018).

However, in this study we focus on early-stage assessment of an emerging technology. The limited data, scale up issues and uncertainties, typical of low TRL (TRL 2-5) technologies, make it very challenging to carry out a standard LCA. In this case, methodological advances in the current LCA framework are necessary (Moni et al., 2020). Early decisions in the technology development phase can strongly reduce the impacts of the future industrial process. It has been estimated that around 80% of the total environmental impacts are associated to the design phase (Tischner and Deutschland, 2000). The study reported by Moni et al. (2020) provides a summary of the main challenges to perform LCA of low TRL technologies and key approaches to overcome these challenges. An eco-design strategy for new technologies is usually ignored in the LCA studies of microalgae systems. This approach evaluates how alternative processes and design parameters can improve the environmental footprint. The methodology used in this work can be adapted and implemented to analyze other processes with low TRLs at early design stages. It allows to analyze and identify the key parameters and to select them according to their environmental loads.

The work of Morales et al. (2020) is so far the only one assessing the impact of a biofilm cultivation technology. Here we extend this analysis to a new generation of rotating biofilm systems, including the water treatment process, which was so far never accounted for in LCA of microalgae processes.

We propose an eco-design approach based on LCA to improve the design and further reduce its environmental impact. The overall environmental impacts are evaluated for different functional units, extending the analysis to identification of hotspots of the processes and an iterative sensitivity analysis of the parameters associated. Finally, we compare the eco-designed technology with classical approaches for producing protein sources (soymeal and fishmeal), and with other microalgae cultivation technologies (ORP and rotating carpet biofilms).

2 Materials and methods

2.1 Research methodology

Figure 1 offers an overall view of the methodology followed for eco-designing the microalgal biofilm production process for an extrapolated industrial process of 20 ha. The main stages are process modelling, environmental assessment, and selection, through a sensitivity analysis, of the eco-design parameters associated to lowest footprint. For the first stage, a preliminary process design is based on pilot data from the Inalve S.A. company. These data are complemented by literature information for the technologies not yet implemented at pilot scale. An excel-based tool

was used for the estimation of mass and energy balances of the large-scale algal cultivation and downstream processing steps. The second stage is based on the process modelling outputs (inventory data). The potential environmental impacts are evaluated through two life cycle impact methods, Environmental footprint (EF) and Cumulative Energy demand (CED). Based on the environmental impacts, the relevant process steps were identified, i.e., process steps with contributions higher than 10% of the total impact in each impact category evaluated. The relevant parameters for the hotspots process steps were then identified and evaluated in a sensitivity analysis. The eco-design parameters influence the process design inventory and the environmental impacts. Thus, this methodology is iterative, i.e., for each eco-design parameter, the process modelling was updated, and the LCA impacts were re-evaluated. The eco-designed process was also compared with conventional protein meals, such as soymeal and fishmeal and with other microalgae cultivation technologies such as open raceways and rotating carpet biofilms.

Figure 1 around here.

2.2 Process design

The inventory data are based on an innovative industrial production pilot and biomass conversion process developed by Inalve S.A. for both biomass and protein production. **Figure 2** illustrates the microalgae production system planned at industrial scale. It consists in a rotating cylinder biofilm under a greenhouse. The microalgae production plant covers an area of 20 ha and involves 6 greenhouse units (205 m long and 151.5 m width). Each greenhouse contains 100 units of ponds. The plant works 320 days per year and is located in the South of France (Cote d'Azur Region). The rain and temperature averages are 894 mm/year and 12.9 Celsius degrees. The estimation of mass and energy balances of the large-scale microalgae cultivation plant were carried out from information related to mass and energy balances (and assumed parameters) to quantify the input and outputs of each subsystem. More technical details of the process design are given in **section 2.3.2.**

Figure 2 around here.

2.3 LCA of rotating biofilms

The LCA methodology is a valuable and standardized tool for determining and quantifying the inputs, outputs, energy demand and environmental impacts associated with a product throughout its life cycle (ISO, 2006a). It covers the whole product lifecycle, also considering transport and compensation of the end-of-life impacts by re-use residues/coproducts. This work follows the requirements defined by the international organization for standardization guidelines series ISO 14040 (ISO, 2006a) and ISO 14044 (ISO, 2006b) .

2.3.1 Goal and scope definition

The main objective is to carry out an LCA study with an eco-design approach to minimize the environmental life cycle impacts of an emerging rotating cylinder biofilm technology for the cultivation of *Tetraselmis suecica*. To develop an eco-designed technology, a contribution analysis of process stages (or substances) to the environmental impacts is implemented. Then, a sensitivity analysis of the key parameters specifying the design of these process stages are evaluated. Finally, the eco-design parameters are determined based on a minimum environmental burden through all the life cycle of this microalgae-based biofilm technology.

A second objective of the study is to compare the environmental impacts of the protein meal produced in the rotating biofilm system with other protein sources, such as soymeal and fishmeal. In addition, the impacts are compared to other microalgae cultivation technologies, such as open raceways and rotating carpet biofilm.

The Unit of analysis (also called Functional Unit by ISO, FU) is used to normalize the results. Inputs and outputs in the inventory and environmental footprint impact assessment profile, are thus relative to the unit of analysis. Three functional units are studied. FU1 is the biomass harvested at the output of subsystem 2, before biomass processing, while FU2 and FU3 are the algae meal after subsystem 4 as shows **Figure 3**. More precisely:

- **FU1:** 1 kg of microalgae biomass at 20%-DW, used for aqua feed.
- **FU2:** 1 kg of algae meal (or protein concentrate) at 10% moisture, used as aqua feed.
- **FU3:** 1000 WNDS (weight nutrient density score) of algae meal (or protein concentrate) at 10% moisture. This unit of analysis is the same as FU2 but considering the nutritional value in the protein concentrate. The WNDS is based on the nutrient score proposed by (Arsenault et al., 2012) considering eight nutrient parameters contributing positively or negatively to the nutrient value (protein, fiber, unsaturated fat, vitamin C, calcium,

sodium, saturated fat and added sugar). The WNDS considered for algae meal is 64.4 WNDS per 100 g algae meal (Morales et al., 2020).

A co-product (biomass residues obtained in the protein recovery subsystem (subsystem 4)) is associated to the production of FU2 and FU3, while FU1 does not induce any coproduct. The system generates 2399.43 kg/day of co-product while 63.31 kg/day of protein algae meal are fabricated. The co-product includes carbohydrates (6.87%), lipids (4.58%), ash (3.10%), starch (0.76%), water (81.12%), and a negligible protein content (3.48%). This co-product has the potential application of nutritional supplement for animal feed.

The system does not exist yet at industrial scale, and neither the product (algae meal) or the coproduct are on the market. Consequently, the price has not been established, so that an economic allocation is not computable. An economic allocation is required when using EF method according to the Product Environmental Footprint Category Rules (PEFCRs) for the classification of feed for food producing animals (European-Commission, 2018).

This study assumes a ratio between the product and co-product, defined as “price ratio” (price of algae meal divided by the price of co-product), to carry out the economic allocation. A price ratio 1 was first considered in the base case, which means an allocation of 2.57% for algae meal and 97.43% for the co-product (Note: for price ratio 1, economic allocation is equivalent to mass allocation). However, the environmental burdens for a price ratio ranging from 1 to 10 in the sensitivity analysis were also determined.

2.3.2 System boundary

A pond-to-gate assessment is performed for the microalgae-based products. These boundaries include the biomass production, infrastructure, machinery production, emissions, and transport related to the chemical and materials used in the process units. The dismantling and final disposal of machinery and infrastructure material are also included. **Figure 3** shows the life cycle boundaries and the four subsystems considered: Subsystem 1- Water treatment and fertilizers addition, Subsystem 2- Cultivation, Subsystem 3- Cell disruption and Subsystem 4-Protein recovery.

Figure 3 around here.

Subsystem 1- Water treatment and fertilizers addition: this subsystem includes the pumping, distribution (pipelines) and water treatment (WT) of large water volumes. In addition, N-based (NH_4NO_3) and P-based (P_2O_5) fertilizers are added into the cultivation ponds.

- *Pipelines*: four independent PVC pipelines are considered: two recycling water pipelines and two recirculation water pipelines. The diameter of the four pipelines is 0.25 m (4.9 mm thickness). Water pipelines recycle the water extracted from each pond to the water treatment area. They are located inside and outside the greenhouses. The pipelines inside the greenhouse have a total length of 149.5 m (per greenhouse), while the ones outside have a total length of 414 m. Recirculation pipelines bring the clarified water from the water treatment area to each pond. The clarified water covers the water lost in the evaporation, harvesting and the water recycled every 2 months (total for all ponds). These pipelines are located inside and outside the greenhouses. The pipelines inside the greenhouse are 149.5 m long in total (per greenhouse), while the pipelines outside are 210.3 m long in total.
- *Water sources*: **Figure 4** shows the water circulation per greenhouse to cover the requirement on the cultivation ponds, including the water for replacement, and the water losses in the evaporation and harvesting.

The recycling water is the replacement of the water content in all the ponds of the facility and sent to WT area, the frequency of recycling is every 2 months (equivalent to $56.25 \text{ m}^3/\text{d}$ per greenhouse). Water is recirculated ($28.13 \text{ m}^3/\text{d}$ per greenhouse) from WT and reused into the ponds, representing a recirculation fraction of 0.5. The remaining water ($28.13 \text{ m}^3/\text{d}$ per greenhouse) is composed of 60% seawater ($16.88 \text{ m}^3/\text{d}$ per greenhouse) and 40% freshwater ($11.25 \text{ m}^3/\text{d}$ per greenhouse) from river water.

In addition, water lost by evaporation and harvesting of the biomass, representing $42.34 \text{ m}^3/\text{d}$ per greenhouse, is covered by 60% seawater ($25.4 \text{ m}^3/\text{d}$) and 40% freshwater from river ($16.94 \text{ m}^3/\text{d}$) to keep the appropriate salinity for *T. suecica*.

Figure 4 around here.

- *Pumping system*: a total of four pump units per greenhouse (24 units total in the facility) of 2.2 kW pumps ($25 \text{ m}^3/\text{h}$ maximum capacity, model MXC-B50-1803/B (Pompen, 2021)) are used for recycling and recirculation water. The distribution is 1 unit of pump per pipelines with an electricity consumption $0.09 \text{ kWh}/\text{m}^3$ each (**Table 1**).

- *Water treatment (WT)*: considers filtration and UV processes. Filter system includes two units per greenhouse with a capacity 6.3 m³/h, functioning 5.6 h/d coupled to a pumping system (1.1 kW pump model MXC-B50-1803/B) (Pompen, 2021) including one unit pump per greenhouse operating around 3 h/d with a capacity of 25 m³/h and an electricity consumption of 0.09 kWh/m³. One unit 0.52 kW UV system per greenhouse was required for the water cleaning with 25 m³/h capacity operating 2.8 h/d and 0.02 kWh/m³ (see **Table 1**). The pumps are mainly made of steel, while the filter is made of polypropylene, and UV system of steel and HDPE.
- *Nutrient addition*: 0.11 kg NH₄NO₃/kg biomass (0.039 kg N/kg biomass) and 0.037 kg P₂O₅/kg biomass (0.016 kg P/kg biomass) are added to the clarified water before its distribution to the ponds.

Table 1. Machinery and equipment description. Subsystems 1, 2, 3 and 4.

Subsystem	Machinery	Mass (kg)	Units per facility	Power (kW/m ³)	Functioning time (h/d)
1	Pumping water	34	24	0.09	2.2 to 4
	Filtration system	1.5	12	-	5.6
	Pumping Filter	34	6	0.09	2.8
	Ultraviolet system	8.5	6	0.02	2.8
2	Rotor	50	600	0.1	24
	Blower	8	1200	0.05	12
	Balancer	10	1200	-	-
3	Homogenization	550	6	3.4	12
4	Centrifuge	50	6	1.35	8
	Pumping	100	18	2.1	6
	Ultrafiltration system	60	18	0.58	6

Note: Data source detailed in section 2.3.3.

Subsystem 2- Culture: the culture subsystem consists in a pond with a rotating cylinder structure, covered by a greenhouse. The rotating system consists of a rectangular pond of 200 m² (200 m length, 1 m width, 0.2 m depth), with a water content of 30.3 m³ (0.15 m depth). The main construction materials are concrete (0.12 m width wall) covered with a liner of PVC (0.9 kg/m² surface density). The rotating structure consists of two main materials: a plastic support and a fabric. The plastic support is made of PVC (0.9 kg/m²), while the fabric includes cotton (30%) and polyester (70%) (245 g/m² density). The rotating biofilm system has a cylinder shape (200 m long and 1 m diameter). The system is rotating around its axis at constant velocity functioning 24 h/d. It is set in motion by one rotor engine (0.1 kW) per pond. Two balancer units per pond (one in each extreme of the cylinder) are required to keep the cylinder aligned. The water movement (to avoid the stagnation of solid matter on the bottom and keep the constant water movement), is carried

out by two blower units per pond (0.05 kW operating 12 h/d) (see more details in **Table 1**). Six greenhouses contain 100 pond units each. The dimension of the greenhouse is 203 m x 151.5 m. It is constructed in concrete, steel, LDPE, polycarbonate, aluminum, and polyester as the model described by Pineda et al. (2020). The soil in the facility consists of sand (0.1 depth) covered by geomembrane and liner PVC.

Subsystem 3- Cell disruption system: A high-pressure homogenization was considered for cell disruption as indicated in the process described by Morales et al. (2020). The homogenizer machine details are shown in **Table 1**.

Subsystem 4- Protein recovery system: Three processing steps were considered for protein recovery: centrifugation, ultrafiltration and drying (Morales et al. (2020). More details about the this subsystem are given in **Table 1**.

2.3.3 Data source and assumptions

The estimation of input and output flows at industrial scale is crucial. The scaling up of the biofilm technology is based on interviews with experts from Inalve completed by proxies and physical calculations as described by Tsoy et al. (2020). The algae plant production simulation at large scale (20 ha) was established by adjusting a pilot plant data (288 m²) with upscaling assumptions.

Primary data provided by Inalve for the foreground processes were compiled as elementary flows for natural resource uses, material uses and emissions profiles. These primary data were obtained through engineering models, direct monitoring from experimental tests, material/product balances, stoichiometry, and other methods. The pilot cultivation plant is located in Nice (South of France). The background inventory data (e.g., electricity production, steel production, PVC production, chemical production and other components cited) is provided by the Ecoinvent database v3.4, using allocation at the point of substitution, also known as APOS system model at market level (including the transport for distribution) as shows **Table A.1 in Appendix A**.

An average targeted productivity of 30 g/m² pond/d is considered, but in the sensitivity analysis 15, 20, 25, and 35 g/m² pond/d are also evaluated. The biochemical composition for *T. suecica* was assumed independent of the seasonality variations, with 24.08% protein, 36.23% carbohydrates, 21.54% lipids, 14.58% ash and 3.57 starch (Morales et al., 2020; Schwenzfeier et al., 2011). The gaseous emissions in the cultivation pond includes nitrogen-based emissions (NO₂ and NH₃ emissions) due to the N-fertilizer consumed. The emissions factors are based on the study reported by Collet et al. (2014), 1.6 and 0.003 g NO₂ per kg N applied, for indirect and direct NO₂

emissions, respectively, while for NH₃ is 120 g NH₃ per kg N applied. The evaporation rates for the cultivation system is the one proposed by Morales et al. (2020) for a biofilm cultivation technology. The plant is operated 320 days per year, assuming an operational lifetime of 50 years. The lifetime assumed for the steel and concrete is 50 years, i.e., it does not consider of material replacement. The lifetime for all machineries is 20 years (e.g., the steel, polypropylene and/or HDPE used in machinery production), and 10 years for the other material used in the infrastructure (including PVC, LDPE, aluminum, polyester, polycarbonate, and geomembrane). Lifetime for the fabric supporting biomass growth is 4 years. Shorter lifetime for fabric and PVC are analyzed in the sensitivity analysis.

2.4 Inventory data analysis

Table 2 details the inventory for microalgae production life cycle considering for the three considered functional units. **Table 2** presents the inputs and outputs per each subsystem and includes all chemicals, machineries, electricity consumption, infrastructure, and emissions involved in the production and processing of microalgae biomass in the rotating cylinder cultivation system. Subsystem 1 includes the water requirements, fertilizers consumption, electricity and machinery involved in the water treatment and distribution. Subsystem 2 considers the infrastructure related to ponds, cylinder, and greenhouse, in addition to the electricity and machinery involved into the rotor and blower. Subsystem 2 also includes the pond emissions (NO₂, NH₃ and water evaporation). Subsystem 3 presents the machinery and electricity related to the homogenization process and Subsystem 4 involves the machinery and electricity consumption regarding the protein recovery.

Table 2. Resource use and emissions profile for 20 ha for 1 kg microalgae biomass (FU1), 1 kg algae meal (FU2) AND 1000 WND5 algae meal (FU3). Biomass productivity: 30 g/m²/d

	FU2	FU1	FU3	UNIT
Subsystem 1				
Inputs				
Freshwater	0.45	9.40·10 ⁻³	0.69	m ³
Seawater	0.67	1.41·10 ⁻²	1.04	m ³
NH ₄ NO ₃ , as N	0.37	7.80·10 ⁻³	0.57	kg
P ₂ O ₅ per GH	0.35	7.33·10 ⁻³	0.54	kg
Steel	4.42·10 ⁻⁴	9.33·10 ⁻⁶	6.86·10 ⁻⁴	kg
Polypropylene	7.40·10 ⁻⁶	1.56·10 ⁻⁷	1.15·10 ⁻⁵	kg
HDPE	5.18·10 ⁻⁵	1.09·10 ⁻⁶	8.05·10 ⁻⁵	kg
PVC	2.92·10 ⁻²	6.17·10 ⁻⁴	4.54·10 ⁻²	kg
Electricity	0.55	1.16·10 ⁻²	0.86	kWh
Outputs				
Clean water to environment	0.44	9.40·10 ⁻³	0.69	m ³
Steel to treatment	4.42·10 ⁻⁴	9.33·10 ⁻⁶	6.86·10 ⁻⁴	kg
Polypropylene to treatment	7.40·10 ⁻⁶	1.56·10 ⁻⁷	1.15·10 ⁻⁵	kg

HDPE to treatment	$5.18 \cdot 10^{-5}$	$1.09 \cdot 10^{-6}$	$8.05 \cdot 10^{-5}$	kg
PVC to treatment	$2.92 \cdot 10^{-2}$	$6.17 \cdot 10^{-4}$	$4.54 \cdot 10^{-2}$	kg
Subsystem 2				
Inputs				
Coton	$5.7 \cdot 10^{-2}$	$1.20 \cdot 10^{-3}$	$8.85 \cdot 10^{-2}$	kg
Polyester	0.13	$2.83 \cdot 10^{-3}$	0.21	kg
PVC	0.65	$1.38 \cdot 10^{-2}$	1.01	kg
Concrete	2.39	$5.04 \cdot 10^{-2}$	3.71	kg
Geomembrane	$1.59 \cdot 10^{-2}$	$3.36 \cdot 10^{-4}$	$2.48 \cdot 10^{-2}$	kg
Sand	4.55	$9.61 \cdot 10^{-2}$	7.07	kg
Steel	$4.66 \cdot 10^{-2}$	$9.84 \cdot 10^{-4}$	$7.24 \cdot 10^{-2}$	kg
LDPE	$1.18 \cdot 10^{-2}$	$2.50 \cdot 10^{-4}$	$1.84 \cdot 10^{-2}$	kg
Polycarbonate	$2.43 \cdot 10^{-2}$	$5.13 \cdot 10^{-4}$	$3.77 \cdot 10^{-2}$	kg
Aluminium	$1.18 \cdot 10^{-3}$	$2.50 \cdot 10^{-5}$	$1.84 \cdot 10^{-3}$	kg
Electricity	5.72	0.12	8.89	kWh
Outputs				
Coton to treatment	$5.7 \cdot 10^{-2}$	$1.20 \cdot 10^{-3}$	$8.85 \cdot 10^{-2}$	kg
Polyester to treatment	0.13	$2.83 \cdot 10^{-3}$	0.21	kg
PVC to treatment	0.65	$1.38 \cdot 10^{-2}$	1.01	kg
Concrete to treatment	2.39	$5.04 \cdot 10^{-2}$	3.71	kg
Geomembrane to treatment	$1.59 \cdot 10^{-2}$	$3.36 \cdot 10^{-4}$	$2.48 \cdot 10^{-2}$	kg
Steel to treatment	$4.66 \cdot 10^{-2}$	$9.84 \cdot 10^{-4}$	$7.24 \cdot 10^{-2}$	kg
LDPE to treatment	$1.18 \cdot 10^{-2}$	$2.50 \cdot 10^{-4}$	$1.84 \cdot 10^{-2}$	kg
Polycarbonate to treatment	$2.43 \cdot 10^{-2}$	$5.13 \cdot 10^{-4}$	$3.77 \cdot 10^{-2}$	kg
Aluminium to treatment	$1.18 \cdot 10^{-3}$	$2.50 \cdot 10^{-5}$	$1.84 \cdot 10^{-3}$	kg
Evaporation water	0.63	$1.33 \cdot 10^{-2}$	0.98	m ³
N ₂ O emissions	$6.02 \cdot 10^{-4}$	$1.27 \cdot 10^{-5}$	$9.35 \cdot 10^{-4}$	kg
NH ₃ emissions	$4.44 \cdot 10^{-2}$	$9.36 \cdot 10^{-4}$	$6.89 \cdot 10^{-2}$	kg
Subsystem 3				
Inputs				
Steel	$1.36 \cdot 10^{-3}$		$2.11 \cdot 10^{-3}$	kg
Electricity	0.65		1.01	kWh
Outputs				
Steel to treatment	$1.36 \cdot 10^{-3}$		$2.11 \cdot 10^{-3}$	kg
Subsystem 4				
Inputs				
Steel	$2.49 \cdot 10^{-3}$		$3.87 \cdot 10^{-3}$	kg
Electricity	0.30		0.47	kWh
Outputs				
Steel to treatment	$2.49 \cdot 10^{-3}$		$3.87 \cdot 10^{-3}$	kg

2.5 Comparison cases

The conventional protein sources considered are soymeal and fishmeal. These inventories were obtained from EF database for soymeal and fishmeal for Europe (EU-28 + EFTA). Also, two alternative technologies to produce algae meal are analyzed: rotating carpet algae biofilm (RCAB) and open raceways (ORP), on the basis of the work of Morales et al. (2020) extended to take water treatment into account. Comparison cases comply with the same allocation (economic allocation), functional unit (1 kg algae meal) and system boundaries (pond-to-gate) of case study (See **Appendix B** for more details).

2.6 Environmental impact assessment methods

SimaPro v9.3.0.3 was used for modelling the inventory data. The characterization factors from two methods were used: EF v3.0 and CED v1.11. EF is a harmonized methodology for environmental footprint normalization at European level. EF method includes 14 impact categories. However, there are 6 impact categories listed that are defined as most relevant for feed products (Skretting Group, internal communication). These categories are Climate change (CC), Particulate matter (Respiratory inorganics, RI), Acidification (terrestrial and freshwater) (A), Land use (LU), Eutrophication terrestrial (ET) and Water scarcity (WS).

CED aims to investigate the energy use throughout the life cycle of a product or a service. This includes the direct uses as well as the indirect or grey consumption of energy from e.g. construction materials or raw materials (Hischier et al., 2010). The CED-indicator is split up into eight categories. This study focuses on the fossil CED subcategory (CED fossil) and the CED total (non-renewable + renewable). More details about the environmental impact assessment methods are given in **Appendix C**.

3 Results and discussion

3.1 Potential environmental impacts

Table 3 presents the EF environmental impacts, CED and electricity consumption for the three functional units. Moreover, **Table 4** details the percentage of contributions per subsystem and its processes involved. Subsystems 1 and 2 are the main contributors in all the impact categories evaluated, around 99%, while for the subsystem 3 and 4 the contributions are lower than 1%.

Table 3. Life cycle impact per impact categories (EF and CED) and electricity consumption, for 1 kg microalgae biomass (FU1), 1 kg algae meal (FU2) and 1000 WNDS algae meal (FU3). Plant area: 20 ha. Biomass productivity: 30 g/m² pond/d.

Impact Categories		FU1	FU2	FU3
Climate change	kg CO ₂ eq	0.18	0.21	0.33
Respiratory inorganics (Particulate matter)	kg PM2.5 eq	3.12·10 ⁻⁸	3.80·10 ⁻⁸	5.93·10 ⁻⁸
Acidification terrestrial and freshwater	mol H+ eq	4.01·10 ⁻³	4.90·10 ⁻³	7.62·10 ⁻³
Eutrophication terrestrial	mol N eq	1.53·10 ⁻²	1.90·10 ⁻²	2.91·10 ⁻²
Land use	Kg C deficit	2.68	3.27	5.11
Water scarcity	m ³ depriv.	3.81·10 ⁻²	4.61·10 ⁻²	7.25·10 ⁻²
CED total	MJ	4.72	5.74	10.1
CED fossil	MJ	2.39	2.91	4.52
Electricity consumption	kWh	0.13	7.23	11.23

Subsystem 1 is one of the main contributors in CC category (49.9%), followed by subsystem 2 (49.3%). The fertilizers production are the main sources of impact in subsystem 1, with a contribution of 0.084 kg CO₂ eq/FU2 coming from NH₄NO₃ production and 0.021 kg CO₂ eq/FU2 coming from P₂O₅ production, while it is the fabric production for subsystem 2, where 0.033 kg CO₂ eq/FU2 results from cotton production and 0.019 kg CO₂ eq/FU2 is coming from polyester production. The production and application of N-based fertilizers, such as NH₄NO₃, contributes to large quantities of greenhouse gases emissions and is based on high energy consumption (Brentrup and Pallière, 2008). N₂O represent 50% and more of all emissions during the fertilizer production affecting CC, followed by CO₂ emissions representing about 1-3% (Hasler et al., 2015). N₂O emissions essentially occur during the production and post-application of fertilizers, while CO₂ emissions are mainly related to the combustion of diesel used for transportation and farm operations (Hasler et al., 2015).

Analyzing RI impact category, subsystem 2 contributes to 81% and subsystem 1 to 18.7%. In Subsystem 2, the ammonia (NH₃) gaseous emissions are the main contributor (2.4·10⁻⁸ kg PM2.5/FU2) representing 63% of the total contribution. The NH₄NO₃ production is the main source in subsystem 1, with a contribution of 4.3·10⁻⁹ kg PM2.5/FU2.

Similar behaviors can be analyzed for the A and ET categories, with the subsystem 2 as the main source of impacts (85% for A and 89.9% for ET), where the emission of NH₃ is the main contributor to both categories, 70.5% of the total impact in A (0.0034 mol H⁺/FU2) and 82.2% for ET (0.015 mol N eq/FU2) as shows **Table 4**. In both categories subsystem 1 represents 14.9% for A and 10% for ET, where most of the contributions are due to NH₄NO₃ production, representing 9.4% and 7.7% of the total impact for A and ET, respectively.

The application of N-based fertilizers implies nitrogen (N) losses, such as ammonia volatility (Pereira et al., 2021). The eutrophication of water bodies and the acid rain are among the environmental impacts caused by the NH₃ emissions, specially contributing to the categories A, ET and RI. (Hasler et al., 2015).

For LU and WS categories the main impact source is subsystem 2, representing 72.7% of LU and 89.9% of WS, mainly due to the cotton production for both impact categories: 56.7% (or 1.85 kg C deficit/FU2) and 85.5% (or 0.040 m³ depriv. water/FU2) of total contribution to LU and WS, respectively. On the other hand, the P₂O₅ production in subsystem 1 is the second main contributor in both categories, with 22.2% of the total LU and 9.5% for WS.

Cotton production requires large quantities of water during the agricultural phase, where fertilizer and pesticides are also consumed, which can lead to WS, ET and toxicity. Global cotton production account for 3% of land use, 3% of global agricultural water, and 5.2% of global pesticide sales (Chen, Fangli et al., 2021). Nevertheless, the most contaminative process in the life cycle of cotton production is dyeing, due to the numerous inputs of chemicals, e.g., dyes, wetting agents, and softener (Chen, Fangli et al., 2021).

Table 4. Percentage of environmental contributions to EF and CED impact categories per process. FU2: 1 kg algae meal (around here)

Subsystem	Process	CC	RI	A	ET	LU	WS	CED total	CED fossil	
S1	S1-Water consumption	Seawater	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
		Freshwater from river	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	S1-Machinery production	Machinery production	0.01	0.01	0.00	0.00	0.00	0.00	0.01	
		Machineries end life treatment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	S1-Pipelines production	Pipelines production	0.00	0.33	0.26	0.10	0.00	0.00	0.92	
		Pipelines end life treatment	0.42	0.01	0.01	0.01	0.04	0.00	0.03	
	S1-Nutrients production	NH4NO3 production	39.48	11.43	9.40	7.73	4.52	0.09	9.98	
		P2O5 production	9.67	6.82	5.10	2.11	22.18	9.49	6.40	
		Filtration pumping	0.06	0.02	0.01	0.01	0.04	0.03	0.57	
	S1-Electricity consumption	Water distribution pumping	0.28	0.09	0.06	0.03	0.17	0.14	2.52	
		UV system	0.01	0.00	0.00	0.00	0.01	0.01	0.14	
									0.01	
S2	S2-Cylinder production	Cotton production	15.42	3.52	3.89	2.36	56.74	85.46	8.59	
		Polyester production	8.93	2.75	1.98	1.30	8.76	2.36	6.91	
		PVC production	0.00	3.17	2.50	0.97	0.00	0.00	8.83	
		Cotton and Polyester end life treatment	1.78	0.13	0.28	0.36	0.04	-0.01	0.16	
		PVC end life treatment	4.04	0.13	0.14	0.09	0.41	0.01	0.29	
	S2-Pond concrete production	PVC production	0.00	1.64	1.30	0.50	0.00	0.00	4.57	
		Concrete production	2.67	0.70	0.46	0.37	2.32	0.17	0.94	
		PVC and concrete end life treatment	2.34	0.27	0.16	0.13	0.85	0.01	0.38	
	S2-Greenhouse production	Geomembrane (soil)	0.12	0.03	0.03	0.01	0.22	0.01	0.08	
		Sand (soil)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
		PVC (soil)	0.00	2.54	2.00	0.78	0.00	0.00	7.06	
		Concrete production	0.01	0.00	0.00	0.00	0.01	0.00	0.00	
		Steel production	0.60	0.28	0.13	0.07	0.19	0.00	0.22	
		LDPE production	0.45	0.10	0.09	0.04	0.19	0.01	0.50	
		Polycarbonate production	2.54	1.05	0.39	0.18	0.03	0.00	1.18	
		Aluminum production	0.13	0.07	0.04	0.02	0.09	0.00	0.08	
		Polyester production	0.08	0.02	0.02	0.01	0.08	0.02	0.06	
		Materials end life treatment	3.85	0.14	0.13	0.09	0.35	0.01	0.26	
		S2-Machinery production	Machinery production	0.50	0.23	0.11	0.06	0.16	0.00	0.19
			Machinery end life treatment	0.02	0.02	0.01	0.01	0.00	0.00	0.01
	S2-Electricity consumption	Rotator motor	2.45	0.79	0.57	0.26	1.46	1.25	22.18	
		Blower	1.25	0.40	0.29	0.13	0.74	0.64	11.31	
	S2-Gaseous emissions	Evaporation water emissions	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
		N2O emissions	2.16	0.00	0.00	0.00	0.00	0.00	0.00	
		NH3 emissions	0.00	63.05	70.49	82.19	0.00	0.00	0.00	
	S3	S3-Machinery production	0.03	0.01	0.01	0.00	0.01	0.00	0.01	
		Homogenizator end life treatment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	S3-Electricity consumption	Homogenizator	0.42	0.14	0.10	0.04	0.25	0.21	3.81	
	S4	S4-Machinery production	Machinery production	0.06	0.03	0.01	0.01	0.02	0.00	0.02
			Machineries end life treatment	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S4-Electricity consumption		Centrifuge	0.03	0.01	0.01	0.00	0.02	0.02	0.30	
		Pumping	0.05	0.02	0.01	0.01	0.03	0.03	0.47	
		Ultrafiltration	0.10	0.03	0.02	0.01	0.06	0.05	0.92	
Drum dryer	0.01	0.00	0.00	0.00	0.01	0.00	0.09			

60% < 30-59.9% 10-29.9% 5-9.9% 1-4.9% 0.01-0.99% 0.01% >

Table 4 also details the contribution per process as percentage of total CED, including renewable and nonrenewable energy carriers; and as percentage of fossil CED. In total CED for FU2, the main contributor was Subsystem 2 (73.8%, 4.24 MJ/FU2), followed by Subsystem 1 (20.6%, 1.18

MJ/FU2). The electricity consumption in Subsystem 2 represents the main source of total CED (1.92 MJ/FU2), with 22.2% and 11.3% from rotator motor and blower, respectively. The second main contributor is the cylinder material (1.40 MJ/FU2): cotton, polyester, and PVC. Finally, fertilizers production represents the third contribution (0.94 MJ/FU2), i.e., NH_4NO_3 (10%) and P_2O_5 (6.4%). Other minor contributions (lower than 7%) result from PVC for subsystem 2 for the soil coverage of greenhouses and ponds.

The most impacting sources to fossil CED come from Subsystem 2 (68.9%, 2 MJ/FU2) and Subsystem 1 (30.6%, 0.89 MJ/FU). The contribution from Subsystem 2 is due to the cylinder production (1.14 MJ/FU2) and especially from the used cotton, polyester, and PVC, with similar contribution between them (ranging from 11.3 to 15% each). For Subsystem 1 the main source of fossil CED is the fertilizer production (0.83 MJ/FU2), where NH_4NO_3 production contributes with 18.8% and P_2O_5 with 9.9% of the total. In addition, contributions of 12% result from PVC required in the subsystem 2 for the soil coverage of greenhouses and ponds.

Table 5 represents the electricity consumption per subsystem. The main electricity consumer is subsystem 2, mainly due to the rotors and blowers. Despite the low power for these machines (0.1 kW per rotor and 0.05 kW per blower), they consume an elevated amount of energy due to the large number of units and long working time. The 100 units of rotors (240 kWh/d) are continuously working, and the 200 units of blowers (122.4 kWh/d) are working 1 minute ON, 1 minute OFF to ensure the fluid movement. This electricity demand can be compared with the homogenizer in subsystem 3 (the only machinery in this subsystem), which has a higher power (3.44 kW), but only one unit in the plant operating 12 h/d, therefore representing 41.1 kWh/d of electricity.

Table 5. Electricity consumption per subsystem (kWh/FU) for 20 ha for 1 kg microalgae biomass (FU1), 1 kg algae meal (FU2) AND 1000 WND5 algae meal (FU3). Biomass productivity: 30 g/m² pond/d

Subsystem	FU1	FU2	FU3
S1-Water treatment/Fertilizers addition	0.012	0.55	0.86
S2-Cultivation	0.12	5.72	8.89
S3-Cell disruption	-	0.65	1.01
S4-Protein recovery	-	0.30	0.47
TOTAL	0.13	7.23	11.23

3.2 Identification of key parameters

The contributions of all the sub-processes to the impacts of 1 kg algae meal are highlighted in **Table 4** to determine the top stressors (or key parameters). The processes with contributions

higher than 10% were identified, and the main parameters driving the key process impact were listed in **Table 6**. The processes with contributions higher than 10% are (see **Table 4**):

- NH_4NO_3 production (subsystem 1): contributing to CC, RI and CED fossil.
- P_2O_5 production (subsystem 1): affecting to LU.
- Cotton production (subsystem 2): critical process to CC, LU, WS and CED fossil.
- Polyester production (subsystem 2): negatively affecting CED fossil
- Plastic production (subsystem 2): represent adverse effect on CED fossil.
- Rotator motor (subsystem 2): electricity consumption affecting CED total category
- Blower motor (subsystem 2): electricity consumption contributing CED total category
- NH_3 emissions (subsystem 2): emissions to atmosphere stressing to RI, A and ET categories.

Table 6 presents the key parameters driving these impacts, which were analyzed into a sensitivity analysis. The sensitivity analysis is used to test the impact of the inventory data and parameters on the results. LCA is an iterative approach, to more efficiently assess and refine the consequences of the process design in the environmental footprint estimate. Then, the best choices for eco-designed parameters can be made to minimize the environmental loads. The most impacting parameters for the biofilm technology are described below together with the eco-design strategy:

- **N-fertilizer source:** NaNO_3 is considered as an alternative source of Nitrogen (instead of NH_4NO_3) since nitrate can be used by *T. suecica*
- **P-fertilizer source:** $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ replaces the initial Phosphorous nutritional source (P_2O_5).
- **Lifespan Fabric:** a fabric of better quality was chosen, so that its lifetime was doubled (8 years instead of 4 years).
- **Fabric material source:** Alternative fabric with better environmental performances (nylon, oleofines and polyester, instead of a mix of cotton and polyester) have been proved to efficiently support biofilm growth (Venable and Podbielski (2019)).
- **Lifespan and thickness plastic used in cylinder:** The amount of plastic used in the cylinder depends on two parameters: the thickness (related to the density) and the lifespan. Both require a joint analysis due to the trade-off between quality and durability. Two lifespans are evaluated, 8 and 16 years instead 10 years. These scenarios were chosen by considering a plastic replacement at the same time than fabric support is replaced (every 8 years). The scenarios of lifespan of 16 years keeps the same thickness as base case (1 mm, 0.9 kg/m² surface density). However, for the scenario of 8 years of lifespan the thickness was reduced by

50%, i.e., 0.5 mm. Thus, the plastic surface density assumed is 0.45 kg/ m². This reduction in the thickness is considered under the assumption that a better quality for plastic is possible.

- **Power rotor:** reduction of the rotor power from 0.1 kW to 0.03 kW, as obtained in theoretical calculations by Morales et al. (2020).
- **Power blower:** reduction of the blower power by 50% reduced to 0.025 kW.
- **Lifespan PVC used in soil:** the lifespan PVC for greenhouse soil coverage was increased to be replaced every 25 or 50 years, instead of every 10 years as in the base case. These assumptions assume a PVC of better quality as described in the study of Gabarrell et al. (2015).
- **NH₃ emission factor:** NH₃ emissions to atmosphere due to N-fertilizer is a theoretical value reported by Collet et al. (2014). However, this parameter can be handled by controlling pH in the medium. To evaluate the effect of this parameter into the environmental footprint, a value 25% lower was assumed, i.e., reducing the NH₃ emission factor from 120 g NH₃/kg N to 90 g NH₃/kg N. It is important to notice that NH₃ emissions are related to the ammonium content in the N-fertilizer. Thus, by using NaNO₃ as a main source of nitrogen this emission is not generated.

Table 6. Eco-design parameters determined in the LCA study.

Process		Parameter	Base case		Alternative		
			Material	Value	Technology or material	Value	
S1	Nutrients production	NH ₄ NO ₃ Production	N-Fertilizer source	NH ₄ NO ₃	0.039 kg N/kg biomass	NaNO ₃	0.039 kg N/kg biomass
		P ₂ O ₅ Production	P-Fertilizer source	P ₂ O ₅	0.016 kg P/kg biomass	NaH ₂ PO ₄ H ₂ O	0.016 kg P/kg biomass
S2	Cylinder production	Cotton production	Lifespan fabric	-	4 years	-	8 years
			Fabric material source	Inalve fabric*	Mix cotton and polyester	Nylon	100 %
		Oleofin				100 %	
	Polyester	100 %					
	Plastic production (cylinder)	Lifespan and Thickness Plastic	-	10 years and 1 mm	-	8 years and 0.5 mm	
					-	16 years and 1 mm	
	Electricity consumption	Rotator motor	Power Rotator	-	0.1 kW	-	0.03 kW
Blower motor		Power Blower	-	0.05 kW	-	0.025 kW	
Greenhouse production	PVC production (soil)	Lifespan PVC	-	10 years	-	25 years	
					-	50 years	
Gaseous emissions	NH ₃ emission	NH ₃ emission factor	-	120 g NH ₃ /kg N	-	90 g NH ₃ /kg N	

*Confidential information

3.3 Sensitivity analysis

Figure 5 shows the sensitivity for 13 scenarios in comparison to the base case for FU2. For CC category, the most sensitive parameters are the nutrients source and the parameters related to fabric production, such as lifespan and material source. Replacing the N-based and P-based

fertilizers increases the CC impact in 100% and 20% respectively. While increasing the lifespan of fabric from 4 years to 8 years decreases the burdens for CC in 13%. Changing the fabric source from cotton and polyester mix to 100% nylon, 100% polyester or 100% oleofin as support materials decreases the CC in the range 4.4% to 18%, the lower environmental loads being obtained with oleofin. Other parameters lead to an increase in the CC impact or have negligible contributions. Similar behavior can be seen in WS and LU categories, where the fabric replacing cotton (by nylon, polyester or oleofin) and lifespan of the fabric are the main parameters to reduce these impacts, while fertilizer replacement negatively affects WS and LU. The extension of the lifespan from 4 to 8 years reduces the impact in WS and LU by 44% and 33%, respectively. However, the most sensitive parameter for these categories is the replacement of the fabric material, with a reduction higher than 84% for WS and 53% for LU.

The A, ET and RI categories have similar performances. They are mainly affected by the fertilizer selection and the NH_3 emission factor. A significant decrease between 48% to 68% is achieved by changing the N-fertilizer (NaNO_3 instead NH_4NO_3) due to leak of NH_3 emissions when NaNO_3 is applied. Also, a reduction ranging from 15.7% to 20.5% is observed in these categories when the NH_3 emission factor decreases by 25%. The variation in the other parameters stays lower than 5% for these impact categories.

Fossil CED and total CED have a similar behavior, i.e., these categories considerably increase with the fertilizer change and decrease for all the other parameters. The reductions are lower than 11% for most of the parameters in the cumulative demand categories. The electricity consumption was only affected when reducing the power in rotor and blower, decreasing by 36.7% and 13.6%, respectively.

Figure 5 around here.

After the sensitivity analysis, the parameters selected for the eco-designed system were:

- N-fertilizer source: NH_4NO_3 .
- P-fertilizer source: P_2O_5 .
- Lifespan fabric: 8 years.
- Fabric material source: 100% Oleofin.
- Lifespan and thickness plastic: 8 years and 0.5 mm.
- Power rotator: 0.03 kW.

- Power blower: 0.025 kW.
- Lifespan PVC: 25 years.
- NH₃ emission factor: 90 g NH₃/kg N.

The base case in this study is price ratio (Protein price/Animal feed price) of 1, i.e., 3% for algae meal and 97% for co-product as economic-based allocation. Price ratio ranging between 1 and 10 were also analyzed. **Figure 6** shows the variation for CC, RI, A, ET, LU and WS at different productivities (between 15 and 35 gr/m² pond/d) according to the price ratios for 1000 WNDS as functional unit. **Figure 6** also presents the environmental impacts for soymeal and fishmeal, the conventional protein sources. **Appendix D** shows this analysis for the base case before eco-design. Lower productivity has a moderated consequence in the environmental impacts at lower price ratios, but the range of impact values increases for different productivities at higher price ratios. At price ratio of 1, CC is 0.32 kg CO₂eq/kg algae for 15 g/m² pond/d (0.24 kg CO₂eq/kg algae meal for 35 g/m² pond/d). However, for a price ratio of 10, CC is not significantly lower than the one for fishmeal (3.34 kg CO₂eq/kg protein). On the other hand, a price ratio higher than 125 for 15 g/m² pond/d and 900 for 35 g/m² pond/d lead to the same CC impact as soymeal (9.0 kg CO₂eq/kg protein).

For RI, A and ET categories, the impact is lower than fishmeal and soymeal when the price ratio is lower than 4. LU and WS impacts are not significantly affected by the price ratio and the values stay lower than soymeal and higher than fishmeal.

Figure 6 around here.

3.4 Eco-designed process and its comparison with other case studies

The eco-designed process is compared on **Figure 7a** with the initial process, with others algae-based processes (rotational carpet algal biofilm and open raceway) and with other protein sources (soymeal and fishmeal) (see **Appendix E** for 1000 WNDS protein meal as functional unit). For all the categories, the conventional protein meals (fish meal and soybean) are the most impacting followed by ORP production system, while the rotational biofilm systems (base case, eco-designed and RCAB) have the lowest impact. The lowest impacts within the rotational biofilm systems are achieved for the eco-designed process in all the impact categories evaluated.

Regarding CC category, the eco-designed process reduces by 27.6% the impacts in comparison to the process before eco-design and by 86.3% with respect to RCAB.

Similar behaviors are obtained in RI, A and ET, where the base case eco-designed achieves impacts 25%, 25.8% and 25.2% respectively lower than base case before eco-design and 45.4%, 46.4% and 33.9% lower than RCAB. For CED total, eco-design reduces by 50.3% and 49.7% compared to the base case and RCAB, respectively. Nevertheless, the highest differences in the impacts are for LU and WS categories. The eco-design leads to almost 3 and 4-fold lower LU impacts than before eco-design and RCAB, respectively and, 9 and 37-fold lower for WS impacts.

When comparing the environmental footprint after eco-design to other protein sources, such as soymeal and fishmeal, a reduction in most of the categories is obtained (**Figure 7a**). The impacts in CC category achieves reduction higher than 93.8% by using algae meal instead of fishmeal or soymeal as aquafeed. Reductions higher than 99.7% are obtained for LU and WS by using algae meal eco-designed instead of soymeal. However, even though the impacts on LU and WS for fishmeal are negligible, the values are similar to the ones obtained by eco-designed algae meal (2.4 kg C deficit/FU3 and 1.7 kg C deficit/FU3 for LU and $-8.9 \cdot 10^{-2} \text{ m}^3 \text{ depriv./FU3}$ and $8.4 \cdot 10^{-3} \text{ m}^3 \text{ depriv./FU3}$ for WS, for fishmeal and eco-designed algae meal, respectively). Reductions between 70.8% and 87.1% are reached for eco-designed algae meal in comparison to fishmeal and soymeal for RI, A, E and CED categories.

Figure 7b compares the impacts of 1 kg algae biomass (FU1). The higher environmental loads are obtained by algae meal produced through open raceways, followed by rotational carpet biofilm. The environmental impact for the algae meal produced by rotational cylinder biofilm shows the lower values, eco-designed technology is the less impacting option for the environment. More details are in **Appendix F**.

Figure 7 around here.

3.5 Eco-design for technologies with low TRL

It is important to note that the scaling up is a crucial aspect for a realistic LCA as many authors have previously discussed (Pechsiri et al., 2023; Sandmann et al., 2021). Usually, many technological choices on the pilot are not rationalized or guided by environmental considerations. This does not have consequences for most of the parameters, but for some it can radically change the process impact. This is typically the objective of the eco-design phase, to track the elements that definitely need an optimization before upscaling. Physical considerations are then used to accurately identify the limits in which these parameters can reasonably lie. The proposed

methodology can be adapted and implemented to analyze processes with low TRLs at early design stages. It allows to analyze and identify the key parameters and selecting them according to their environmental loads.

4 Conclusions

This study proposes an LCA with an eco-design approach dedicated to emerging technologies at early design stages, for which extrapolation at industrial scale is necessary. This study represents a methodological approach for the identification and evaluation of the main eco-design parameters. When applied to the microalgal production process in biofilm, the most impacting eco-design parameters are NH₃ emission factor, power blower, power rotator, thickness and lifespan for plastic used in the cylinder, fabric material source and lifespan fabric. They were targeted through a sensitivity analysis, resulting in an environmental impact reduction between 25% and 27.6% for CC, RI, A, ET and a reduction of 65.7% for LU, 88.3% for WS and 50.3% CED. In the end, algae meal from eco-designed rotating biofilm has an environmental impact reduced by at least 70.8% in comparison to conventional aquafeeds (fishmeal and soymeal).

However, all the impacts evaluated for this innovative technology remain highly dependent of the economic price of the main product (algae meal) and its co-product, due to the economic allocation. The trickiest step in the extrapolation phase is therefore to assess a realistic future market price for these non-conventional feeds and their co-products, at a stage where they are not produced. The assumptions on the process economics must therefore be later updated to consolidate the impact analysis. The methodology proposed can guide technology developers to understand the implications of design choices on the future environmental impacts.

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Abbreviations in this paper

A: Acidification
APOS: Allocation at the Point Of Substitution
CC: Climate Change
CED: Cumulative Energy Demand
EF: Environmental Footprint
ET: Eutrophication Terrestrial
FU: Functional Unit
LCA: Life Cycle Assessment
LU: Land Use
ORP: Open Raceway Pond
PEFCRs: Product Environmental Footprint Category Rules
RCAB: Rotating Carpet Algae Biofilm
RI: Respiratory Inorganic
TRL: Technology Readiness Level
WNDS: Weight Nutrient Density Score
WS: Water Scarcity
WT: Water Treatment